



**RÉPUBLIQUE
FRANÇAISE**

*Liberté
Égalité
Fraternité*

EXAMEN DE QUALIFICATION EN VUE DE L'INSCRIPTION SUR LA LISTE DES PERSONNES QUALIFIÉES EN PROPRIÉTÉ INDUSTRIELLE

MENTION BREVETS D'INVENTION

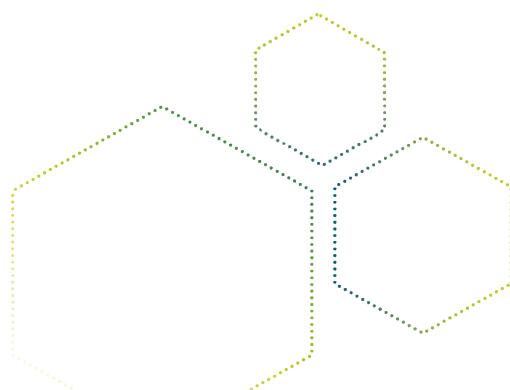
SESSION 2022

02.03.2023

Secteur chimie/pharmacie

ÉPREUVES ÉCRITES et ORALES

inpi



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AVERTISSEMENT

L'Institut national de la propriété industrielle publie pour chaque session d'examen des annales destinées à donner aux candidats une base pour leur préparation à cet examen.

Ces annales regroupent les textes des épreuves écrites de l'examen.

Ces annales sont publiées par secteur technique.

Cet examen est mis en place conformément à l'arrêté du 23 septembre 2004 modifié portant application des dispositions des articles R. 421-1, R. 421-2 et R. 421-5 à R. 421-8 du code de la propriété intellectuelle.

Instructions aux candidats

PREMIÈRE ÉPREUVE ÉCRITE

Dans cette épreuve, le candidat doit supposer qu'il a reçu de son client le courrier annexé au sujet, qui comporte la description d'une invention pour laquelle son client souhaite obtenir un brevet français, ainsi que des renseignements et/ou documents relatifs à l'état de la technique le plus pertinent dont son client a connaissance.

Le candidat doit accepter les faits exposés dans le sujet de l'épreuve et fonder ses réponses sur ces faits. Il décide sous sa propre responsabilité s'il fait usage de ces faits, et dans quelle mesure.

Le candidat doit admettre que l'état de la technique, dans le domaine spécifique de l'invention que lui soumet son client, est effectivement celui qui est indiqué dans l'épreuve et/ou ses documents annexes, et que cet état de la technique, le cas échéant complété des connaissances générales nécessaires sur lesquelles il pourrait s'appuyer de façon implicite, est exhaustif.

Il est demandé au candidat de rédiger sauf instruction contraire, en les présentant dans cet ordre : (1) la partie introductive de la description de la demande de brevet souhaitée par le client, et (2) un jeu de revendications comprenant au moins une revendication indépendante et quelques revendications dépendantes.

Il est entendu par partie introductive :

- l'indication du domaine technique auquel se rapporte l'invention ;
- l'indication de l'état de la technique antérieure, connu du demandeur, pouvant être considérée comme utile pour l'intelligence de l'invention et pour l'établissement du rapport de recherche ; les documents servant à refléter l'état de la technique antérieure sont, autant que possible, cités ;
- un exposé de l'invention, telle que caractérisée dans les revendications, permettant la compréhension du problème technique ainsi que la solution qui lui est apportée ; sont indiqués, le cas échéant, les avantages de l'invention par rapport à l'état de la technique antérieure.

La ou les revendication(s) indépendante(s) sera(ont) rédigée(s) de façon à donner au client la protection la plus étendue possible, tout en respectant les critères de brevetabilité et les exigences formelles applicables.

Les revendications dépendantes, seront rédigées de façon à définir une position de repli utile pour le cas où un art antérieur affectant la généralité de chaque revendication indépendante serait découvert après le dépôt de la demande brevet.

L'exercice de rédaction demandé se limite à une seule demande de brevet français, qui devra satisfaire aux exigences d'unité d'invention. Au cas où, dans la pratique, il demanderait la protection d'autres inventions en déposant une ou plusieurs autres demandes distinctes, le candidat devra indiquer succinctement, dans une troisième partie, l'objet de la principale revendication indépendante de chaque autre demande distincte, la rédaction détaillée de telles revendications indépendantes n'étant cependant pas requise.

Enfin le candidat peut, sauf instruction contraire du sujet, indiquer dans une note séparée les raisons du choix de sa solution, et par exemple expliquer pourquoi il a choisi telle ou telle forme de revendication, telle ou telle caractéristique pour une revendication indépendante, tel ou tel élément particulier de l'état de la technique comme point de départ, toute note de ce genre devant cependant rester brève.

SUJET DE LA PREMIERE EPREUVE ECRITE

Vous trouverez, ci-joint, une note technique transmise par votre client, la société UVS SA, spécialisée dans le domaine des produits cosmétiques.

Une recherche d'antériorités a été effectuée par votre client, qui a permis d'identifier les documents D1 (WO85/0111) et D2 (US 4,000,000).

Après avoir pris connaissance de l'ensemble des documents, vous rédigerez le jeu de revendications d'une demande de brevet français ainsi que la partie introductive de sa description, en veillant à protéger au mieux les intérêts de votre client tout en respectant les critères de brevetabilité, de clarté et d'unité d'invention.

Dans la lettre à votre client, accompagnant votre projet, vous lui indiquerez les raisons du choix de la solution retenue, lui ferez part de toutes vos suggestions et le cas échéant, répondrez succinctement aux questions posées par la note technique.

Si vous estimez que plus d'une demande de brevet est nécessaire pour protéger au mieux les intérêts de votre client, vous indiquerez brièvement, dans la lettre au client, l'objet de la principale revendication indépendante de chaque autre demande distincte, une rédaction détaillée de telles revendications indépendantes n'étant cependant pas requise.

Annexes:

- Lettre du client
- Annexe 1 (Compte Rendu du Groupe de Travail 'Ecrans Solaires')
- Document D1: WO85/0111
- Document D2: US 4,000,000

UVS SAS
Impasse Beausoleil
40130 Cap Breton

Le 20 juin 2022

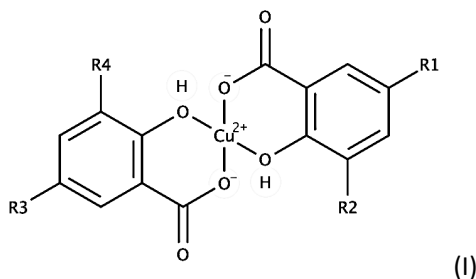
Bonjour,

Nous vous consultons en tant que conseil en propriété industrielle.

Comme nous vous l'avons indiqué lors de notre entretien téléphonique, notre société « Une Vie Saine » (UVS SAS), petite entreprise de 25 salariés, est spécialisée dans la production et la commercialisation de produits cosmétiques anti-UV ainsi que de crèmes hydratantes.

Notre activité a débuté en 2000 lorsque notre fondateur, un ancien chimiste de la société « L'Aurore », a découvert que certains dérivés d'un composé utilisé pour la synthèse de l'aspirine pouvaient dans certaines conditions particulières de formulation jouer un rôle de protection de la peau contre les rayonnements UV et donc pouvaient être formulés en tant que crèmes de protection solaire.

Dans le cadre de nos recherches, une famille de complexes organométalliques - des dérivés d'acide salicylique cuivrique – couverte par la formule générale (I) a fait l'objet d'investigations scientifiques pour ses propriétés de protection de la peau contre le rayonnement UV (c'est-à-dire de filtration des UV) :



Les radicaux R1, R2, R3 et R4 sont des radicaux alkyles dont les définitions seront précisées ci-après, notamment dans les exemples et tests.

Récemment, un étudiant, Sellah Khata a effectué un stage pour son master de chimie dans nos laboratoires de l'usine de Cap Breton. Après plusieurs semaines, Sellah est devenu plus autonome et des missions moins encadrées lui ont été confiées. Il avait notamment la charge, lors du week-end du 15 août 2021, de la surveillance des synthèses de nos composés.

Mais vous connaissez les étudiants, ils sont plus motivés par le contact sur les réseaux sociaux que par leur activité professionnelle. Après une matinée à discuter avec ses amis sur les réseaux, la batterie de son téléphone portable étant déchargée et désirant recharger son téléphone portable, il l'a branché sur la première prise de courant du laboratoire à sa portée et a malencontreusement arrêté le chauffage et l'agitation du milieu réactionnel dans lequel avaient été introduits les réactifs pour la synthèse de l'un de nos produits les plus avancés : UV STOP 007.

Le chauffage et l'agitation ont été arrêtés pendant 3h, et la température du mélange réactionnel est redescendue à température ambiante.

Notre ingénieur en chef Sam Ainerve - qui a supervisé la mise au point du procédé – est passé au laboratoire pour vérifier que tout se passait bien et s'est rendu compte de l'arrêt de la synthèse. Sam a immédiatement relancé le chauffage et l'agitation du milieu réactionnel pendant 1h.

Évidemment, Sellah a passé un mauvais moment, mais *in fine* Sam s'est rendu compte que le produit UV STOP 007 obtenu suite à cette interruption du chauffage et de l'agitation, était conforme aux critères de pureté, de qualité, de stabilité et de protection exigés, mais avec un rendement extraordinairement élevé.

Grâce à cela – en ayant reproduit les conditions d'arrêt de chauffage et d'agitation sur l'ensemble des produits préparés - le procédé se révèle particulièrement économiquement viable car moins consommateur d'énergie et en particulier du fait du rendement amélioré.

Suite à d'autres simulations effectuées par ordinateurs ('in silico') pour la mise en œuvre à l'échelle industrielle, il a été défini que la première phase de chauffage et d'agitation peut également être réduite. Au lieu de 2h, on peut réduire le temps de cette étape de 25%. De même, on peut réduire la période d'arrêt de 50% et la période de relance de 20%.

Il nous semble donc important de protéger au mieux ces aspects du procédé. D'autres informations techniques – y compris le livre de procédé (process book) - sont disponibles si nécessaire.

Nos composés sont utilisés en l'état pour la fabrication de compositions anti-UV. Nous avons également remarqué, qu'en raison du caractère liposoluble des composés cuivriques de formule (I), les compositions anti UV mises au point peuvent comprendre une phase grasse.

Les compositions peuvent ainsi plus facilement être formulées sous différents conditionnements : huiles, lotions oléocalcooliques, émulsions, telles que des crèmes ou des laits, gels gras ou oléocalcooliques, bâtonnets solides ou conditionnement en aérosol.

Une grande partie de nos clients souhaitent disposer de produits contre les UV au toucher gras, car ils nous indiquent qu'ils peuvent observer l'application sur la peau. Toutefois, quelques clients nous ont indiqué que des produits gras tachent les vêtements.

A titre d'exemple, la phase grasse comprend au moins un constituant choisi parmi les huiles ou cires minérales, animales et végétales, les acides gras, les triglycérides d'acides gras ayant de 6 à 18 atomes de carbone et les alcools gras. La sélection des constituants - tous connus par l'homme du métier- est dictée par le conditionnement souhaité.

Pour la première étape, on dissout de 0,01% à 5% en poids, sur la base du poids total de la composition, du produit de formule (I) dans la phase grasse préalablement chauffée, maintenue à 40-90°C et agitée. Puis, le milieu réactionnel n'est plus agité ni chauffé pour revenir à la température ambiante. Puis le mélange est de nouveau chauffé à 40-90°C pendant une durée supplémentaire.

En fait, nous nous sommes rendu compte que lorsque nous formulons nos compositions, très peu de composé cuivrique peut être utilisé. Aussi, il est avantageux de n'en utiliser que de 0,01% à 5% en poids par rapport au poids total de la composition, qu'elle contienne ou non une phase grasse. Il est vrai qu'à 0,01% les effets ne sont pas optimaux, mais nous avons des résultats intéressants. La gamme préférée est de 0,05 à 1% en poids.

A l'issue de cette 1ère étape en 3 temps, on ajoute classiquement au moins un adjuvant cosmétique choisi parmi les monoalcools ou polyalcools inférieurs comportant 1 à 6 atomes de carbone, l'eau ou

les mélanges de ces composés, les émulsifiants non ioniques, anioniques, cationiques ou amphotères, les épaississants, les produits hydratants, les adoucissants, les conservateurs, les colorants, les opacifiants, les agents de régulation du pH, les propulseurs et les parfums.

Les émulsions se révèlent être une forme de conditionnements préférée du fait du nombre limité d'ingrédients nécessaires et de la possibilité d'utiliser des produits naturels, notamment des émulsifiants naturels tout à fait compatibles avec le composé de formule (I). Ces compositions sous forme d'émulsions sont ainsi plus stables dans le temps à la chaleur. Les techniques de préparations sont classiques.

Pour préparer notre émulsion de la composition anti-UV sous forme de crème ou de lait protecteur, nous avons ajouté à la phase grasse préalablement chauffée à 75-80°C dans laquelle est dissous le composé de formule (I) et au moins un émulsifiant, la phase aqueuse chauffée à la même température. On maintient l'agitation pendant 10 à 15 minutes, puis on laisse refroidir sous agitation à 40°C, et on ajoute éventuellement un parfum et un conservateur.

Pour une composition cosmétique anti-UV sous forme d'un gel gras, d'une huile ou d'une lotion ou d'un gel oléo-alcoolique, on chauffe à 40-50°C le mélange de composé de formule (I), de la phase grasse ou oléo-alcoolique et éventuellement des adjuvants cosmétiques.

Nos compositions sont destinées à protéger la peau des rayonnements ultraviolets. Le but est d'éviter le fameux « coup de soleil ». Aussi, et surtout car nos concurrents le font, nous ajoutons parfois des filtres solaires absorbant les rayons ultraviolets dans la gamme de longueurs d'onde allant de 280 à 400 nm. Ces filtres sont intéressants car comme leur nom l'indique, ils absorbent les rayons UV. En association avec nos composés cuivriques, qui eux réfléchissent les rayons UV, la protection est améliorée.

Pour qu'un produit soit considéré comme un « écran solaire » il doit réduire l'exposition de la peau aux UV d'au moins 95%. C'est le cas d'UV STOP 007, et nous en sommes très fiers. Toutefois d'autres dérivés cuivriques sont parfois un peu moins efficaces (90% de réduction), et dans ce cas nous les combinons avec des filtres absorbant les rayons ultraviolets.

Afin que vous vous fassiez une idée de l'efficacité de nos produits nous vous présentons ci-après les résultats que nous avons accumulés et qui nous semblent particulièrement pertinents.

Notre produit UV STOP 007 va être commercialisé dans 1 mois. Nous avons déposé notre marque en France, et avons même protégé le design du tube. Ce produit est notre fierté, nous le souhaitons le plus élégant et le plus attirant possible pour les consommateurs. Nous avons même poussé le perfectionnisme jusqu'à proposer une jolie notice d'utilisation où il est expliqué comment le produit doit être étalé sur la peau, en le répartissant de manière homogène sur la zone à protéger, afin que les utilisateurs bénéficient des effets maximums. C'est important car souvent les consommateurs négligent l'application et reviennent rouges de leur exposition au soleil, simplement car ils n'ont pas utilisé le produit correctement.

Afin d'évaluer nos écrans solaires, nous procédons à deux types de tests :

- des tests sur la peau de volontaires, qui sont souvent plusieurs de nos employés mais également des clients fidèles qui souhaitent nous aider à améliorer nos compositions, et
- des tests *in vitro* sur recommandation de la S.F.D.C, utilisant un modèle de peau reconstituée (voir Annexe 1, ci-jointe).

Les tests sur la peau sont réalisés à l'aide d'un appareil émettant de la lumière UV à raison de 90 mJ/cm².

Chaque individu est son propre témoin.

Sur une surface de 4 cm² du bras gauche, on applique une composition à tester (environ 500 µm d'épaisseur). Une zone de taille identique est définie sur le bras droit, mais on y applique de la vaseline (même épaisseur).

Les régions de peau sont exposées pendant 1 min au rayonnement UV, puis sont laissées au repos pendant 20 min.

Les zones exposées aux UV sont alors photographiées et à l'aide d'un logiciel CIELab, l'intensité de la coloration rouge (pour les coups de soleil) est évaluée.

Pour chaque composé, les résultats sont donnés sous la forme d'un résultat normalisé correspondant à la valeur :

$$C_{\text{rouge}} = \frac{\text{Intensité sans composition}}{\text{Intensité avec composition}}$$

Les résultats sont normalisés de sorte que seront notés

(-) des composés ne protégeant pas la peau des UV (non filtrants), c'est-à-dire des composés permettant d'obtenir un C_{rouge} inférieur à 1,5

(+) des composés protégeant la peau des UV (filtrants), c'est-à-dire des composés permettant d'obtenir un C_{rouge} compris entre 1,5 et 3, et

(++) des composés protégeant très bien la peau des UV (très filtrants), c'est-à-dire des composés permettant d'obtenir un C_{rouge} supérieur à 3.

Évidemment les composés (++) sont ceux que nous recherchons en priorité, mais les composés (+) sont également d'intérêt.

Exemple 1

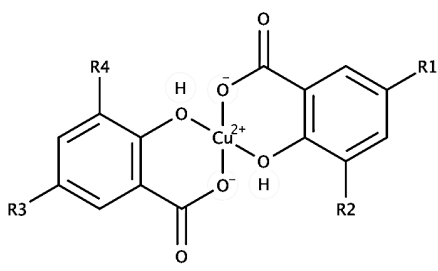
Dans cet exemple, 18 individus ont participé aux tests.

6 séries de 9 composés ont été testés. Chaque individu a reçu 9 composés sur un bras. 3 individus sont testés par série.

Les résultats obtenus sont la moyenne de trois individus par série.

Le tableau 1 suivant récapitule l'ensemble de ces résultats dans lequel C1 signifie méthyl, C2 signifie éthyle, C3 signifie propyle et C4 signifie butyle.

Tableau 1

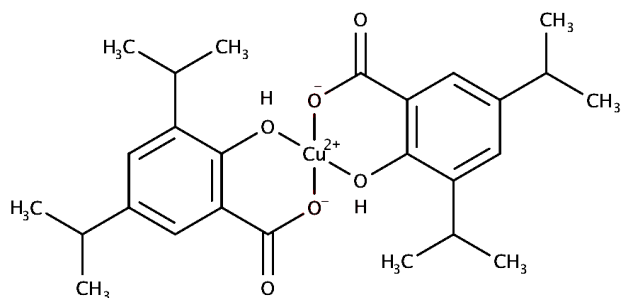
		Série 1	Série 2	Série 3	Série 4	Série 5	Série 6
		R1 = C1 R2= C1	R1=C2 R2=C2	R1=C3* R2=C3*	R1=C4** R2=C4**	R1=C2 R2=C3*	R1=C3* R2=C2
#1	R3= C1 ; R4= C1	-	-	++	-	-	-
#2	R3= C1 ; R4= C2	-	-	+	-	-	-
#3	R3= C1 ; R4= C3*	-	-	+	-	-	-
#4	R3= C2 ; R4= C1	-	-	+	+	-	-
#5	R3= C2 ; R4= C2	-	-	++	-	-	-
#6	R3= C2 ; R4= C3*	-	-	+	-	-	-
#7	R3= C3* R4= C1	-	+	+	-	-	-
#8	R3= C3* R4= C2	-	-	++	-	-	-
#9	R3= C3* R4= C3*	-	-	++	-	-	+

C3* : signifie que les résultats obtenus pour *n*-propyle (-CH₂-CH₂-CH₃) ou isopropyle (-CH(CH₃)₂)

sont identiques.

C4** : signifie que seul le n-butyle (-CH₂-CH₂-CH₂-CH₃) a été testé. Les autres C4 (ter-butyle, sec-butyle et isobutyle) réduisant la solubilité des composés dans un solvant (eau ou solvant organique ou graisse), n'ont pas été testés.

Le composé Série 3#9 de formule (II) suivante :



(II),

est désormais notre produit phare (UV STOP 007) car sa synthèse est extrêmement peu coûteuse et le rendement est excellent (>85%).

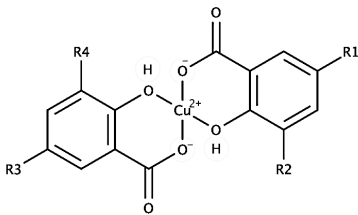
Exemple 2

Nous avons également testé l'influence du solvant sur l'efficacité des produits de la Série 3. Après synthèse, nous avons dissous des composés de la série 3 soit dans une solution aqueuse, soit dans une phase grasse (huile végétale).

Les composés ont été testés sur la peau de la même manière que pour l'exemple 1.

Le tableau 2 suivant récapitule l'ensemble de ces résultats :

Tableau 2

		Série 3					
		Solution aqueuse			Base grasse		
		R1 = nPr R2 = nPr	R1 = nPr R2 = iPr	R1 = iPr R2 = iPr	R1 = nPr R2 = nPr	R1 = nPr R2 = iPr	R1 = iPr R2 = iPr
#1	R3= C1 ; R4= C1	++	++	++	++	++	++
#2	R3= C1 ; R4= C2	+	+	++	+	++	++
#3	R3= C1 ; R4= C3*	++	+	+	++	++	+
#4	R3= C2 ; R4= C1	++	++	+	+	++	+
#5	R3= C2 ; R4= C2	++	++	++	++	++	+
#6	R3= C2 ; R4= C3*	+	+	+	+	+	+
#7	R3= C3* R4= C1	++	+	+	++	++	+
#8	R3= C3* R4= C2	++	++	+	+	++	+
#9	R3= C3* R4= C3*	++	++	++	++	++	++

C3* : signifie que les résultats obtenus pour n-Pr (n-propyle ou -CH₂-CH₂-CH₃) ou iPr (isopropyle ou

-CH(CH₃)₂) sont identiques.

On constate que la phase grasse n'a pas vraiment d'influence sur l'efficacité des produits de la série 3.

Exemple 3

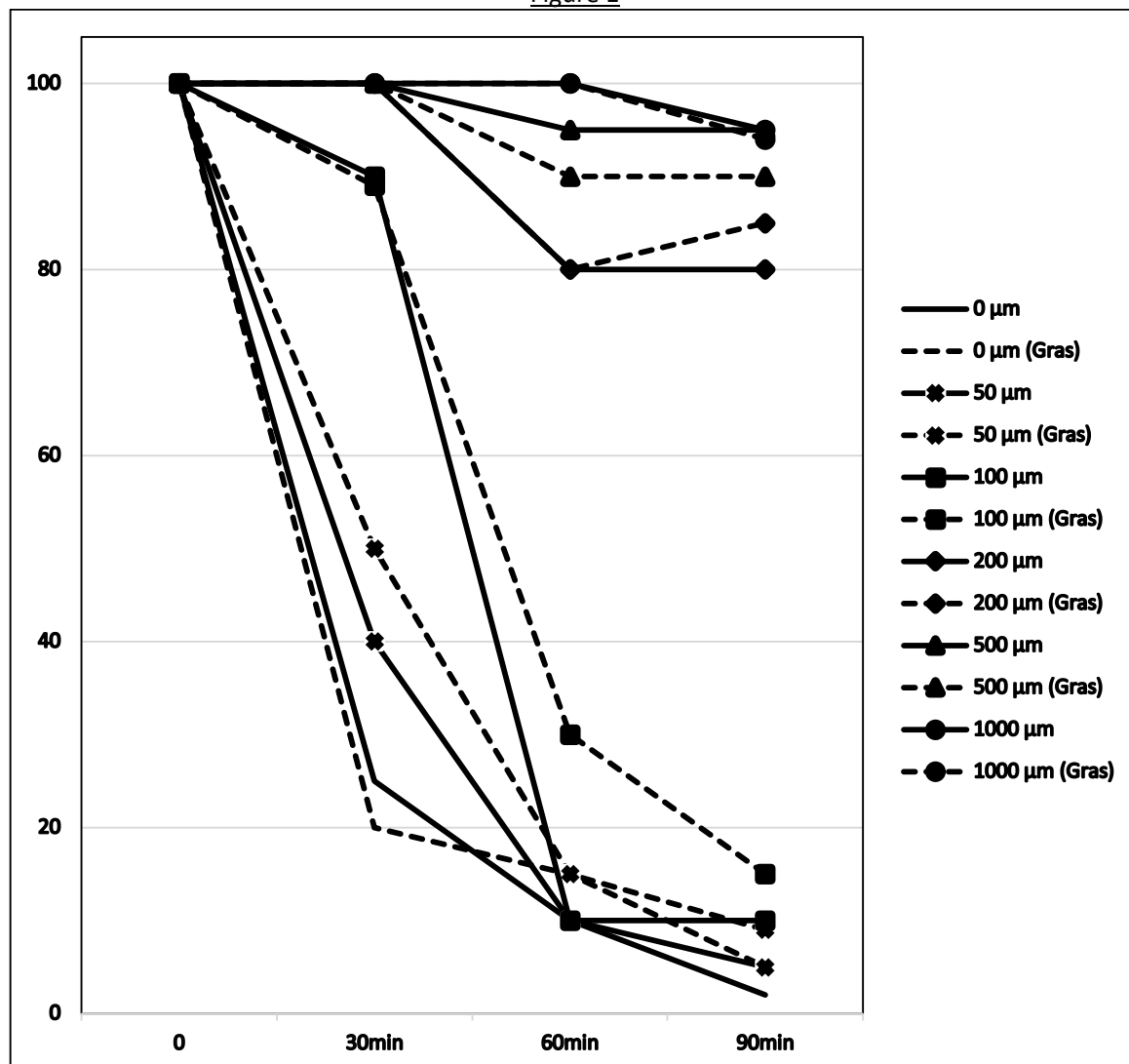
Nous avons ensuite évalué la quantité nécessaire de composé pour obtenir une protection efficace contre les UV.

Pour cela nous avons appliqué sur la peau des quantités différentes du composé S3#9 afin d'obtenir des épaisseurs de composition appliquées sur la peau de 50, 100, 200, 500 et 1000 µm. Le composé S3#9 est repris soit dans une phase grasse soit dans une composition saline.

Le test sur la peau a été répété 3 fois, toutes les ½ heures par individu, afin de mesurer l'effet dans le temps.

Les résultats sont présentés sur la figure 1 (Y : % de protection/filtration ; X temps en min)

Figure 1



Pour respecter la réglementation, il est nécessaire que le produit soit stable au moins pendant 1h d'exposition, que cette exposition soit continue ou intermittente.

On notera qu'il a été plus facile d'appliquer des épaisseurs d'au moins 200 µm avec le composé dans une phase grasse qu'avec les composés en solution aqueuse, bien que cela soit possible pour des épaisseurs plus faibles. Il apparaît donc inapproprié de proposer des compositions dépourvues de phase grasse, les clients ne pouvant pas l'appliquer correctement afin de se protéger efficacement des méfaits du soleil.

Exemple 4

Les composés ont également été testés selon la technique de la peau reconstituée considérée comme étant le test de référence par la S.F.D.C.

Tous nos composés considérés comme étant actifs sont des composés permettant de protéger la peau contre les effets néfastes des rayonnements UV (c'est-à-dire des composés filtrants). Cette information est capitale car cela nous donne un très bon positionnement commercial.

Il faut donc protéger notre méthode de protection de la peau contre les rayonnements UV. C'est un aspect important pour nous.

Exemple 5

Nous vous communiquons la formule de notre composition phare.

Cette formule est secrète et nous ne souhaitons en aucun cas la divulguer car nos concurrents pourraient avoir l'idée de la reproduire, au vu des propriétés surprenantes. Notre fondateur souhaite que cette formule reste au moins secrète jusqu'à son départ en retraite en 2035.

Toutefois nous vous la divulguons pour que vous compreniez mieux comment sont formulées nos compositions. Devons-nous vous faire signer un *Non Disclosure Agreement* (Accord de confidentialité – NDA) ?

Alcool cétylstéarylique oxyéthylène a 15 moles d'oxyde d'éthylène	3 g
Mélange de mono et distéarate de glycérol non auto-émulsionnable	4,8 g
Alcool myristique	4,5 g
Huile de vaseline	18 g
Bis (3,5-diisopropylsalicylate) de Cu II	0,5 g
p-(diméthylamino) benzoate de 2-éthylhexyle "ESCALOL 507" de VAN DYK	2,75g
3-benzylidène-DL-camphre	2 g
Propylène glycol	6 g
Conservateur, parfum q.s.	
Eau	qsp 100 g

La formule ci-dessus est la formule pour une crème.

Nous comprenons de notre discussion téléphonique qu'il serait envisageable de protéger nos travaux d'une quelconque manière, et notamment en déposant une demande de brevet.

Il semble également qu'un nouvel investisseur, la société NOZTRAISSSE, soit intéressé pour entrer au capital de notre entreprise et nous comprenons qu'une demande de brevet serait un atout.

S'il vous plaît, si vous pensez que nous pouvons déposer électroniquement une demande de brevet sur nos travaux, ne dépensez que le strict nécessaire en matière de taxes (toutes taxes payées à l'INPI), le budget pour ces travaux a été largement dépassé pour le marketing, et nous n'avons pas la possibilité de dépenser plus de 280 € sur cette ligne budgétaire.

Rassurez-vous, vos honoraires seront pris sur une autre ligne budgétaire, mais ce second budget ne peut pas intégrer des taxes, notre comptable Mademoiselle Tick a été très claire sur ce point, au risque d'un redressement fiscal. Ce ne serait pas bon au vu de nos négociations avec NOZTRAISSSE.

Avant de terminer, j'allais oublier un aspect. NOSTRAISSE est spécialisée dans les produits anti-ulcéreux.

Le chef de notre service innovation Mr Prunel de Maizieux, a mis en place un petit groupe de travail BLIND, qui a évalué l'effet biologique, dans quelques modèles animaux, de différents composés dérivés de l'acide salicylique. Nous n'avons normalement pas de connexion entre notre production destinée aux produits anti-UV et les produits destinés à d'autres applications, et testés par le groupe BLIND.

Le stagiaire dont nous vous avons parlé, Sellah Khata, qui devait pour la fin de son stage travailler dans le groupe BLIND, n'a pas respecté la séparation que nous avons imposée entre la production d'agents anti-UV et le groupe BLIND.

Il a, pour des essais dans le groupe BLIND, formulé le mauvais dérivé d'acide salicylique et a utilisé le composé bis(3,5-diisopropyl-salicylate) de cuivre II (en anglais, *Bis(3,5-diisopropylsalicylato (O,O)copper(II)* ou *Cu(II) (3,5-diisopropylsalicylate)₂*, ou encore *Cu(II)(3,5-dips)₂*), c'est à dire le composé S3#9.

Ceci est passé inaperçu jusqu'à ce que les tests réalisés sur un modèle d'ulcère gastrointestinal, utilisant des souris infectées par les bactéries *Helicobacter pilori* (*H. pilori*), bactéries responsables d'ulcères chez l'animal, soient complètement différents des résultats précédemment obtenus. Ces résultats précédents sont très reproductibles, et donnent toujours la même réponse.

En effet, nous utilisons habituellement le composé bis(3,5-dibutyl-salicylate) de fer II, composé bien connu, et maintenant commercialisé pour le traitement de l'ulcère par Basalt Pharmaceuticals, une société basée à Clermont-Ferrand. Ce composé permet, chez la souris, d'éliminer les *H. pilori* en 5 j (jour(s)), par une prise quotidienne sous forme d'une solution saline. A 5 j, plus aucune trace de bactérie n'est retrouvée dans les fèces.

Il est d'ailleurs montré, par Basalt Pharmaceuticals, qu'une autre posologie n'a pas d'effet ou qu'elle induit des saignements gastrointestinaux chez l'animal, entraînant sa mort par d'hémorragie interne en 7 j.

Avec le composé préparé par Sellah, aucune amélioration à 5 j n'était observée ; la mort des souris étant constatée à 7 j.

Sans en parler à Mr Prunel de Maizieux, Sellah a réalisé des tests chez des souris infectées par *H. pilori* en leur administrant *per os* (voie orale) :

- Soit deux doses par jour de composition comprenant le composé S3#9 avec une phase grasse,
- Soit trois doses par jour de composition comprenant le composé S3#9 avec une phase grasse,
- Soit deux doses par jour d'une solution saline comprenant le composé S3#9.

Il souhaitait avoir plus de résultats pour son rapport.

De manière très surprenante, les souris ayant reçu deux doses par jour de composition comprenant le composé S3#9 avec une phase grasse n'ont plus aucune trace de bactéries *H. pilori* dans leurs fèces après 4 j au lieu de 5 pour le composé habituel (composé Fer II).

Des résultats similaires sont obtenus chez les souris ayant reçu trois doses par jour de composition comprenant le composé S3#9 avec une phase grasse. En revanche, les souris ayant reçu deux doses par jour du composé S3#9 dans une solution saline meurent d'ulcère après 7 j.

Sellah a présenté ses résultats à Mr Prunel de Maizieux, qui fut fort surpris. Mr Prunel de Maizieux a décidé d'analyser par spectrométrie le composé utilisé et a découvert que celui-ci était en fait notre composé S3#9. Sellah a de nouveau passé un mauvais moment pour avoir mélangé les travaux entre la production de composés anti-UV et ceux du groupe BLIND et pour avoir réalisé des expériences chez la souris sans supervision. Mais son erreur semble *in fine* très importante pour nous.

Au vu de ces résultats importants, nous envisageons de développer une forme galénique retard permettant la distribution au moins deux fois par jour du composé S3#9. Des premiers résultats devraient être disponibles d'ici 7 à 8 mois.

Nous avons d'ailleurs indiqué à NOZTRAISSSE que nous avons des résultats très intéressants sur les ulcères. Ils ont eu l'air très intéressés. Nous les voyons demain. Rassurez-vous nous allons signer un NDA avec eux.

Pensez-vous que nous devrions protéger ce nouvel aspect ? Si oui, dans la même demande de brevet ? Si ce nouvel aspect est protégeable, nous privilégions cette deuxième possibilité, afin de limiter les coûts. D'ailleurs pour les raisons invoquées plus haut, si nous déposons deux demandes de brevets, nous serions confrontés au problème de paiement des taxes pour la deuxième demande. Si cela est possible, une protection dans plusieurs États de l'Union européenne serait souhaitable d'ici 4 ans, car cela renforcerait notre position lors des négociations avec NOZTRAISSSE. Nous ne sommes intéressés ni par le marché américain, ni par le marché asiatique.

Je pense vous avoir tout dit.

Nous partons demain à 16h30 avec Mr Prunel de Maizieux dans la maison de campagne du PDG de NOZTRAISSSE, à 100 km d'Helsinki, présenter ces travaux sur les ulcères au comité directeur. Nous ne serons pas joignables avant 1 semaine, la maison a l'air d'être très reculée et les réseaux de téléphonie mobile semblent inexistantes.

Contactez-moi d'urgence aujourd'hui après 14h avec vos propositions, que nous ayons de la matière pour nos réflexions internes et nos discussions avec NOZTRAISSSE.

Merci de votre aide,

Bien cordialement,

T. TOURENESSOLE

ANNEXE 1

SOCIETE FRANCAISE DES DERMATOSES CUTANNEES (S.F.D.C.)

Groupe de travail sur les écrans solaires : 21ème Session
Maison de la cosmétique
17 avenue de l'opéra
75001 Paris

Paris, le 25 novembre 2011

Compte rendu de la 21ème session du groupe de travail sur les écrans solaires.

A la demande du Ministère de la Santé et des Solidarités, la S.F.D.C. a été consultée afin d'établir une classification technique des compositions, crèmes, lotions, huiles et autres formes galéniques disponibles sur le marché déclarées comme ayant des propriétés de protection contre les rayons ultraviolets, et commercialisées avec une mention « écran solaire ».

Après étude de 1200 produits commerciaux, et des études menées par le comité d'experts du groupe, sous l'égide de la Professeure Eryn Theme-Coutanet, le groupe a adopté la classification suivante des « écrans solaires » selon deux types :

- 1** - Les « écrans solaires » de type filtrants,
- 2** - Les « écrans solaires » protecteurs cellulaires.

Le test de référence choisi par le groupe de travail est le test de « peau reconstituée » conçu par la Professeure Eryn Theme-Coutanet et qui comprend une souche de fibroblastes recouverte de matrice mimant la couche cornée de la peau. Au sein de la couche de fibroblastes sontensemencés des mélanocytes, cellules productrices de mélanine colorant de la peau en réponse à l'exposition aux U.V.

La peau artificielle forme un film fin. Chaque « écran solaire » à tester est appliqué sur la matrice à une température ambiante comprise entre 17°C à 24°C.

Le film avec « écran solaire » appliqué est disposé sur une boîte de pétri transparente, et exposé à un rayonnement UV de 90 mJ/cm².

Lors de l'exposition, on mesure le rayonnement au travers de la peau reconstituée (test de filtrage).

En parallèle, la peau est observée au microscope muni d'une caméra CCD filmant la migration des mélanocytes (test physiologique).

Il ressort de l'évaluation des 1200 produits commerciaux trois catégories d'écrans solaires :

- a.** les écrans solaires ne déviant ou ne bloquant que les rayons U.V. : la quantité de rayonnement au travers de la peau reconstituée est réduite d'au moins trois fois (soit 30 mJ/cm² ou moins)
- b.** les écrans solaires de type filtrant influant sur la migration des mélanocytes : alors que les mélanocytes sont peu mobiles ou ont une migration aléatoire, les mélanocytes vont converger vers un point afin de créer une barrière aux U.V. lorsqu'ils ont un effet protecteur cellulaire, et
- c.** les écrans mixtes qui filtrent les U.V. et influent sur la migration des mélanocytes.

Selon la Norme DERMASOL n°987/2019, tout produit ayant un effet sur la migration des mélanocytes doit être soumis à la réglementation relative aux agents thérapeutiques.

Les autres produits, sans migration, sont dans la classification technique des cosmétiques.

Selon la Norme MaL-2-T3T3 n°670/11/24, les compositions comprenant tout type de dérivés d'acide salicylique doivent être formulés à une température comprise entre 17°C à 24°C. En dehors de cette gamme, lesdits acides se dégradent et sont impropres pour l'utilisation cosmétique ou thérapeutique.

PCT

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(21) International Application Number: PCT/US84/0012 (22) International Filing Date: 10 June 1984 (71) Applicant: (72) Inventors: (74) Agent: (81) Designated States: AT (European patent), AU, BE (European patent), CH (European patent), DE (European patent), DK, FR (European patent), GB (European patent), JP, LU (European patent), NL (European patent), SE (European patent).		Published <i>With international search report.</i>
(54) Title: COPPER COMPLEX FOR TREATING CANCER (57) Abstract Copper complexes exhibiting superoxide dismutase-like activity in mammalian cells. The copper complexes, which act as superoxide radical (O₂⁻) scavengers, are used in treating cancer by replacing the lost superoxide dismutase activity which characterizes tumor systems.		

COPPER COMPLEX FOR TREATING CANCER

Technical Field

The present invention relates to a copper complex for treating cancer by decreasing tumor growth, increasing survival of a host, decreasing tumor metastasis and inducing morphological differentiation of cancerous cells. A method for treatment of cancer using this complex is also disclosed.

Background of the Invention

Oxygen toxicity in aerobic organisms has been widely studied. The enzyme SOD - superoxide dismutase - plays a significant role in the defense against such toxicity. Superoxide radical (O_2^-) is generated in various biological reactions; this free radical anion is potentially damaging either because it is extremely reactive or because it can generate highly toxic species like OH. Superoxide dismutase catalyses the dismutation of O_2^- :



In a mammalian cell, two types of SOD are found. One contains both copper (Cu) and zinc (Zn): CuZnSOD. The other enzyme contains manganese (Mn): MnSOD. All normal mammalian cell types investigated contain these two types of the enzyme.

In nearly all of the tumor systems studied so far, the level of CuZnSOD and MnSOD activities were diminished. There are a few exceptions for CuZnSOD activity, but no exception has been found in the case of MnSOD activity. In many tumor samples, the MnSOD content is reduced to a level where it cannot be detected.

The role of SOD in cancer has been the topic of a recent review in Oberley, L.W. and Buettner, G.R., The Role of Superoxide Dismutase in Cancer: A Review, Cancer Res. 39:1141-1149 (1979) and in 2 Oberley, L.W., Superoxide Dismutase, Chapter 6, (CRC Press 1982), both hereby incorporated by reference. SOD activities for numerous cancer types are specified.

Reduced MnSOD activity has been found in over fifty human, rat, mouse, spontaneous, transplanted virally- induced, chemically induced, in vivo and in vitro tumors. In addition, both CuZnSOD and MnSOD activities in over thirty types of human tumors have been found to be less than those in control organs, Westman, N.G. and Marklund, S.L., Copper and Zinc-Containing Superoxide Dismutase and Manganese-Containing Superoxide Dismutase in Human Tissues and Human Malignant Tumors, Cancer Res. 41:2962 (1981), hereby incorporated by reference. Thus, it has been demonstrated that loss of SOD activity is characteristic of a wide variety of human tumors and is not a phenomenon restricted to mouse and rat tumors.

Examples of cancer types characterized by low SOD activity levels include: Morris hepatomas, H6 hepatoma, Novikoff hepatoma, C3H carcinoma, Lewis lung carcinoma, Walker carcinoma, Mammary adenocarcinoma, Ehrlich ascites, Web 4 ascites, Guerin TR ascites, S91 melanoma, B16 melantic melanoma, Sarcoma 180, L1210 leukemia, L1210/6MP lymphoid leukemia, L1210/5FU lymphoid leukemia, P388 lymphoid leukemia, L1210/0 lymphoid leukemia, and Manning leukemia. This theory of excess free radicals with a deficiency of free radical scavengers in cancer cells has also been

proposed by Georgieff, K.K., Free Radical Inhibitory Effect of Some Anticancer Compounds, Science 173:530 539 (1971), hereby incorporated by reference.

Georgieff showed that various types of chemotherapeutic agents exhibit free radical inhibitory activity.

It has been discovered that copper complexes, which act as O_2^- scavengers (capture O_2^-), allow cells to revert to its non-cancerous stage. These complexes also inhibit cancer cell growth in vivo, thereby prolonging the survival of tumor-bearing host organisms. Tumor metastasis is also reduced. The discovery is not only a significant advance but also an unexpected discovery in the art of treating cancer.

Disclosure of Invention

Native SOD has a molecular weight of about 32,000 and, as a result, does not penetrate well into cell membranes. $Cu(II)$ (3,5-diisopropylsalicylate)₂, hereinafter **CuDIPS**, and other **copper salicylate** complexes are also O_2^- scavengers and have a molecular weight that facilitates penetration into cell membranes. Their smaller molecular size (molecular weight between about 340 and about 1000, preferably between about 340 and about 600 for copper salicylate complexes and molecular weight between about 845 and about 1800, preferably between about 845 and about 1400 for copper acetylsalicylate complexes), that is, low molecular weight, and their lipid solubility allow better penetration of cell membranes,. See Leuthauser, W.C., Oberley, L.W., Oberley, T.D., Sorenson, J.R.J., Ramakrishna, K., Antitumor Effect of a Copper Coordination Compound With Superoxide Dismutase Like Activity, JNCI 66:107F 1081 (1981), hereby incorporated by reference. Thus, CuDIPS can mimic the action of lost SOD activity in tumor cells. The use of copper salicylate complexes, their solvates, as well as mixtures thereof, for the treatment of cancer according to this invention is advantageous in that the overall treatment mechanism does not, as with various known methods, involve only cytotoxicity but, instead, focuses on the replacement of lost SOD activity in tumor cells to effectively halt cell division and subsequent tumor growth. Such a non-cytotoxic approach to cancer therapy allows a continued regimen of treatment which is not, as with methods using cytotoxic compounds, limited by potential compounding of toxic effects.

This invention involves the effect of compounds with SOD activity on tumor growth and survival of tumor bearing mammals. Natural SOD and the subject **copper salicylate** complexes were found to have surprisingly different effects on these parameters.

Low SOD activity, which is defined as SOD activity below that exhibited in a given normal, non-cancerous cell, characterizes various types of cancer. **Copper salicylate** complexes, their solvates, as well as mixtures thereof, are useful in treating such types of cancer. The purpose of tumor therapy is to eliminate the tumor or to retard its growth, prevent metastasis and at the same time, prolong survival of a host. Inhibition of tumor metastasis is an important parameter in cancer control. Most primary tumors can be easily treated by various therapy regimens, but metastasis is usually difficult to prevent.

The tumor model used in the following experiment is Ehrlich carcinoma cells, which metastasized to the lungs, forming macroscopic nodules. Microscopic metastases are observed as early as the 10th day after transplantation of tumor cells in Tween 80 saline treated control animals. When mice treated

with 5 daily doses of CuDIPS were killed on the 45th day after transplantation, only microscopic nodules were observed in the lungs.

CuDIPS had delayed the process of metastasis in these Ehrlich tumor mice. However, lung metastases were the cause of death of these treated mice. Prolonged treatment further increased survival, possibly by suppressing metastases longer and thereby retarding tumor growth.

The results of the experimentation of this invention indicate that CuDIPS does indeed exert in vivo antitumor activity in the solid form of Ehrlich carcinoma. A greater effect was observed with an increased number of treatments when the total dose did not exceed the median lethal dose LD50 of the compound. It has also been observed that in vitro, CuDIPS inhibits growth of cultured neoplastic cells and induces morphological differentiation in cultured neuroblastoma cells.

It has also been discovered that cancer types characterized by low SOD activity are advantageously treated using a copper salicylate complex in combination with known anti-cancer drugs. For example, a treatment mixture containing both CuDIPS and bis-dichloroethyl N-nitroso urea (BCNU) is effective in prolonging survival of tumor-bearing host organisms, 2 Oberley, L.W., (unpublished observation).

It has not been possible to discern the mechanism by which CuDIPS effects is antitumor activity. The mechanism is just not known. However, this mechanism is not crucial to the advantageous practice of this invention. The following discussion merely postulates what are believed to be mechanisms that could describe the activity.

There are at least five different mechanisms by which CuDIPS could work: 1) cytotoxicity; 2) immune cell mediated effects; 3) antibody mediated effects; 4) inhibition of cell proliferation without cell differentiation; 5) inhibition of cell proliferation with differentiation. Cytotoxicity appears to be unimportant.

Best Mode for Carrying Out the Invention

Orgotein, a generic name for CuZnSOD derived from bovine liver, is obtained from Diagnostic Data Incorporated, Mountain View, California. It is 98% pure CuZnSOD isolated from bovine liver and had 3,300 units of pure erythrocyte SOD activity/mg protein when assayed by the method of Beauchamp, C. and Fridovich, I., Superoxide Dismutase: Improved Assays and an Assay Applicable to Acrylamide Gel., Anal. Biochem. 44:276-287 (1971), hereby incorporated by reference. Orgotein was dissolved in USP grade nonpyrogenic sterile saline.

CuDIPS is synthesized using known methods.

Sorenson, J.R.J., Copper Chelates as Possible Active Forms of the Antiarthritic Agents, J. Med. Chem. 19:13S 148 (1976); Sorenson, J.R.J., Some Copper Coordination Compounds and their Anti inflammatory and Antiulcer Activities, Inflammation 1:317- 331 (1976), hereby incorporated by reference. CuDIPS is lipid soluble, and the median lethal dose LD50 is 240 + 33 mg/kg when administered subcutaneously to rats. CuDIPS is suspended in 10% Tween 80-saline ((Z) Sorbiton mono-9-ocladecinoate poly(ox 1,2- ethonediy)) solution.

The SOD activity of Orgotein and of tumor homogenates is assayed by the method of Beauchamp and Fridovich. Reaction mixtures contain 0.05M potassium phosphate buffer (pH 7.8) with 10^{-3} M

diethylene triamine pentaacetic acid, 10^{-4} M of xanthine, 5.6×10^{-5} M of nitro blue tetrazolium, 10^{-3} units of xanthine oxidase and 1 unit of catalase. Addition of 5×10^{-3} M NaCN to this mixture inhibits CuZnSOD activity and allows the measurement of the activity of the cyanide insensitive MnSOD alone. The rate of blue formazan formation is measured by following the change of absorbance at 560 nm with a Cary Model 15 spectrophotometer. The sensitivity of the assay was such that 1 unit equaled 14 ± 1 ng of pure erythrocyte SOD.

The amount of protein in each sample is assayed using a conventional method.

Tissue homogenates are prepared by excising tumor mass from mice implanted with Ehrlich carcinoma cells. After bones and muscles are removed, the tumor tissue is rinsed and minced in 0.05 M potassium phosphate buffer (pH 7.8) and homogenized in a motor-driven Teflon pestle homogenizer. 2 scavenging ability of **CuDIPS** is determined by a similar assay method; the only difference is that dimethyl sulfoxide (DMSO) solvated potassium superoxide (KO_2) is used to generate O_2^- instead of xanthine and xanthine oxidase. **CuDIPS** is dissolved in 10% Tween 80 saline.

This alternate method is chosen due to the length of previous procedure and lipid insolubility of some of the reactants of the previous assay. Free O_2^- is released when DMSV KO_2 is added to an aqueous solution. Reaction mixtures contain 1:20 dilution of a saturated KO_2 solution (20 mg KO_2 /ml of dimethyl sulfoxide), 5.6×10^{-5} M of nitro blue tetrazolium, 1 unit of catalase and 0- 500 μg of **CuDIPS** in 0.5 M potassium phosphate buffer, pH 7.8. The formation of blue formazan is determined by recording the final absorbance of the assay mixture at 560 nm with a Cary Model 15 spectrophotometer. The sensitivity of this assay is found to be 0.85 μg of pure bovine SOD per unit of activity.

Tumor models used in these experiments are Sarcoma 180 cells implanted in 8- 12 week old standard CF1 female mice (Charles River Breeding Laboratory, Wilmington, Massachusetts) and Ehrlich carcinoma cells implanted in standard CBA/J female mice (Jackson Laboratory, Bar Harbor, Maine). The tumor cells are maintained intraperitoneally in their respective hosts and are harvested and transferred weekly. Peritoneal fluid from these tumor hosts are centrifuged at 420xg for 2 minutes; the pellet is resuspended and washed three times in isotonic saline. The resulting pellet is diluted 1:5 with isotonic saline. Cell counts are done using a Coulter Model B cell counter. Solid tumors are induced by implanting 5×10^6 cells intramuscularly into the right thigh of their respective hosts. Tumor growth is estimated by two dimensional measurements of the tumor thigh; group means and standard error of the means are plotted. The significance of differences between tumor growth curves is determined by a conventional Student's t-tests. Survival curves are analyzed by a conventional Mantel-Cox's Generalized Savage Test and a conventional Breslow's Generalized Wilcoxon test. The p values (probability that observation is due to chance alone) reported are the higher of the p values from these 2 tests.

The antitumor activity of Orgotein is studied in both tumor models. A dose of 1.2 mg of Orgotein in 0.15 ml Tween 80-saline is injected intramuscularly at the tumor site. A single dose is given to animals implanted with Sarcoma 180 cells at 1 hr, 5 days or 10 days after transplantation. Mice implanted with Ehrlich carcinoma cells are also treated with Orgotein. 5 or 10 daily doses are administered instead of a single dose; Tween 80-saline treated mice are used as control. Antitumor activity of **CuDIPS** is examined in mice with Ehrlich carcinoma only. A daily dose of 0.5 or 1.0 mg in 0.2 ml of Tween 80-saline is administered intramuscularly at the tumor site for 5 days, 10 days, or every Monday, Wednesday, and Friday for a total of 10 or 18 doses with the first dose administration at 1 hr following

implantation of tumor cells. Another treatment schedule includes 5 daily doses beginning immediately after transplantation and an additional 5 daily doses starting on the 35th day after transplantation. Each treatment group consists of 24 tumor bearing mice. Tween 80-saline injected mice are used as controls. Autopsies are performed on all dead animals. Tissues from the tumor masses and organs are excised and processed by known histologic techniques and stained with hematoxylin and eosin.

Antitumor Activity of Orgotein

Orgotein is administered to CF1 and CBA mice one hour after intramuscular implantation of Sarcoma 180 cells and Ehrlich carcinoma cells, respectively. As shown in Table I, tissue homogenates from these two types of solid tumor contain reduced amounts of total SOD activity and diminished amounts of MnSOD activity as compared to that from normal leg muscle of the mice without tumor cell implantation. Thus, these models appear to be representative in their SOD activity of the total class of tumors.

TABLE I : SOD ACTIVITIES IN TISSUE HOMOGENATES (Units/mg protein)*

	Total SOD	MnSOD
Normal leg muscle	45 + 3(4)**	14+1
Sarcoma 180	39 + 6(7)	less than 2
Ehrlich carcinoma	27 + 2(13)	less than 2

* Sarcoma 180 and Ehrlich carcinoma tissues are excised between 5 and 45 days after transplantation, minced and homogenized in 0.05M potassium phosphate buffer (pH 7.8). Little differences in activity are observed between days 5 and 45, so the values obtained are averaged. Normal leg muscle tissue is treated in a similar fashion. SOD activity is measured by the conventional xanthine-xanthine oxidase nitro blue tetrazolium method. Errors represent standard error.

** Number of animals sampled.

Orgotein is assayed to have 49,500 + 300 units of SOD activity/mg protein by the xanthine-xanthine oxidase method. When 1.2 mg of Orgotein is given to mice with Sarcoma 180 tumors at 1 hour after tumor cell implantation, a reduction in tumor size is observed, as indicated in Chart I (p less than 0.05 between 11 and 27 days); after 27 days, the growth curves are the same.

CHART I

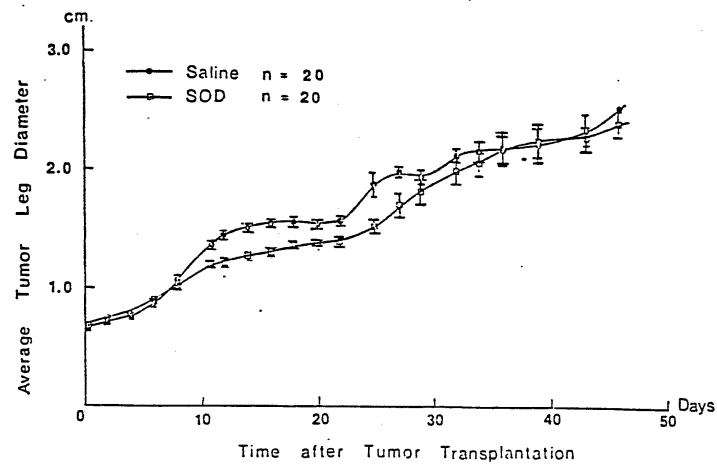


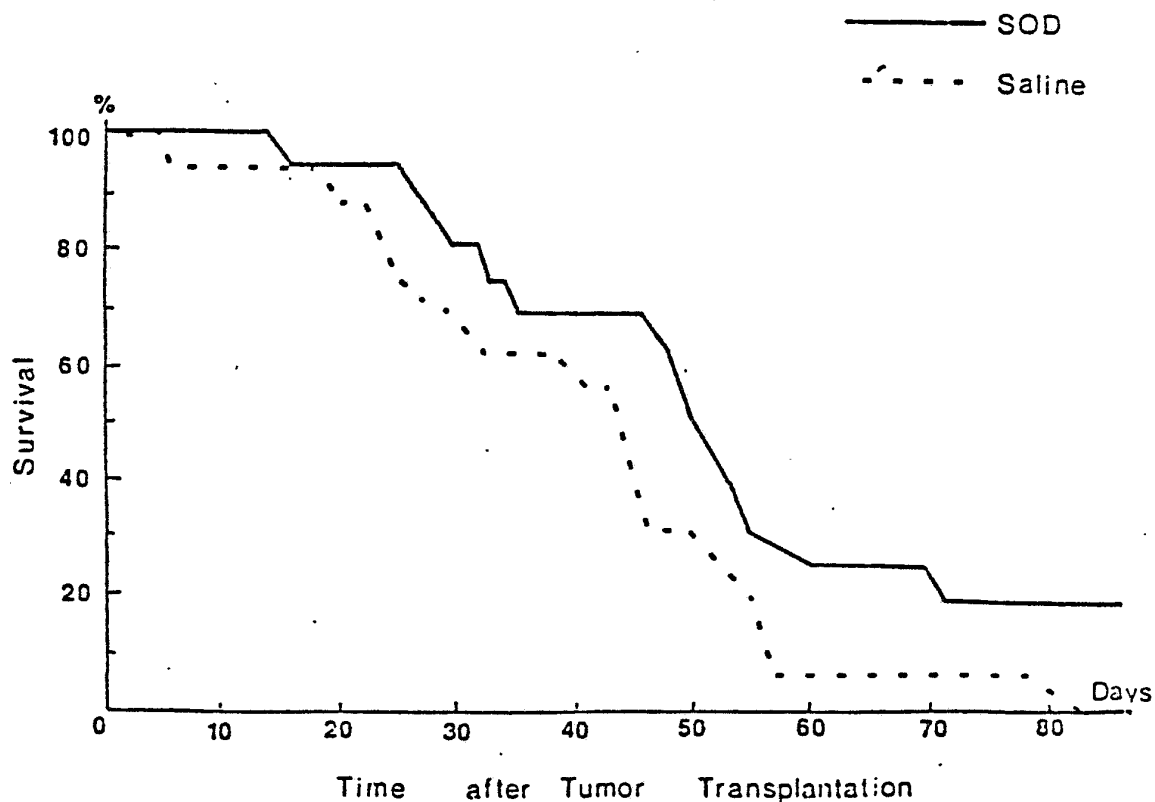
Table II and Chart II show the survival times of these tumor bearing mice; there is a 23% increase in mean survival time in the Orgotein treated group (p less than 0.1). Viability of the Sarcoma 180 cells (2.5 x 10⁷ cells/ml) is unchanged when they are incubated in 0.8 mg/ml Orgotein for one hour, showing that the effect of Orgotein is probably not due to cytotoxicity.

TABLE II
EFFECT OF ORGOTEIN TREATMENT ON SURVIVAL
OF CF1 MICE IMPLANTED WITH SARCOMA 180 CELLS

Treatment Group	Mean Survival Time in Days	Median Survival Time in Days	Maximum Survival Time in Days
Saline	41 ± 5*	46	82
Orgotein	53 ± 5	53	90

* standard error

Chart II



Tumors developed from those cells treated with Orgotein show no difference histologically from those developed from untreated cells. Histologic studies of the tumors from the Orgotein treated group show evidence of the development of a prominent fibroblast response in the area surrounding the tumor in some, but not all, of these animals on the 5th day after inoculation of tumor cells.

An infiltrative pattern of growth is noted from tumors in the saline control mice.

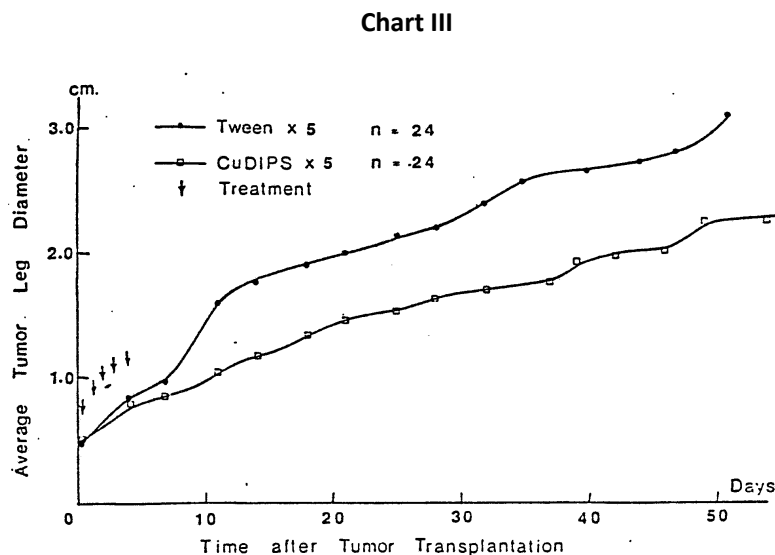
When Orgotein at the same dose is given to mice transplanted 5 or 10 days earlier with Sarcoma 180 solid tumor, no significant decrease in tumor growth or increase in survival is observed compared to the saline treated control. Ehrlich carcinoma cells implanted in cBA mice also do not respond to Orgotein treatment administered on the day of implantation or for 5 or 10 consecutive days afterwards.

Antitumor Activity of CuDIPS

When SOD activity is measured by the method of DSV KO_2 , **CuDIPS** has a mean of $17,400 \pm 1,800$ units of SOD activity/mg of the compound, but the ligand-DIPS alone, possesses no O_2 scavenging ability.

When Ehrlich carcinoma cells (25×10^7 cells/ml) are incubated with 5 mg/ml of **CuDIPS** for one hour, no toxicity is indicated. Preliminary dose study indicates that about 0.5 to 1.0 mg of **CuDIPS** gives a maximum effect without causing toxicity to animals. For this reason, a dose of 0.5 to 1.0 mg is always used. Chart III shows the difference in tumor growth when the tumor bearing mice are treated with

5 **CuDIPS** (p less than 0.001) for 5 days.



Approximately the same growth curve is obtained for all other treatment groups.

Table III summarizes the differences in survival times in the various treatment groups (p less than 0.0001 for all groups). Chart IV shows the survival curves of **CuDIPS** treated groups.

TABLE III

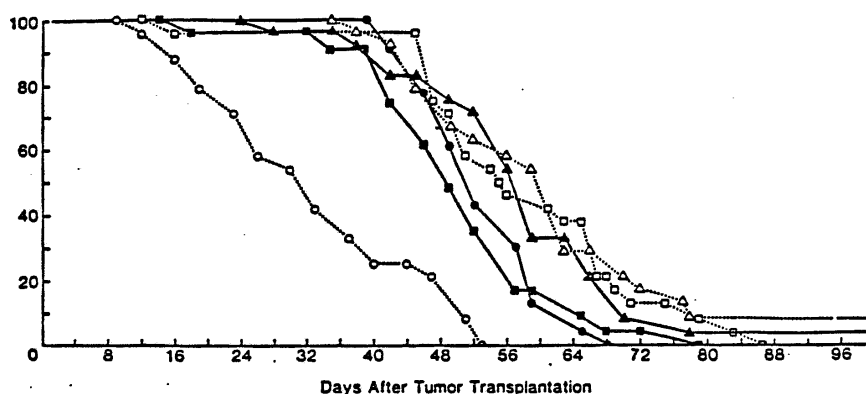
EFFECT OF CuDIPS TREATMENT ON SURVIVAL OF CBA MICE IMPLANTED
WITH EHRlich CARCINOMA CELLS

Treatment Group	Mean Survival Time in Days	Median Survival Time in Days	Maximum Survival Time in Days
Tween- 80 x 5** (Daily)	33 $\pm$ 4*	33	53
CuDIPS x 5 (Daily)	54 $\pm$ 3	52	68
CuDIPS x 10 (Daily)	58 $\pm$ 5	55	87
CuDIPS x 18 (MWF x 6)	50 $\pm$ 4	49	79
CuDIPS x 10 (MWF)	61 $\pm$ 6	62	124
CuDIPS x 10 (Daily, days, 0- 4 and 35- 39)	58 $\pm$ 4	59	greater than 124

* standard error

** total number of injections

CHART IV*



* Survival of CBA mice after implantations of Ehrlich carcinoma cells. Mice were given 5 doses of Tween 80 (○); 5 daily doses of **CuDIPS** (●); 10 daily doses of **CuDIPS** (□); **CuDIPS** on Mondays, Wednesdays, and Fridays for a total of 10 (△) or 18 (■) doses; or 10 daily doses of **CuDIPS** on days 0- 4 and 35- 39 (▲).

All treatments showed a similar effect on survival of the mice, but the group that received 0.5 mg of **CuDIPS** on Mondays, Wednesdays, and Fridays for a total of 10 doses showed a greater increase in mean survival. Out of 24 mice in this group, two of them had regressed tumors; however, the tumors recurred by 90 days after the initial implantation. Autopsies are performed on all dead mice. It is found that all animals died from massive lung metastases. Histologic studies of the organs of the tumor bearing mice revealed that microscopic nodules of tumor cells are present in the lung of the control mice as early as 10 days after implantation of cells, but microscopic lung metastasis are not observed in the **CuDIPS** treated mice until 45 days after transplantation of the tumor cells. Histologic studies of the tumor from the **CuDIPS** treated mice show tumors are not infiltrated by any type of inflammatory cell.

CuSO_4 at a dose of 0.5 mg in 0.1 ml of Tween 80- saline is also given to cBA mice implanted with Ehrlich carcinoma cells for 5 days after transplantation. A continuous loss of weight of the tumor bearing mice is observed and death is caused by massive lung metastasis. In contrast to the above results, no weight loss was observed when mice are treated with various doses of **CuDIPS**, indicating that its effects are most likely not due to toxicity of the copper compounds.

Another group of CBA mice implanted with Ehrlich carcinoma cells received intramuscular injections of single doses of DIPS. No apparent SOD like activity is exhibited by the DIPS and loss of body weight followed by death due to extensive lung metastasis is observed in the hosts.

CuSO_4 or DIPS alone have no anti-tumor activity. These observations demonstrate that the anti-tumor effects of **CuDIPS** are independent of and not attributable to any activity of its respective Cu or DIPS components.

The results indicate that the two tumor models chosen did indeed have a reduced level of both CuZnSOD and MnSOD activity. When this lost SOD activity is replaced by the local injection of Orgotein, a slight effect on the tumor growth and survival is observed in case of Sarcoma 180 tumor cells. Ehrlich carcinoma in OBA mice, however, did not respond to Orgotein treatment. Preliminary experiments

with standard Swiss mice implanted with Ehrlich carcinoma cells indicated that a reduction in tumor size occurs in the Orgotein treated mice. CBA mice are able to acquire immunologic tolerance; this may have contributed to the lack of response to the Orgotein treatment. Another possible explanation for the observed differences in response is that under certain conditions, the tumor cells may be able to take in SOD by phagocytosis. Thus, a small amount of the enzyme may get in the cell or Orgotein may represent a source of copper complexes which can be utilized by cells. In any case, Orgotein does not have a large effect on the growth of tumor cells in any of the models studied. Other copper compounds have antineoplastic activity [i.e.: blocking the formation of neoplasms (growths that may become cancer)] which prolongs survival of tumor bearing mice. Most of these compounds are cytotoxic; they prevent the development of tumors by cell killing.

CuDIPS has been shown to be a potent anti-inflammatory agent. This compound is also non-toxic to the host at the dose levels used. The advantage of this 2 scavenger over Orgotein is its small molecular size, which enables it to penetrate cell membranes and enter organelles, thus replacing the lost SOD activity within the tumor cells. The reduction in the size of the Ehrlich carcinomas in CBA mice after treatment with **CuDIPS** means that **CuDIPS** has a marked beneficial effect on this tumor model. Because Orgotein exerts no effect on tumor growth in this model, and both compounds are 2 scavengers, these different antitumor activities may be due to differences in ability to penetrate cell membranes. Thus, intracellular localization of the added O_2 scavengers may be responsible for the observed anti-tumor effect.

Due to the heterogeneous nature of tumors, various types of tumors respond to the **CuDIPS** therapy differently. In all **CuDIPS** treated tumor mice, tumor growth retardation occurs; however, the reduction of tumor size is unaffected by the total dose administered. This could be due to the fact that the **CuDIPS** sensitive cell populations are eliminated at a certain dose level; different regimens may be required to achieve additional beneficial therapeutic effects.

Growth Inhibition and Morphological Differentiation Effects of CuDIPS On Neuroblastoma Cells

CuDIPS inhibits cell population growth and induces morphological differentiation of mouse neuroblastoma cells in vitro.

The neuroblastoma cell clone NBP2 provided by Dr. K. N. Prasad (University of Colorado), is maintained according to the procedure of Prasad and Hsie, Prasad, K. N. and Hsie, A. N., Nature New Biol. 233:141 (1971), hereby incorporated by reference. Cells are grown as a monolayer in 75 cm² Falcon plastic tissue culture flasks containing F-12 medium supplemented with 10% gamma globulin free newborn calf serum, penicillin (100 U/ml) and streptomycin (100 µg/ml). Cultures are maintained in a humidified atmosphere of 5% CO₂ in a water jacketed incubator at 37°C. The cells are harvested with 0.25% Pancreatin and washed twice with fresh medium for subsequent plating. Only exponentially growing cells are used in the experiment. The viability of attached cells, determined 3 days after treatment by exclusion of trypan blue dye in control and treated cells, regularly exceeds 90%. Medium, serum, antibiotics, and pancreatin are purchased from Grand Island Biological Company. **CuDIPS** was synthesized using known methods. Fifty-thousand cells were seeded in each 60 mM plastic petri dish containing 5 ml of the growth medium. Cells are treated with **CuDIPS** 24 hours after seeding. **CuDIPS** is dissolved in ethanol to give a final concentration of 10, 25, or 50 µg/ml of copper compound in the medium. The concentration of ethanol in the medium is kept constant at 1% in all the treated and control dishes. Culture dishes containing 1% ethanol serve as controls. One day after the start of

treatments and daily afterwards, fresh drug and medium are provide in each dish. To avoid possible degradation, **CuDIPS** is dissolved in ethanol immediately before use. The experiment is done in triplicate. Three days after the initial treatments the number of cells and percent differentiation of cells are scored in each dish.

- 5 Four hundred cells per culture dish from at least three different regions are examined with a Leitz Divert phase contrast microscope. Cells with one or more processes at least twice as long as the soma diameter (processes usually longer than 50 microns) are scored as differentiated. To count the number of cells, cells are harvested with Pancreatin and the number of cells in each dish is determined by counting cells in a hemocytometer. The effects on cell population and the percentage of morphological
- 10 differentiation after three days of treatment with various concentrations of **CuDIPS** are observed and are summarized in Table IV.

TABLE IV

Effect of Various Concentration of CuDIPS on Cell Population
and Differentiation of Neuroblasma in Culture

Treatments	Cell number* $\times 10^5$	% differentiation**
Control	17.1 ± 1.9	8.0 ± 3.7
Cu(II) (3,5-diisopropyl-salicylate) ₂		
10 $\mu\text{g/ml}$	12.7 ± 1.4	7.4 ± 2.6
25 $\mu\text{g/ml}$	7.1 ± 1.2	22.4 ± 2.6
50 $\mu\text{g/ml}$	1.4 ± 0.6	74.2 ± 4.9

5

* Each value represents mean of 24 determinations

** Each value represents 9 determinations, at least 300 cells were counted in each dish.

The experiment is conducted in triplicate and each value is given as mean + S.D.

- 10 Three days after treatment with the **CuDIPS** at 10 $\mu\text{g/ml}$ concentration, a small reduction in the number of cells per culture dish is observed. The decrease in cell population is significant (p less than 0.05, Student's t -test). This level of concentration of **CuDIPS** caused no significant change in the percentage of differentiated cells. Treatment with 25 $\mu\text{g/ml}$ of **CuDIPS** reduces the cell population to $(7.1 \pm 1.2) \times 10^5$ cells/culture dish, a figure which represents less than half of the cell population found
- 15 in control. At a concentration of 50 $\mu\text{g/ml}$, **CuDIPS** causes a remarkable reduction in cell population. Higher concentrations of **CuDIPS** (75 or 100 $\mu\text{g/ml}$) are toxic to cells and kills almost all cells in 3 days of treatments. No floating or dead cells are observed in the culture dishes which receive the copper compound to a maximum concentration of 50 $\mu\text{g/ml}$.

- CuDIPS** also induces morphological differentiation in neuroblastoma cells. Although the induction of
- 20 differentiation is not observed at a concentration of 10 $\mu\text{g/ml}$, at a concentration level of 25 $\mu\text{g/ml}$, 22.4 + 2.6 percent of the neuroblastoma cells differentiated. A treatment concentration of 50 $\mu\text{g/ml}$, produces an average of 74.2 + 4.9 percent differentiated cells after 3 days of treatment. This represents more than 9 times the percentage of differentiated cells in controls. The majority of cells treated with 50 $\mu\text{g/ml}$ of **CuDIPS** morphologically resembles mature neural cells.

- 25 **Antineoplastic Activities of Copper salicylates**

A variety of **copper salicylate** complexes exhibit antineoplastic activity in CBA/J female mice implanted with Ehrlich carcinoma cells. The degree of tumor growth inhibition positively correlated with the degree of lipid solubility of the particular **copper salicylate** complex.

Tumors are induced in inbred CBA/J female mice (The Jackson Laboratory, Bar Harbor, Maine) by implantation of Ehrlich carcinoma cells. Tumor cells are maintained intraperitoneally in their hosts and are harvested and transferred weekly. Solid tumors are induced by intramuscular injections of 5×10^6 cells into the right thighs of hosts. Cells are enumerated with a Coulter Model B counter. Tumor growth is estimated by two-dimensional measurements of the thighs; group means and standard errors are calculated. The significance of differences between tumor growth curves is determined by Student's t-Tests for particular times. Survival curves are analyzed by Mantel-Cox's generalized Savage Test and Brelow's generalized Wilcoxon Test. The p- values reported are the higher values from these two tests. For survival data, p- values represent differences between complex treated and Tween 80-saline treated control groups over the whole curve and not at a particular time.

Copper salicylates are synthesized by known methods. Aqueous solutions of sodium salicylates are prepared by combining equivalent amounts of sodium hydroxide and the corresponding salicylic acid. To this solution is added 0.5 equivalents of copper chloride. The resulting precipitates are collected by filtration, washed with water, dried in vacuum for 24 hours at 50°C, and submitted for elemental analysis. The analytical values agree with the calculated values within + 0.4%. Mice implanted with Ehrlich carcinoma cells are treated with copper chelates suspended in 10% Tween 80 saline solution just prior to administration. All treatments are delivered by 0.1 ml intramuscular injections at the site of cell implantation. A daily dose of 0.5 to 1.0 mg copper chelate in 0.1 ml of 10% Tween 80 saline is administered for 5 days, 10 days, or every Monday, Wednesday, and Friday for a total of up to 12 doses, with the first dose given 1 hour after inoculation of cells.

Another schedule includes five daily doses beginning 1 hour after transplantation. Each treatment group consists of 24 tumor-bearing mice. Mice given Tween 84 saline injections are used as controls. The data presented in Charts **V** and **VI** show that each of the following copper compounds:

- Cu(II) (salicylate)₂ – CuS
- **CuDIPS**
- Cu(II) (3,5-ditertiarybutylsalicylate)₂ - CuDTBS

dramatically reduced tumor growth and prolonged survival of the host organisms.

Chart V

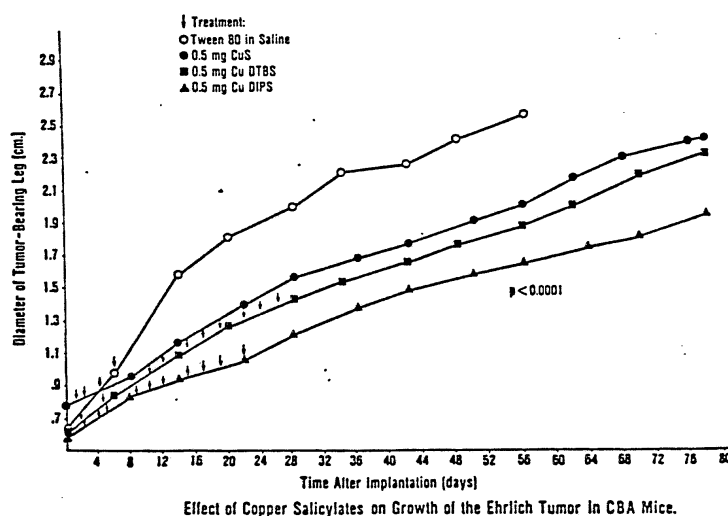
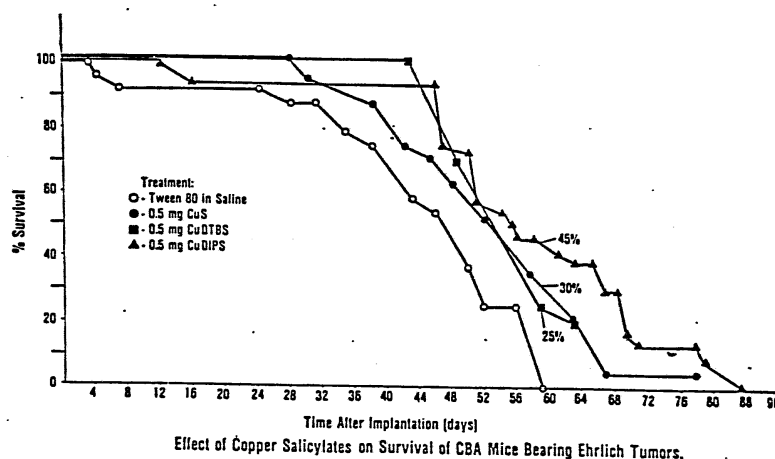


Chart VI



On the 58th day after implantation, all of the control animals died, but there were surviving hosts in each of the complex-treated groups. 25% of the CuDTBS-treated, 30% of the CuS-treated, and 45% of the **CuDIPS** treated mice survived beyond the time at which all of the control mice died. In addition, the period for 100% survival increased as compared to controls for all of the treated groups and the longest period was ten times longer, 44 days, for the CuDTBS-treated group. More than 90% of the animals treated with **CuDIPS** survived for 48 days. Lastly, when **CuDIPS** was given every day for 10 days, 10% of the animals survived for over 98 days, Leuthauser, S.W.C., Oberley, L.W., Oberley, T.D., Sorenson, J.R.J., and Ramakrishna, K., Antitumor Effect of a Copper Coordination Compound with Superoxide Dismutase-Like Activity, J. Nat'l. Cancer Inst. 66:1077-1081 (1981), hereby incorporated by reference. Lung metastasis was markedly reduced in the copper complex treated mice.

The observed antineoplastic activity must be attributed to the complexed form of copper because copper sulfate and diisopropylsalicylic acid are shown to have no anti-tumor effect in this Ehrlich tumor model, Oberley, L.W., Leuthauser, S.W.C., Oberley, T.D., Sorenson, J.R.J. and Pasternack, R.F., "Antitumor Activities of Compounds with Superoxide Dismutase Activity in Roles of Copper and Other

Essential Metalloelements in Inflammatory Diseases, Humana Press, Clifton, New Jersey, pp. 42) 433 (1982), hereby incorporated by reference.

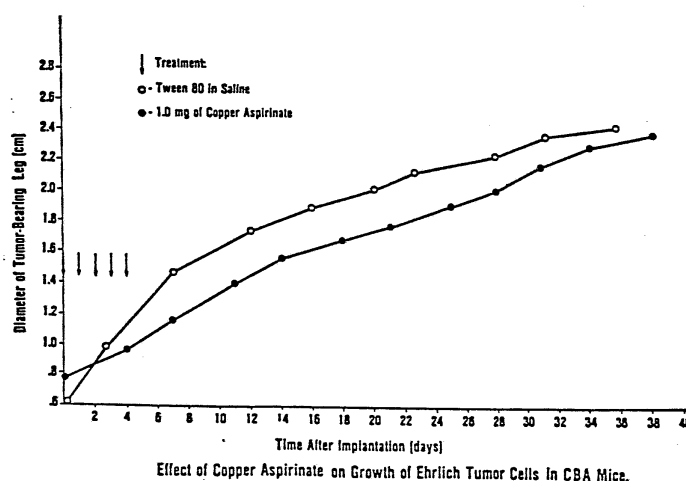
Antitumor Activity of Copper

Acetylsalicylate Complexes.

- 5 The activity of various copper acetylsalicylate compounds is also tested. As shown in Chart VII, the copper complex of aspirin, $\text{Cu(II)}_2(\text{aspirinate})_4$, reduces tumor growth and increases survival of the host. This complex is more active than SOD, because the latter has no effect against this solid Ehrlich tumor.

10

Chart VII



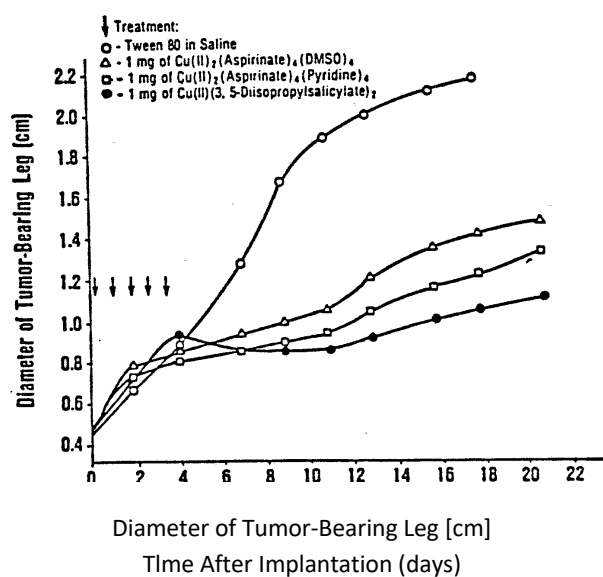
Time After implantation [days]

Effect of Copper Aspirinate on Growth of Ehrlich Tumor Cells In CBA Mice.

- 15 Tests involving other **copper salicylate** complexes reveal that they are more effective than the copper aspirinate compound, producing a further inhibition of tumor growth, as shown in Chart VII ~~Chart IX~~. The **copper salicylate** complexes compounds tested include:

- $\text{Cu(II)}_2(\text{aspirinate})_4(\text{DMSO})_4$,
- $\text{Cu(II)}_2(\text{aspirinate})_4(\text{pyridine})_4$;
- 20 - and $\text{Cu(II)} (3,5\text{-diisopropylsalicylate})_2$.

Chart VII



Effect of Copper Coordination Compounds
On Growth of Ehrlich Tumor Cells In CBA Mice.

Among the copper complexes shown in **Chart VII**, increased lipid solubility appears to be related to increased antitumor activity. Cu(II) (3,5-diisopropyl- salicylate)₂ (**CuDIPS**), having the highest agree of lipid solubility of the tested compounds, is found to be most effective in reducing tumor growth.

- 10 Cytoxicity does not appear to account for the effectiveness of these complexes, because their LD50 values are much larger than the dosages used in the experiments. The effective dose of Cu(II)₂(aspirinate)₄ is 50 mg/kg following intramuscular administration, while its LD50 is determined to be 760 mg/kg in rats and greater than 320 mg/kg in mice, following subcutaneous injection. An intramuscular dose of 25 mg/kg of CuS is found to be an effective dose, while LD50 values of 440
- 15 mg/kg, and greater than 320 mg/kg were reported following subcutaneous administration to mice, Sorenson, J.R.J., Rauls, D.O., Ramakrishna, K. Stull, R.E. and Voldeng, A. N., "Anticonvulsant Activity of Some Copper Complexes" in Trace Substances in Environmental Health, XIII University of Missouri Press, Columbia, Mo, pp. 360-367 (1979).

- 20 Similarly, the effective intramuscular dose of **CuDIPS** is 25 mg/kg while reported LD50 values are 240 mg/kg in rats and greater than 320 mg/kg in mice following subcutaneous administration. Also, if these complexes cause cell death, a significant number of normal muscle cells would have been killed with treatment. Finally, there is no difference in the number of cells killed when Ehrlich cells were incubated at 37°C for one hour with medium containing 5 mg of **CuDIPS** per milliliter of saline.

- 25 In the treatment of human cancer patients, the copper complexes would be administered to patients in dosages and according to regimens that would be therapeutically effective in replacing lost SOD activity in the cancerous cells.

CLAIMS

1. A compound having superoxide dismutase-like activity comprising a copper salicylate complex, solvate or mixture thereof.
- 5 2. A compound for treating cancer in mammals comprising a copper salicylate complex, solvate or mixture thereof.
3. The compound according to claim 2 wherein the copper salicylate complex is $\text{Cu(II) (salicylate)}_2$.
- 10 4. The compound according to claim 2 wherein the copper salicylate complex is $\text{Cu(II) (3,5-diisopropylsalicylate)}_2$.
5. The compound according to claim 2 wherein the copper salicylate complex is $\text{Cu(II) (3,5-ditertiar butylsalicylate)}_2$.
- 15 6. The compound according to claim 2 wherein the copper salicylate complex is $\text{Cu(II)}_2(\text{aspirinate})_4$.
7. The compound according to claim 2 wherein the compound is soluble in lipids.
- 20 8. The compound according to claim 2 wherein the compound is and scavenger.

[54] ANTI-INFLAMMATORY AND ANTI-ULCER COMPOUNDS AND PROCESS

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[22] Filed: May 30, 1978

Related U.S. Application Data

[62] Division of Ser. No. 563,778, Mar. 31, 1975, abandoned.

[51] Int. Cl.² **A61K 31/60**

[52] U.S. Cl. **424/230**

[58] Field of Search 424/294, 141, 230

[56] References Cited**PUBLICATIONS**

Die Med. Welt, 25(N.F.), Stuttgart, Nov. 22, 1974, Hangerter.

Primary Examiner—Frederick E. Waddell
Attorney, Agent, or Firm—Wood, Herron & Evans

[57] ABSTRACT

Disclosed herein are anti-inflammatory and anti-ulcer copper coordination compounds and a process for using them in the treatment of arthritis and gastrointestinal ulcers in animal bodies. The copper coordination compounds utilized are the reaction products of copper salts with:

1. aromatic carboxylic acids or their alkaline earth salts;
2. heterocyclic carboxylic acids or their alkaline earth salts;
3. amino acids or their alkaline earth salts;
4. amines; and
5. suitably substituted steroids.

The process disclosed comprises administering to experimental animals, orally or parentally (subcutaneously), in controlled dosages, the aforementioned copper coordination compounds for the treatment of inflammation (i.e., arthritis) and ulcers of the gastrointestinal tract.

7 Claims, No Drawings

ANTI-INFLAMMATORY AND ANTI-ULCER COMPOUNDS AND PROCESS

RELATED APPLICATION

This application is a division of application Ser. No. 563,778 filed Mar. 31, 1975, now abandoned.

BACKGROUND OF THE INVENTION

The prior art is replete with attempts to discover new anti-inflammatory drugs that are useful in the treatment of arthritis. Some of the more widely published results relate to the use of aspirin, indomethacin, penicillamine, hydrocortisone and dexamethasone. While all have demonstrated, to varying degrees, anti-inflammatory activity, they all exhibit undesirable side effects.

Such undesirable side effects include, for some of these drugs, toxicity problems; the production of fatty liver problems; and the creation of Cushing Syndrome. For a further and more complete discussion see Bach, "Adverse Reactions of Antirheumatic Drugs", *Int. J. Clin. Pharmacol* 7 2/3 (1973) 198-205.

Of even more importance, all of the above anti-inflammatory drugs produce gastrointestinal ulceration in experimental animals and in humans. Volume VII, p. 160, *Side Effects of Drugs*, (1971 Excerpta Media, Amsterdam); G. L. Bach, "Adverse Reactions of Antirheumatic Drugs", *supra*. Insofar as the state of the art is concerned, it is widely recognized that "it has not been possible to dissociate gastrointestinal toxicity from anti-inflammatory activity." *Side Effects of Drugs*, *supra* at p. 100.

It is therefore the principal objectives of my invention to provide anti-inflammatory drugs for use in treating arthritis in experimental animals which exhibit improved anti-inflammatory activities while at the same time providing drugs that are antiulcerogenic and have acceptable levels of toxicity.

SUMMARY OF THE INVENTION

It has been quite unexpectedly determined that copper coordination compounds produced by reacting copper salts with the following classes of organic compounds produce products which when used in accordance with the following processes, exhibit excellent anti-inflammatory activity in animals, i.e., a warmblooded animal or mammalian subject, and which are anti-ulcer:

1. aromatic carboxylic acids or their alkaline earth salts;
2. heterocyclic carboxylic acids or their alkaline earth salts;
3. amino acids or their alkaline earth salts;
4. amines; and
5. suitably substituted steroids.

It has been empirically determined that the copper coordination compounds disclosed herein not only demonstrate excellent anti-inflammatory activity but that they are anti-ulcer and may be utilized both as anti-inflammatory agents in the treatment of arthritis and in the treatment of gastrointestinal ulcers in animals.

In the treatment of inflammation and/or ulcers, the compounds are administered orally or parentally. The copper coordination compounds, being relatively insoluble in water, are administered by dissolving them in saline solution to which a suitable suspending agent has been added.

In treating inflammation by subcutaneously injecting test animals with the copper coordination compound so prepared it has been found that excellent anti-inflammatory results, in the test models hereinafter described, may be obtained if the dosages administered comprise about 2.5-165 mg. per kilogram of body weight.

In treating gastrointestinal ulcers by orally introducing into test animals the copper coordination compounds of this invention it has been found that excellent results, in the test models hereinafter described, are obtained if the dosages administered comprise about 2 to 125 mg. per kilogram of body weight.

DETAILED DESCRIPTION OF THE INVENTION

For the purpose of more fully understanding the present invention, a copper coordination compound is intended to mean a compound whose molecular structure contains one or more copper atoms bonded to one or more atoms of one or more molecules or ions by coordinate covalent bonds.

The copper coordination compounds of the present invention are prepared by reacting copper salts, preferably cupric chloric or cupric acetate with a member of the following classes of organic compounds:

1. aromatic carboxylic acids or their alkaline earth salts;
2. heterocyclic carboxylic acids or their alkaline earth salts;
3. amino acids or their alkaline earth salts;
4. amines; and
5. suitably substituted steroids.

More specifically, it has been found that suitable compounds may be produced by reacting cupric chloride with the sodium salts of L & D tryptophan, anthranilic acid, 3,5-diisopropylsalicylic acid, acetylsalicylic acid, hydrocortisone-21-phosphate, dexamethasone-21-phosphate, salicylic acid, 3-p-chlorophenyl-3,4,5,6-tetrahydro- β -carboline-5-carboxylic acid, 3, 4, 5, 6-tetra-hydro- β -carboline-5-carboxylic acid, and 1-(p-chlorobenzoyl)-5-methoxy-2-methylindole-3-acetic acid; by reacting cupric chloride with 1-phenyl-5-aminotetrazole, ϵ aminocaproic acid, pyridine, a mixture of D and L-tryptophan, morpholine, and histamine; by reacting cupric chloride with the ammonium salt of hydrocortisone-21-hemisuccinic acid; by reacting cupric acetate with the sodium salts of 2[3(trifluoromethyl)phenyl] aminonicotinic acid (sometimes referred to hereinafter as tpan), 1-carboxyisoquinoline, phenylcinchoninic acid, hydrocortisone-21-phosphate, and 4-n-butyl-1,2-diphenyl-3,5-pyrazolidinedione; by reacting cupric acetate with the ammonium salt of nicotinic acid; by reacting cupric acetate with D-penicillamine, 1-phenyl-5-aminotetrazole, D or L-aspartic acid, L-lysine, 2-carboxyindole; and by reacting cupric acetate with the potassium salt of 17-hydroxy-3-oxo-17 α -pregn-4,6-diene-21 carboxylic acid.

It is preferable to produce copper coordination solvates rather than anhydrous compounds as will be more fully appreciated by the following description. The compounds may be solvated with a lower alcohol (methanol or ethanol), acetone, pyridine, water or dimethyl sulfoxide.

Following is a more detailed description of how the copper coordination compounds of the present invention may be prepared. Table V contains suggested structural formulae for some of my coordination compounds. Not all have been empirically determined.

EXAMPLE 1:

Bis-L-tryptophanato(O,N)copper(II), [Cu(II)(L-tryptophan)₂]

L-tryptophan (5.0 g, 0.025 mol) was dissolved in 100 ml of H₂O with a solution of NaOH (50%), filtered and back titrated if necessary with a solution of HCl (10%) until indicator paper showed the solution to be weakly basic. This solution was then dropped into 100 ml of H₂O containing CuCl₂ dihydrate (3.3 g, 0.021 mol). After stirring for about one hour a precipitate formed and was collected by filtration. This blue precipitate was washed with H₂O and diethylether, dried at 100° and 15 mm Hg overnight and weighed (4.7 g, 82% yield). A sample of this material on heating turned brown at 240° C. and finally decomposed at 260° C. Analysis Calcd. for C₂₂H₂₂N₄O₄Cu: C, 56.22; H, 4.72; N, 11.92. Found: C, 56.07; H, 4.89 and N, 12.16.

EXAMPLE

2:BIS-D-tryptophanato(O,N)copper(II)[Cu(II)(D-tryptophan)₂].

This coordination compound was prepared as described for the L isomer (example 1) using 5.0 g, 0.021 mol of D-tryptophan. After collecting the precipitate by filtration, washing with H₂O, diethylether and acetone (250 ml), the precipitate was dried overnight at 100° and 15 mm Hg and weighed (4.3 g, 75% yield). A sample of this material decomposed slowly on heating to 269° C. Analysis Calcd. for C₂₂H₂₂N₄O₄Cu: C, 56.22; H, 4.72; N, 11.92. Found: C, 56.10, H, 4.72 and N, 12.00.

EXAMPLE 3:

Bisanthranilato(O,N)copper(II), [Cu(II)(anthranilate)₂]

The sodium salt of anthranilic acid (5 g 0.04 mol) was prepared as described in example 1 in 150 ml of H₂O with 50% NaOH. This solution was dropped into 300 ml of a stirred aqueous solution of CuCl₂ dihydrate (2.5 g, 0.016 mol). The precipitate which formed was removed by filtration and washed with H₂O and diethylether (5×50 ml). After drying overnight @120° C. and 15 mm Hg the material weighed 6.1 g, 99% yield. A sample of this greenishblue material decomposed on heating to 240° C. and continued to decompose on heating to 290° C. Analysis Calcd. for C₁₄H₆N₂O₄Cu: C, 50.07; H, 3.60; N, 8.35. Found: C, 50.07; H, 3.77; N, 8.42.

EXAMPLE 4:

Bis(3,5-diisopropylsalicylato(O,O)copper(II)[Cu(II)(3,5-dips)₂]

A solution of the sodium salt of 3,5-diisopropylsalicylic diisopropylsalicyclic acid (5 g, 0.023 mol) was prepared as described in example 1 and added to 300 ml of a stirred aqueous solution of CuCl₂ dihydrate (1.59 g, 0.0336 mol). A brown precipitate formed which when recrystallized from ether gave green crystals. These crystals were filtered and dried at 125° C. and 15 mm Hg for three hours. The resulting brown crystalline material melted with decomposition over the range of 142°–144° C. Analysis Calcd. for C₂₆H₃₄O₃Cu: C, 61.70; H, 6.77. Found: C, 61.49; H, 6.83.

EXAMPLE 5:

Tetra(μ-acetylsalicylato)biscopper(II), [Cu(II)₂(aspirinate)₄]

The sodium salt of acetylsalicylic acid was prepared by dissolving acetylsalicylic acid (30 g, 0.165 mol) in 200 ml of H₂O at 0° C. with 50% NaOH so that the pH

did not go above 11.0 and rarely reached 11.0. This was done over a period of 45 to 60 minutes. The final pH of the solution was about 8.7. The CuCl₂ solution prepared by adding 56.5 g, 0.330 mol of CuCl₂ dihydrate to 500 ml of water, was added to a stirred solution of sodium acetylsalicylate during a period of 10 to 15 minutes. Following the completion of this addition the blue precipitate was collected by filtration; washed with H₂O (500 ml×2), acetone (400 ml×2) and diethylether (300 ml) and left to dry on a filter funnel attached to a water aspirator. After two days the powder was dried at 50° C. for 6–7 hours and weighed (31.3 g, 90.6% yield). Analysis calculated for C₃₆H₂₈O₁₆Cu₂: C, 51.25; H, 3.35; Found: C, 51.20; H, 3.51.

EXAMPLE 6:

[2[3(trifluoromethyl)phenyl]aminonicotinato]_{2n}-(aqua)_n-copper (II)_n[Cu(II)_n(tpan)_{2n}(H₂O)_n]

The sodium salt of 2[3 (trifluoromethyl)phenyl]aminonicotinic acid (20 g, 0.0708 mol) was prepared as described in example 1. The solution of this salt was then added to about 300 ml of a saturated, stirred solution of cupric acetate monohydrate. The resultant greenish precipitate was collected by filtration and dissolved in 200 ml of diethylether. The ether solution was then dropped into about 4 liters of boiling skellysolve A. The resultant precipitate was collected from the hot solution by filtration, dried at 125° C. for three hours at 15 mm Hg and weighed (16 g, 70% yield). A sample of this material melted with decomposition over the range of 201° to 208° C. Analysis Calcd. for C₅₂H₃₆O₁₀N₈F₁₂Cu: C, 48.49; H, 2.82; N, 8.70. Found: C, 48.53; H, 2.66; N, 8.91.

EXAMPLE 7:

D-penicillaminato-(aqua)_{1.5}-copper(I), [Cu(I)_n(D-pen)_n(H₂O)_{1.5n}]

D-penicillamine (5 g, 0.0335 mol) was dissolved in 50 ml of water. The solid cupric acetate monohydrate (6.68 g, 0.017 mol) was then added to the solution at such a rate so as to not exceed its rate of solution. Upon the completion of this addition the solution was dark gray. About 50–100 ml of H₂O was then added and the mixture left to stir for about 30 minutes. The resultant gray precipitate was collected by filtration leaving a blue filtrate (125 ml). This blue filtrate was subsequently diluted with about 375 ml of acetone and set aside for use in example 8. The air-dried gray powder weighed 4.3 g, 54.0% yield. A sample of this material decomposed over the range of 155°–157° C. Analysis Calcd. for C₅H₉SNO_{3.5}Cu: C, 25.26; H, 5.10; N, 5.89. Found: C, 25.32; H, 5.03; N, 5.47.

EXAMPLE 8:

(D-penicillaminato)_{2n}-(aqua)_{2n}-copper(II), [Cu(II)_n(D-pen)_{2n}(H₂O)_{2n}]

On standing, the acetone diluted blue filtrate described in the preparation of Example 7 gave a gray precipitate which was collected by filtration and this filtrate also set aside. The gray solid was washed with 60 ml of water and the remaining light tan solid washed with 60 ml of acetone air dried and weighed (1.15 g, 17.4% yield). A sample of this solid melted with decomposition over the range of 155° to 157° C. Analysis Calcd. for C₁₀H₂₄O₆N₂Cu: C, 30.33; H, 6.11; N, 7.08. Found: C, 30.42; H, 6.49; N, 6.72.

EXAMPLE 9: (D-pencillamine disulfide)_n(aqua)_{3n}copper(II)_n[Cu(II)_n(D-pen disulfide)_n(H₂O)_{3n}]

The acetone-water filtrate obtained after removing example 8 from the blue acetone filtrate, described above, was concentrated to about 100 ml and diluted with 400 ml of acetone. A blue precipitate (1.3 g, 9.4% yield) was obtained following filtration, washing with acetone and air drying. A sample of this material decomposed over the range of 157° to 158° C. After drying twice at 73° and 15 mm Hg overnight a sample of this material decomposed over the range of 173° to 175° C. Analysis Calcd. for C₁₀H₂₄N₂S₂O₇Cu: C, 29.15; H, 5.87; N, 6.80. Found: C, 29.43; H, 5.76; N, 6.36.

EXAMPLE 10:

(Acetato)_{2n}(1-phenyl-5-aminotetrazolato)_ncopper(II)_n[Cu(II)_n(pat)_n(acetate)_{2n}]

Five grams of cupric acetate monohydrate (0.012 mol) was dissolved in 20 ml of H₂O. This solution was diluted with 100 ml of methanol. 5 g (0.31 mol) of 1-phenyl-5-aminotetrazole was added to obtain a blue gel. This gel was filtered and the resulting blue flakes were washed with about 400 ml of methanol until the washings were no longer blue. The filtrate was then concentrated to about 150 ml and stored for about one week in the refrigerator. A precipitate formed and was removed by filtration. This green crystalline solid was air dried and weighed (3.8 g, 17.9% yield). A sample of this solid decomposed over the range of 186°–189° C. Analysis Calcd. for C₂₂H₂₆N₁₀O₈Cu₂: C, 38.54; H, 3.82; N, 20.43. Found: C, 38.42; H, 3.94; N, 20.92.

EXAMPLE 11:

(1-phenyl-5-aminotetrazolato)_{2n}(chloride)_{2n}copper(II)_n[Cu(II)_n(pat)_{2n}(HCl)_{2n}]

Five grams (0.012 mol) of 1-phenyl-5-aminotetrazole was dissolved in 30 ml of methanol, then 5 g (0.029 mol) of CuCl₂ dihydrate dissolved in 25 ml of methanol was added to the stirred solution of tetrazole. The resulting solution was filtered and set aside. Three subsequent crops of a green solid were obtained following filtration and concentration of the filtrate. The combination of these were air dried and weighed (5 g, 17.7% yield). A sample of this material decomposed on heating over the range of 184° to 185° C. Analysis Calcd. for C₁₄H₁₄N₁₀CuCl₂: C, 36.81; H, 3.09; N, 30.67. Found: C, 36.65; H, 3.17; N, 31.03.

EXAMPLE 12:

(D-Aspartato)_n(aqua)_{3.5n}copper(II)_n[Cu(II)_n(D-aspartate)_n(H₂O)_{3.5n}]

This material is made in a manner similar to the preparation of example 1 using D-aspartic acid in place of L-tryptophan. Analysis calculated for C₄H₁₂N O_{7.5}Cu: C, 18.69; H, 4.66. Found: C, 18.55; H, 4.92.

EXAMPLE 13:

(L-Aspartato)_n(aqua)_{3.5n}copper(II)_n[Cu(II)_n(L-aspartate)_n(H₂O)_{3.5n}]

This material is made in a manner similar to the preparation of example 1 using L-aspartic acid in place of L-tryptophan. Analysis calculated for C₄H₁₂N O_{7.5}Cu: C, 18.69; H, 4.66. Found: C, 18.41; H, 4.73.

EXAMPLE 14:

(L-Lysino)_n(chloro)_{2n}(aqua)_ncopper(II)_n[Cu(II)_n(L-lysinate)_n(Cl)_{2n}(H₂O)_n]

This material is made in a manner similar to example 1 using L-lysine in place of L-tryptophan. A sample of this material decomposed on heating over the range of 169° to 170° C. Analysis calculated for C₆H₁₆N₂O₃. CuCl₂: C, 24.10; H, 5.40; Cl, 23.70; N, 9.38. Found: C, 24.54; H, 5.07; Cl, 24.00; N, 9.36.

EXAMPLE 15:

(L-Lysino)_{2n}(chloro)_{2n}(aqua)_ncopper(II)_n[Cu(II)_n(L-lysinate)_{2n}(Cl)_{2n}(H₂O)_n]

This material is made in a manner similar to example 1 using L-lysine in place of L-tryptophan. A sample of this compound decomposed on heating up to and over the range 210° to 214° C. Analysis calculated for C₁₂H₃₂N₄O₅CuCl₂: C, 32.40; H, 6.80; Cl, 12.60. Found: C, 32.56; H, 7.04; Cl, 12.24.

EXAMPLE 16:

Bis(D,L-tryptophanato(O,N)copper(II)₂[Cu(II)(D,L-tryptophan)₂]

This coordination compound was prepared and isolated as described in example 1 using a mixture of D and L-tryptophan in place of L-tryptophan. Analysis Calcd. for C₂₂H₂₂N₄O₄Cu: C, 56.22; H, 4.72. Found: C, 55.58; H, 4.87.

EXAMPLE 17:

(ε-aminocaproato)_n(chloro)_{1.5n}(methanol)_{0.5n}copper(II)_n[Cu(II)_n(ε-aminocaproate)_n(Cl)_{1.5n}(CH₃OH)_{0.5n}]

This coordination compound was prepared by stirring a suspension of 10 g (0.08 mol) of ε-aminocaproic acid in 200 ml of methanol and slowly adding 10 g (0.065 mol) of solid cupric chloride dihydrate. The resultant green precipitate was collected by filtration washed with methanol, dried at 25° C. and 15 mm Hg overnight, and weighed (10.5 g, 51% yield). A sample of this material decomposed over the range of 157° to 158° C. Analysis Calcd. for C₇H_{15.5}O_{2.5}CuCl_{1.5}: C, 29.53; H, 5.91; Cl, 20.11. Found: C, 29.97; H, 6.06; Cl, 20.36.

EXAMPLE 18:

(ε-aminocaproato)_n(chloro)_{2n}(aqua)_{0.5n}copper(II)_n[Cu(II)_n(ε-aminocaproate)_n(Cl)_{2n}(H₂O)_{0.5n}]

This coordination compound was obtained from the filtrate described in example 17. Following concentration of the filtrate and methanol washings to about 100 ml of bluish-green precipitate formed. This precipitate was collected by filtration dried at 25° C. at 15 mm Hg and weighed (4.6 g, 21% yield). A sample of this material decomposed on heating over the range of 193° to 194° C. Analysis Calcd. for C₆H₁₄NO_{2.5}Cl₂Cu: C, 26.24; H, 5.14; Cl, 25.82. Found: C, 26.29; H, 5.28; Cl, 25.39.

EXAMPLE 19:

tetra(μ-acetato)bis(monopyridino)copper(II)₂[Cu(II)_n(pyridine)_n(acetate)_{2n}]

This coordination compound was prepared by adding 10 g (0.025 mol) of cupric acetate monohydrate to 70 ml of pyridine and the mixture heated while stirring at 100° C. The hot suspension was filtered and the resulting precipitate collected by filtration and washed with 200 to 300 ml of diethylether. A sample of this green solid

decomposed on heating over the range of 214° to 216° C. When the ether-pyridine filtrate cooled a second precipitate, which was bluish, was obtained. Removal by filtration and washing with ether gave a second crop of the green material in the filtrate. This green solid had a decomposition range of 216° to 218° C. The mixture decomposition range of 216° to 218° C. was observed for a sample of the combination of the two green solids. Total yield was 12 g, 92%. Analysis Calcd. for $C_{18}H_{22}N_2O_8Cu_2$: C, 41.46; H, 4.25; N, 5.37. Found: C, 41.87; H, 4.54; N, 5.23.

EXAMPLE 20:

Bispyridinobischlorocopper(II), $[Cu(II)(pyridine)_2(Cl)_2]$

This composition was prepared by dissolving 9.42 g (0.062 mol) of $CuCl_2$ dihydrate in 95% ethanol and adding 15 g (0.19 mol) of pyridine slowly to the stirred solution. The resultant blue precipitate was removed by filtration, washed with 95% ethanol (200 ml), dried at about 50° C. for 24 hours and weighed (19.8 g, 35.6% yield). A sample of this material decomposed over the range of 225° to 275° C. Analysis Calcd. for $C_{10}H_{10}N_2CuCl_2$: C, 41.32; H, 3.44. Found: C, 41.25; H, 3.52.

EXAMPLE 21:

Bismorpholoniumtetrachlorocopper(II), $[Cu(II)(morpholine)_2(Cl)_2(HCl)_2]$

This coordination compound was prepared according to the published procedure of W. H. C. Rueggeberg, G. N. Jarman and R. B. Wearn, J.A.C.S., 69, 1222 (1947) incorporated by reference herein. Starting with 14.5 g (0.167 mol) of morpholine the coordination compound was obtained in 41% yield. A sample of this green crystalline melted with decomposition over the range of 167°–170° C. Analysis Calcd. for $C_8H_{20}N_2O_2 \cdot CuCl_4$: C, 25.17; H, 5.28; N, 7.34. Found: C, 25.17; H, 5.41; N, 7.21.

EXAMPLE 22:

(Histamino) $_n$ -(chloro) $_{2n}$ -(hydrochloro) $_{2n}$ -copper(II) $_n$, $[Cu(II)_n(histamine)_n(Cl)_{2n}(HCl)_{2n}]$

This coordination compound was prepared by mixing 5 g (0.048 mol) of cupric chloride dihydrate in 200 ml of methanol and concentrating to 135 ml. On standing a tan solid precipitated. This was removed by filtration and the filtrate concentrated to 80 ml. Upon addition of 40 ml of diethylether to this concentrate a light green solid precipitated. After removal by filtration and air drying this material was weighed (4.0 g, 23% yield). A sample decomposed over the range of 185° to 189° C. with softening at 182° C. Analysis Calcd. for $C_5H_{10}N_3Cl_4Cu$: C, 18.91; H, 3.17; N, 13.24. Found: C, 18.90, H, 3.30; N, 13.30.

EXAMPLE 23:

(Sodium) $_4$ -(salicylato) $_4$ -copper(II) $_2$, $[Cu(II)_2(salicylate)_4(Na)_4]$

This material was prepared from the material obtained in example 24 with the addition of sodium ethoxide in suitable solvent. Analysis calculated for $C_{28}H_{16}O_{12}Cu_2Na_4$ were found to be within $\pm 0.4\%$ of the theoretical values.

EXAMPLE 24:

(Salicylato) $_{2n}$ -(aqua) $_{4n}$ -copper(II) $_n$, $[Cu(II)(Salicylate)_2(H_2O)_4]$

This material may be prepared as described in example 1 using salicylic acid in place of L-tryptophan. Analysis calculated for $C_{14}H_{18}O_{10}Cu$: C, 41.03; H, 4.43. Found: C, 41.24; H, 4.52.

EXAMPLE 25:

(Pyridine-3-carboxylato) $_{2n}$ -(aqua) $_{1.5n}$ -copper(II), $[Cu(II)_{2n}(nicotinate)_{4n}(H_2O)_{3n}]$

This coordination compound was prepared by dissolving 10 g (0.08 mol) nicotinic acid in 100 ml of water with concentrated NH_4OH so that the final pH was 7.0. A cupric chloride solution, prepared by dissolving 21.6 g (0.14 mol) of cupric chloride dihydrate in 200 ml of water, was stirred while the ammonium salt of nicotinic acid was added dropwise. The blue precipitate was collected by filtration, washed with 500 ml of water and air dried. The resulting material was dried at 80° C. and weighed (10.7 g, 80% yield). A sample of this material decomposed on heating up to and through the range of 265° to 266° C. Analysis Calcd. for $C_{24}H_{22}O_{11}N_4Cu_2$: C, 43.05; H, 3.31; N, 8.37. Found: C, 43.25; H, 3.00; N, 8.12.

EXAMPLE 26:

(Isoquinoline-1-carboxylato) $_{2n}$ -copper(II) $_n$, $[Cu(II)_n(1-carboxyisoquinoline)_{2n}]$

The copper coordination compound of 1-carboxyisoquinoline (5 g 0.029 mol) was prepared by adding to its solution of the sodium salt, prepared as in example 1 in 200 ml of water using 1-carboxyisoquinoline in place of L-tryptophan, 60 ml of a saturated aqueous solution of cupric acetate monohydrate. The resultant purple precipitate was collected by filtration, washed with 500 ml of water and dried overnight at 100° C. and 15 mm Hg. A sample of this material (4.0 g, 70.2% yield) decomposed over the range of 295° to 296° C. Analysis Calcd. for $C_{20}H_{12}N_2O_4Cu$: C, 58.84; H, 2.97; N, 6.87. Found: C, 58.49; H, 3.14; N, 6.79.

EXAMPLE 27:

(2-Phenyl-4-isoquinoline-carboxylato) $_{2n}$ -(aqua) $_{2n}$ -copper(II) $_n$, $[Cu(II)_n(2-phenyl-4-carboxyisoquinoline)_{2n}(H_2O)_{2n}]$

This coordination compound was synthesized from the sodium salt of phenylcinchoninic acid (25 g, 0.15 mol), which was prepared as described in example 1 using "2-phenyl-4-isoquinoline-carboxylic acid" in place of L-tryptophan in 550 ml of water. The solution of the sodium salt was dropped into a stirred solution of cupric chloride dihydrate (14.2 g, 0.09 mol). The resulting green precipitate was collected by filtration, washed with methanol, water and then air dried and weighed (29.5 g, 67% yield). A sample of this material decomposed on heating over the range of 228° to 229° C. Analysis Calcd. for $C_{64}H_{48}N_4O_{12}Cu_2$: C, 64.48; H, 4.06; N, 4.70. Found: C, 64.55; H, 3.80; N, 4.61.

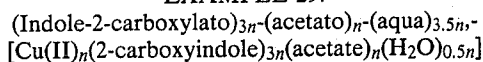
EXAMPLE 28:

(Indole-2-carboxylato) $_{3n}$ -(acetato) $_n$ -aqua) $_{0.5n}$, $[Cu(II)_n(2-carboxyindole)_{3n}(acetate)_n(H_2O)_{0.5n}]$

This copper coordination compound was prepared from the parent acid 2-carboxyindole (4.5 g, 0.028 mol) as in example 1, using cupric acetate. The green precipitate was collected by filtration, air dried for several

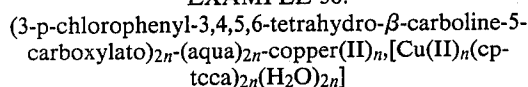
days, suspended in boiling methanol and again collected by filtration. It was then dried at 100° C. and 15 mm Hg overnight and at 125° and 15 mm Hg for 3 hours. A sample of this material (3.0 g, 23.3% yield) decomposed over the range of 249°–255° C. Analysis Calcd. for $C_{29}H_{22}N_3O_9Cu$: C, 56.91; H, 3.59; N, 6.86. Found: C, 56.87; H, 4.03; N, 6.62.

EXAMPLE 29:



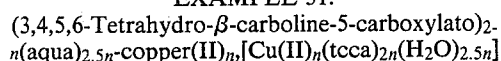
This material was prepared as described in example 1 using 2-carboxyindole in place of L-tryptophan and dried at 100° C. and 15 mm Hg over the weekend. A sample of this material did not melt but did turn brown, as did the material in example 28, on heating to 260°. Analysis Calcd. for $C_{29}H_{28}N_3O_{12}Cu$: C, 52.29; H, 4.20; N, 6.30. Found: C, 51.85; H, 3.78; N, 6.59.

EXAMPLE 30:



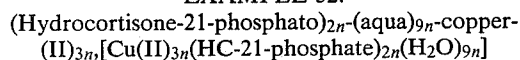
The copper coordination compound of the parent acid (5 g, 0.015 mol) was prepared as described for example 1 except 3-p-chlorophenyl-3,4,5,6-tetrahydro- β -carboline-5-carboxylic acid was substituted for L-tryptophan. An olive drab precipitate was collected by filtration, washed with 500 ml of H_2O , 300 ml of diethylether and then with acetone until the washings were colorless. This material was dried at 100° C. overnight and 110° C. at 15 mm Hg for 3 hours before dissolving in acetone and precipitated with Skellysolve B. This material (2 g, 40% yield) was then dried overnight at 60° C. and 15 mm Hg and again at 125° C. and 15 mm Hg. A sample of this material decomposed over the range of 205° to 210° C. Analysis Calcd. for $C_{36}H_{32}Cl_2N_4O_6Cu$: C, 57.56; H, 4.30; N, 7.46. Found: C, 57.16; H, 4.15; N, 6.96.

EXAMPLE 31:



The copper coordination compound of the parent acid (5 g, 0.023 mol) was prepared as described for example 1 except that 3,4,5,6-tetrahydro- β -carboline-5-carboxylic acid was substituted for L-tryptophan. This dark green solid was washed with 500 ml of water, then suspended in 500 ml of boiling acetone and collected by filtration. Drying was done at 100° C. at atmospheric pressure for 24 hours and then at 110° C. and 15 mm Hg for 3 hours. Subsequent leaching with hot propylene glycol gave an insoluble material (3.3 g, 52.8% yield) which rapidly decomposed on heating to 294° C. Analysis Calcd. for $C_{24}H_{27}N_4O_{6.5}Cu$: C, 53.47; H, 5.05 and N, 10.40. Found: C, 53.54; H, 4.69 and N, 10.58.

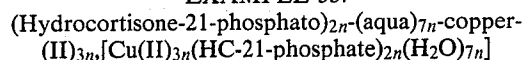
EXAMPLE 32:



This coordination compound was prepared by dissolving 1 g (0.002 mol) of the disodium salt of hydrocortisone-21-phosphate in 25 ml of water and adding this solution dropwise to a stirred solution of cupric acetate monohydrate, prepared by adding 0.79 g (0.004 mol) of cupric acetate monohydrate to 25 ml of water. After the addition was complete, stirring was continued for one-half hour before the light blue precipitate was collected

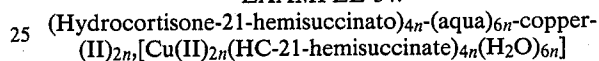
by filtration and washed with 500 ml of water before air drying. The yield was 0.185 g, 34%. On heating a sample of this material to 209° C. it decomposed. Analysis Calcd. for $C_{42}H_{78}O_{25}P_2Cu_3$: C, 40.82; H, 6.36. Found: C, 40.59; H, 6.18.

EXAMPLE 33:



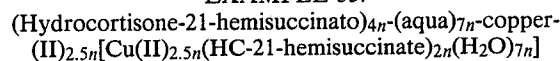
This coordination compound was prepared by dissolving 1 g (0.002 mol) of the disodium salt of hydrocortisone-21-phosphate in 100 ml of water, adding 1 drop of concentrated hydrochloric acid to give a pH of 6.6 and adding this solution dropwise to a stirred solution of cupric chloride dihydrate (1 g, 0.006 mol) in 50 ml of water. After the addition was complete the mixture was allowed to stir for one hour and the light blue precipitate collected by filtration, washed with 200 ml of water, air dried and weighed (400 mg, 33% yield). A sample of this material gradually decomposed on heating to 210° C. Analysis Calcd. for $C_{42}H_{74}O_{23}P_2Cu_3$: C, 42.05; H, 6.22. Found: C, 42.00; H, 6.21.

EXAMPLE 34:

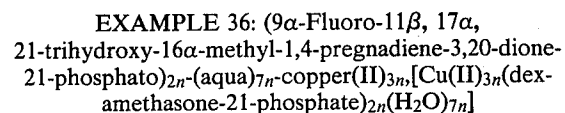


This coordination compound was prepared by dissolving 1 g (0.002 mol) of hydrocortisone-21-hemisuccinic acid in 250 ml of water with concentrated ammonium hydroxide. The resulting pH was 9.0 and was adjusted to pH 7.0 with a 10% solution of hydrochloric acid. This solution was then added dropwise to a stirred solution of cupric chloride dihydrate (1 g, 0.006 mol) dissolved in 250 ml of water. The resulting light blue-green precipitate was collected, air dried and weighed (1 g, 96% yield). A sample of this material decomposed on heating over the range of 191° to 195° C. Analysis Calcd. for $C_{100}H_{144}O_{38}Cu_2$: C, 57.71; H, 6.97. Found: C, 57.41; H, 7.26.

EXAMPLE 35:



This coordination compound was prepared by dissolving 1 g (0.002 mol) of hydrocortisone-21-hemisuccinic acid in 20 ml of water with concentrated ammonium hydroxide. The resulting pH was 9.5. This solution was then added dropwise to a stirred solution of cupric chloride dihydrate (1 g, 0.006 mol) dissolved in 15 ml of water. The light blue precipitate which formed was collected by filtration, air dried and weighed (1.2 g, 99% yield). A sample of this material decomposed on heating over the range of 196° to 197° C. Analysis Calcd. for $C_{100}H_{160}O_{46}Cu_5$: C, 49.71; H, 6.68. Found: C, 49.91; H, 6.63.



This coordination compound was prepared by dropping a solution of the disodium salt of dexamethasone-21-phosphate (9 g, 0.017 mol) dissolved in 100 ml of water, into a stirred solution of 100 ml of water containing 4.6 g (0.003 mol) of cupric chloride dihydrate. After the addition was completed an additional 300 ml of water was added. The resulting light blue precipitate was collected by filtration, washed with water, air dried

and weighed (8.1 g, 75% yield). A sample of this material gradually decomposed on heating to 300° C. Analysis Calcd. for $C_{88}H_{140}O_{46}P_4F_4Cu_6$: C, 42.02; H, 5.61. Found: C, 42.04; H, 5.5.

EXAMPLE 37: (9 α -Fluoro-11 β , 17 α -21-trihydroxy-16 α -methyl-1,4-pregnadiene-3,20-dione-21-phosphato) $_{2n}$ -(aqua) $_{1.5n}$ -copper(II) $_{3n}$ -[Cu(II) $_{3n}$ (dexamethasone-21-phosphate) $_{2n}$ (H $_2$ O) $_{1.5n}$]

This coordination compound was prepared by taking 2 g (0.0008 mol) of the material prepared in example 36 and suspending it in a stirred methanol for two hours to remove some of the water of hydration. After air drying this material was dried at 45° C. and 15 mm Hg overnight. A sample of this material also decomposed on heating to 300° C. Analysis Calcd. for $C_{88}H_{118}O_{35}P_4F_4Cu_6$: C, 45.62; H, 5.13. Found: C, 45.51; H, 5.48.

EXAMPLE 38:

[1-(p-Chlorobenzoyl)-5-methoxy-2-methylindole-3-acetato] $_{4n}$ -(aqua) $_{4n}$ -copper(II) $_{2n}$, Cu(II) $_{2n}$ [1-(p-chlorobenzoyl)-5-methoxy-2-methylindole-3-acetate] $_{4n}$ (H $_2$ O) $_{4n}$]

This coordination compound was synthesized from the sodium salt of the parent acid (5 g, 0.014 mol), prepared as in example 1 except 1-(p-chlorobenzoyl)-5-methoxy-2-methylindole-3-acetic acid was used in place of L-tryptophan, in 200 ml of water. The solution of the sodium salt was dropped into a stirred 300 ml water solution of cupric chloride dihydrate (1.95 g, 0.013 mol). The resultant green precipitate was collected by filtration, washed with water, air dried and weighed (5.6 g, 98% yield). A sample of this material decomposed on heating to 190° C. Analysis Calcd. for $C_{76}H_{68}O_{20}N_4Cl_4Cu_2$: C, 56.13; H, 4.21; N, 3.44. Found: C, 56.00; H, 3.78; N, 3.40.

EXAMPLE 39:

[1-(p-Chlorobenzoyl)-5-methoxy-2-methylindole-3-acetato] $_{4n}$ -(acetone) $_{2n}$ -copper(II) $_{2n}$, Cu(II) $_{2n}$ [1-(p-chlorobenzoyl)-5-methoxy-3-methylindole-3-acetate] $_{4n}$ (CH $_3$ COCH $_3$) $_{2n}$]

This coordination was prepared in a manner similar to that described for example 38, using twice the amount of parent acid and cupric chloride dihydrate. However, after the green precipitate was collected by filtration it was leached with 1 liter of acetone and the leachate concentrated to 500 ml. On standing, additional green crystals formed in the acetone solution. These were collected by filtration, air dried and weighed (6.9 g, 62% yield). A sample of this material decomposed on heating up to and over the range of 190° to 193° C. Analysis Calcd. for $C_{82}H_{72}O_{18}N_4Cl_4Cu_2$: C, 58.81; H, 4.79; N, 3.21. Found: C, 58.96; H, 4.34; N, 3.35.

EXAMPLE 40:

(4-n-Butyl-1,2-diphenyl-3,5-pyrazolidinedione) $_{2n}$ -copper(II) $_{n}$ [Cu(II) $_{n}$ (4-n-butyl-1,2-diphenyl-3,5-pyrazolidinedione) $_{2n}$]

A solution of the sodium salt of the parent compound 4-n-butyl-1,2-diphenyl-3,5-pyrazolidinedione, (5 g, 0.015 mol) dissolved in 50 ml of 95% ethanol was diluted with 150 ml of H $_2$ O. To this stirred solution was added 2.73 g (0.007 mol) of cupric acetate monohydrate, in small aliquats. The greenish precipitate which formed was collected by filtration, dried at 95° C. and 15 mm Hg overnight and weighed (4.5 g, 94.5% yield). A sample of this material softened and melted over the

range of 65° to 75° C. Analysis Calcd. for $C_{38}H_{38}N_4O_4Cu$: C, 67.29; H, 5.65; N, 8.26. Found: C, 67.61; H, 5.43; N, 8.28.

EXAMPLE 41:

(17-Hydroxy-3-oxo-17 α -pregn-4,6-diene-21-carboxylato) $_{2n}$ -(aqua) $_{2n}$ -copper(II) $_{n}$ [Cu(II) $_{n}$ (17-hydroxy-3-oxo-17 α -pregn-4,6-diene-21-carboxylato) $_{2n}$ (H $_2$ O) $_{2n}$]

The potassium salt of the parent acid (17-hydroxy-3-oxo-17 α -pregn-4,6-diene-21-carboxylic acid) (5 g, 0.013 mol) was dissolved in 50 ml of water. This solution was dropped into a stirred solution of cupric acetate monohydrate, prepared by dissolving 5 g (0.012 mol) in 50 ml of water. After the addition was completed the mixture was left to stir for an additional one-half hour before removing the precipitate by filtration. This precipitate was washed with 500 ml of water before air drying followed by drying at 30° and 15 mm Hg over the weekend. A 5 g, 24% yield was obtained. A sample of this material decomposed on heating over the range of 168°-169° C. This material was redried at 40° and 15 mm Hg before obtaining elemental analysis. Analysis Calcd. for $C_{88}H_{128}O_{22}Cu_2$: C, 64.88; H, 7.67. Found: C, 64.44; H, 7.87.

Inflammation Test Models Employed

In order to test the anti-inflammatory activities of my copper coordination compounds, the following test models were employed:

(1) Carrageenin Foot Edema Model as described in Experientia, 6, pp. 469-71, "Zur Frage des Mechanismus der Hemmung des Bindegebswachstums durch Cortisone" (1950);

(2) Cotton Wad Granuloma Model as described in J. Pharmacol. Expt'l. Ther., 141, pp. 369-76, "Anti-inflammatory and Antipyretic Activities of Indomethacin, 9-(p-chlorobenzoyl)-5-Methoxy-2-Methyl-Indole-3-Acetic Acid" (1963); and

(3) Polyarthrititis Model as described in Nature, 224, pp. 1320-21, "Effect of Prostaglandin E $_2$ on Adjuvant Arthritis" (1969).

The foregoing literature references were followed in the following tests unless otherwise stated and they are incorporated by reference herein. Each test model will only be briefly described. Each test model is a recognized and accepted model for testing drugs for the treatment of arthritis.

Carrageenin Foot Edema Model

In this test model test rats were injected with carrageenin in a hind paw one hour after administration of the copper coordination compound. The carrageenin induced inflammation. The rats were male, of the Sprague Dawley variety and weighed on the average 120 grams. The copper coordination compounds were first introduced subcutaneously. The dosages were compounded as follows:

TABLE A

Copper Coordination Compound Weight in Milligrams	Saline Solution	Suspending Agent
0.5	0.2 ml	several drops
1	0.2 ml	per 10 ml
2	0.2 ml	
5	0.2 ml	
25	0.2 ml	

The suspending agent was Tween 80, a nonionic surfactant sold by the Atlas Powder Company. A full description of it is contained at p. 648 of *Remingtons Practice of Pharmacy* (11th ed., Martin & Cook, The Mack Publishing Co., Easton Pa. 1956). Generally they may be described as a complex mixture of polyoxyethylene ethers of mixed oleic esters of sorbitol anhydrides. The initial screening dosage was 25 mg of the copper coordination compound given in one injection to ten rats. Compounds which were active received further testing at 5.0 mg and lower dosages. Each dosage was administered to at least ten rats. A dose was rated active if it caused a significant decrease ($P < 0.05$) in the circumference of the feet injected with carrageenin six hours after administration. Compounds were rated active if they possessed a subcutaneous potency of $\geq 1\%$. As a reference standard, saline solution plus suspending agent was subcutaneously administered to each of the ten rats.

Cotton Wad Granuloma

This test utilizes the discovery that when cotton is introduced subcutaneously into a rat's skin it becomes encapsulated with connective tissue forming a granuloma. This is a manifestation of a local inflammatory response.

The rats utilized were male, of the Sprague Dawley variety and weighed on the average 175–200 gm. The rats were given an adrenalectomy one day prior to implantation. The cotton pellets, sterilized, each weighing 32–50 mg but for each experiment not varying ± 1 mg. were inserted in each animal adjacent to the abdomen. The copper coordination compounds were subcutaneously introduced in various dosages daily for two days. The dosages were prepared in accordance with Table A and the description given in explanation thereof. Each dose was administered to at least ten rats. The initial screening dose was 20 mg. The compounds were rated active if they caused a significant decrease ($P < 0.05$) in the adjusted weight of the granuloma tissue encapsulating the implanted cotton. In order to arrive at the adjusted weight a reference standard, hydrocortisone, 0.5 mg. was administered subcutaneously to ten reference standard rats. After two days, the rats, in the test model and reference standard were sacrificed and the granulomas from each were removed, weighed, dried and reweighed. The adjusted weights were calculated as wet granuloma from test model compared to wet granuloma from reference standard and dry granuloma from test model compared to dry granuloma from reference standard.

Polyarthrititis Model

In this model the test rats, male Sprague Dawley rats, 160–180 g, were inoculated intradermally at the base of the tail with a Freund type adjuvant as described in Nature, supra, to wit: 0.6 mg dry, heat-killed *Mycobacterium butyricum* (purchased from Difco) suspended in 0.05 ml of paraffin oil. The test rats were injected subcutaneously daily for 16 days with varying dosages of copper coordination compound prepared by mixing the copper compound with saline solution and suspending agent in the proportion set forth in Table A and in the manner described in connection therewith. The initial screening dose was 5 mg. Each dose was administered to at least twelve rats. As a control group, twelve rats were also injected subcutaneously with saline solution plus suspending agent.

On day 16 (day 1 = day of inoculation) the rats were sacrificed. The circumference of the tibiotarsal (ankle) joint was used as a measure of the severity of the inflammation. Table I and Table IV set forth the results of the foregoing tests and Table I includes in addition comparisons of the anti-inflammatory activities of my compound vs. hydrocortisone.

Ulcer Test Models Employed

To determine the ulcerogenicity of my copper coordination compounds, two different test models were employed--the Shay rat test and the Corticoid Induced Rat Ulcer Test. These are commonly employed tests described in the literature. Shay, et al, "A Simple Method for the Uniform Production of Gastric Ulceration in the Rat," *Gastroenterology* 5, 43–61(1945); and Robert, A. et al., Proc. Soc. Expt'l. Biol. 99, pp. 443–47, "Ulcerogenic Properties of Steroids" (1958), incorporated by reference herein.

Shay Rat Test

The procedure set forth in the above described method by Shay et al was followed. The rats were males of the Sprague Dawley variety, weighing 200–250 grams, which had been fasted for 72 hours. The control group of twelve rats received a saline and Tween 80 solution introduced intragastrically. An initial screening dose of 50 mg copper coordination compound, mixed with saline solution and Tween 80 suspending agent was introduced intragastrically into twelve rats. If the compounds displayed activity, as determined in the manner described below, dosages of either 0.5 mg, 1 mg, 2.5 mg, or 10 mg were administered. Each dose was given to at least twelve rats. The following Table B shows how the dosages were formulated.

TABLE B

Copper Compound (mg)	Saline Solution ML	Suspending Agent
0.5	1	1 drop
1	1	1 drop
2.5	1	1 drop
10	1	1 drop
50	1	1 drop

The activity of the dosages was arrived at by sacrificing the rats after 19 hours and determining the size and number of ulcers present. The incidence of ulcers in rats receiving the dosages of copper compounds were compared with the incidence of ulcers in rats in the control group. The compounds were rated active if the comparison showed that the compound significantly inhibited ulceration ($P < 0.05$).

Corticoid-Induced Rat Ulcer Test

8 mg of Δ^1 -cortisol in about 0.2 ml corn oil was injected subcutaneously into each rat in two groups of male Sprague Dawley rats (about 150 to 165 g) daily for four days. The rats in the control group received no other compound, while the rats in the treated group received orally 25 mg of various copper coordination compounds of my invention (1 ml saline solution - 1 drop Tween 80) three times daily.

The results of this test, using the ulcer index as found in Robert, A. et al, Proc. Soc. Expt'l. Biol. 99, pp. 443–47, "Ulcerogenic Property of Steroids" (1958) (in-

corporated by reference herein) are included in Tables III and IV.

In addition to the foregoing anti-inflammatory and anti-ulcer tests, several LD₅₀ tests [performed in accordance with Miller et al, Proceed. of The Society of Expt'l. Medicine, 57, p. 261 (1944)] were performed with some copper coordination compounds and compared to cupric acetate, aspirin and tpan. The results are set forth in Table II along with TI data which is the ratio of the lethal dose to effective dose.

The following surprising results may be gleaned quickly by reviewing the results set forth in Tables I-IV.

(1) A marked increase in anti-inflammatory activity was observed for most of the compounds of the present invention as compared to prior art compounds.

(2) Many parent compounds used to prepare the copper coordination compounds possess no anti-inflammatory activity while their copper coordination compounds did.

(3) The LD₅₀ of the copper coordination compounds of my invention were substantially less than prior art anti-inflammatory agents.

(4) While all known prior art compounds useful as anti-inflammatory agents are known ulcerogenic compounds, my copper coordination compounds displayed anti-ulcer activity.

Upon further and more extensive study of the results the following observations are of interest to those skilled in the art.

From a review of the Tables it can be noted that activity in the various test models is apparently not a function of the amount of the percent of copper present in the copper coordination compound. For example, activity in the Shay Rat Anti-Ulcer activity model was displayed in compounds at similar dosages having about 7.7% be weight copper and in compounds having about 19% by weight copper.

While examples 1, 8, 16, 30 and 41 showed no anti-inflammatory activity at the initial screening tests and using only one test model, it is believed that they would display anti-inflammatory activity under other conditions.

From the foregoing description it can be noted that all of the compounds of the present invention have the formula Cu_yX_n wherein y and n are numerals and wherein x is derived from at least one group of coordinating elements. By group of coordinating elements I mean a group of elements which are not attached to each other in a cyclic manner. The coordinating compounds may contain within them carbocyclic or heterocyclic structural components. By coordinating elements I mean elements which contribute electrons to form a covalent bond with copper, which may have a valence state of +1 or +2. In most instances, such coordinating elements are selected from the group of oxygen, nitrogen, halogen and sulfur.

In most instances my copper coordination compounds have the formula $Cu_{1y}X_{2n}$. In this case the coordinating groups may be the same or different. Another class of my copper coordination compounds has the formula $Cu_{3y}X_{2n}$ and the coordinating groups may be the same or different. A still further class has the formula $Cu_{2y}X_{4n}$ and the coordinating groups may be the same or different.

From the foregoing description it will also be understood that in the treatment of inflammation (arthritis) and gastrointestinal ulcers in animals my compounds may be administered in customary ways. While the dosages recommended herein for use are preferred, other dosages may also provide beneficial results.

Referring now to Table V, which sets out some of the structural formulae of my copper coordination compounds, the following is given in explanation thereof.

Example 1 has the structure d when the ligand is derived from L-tryptophan (a) and x is a amino. Example 2 has the structure d when the ligand is derived from D-tryptophan (a) and x is amino. Example 16 has the structure d when the ligand is derived from D,L-tryptophan (a) and x is amino. Example 3 has the structure d when the ligand is derived from anthranilic acid (b) and x is o-amino.

Example 4 has the structure d when the ligand is derived from 3,5-diisopropylsalicylic acid (c) and x is o-hydroxyl.

TABLE I

THE ANTI-INFLAMMATORY ACTIVITIES OF SOME COPPER COORDINATION COMPOUNDS COMPARED WITH SOME OF THEIR PARENT COMPOUNDS						
EX-AMPLE	COMPOUND	CARRA-GEENIN FOOT EDEMA	COTTON WAD GRANULOMA	POLYARTHRITIS	% POTENCY COMPARED WITH HYDRO-CORTISONE	% COPPER
	cupric acetate	A@1 mg SC	I@20 mg SC	I@5 mg SC	340	31.8
1	L-tryptophan	I@25 mg SC	NT	NT		
	Cu(II)(L-tryptophan) ₂	I@25 mg SC	I@20 mg SC	NT		13.5
	D-tryptophan	I@25 mg SC	NT	NT		
2	Cu(II)(D-tryptophan) ₂	A@25 mg SC	I@20 mg SC	NT		13.5
	anthranilic acid	I@25 mg SC	NT	I@5 mg SC		
3	Cu(II)(anthranilate) ₂	A@1 mg SC	A@5 mg SC	A@0.2 mg SC(+25)	60	18.9
	3,5-dips acid	I@25 mg SC	NT	I@5 mg SC		
4	Cu(II)(3,5-dips) ₂	A@1 mg SC	A@1 mg SC	A@0.2 mg SC(+24)	50	12.5
	aspirin	A@8 mg SC	A@40 mg IG	A@1 mg SC(+5)	6	
5	Cu(II) ₂ (aspirinate) ₄	A@1 mg SC	A@2 mg SC	A@0.2 mg SC(+27)	130	15.0
	tpan	A@5 mg IG	A@5 mg IG	A@1 mg IG(+5)	31	
6	Cu(II) _n (tpan) _{2n} (H ₂ O) _n	A@1 mg SC	A@2 mg SC	A@0.2 mg SC(+18)	180	10.1
	D-penicillamine	I@25 mg SC	I@20 mg SC	I@5 mg SC		
7	Cu(I) _n D-pen(H ₂ O) _{1.5n}	A@1 mg SC	A@2 mg SC	NT		26.7
8	Cu(II) _n (D-pen) _{2n} (H ₂ O) _{2n}	I@25 mg SC	NT	NT		16.1
9	Cu(II) _n (D-pen disulfide) _n (H ₂ O) _{3n}	A@1 mg SC	A@5 mg SC	A@5 mg SC(+18)		15.4
	pat	I@25 mg SC	A@20 mg IG	A@20 mg IG(+5)		
10	Cu(II) _n (pat) _n (acetate) _{2n}	A@1 mg SC	A@5 mg SC	A@5 mg SC(+5)		18.5
11	Cu(II) _n (pat) _{2n} (HCl) _{2n}	A@2 mg SC	A@2 mg SC	NT		13.9

TABLE II

THE LD ₅₀ AND CALCULATED TI DATA FOR SOME COPPER COORDINATION COMPOUNDS				
EX- AMPLE	COMPOUND	LD ₅₀	TI	
			CFE	PA
3	cupric acetate	350 mpk SC	70	0
	Cu(II)(anthranilate) ₂	750 ± 106 mpk SC	150	750
4	Cu(II)(3,5-dips) ₂	240 ± 33 mpk SC	48	240
	aspirin	1500 mpk IG		
		790 mpk RT		

TABLE II-continued

THE LD ₅₀ AND CALCULATED TI DATA FOR SOME COPPER COORDINATION COMPOUNDS				
EX- AMPLE	COMPOUND	LD ₅₀	TI	
			CFE	PA
5	Cu(II) ₂ (aspirinate) ₄	760 ± 100 mpk SC	150	760
	tpan	370 ± 25 mpk IG	15	74
10	Cu(II) _n (tpan) _{2n} (H ₂ O) _n	650 ± 80 mpk SC	130	650

TABLE III

A COMPARISON OF THE ANTI-ULCER ACTIVITIES AMONG THE
COPPER COORDINATION COMPOUNDS OF
TABLE I AND SOME OF THEIR PARENT COMPOUNDS

EXAMPLE	COMPOUND	SHAY RAT: ANTI-ULCER ACTIVITY	CORTICOID INDUCED: ANTI-ULCER ACTIVITY
	Cu(II) ₂ (acetate) ₄ (H ₂ O) ₂	A@50 mg IG	NT
1	Cu(II)(L-tryptophan) ₂	A@0.1 mg IG	A@25 mg IG
2	(Cu(II)(D-tryptophan) ₂	A@1 mg IG	NT
	D-tryptophan	I@50 mg IG	NT
3	Cu(II)(anthranilate) ₂	A@1 mg IG	A@25 mg IG
	anthranilic acid	A@50 mg IG	NT
4	Cu(II)(3,5-dips) ₂	A@0.5 mg IG	A@25 mg IG
5	Cu(II) ₂ (aspirinate) ₄	A@2.5 mg IG	NT
	aspirin	A@50 mg IG	NT
6	Cu(II) _n (tpan) _{2n} (H ₂ O) _n	A@1 mg IG	NT
	tpan	I@50 mg IG	NT
7	Cu(I) _n D-pen(H ₂ O) _{1.5n}	NT	NT
8	Cu(II) _n (D-pen) _{2n} (H ₂ O) _{2n}	A@1 mg IG	NT
9	Cu(II) _n (D-pen disulfide) _n (H ₂ O) _{3n}	A@1 mg IG	NT
10	Cu(II) _n (pat) _n (acetate) _{2n}	A@1 mg IG	NT
11	Cu(II) _n (pat) _{2n} (HCl) _{2n}	A@1 mg IG	NT

TABLE IV

THE ANTI-INFLAMMATORY AND ANTI-ULCER ACTIVITIES OF SOME ADDITIONAL COPPER COORDINATION
COMPOUNDS COMPARED TO SOME OF THEIR PARENT COMPOUNDS

Ex.	Compound	Carrageenin Foot Edema	Cotton Wad Granuloma	Polyarthrits	Percent Copper	Shay Rat: Anti-Ulcer Activity
Amino Acids						
	Cu(II) _n (D-aspartate) _n (H ₂ O) _{3.5n}	A@1 mg SC	A@2 mg SC	NT	32.6	A@0.5 mg IG
	Cu(II) _n (L-aspartate) _n (H ₂ O) _{3.5n}	A@1 mg SC	NT	NT	32.6	A@0.5 mg IG
	Cu(II) _n (L-lysinate) _n (Cl) _{2n} (H ₂ O) _n	A@0.5 mg SC	A@2 mg SC	NT	21.9	A@10 mg IG
	Cu(II) _n (L-lysinate) _{2n} (Cl) _{2n} (H ₂ O) _n	A@1 mg SC	NT	NT	14.3	NT
16	Cu(II)(D,L-tryptophan) ₂	I@25 mg SC	NT	NT	13.5	A@1 mg IG
17	Cu(II) _n (ε-aminocaproate) _n (Cl) _{1.5n} (CH ₃ OG) _{0.5n}	NT	NT	NT	24.0	A@2.5 mg IG
18	Cu(II) _n (ε-aminocaproate) _n (Cl) _{2n} (H ₂ O) _{0.5n}	NT	NT	NT	23.2	A@1 mg IG*
Amines						
19	Cu(II) ₂ (pyridine) ₂ (acetate) ₄	A@2 mg SC	NT	NT	24.4	A@0.5 mg IG
20	Cu(II) (pyridine) ₂ (Cl) ₂	A@1 mg SC	NT	NT	21.6	A@10 mg IG
21	Cu(II)(morpholine) ₂ (Cl) ₂ (HCl) ₂	A@0.5 mg SC	A@5 mg SC	NT	16.6	NT
22	Cu(II) _n (histamine) _n (Cl) _{2n} (HCl) _{2n}	NT	NT	NT	20.0	A@10 mg IG*
Salicylates						
	Cu(II) ₂ (salicylate) ₄ (Na) ₄	A@2 mg SC	A@2 mg SC	NT	17.8	NT
	Cu(II) _n (salicylate) _{2n} (H ₂ O) _{4n}	A@1 mg SC	A@5 mg SC	NT	15.5	A@1 mg IG
Heterocyclic Carboxylic Acids						
25	Cu(II) _{2n} (nicotinate) _{4n} (H ₂ O) _{3n}	NT	NT	NT	19.0	A@1 mg IG
26	Cu(II) _n (1-carboxyisoquinoline) _{2n}	A@5 mg SC	A@5 mg SC	NT	15.6	A@50 mg IG
27	Cu(II) _n (2-phenyl-4-carboxyisoquinoline) _{2n} (H ₂ O) _{2n}	NT	NT	NT	10.6	A@1 mg IG*
28	Cu(II) _n (2-carboxyindole) _{3n} (acetate) _n (H ₂ O) _{0.5n}	A@1 mg SC	A@2 mg SC	NT	16.5	A@0.5 mg IG
29	Cu(II) _n (2-carboxyindole) _{3n} (acetate) _n (H ₂ O) _{3.5n}	NT	NT	NT	15.4	A@1 mg IG*
30	Cu(II) _n (cp-tcca) _{2n} (H ₂ O) _{2n}	I@25 mg SC	NT	NT	8.8	A@1 mg IG
31	Cu(II) _n (tcca) _{2n} (H ₂ O) _{2.5n}	I@5 mg SC	NT	NT	11.8	A@50 mg IG
Corticoids						
	hydrocortisone-21-phosphate(Na) ₂	NT	NT	I@1 mg SC**		I@10 mg IG**
32	Cu(II) _{3n} (HC-21-phosphate) _{2n} (H ₂ O) _{9n}	A@2 mg SC	A@5 mg SC	A@1 mg SC (+7)**	15.4	A@5 mg IG
33	Cu(II) _{3n} (HC-21-phosphate) _{2n} (H ₂ O) _{7n}	NT	NT	I@1 mg SC**	15.9	A@2.5 mg IG
	hydrocortisone-21-hemisuccinate	NT	NT	A@1 mg SC (+7)**		I@10 mg IG**
34	Cu(II) _{2n} (HC-21-hemisuccinate) _{4n} (H ₂ O) _{6n} [Green]	A@2 mg SC	A@5 mg SC	A@1 mg SC (+7)**	6.1	A@5 mg IG
35	Cu(II) _{2.5n} (HC-21-hemisuccinate) _{2n} (H ₂ O) _{7n} [Blue]	A@2 mg SC	A@1 mg SC	A@1 mg SC (+11)**	13.5	A@10 mg IG*

TABLE IV-continued

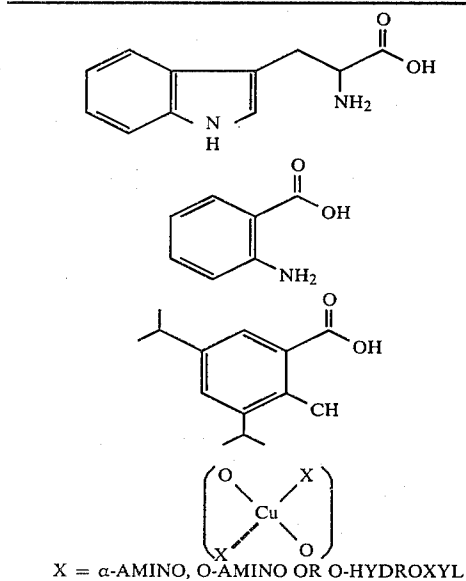
THE ANTI-INFLAMMATORY AND ANTI-ULCER ACTIVITIES OF SOME ADDITIONAL COPPER COORDINATION COMPOUNDS COMPARED TO SOME OF THEIR PARENT COMPOUNDS					
Ex.	Compound	Carrageenin Foot Edema	Cotton Wad Granuloma	Polyarthritits	Shay Rat: Anti-Ulcer Activity
	Dexamethasone-21-phosphate(Na) ₂	NT	NT	NT	NT
36	Cu(II) _{3n} (dexamethasone-21-phosphate) _{2n} (H ₂ O) _{7n}	NT	NT	NT	15 A@5 mg IG
37	Cu(II) _{3n} (dexamethasone-21-phosphate) _{2n} (H ₂ O) _{1.5n}	NT	NT	NT	16 A@5 mg IG
Arylacetic Acids					
38	Cu(II) _{2n} [1-(p-chlorobenzoyl)-5-methoxy-2-methylindole-3-acetate] _{4n} (H ₂ O) _{4n}	NT	NT	NT	7.8 A@10 mg IG*
39	Cu(II) _{2n} [1-(p-chlorobenzoyl)-5-methoxy-2-methylindole-3-acetate] _{4n} (CH ₃ COCH ₃) _{2n}	NT	NT	NT	7.7 A@1 mg IG
Pyrazolidinedione					
40	Cu(II) _n (4-n-butyl-1,2-diphenyl-3,5-pyrazolidinedione) _{2n}	NT	NT	NT	11.8 A@10 mg IG
Steroidal Acids					
41	Cu(II) _n (17-hydroxy-3-oxo-17-pregn-4,6-diene-21-carboxylate) _{2n} (H ₂ O) _{2n}	I@5 mg SC**	NT	I@5 mg SC**	7.8 A@5 mg IG

*Not tested at lower dose

**Only dose tested

NT = Not Tested

TABLE V



EXAMPLES 1, 2, 16; 3 and 4.

EXAMPLE 5

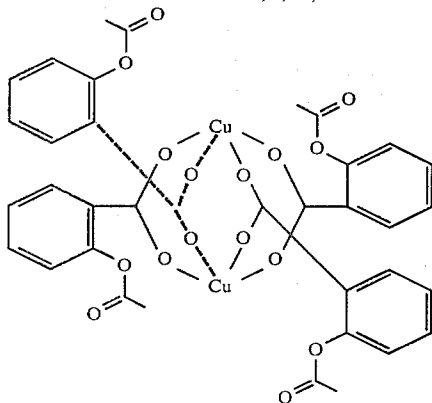
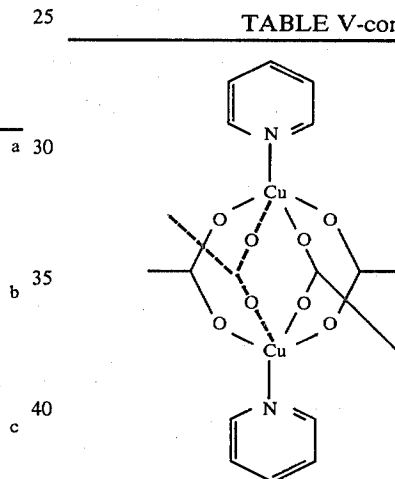
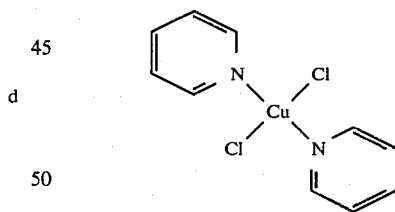


TABLE V-continued

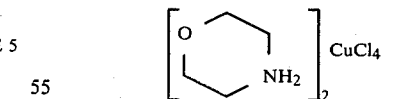
EXAMPLE 19



EXAMPLE 20



EXAMPLE 21



EXAMPLE 23

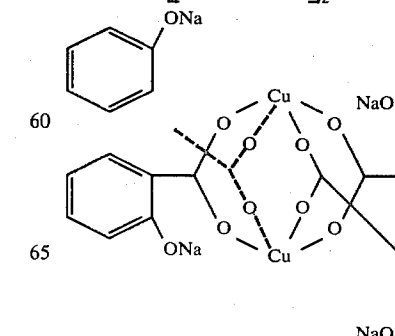
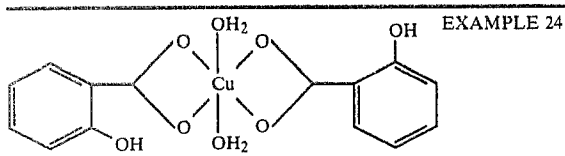


TABLE V-continued



Having thus described my invention, I claim:

1. A method for treating gastrointestinal ulcers in an animal body comprising:

administering to said animal body, orally or parenterally, a copper coordination compound having anti-ulcerogenic properties produced by reacting a copper salt with an aromatic carboxylic acid or alkaline earth salt thereof selected from the group consisting of 3,5-diisopropylsalicylic acid, acetylsalicylic acid and salicylic acid and alkaline earth salts thereof, the amount of said copper coordination compound administered to said animal body being about 2.0 to 165 mg per kilogram of body weight.

2. The method of claim 1 wherein the copper salt is selected from the group consisting of cupric chloride and cupric acetate.

3. A method for treating gastrointestinal ulcers in an animal body comprising:

administering to said animal body, orally or parenterally, a copper coordination compound having anti-ulcerogenic properties produced by reacting a copper salt with 3,5-diisopropylsalicylic acid, acetyl-

salicylic acid or salicylic acid and the alkaline earth salts thereof, the amount of said copper coordination compound administered to said animal body being about 2.0 to 165 mg per kilogram of body weight.

4. The method of claim 3 wherein the copper salt is selected from the group consisting of cupric chloride and cupric acetate.

5. A method for treating gastrointestinal ulcers in an animal body comprising:

administering to said animal body, orally or parenterally, a copper coordination compound having anti-ulcerogenic properties produced by reacting a copper salt with 3,5-diisopropylsalicylic acid or its alkaline earth salts.

6. A method for treating gastrointestinal ulcers in an animal body comprising:

administering to said animal body, orally or parenterally, a copper coordination compound having anti-ulcerogenic properties produced by reacting a copper salt with acetylsalicylic acid or its alkaline earth salts.

7. A method for treating gastrointestinal ulcers in an animal body comprising:

administering to said animal body, orally or parenterally, a copper coordination compound having anti-ulcerogenic properties produced by reacting a copper salt with salicylic acid or its alkaline earth salts.

* * * * *

Instructions aux candidats

DEUXIEME ÉPREUVE ÉCRITE

Dans cette épreuve, le candidat doit supposer qu'il a reçu de son client le courrier annexé au sujet, qui comporte la description d'un problème relatif à la validité, à la contrefaçon et/ou à la procédure de délivrance d'un brevet applicable au territoire français, ainsi qu'une copie au moins partielle de ce brevet, le cas échéant, des renseignements et/ou documents reflétant l'état de la technique le plus pertinent et des agissements contestés dont le client a connaissance à l'égard du brevet en question.

Le candidat doit accepter les faits exposés dans le sujet de l'épreuve et fonder ses réponses sur ces faits. Il décide sous sa propre responsabilité s'il fait usage de ces faits, et dans quelle mesure.

Le candidat doit admettre que l'état de la technique, dans le domaine spécifique de l'invention qui fait l'objet du brevet précédemment évoqué, est effectivement celui qui est indiqué dans le sujet et/ou les documents annexes, et que cet état de la technique, le cas échéant complété des connaissances générales nécessaires sur lesquelles il pourrait s'appuyer de façon implicite, est exhaustif.

Il est demandé au candidat de rédiger, sous la forme d'une consultation, un avis sur le problème soumis par son client, en y incluant l'indication de toutes solutions et procédures qu'il pourrait recommander à ce dernier.

Le candidat devra, dans la rédaction de cet avis, identifier de façon complète et non ambiguë les bases factuelles et juridiques de ses conclusions, veiller à exposer clairement le raisonnement qui l'y conduit, et évaluer l'efficacité prévisible de chacune des voies et/ou possibilités de solution qu'il aura envisagée, en les hiérarchisant par degré de pertinence et d'efficacité, afin d'aider son client dans sa prise de décision.

Pour des raisons d'efficacité de rédaction et de lisibilité de cette consultation, il est recommandé au candidat d'éviter de recopier de longs extraits des documents annexés au sujet ou de textes législatifs ou réglementaires, les éléments de fait ou de droit nécessaires à la compréhension de l'argumentation étant de préférence identifiés par localisation des pages et paragraphes pertinents de ces documents et par référence aux numéros des articles applicables.

Examen de Qualification Français

Session 2022 - Mention brevet d'invention

Sujet de Chimie - Deuxième épreuve écrite

Durée : 5 heures

(Début du sujet).

[1] La société holding GRATTON, ayant son siège social à Paris (France) et spécialisée dans le traitement des maladies dermatologiques vient demander votre conseil et vous livre les informations suivantes.

[2] Le psoriasis est une maladie inflammatoire chronique de la peau qui se manifeste par des plaques rouges présentant des squames. Il s'agit le plus souvent d'une maladie bénigne, mais il existe des formes sévères (environ 20% des cas), associées à une atteinte généralisée et/ou à des douleurs articulaires. A l'heure actuelle, on ne sait pas guérir cette maladie mais des traitements permettent d'en réduire les symptômes et d'améliorer la qualité de vie des patients.

[3] Pour développer de nouveaux médicaments plus spécifiques et mieux tolérés, les laboratoires tentent de mieux comprendre les mécanismes pathologiques comme les interactions entre les cellules inflammatoires et les cellules cutanées, ou encore la nature et le rôle des différentes molécules présentes dans l'inflammation.

[4] La société holding GRATTON a décidé d'apporter sa pierre à l'édifice en lançant une étude de recherche et développement de formulations dermatologiques pour améliorer la vie des patients souffrant de psoriasis.

[5] Pour cette étude, la société holding GRATTON a constitué, à compter du 1^{er} janvier 2019, une équipe dédiée faisant intervenir des spécialistes dans différents domaines, salariés de la société holding GRATTON ou de sociétés filiales dont la société holding GRATTON détient l'intégralité du capital.

[6] Cela a abouti à une proposition de formulations dermatologiques pour le traitement du psoriasis telles que présentées en Annexe 1, dont les études sur modèle de peau ont été finalisées en juin 2020. Ce modèle de peau *ex vivo* permet de mimer les lésions psoriasiques. Il est issu d'explants de peau humaine. Divers outils d'analyses histologiques et biochimiques de marqueurs spécifiques permettent de donner un score d'efficacité sur le psoriasis. Ces outils appartiennent à la filiale GratSol de GRATTON et sont confidentiels.

[7] Plus spécifiquement, la société holding GRATTON a quatre filiales :

- la société GratArt, située dans les locaux de la société GRATTON, spécialisée dans le design des packagings,
- la société GratDev, située à Orléans (France), spécialisée dans la recherche et le développement de formulations,
- la société GratSol, située à Metz (France), spécialisée dans les essais sur modèle de peau des formulations développées par la société GratDev, et

- la société GratFab, située à Vierzon (France), spécialisée dans la production à échelle industrielle des formulations conçues par la société GratDev.

[8] Les formulations décrites en Annexe 1 comprennent :

- du calcipotriol en tant que principe actif,
- de la bétaméthasone ou un de ses esters en tant que principe actif,
- un ou plusieurs diluants et/ou solvants pharmaceutiquement acceptables.

[9] Les formulations ont été étudiées et définies par Mme Rousseau, chercheur salariée de la société GratDev. Mlle Boileau, salariée de la société GratSol, les a testées sur modèle de peau.

[10] La société holding GRATTON se fournit en principes actifs auprès d'un fournisseur indien.

[11] GRATTON a également développé un packaging spécifique avec un bouton poussoir qui délivre une dose exacte et qui permet de conserver la formulation dans un environnement stérile.

[12] Pour développer ce nouveau packaging, M. Diderot, ingénieur salarié de la société GratArt, a collaboré avec M. D'Allembert, employé de la société PACK+ localisée à Nancy (France). Cette coopération a permis l'élaboration de ce nouveau packaging particulièrement adapté à la conservation des formulations.

[13] Afin de poursuivre l'étude des formulations de l'Annexe 1, la société holding GRATTON débute la semaine prochaine des essais cliniques en France sur 50 volontaires sains. Les volontaires vont recevoir le traitement pendant 6 semaines. Les résultats sont attendus pour fin août 2022. Ces essais ont nécessité la production de 25 kgs de chaque formulation.

[14] Si ces essais sont concluants, à savoir que les formulations sont bien tolérées, des essais cliniques sur 200 patients atteints de psoriasis seront réalisés. Les résultats sont attendus pour fin juin 2023.

[15] Lors de sa recherche bibliographique, Mme Rousseau a identifié le brevet EP 2 000 000 au nom de la société PSOPHARM qui lui paraît pertinent vis-à-vis des formulations selon Annexe 1.

[16] Vous avez découvert que ce brevet est validé en France et en vigueur en France. L'examen de sa procédure a mis à jour quatre documents E1 à E4 qui sont joints.

Les questions suivantes sont indépendantes les unes des autres.

I – Validité du brevet EP 2 000 000

Compte tenu du présent contexte et sur la base des informations actuellement à votre disposition, votre client, la société GRATTON, souhaite obtenir votre analyse quant à la validité du brevet EP 2 000 000.

Donnez votre avis circonstancié sur la validité du brevet EP 2 000 000.

Si vous aviez été le CPI du déposant PSOPHARM, qu'auriez-vous conseillé lors de la rédaction de cette demande de brevet en termes de brevetabilité ?

II – Analyse de contrefaçon et des risques associés :

Considérant que le brevet est valide, donnez votre analyse complète sur la matérialité et les éventuels actes de contrefaçon actuels ou à venir dont pourrait être accusée la société holding GRATTON et citez les bases légales sur lesquelles vous vous fondez.

III – Actions judiciaires et évaluation des sanctions potentielles :

III.a – Par ailleurs, la société holding GRATTON désire disposer de votre analyse sur les possibles actions judiciaires qui pourraient lui être intentées.

Détaillez les actions possibles qui pourraient être engagées à l'encontre de la société holding GRATTON et les sanctions possibles qui pourraient être prononcées en conséquence.

III.b – Enfin, la société holding GRATTON voudrait disposer de votre analyse sur les actions susceptibles d'être engagées à l'encontre du brevet EP 2 000 000

Listez les actions possibles qui peuvent être engagées par la société holding GRATTON à l'encontre du brevet EP 2 000 000

IV – Recommandations et préconisations

Au vu de vos conclusions précédentes, la société holding GRATTON souhaite disposer de recommandations et préconisations quant aux formulations telles que présentées en Annexe 1.

Formulez vos recommandations à la société holding GRATTON sur les éventuelles mises en œuvre des formulations telles que présentées en Annexe 1

V – Question supplémentaire :

La société holding GRATTON souhaite pouvoir protéger à son nom les diverses innovations réalisées dans le cadre du développement d'une formulation pour traiter le psoriasis.

Elle désire vos conseils juridiques sur les mesures à entreprendre à cet effet.

Documents joints :

Brevet EP 2 000 000

E1

E2

E3

E4

Annexe 1

(Fin du sujet).



(11) **EP 2 000 000 B1**

(12) **EUROPEAN PATENT SPECIFICATION**

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A61P 17/06 ^(2006.01)

(21) Application number: **15196888.0**

(22) Date of filing: **27.01.2015**

(54) **Pharmaceutical composition for dermal use comprising calcipotriol and betamethasone for treating psoriasis**

Pharmazeutische Zusammensetzung zur Anwendung auf der Haut enthaltend Calcipotriol und Betamethason zur Behandlung von Psoriasis

Composition pharmaceutique pour application dermique comprenant du calcipotriol et de la bétaméthasone pour le traitement du psoriasis

(84) Designated Contracting States:
**AT BE CH CY DE DK ES FI FR GB GR IE IT LI LU
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(62) Document number(s) of the earlier application(s) in

(73) Proprietor: **PSOPHARM**

(72) Inventors:

(73) **DADY, Erik**

(74) Representative: **CBD LLP**

(56) References cited:

Note: Within nine months of the publication of the mention of the grant of the European patent in the European Patent Bulletin, any person may give notice to the European Patent Office of opposition to that patent, in accordance with the Implementing Regulations. Notice of opposition shall not be deemed to have been filed until the opposition fee has been paid. (Art. 99(1) European Patent Convention).

Description

FIELD OF THE INVENTION

[0001] The present invention concerns pharmaceutical compositions for dermal use which contain at least one vitamin D or vitamin D analogue and at least one corticosteroid. More specifically, the invention relates to pharmaceutical compositions containing two or more pharmacologically active compounds which have low compatibility with respect to the pH value of optimum stability, preferably, said pharmacologically active compounds are at least one vitamin D analogue and at least one corticosteroid.

BACKGROUND OF THE INVENTION

[0002] In the treatment of a number of conditions using dermal application, e.g. in the treatment of psoriasis, it is often indicated to employ a combination treatment incorporating two or even more different pharmacologically active compounds. Thus, in the treatment of e.g. psoriasis, it is common to use a combination treatment involving a steroid compound, such as a corticosteroid compound, and a vitamin D analogue such as calcipotriol, and where each of the active compounds are formulated in separate preparations.

[0003] Clinical studies have been conducted in psoriasis patients wherein calcipotriol was administered in the morning and betamethasone dipropionate or valerate in the evening. Combination therapy was more effective than monotherapy. (Br. J. Dermatol. 1996, 135(3), 379-84; Br. J. Dermatol. 1998, 138, 254-8; Nouv. Dermatol. 1994 Jan, 13(10), 746-51; Br. J. Dermatol. 1998 Jan, 139, 649-54).

[0004] Until now a topical pharmaceutical composition comprising a combination of a vitamin D analogue and a topical steroid has not been described. Moreover, these two types of compounds often have optimum stability values of pH that differ significantly from one another making it non-obvious to attempt to prepare a topical pharmaceutical preparation containing a steroid compound together with a vitamin D analogue. US patent No. 5,565,462 relates to topical pharmaceutical compositions containing certain xanthine compounds, and where said compositions may additionally contain active compounds, such as steroids and vitamin D and its derivatives. However, there is no disclosure of a topical composition containing both a steroid and a vitamin D or vitamin D analogue or derivative, nor is there any description of a method of preparing such a composition.

[0005] The following example describes the difficulties encountered when the skilled person wishes to prepare a combination composition for topical use comprising both a vitamin D or a vitamin D analogue or derivative and a topical steroid: The vitamin D analogue calcipotriol, as well as other examples of vitamin D analogues, requires a pH value above 8 for maximum stability, whereas corticosteroids such as Betamethasone (9- fluoro- 11, 17, 21- trihydroxy- 16- methylpregna- 1, 4- diene- 3, 20- dione) require pH values in the range of 4- 6 for maximum stability. Since the base auxiliary materials and additives traditionally used in preparing topical formulations, such as creams and/or ointments, involve having some kind of acid or alkaline nature or reaction ability, it has therefore hitherto not been possible to combine the two active compounds in one single formulation while maintaining good stability of the active compounds.

[0006] Consequently, physicians have had to resort to letting patients under this type of two- component regimen perform sequential application of two creams/ ointments, each containing one of the compounds formulated at its maximum stability pH. This may lead to incompatibility of the preparations so that patients must, e.g., apply one cream/ ointment in the morning and the other in the evening. Needless to say, patient compliance as well as correct administration dosage is a problem under such circumstances. Richards, H.L. et al. report in J Am Acad Dermatol 1999 Oct; 41 (4) : 581- 3 on a study of patients with psoriasis and their compliance with medication. They report that poor compliance with treatment advice in chronic conditions, such as psoriasis, represents a major challenge to health care professionals: Thirty- nine percent of participants reported that they did not comply with the treatment regimen recommended. The noncompliant group had a higher self- rated severity of psoriasis, were younger, and had a younger age at onset than those who were compliant. The noncompliant group reported that psoriasis had a greater impact on daily life.

[0007] It is therefore an object of the present invention to provide a pharmaceutical composition for dermal use where said composition alleviates the inconveniences of a two-component or multi-component regimen for the treatment of psoriasis and other inflammatory skin diseases including nail diseases. The provision of said composition will result in a substantial improvement in quality of life for a large population of psoriasis patients, especially the noncompliant group having a higher self-rated severity of psoriasis, being younger, and having a younger age at onset than those who are compliant.

SUMMARY OF THE INVENTION

[0008] In order to solve the above mentioned problems, the invention of the present Application provides a non-aqueous topical pharmaceutical composition in the form of an ointment, a cream, a lotion, a liniment or other spreadable

liquid or semi-liquid preparation for dermal use in the treatment of psoriasis, sebopsoriasis or seborrheic dermatitis in humans and other mammals, said composition comprising a first pharmacologically active component A consisting of calcipotriol and a second pharmacologically active component B consisting of betamethasone or an ester thereof and at least one pharmaceutically acceptable carrier, solvent, or diluent.

DETAILED DESCRIPTION OF THE INVENTION

[0009] Moreover, the invention relates to pharmaceutical compositions for dermal use which contain at least one vitamin D or vitamin D analogue and at least one corticosteroid and which exhibits a higher efficacy in the treatment of psoriasis and other inflammatory skin diseases in humans and other mammals than any of the pharmacologically active components used alone. Said efficacy is preferably measured as percentage change in PASI score in psoriasis and related skin diseases, such as sebo- psoriasis and seborrheic dermatitis.

[0010] PASI (Psoriasis Area and Severity Index) score assesses the extent and severity of the patient's psoriasis. The following formulae are used to calculate the PASI score:

$$\text{Arms} \quad 0.2(R+T+S)E=X$$

$$\text{Trunk} \quad 0.3(R+T+S)E=Y$$

$$\text{Legs} \quad 0.4(R+T+S)E=Z$$

[0011] Where R=score for redness, T=score for thickness, S=score for scaliness, and E=score for extent where the score is assessed according to a scale from 0 to 4 as follows:

0=no involvement, 1=<10%, 2=10-29%, 3=30-49%, and 4=50-69%. The sum of X+Y+Z gives the total PASI score which can range from 0 to 64.8.

DESCRIPTION OF THE DRAWINGS

[0012]

Fig. 1 is a graphic illustration of the percentage change in PASI score obtained during 4 weeks of clinical trial where the efficacy of a preparation according to the invention containing calcipotriol hydrate (52.2µg/g) and betamethasone dipropionate (0.643mg/g) is compared to that of a preparation in the same vehicle containing only calcipotriol hydrate (52.2µg/g) and a preparation in the same vehicle of betamethasone dipropionate (0.643mg/g). Fig. 1 shows an efficacy of the preparation of the invention which by far exceeds the efficacy obtainable by the two single component preparations. The change in PASI score reflects in the group of patients treated with the preparation of the invention a success of treatment of psoriasis hitherto unattainable by treatment with commercial preparations containing either calcipotriol or betamethasone, or by alternating treatment with such commercial preparations (cf.) thus proving the advantage of having the two active components present in the same preparation. (EOT=end of treatment).

Fig. 2 is a table showing the figures for percentage change in PASI score at each visit and end of treatment for the same clinical trial as described for Fig. 1.

Fig. 3 is a bar diagram showing percentage of responders as a result of investigators' assessment of overall efficacy at each visit and end of treatment in the same clinical trial as for Fig. 1. Responders are defined as patients with marked improvement or clearance

Fig. 4 is a table showing the figures for percentage of responders as a result of investigators' assessment of overall efficacy at each visit and end of treatment, cf. Fig. 3, in the same clinical trial as for Fig. 1.

TOPICAL FORMULATIONS

[0013] In a preferred embodiment the invention provides a topical pharmaceutical composition in the form of an ointment, a cream, a lotion, preferably a scalp lotion, a liniment or other spreadable liquid or semi liquid preparation which is non-aqueous. In one preferred embodiment, the composition of the invention is a mono-phase composition, i.e. a composition comprising a single solvent system, such as an ointment.

[0014] In a further preferred embodiment the invention provides a non-aqueous pharmaceutical composition for dermal use, said composition comprising at least one solvent component C selected from the group consisting of:

- 5 (i) compounds of the general formula $R^3 (OCH_2C(R^1)H)_xOR^2$ (I) wherein x is in the range of 2- 60, R^1 in each of the x units independently is H or CH_3 , R^2 is straight chain or branched C_{1-20} alkyl or benzoyl, and R^3 is H or phenyl-carbonyloxy;
- (ii) di- (straight or branched)- C_{4-10} alkyl esters of C_4 - C_8 dicarboxylic acids;
- (iii) straight or branched C_{12-18} - alkyl benzoates;
- 10 (iv) straight or branched C_{2-4} - alkyl esters of straight or branched C_{10-18} - alkanolic or- alkenolic acids;
- (v) propylenglycol diesters with C_{8-14} - alkanolic acids; and
- (vi) branched primary C_{18-24} alkanols.

[0015] It has been found that in such combination compositions containing a solvent component C, the active components can co-exist without degradation, despite their different pH/stability profiles. The tendencies of the active compounds to affect one another with regard to pH is minimised or eliminated.

[0016] In the general formula (I) defined above, it is preferred that the factor x (which designates the number of the units within the parentheses) is in the range 4-50, more preferably 4-40, in particular 4-30, especially 5-25, more especially 10-20, such as about 15. It is further preferred that R^1 is CH_3 .

20 **[0017]** It is preferred that said component C is selected from compounds of the general formula $H(OCH_2C(R^1)H)_xOR^2$ (II) where R^1 , x, and R^2 are as defined above, and mixtures thereof.

[0018] As non-limiting specific examples of the types (i)-(vi) of the solvent component C defined above may be mentioned the following, including trade names:

- 25 Arlamol E (polyoxypropylene (15) stearyl ether) ;
- Arlamol DoA (diisooctyl ester of adipic acid);
- Arlasolve 200 (Polyoxyethylene- 20- isohexadecyl ether) ;
- Eutanol G (2- octyldodecanol) ;
- Finsolv (Isostearyl benzoate);
- 30 Finsolv P (polyoxypropylene- 15- stearyl ether benzoate) ;
- Isopropylesters of straight or branched C_{10} - C_{18} alkanolic or alkenolic acids such as isopropyl myristate, isopropyl palmitate, isopropyl isostearate, isopropyl linolate and isopropyl monooleate;
- Miglyol 840 (Propylene glycol diester of caprylic and caprinic acid);
- DPPG (propylene glycol dipelargonate);
- 35 Procetyl AWS $(CH_3(CH_2)_{14}CH_2(OCH(CH_3)CH_2)_5(OCH_2)_{20}OH)$.

[0019] The compositions of the present invention may be prepared in accordance with methods well known to the person skilled in the field of pharmacy. Thus, the non-aqueous compositions may be prepared by incorporating the components into a well known ointment or lotion base excipient such as white soft paraffin (also known as vaseline) or Plastibase™ (a base prepared from polyethylene (average MW about 21,000) and paraffin liquid) or ESMA-P™ (a microcrystalline wax). As an example, preparation of a composition according to the invention is typically performed by melting white soft paraffin, adding a solution (typically at a concentration in the range of 0.0005-2.5% w/w) of the vitamin D analog in the required amount of solvent component C, e.g. Arlamol E, followed by addition of a dispersion of the corticosteroid component B in paraffin oil, typically with a particle size of from 0.1 to 20 μm , and then cooling the mixture.

40 Typical content ranges of the various components in the finished composition according to the invention are 0.005 to 0.1 %w/w of the corticosteroid component B, from 0.0001 to 0.025 %w/w of the vitamin D analog component A, and from 1 to 20% w/w of the solvent component C, the remainder typically being primarily base excipient such as the above-mentioned white soft paraffin and/or paraffin oil. The composition may also contain other commonly used additives such as antioxidants (e.g. α -tocopherol).

50 **[0020]** The composition according to the invention provides the following therapeutic advantages in the treatment of skin diseases, such as psoriasis, sebo-psoriasis and related disorders, compared to the single compound therapy or combination therapy of the prior art:

[0021] A clinical investigation has showed that treatment of psoriasis patients with a composition according to the invention comprising calcipotriol and betamethasone resulted in a faster onset of healing and a more effective healing of plaques than patients treated with only one of the active compounds.

55 **[0022]** The composition of the invention, which combines a vitamin D analogue and a topical steroid, provides synergy in the form of additional benefit to the patient apart from the direct therapeutic value of the active substances. It has been shown that the skin irritative side effects of a vitamin D analogue, such as calcipotriol, is alleviated by the simul-

taneous application of a steroid, such as betamethasone, onto psoriatic skin, an effect that is only attainable using a two- component or multi- component treatment regimen where a vitamin D analogue and a steroid cannot be applied simultaneously to affected skin due to incompatibility of the preparations. When both a vitamin D analogue and a topical steroid are used in a combination treatment of psoriasis it has hitherto been necessary to use separate applications, typically one in the morning and the other in the evening, making it impossible to obtain any synergistic effect of the two types of active compounds (cf. Ortonne, J.P., *Nouv. Dermatol.*, 1994, 13 (10) , p. 746- 751) , or where a certain degree of synergistic effect, such as less skin irritation, has been reported for a two- component regimen (cf. Kragballe, K. et al. *Br J Dermatol* 1998 Oct; 139 (4) : 649- 54, and Ruzicka, T. et Lorenz, B. *Br J Dermatol* 1998, 138 (2) , 254- 58) a substantial proportion of psoriasis patients will not benefit due to non- compliance with the treatment regimen.

[0023] Satisfactory medical treatment of skin disorders, such as psoriasis, can be attained in a shorter period of time using the composition according to the invention resulting in a reduction of steroid side effects, such as skin atrophy and rebound. Besides, it can be anticipated that even a milder acting steroid of group I, such as hydrocortisone which is presently not administered for psoriasis treatment, will be efficient in reducing or even eliminating the skin irritation which often follows calcipotriol treatment.

[0024] Thus, the tolerance of the treatment will be considerably improved due to reduction of side effects of the active compounds.

[0025] Instructions for treatment will be simpler when a single preparation is needed resulting in improved compliance for the patient and the possibility of efficient treatment of a much larger population of psoriasis patients.

[0026] Instructions for treatment will be simpler when a single preparation is needed resulting in improved safety for the patient.

[0027] The invention also relates to a preferred pharmaceutical preparation according to the invention which is especially useful for the treatment of psoriatic skin diseases which are complicated by additional fungal infections, and which further contains an anti-fungal agent selected, e.g., from the group consisting of miconazol, clotrimazol, terbinafin, ciclopirox, bifonazol, nystatin, ketoconazol, econazol, and amorolfine.

[0028] Preferably, the compositions according to the invention do not contain other therapeutically effective compounds selected from the group consisting of the xanthine derivatives pentoxifylline, propentofyllin, and torbafylline, or any other xanthine or xanthine derivative.

[0029] The composition of the invention is used in a method of treatment of psoriasis and related skin diseases comprising topically administering an effective amount of a composition according to the invention to a patient in need of such treatment. Said method preferably comprises topical administration once or twice daily of a medically sufficient dosage of said composition.

[0030] The composition according to the invention preferably contains 0.001-0.5mg/ g or ml or more preferably 0.001-0.25mg/g or ml of said component A and 0.05-0.1 mg/g or ml of said component B.

[0031] The invention is further illustrated by the following examples.

EXAMPLE 1

Ointment containing Calcipotriol and Betamethasone dipropionate

[0032] 919, 3 g of White Soft Paraffin is melted at 80°C followed by cooling to 70°C and maintaining that temperature. Thereafter, 52.2 mg Calcipotriol hydrate (50 mg Calcipotriol) is dissolved in 50 g Arlamol E (polyoxypropylene- 15- stearyl ether) to form a solution (Solution 1) . Solution 1 is then added slowly into the molten paraffin while stirring.

[0033] Betamethasone (0,5 g, in the form of 0.643g of its dipropionate) in particulate form (99% <15µm) is dispersed in 30 g Paraffin Liquid to form Dispersion 1. Dispersion 1 as well as 20 mg α-tocopherol are added to the Calcipotriol-containing paraffin mixture of while stirring, after which the mixture is cooled to below 30°C to give a composition according to the invention with the following composition:

1g of ointment contains: Betamethasone (as dipropionate 0.643 mg)	0.5mg
Calcipotriol (as hydrate 52.2 µg)	50 µg
Paraffin, Liquid	30 mg
Polyoxypropylene-15-Stearyl Ether	50 mg
α-Tocopherol	20 µg
White Soft Paraffin	to make 1 g

EXAMPLE 2**Stability test**

5 **[0034]** The chemical stability of the two active components was tested after storage for 1 month at 40°C and 3 months at 25°C and 40°C, respectively. The quantitative content of Calcipotriol was determined by HPLC.

10 **[0035]** The Calcipotriol was extracted from the preparation into a mixture of methanol and 0.01 M diammonium hydrogenphosphate (70:30) and quantified under the following HPLC conditions: Column: about 125 mm Ø 4 mm (i.d.) stainless steel column with LiChrospher RP-18, 5 µm; mobile phase: acetonitrile-methanol-0.01 M aqueous ammonium phosphate pH 6 (20:50:30); flow: about 2 ml/min; detection: variable wavelength UV-detector set at 265 nm. Calcipotriol and the related substances were separated by the reverse phase HPLC-method described above; Column: Superspher RP-18, 4 µm; Flow: 1.2 ml/min. The quantitative content of Betamethasone Dipropionate was determined by HPLC.

15 **[0036]** The Betamethasone Dipropionate was extracted from the preparation into a mixture of acetonitrile:water (50 : 55) and quantified under the following HPLC conditions: Column: About 125 mm Ø 4 mm (i.d.) stainless steel column packed with LiChrospher RP-18, 5µm. Mobile phase: Acetonitrile: water (50 : 55). Flow: 2 ml/min. Detection: Variable wavelength UV-detector set at 240 nm. The related substances besides betamethasone were determined by a reverse phase HPLC-method analogous to the above. Betamethasone: Determined as above with the exception of the mobile phase: Acetonitrile/methanol/0.05M buffer pH7 (25:5:70).

20 **[0037]** The results are shown in the following Table 1.

Table 1

	Calcipotriol µg/g	Calcipotriol related substances %	Betamethasone dipropionate Mg/g	Betamethasone related substances %
25 Start	50.0	1.6	0.63	1.2
25°C				
3 months	50.5	1.4	0.64	0.2
30				
40°C				
1 month	48.0	2.1	0.64	0.6
35 3 months	49.7	1.8	0.64	0.2

[0038] It will be seen from Table 1 that both Calcipotriol and Betamethasone ester are very stable under the test conditions.

40 **[0039]** The stability of Calcipotriol was compared to a similar ointment where propylene glycol was used as the solvent and lanolin used as an emulsifier. The composition of the comparison ointment was the same as the above with respect to Calcipotriol and Betamethasone dipropionate, as well as 10% w/w propylene glycol, 10% w/w anhydrous lanolin and 80% w/w White Soft Paraffin. The comparison ointment was stored for 2.5 months at 5°C and 40°C, respectively. Only the content of Calcipotriol-related substances was determined in the manner described above. The results are shown in Table 2.

45

Table 2

Calcipotriol related substances %	
50 5°C	20
40°C	96

[0040] As it will be seen from the results, Calcipotriol is degraded almost completely in the comparison composition under the test conditions as opposed to a composition of the invention, where the Calcipotriol is retained with essentially no degradation.

55

EXAMPLE 3

Medical skin lotion comprising a two phase solvent system (reference)

5 **[0041]** 1 g contains:

	Betamethasone (As Dipropionate 0,643 mg)	0.5 mg
	Calcipotriol (as Hydrate 52.2 µg)	50 µg
	Disodium Phosphate Dihydrate	2.5 mg
10	Diazolidinyl Urea	3 mg
	Polyoxypropylene-15-Stearyl Ether (Arlamol® E)	50 mg
	Isohexadecan (Arlamol® HD)	200 mg
	Polyoxyethylene-2-Stearyl Ether® (Brij 72)	30 mg
15	Water, purified to make	1 g

Procedure for the preparation of 1 kg of lotion:

20 **[0042]** 2.5 g Disodium Phosphate and 3 g Diazolidinyl Urea are dissolved in about 714 g water. The solution is heated to 60 - 70°C to obtain the water phase. 30 g Polyoxyethylene-2-Stearyl Ether is melted together with 200 g Isohexadecan at 60 - 70°C and a solution of 52.2 mg Calcipotriol Hydrate in 50 g Polyoxypropylene-15-Stearyl Ether is added to obtain the oil phase. The two phases are mixed during homogenisation, 643 mg Betamethasone Dipropionate is dispersed into the mixture, and the lotion is cooled during mixing to room temperature. The preparation is stable at 25°C for > 14 days.

Claims

- 25
1. A non-aqueous topical pharmaceutical composition in the form of an ointment, a cream, a lotion, a liniment or other spreadable liquid or semi-liquid preparation for dermal use in the treatment of psoriasis, sebopsoriasis or seborrheic dermatitis in humans and other mammals, said composition comprising
30 a first pharmacologically active component A consisting of calcipotriol and a second pharmacologically active component B consisting of betamethasone or an ester thereof and at least one pharmaceutically acceptable carrier, solvent, or diluent.
 - 35 2. A pharmaceutical composition for use according to claim 1, wherein component B consists of a betamethasone ester, such as the 17-valerate or 17,21-dipropionate.
 3. A pharmaceutical composition for use according to any one of the preceding claims in the form of a mono-phase composition.
40
 4. A pharmaceutical composition for use according to the preceding claim which is an ointment.
 5. A pharmaceutical composition for use according to claim 1 **characterised in that** the difference between the optimum stability pH of said first component A and the optimum pH of said second component B is at least 1 further comprising
45 at least one solvent component C selected from the group consisting of:
 - (i) compounds of the general formula $R^3(OCH_2C(R^1)H)_xOR^2$ (I) wherein x is in the range of 2-60, R^1 in each of the x units independently is CH_3 , R^2 is straight chain or branched C_{1-20} alkyl or benzoyl, and R^3 is H or phenyl-carbonyloxy;
 - 50 (ii) di-(straight or branched)- C_{4-10} alkyl esters of C_4 - C_8 dicarboxylic acids;
 - (iii) straight or branched C_{12-18} -alkyl benzoates;
 - (iv) straight or branched C_{2-4} -alkyl esters of straight or branched C_{10-18} -alkanoic or -alkenoic acids;
 - (v) propylenglycol diesters with C_{8-14} -alkanoic acids; and
 - 55 (vi) branched primary C_{18-24} alkanols.
 6. A composition for use according to the preceding claim, wherein said component C is selected from compounds of the general formula $H(OCH_2C(R^1)H)_xOR^2$ (II) where R^1 , x, and R^2 are as defined in claim 5, and mixtures thereof.

7. A composition for use according to claim 6, wherein said component C is polyoxypropylene-15-stearyl ether.
8. A pharmaceutical composition for use according to claim 5, containing 0.0001 to 0.025% w/w of said component A, 0.005 to 0.1% w/w of said component B, and 1 to 20% w/w of said solvent component C.
9. A pharmaceutical composition for use according to claim 1, wherein, in the treatment, the composition is applied topically once or twice daily in a medically sufficient dosage.

Patentansprüche

1. Nichtwässrige topische pharmazeutische Zusammensetzung in Form einer Salbe, einer Creme, einer Lotion, eines Liniments oder einer anderen spreitbaren flüssigen oder halbflüssigen Zubereitung zur dermalen Verwendung bei der Behandlung von Psoriasis, Sebopsoriasis oder seborrhoischer Dermatitis bei Menschen und anderen Säugtieren, wobei die Zusammensetzung Folgendes umfasst:

eine erste pharmakologisch wirksame Komponente A, die aus Calcipotriol besteht, und eine zweite pharmakologisch wirksame Komponente B, die aus Betamethason oder einem Ester davon besteht, und mindestens einen pharmazeutisch unbedenklichen Träger, mindestens ein pharmazeutisch unbedenkliches Lösungsmittel oder mindestens ein pharmazeutisch unbedenkliches Verdünnungsmittel.

2. Pharmazeutische Zusammensetzung zur Verwendung nach Anspruch 1, wobei Komponente B aus einem Betamethasonester, wie dem 17-Valerat oder 17,21-Dipropionat, besteht.

3. Pharmazeutische Zusammensetzung zur Verwendung nach einem der vorhergehenden Ansprüche in Form einer einphasigen Zusammensetzung.

4. Pharmazeutische Zusammensetzung zur Verwendung nach dem vorhergehenden Anspruch, bei der es sich um eine Salbe handelt.

5. Pharmazeutische Zusammensetzung zur Verwendung nach Anspruch 1, die ferner mindestens eine Lösungskomponente C aus der Gruppe bestehend aus:

- (i) Verbindungen der allgemeinen Formel $R^3(OCH_2C(R^1)H)_xOR^2$ (I), worin x im Bereich von 2-60 liegt, R¹ in jeder der x Einheiten unabhängig CH₃ ist, R² ein geradkettiges oder verzweigtes C₁₂₀-Alkyl oder Benzoyl ist und R³ H oder Phenylcarbonyloxy ist;
- (ii) di- (geradkettigen oder verzweigten) -C₄₋₁₀-Alkylestern von C₄-C₈-Dicarbonsäuren;
- (iii) geradkettigen oder verzweigten C₁₂₋₁₈-Alkylbenzoaten;
- (iv) geradkettigen oder verzweigten C₂₋₄-Alkyl-estern geradkettiger oder verzweigter C₁₀₋₁₈-Alkansäuren oder -Alkansäuren;
- (v) Propylenglycoldiestern mit C₈₋₁₄-Alkansäuren und
- (vi) verzweigten primären C₁₈₋₂₄-Alkanolen umfasst.

6. Zusammensetzung zur Verwendung nach dem vorhergehenden Anspruch, wobei die Komponente C aus Verbindungen der allgemeinen Formel $H(OCH_2C(R^1)H)_xOR^2$ (II), wobei R¹, x und R² wie in Anspruch 5 definiert sind, und Mischungen davon ausgewählt ist.

7. Zusammensetzung zur Verwendung nach Anspruch 6, wobei es sich bei Komponente C um Polyoxypropylen-15-stearylether handelt.

8. Pharmazeutische Zusammensetzung zur Verwendung nach Anspruch 5, die 0,0001 bis 0,025 Gew.-% der Komponente A, 0,005 bis 0,1 Gew.-% der Komponente B und 1 bis 20 Gew.-% der Lösungskomponente C enthält.

9. Pharmazeutische Zusammensetzung zur Verwendung nach Anspruch 1, wobei die Zusammensetzung bei der Behandlung ein- oder zweimal täglich in einer medizinisch ausreichenden Dosierung topisch verabreicht wird.

Revendications

1. Composition pharmaceutique topique non aqueuse sous forme d'un onguent, d'une crème, d'une lotion, d'un liniment ou d'une autre préparation liquide ou semi-liquide pouvant être étalée, pour utilisation cutanée dans le traitement du psoriasis, du sébopsoriasis ou de la dermatite séborrhéique chez l'humain et d'autres mammifères, ladite composition comprenant un premier composant actif pharmacologique A constitué de calcipotriol et un second composant actif pharmacologique B constitué de bétaméthasone ou de l'un de ses esters et au moins un vecteur, solvant ou diluant de qualité pharmaceutique.
2. Composition pharmaceutique pour emploi conforme à la revendication 1, où le composant B est constitué d'un ester de bétaméthasone, comme le 17-valérate ou le 17,21-dipropionate.
3. Composition pharmaceutique pour emploi conforme à l'une quelconque des revendications précédentes, sous forme d'une composition monophasique.
4. Composition pharmaceutique pour emploi conforme à la revendication précédente, qui est un onguent.
5. Composition pharmaceutique pour emploi conforme à la revendication 1, comprenant en outre au moins un composant solvant C choisi dans le groupe constitué par :
 - (i) les composés de formule générale $R^3(OCH_2C(R^1)H)_xOR^2$ (I) où x est compris entre 2 et 60, R^1 dans chacun des x motifs représente indépendamment CH_3 , R^2 représente un groupement alkyle en C_{1-20} linéaire ou ramifié ou un groupement benzoyle, et R^3 représente H ou un groupement phénylcarbonyloxy ;
 - (ii) les esters de di-(alkyle en C_{4-10} linéaire ou ramifié) d'acides dicarboxyliques en C_4-C_8 ;
 - (iii) les benzoates d'alkyle en C_{12-18} linéaire ou ramifié ;
 - (iv) les esters d'alkyle en C_{2-4} linéaire ou ramifié d'acides alcanoïques ou alcénoïques en C_{10-18} linéaires ou ramifiés ;
 - (v) les diesters de propylène glycol avec des acides alcanoïques en C_{8-14} ; et
 - (vi) les alcools primaires en C_{18-24} ramifiés.
6. Composition pour emploi conforme à la revendication précédente, où ledit composant C est choisi parmi les composés de formule générale $H(OCH_2C(R^1)H)_xOR^2$ (II) où R^1 , x et R^2 sont tels que définis dans la revendication 5, et leurs mélanges.
7. Composition pour emploi conforme à la revendication 6, où ledit composant C est l'éther de polyoxypropylène-15-stéaryle.
8. Composition pharmaceutique pour emploi conforme à la revendication 5, comportant entre 0,0001 et 0,025 % en masse dudit composant A, entre 0,005 et 0,1 % en masse dudit composant B, et entre 1 et 20 % dudit composant solvant C.
9. Composition pharmaceutique pour emploi conforme à la revendication 1, où, dans le traitement, la composition est appliquée de façon topique une ou deux fois par jour à une dose médicalement suffisante.

Percentage change in PASI

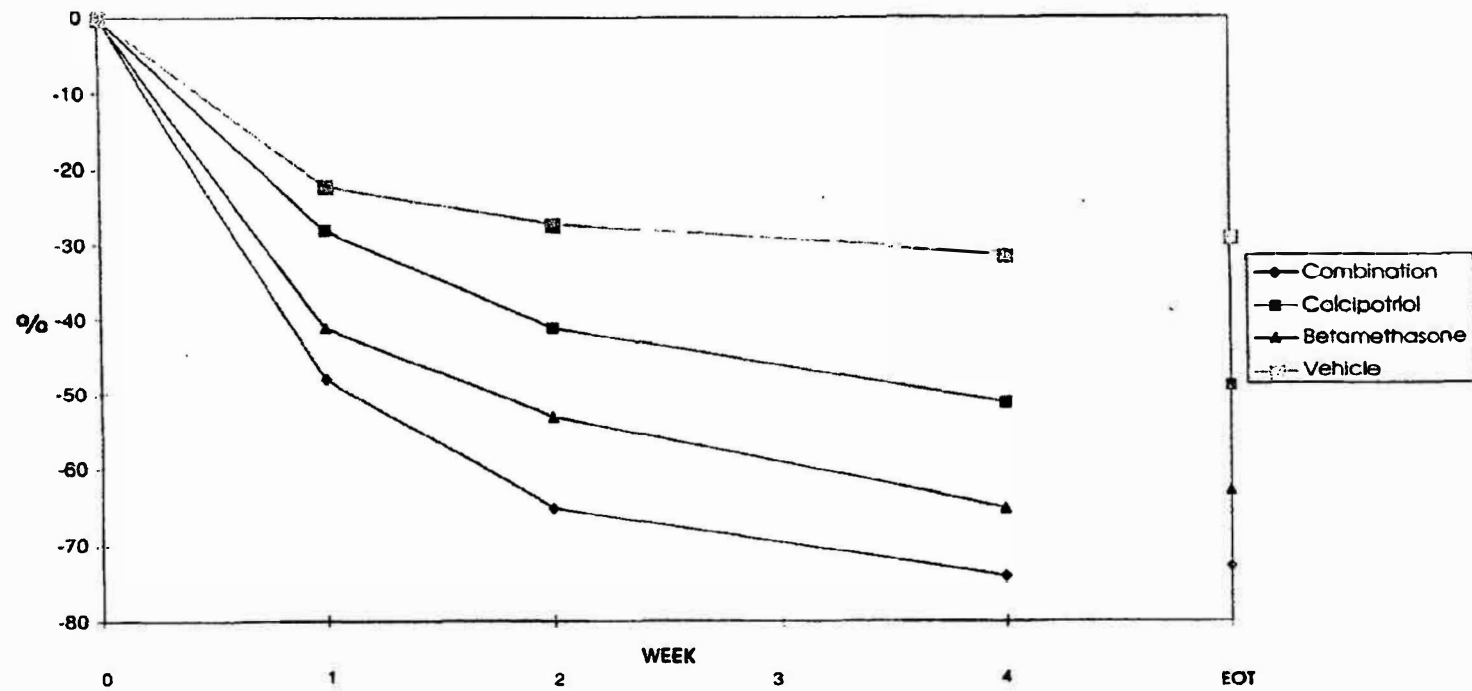


FIGURE 1

**Percentage change in PASI score at each visit and
end of treatment**

Percentage change in PASI score	COMB (n=301)	CALC (n=308)	BETA (n=312)	VEHICLE (n=107)
Visit 1				
Mean	10.9	10.9	10.7	10.6
Percentage change				
To visit 2				
Mean	-48.1	-28.4	-41.4	-21.5
To visit 3				
Mean	-64.9	-40.8	-53.2	-27.4
To visit 4				
Mean	-73.9	-51.3	-64.5	-31.3
To end of treatment				
Mean	-73.2	-48.8	-63.1	-28.8

FIGURE 2

Responders (Investigator's assessment)

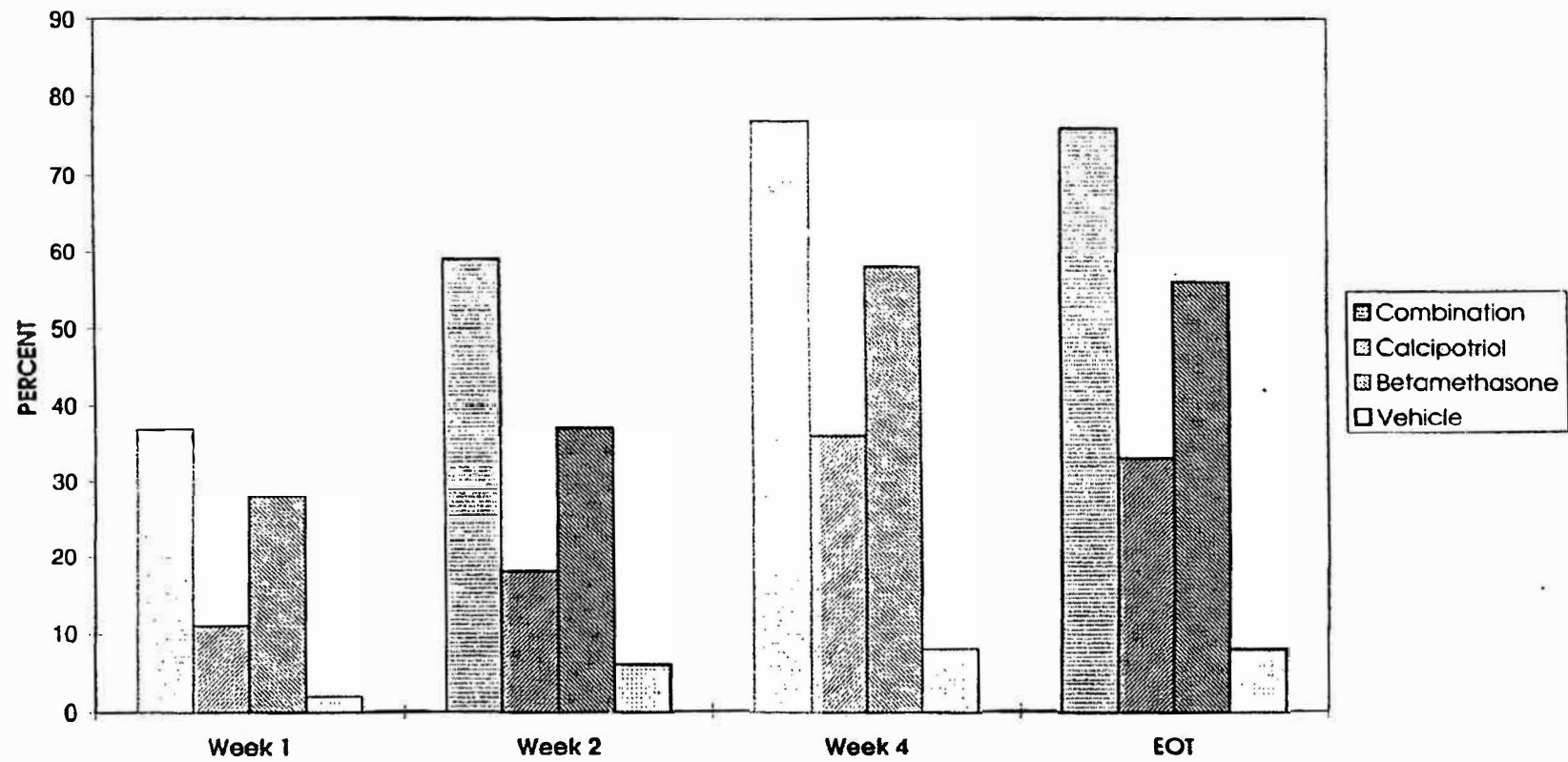


FIGURE 3

Investigator's assessment of overall Efficacy at each visit and end of treatment

Investigator's overall efficacy assessment	COMB (n=301) %	CALC (n=308) %	BETA (n=312) %	VEHICLE (n=107) %
Visit 2				
Non responder	63.5	89.5	72.5	98.1
Responder	36.5	10.5	27.5	1.9
Total	100.0	100.0	100.0	100.0
Visit 3				
Non responder	41.5	82.2	62.7	94.2
Responder	58.5	17.8	37.3	5.8
Total	100.0	100.0	100.0	100.0
Visit 4				
Non responder	23.1	64.4	42.4	91.9
Responder	76.9	35.6	57.6	8.1
Total	100.0	100.0	100.0	100.0
End of treatment				
Non responder	23.9	66.6	44.2	92.5
Responder	76.1	33.4	55.8	7.5
Total	100.0	100.0	100.0	100.0

FIGURE 4

REFERENCES CITED IN THE DESCRIPTION

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Comparison of calcipotriol monotherapy and a combination of calcipotriol and betamethasone valerate after 2 weeks' treatment with calcipotriol in the topical therapy of psoriasis vulgaris: a multicentre, double-blind, randomized study

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Summary

A clinical study was conducted to determine whether, in the topical treatment of psoriasis, a combination of calcipotriol and betamethasone valerate after previous treatment with calcipotriol alone was more effective than the continuation of the monotherapy with calcipotriol, especially in 'low responders'. Patients ($n = 169$) with the clinical diagnosis 'chronic plaque-type psoriasis' were treated twice daily for 2 weeks with calcipotriol, followed by a 4-week treatment with calcipotriol monotherapy in 87 patients or combined calcipotriol/betamethasone valerate in 82 patients; all patients were followed for 8 weeks. The psoriasis area and severity index (PASI) was used to compare the two treatment groups. The overall therapeutic result was also assessed by the investigators and patients. The combination therapy was more effective, as assessed by all evaluated variables; moreover, patients showing insufficient response to calcipotriol alone after 2 weeks showed a regression of psoriatic lesions using the combination regimen. Thus, the combination of calcipotriol and topical steroids is recommended as the therapy of first choice for patients who do not respond well to treatment with 2 weeks of calcipotriol alone. Furthermore, this combination reduces the hazards associated with the long-term use of topical corticosteroids (atrophy and rebound) as well as the irritation associated with calcipotriol.

Several drugs are currently available for the treatment of psoriasis. Substances like tar, dithranol (anthralin), steroids, retinoids, methotrexate and cyclosporin A, as well as ultraviolet (UV) radiation and their combinations, have been effective treatments for many years. Calcipotriol, the first vitamin D₃ analogue, was introduced into antipsoriatic therapy in the early 1990s and since then it has been used for mild and moderate plaque-type psoriasis. Many clinical studies report the favourable effect of calcipotriol or other calcitriols on the various clinical features of psoriasis.^{1–6} Further clinical studies showed that calcipotriol is as clinically effective as topical steroids and short-contact therapy with dithranol.^{7–15} Clinical experience and a re-analysis of the clinical comparisons of calcipotriol with topical steroids have shown that about two-thirds of patients respond with a marked improvement in psoriatic lesions.¹⁶ Further experience has been gained with calcipotriol in combination with UVB, PUVA and cyclosporin A. The combinations were more effective and

the response showed a more rapid onset than the monotherapy; fewer calcipotriol-induced side-effects were also reported.^{17–24}

The present study is the first to determine whether a combination of topical calcipotriol and betamethasone valerate after a 2-week course of treatment with calcipotriol is better than continued monotherapy alone.

Patients and methods

The study was conducted as a multicentre, double-blind, randomized comparison between monotherapy with 0.005% calcipotriol ointment (Psorcutan[®] Salbe, Schering AG, Berlin, Germany) and a combination of calcipotriol and 0.1% betamethasone valerate ointment (GlaxoWellcome, Hamburg) after 2 weeks of treatment with calcipotriol alone. The study was carried out according to good clinical practice (GCP) guidelines.

Included were men and women patients older than 18 years having chronic plaque-type psoriasis with

lesions on the lower and/or upper extremities and/or trunk, with an affected area not exceeding 30% of the total body surface. No systemic antipsoriatic treatment or UV therapy had been administered during the previous 2 months. The serum calcium levels, renal and liver function were in the normal range; the patients were neither pregnant nor nursing.

Treatment schedule

The study treatment was carried out in four consecutive phases: (i) a 2-week wash-out in which the patients were only allowed to apply an ointment base; (ii) a 2-week monotherapy when calcipotriol was applied twice daily; (iii) a 4-week treatment continuation with either calcipotriol twice daily (monotherapy group) or calcipotriol once daily (morning) and betamethasone valerate once daily (evening) comprising the combination therapy group; and (iv) an 8-week follow-up when the patients applied only the ointment base. Patients with exacerbated disease during the first 2-week treatment phase were excluded from the study. Complete remission before the end of therapy led to the early termination of treatment.

Clinical assessment

Patients were examined 2 weeks before the start of the treatment, after the wash-out period and at 2, 6 and 14 weeks after the start of treatment. At these intervals the severity of the psoriasis was evaluated according to the psoriasis area and severity index (PASI).²⁵ Additionally, at 2 and 6 weeks the investigators and patients rated the global response according to six- and five-point scales, respectively. The investigator's scale was used to classify patients into high (those graded as 'complete healing' or 'distinct improvement') and low (graded as 'moderate' or 'slight improvement', 'no change' or 'deterioration') responders. A safety evaluation was performed before the start of the wash-out period and after 2 and 6 weeks of treatment, which included serum measurements of creatinine, urea, total calcium, inorganic phosphate, total protein and albumin. All adverse events, whether subjective or objective, were recorded at each visit and particular attention given to any increase in calcium levels above the normal range.

Statistical analysis

The primary end-point was the reduction of the PASI at the end of the therapy. The evaluation of the primary

variable was performed as an intention to treat (ITT) analysis. The two treatments were compared using the *t*-test or the non-parametric Mann-Whitney *U*-test at $\alpha = 0.025$. The number of patients who remained low responders after the end of the therapy was compared using the one-sided Fisher's exact test at $\alpha = 0.025$, so an overall α of 0.05 was maintained. For additional variables, descriptive *P*-values were calculated with the *U*-test or Fisher's test.

Results

Of the 178 patients recruited into the trial, the data for 169 patients from 20 centres in Germany were analysed (94 men and 75 women, mean age 42 years, range 18–80); 87 patients were treated with calcipotriol monotherapy and 82 with the combination therapy. Eleven patients discontinued therapy (five monotherapy and six combination therapy); the reasons for the premature discontinuation were: pathological laboratory values at the beginning, insufficient healing, adverse events, healing of psoriasis or non-medical reasons. Nearly all patients had previously used antipsoriatic medication, with topical glucocorticoid steroids the most frequent. An ITT analysis was carried out of all patient data available after the beginning of treatment.

Psoriasis area and severity index

In the monotherapy group, the mean PASI decreased from 6.2 at the beginning of therapy to 3.5 after 2 weeks, and to 1.9 after 6 weeks; 8 weeks after the end of therapy the PASI increased to 2.6. In the combination

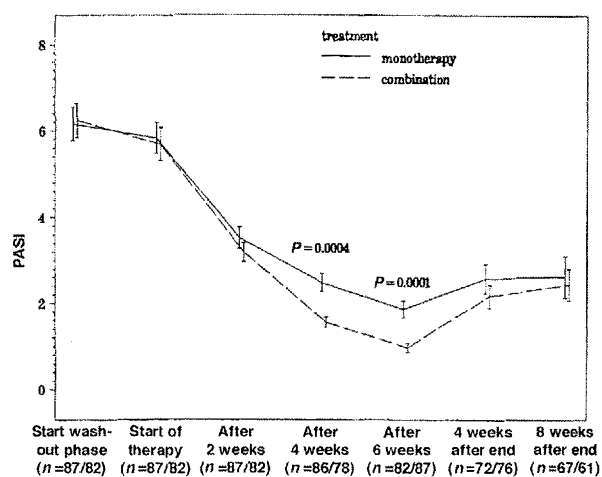


Figure 1. The mean ($\pm$ SD) changes in the PASI, by 'intention to treat' analysis.

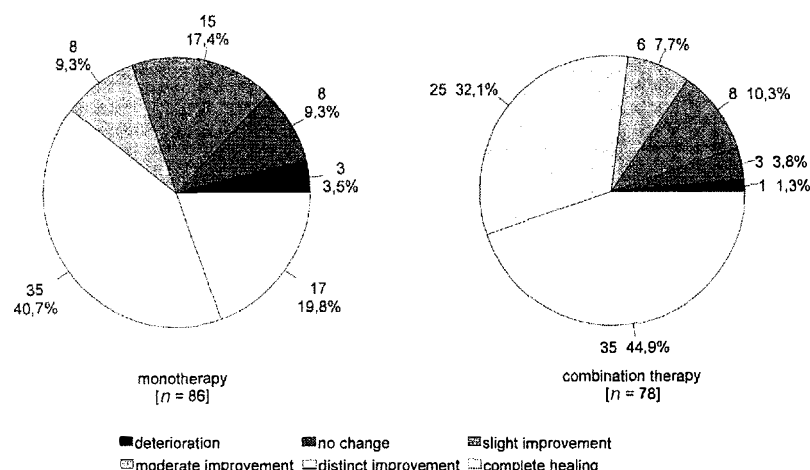


Figure 2. Assessment of the clinical symptoms of psoriasis by the investigator: intention to treat analysis after 6 weeks therapy or at premature withdrawal (includes only patients with at least 4 weeks therapy).

therapy group, the mean PASI decreased from 5.7 at the beginning of therapy to 3.2 after the initial 2-week monotherapy. During the next 4 weeks (under combination therapy) the PASI decreased to 1.0; at 8 weeks after the end of therapy the PASI increased to 2.4. Comparing the two treatments showed a significant advantage for the combination therapy after 2 and 6 weeks ($P < 0.001$; Fig. 1).

Global assessment

For the investigator's assessment at the end of therapy, the monotherapy group comprised 17 patients (20%) with 'complete healing' and 35 (41%) with 'distinct improvement'. Thus, there were 52 high responders (60.5%). The other patients in this group showed 'moderate improvement' at best (low responders). In the combination therapy group, 35 patients (45%) showed 'complete healing' and 25 (32%) a 'distinct improvement': thus 60 patients (77%) were classified as high responders and the others as low responders (Fig. 2). There was no major difference between the global assessment by the patients and that of the investigators.

High and low responders

After the initial 2 weeks of treatment (which was the same in both groups, i.e. calcipotriol monotherapy), there were 50 low responders (58%) in the monotherapy group and 41 (50%) in the combination therapy group (Fig. 3). Of the 50 low responders in the former, 49 continued therapy and at the end of 6 weeks of calcipotriol monotherapy, there were 22 (25%)

low responders. Of the 41 low responders in the combination therapy group, 39 continued treatment with the combination and at the end of 6 weeks calcipotriol treatment (2 weeks monotherapy and 4 weeks combination) there were only 12 (15%) low responders. Comparing the two treatment groups showed significant advantages for the combination therapy ($P = 0.0189$).

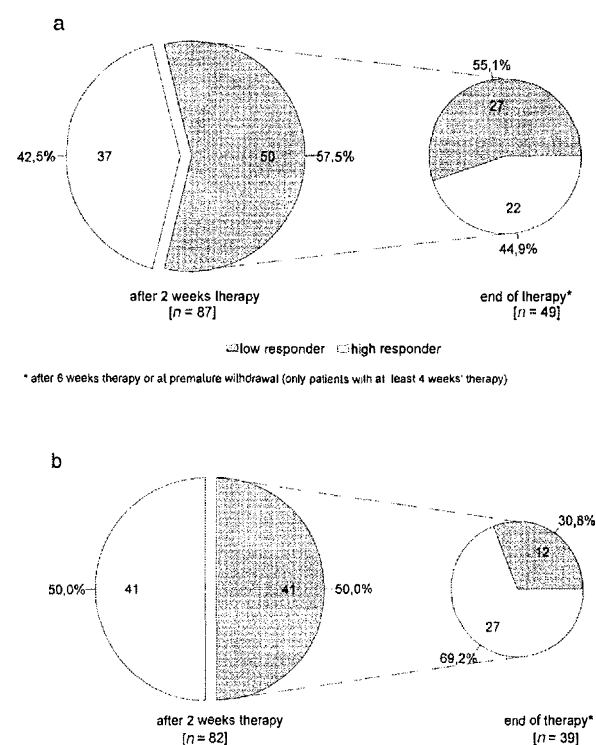


Figure 3. High and low responders after 2 weeks of therapy and at the end of therapy: (a) monotherapy and (b) combination therapy group.

Evaluation of safety and tolerability

Twenty patients (23%) in the monotherapy and 13 (16%) in the combination therapy group reported local or general adverse events (AEs) during therapy. Local AEs were reported in 13 patients (15%) in the monotherapy and in six (7%) in the combination therapy group. The most frequently reported local AEs were erythema, burning and pruritus. Nearly all local AEs were mild or moderate except in two patients (monotherapy) with severe AEs (pruritus, burning sensation of the skin and erythema). Two patients withdrew from the study because of the burning sensation after wiping sweat off their face with contaminated hands (monotherapy) and after an eczematous reaction (combination therapy).

General AEs were documented for 11 patients (12%) in the monotherapy and for seven (8.5%) in the combination therapy group. Most of these AEs could not be related to the study medications. Two patients were withdrawn from the study as their serum calcium levels were above the upper normal range. Both monotherapy and combination therapy were safe treatments, causing no serious AEs.

Discussion

As calcipotriol and betamethasone valerate work by interacting with different receptor subtypes (vitamin D or glucocorticoid receptors), an additive or synergistic effect could theoretically be expected. Therefore, the combination of topical calcipotriol and betamethasone valerate was assessed to determine whether there is such an effect in those patients who do not respond to calcipotriol alone. The results clearly show that the combination of both drugs leads to an additive clinical effect in terms of reduced psoriatic symptoms. Fewer local AEs were reported with combination therapy than in the monotherapy group. In particular, erythema, the most frequently reported local AE, was significantly reduced by an alternate application of calcipotriol and betamethasone valerate. Erythema is thought to be caused by the irritative potential of calcipotriol and might be neutralized by the anti-inflammatory action of betamethasone valerate.

This combined regimen reduced the hazards associated with the long-term use of topical corticosteroids (atrophy, rebound), as well as the irritation associated with calcipotriol. Thus the combination therapy can be recommended for 'low responders', i.e. those who do not respond well to treatment with 2 weeks of calcipotriol alone.

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Development of a New Formulation Combining Calcipotriol and Betamethasone Dipropionate in an Ointment Vehicle

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ABSTRACT

Calcipotriol and betamethasone dipropionate are widely used effective treatments for psoriasis. Combined therapy is known to be superior to monotherapy, but current formulations do not permit simultaneous application as the drug substances will degrade when mixed. The purpose of the study was to develop a formulation which combines calcipotriol and betamethasone dipropionate in a single vehicle hereby achieving optimal delivery of both substances into the skin. As the two substances are incompatible in aqueous and alcoholic medias, different non-aqueous formulations were prepared. Skin permeation studies were investigated using Franz-type diffusion cells. Formulations based on isopropyl myristate were found to decrease the permeation rate (25–35%) as compared with marketed monotherapy products (set to 100%). Lanolin had no overall effect on skin permeability. However, polyoxypropylene-15 stearyl ether (PSE) had a marked effect. A 5% PSE formulation resulted in a permeation rate comparable to the marketed products. Thus, by using PSE as solvent, it was possible to combine calcipotriol and betamethasone dipropionate in a single formulation while optimal skin permeability was attained. Recently, the efficiency of this formulation (Daivobet[®]) has been verified in clinical studies showing an improved efficacy in the treatment of psoriatic patients.

Key Words: Calcipotriol; Betamethasone dipropionate; Daivobet[®]; Skin permeation; Formulation.

INTRODUCTION

Psoriasis is a chronic, inflammatory skin disease affecting 1–3% of the world's population.^[1] Despite decades of active research, there is currently no cure and different treatment strategies focus on relieving the symptoms and restricting its severity.

Topical corticosteroids and the vitamin D analogue, calcipotriol, have become mainstays in psoriatic treatment,^[2] each having different mechanisms of action.^[3,4] Several studies have shown that combined therapy with products containing these two active substances is more effective than monotherapy, and that additive clinical effects and reduced skin irritation may be achieved.^[5–8] However, current corticosteroid products cannot be applied together with a calcipotriol product as the drugs are incompatible and will degrade when mixed.^[9,10] Consequently, patients have been applying one product in the morning and the other in the evening. To explore the effect of simultaneous treatment and to improve patient compliance alleviating the inconvenience of separate applications, it is desirable to have a single product in which both substances are stable and can be applied simultaneously.

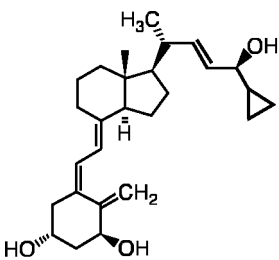
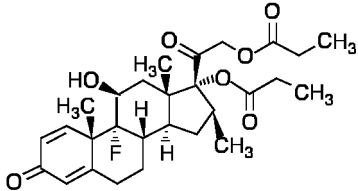
Due to differences in physico-chemical properties and stabilities it has not previously been possible to successfully combine the two drug substances in a single formulation. Calcipotriol is sensitive to oxidising agents and acidic residues; it reacts with alcohols, and

easily undergoes epimerisation processes. Contrary to this, corticosteroids like betamethasone dipropionate are stable under acidic conditions, but sensitive to alkaline residues and oxidising agents. If water is present in the formulation, calcipotriol requires a pH value above 8 for maximum stability, whereas betamethasone dipropionate requires pH-values in the range of 4–6 (Table 1).

An optimal vehicle combining the two drug substances must therefore be non-aqueous in order to avoid pH-dependent degradation. In addition, the choice of the pharmaceutical excipients is limited as many have acidic or alkaline residues, or oxidising abilities. Furthermore, as calcipotriol and betamethasone dipropionate are very potent drugs and only a small amount is needed in a formulation (0.005% and 0.05%, respectively), a homogeneous distribution of the drugs must be ensured. Betamethasone dipropionate can be suspended homogeneously as it is available as micronised particles. However, calcipotriol is present in a much lower concentration and needs to be dissolved in a carefully selected vehicle component to ensure an even distribution.

The aim of the present study was to combine the two drug substances in a single formulation, and in addition achieve a skin delivery similar to the marketed monotherapy products; Daivonex[®] ointment and Diproderm[®] ointment. In particular, we wished to investigate the effect of different vehicle components

Table 1. Physico-chemical properties of the drug substances.

Drug substances		Mw (g/mol)	Log P ^a	Water solubility (mg/L) 25°C	Maximum stability at
Calcipotriol		412.6	5.43	1.3 ± 0.7 at pH 10 ^b	pH > 8
		504.6	4.23	1.2 at pH 7.4	pH 4–6
Betamethasone dipropionate					

^aOctanol/water partition coefficient.

^bData obtained from Ref. [11].

on the skin permeability of both substances. It is the overall goal that a formulation combining calcipotriol and betamethasone dipropionate will increase treatment efficacy and patient satisfaction and thus become a valuable new approach to the treatment of psoriasis.

MATERIALS AND METHODS

Chemicals

Calcipotriol hydrate was supplied by LEO Pharma (Ballerup, Denmark) and betamethasone dipropionate (BDP) was from Sicor (Milan, Italy). Isopropyl myristate (IPM) and polyoxypropylene-15 stearyl ether (PSE) were obtained from Uniqema (Gouda, Netherlands), propylene glycol (PG) from Lyondell (Fos-sur-Mer, France), and lanolin from Croda/Westbrook (Rawcliffe Bridge, UK). Liquid paraffin and white soft paraffin were purchased from Witco (Middlebury CT, US). Diammonium hydrogen phosphate and 2-propanol were of analytical grade and obtained from Merck (Darmstadt, Germany). For physico-chemical properties of the drug substances, see Table 1.

Test Formulations

The ointments were prepared by dissolving calcipotriol in the test solvents (IPM, PG, or PSE) and then adding melted white soft paraffin and the test component lanolin (both phases were 70°C). Betamethasone dipropionate was suspended as micronised particles in liquid paraffin and added to the mixture. The ointments were continuously stirred while cooling. It was ensured that both drug substances were homogeneously distributed in the intended concentrations of 0.05 mg/g for calcipotriol (as hydrate) and 0.5 mg/g for betamethasone (as dipropionate). The test formulations were based on the following solvent variations; 5% and 10% IPM (oint. 1 and 2); 5% PSE and 5% lanolin (oint. 4); and 2.5% PSE and 5% lanolin (oint. 6); 10%, 5%, and 2.5% PSE (oint. 3, 5 and 7); 10% PG and 5% IPM (oint. 8). The amount of the inert ointment base, white soft paraffin, was varied accordingly. All percentages are weight to weight-ratios (w/w). The test ointment based on 5% PSE (oint. 5) is identical to the recently marketed Dovobet[®] (Daivobet[®]) ointment (LEO Pharma). As reference products, Dovonex[®] (Daivonex[®]) ointment (calcipotriol 0.05 mg/g; LEO Pharma) and Diprosone[®] (Diprodern[®]) ointment (betamethasone (as dipropionate) 0.5 mg/g; Schering-Plough) were used.

In Vitro Skin Permeation Studies

Full-thickness skin was removed from the back of pig ears obtained from a local abattoir. The excised skin was cut into appropriate pieces after careful removal of the subcutaneous tissue. The skin samples were mounted in open two-chamber Franz-type diffusion cells with an available diffusion area of 3.14 cm² and a recipient volume of 10 ml. The diffusion cells were placed in a thermostated water bath resulting in a temperature of 32°C on the skin surface. A mixture of 0.04 M isotonic phosphate buffer pH 7.4 and 2-propanol (70:30) was used as recipient phase. After a 2 hour equilibrium period, the test formulation (5 mg/cm²) was applied to the stratum corneum side of the skin and spread with a tared glass spatula. At appropriate time intervals an exact amount of the recipient phase was withdrawn and replaced by thermostated fluid. Throughout the study, the formulations were reapplied corresponding to a twice daily dosage regime. The permeation was followed for 72 h and it was ensured that sink conditions were present. As a minimum, all permeation experiments were carried out in triplicates. The samples were kept at 4°C for a maximum of two days until HPLC analysis, see below.

HPLC Analysis of the Drug Substances

A high-performance liquid chromatographic (HPLC) method was developed for the analysis of calcipotriol and betamethasone dipropionate in the recipient phase samples. The concentrations were quantified using a Merck-Hitachi HPLC system (L-7250 Programmable Autosampler, L-7300 Column Oven, L-7400 UV Detector, L-7100 Pump and D7000 Interface). The substances were separated on a LiChrospher 60 RP-select B (125 mm × 4 mm, 5 µm) from Merck (Darmstadt, Germany) using a column temperature of 40°C, a detector wave length of 264 nm, an injection volume of 1000 µl, and a flow rate of 1.0 ml/min. The mobile phase consisted of 2-propanol and 0.005 M ammonia phosphate buffer pH 7.4 in a step gradient mixture of 26:74 (2-propanol:buffer) at an interval of 0 to 1 min, and 36:64 from 1 to 16 min. The retention time was 10 min. for betamethasone dipropionate and 12 min. for calcipotriol. The detection limit was 10 ng/ml for both calcipotriol and betamethasone dipropionate, and the quantitation limit was 25 ng/ml for both substances. Calibration curves were linear over a concentration range of 25–1000 ng/ml ($R^2 > 0.9990$) and validated for intra- and inter-day variation (CV_{intra} : 2%, CV_{inter} : 4%). The recovery

was 92% for calcipotriol and 100% for betamethasone dipropionate.

Data Analysis

Based on the obtained concentrations and the amount of recipient phase withdrawn at each specific point of time, the permeated cumulated amount of drug was calculated for each diffusion cell (ng/cm^2) and plotted as a function of time (h). For all individual cells, steady state flux was determined from the slope of the linear part of the curve, and lag time as the x-intercept using linear regression analysis. Post-study data analysis was used to check the integrity of the skin comparing the individual cell data with the mean data for the other cells in the specific group (ANOVA). No damaged skin preparations were identified.

For statistical comparison of the formulations, a two-way ANOVA model was applied. The observa-

tions were logarithmically transformed to fulfil the assumption about normality and variance homogeneity. Pairwise comparisons of the ointments were performed using the Students' t-test within the ANOVA. P-values below 0.05 are considered significant.

RESULTS

The in vitro skin permeation profiles of calcipotriol and betamethasone dipropionate in the different test formulations are shown in Fig. 1A and 1B. It is seen that the different solvent systems had a marked effect on the cumulated permeated amount of both drug substances. Based on these permeation profiles, the steady state fluxes and lag times were calculated (Table 2). In order to make a direct comparison of the different formulations with the reference products, all flux values were normalised by dividing the individual

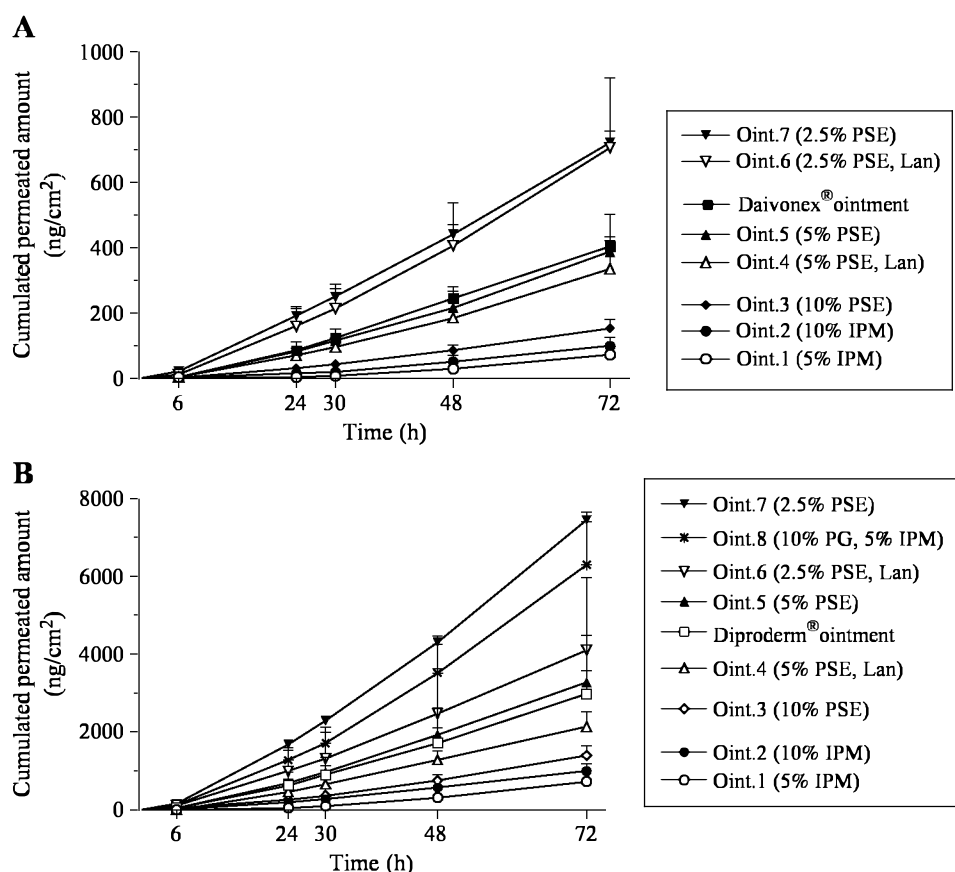


Figure 1. In vitro skin permeation profiles of calcipotriol (A), and betamethasone dipropionate (B) from the test formulations based on different vehicle components (Lan: lanolin; IPM: isopropyl myristate; PG: propylene glycol, PSE: polyoxypropylene-15 stearyl ether) expressed as mean values + SE ($n=3-7$ cells per formulation).

Table 2. In vitro skin permeation results (mean values $\pm$ SE; n=3–7 cells).

Test formulations	Steady state flux (ng/cm ² ·h ⁻¹)		Lag time (h)	
	Calcipotriol	BDP	Calcipotriol	BDP
Ointment 1 (5% IPM)	1.6 $\pm$ 0.1	15 $\pm$ 1	26 $\pm$ 3	24 $\pm$ 6
Ointment 2 (10% IPM)	1.9 $\pm$ 0.4	17 $\pm$ 3	21 $\pm$ 4	15 $\pm$ 2
Ointment 3 (10% PSE)	2.6 $\pm$ 0.5	25 $\pm$ 5	13 $\pm$ 3	15 $\pm$ 4
Ointment 4 (5% PSE, Lan)	6 $\pm$ 1	36 $\pm$ 6	14 $\pm$ 3	12 $\pm$ 3
Ointment 5 (5% PSE)	7 $\pm$ 2	52 $\pm$ 17	13.6 $\pm$ 0.6	13 $\pm$ 1
Ointment 6 (2.5% PSE, Lan)	12 $\pm$ 3	66 $\pm$ 28	13 $\pm$ 2	11 $\pm$ 2
Ointment 7 (2.5% PSE)	11.3 $\pm$ 0.5	123 $\pm$ 2	8 $\pm$ 2	12 $\pm$ 1
Ointment 8 (10% PG, 5% IPM)	n.a.	110 $\pm$ 17	n.a.	16 $\pm$ 2
Daivonex [®] ointment	6.7 $\pm$ 0.4	–	12 $\pm$ 1	–
Dipoderm [®] ointment	–	50 $\pm$ 9	–	13 $\pm$ 2

n.a.: Data not available due to degradation (see results).

fluxes with the mean flux of the reference product (Fig. 2).

The otherwise well known skin penetration enhancer IPM (oint. 1 and 2) was found to have no enhancing effect itself on either of the drug substances compared with the reference products as the total cumulated permeated amount was significantly lower (t-test; $p < 0.05$). As seen from Table 2, the fluxes were significantly lower and the lag times longer than for the references. From Fig. 2 it is seen that the permeability of both drug substances ranged 25–35% of that of the reference products (set to 100%). There

was a tendency to when increasing the amount of IPM from 5% to 10%, the permeability increased slightly but no statistically significant difference was found ($p > 0.21$).

PG was added as a co-solvent to a formulation containing 5% IPM (oint. 8). This resulted in a significantly increased skin permeability for betamethasone dipropionate (Fig. 1B, Table 2), and a normalised flux ratio of 219% compared with the reference. No data is shown for calcipotriol as the drug substance was found to be very unstable in the formulation and rapidly transformed into degradation substances.^[9]

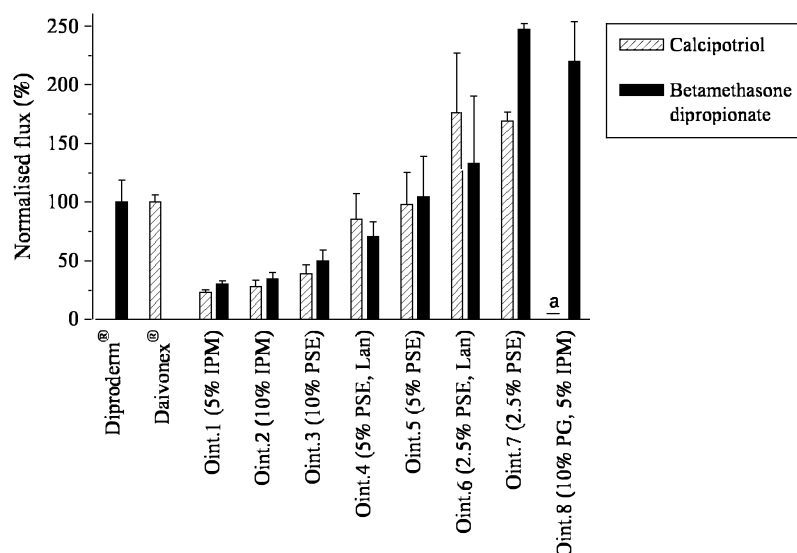


Figure 2. Normalised flux ratios for calcipotriol and betamethasone dipropionate in the test formulations based on different vehicle components (Lan: lanolin; IPM: isopropyl myristate; PG: propylene glycol; PSE: polyoxypropylene-15 stearyl ether). Normalisation was performed by dividing the fluxes with the mean flux of the reference products; Daivonex[®] ointment and Dipoderm[®] ointment (mean values $\pm$ SE). ^aData on calcipotriol was not available due to degradation (see results).

In contrast to IPM, the solvent PSE (oint. 3–7) proved to increase the skin permeability of both drug substances. Within the tested range, an inverse relationship was found between the amount of PSE in the formulation and the skin permeability. When comparing the total cumulated permeated amount of both drug substances, there was a statistically significant difference between the formulations containing 2.5%, 5%, and 10% PSE (two-way ANOVA; $p < 0.0001$). As seen from Table 2, the fluxes decreased as the content of PSE increased. In general, the lag times were lower than those of the IPM formulations, and within the range of the reference products. However, within the group, the varying amount of PSE had no significant effect on the lag times (two-way ANOVA; $p > 0.41$). The formulations containing 5% PSE (oint. 4 and 5) had fluxes comparable to those of the reference products (t-test; $p > 0.29$) corresponding to 100% for both drug substances (Fig. 2). Increasing the amount of PSE in the formulation to 10% (oint. 3), caused a reduction in the normalised flux ratio to 39% for calcipotriol and to 49% for betamethasone dipropionate. When the amount of PSE in the formulation was reduced to 2.5% (oint. 7), the flux ratio increased to 170% and 247%, respectively.

The skin emollient lanolin was added to ointment 4 and 6, and by pairwise comparison to ointment 5 and 7, respectively, no significant effect of lanolin was found for calcipotriol ($p > 0.72$). However, for betamethasone dipropionate, the formulation containing 2.5% PSE had a statistically significant lower flux when lanolin was present (t-test: $p = 0.009$). This is also reflected in Fig. 2 showing that normalised flux ratio decreased from 247 to 133%, when lanolin was added to the formulation.

DISCUSSION

The present study demonstrated that it was possible to combine calcipotriol and betamethasone dipropionate in a formulation and by careful selection of the vehicle components also attain a skin delivery similar to the marketed monotherapy products.

Several factors had to be considered when combining the two otherwise incompatible drug substances. The vehicle had to be non-aqueous, consisting of compatible components which ensured stability of both substances, and at the same time delivered both drug substances into the skin at a rate comparable to the marketed reference products. For this purpose, the applied *in vitro* set-up with skin samples mounted in Franz-type diffusion cells proved to be a very valuable

tool for testing the effect of the different vehicle components on the skin permeability.

IPM is a well known solvent generally acting as a skin permeation enhancer. However, in the present study it had no increasing effect as it only lead to a skin permeation rate of about 25–35% compared with the references. IPM facilitates the passage of the drugs through the skin barrier often in conjunction with a cosolvent serving as a drug carrier. In the investigated formulations, IPM had no effect presumably because none of the other components in the formulation served as a carrier. To test this, we added PG to the formulation and found a significantly increased skin permeability of betamethasone dipropionate (oint. 8, Table 2). Unfortunately, this led to a rapid degradation of calcipotriol.^[9]

The solvent PG is successfully used in some of the marketed calcipotriol products; Daivonex[®] ointment and Daivonex[®] solution, and was also initially considered in the present study as described above. PG may, however, contain some acidic impurities, which will catalyse an epimerisation process of calcipotriol and lead to the formation of mainly calcipotriol propylene glycol ethers. To avoid this, PG has to be buffered to a pH above 8. In a combination product this would lead to hydrolysis of the ester side chain of betamethasone dipropionate and further degradation of the acetone–alcohol group. Therefore, it was not possible to use PG in the formulation.

PSE is used as an emollient in cosmetic products and also as a drug solvent in pharmaceuticals. It was found to be a very effective solvent for both drug substances having a significant effect on the skin permeability. PSE is a lipophilic component and thus, when a high amount is used in the vehicle, the skin-vehicle partition coefficient is expected to decrease. This was also reflected in the inverse relationship between the amount of PSE in the formulation and the total cumulated permeated amount (Fig. 1) and the flux values (Table 2).

Lanolin was added to the ointment base (oint. 4 and 6) due to its emulsifying and skin emollient properties. When an extra component is added to a dermatological vehicle, it may affect the skin permeability owing to a change in the partition coefficient. In the present study, lanolin was found to have no effect on the skin permeation of calcipotriol and actually decreased the skin permeation of betamethasone dipropionate. As lanolin contains lanosterols, which have a chemical structure similar to betamethasone dipropionate (Table 1), it may be speculated that betamethasone dipropionate has a special affinity to lanolin. This may have caused a reduction of the skin-vehicle partition

coefficient and may explain the decreased flux ratios (Fig. 2). As the primary objective is to deliver the optimal amount of drug substance to the skin, the intention of adding a supplementary emollient to the ointment base was abandoned.

High skin permeabilities are not necessarily desirable for a corticosteroid product due to the possibility of local side effects such as skin atrophy, stria, telangiectasia, or the more rare side effect; suppression of the hypothalamic-pituitary-adrenal axis.^[8] For calcipotriol, the side effects are fewer and restricted to local skin irritation.^[5] The two marketed monotherapy products were therefore used as references serving as valuable bioequivalence markers to attain comparable skin permeabilities. The formulation containing 5% PSE (oint. 5) had fluxes and lag time values similar to both references (Table 2). Furthermore, excellent stability of both drug substances has been documented,^[9] and this formulation has now become available as Daivobet[®] ointment.

The atrophogenic potential of this new formulation has recently been investigated in a human study.^[12] The clinical results support the present skin permeation results as no difference in skin atrophy was found between the new combination product and the reference product; Diproderm[®] ointment. In addition, skin atrophy was similar for the two products following twice daily application over a 4-week treatment period.

It is emphasized that the present permeation results are based on in vitro experiments performed on excised skin from pig ears. In vitro studies using skin samples mounted in diffusion cells are, however, a well-established method to predict skin permeability.^[13,14] Moreover, skin from pig ear has proved to have properties similar to those of human skin and has been suggested a good model for human skin permeability.^[15] Nevertheless, we repeated parts of the present study (oint. 3–7) using excised human abdominal skin (data not shown) and found the exact same rank order of the formulations, and flux values within the same range as the present results. This shows that the present set-up is very predictive for human skin absorption as also demonstrated by the correlating results on skin atrophy (see above). In addition, the new combination product has recently been tested in large clinical trials showing an improved efficacy after application once daily for the treatment of psoriasis compared to either drug used alone, and also showing that the treatment was well tolerated by the patients.^[16–19] These results clearly support the rationale for developing a combined formulation and that the permeability of the drug substances from this new vehicle was as intended.

CONCLUSION

The study proved it possible to develop a formulation which combined calcipotriol and betamethasone dipropionate in a single vehicle achieving optimal delivery of both substances into the skin. The results demonstrated that the choice and the amount of the solvent had a significant influence on the skin permeability. Especially, the solvent PSE had a marked influence. The formulation containing 5% PSE resulted in a skin permeation rate similar to that of the marketed reference products; Daivonex[®] and Diproderm[®] ointment. This new combination product has recently been tested in clinical studies showing an improved efficacy in the treatment of psoriasis and has now been marketed as Daivobet[®] ointment.

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Psoriasis: nouvelle modalité thérapeutique par le calcipotriol plus le dipropionate de bétaméthasone

ENGLISH TRANSLATION INCLUDED.

Psoriasis: new therapeutic modality by calcipotriol and betamethasone dipropionate

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RÉSUMÉ/SUMMARY

Dans cette étude multicentrique randomisée conduite en double aveugle, 188 patients atteints de psoriasis vulgaire ont été traités durant six semaines soit par une application de pommade calcipotriol le matin et une application de pommade dipropionate de bétaméthasone le soir, soit par l'application matin et soir de pommade calcipotriol.

En fonction des résultats constatés sur le critère principal retenu pour juger de l'efficacité de l'une ou l'autre de ces modalités thérapeutiques, l'évolution du score PASI, il a été noté une diminution plus rapide et plus importante du score pour le traitement associant dipropionate de bétaméthasone et calcipotriol ($p = 0,001$). En outre, la tolérance de ce traitement s'est également révélée meilleure sur les plans local et général de façon significative ($p = 0,04$).

In this multicentric randomized double blind study, 188 patients with psoriasis vulgaris were treated for six weeks either one application of calcipotriol ointment in the morning and one application of betamethasone dipropionate in the evening, or with application of calcipotriol ointment B.I.D.

Using the Psoriasis Area Severity Index (PASI) as the main criterion to evaluate treatment efficacy, a faster and more important improvement was seen with the regimen associating betamethasone dipropionate and calcipotriol ($p = 0.001$). This regimen was also better tolerated both locally and systemically ($p = 0.04$).

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Introduction

La lésion psoriasique associe une hyperprolifération des kératinocytes, une anomalie de leur différenciation et une réaction immunoinflammatoire dermique.

Le mode d'action des dermocorticoïdes est multiple (1), action anti-inflammatoire (vaso-constriction, prostaglandines), anti-proliférative, modulatrice de la différenciation des kératinocytes, immunosuppressive sur les cellules de Langerhans, les lymphocytes T et les polynucléaires neutrophiles des infiltrats dermiques.

Le calcipotriol inhibe (2, 3) la prolifération cellulaire et module la différenciation des kératinocytes agissant par l'intermédiaire d'un récepteur nucléaire spécifique. De nombreuses publications ont souligné son efficacité dans le traitement du psoriasis. Toutefois, son utilisation locale s'accompagne fréquemment d'une irritation se manifestant par des sensations de cuisson et de picotement sur les zones traitées.

Le mécanisme d'action des corticoïdes locaux et du calcipotriol impliquant des mécanismes moléculaires distincts, l'hypothèse d'une complémentarité d'action méritait d'être examinée. Il est donc apparu logique de rechercher une éventuelle potentialisation de l'efficacité de ces agents par une utilisation concomitante et d'autre part, une amélioration de la tolérance locale du calcipotriol du fait de l'activité anti-inflammatoire des dermocorticoïdes.

Aucune étude clinique n'a étudié à ce jour l'efficacité et la tolérance de l'association quotidienne d'un dérivé de la vitamine D à la corticothérapie locale (application le matin de l'un, le soir de l'autre). Une étude seulement a suggéré que ces deux

traitements locaux pourraient être utilisés de manière séquentielle (4).

Ce travail rapporte les résultats d'un essai comparatif multicentrique (20 centres en France), randomisé en double aveugle mené sur groupes parallèles de l'utilisation concomitante du dipropionate de bétaméthasone et du calcipotriol versus le calcipotriol, utilisé pendant 6 semaines chez des patients atteints de psoriasis vulgaire.

Malades et méthode

1- Population étudiée

Ont été inclus dans l'étude, les malades ambulatoires des deux sexes répondant aux critères d'inclusion suivants: diagnostic confirmé de psoriasis vulgaire, stable ou en cours d'aggravation depuis plus d'une semaine; atteinte cutanée comprise entre 10% et 40% de surface corporelle (tables des "9" de Wallace); score Psoriasis Area and Severity Index (PASI) compris entre 1 et 30, ce qui a toujours été le cas, et capacité du patient à se soumettre aux contraintes du protocole de l'étude.

N'ont pas été inclus dans l'étude les patients répondant aux critères suivants: femmes enceintes ou allaitant; psoriasis pustuleux, en gouttes, érythrodermique, associé à une lésion cutanée, bactérienne, fongique ou virale; pathologie intercurrente ainsi que tout traitement susceptible d'interférer avec le traitement étudié; anomalie biologique connue; hypersensibilité à la vitamine D ou à ses analogues; exposition solaire prolongée dans le mois précédant ou devant avoir lieu durant l'étude; traitements administrés, dans la période précédant l'inclusion: dermocorticoïdes

► dans les deux semaines, calcipotriol et dérivés des goudrons, dérivés des rétinoïdes, cyclosporine, méthotrexate et thérapie par rayonnements (PUVA) dans les quatre semaines.

2- Méthode

L'étude randomisée, comparative, en double aveugle a été menée sur deux groupes parallèles dans 20 centres en France entre septembre 1993 et juin 1994. L'attribution du traitement a été faite dans l'ordre numérique croissant alloué par tirage au sort selon l'ordre d'inclusion des patients.

Les patients ont reçu soit une application de pommade calcipotriol/Daivonex® (laboratoires Léo) le matin et de dipropionate de bétaméthasone/Diprosone® (Schering Plough) sous forme de pommade le soir, soit une application de pommade calcipotriol matin et soir durant six semaines.

Les deux médicaments ont été conditionnés de façon rigoureusement identique par Schering-Plough de telle sorte qu'il n'était pas possible de les identifier. Pour respecter le schéma d'administration, les tubes portaient la mention "matin" (étiquette jaune) ou "soir" (étiquette bleue).

L'investigateur demandait au patient de s'appliquer une mince couche de pommade sur les lésions à traiter et de rapporter les tubes utilisés.

3- Critères cliniques d'évaluation de la sévérité du psoriasis

A chaque visite, l'investigateur a évalué l'étendue des lésions (pourcentage de surface corporelle atteinte) et l'intensité des symptômes érythème, induration et desquamation selon l'échelle graduée de 0 (absence de symptôme) à 4 (symptôme très sévère).

Afin d'assurer le suivi de l'évolution du psoriasis sous traitement, le score PASI (Psoriasis Area and Severity Index) (5) a été déterminé à partir de ces paramètres tenant compte également de la localisation des lésions.

L'analyse de l'efficacité a été basée essentiellement sur le score PASI exprimé à la fois en valeur absolue aux cinq temps de contrôle, en valeur relative par rapport à la valeur initiale (indice) et sous la forme d'une aire sous la courbe englobant l'ensemble des valeurs relatives, calculée selon la méthode des trapèzes, cette dernière expression (AUC) constituant le critère principal.

De plus, à la fin des six semaines de traitement, l'investigateur a apprécié l'efficacité globale de la réponse thérapeutique selon l'échelle: 1 = guérison, 2 = amélioration marquée, 3 = amélioration modérée, 4 = amélioration légère, 5 = sans changement, 6 = exacerbation.

4- Critères cliniques d'évaluation de la tolérance

A chaque visite un interrogatoire et un examen attentifs se sont attachés à dépister un événement indésirable dont l'imputabilité au traitement a été cotée: probable, possible, douteuse, indéterminée. Il n'y a pas eu de liste pré-établie d'effets secondaires susceptibles de survenir.

5- Observance thérapeutique

A chaque visite de suivi, les tubes utilisés ont été collectés par l'investigateur afin de vérifier que le traitement a bien été administré conformément au schéma thérapeutique.

Au terme de l'étude les tubes de pommade ont été pesés dans le but de déterminer la consommation médicamenteuse par patient.

6- Traitements concomitants

Les patients ont pu continuer à recevoir une thérapeutique qui n'influence pas l'évolution de l'affection traitée. N'ont pas été autorisés: les corticoïdes oraux, parentéraux ou topiques, le calcium, la vitamine D, les anti-acides, les digitaliques, les diurétiques thiazidiques, la PUVAthérapie, les rétinoïdes.

7- Ethique

Cette étude a été approuvée par le Comité Consultatif de Protection des Personnes dans la Recherche Biomédicale du Groupe Hospitalier Pitié-Salpêtrière le 7 juillet 1993.

L'essai a été mené conformément aux Bonnes Pratiques Cliniques.

Chaque malade a signé un formulaire de consentement éclairé après avoir reçu toutes les informations utiles et pris connaissance de la notice d'information.

8- Analyse statistique

- **Nombre de patients analysés:** l'effectif des patients à enrôler a été calculé de manière à pouvoir mettre en évidence une différence d'au moins 8 % (en indice) entre les deux groupes, sur la variation du PASI par rapport à J0 avec une déviation standard de 20 %. Le risque alpha a été fixé à 0,05, la puissance du test (1-béta) à 80%.

- **La comparabilité des groupes** a été mesurée par le test du CHI² pour les critères qualitatifs, par le test de Student pour les critères quantitatifs discrets ou continus et par le test de U de Mann Whitney pour les critères ordinaux (scores).

- **Pour l'analyse de l'efficacité** basée sur le score PASI, le test U de Mann-Whitney a été utilisé. L'évaluation globale de la réponse thérapeutique à J42 a été analysée par le test du CHI² et le test U de Mann Whitney.

- **Tolérance:** le nombre de patients ayant présenté au moins un effet secondaire a été comparé par le test du CHI².

Résultats

L'étude a été réalisée de septembre 1993 à juin 1994. La population étudiée a été de 188 malades, 125 hommes et 63 femmes d'âge compris entre 20 et 85 ans moyenne: 47 ± 15 ans dans le groupe I (calcipotriol) et 45 ± 14 ans dans le groupe II (dipropionate de bétaméthasone-calcipotriol) avec un poids de $71,8 \pm 13,4$ kg (groupe I) et $73,8 \pm 14$ kg (groupe II) et une taille de 170 ± 8 cm (groupe I) et 168 ± 8 cm (groupe II).

Le score PASI au départ a été de $10,99 \pm 5,75$ (groupe I) et de $11,82 \pm 6,03$ (groupe II) avec un pourcentage de surface corporelle atteinte de $18,45 \pm 8$ et $20,18 \pm 9$ respectivement. Le psoriasis a été en phase d'aggravation dans 46 cas et dans 48 cas, stable dans 48 et 40 cas respectivement, en amélioration dans deux cas dans chacun des groupes.

Le psoriasis est apparu en moyenne $15,85 \pm 13,97$ (groupe I) et $13,81 \pm 9,77$ (groupe II) auparavant (valeurs mini: deux mois, maxi: 70 ans). 76 patients du premier groupe et 68 du deuxième groupe avaient déjà reçu précédemment un dermo-corticoïde lors de poussées antérieures de psoriasis.

Il n'y avait aucune différence statistiquement significative entre les groupes en ce qui concerne l'ensemble des données recueillies avant le début du traitement hormis le nombre d'hommes plus important ($p = 0,04$) dans le groupe dipropionate de bétaméthasone + calcipotriol (67 contre 58). Le tableau résume les paramètres dermatologiques des 156 patients analysables sur le plan de l'efficacité.

Patients	Calcipotriol (n = 81)	Dipropionate de bétaméthasone + calcipotriol (n = 75)	P
Age moyenne (ans)	47 ± 16 (20 - 85)	45 ± 14 (20 - 79)	0,40 *
Sexe			
hommes	49 (60,5 %)	56 (74,7 %)	0,06
femmes	32 (39,5 %)	19 (25,3 %)	***
Poids (kg)	72,1 ± 13,1	74,5 ± 14,4	0,27 *
Taille (cm)	169 ± 9	170 ± 8	0,24 *
Psoriasis stade d'évolution à J0			
aggravation rapide	6 (7,5 %)	3 (4 %)	0,57
aggravation lente	33 (41,2 %)	38 (51,4 %)	***
stable	40 (50 %)	32 (43,2 %)	
amélioration lente	1 (1,3 %)	1 (1,4 %)	
Durée de l'affection primaire (mois)	190 ± 168	166 ± 117	0,29 *
Pourcentage de surface atteinte	18,05 ± 8,16	19,81 ± 9,51	0,22 *
PASI	11,22 ± 5,76	12,15 ± 6,28	0,42 **

Tableau 1a: Résumé des paramètres démographiques des patients atteints de psoriasis (* Test T de Student; **, Test U de Mann-Whitney; *** Test du Chi2)
Demographic parameters of psoriatic patients

Groupes	Nombre de visites	1	2	3	4
Calcipotriol n = 16	Non revu	1	2		1
	Effets secondaires	1	4		3
	Autres	1	1	1	1
	TOTAL	3	7	1	5
Dipropionate de bétaméthasone + calcipotriol n = 16	Non revu	3	1	2	
	Effets secondaires			2	1
	Autres		1		6
	TOTAL	3	2	4	7

Tableau 1b: Nombre de visites réalisées par les patients exclus de l'analyse de l'efficacité (n'ayant pas de score PASI aux cinq temps)
Number of visits done by patients excluded from the efficacy analysis (for not having five PASI scores)

1- Evaluation de l'efficacité du traitement

Dans un premier temps, seuls les patients ayant un score PASI établi à chacune des cinq visites ont été analysés du point de vue de l'efficacité (81 patients dans le groupe calcipotriol et 75 patients dans le groupe dipropionate de bétaméthasone + calcipotriol). Le tableau 1b liste les patients ayant des valeurs manquantes du PASI.

L'analyse de la variation du score PASI, exprimée sous forme d'indice, montre une diminution progressive au cours du temps dans les deux groupes. Il existe constamment une différence dans l'ampleur de cette diminution à chaque visite, en faveur du groupe dipropionate de bétaméthasone + calcipotriol, et ceci de façon statistiquement significative (Tableau II, Fig. 1).

Cette différence d'intensité de variation, constatée dès le septième jour en faveur du groupe dipropionate de bétaméthasone + calcipotriol ($p = 0,007$) se confirme au quatorzième jour à la fois en ce qui concerne la valeur absolue du score PASI ($p = 0,04$) et d'une partie de ses composants: érythème, desquamation (Tableau III). En effet, bien que le score de départ (J0) soit iden-

tique pour les deux groupes, il existe une différence constante (Tableau II) illustrant le fait que l'action du calcipotriol est renforcée et complétée par la présence du dipropionate de bétaméthasone (Fig. 2).

D'autre part, il existe une différence ($p = 0,001$) entre les AUC des deux groupes, démontrant l'évolution plus favorable en fonction du temps, de l'ensemble des scores dans le groupe dipropionate de bétaméthasone + calcipotriol, 175 ± 62,07 contre 208,58 ± 62,89 dans le groupe calcipotriol seul (Tableau IV).

Cette différence est encore plus forte si on tient compte dans un dernier temps de toute la population traitée dans l'étude, c'est à dire en estimant les valeurs manquantes sur la base des dernières valeurs présentes ($p = 0,0002$) (Tableau IV).

En outre à J42, l'investigateur a apprécié la réponse globale au traitement administré. C'est ainsi que le nombre de patients blanchis complètement ou de façon marquée est de 64 (86,5 %) avec les deux traitements en alternance et de 51 (63,7 %) avec le calcipotriol utilisé seul ($p = 0,001$) (Tableau V, Fig. 3).

Indices	Groupe calcipotriol (n = 81)	Groupe dipropionate de bétaméthasone + calcipotriol (n = 75)	P*
Indice J7/J1 X ± S médiane mini - maxi	66,54 ± 21,88 68,97 17,20 - 113,79	57,92 ± 21,67 57,78 19,15 - 129,47	0,0068
Indice J14/J1 X ± S médiane mini - maxi	45,80 ± 21,28 42,86 9,57 - 87,50	36,26 ± 23,16 33,16 4,03 - 102,11	0,0037
Indice J28/J1 X ± S médiane mini - maxi	33,43 ± 21,05 28,81 3,42 - 87,30	22,83 ± 17,81 16,67 62,96	0,0008
Indice J42/J1 X ± S médiane mini - maxi	25,63 ± 22,38 20,59 0,00 - 120,83	17,45 ± 16,41 11,77 0,00 - 66,67	0,0092

Tableau II: Variations relatives du PASI par rapport à la valeur initiale (patients ayant eu cinq valeurs de PASI) (*Test U de Mann-Whitney)
Relative variations of PASI in comparison of initial values (patients with five values of PASI) (*Mann-Whitney's U-test)

Symptômes	Groupe calcipotriol (médiane)	Groupe dipropionate de bétaméthasone + calcipotriol (médiane)	P*
	J14	J14	
Erythème	4	3	0,02
Desquamation	2	1	0,007
Induration	3	2	0,12

Tableau III: Comparaison des scores des symptômes à J14 (*Test U de Mann-Whitney)
Comparison of symptoms scores at D14 (*Mann-Whitney's U-test)

2- Tolérance

Il y a une différence statistiquement significative ($p = 0,04$) entre le nombre de patients présentant au moins un effet secondaire: 24 patients (25,5 %) dans le groupe calcipotriol seul et 11 patients (12,5 %) dans le groupe dipropionate de bétaméthasone + calcipotriol.

De plus, dans le groupe calcipotriol seul, le nombre d'effets secondaires est de 31 (dont 27 locaux et 4 généraux) contre 12 (tous locaux) dans le groupe dipropionate de bétaméthasone + calcipotriol (Tableau VI).

Il s'agit la plupart du temps d'irritation locale provoquée par les applications de calcipotriol (20 patients sur les 24) alors que seuls trois patients ont souffert de cet effet secondaire dans le groupe dipropionate de bétaméthasone + calcipotriol.

Dans le groupe calcipotriol seul, 11 patients ont reçu un traitement approprié à l'effet secondaire. Six effets secondaires ont été jugés sévères chez cinq patients. Au total, six patients sont sortis dans ce groupe pour intolérance.

Dans le groupe dipropionate de bétaméthasone + calcipotriol, deux patients ont reçu un traitement approprié à l'effet secondaire. Aucun n'a été jugé sévère; trois patients sont sortis d'étude.

3- Observance thérapeutique

Pour l'ensemble des patients, il n'a pas été observé d'écarts au schéma thérapeutique. Aucune différence n'a été mise en évidence entre les groupes en ce qui concerne le nombre de grammes de pommade utilisés. En aucun cas, leur utilisation n'a dépassé 100g par semaine.

4- Evaluation des coûts de traitement

Cette étude clinique comparative du calcipotriol à deux applications quotidiennes versus l'application alternée le matin du calcipotriol, le soir du dipropionate de bétaméthasone, autorise une analyse concernant les coûts de traitement respectifs de chaque option; en effet, les populations traitées dans les deux groupes furent homogènes.

Le protocole ne permettait pas une mesure du coût des effets secondaires (visites et traitements supplémentaires) dans la mesure où il s'agissait d'une étude en double aveugle avec durée prédéterminée de traitement et visites de contrôle obligatoire à intervalles fixés.

Dans une situation "empirique", la fréquence plus élevée d'effets secondaires observée dans le groupe traité par le calcipotriol deux fois par jour, contribuerait à augmenter le coût de la pathologie mais cette mesure ne peut être prise en compte dans la présente analyse.

Les quantités de pommade consommées ont été mesurées par pesage des tubes ramenés par les patients. Ces conditions étant remplies les calculs hebdomadaires de consommation montrent:

- dans le groupe traité par le calcipotriol pommade deux fois par jour, une consommation hebdomadaire de 35,41g;
- dans le groupe traité, le matin par le calcipotriol et le soir par le dipropionate de bétaméthasone respectivement 17,37 g et 19,15 g par semaine.

AUC	Groupe calcipotriol	Groupe dipropionate de bétaméthasone calcipotriol	P*
X ± S médiane mini - maxi	PATIENTS AYANT EU CINQ VALEURS DE PASI n = 81 208,58 ± 62,89 200,85 106,90 - 343,75	n = 75 175,73 ± 62,07 161,11 84,38 - 362,11	0,001
X ± S médiane mini - maxi	ECHANTILLON TOTAL AVEC VALEURS MANQUANTS ESTIMÉES n = 97 223,15 ± 75,34 209,62 106,90 - 456,00	n = 91 185,15 ± 73,58 167,61 84,38 - 400,00	0,0002

Tableau IV: Valeurs des aires sous la courbe entre J1 et J42 (*Test U de Mann-Whitney)
AUC values between D0 and D42 (*Mann-Whitney's U-test)

Evaluation globale à J 42	Calcipotriol (n = 81)		Dipropionate de bétaméthasone calcipotriol (n = 75)	
Guérison	10 (12,5 %)	51* (63,7 %)	64* (86,5 %)	18 (24,3 %)
Amélioration marquée	41 (51,2 %)			46 (62,2 %)
Amélioration modérée	16 (20 %)			7 (9,5 %)
Amélioration légère	11 (13,7 %)	29* (36,3 %)	10* (13,5 %)	2 (2,7 %)
Sans changement	1 (1,3 %)			1 (1,3 %)
Aggravation	1 (1,3 %)			0
Total	80**		74**	

Tableau V: Efficacité/Efficacy [Test du Chi2: p = 0,02. Test U de Mann-Whitney: p = 0,0008. - * Test du Chi2: p = 0,001 (guérison + amélioration marquée vs amélioration modérée + amélioration légère + sans changement + aggravation) - ** Pour un patient dans chaque groupe. l'évaluation finale n'a pas été documentée]

Tolérance	Calcipotriol (n = 94*)	Dipropionate de bétaméthasone calcipotriol (n = 88*)	P*
Nombre de patients	24 (25,5 %) ¹	11 (12,5 %)	0,04
Nombre d'effets secondaires	31	12	

Tableau VI: Nombre de patients présentant au minimum un effet secondaire (* Test du Chi2). Pour trois patients dans chaque groupe l'investigateur n'a pas complété la rubrique effets secondaires

Nombre total d'effets secondaires par traitement

Number of patients presented at least one side-effect (*Chi2 test). For three patients in each group, the investigator did not complete the side-effects

Total number of side-effects per treatment

Comme indiqué dans le tableau VII, il apparaît par ailleurs que le traitement par l'association est nettement plus économique que le traitement par le calcipotriol seul.

Coûts	Daivonex® pommade matin et soir	Daivonex® pommade matin Diprosone® pommade soir
- pour une semaine calcul en grammes calcul en tubes	F 84,31 F 188,20*	F 56,99 F 118,60*
- pour six semaines calcul en grammes calcul en tubes	F 505,89 F 570,30*	F 341,96 F 430,20*

* Les calculs ont été effectués au prix public avec le conditionnement le moins onéreux.

Tableau VII: Coût des traitements/Cost of the treatments

Le traitement du psoriasis par calcipotriol le matin et dipropionate de bétaméthasone le soir est une alternative à moindre coût pour des résultats cliniques meilleurs.

Dans les conditions d'utilisation usuelle, l'économie est de l'ordre de 70,00 Frs pour un traitement d'une semaine et de l'ordre de 140,00 Frs pour un traitement prescrit pendant 6 semaines.

Discussion

Cette étude démontre sans ambiguïté qu'un traitement concomitant de dermocorticoïde et de calcipotriol est plus efficace et mieux toléré que le calcipotriol utilisé seul. Elle établit pour la première fois le bénéfice thérapeutique de l'association des deux familles d'agents thérapeutiques locaux pour la prise en charge du psoriasis.

En effet, la comparaison des deux groupes de patients montre la supériorité du traitement dipropionate de bétaméthasone +

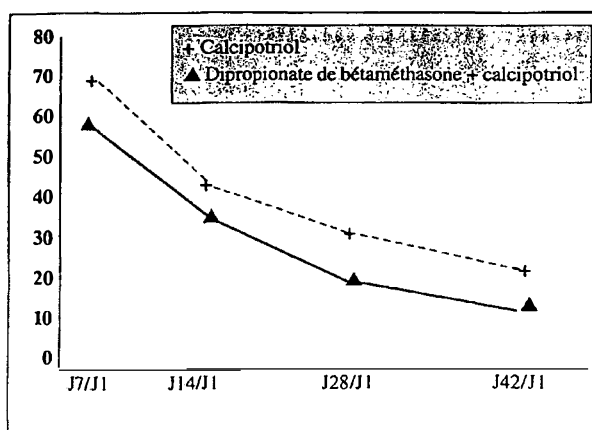


Figure 1: Indices: valeurs relatives du PASI par rapport à la valeur initiale (médianes)

Relative PASI values in comparison with initial value (medians)

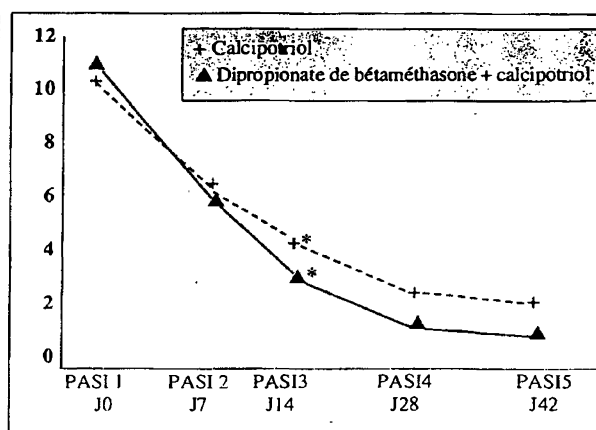


Figure 2: PASI: valeurs absolues (médianes) (* $p = 0,04$ - test U de Mann-Whitney)

PASI: absolute values (medians) (* $p = 0,04$ - Mann-Whitney's U-test)

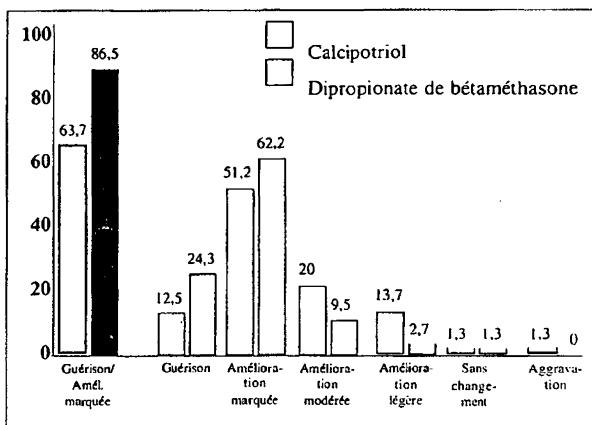


Figure 3: Evaluation globale de la réponse thérapeutique à J42 (%)

Global evaluation of therapeutic response at J42 (%)

Mots-clés:

Psoriasis
Calcipotriol
Dipropionate de bétaméthasone

Key words:

Psoriasis
Calcipotriol
Betamethasone dipropionate

calcipotriol de façon statistiquement significative par rapport au traitement calcipotriol seul quels que soient les critères choisis pour évaluer l'efficacité (variation du score PASI à chaque visite, aire sous la courbe de l'ensemble des indices aux différentes visites, évaluation globale de la réponse thérapeutique de la sixième semaine).

En ce qui concerne la tolérance, le nombre de patients présentant des effets secondaires est significativement réduit dans le groupe du dipropionate de bétaméthasone par comparaison au calcipotriol seul. Ce résultat confirme donc notre hypothèse de départ et suggère que l'activité anti-inflammatoire des corticoïdes a diminué la réaction inflammatoire locale induite par le calcipotriol.

Jusqu'à présent, seules des études comparatives de dermocorticoïde et de calcipotriol ont été publiées qui ne permettaient pas d'évaluer l'association. Ainsi, l'étude de W.J. Cunliffe (6), menée sur 409 patients répartis en groupes parallèles, ne met pas en évidence de différence statistiquement significative entre le groupe traité par le valérate de bétaméthasone et le calcipotriol. Dans une autre étude de comparaison bilatérale, chez 347 patients, K. Kragballe (7) a mis en évidence une supériorité du calcipotriol (réduction du PASI à six semaines de 68,8 %) sur le valérate de bétaméthasone (réduction du PASI à six semaines de 61,4 %).

Notre étude avait pour objectif d'apprécier la complémentarité éventuelle du dipropionate de bétaméthasone et du calcipotriol. Les résultats montrent que cette complémentarité existe en termes d'efficacité et de tolérance. La durée de notre étude ne permet toutefois pas de recommander cette association au-delà de six semaines. Il convient de souligner enfin que l'association dipropionate de bétaméthasone calcipotriol permet une économie de coût de traitement par rapport à l'administration bi-quotidienne du calcipotriol.

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**Psoriasis: new treatment modality with calcipotriol
and betamethasone dipropionate**

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SUMMARY

In this multicentre randomised double-blind trial, 188 patients with psoriasis vulgaris were treated for six weeks either with one application of calcipotriol ointment in the morning and one application of betamethasone dipropionate ointment in the evening, or by application of calcipotriol ointment morning and evening.

According to the results observed on the principal efficacy criterion, the change in the PASI (Psoriasis Area Severity Index) score, a faster and more substantial fall in the score was observed for the treatment combining betamethasone dipropionate and calcipotriol ($p = 0.001$). In addition, general and local tolerability of this treatment was significantly better ($p = 0.04$).

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Introduction

Psoriasis associates excessive proliferation of the keratinocytes, an anomaly of their differentiation, and a dermal immuno-inflammatory reaction.

The dermal corticosteroids act in many ways [1]: they have an anti-inflammatory action (vaso-constriction, prostaglandins), an anti-proliferative action, a modulatory action on keratinocyte differentiation, and an immunosuppressive action on Langerhans' cells, the T lymphocytes and the neutrophils of the dermal infiltrates.

Calcipotriol inhibits [2, 3] cell proliferation and modulates keratinocyte differentiation, acting by means of a specific nuclear receptor. Numerous publications have emphasised its efficacy in the treatment of psoriasis. However, local use is frequently accompanied by irritation in the shape of burning and prickling sensations on the treated areas.

As the mechanism of action of local corticosteroids and of calcipotriol involves different molecular mechanisms, the hypothesis that their action was complementary was worth examination. It therefore seemed logical to look for possible potentiation of the efficacy of these agents by using them concomitantly and, on the other hand, for an improvement in the local tolerability of calcipotriol because of the anti-inflammatory activity of the dermal corticosteroids.

No clinical trial to date has investigated the efficacy and tolerability of the daily association of a vitamin D derivative with local corticosteroid therapy (applying one in the morning and the other in the evening). One study only has suggested that these two local treatments could be used sequentially [4].

This study reports the results of a multicentre (20 centres in France) double-blind randomised comparative trial, on parallel groups, of the concomitant use of betamethasone dipropionate and calcipotriol vs. calcipotriol, used for six weeks in patients with psoriasis vulgaris.

Patients and methods

1. Population investigated

Out-patients of both sexes who met the following inclusion criteria were included in the study: confirmed diagnosis of psoriasis vulgaris, either stable or getting worse for more than a week: skin area affected between 10% and 40% of body surface (Wallace "9"s tables); Psoriasis Area and Severity Index (PASI) score between 1 and 30, which was always the case, and the patient's ability to submit to the constraints of the trial protocol. Patients who met the following criteria were not included in the trial: women who were pregnant or breast-feeding; pustular, guttata, or erythrodermic psoriasis, associated with a

bacterial, fungal or viral skin lesion; intercurrent disease, and any treatment liable to interfere with the treatment investigated: known laboratory anomaly; hypersensitivity to vitamin D or to its analogues; prolonged exposure to the sun in the month preceding or during the trial; treatments administered in the period preceding inclusion: dermal corticosteroids ...

Patients	Calcipotriol (n = 81)	Betamethasone dipropionate + calcipotriol (n = 75)	P
Age	47±16	45±14	0.40
mean (years)	(20 - 85)	(20-79)	*
Sex			
men	49 (60.5%)	56(74.7%)	0.06
women	32 (39.5%)	19(25.3%)	***
Weight (kg)	72.1±13.1	74.5±14.4	0.27
			*
Height (cm)	169±9	170±8	0.24
Psoriasis			*
stage of evolution on D0			0.57
rapid worsening	6 (7.5%)	3 (4%)	***
slow worsening	33 (41.2%)	38 (51.4%)	
stable	40 (50%)	32 (43.2%)	
slow improvement	1 (1.3%)	1 (1.4%)	
Duration of the primary disorder (months)	190±168	166±117	0.29
Percentage of area affected	18.05±8.16	19.81 ± 9.52	*
			0.22
PASI	11.22±5.76	12.15±6.28	0.42
			**

Table 1a: Summary of the demographic parameters of the patients with psoriasis (*Student's t test; ** Mann-Whitney U test; Chi2 test).

Groups	Number of visits	1	2	3	4
Calcipotriol n = 16	Not reviewed	1	2		1
	Adverse effects	1	4		3
	Others	1	1	1	1
	TOTAL	3	7	1	5
Betamethasone dipropionate + calcipotriol n = 16	Not reviewed	3	1	2	
	Adverse effects			2	1
	Others		1		6
	TOTAL	3	2	4	7

Table 1b: Number of visits made by the patients excluded from the analysis of efficacy (for not having five PASI scores).

1. Evaluation of the efficacy of the treatment

At first, only the patients with a PASI score established at each of the five visits were analyzed from the point of view of efficacy (81 patients in the calcipotriol group and 75 patients in the betamethasone dipropionate + calcipotriol group). Table 1b lists the patients with missing PASI values.

The analysis of the variation in the PASI score, expressed in the form of an index, shows a progressive reduction with time in both groups. There was always a difference in the scale of this reduction at each visit, in favour of the betamethasone dipropionate + calcipotriol group, and this was statistically significant (Table II, Figure 1).

This difference in scale of variation, found as early as Day 7 in favour of the betamethasone dipropionate + calcipotriol group ($p = 0.007$), was confirmed on Day 14 both where the absolute value of the PASI score ($p = 0.04$) and some of its components were concerned - erythema, desquamation (Table III). Although the initial score (D0) was identical for both groups, there was a consistent difference (Table II) illustrating the fact that the action of calcipotriol was reinforced and supplemented by the presence of the betamethasone dipropionate (Figure 2).

Moreover, there was a difference ($p = 0.001$) between the AUCs for the two groups, demonstrating a more favourable time-related change in all the scores in the betamethasone dipropionate + calcipotriol group, 175 ± 62.07 against 208.58 ± 62.89 in the calcipotriol only group (Table IV).

This difference was even greater if one took account lastly of the whole population treated in the trial, that is to say by estimating the missing values on the basis of the last value present ($p = 0.0002$) (Table IV).

In addition, on Day 42, the investigator assessed the global response to the treatment. Thus the number of patients completely or considerably controlled was 64 (86.5%) with the two alternating treatments and 51 (63.7%) with calcipotriol used on its own ($p = 0.001$) (Table)

AUC	Calcipotriol group	Betamethasone dipropionate + calcipotriol group	P*
	PATIENTS WITH n = 81	FIVE PASI VALUES n = 75	
X ± S	208.58 ± 62.89	175.73 ± 62.07	0.001
Median	200.85	161.11	
Min.- Max.	106.90 - 343.75	84.38 - 362.11	
	TOTAL SAMPLE WITH n = 97	MISSING VALUES ESTIMATED n = 91	
X ± S	223.15 ± 75.34	185.15 ± 73.58	0.0002
Median	209.62	167.61	
Min.- Max.	106.90 - 456.00	84.38 - 400.00	

Table IV. AUC values between D1 and D42 (*Mann-Whitney U test)

Global evaluation on D 42	Calcipotriol (n = 81)		Betamethasone dipropionate + calcipotriol (n = 75)	
Cure	10 (12.5%)	51* (63.7%)	64* (86.5%)	18 (24.3%)
Marked improvement	41 (51.2%)			46 (62.2%)
Moderate improvement	16 (20%)	29* (36.3%)	10* (13.5%)	7 (9.5%)
Slight improvement	11 (13.7%)			2 (2.7%)
No change	1 (1.3%)			1 (1.3%)
Worse	1 (1.3%)			0
Total	80**		74**	

Table V: Efficacy (Chi2 test: p = 0.02. Mann-Whitney U test: p = 0.0008. * Chi2 test: p = 0.001 (cure + marked improvement vs. moderate improvement + slight improvement + no change + worsening). ** The final evaluation was not documented for one patient in each group.

Tolerability	Calcipotriol (n = 94)*	Betamethasone dipropionate + calcipotriol (n = 88)*	P*
Number of patients	24 (25.5%) ¹	11 (12.5%)	0.04
Number of adverse effects	31	12	

Table VI. Number of patients presenting at least one adverse effect (*Chi2 test). The investigator did not complete the adverse effects heading for three patients in each group.

Total number of adverse effects per treatment.

As Table VII shows, it also appears that treatment with the combination is much more economical than treatment with calcipotriol only.

Costs	Daivonex [®] ointment morning and evening	Daivonex [®] ointment a.m. Diprosone [®] ointment p.m.
- For one week		
Calculated in grammes	F84.31	F 56.99
Calculated in tubes	F188.20*	F118.60*
- For six weeks		
Calculation in grammes	F505.89	F341.96
Calculation in tubes	F570.30*	F430.20*

* The calculations were made on the price to the public with the cheapest packaging.

Table VII. Cost of the treatments.

The treatment of psoriasis with calcipotriol in the morning and betamethasone dipropionate in the evening is a cheaper alternative with better clinical results.

Under customary conditions of use, the saving is of the order of 70.00 francs for one week's treatment and of the order of 140.00 francs for treatment prescribed for six weeks.

Discussion

This study shows unambiguously that concomitant treatment with dermal corticosteroid and calcipotriol was more efficacious and better tolerated than calcipotriol used on its own. It establishes for the first time the therapeutic benefit of the combination of the two families of local agents for the care of psoriasis.

The comparison of the two groups shows the superiority of the betamethasone dipropionate + calcipotriol treatment.

Original article

Mixed treatment comparison of a two-compound formulation (TCF) product containing calcipotriol and betamethasone dipropionate with other topical treatments in psoriasis vulgaris

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Key words:

Mixed treatment comparison – PASI 75 – Psoriasis – Psoriasis treatment

Abstract

Objective:

The efficacy of the two-compound formulation (TCF) product containing calcipotriol and betamethasone dipropionate applied once daily in psoriasis has been demonstrated in phase III trials but no randomised clinical trial comparing all commonly used topical treatments exists. The aim of the study was to compare the efficacy of once-daily use of the TCF product relative to other commonly used topical agents in plaque psoriasis.

Research design and methods:

Data on change in Psoriasis Area and Severity Index (PASI) score from baseline and PASI 75 (percentage of patients achieving a 75% reduction in PASI score), after 4 weeks of treatment were obtained by means of a systematic literature review of randomised controlled trials and synthesised with a Bayesian mixed treatment comparison meta-analysis.

Results:

Relative to all active interventions, except for the unlicensed twice-daily application of the TCF product, the TCF once daily showed a greater efficacy based on PASI 75 response (relative risk ranging from 1.22 to 3.18) and improvement in PASI score from baseline (difference in % CFB PASI between TCF once daily and other active interventions ranged from 4.01 to 49.68).

Conclusion:

Among topical therapies evaluated, TCF once daily can be considered the most efficacious treatment for plaque psoriasis.

Introduction

Psoriasis vulgaris is a chronic relapsing and remitting inflammatory and scaling skin disease thought to have a prevalence of between 0.6% and 4.8% in industrialised Western countries^{1,2}. Disease severity is usually classified as mild, moderate or severe, according to the proportion of skin affected, the extent of redness, thickness and scaling of the plaques, the impact on quality of life (QOL) and the historical severity in terms of response to previous treatments.

The primary objective of therapy is to reduce symptoms and attain long-term remission³. Treatment choice depends on factors such as severity of disease, extent of body surface area involvement and prior response to treatment. Treatment usually starts with topical preparations. Patients with large

involvement of the skin or those not responding to topical therapy are candidates for phototherapy, traditional systemic therapy or biologic therapy with immunomodulatory drugs³. Current treatment strategies also involve combinations of agents. One of the most commonly used topical combinations is the vitamin D analogue calcipotriol with a corticosteroid.

The two-compound formulation (TCF) product is a topical ointment containing calcipotriol (50 µg/g) and betamethasone dipropionate (BDP) (0.5 mg/g) and is licensed to be applied once daily. Clinical studies showed that the TCF is more effective than its individual constituents and is associated with less skin irritation than calcipotriol monotherapy⁴⁻⁸. No published head-to-head comparisons between the TCF and the non-fixed combination of calcipotriol and BDP, or other commonly used treatments are available. However, an indirect unadjusted comparison indicated that the TCF product provided higher PASI 75 response rates than the non-fixed combination⁹.

In recent years, the role of meta-analysis has developed substantially in medical applications¹⁰⁻¹² and is considered a well accepted method of generating evidence in the context of healthcare decision making¹³⁻¹⁶. In the absence of randomised comparative studies, alternative approaches are required to assess the relative value of a particular intervention versus other relevant options. A mixed treatment comparison (MTC) can be considered a valuable alternative. In traditional meta-analysis all included studies compare the same intervention with the same comparator. MTC meta-analysis extends this concept by including multiple pair-wise comparisons across a range of interventions and provides comparative effectiveness information on multiple treatment comparisons¹⁷⁻¹⁹.

A MTC was conducted to assess the efficacy of the once-daily application of the TCF product containing calcipotriol and BDP relative to other commonly used topical agents in the management of psoriasis vulgaris.

Methods

Identification and study selection

In order to identify all relevant publications an extensive systematic literature review was performed. Computerised bibliographic databases (MEDLINE 1996 – January 2008, EMBASE 1974 – January 2008, BIOSIS until January 2008) were searched. In addition, the Cochrane Controlled Trial Register and the Health Technology Assessments of the National Institute for Health and Clinical Excellence (NICE) were reviewed. Studies were then included according to the following predetermined conditions.

Study design

Any randomised clinical trial (RCT) meeting the criteria for participants, interventions or outcomes listed below. Only fully published reports were considered, and letters and abstracts were excluded.

Interventions

The interventions of interest were selected based on recently published treatment guidelines²⁰ and included: the TCF product, vitamin D analogues (tacalcitol, calcitriol, calcipotriol), steroids (betamethasone valerate, betamethasone dipropionate, clobetasol, mometasone and fluocinolone), any non-fixed combination of a vitamin D analogue and a corticosteroid, coal tar, tazarotene, dithranol, pimecrolimus, tacrolimus. Studies of combination regimens other than the combination of a corticosteroid and a vitamin D analogue were excluded as these are considered of limited importance in clinical practice.

Study population

Patients above 18 years with psoriasis vulgaris. Studies of patients with scalp or nail psoriasis were excluded.

Outcome measures

The most commonly used tool to assess treatment outcomes is the Psoriasis Area and Severity Index (PASI)²¹. Traditionally, the percentage change from baseline in PASI score has been used as a measure of efficacy in clinical studies. However, the PASI 75 (percentage of patients with at least 75% improvement in PASI score) is the currently recognised benchmark endpoint used in clinical trials^{22,23}. Hence outcome measures of interest were PASI 75 response and percentage change from baseline (CFB) in PASI score.

Language

Full publications in English, German, Dutch and French were considered.

Data extraction

For each selected study, details were extracted on design, inclusion criteria, study population characteristics, interventions, outcome measures and results, which were subsequently checked by a second reviewer. The key outcome measures of interest were the PASI 75 response and % CFB PASI after 4 weeks of treatment.

Full publications were used for data extraction. However, as PASI 75 response is a more recently used outcome, PASI 75 data were also based on data on file at LEO Pharma as these provided more comprehensive data than those provided in the related publications. In essence,

from all studies included in the PASI 75 analysis equivalent data were available. Data on % CFB PASI and uncertainty by treatment relative to placebo or other comparators was directly taken from the publications if available. Recalculations were carried out for studies reporting absolute PASI scores rather than percentage change. In cases where standard error of the change was not reported, these were calculated based on the standard deviations in combination with sample size, or alternatively by conversion of the reported *p*-value to a *Z*-score reflecting the number of standard deviations the observed mean is different from the expected mean.

Analysis

Since the available evidence consists of a network of multiple RCTs involving treatments compared directly or indirectly or both, it was synthesised by means of a Bayesian MTC meta-analysis with linear and logistic regression models^{10,12,17,24}. (See Appendix for more detail). The analyses were performed for all treatments individually and for treatments by drug class.

MTC within the Bayesian framework involve data, a likelihood distribution, a model with parameters, and prior distributions. The model relates the data from the individual studies to basic parameters reflecting the pooled relative treatment effects of each intervention compared to an overall reference treatment (i.e., placebo). Based on these basic parameters, the relative efficacy estimates between the TCF product and the competing interventions of interest were obtained according to functional relations between the basic parameters of each intervention (see Appendix for details). For each of the outcomes, a fixed- and a random-effects model was evaluated. With a fixed-effects model, it is assumed that there is no variation in relative treatment effects across studies for a particular pair-wise comparison. However, if effect size estimates vary between studies to a greater extent than expected on the basis of chance alone, the studies are considered to be heterogeneous, and it is necessary to account for the extra variation, through use of a random-effects model. With a random-effects MTC model the heterogeneity is often assumed to be constant for all pair-wise comparisons. According to the 'goodness of fit' of the models, the random-effects model was the most appropriate approach for all analyses with the exception of the PASI 75 analysis by treatment.

The Bayesian approach involves a formal combination of a prior probability distribution (that reflects a prior belief of the possible values of the basic parameters) with a (likelihood) distribution of the pooled effect based on the observed data to obtain a posterior probability distribution for the estimate of the basic parameters. In order not to influence the estimates by the choice of the prior

distribution, a non-informative (i.e., 'flat') distribution was used for the parameters of the model. With such a prior distribution, results as reflected with the posterior distribution are solely driven by the data. The posterior distribution can be interpreted in terms of probabilities (e.g., 'There is an *x*% probability that treatment A results in a greater response than treatment B'). The posterior distribution is summarised with a point estimate reflecting the most likely value and the 2.5th and 97.5th percentile reflecting the 95% credible interval (CrI). In contrast to a 95% confidence interval obtained with a frequentist statistical analysis, the 95% CrI indicates the 95% probability that the 'true' value lies in the interval^{18,20}. The posterior distribution also allowed calculation of the probability that a certain treatment is best out of those compared, or second best, or third best, etc. Combining these results provides a rank ordering of the treatments regarding their efficacy estimates.

Analyses were performed with WinBUGS 1.4 statistical software (MRC Biostatistics Unit, Cambridge, UK) and relative efficacy estimates were presented for the TCF product once daily relative to other treatments. For PASI 75 results were presented with the relative risk (RR) of a response along with the corresponding 95% CrI, and for CFB PASI, the differences in % CFB PASI along with 95% CrI were presented. The expected PASI 75 response with placebo (defined as the average of all results observed with placebo in the placebo controlled trials) in combination with the relative efficacy estimates of each treatment versus placebo allowed calculation of the expected PASI 75 response as well.

Results

A flow chart summarising the study selection process is shown in Figure 1.

Of the 843 abstracts retrieved from the electronic databases, 195 abstracts met the inclusion criteria. Three publications were added from other sources (identified in review papers; these publications were not indexed in the computerised databases) resulting in 198 publications screened for eligibility. In all, 136 publications reported on topical therapies and, of these, 110 publications were excluded. Data extraction was therefore performed for a total of 26 original research studies of which seven studies (eight publications since two publications referred to the same study) were excluded from the analysis for not reporting the time point of interest^{25–28} or because of another study design^{29–32}. As a consequence, data from 19 studies were included, seven describing PASI 75 outcomes and all reporting CFB PASI outcomes. Figure 2 reflects the network of RCTs. Every node reflects a treatment, and every line reflects a direct comparison performed in one or more RCTs. The total network reflects the available

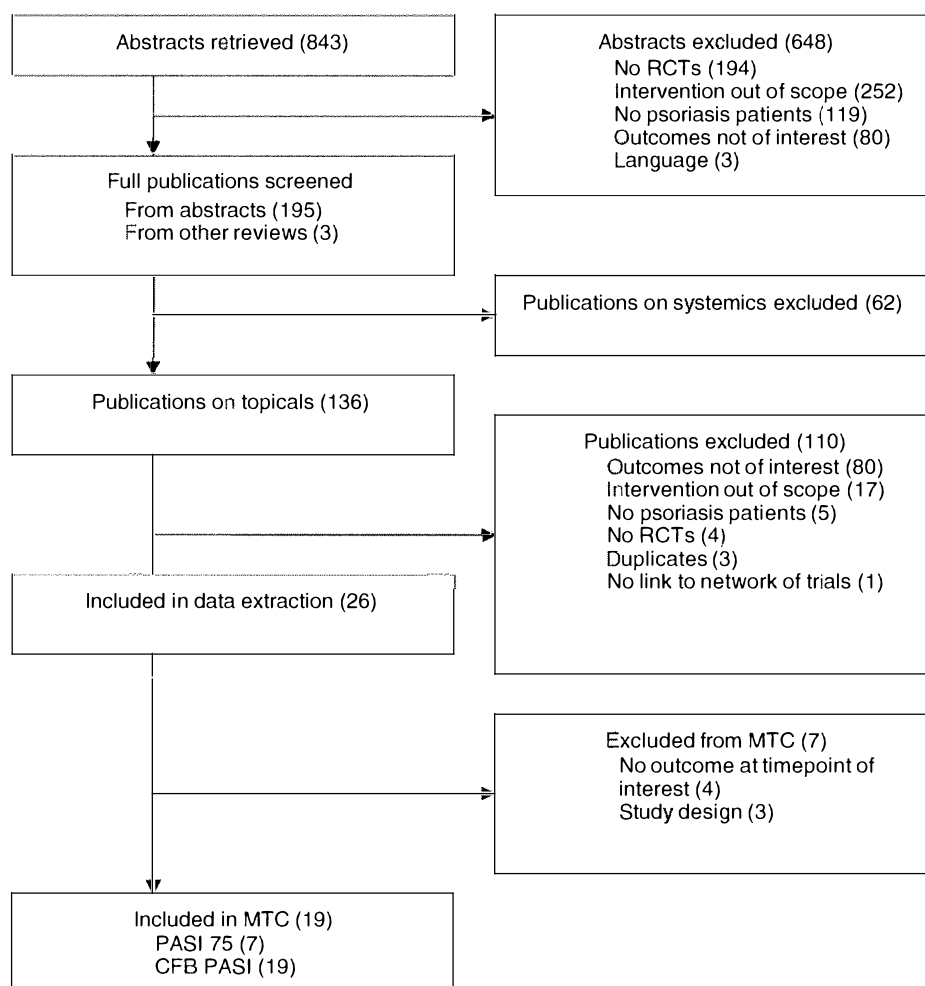


Figure 1. Flow chart outlining results of study selection.

evidence for CFB PASI, and the black nodes and edges reflect the network available for PASI 75 response. Table 1 summarises study and patient characteristics of the 19 studies included in the analysis.

The therapies evaluated in the different studies were: TCF product (seven studies)^{4–8,33,34}, vitamin D analogue (18 studies)^{4–8,33–45}, corticosteroid (seven studies)^{4,6,8,36,39,41,46}, the non-fixed combination of a vitamin D analogue and a corticosteroid (three studies)^{40,43,44}, dithranol (three studies)^{35,38,42} and coal tar (two studies)^{45,46}. There were four placebo-controlled studies among which three included the TCF product^{5,6,8} and one compared calcipotriol with placebo³⁷. Four studies were so called ‘left-right’ studies, where one of the treatments of interest was applied on one half of the body, and the other treatment of interest was applied on the other half^{37,39,44,45}. Although of a different design than the other RCTs (within-patient randomisation rather than between-patient randomisation) these studies were included in the analysis. The duration of the (randomised)

treatment phase in the various studies ranged from 4 to 10 weeks. In general, the studies randomised more males than females, and the mean age range was 40–52 years. The patients had a diagnosis of psoriasis for 10–20 years prior to randomisation. The mean baseline PASI scores reported ranged from 5.7 to 14, with the exception of one small study reporting a baseline PASI score of 17.1 and 17.7 in the two treatment arms⁴⁶. Although the various patient characteristics were not consistently reported across the trials, the included population was considered comparable regarding key baseline characteristics such as baseline PASI score and inclusion criteria.

Table 2 and 3 summarise the reported outcomes for PASI 75 (seven RCTs) and % CFB PASI (all 19 RCTs), respectively. Note that the number of patients included varied from treatment to treatment. In particular, the non-fixed combinations of vitamin D analogue and corticosteroids, tar products and calcitriol twice daily are characterised by a relatively small amount of patients.



Figure 2. Network of evidence for PASI 75 response (black network) and % CFB PASI (total network). Numbers in figure reflect study reference.

Results of the mixed treatment comparison

PASI 75

Seven studies randomising a total of 6708 patients reported PASI 75 data at week 4. All interventions were more efficacious than placebo. Figure 3 depicts the absolute expected PASI 75 response associated with each individual treatment as estimated with the MTC analysis. The TCF product applied twice and once daily were ranked as the two most efficacious treatment options, achieving an expected PASI 75 response of 62% and 51%, respectively. The expected PASI 75 response with the competing interventions ranged from 19% with calcipotriol do to 42% with betamethasone dipropionate bid.

Figure 4 present the relative probability of a PASI 75 response with TCF product once daily versus each of the

competing interventions expressed with a RR and shows that TCF product once daily is more efficacious than all the other interventions with the exception of the TCF product applied twice daily. Despite the relatively large uncertainty in the expected PASI 75 response estimate for the combination of calcipotriol once daily with betamethasone valerate once daily as presented in Figure 3, the RR estimate of TCF product once daily versus this intervention is 1.30 (95% CrI 0.94–1.94) indicating a 94% probability that TCT once daily is more efficacious.

The analysis by drug class shows the TCF product applied once daily to have a greater PASI 75 response than the class of vitamin D analogues, corticosteroids or their non-fixed combinations (Figure 5).

Table 1. Study and patient characteristics.

Study	Study design	Dosage regimen	No. of pts randomised	Male (%)	Mean age (years)	Years since diagnosis	Baseline PASI score mean (range)	Severity of psoriasis (% mild/moderate/severe)
Kaufmann <i>et al.</i> 2002 ^b	RCT, PC, DB, MC	Placebo od	157	56	50	18	9.5 (2.3–36.9)	nr/63.1/nr
		Calcip 50 µg/g od	480	59	49	20	10.4 (1.2–44.5)	nr/62.5/nr
		BDP 0.05% od	476	61	48	19	9.8 (1.2–49.5)	nr/62.4/nr
		TCF od	490	63	48	18	9.9 (1.2–42.8)	nr/63.6/nr
Douglas <i>et al.</i> 2002 ⁴	RCT, PC, DB, MC	Calcip 50 µg/g bd	369	60	48	19	10.9 (2.4–38.8)	nr
		BDP 0.05% bd	365	61	46	18	10.5 (2.4–39.6)	nr
		TCF bd	372		48	19	10.8 (2.1–38.0)	nr
							10.8 (1.0–36.0)	nr
Papp <i>et al.</i> 2003 ⁸	RCT, PC, DB, MC	Placebo bd	108	60	48	18		nr
		Calcip 50 µg/g bd	308	59	46	18		nr
		BDP 0.05% bd	313	59	47	19		nr
		TCF bd	304	57	48	19		nr
Guenther <i>et al.</i> 2002 ⁵ ; van de Kerkhof <i>et al.</i> 2004 ⁵³	RCT, PC, DB, MC	Placebo bd	208	64	47	18	10.4 (nr)	nr
		TCF od + placebo od	152	59	48	18	9.9 (nr)	nr
		TCF bd	237	70	49	18	10.6 (nr)	nr
		Calcip 50 µg/g bd	231	62	49	19	10.8 (nr)	nr
Ortonne <i>et al.</i> 2004 ³⁴	RCT, DB, MC	Tacal 4 µg/g od	252	56	52	19	9.9 (nr)	nr
		TCF od for 4 weeks then calcip 50 µg/g od for 4 weeks	249	54	51	20	9.7 (nr)	nr
Kragballe <i>et al.</i> 2004 ⁷	RCT, partly DB, MC	Calcip 50 µg/g bd	327	65	48	18	10.9 (2.0–41.0)	nr/65.4/nr
		TCF od for 4 weeks then calcip 50 µg/g od for 8 weeks	323	64	48	19	10.4 (2.0–42.0)	nr/62.8/nr
		TCF od for 8 weeks then calcip 50 µg/g od for 4 weeks	322	62	48	18	10.3 (2.0–49.0)	nr/64.6/nr
Dubertret <i>et al.</i> 1992 ³⁷	RCT, PC, DB, MC	Placebo bd	66	70	43	13	14.1 (nr)	nr
		Calcip 50 µg/g bd					14.2 (nr)	
Kragballe <i>et al.</i> 1991 ³⁹	RCT, DB, MC	Calcip 50 µg/g bd	345	59	45	20	8.4 (0.6–48.5)	nr
		BV 0.1% bd					8.3 (0.6–48.5)	
Cunliffe <i>et al.</i> 1992 ³⁶	RCT, DB, MC	BV 0.1% bd	204	56	46	17	9.4 (0.6–36.0)	nr
		Calcip 50 µg/g bd	205	55	44	16	8.7 (0.6–41.2)	nr
Molin <i>et al.</i> 1997 ⁴¹	RCT, DB, MC	BV 0.1% bd	211	nr	nr	nr	nr	60.7/26.1/13.4
		Calcip 50 µg/g bd	210	nr	nr	nr	nr	58.1/30.5/11.4
Ortonne <i>et al.</i> 1994 ⁴³	RCT, DB, MC	Calcip 50 µg/g od	188	75	45	14	12.1 (nr)	nr
		(morning) + BDP 0.05% nightly						
Salmhofer <i>et al.</i> 2000 ⁴⁴	RCT, DB, LR, MC	Calcip 50 µg/g bd		61	47	16	11.2 (nr)	nr
		Calcip 50 µg/g bd	63	54	47	12	5.7 (nr)	nr
		Calcip 50 µg/g od (morning) + diflu 0.1% nightly						
Kragballe <i>et al.</i> 1998 ⁴⁰	RCT, PC, DB, MC	Calcip 50 µg/g od + placebo od	174	nr	nr	nr	8.4 (nr)	nr
		Calcip 50 µg/g bd	174	nr	nr	nr	8.6 (nr)	nr
		Calcip 50 µg/g od (morning) + CB 0.05% nightly	175	nr	nr	nr	8.1 (nr)	nr

		Calcip 50 µg/g od (morning) + BMV 0.1% nightly	176	nr	nr	nr	8.4 (nr)	nr
Hutchinson <i>et al.</i> 2000 ³⁸	RCT, OL, MC	Calcitriol 3 µg/g bd	60	75	42	14	11.6 (nr)	0.0/66.3/26.7
Berth-Jones <i>et al.</i> 1992 ³⁵	RCT, OL, MC	Dithra od for 30 minutes	54	83	43	17	12.0 (nr)	0.0/63.0/31.5
		Calcip 50 µg/g bd	239	57	45	17	9.4 (nr)	nr
Tham <i>et al.</i> 1994 ⁴⁵	RCT, SB, LR	Dithra od for 30 minutes	239	52	43	18	9.1 (nr)	nr
		Calcip 50 µg/g bd	30	57	40	10	6.6 (nr)	nr
		Tar 15% od + placebo od					6.5 (nr)	nr
Thawornchaisit <i>et al.</i> 2007 ⁴⁶	RCT, OL, SC	Tar 10% bid	28	61	40	nr	17.1 (10.8–21.6)	nr
		BV 0.1% bid	30	63	42	nr	17.7 (9.2–21.8)	nr
Monastirli <i>et al.</i> 2000 ⁴²	RCT, OL, SC	Calcip 50 µg/g bd	35	63	48	18	6.9 (nr)	nr
		Dithra od for 30 minutes	35	51	47	16	7.3 (nr)	nr
Saraceno <i>et al.</i> 2007 ³³	RCT, OL, MC	Calcip 50 µg/g bid for 12 weeks	75	72	46	16	8.9 (1.6–17.7)	All pts mild-to-moderate
		TCF 4 weeks then calcip 50 µg/g bd 8 weeks	75	60	49	12	9.4 (2.4–33.0)	

BDP, betamethasone dipropionate; bd, twice daily; BV, betamethasone valerate; calcip, calcipotriol; CB, clobetasol butyrate; DB, double-blind; diflu, diflucortolone; dithra, dithranol; LR, left-right study; MC, multicentre; nr, not recorded; od, once daily; OL, open label; PC, placebo controlled; RCT, randomised clinical trial; SB, single-blind; SC, single centre; Tacal, tacalcitol; TCF, Two compound formulation.

Table 2. PASI 75 response by treatment in the individual studies.

Study	Placebo		TCF od		Calcip od		BDP od		TCF bd		Calcip bd		BDP bd		Tacal od		Calcip od		CB od		Calcip od		BV od	
	r	n	r	n	r	n	r	n	r	n	r	n	r	n	r	n	r	n	r	n	r	n	r	n
Kaufmann 2002 ⁶	14	157	270	490	103	480	161	476																
Douglas <i>et al.</i> 2002 ⁴									219	366	95	365	128	362										
Papp <i>et al.</i> 2003 ³	6	107							175	301	78	308	129	312										
Guenther <i>et al.</i> 2002 ⁵	16	207	72	150					134	234	74	227												
Ortonne <i>et al.</i> 2004 ³⁴			116	249											35	252								
Kragballe <i>et al.</i> 2004 ⁷			155	323							87	327												
			158	322																				
Kragballe <i>et al.</i> 1998 ⁴⁰					11	173					17	172					17		174		26		174	

bd, twice daily; BDP, betamethasone dipropionate; BV, betamethasone valerate; Calcip, calcipotriol; CB, clobetasol butyrate; M, number of PASI 75 responders; n, number of patients randomised; od, once daily; PASI 75, patients with 75% improvement in psoriasis area and severity index score; Tacal, tacalcitol; TCF, two-compound formulation.

Table 3. Mean CFB PASI by treatment as reported in the individual studies.

Study	Placebo	TCF od	Calcip od	BDP od	TCF bd	Calcip bd	BDP bd	Tacal od	BV bd	Calcip od + BV od	Calcip od + BDP od	Calcip of + acifu od	Calcip od + CB od	Calcitriol 3 µg/g bd	Dithra od	Tar 15% od	Tar 10% od
Kaufmann 2002 ⁶	–22.7 ^a	–71.3 ^a	–46.1 ^a	–57.2 ^a													
Douglas <i>et al.</i> 2002 ⁴					–74.4 ^a	–55.3 ^a	–61.3 ^a										
Papp <i>et al.</i> 2003 ⁸	–30.8 ^a				–73.9 ^a	–50.5 ^a	–65.2 ^a										
Guenther <i>et al.</i> 2002 ⁵	–26.6 ^a	–68.6 ^a			–73.8 ^a	–58.8 ^a											
Ortonne <i>et al.</i> 2004 ³⁴		–65.0 ^a						–33.3 ^a									
Kragballe <i>et al.</i> 2004 ⁷		–68.0 ^a															
		–70.0 ^a				–56.0 ^a											
Dubertret <i>et al.</i> 1992 ³⁷	–35.4					–58.6											
	(37.2) ^b					(31.7) ^b											
Kragballe <i>et al.</i> 1991 ³⁹						–61.5 ^c			–55.2 ^c								
Cunliffe <i>et al.</i> 1992 ³⁶						–50.4			–48.1								
						(3.8)			(4.1)								
Molin <i>et al.</i> 1997 ⁴¹						–34.5			–32.4								
						(1.9)			(2.5)								
Ortonne <i>et al.</i> 1994 ⁴³						–66.6 ^c					–77.2 ^c						
Salmhofer <i>et al.</i> 2000 ⁴⁴						–66.7						–68.4					
						(51) ^b						(50) ^b					
Kragballe <i>et al.</i> 1998 ⁴⁰			–34.0			–40.1				–47.6			–46.3				
			(2.5)			(2.2)				(2.0)			(1.9)				
Hutchinson <i>et al.</i> 2000 ³⁸														–57.1	–51.7		
														(8.0)	(12.1)		
Berth-Jones <i>et al.</i> 1992 ³⁵						–54.5									–36.3		
						(5.0)									(5.0)		
Tham <i>et al.</i> 1994 ⁴⁵						–57.5										–22.3	
						(19.4) ^b										(24.2) ^b	
Thawornchaisit <i>et al.</i> 2007 ⁴⁶									–51.4								–28.2
									(18.2) ^b								(16.5) ^b
Monastirli <i>et al.</i> 2000 ⁴²						–34.4									–56.8		
						(4.3)									(4.7)		
Saraceno <i>et al.</i> 2007 ³³		–73.7 ^c				–55.3 ^c											

bd, twice daily; BDP, betamethasone dipropionate; BV, betamethasone valerate; Calcip, calcipotriol; CB, clobetasol butyrate; *N*, number of PASI 75 responders; *n*, number of patients randomised; od, once daily; PASI, psoriasis area and severity index; PCB, percentage change from baseline; Tacal, tacalcitol; TCF, two-compound formulation; Standard error for the change from baseline by intervention is reported in brackets if reported in the actual study. For many studies only the *p*-value or standard error for the difference in change from baseline between interventions compared was reported and used as such in the MTC. These are not reported in this table. ^a, confidence interval reported for differences; ^b, standard deviation; ^c, standard error of differences based on *p*-values.

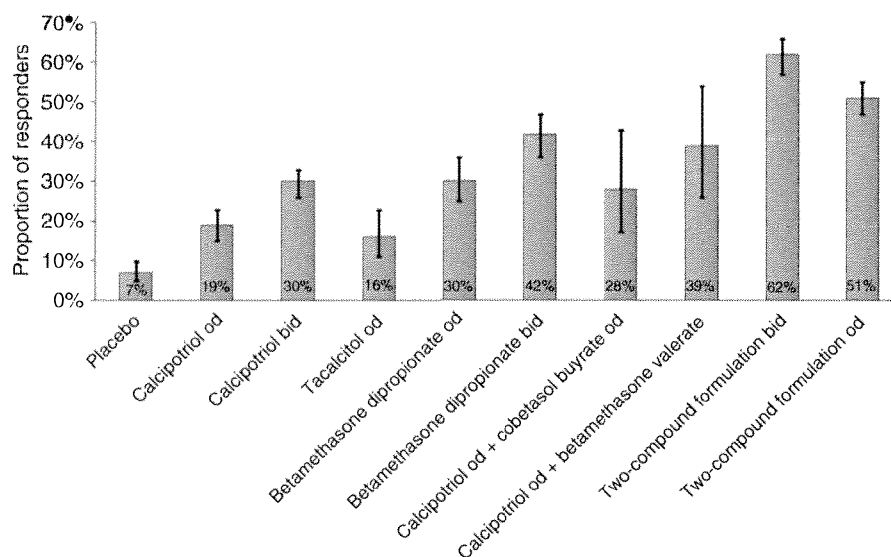
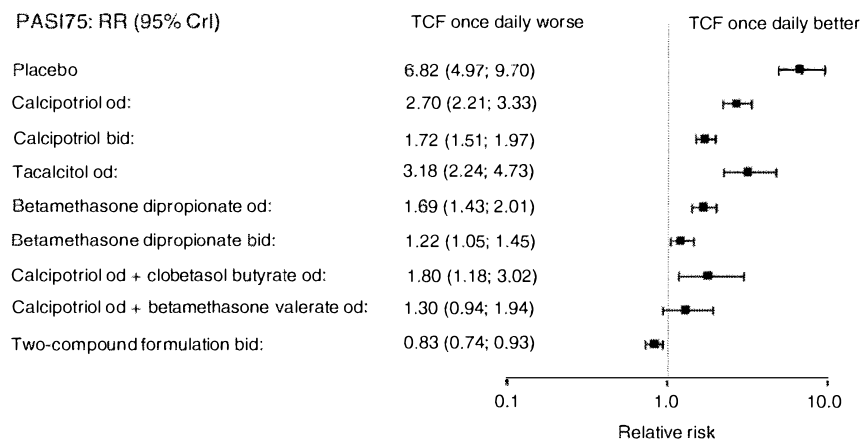


Figure 3. Absolute expected proportion of PASI 75 responders as estimated by MTC. Fixed-effects model (mean and 95% CrI). PASI 75 is defined as percentage of patients achieving at least 75% reduction in PASI.



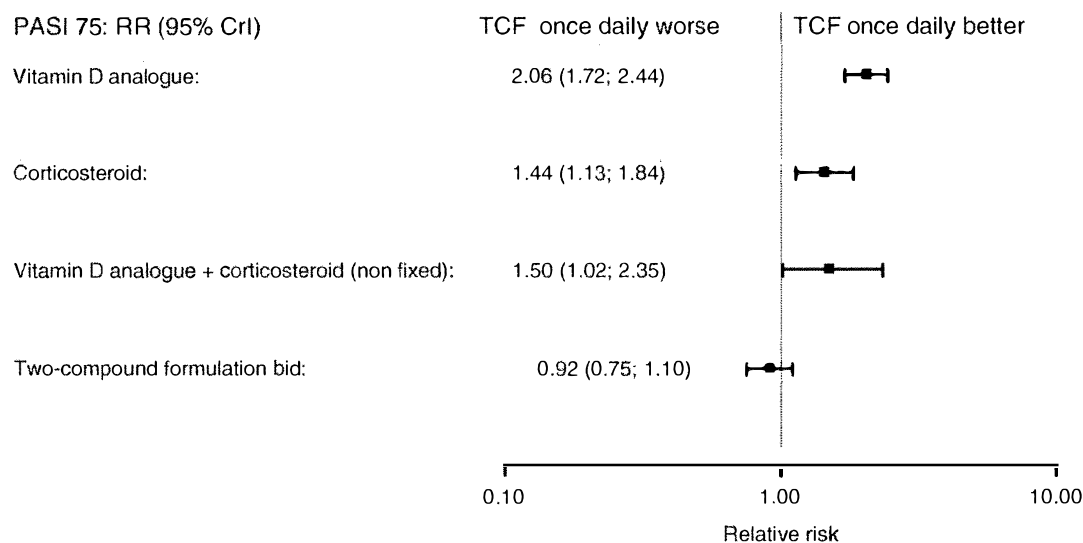
When the 95% bars exclude the 1, the two-compound formulation once daily is better than the comparators (with a > 97.5% probability).

Figure 4. Relative treatment effect for PASI 75 response of the TCF once daily versus comparators. Relative risk and 95% CrI (fixed-effects model).

Percentage change from baseline PASI at week 4

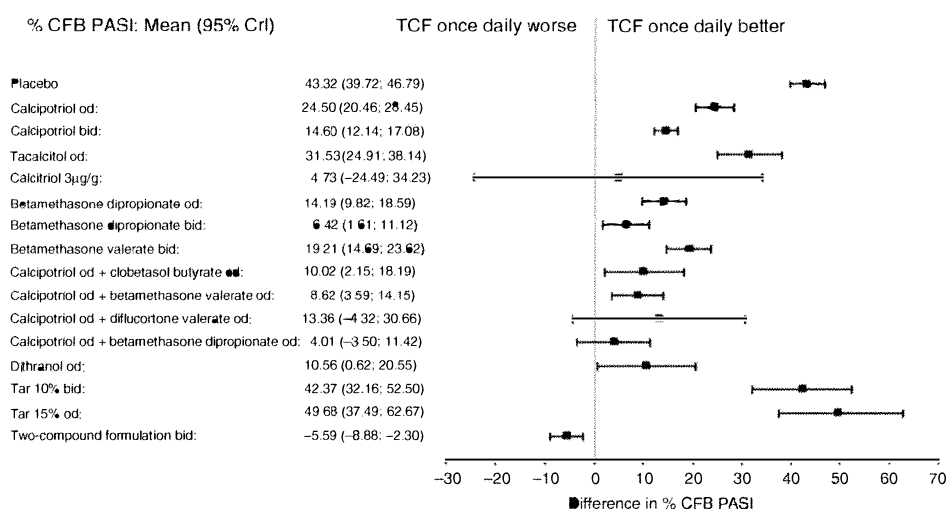
Nineteen studies including data from 9489 patients reported % CFB PASI at week 4. Both the twice-daily application and the once-daily application of the TCF product provided the greatest reduction in PASI. In Figure 6 the difference in % CFB PASI after 4 weeks of treatment with the TCF product once daily relative to each of the other interventions is presented. TCF once daily results in greater PASI reduction than most of the competing interventions. TCF twice daily is more efficacious. Although the relative effect estimates of TCF once daily versus the non-fixed combination of calcipotriol

once daily with betamethasone dipropionate once daily as well the non-fixed combination of calcipotriol once daily with diflucortone valerate imply some ambiguity, there is still an 85% and 93% probability that TCF once daily results in a greater PASI reduction than these two interventions respectively. No difference in PASI improvement was observed between the TCF product and calcitriol 3 µg/g twice daily. The large uncertainty in the relative efficacy estimate is noteworthy, and is the result of the small sample size in the calcitriol study, in combination with the relatively 'long chain' for the comparison of calcitriol with TCF once daily in the network



When the 95% bars exclude the 1, the two-compound formulation once daily is better than the comparators (with a > 97.5% probability).

Figure 5. Relative treatment effect PASI 75 response of TCF once daily versus other drug classes. Relative risk and 95% CrI (random-effects model).



When the 95% bars exclude the zero, the two-compound formulation once daily is better than the comparators (with a > 97.5% probability).

Figure 6. Relative treatment effect % CFB PASI of the TCF once daily versus comparators. Difference in % CFB PASI and 95% CrI (random-effects model).

adding additional uncertainty to this indirect estimate (See Figure 2).

The analysis by drug class showed that the TCF product applied once daily results in a greater PASI reduction after 4 weeks in comparison to all other drug classes except for the twice-daily application of the TCF product (Figure 7).

Comparative analysis for PASI reductions after 2 weeks of treatment showed results similar to those at week 4 (data not shown) suggesting that the TCF product is associated with a fast onset of action.

Discussion

The objective of this study was to assess the efficacy of the once-daily application of the TCF product containing calcipotriol and BDP relative to other commonly used interventions for the treatment of psoriasis vulgaris. Although the MTC also evaluated the efficacy of the TCF product applied twice daily, the relative value of the once daily use, as being the licensed indication, versus other topical treatments was the main interest. An RCT comparing all relevant topical treatments is

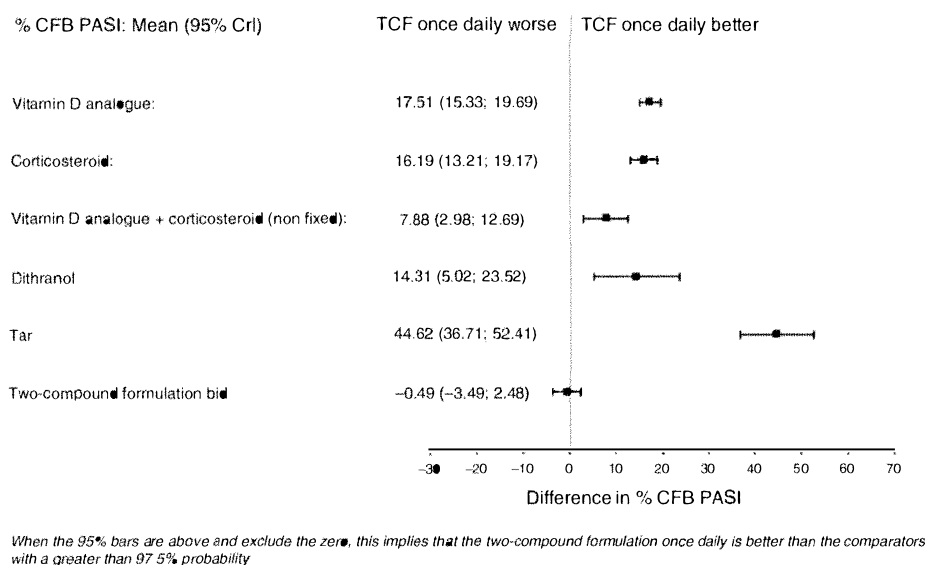


Figure 7. Relative treatment effect % CFB PASI of TCF once-daily versus other drug classes. Difference in % CFB PASI and 95% CrI (random-effects model).

always preferable. However, such a trial does not exist and is not considered feasible. A MTC is then considered a good alternative to get insight in the relative efficacy of a particular treatment versus the alternatives.

The main findings of the MTC were that the TCF product was ranked as the most efficacious treatment relative to all other commonly used topical anti-psoriatic therapies in terms of PASI 75 response and % CFB PASI over 4 weeks of use. The unlicensed twice-daily use of the TCF product was more efficacious than once-daily application. Of particular interest is the finding that in almost all analyses (both by individual treatment as well as by drug class) the TCF product applied once daily is likely to be more efficacious than the non-fixed combination of a vitamin D analogue once daily plus a corticosteroid once daily. For all comparisons, it was observed that administration of the unregistered TCF product twice daily was more efficacious (per treatment analysis) or at least as efficacious (analysis by drug class) as once daily administration. Results after 2 weeks of treatment not only confirm the efficacy of the TCF product but also show the ability of inducing a fast response.

Previously performed meta-analyses on topical treatment of psoriasis are available but are limited with respect to interventions and/or outcomes evaluated and do not involve the TCF product^{47–49}. The current MTC involves both the main topical therapies of interest as well as important outcome measures such as PASI 75 response. It should however be realised that as a consequence of inclusion of publications over a large time span (1976–2007), outcomes reported in the various studies changed over time. PASI 75, for example, nowadays being considered as one of the key outcome measures^{22,23} was not reported in the

earlier trials. As a result, the actual analyses were based on a more limited amount of publications than originally identified.

The current MTC includes a range of interventions from different RCTs. Although RCTs were included, randomisation does not hold across studies. As a result, there is the risk that patients assigned to the different trials were not comparable regarding certain demographics and clinical characteristics that act as effect modifiers of the relative treatment effect. Therefore, only those studies that seemed well matched regarding baseline patient characteristics were included in this MTC (e.g., gender, age, duration of disease, baseline PASI score), although baseline characteristics have not always been consistently reported. It cannot be ignored that inclusion of studies that used different designs (e.g., left-right studies) to measure treatment effects, and the large time-span over which the studies were published (1976–2007), may have impacted the results. For example, data on PASI 75, being the primary success criterion in more recent studies, were only available from the studies with the TCF^{4–8,34} and the study by Kragballe *et al.* 1998⁴⁰.

Despite all efforts to have similar studies and thereby limiting the risk of biased estimates, heterogeneity is still present for most of the outcomes. To capture this, a random-effects approach was performed for all analyses other than PASI 75 per treatment. Although a random-effects approach does not necessarily remove bias in the actual estimates of the treatment effect, it reflects uncertainty in the indirect estimates of the MTC due to variation between studies. Accordingly, with a random-effects approach, the relative treatment effect has a wider credible interval and the interpretation can therefore be considered more conservative.

Apart from efficacy of therapies, both safety and compliance are important outcome measures to consider. The current MTC only focused on efficacy since compliance data were not reported in the RCTs evaluated. Adverse events were reported in the RCTs but an analysis of safety was not undertaken due to differences in reporting across trials (e.g., treatment-related adverse events (as judged by the investigator), adverse events with an incidence of at least 5%, etc.).

The results generated by this MTC analysis are consistent with the findings of two meta-analyses, which demonstrated that the non-fixed combination of a potent topical corticosteroid plus calcipotriol was more efficacious than calcipotriol alone^{47,48}. The current MTC also confirm and extend the results of three randomised controlled studies showing that the TCF product with the fixed-dose calcipotriol plus BDP had superior efficacy to monotherapy with this corticosteroid^{4,6,8}. Accordingly, current psoriasis treatment guidelines indicate the value of the combination of calcipotriol and a corticosteroid in the treatment of mild-to-moderate psoriasis^{20,50}. Until the development of the TCF, patients had to apply each component at separate times of the day to avoid drug-drug incompatibility⁵¹. Zaghoul and Goodfield⁵² have shown that psoriasis patients treated with a once-daily topical were twice as compliant as patients treated with a twice-daily topical. The effect of improved compliance is probably not fully incorporated in the respective clinical trials. The compliance in clinical trials is often higher than what can be seen in real life settings⁵².

Although an MTC has certain inherent limitations, the authors believe that the performed analysis has relevance for clinical decision making in the area of psoriasis. First, evidence of efficacy is identified for all relevant interventions by means of a systematic review of RCTs and the synthesis of evidence is based on current state-of-the-art methods. Second, the endpoints considered encompass the outcomes currently recommended in clinical trials and the analysis allowed for interpreting the estimated effect sizes regarding their clinical relevance. Third, it is unrealistic that a clinical trial including all relevant treatments can be conducted and the MTC can therefore be considered the best available evidence.

Conclusion

The current study demonstrates that the (licensed) once-daily use of the TCF product containing calcipotriol and BDP is an effective treatment for psoriasis and has a greater efficacy in comparison to the other common topical treatments used to treat psoriasis vulgaris.

Transparency

Declaration of funding

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Declaration of financial/other relationships

P.C.M. van de Kerkhof has disclosed he has received consultancy fees from: Schering Plough, Celgene, Centocor, Allmirall, UCB, Wyeth, Pfizer, Soffinova, Abbott, Actelion, Galderma, Novartis, Jansen Cilag, LEO Pharma and carries out clinical trials for: Centocor, Wyeth, Schering Plough, Merck Serono, Abbott, Philips Lighting, Ria de Peuter, and Jeroen Jansen are employees of Mapi Values. Mapi Values received consultancy fees from LEO Pharma A/S related to this work. Jacob Rytto is an employee of LEO Laboratories.

Some peer reviewers receive honoraria from CMRO for their review work. The peer reviewers of this paper have disclosed that they have no relevant financial relationships.

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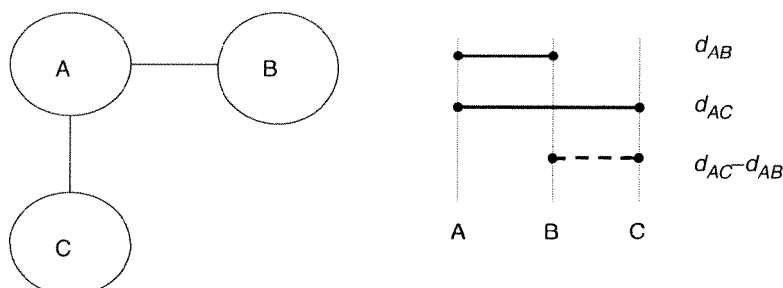
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Appendix

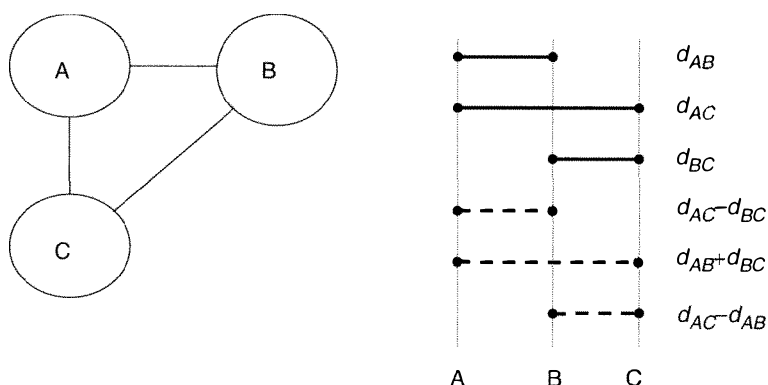
Mixed treatment comparisons

In order to synthesise the results of multiple different RCTs in a network and obtain relative efficacy estimates for all possible pair-wise comparisons, an analysis method needs to be used that preserves randomisation within trials and minimise bias due to lack of randomisation across trials. Given the presence of AB studies and AC studies, an unbiased indirect estimate of C versus B can be obtained by means of an indirect comparison, as illustrated below. If the network of interventions consists of a loop, as illustrated with the second figure, then the analysis method needs to combine estimates of the direct comparisons with estimates of the indirect comparisons and is called a mixed-treatment comparison (MTC). Given a network of A, B, and C comparisons the relative effect estimates of C versus B can be expressed as follows: $d_{BC} = d_{AC} - d_{AB}$ assuming there are no differences in effect modifiers of the relative effect across indirect comparisons. When this expression is generalised to any network with multiple different interventions the following is obtained: $d_{bk} = d_{Ak} - d_{Ab}$ with k the intervention and b the comparator. Depending on the network k can be intervention B, C, D, E, etc. Comparator b can be A, B, C, D, etc, as long as k is alphabetically after b . This expression implies that any estimate for a particular pair-wise comparison can be expressed by the relative efficacy estimates of the intervention and comparator relative to an overall reference treatment A as long as all interventions are connected in one network. d_{AB} , d_{AC} , d_{AD} , ..., d_{Ak} , are called basic parameters of the used regression model that are estimated based on the available studies. d_{BC} , d_{BD} , d_{CD} , etc. are functional parameters and can be calculated based on the (pooled) estimates for the basic parameters from the regression model. For a network involving K treatments and T types of comparisons, there are $K - 1$ basic parameters and $T - K + 1$ functional parameters. To summarise, an MTC meta-analysis model is an extension of a traditional meta-analysis model consisting of not one, but $K - 1$ parameters that need to be estimated with the statistical model to allow for multiple pair-wise comparisons across a range of K interventions.

Indirect treatment comparison



Mixed treatment comparison: 'closed loop' of evidence results in direct and indirect evidence for the comparisons



d_{AC} = relative efficacy of C versus A

- — ● Direct effect between two treatments as available from RCT
- - - - ● Indirect effect that can be obtained from network when all trials are analyzed simultaneously

Annexe 1

N° de formule	A	Abis	B	C	D	E	F	G	Gbis
% en poids de calcipotriol	0,005	0,005	0,005	0,005	0,005	0,005	0,005	0,005	0,005
% en poids de 17-valérate de bétaméthasone	0,05	0,05	0,05	0,05	0,05	0,05	0,05	0,05	0,05
Paraffine liquide	3	3	3	3	3	3	3	3	3
Arlamol E	5	5	2,5	5	2,5				
Isopropyl myristate						5	2,5	5	5
Miglyol 840								10	10
Lanoline				5	5				
Eau		5							5
Vaseline	Qsp 100%	Qsp 100%	Qsp 100%	Qsp 100%	Qsp 100%	Qsp 100%	Qsp 100%	Qsp 100%	Qsp 100%
Score	15	15	12	14	13	9	7	18	18

Qsp = quantité suffisante pour

Score = résultat du test sur modèle de peau

Résultats satisfaisants si score > 10

Instructions aux candidats

Épreuve orale

Le choix du secteur technique est effectué par le candidat au moment de l'inscription (mécanique/électricité ou chimie/pharmacie).

Pour cette épreuve, il est remis au candidat le sujet composé soit d'une note décrivant les éléments du contexte à étudier, soit d'une décision de justice à commenter. Il peut être remis également le texte du brevet en cause, les documents de l'art antérieur (en langue française, anglaise ou allemande) et l'objet suspecté d'être contrefaisant ou une description ou une représentation de celui-ci.

L'épreuve orale consiste en un exposé, suivi d'un entretien avec la commission d'examen, sur l'acquisition et l'exploitation d'un brevet en France, notamment sur les aspects techniques, juridiques et/ou contentieux d'un problème de validité, de propriété et/ou de contrefaçon. Lors de l'entretien, des questions concernant la déontologie professionnelle, l'application des conventions européennes ou internationales et des règlements et directives communautaires ainsi que les droits étrangers prévus au règlement de l'examen pourront être posées. Pour la session 2020 les pays sont : Allemagne et Etats-Unis d'Amérique.

Le candidat dispose de 1h30 pour préparer le sujet qu'il traitera devant le jury pendant environ 30 minutes, sans toutefois que cela excède 45 minutes, questions comprises.

Enfin, à la fin de l'épreuve, le candidat ne devra conserver aucun document écrit ou note personnelle, et devra restituer les documents ou objets qui lui ont été éventuellement remis pour analyse.

SUJET ORAL EQF Brevets 2022 – CHIMIE / PHARMACIE

Article 19 - instructions épreuve orale

Le secteur technique du sujet est celui choisi au moment de l'inscription (mécanique/électricité où chimie/pharmacie).

Pour cette épreuve virgule il est remis au candidat une note décrivant les éléments du contexte à étudier. Il peut être remis également le texte du brevet en cause, les documents de l'art antérieur (en langue française ou anglaise) et l'objet suspecté d'être contrefaisant ou une description ou une représentation de celui-ci.

L'épreuve orale consiste en un exposé, suivi d'un entretien avec la commission d'examen, sur l'axe l'acquisition L'exploitation d'un brevet en France, notamment sur les aspect techniques, juridique et/ou contentieux d'un problème de validité, de propriété et/ou de contrefaçon. lors de l'entretien, des questions concernant la déontologie professionnelle, l'application des conventions européennes où internationales et des règlements et directives communautaires et de l'Union européenne ainsi que les droits étrangers prévus à l'article 9 de ce règlement de l'examen pourront être posées.

Le candidat dispose de 1h30 pour préparer le sujet qu'il traitera devant le jury pendant environ 30 minutes, sans toutefois que cela excède 45 minutes, questions comprises.

Enfin, à la fin de l'épreuve le candidat ne devra conserver aucun document écrit ou note personnelle, et devra restituer les documents ou objets qui lui ont été éventuellement remis pour analyse.

- Lettre du client	pages 2 à 4
- revendications des brevets français et européen POX	page 5
- revendications de la demande de brevet Monkey POX	page 6
- revendications des brevets français et européen Marbourg	page 7

Lettre du client

Bonjour,

Aujourd'hui, le 17 septembre 2022, nous souhaitons, sur recommandation de notre avocate Me Lacand, votre assistance concernant les problèmes en matière de brevets que nous rencontrons actuellement.

Nous développons au sein de notre société, VACCI-CURE, diverses solutions injectables contre les infections anciennes qui resurgissent actuellement. Nous avons, depuis de nombreuses années, développé des candidats vaccins contre les virus de la variole (POX virus), contre la toxine botulique, contre le virus de Marbourg et contre le virus de la poliomyélite. concernant les virus, notre technologie est basée sur des ARN messagers codant des protéines virales, ARN que nous encapsulons dans des vésicules lipidiques à différents pH basiques. Ces vésicules ciblent spécifiquement les cellules du système immunitaire permettant une réponse adaptée et très efficace. les vésicules lipidiques sont développées par la société ANCIENNA, spécialisée dans les lipides modifiés sous forme de nanoparticules.

Historiquement, nous travaillons sur les virus de la famille POX, virus responsables de la variole dite « classique », et les varioles plus récentes dites « variole du singe » (Monkey POX) et « variole du pangolin » (Pango POX). Ces trois virus sont assez similaires, mais ont cependant quelques spécificités. Tous les virus POX expriment la protéine membranaire 6K2, qui est un très bon antigène viral, permettant de développer un vaccin.

Le virus Monkey POX est quant à lui le seul à exprimer une autre protéine membranaire, la protéine M-7K3. Cette protéine découverte fin 2018 est également un très bon antigène viral, et permet de fabriquer un vaccin spécifique de cette forme de variole.

Nous avons déposé le 13 janvier 2003 une demande de brevet français concernant un vaccin anti POX virus, avec ANCIENNA. Le brevet français est délivré depuis 2006 et nous le maintenons en vigueur depuis. Le 13 janvier 2004.

Nous avons déposé une demande de brevet européen, sous priorité de la demande française, pour laquelle nous avons désigné tous les États contractants disponibles sauf l'Italie. Cette demande a donné lieu à la délivrance d'un brevet le 16 janvier 2022. Ce brevet a été validé en Espagne, et nous avons désigné des mandataires locaux en France, Allemagne, au Royaume Uni, en Belgique et en Suisse.

Une autorisation de mise sur le marché (AMM) nous a été accordée le 1^{er} mai 2022 pour le vaccin avec la protéine 6K2 de séquence SEQ ID NO : 3, et nous commercialisons ce vaccin depuis juin 2022.

Le marché du virus contre la variole est important car cette maladie refait son apparition depuis mai 2022, notamment en France. Nous avons appris qu'un concurrent, KILLPOX, allait commercialiser un vaccin en février 2024. Ce vaccin est semble-il le même que le nôtre. Nous sommes certains qu'ils fabriquent depuis 6 mois leur vaccin en vue de l'obtention d'une AMM. Nous voulons les arrêter.

Nous avons également déposé une demande de brevet français le 13 avril 2010 sur un vaccin contre le virus Marbourg, toujours en copropriété avec ANCIENNA. Le brevet français est

délivré depuis fin 2013 et maintenu en vigueur. Sous priorité, nous avons déposé en 2011 une demande de brevet aux États Unis et en Europe. La demande américaine est rejetée au titre de l'article 35 U.S.C. 101, mais nous ne savons pas pourquoi, car les revendications sont les mêmes qu'en Europe.

Pour le brevet européen, nous avons retiré la désignation de l'Espagne et de la Belgique et le brevet délivré le 17 janvier 2015 a été validé en Italie et nous avons désigné des mandataires locaux en Allemagne, au Royaume Uni et en Suisse.

Pour tous nos brevets européens, nous ne maintenons en vigueur les brevets dans les États ayant ratifié les accords de Londres que si nous avons désigné un mandataire local.

Nous avons déposé en 2019 une demande de brevet français spécifique au traitement des maladies liées au virus de la variole du singe, dont la protection est basée sur une posologie particulière : 3 doses séparées par 28 jours chacune. Les tests montrent que si une autre posologie est envisagée, le vaccin n'est efficace que dans 10% des cas, contre 95% selon cette posologie.

ANCIENNA avait d'ailleurs développé une forme galénique particulière de ses lipides permettant une telle posologie. Il s'agit d'une forme de capsule à effet retard, calibrée pour diffuser les antigènes en trois fois.

Cette demande de brevet est en tous points similaire à une demande de brevet belge de 2018, dont la priorité est valablement revendiquée, et pour laquelle le rapport de recherche établi par l'OEB était très positif. Le brevet français n'est pas encore délivré et nous attendons le rapport de recherche préliminaire.

L'INPI nous a demandé il y a un mois de fournir une copie de la recherche antérieure réalisée en Belgique. Nous sommes assez confiants concernant la procédure de délivrance de cette demande française.

Aujourd'hui comme vous le savez, il n'y a pas assez de doses de vaccin contre la variole du singe. Nous avons été approchés par une société, MONKEYKILL qui souhaite développer un vaccin avec notre technologie. Nous avons entamé des négociations de licence, mais la société n'avait pas une bonne santé financière selon nous, de sorte que les négociations ont échoué à notre initiative.

Nous sommes donc aujourd'hui les seuls à exploiter en France un vaccin, soit contre tous les virus POX, soit spécifique au Monkey POX.

Nous avons reçu hier un courrier du ministère de la Santé nous informant que nous devons produire plus de vaccins, accompagné d'une copie du journal officiel de la république française publiant un arrêté ministériel. Notre chef de production est très inquiet de la teneur de ce courrier qui a l'air menaçant, et il ne comprend ce que cela signifie réellement.

Au surplus, nous avons identifié une petite startup basée à Liège en Belgique, VACCINORD, qui produit, en Belgique, et vend depuis son site de Yport en France pour la Somalie (territoire où nous vendons le plus notre vaccin) des doses de vaccin contre le virus Marbourg. Nous sommes convaincus que le vaccin est exactement le même que le nôtre. D'ailleurs, leur chef de projet est un ancien salarié de chez nous.

Nous avons au préalable envoyé une lettre de mise en demeure à VACCINORD, pour l'informer de nos droits sur les vaccins Marbourg, et cette dernière nous a indiqué avoir une licence

exclusive que lui a concédé ANCIENNA sur notre brevet. Ils nous disent donc qu'ils ne peuvent pas être pas contrefacteurs.

Notre directeur juridique est déçu que nous ne puissions rien faire contre VACCINORD.

Tout ceci nous paraît tellement compliqué, et notre avocate qui est spécialisée en droit des affaires, nous a recommandé de vous contacter. Pourriez-vous nous aider à y voir plus clair et nous indiquer ce que nous pouvons faire

- concernant nos brevets POX
 - vis-à-vis de KILLPOX
 - concernant MONKEYKILL
 - avec la lettre du ministère de la santé,
- concernant nos brevets Marbourg, et en particulier vis-à-vis de VACCINORD, et
- plus généralement ce que nous pouvons faire avec ANCIENNA ?

Pour vous aider, nous vous transmettons les revendications de nos brevets ou demandes de brevets.

Bien cordialement,

Louis Marbourg-Pasteur , CEO VACCI-CURE

P.J. : revendications de nos brevet et de notre demande de brevet

REVENDEICATIONS BREVET FR POX

- 1- Composition comprenant un acide nucléique codant la protéine 6K2 de séquence consensus SEQ ID NO : 1, ledit acide nucléique étant encapsulé dans une vésicule lipidique constituée d'acides gras hydrogénés et epoxydés, ladite vésicule ayant un pH basique, ladite vésicule lipidique ayant une taille de 1 à 5 nm.
 - 2- Composition selon la revendication 1, où l'acide nucléique est un acide ribonucléique ou ARN.
 - 3- Composition selon la revendication 1 ou 2, où l'acide nucléique code les protéines de séquence SEQ ID NO : 2 à 7.
 - 4- Composition selon l'une quelconque des revendications 1 à 3, où l'acide nucléique consiste en l'une des séquences SEQ ID NO : 8 à 13.
 - 5- Composition selon l'une quelconque des revendications 1 à 4, en tant que médicament.
 - 6- Composition selon l'une quelconque des revendications 1 à 4, pour son utilisation pour le traitement de la variole induite par les virus POX.
-

REVENDEICATIONS BREVET EP POX

- 1- Composition comprenant un acide nucléique codant la protéine 6K2 de séquence consensus SEQ ID NO : 1, ledit acide nucléique étant encapsulé dans une vésicule lipidique constituée d'acides gras hydrogénés et epoxydés, ladite vésicule ayant un pH basique, ladite vésicule lipidique ayant une taille de 1 à 5 nm, où l'acide nucléique code les protéines de séquence SEQ ID NO : 2 à 7.
- 2- Composition selon la revendication 1, où l'acide nucléique est un acide ribonucléique ou ARN.
- 3- Composition selon la revendication 1 ou 2, où l'acide nucléique consiste en l'une des séquences SEQ ID NO : 8 à 13.
- 4- Composition selon l'une quelconque des revendications 1 à 3, en tant que médicament.
- 5- Composition selon l'une quelconque des revendications 1 à 4, pour son utilisation pour le traitement de la variole induite par les virus POX, sous réserve que ledit virus POX ne soit pas le virus responsable de la variole du singe.

REVENDEICATIONS DEMANDE FR MONKEY POX

- 1- Composition comprenant une vésicule lipidique constituée d'acides gras hydrogénés et epoxydés, ladite vésicule ayant un pH basique, ladite vésicule lipidique ayant une taille de 1 à 5 nm, ladite vésicule comprenant un acide nucléique codant la protéine M-7K3 du virus de la variole du singe, pour son utilisation pour le traitement de la variole du singe, ladite composition étant administrée sous la forme de 3 doses séparées de 28 jours.
- 2- Composition pour son utilisation selon la revendication 1, où ledit acide nucléique est un acide ribonucléique.
- 3- Composition pour son utilisation selon la revendication 1 ou 2, en association avec un agent antiviral.

REVENDEICATION BREVET FR MARBOURG

- 1- Composition comprenant un acide nucléique codant la protéine VP24 de séquence SEQ ID NO : 1, ledit acide nucléique étant encapsulé dans une vésicule lipidique constituée d'acides gras monoinsaturés, hydrogénés et époxydés, ladite vésicule ayant un pH basique, ladite vésicule lipidique ayant une taille de 7 à 15 nm.
 - 2- Composition selon la revendication 1, où l'acide nucléique est un acide ribonucléique ou ARN.
 - 3- Composition selon la revendication 1 ou 2, où l'acide nucléique consiste en l'une des séquences SEQ ID NO : 8 à 13.
 - 4- Composition selon l'une quelconque des revendications 1 à 3, en tant que médicament.
 - 5- Composition selon l'une quelconque des revendications 1 à 3, pour son utilisation pour le traitement des fièvres hémorragiques à virus de Marbourg.
-

REVENDEICATION BREVET EP MARBOURG

- 1- Composition comprenant un acide nucléique codant la protéine VP24 de séquence SEQ ID NO : 1, ledit acide nucléique étant encapsulé dans une vésicule lipidique constituée d'acides gras monoinsaturés, hydrogénés et époxydés, ladite vésicule ayant un pH basique, ladite vésicule lipidique ayant une taille de 7 à 15 nm.
- 2- Composition selon la revendication 1, où l'acide nucléique est un acide ribonucléique ou ARN.
- 3- Composition selon la revendication 1 ou 2, où l'acide nucléique consiste en l'une des séquences SEQ ID NO : 8 à 13.
- 4- Composition selon l'une quelconque des revendications 1 à 3, en tant que médicament.
- 5- Composition selon l'une quelconque des revendications 1 à 3, pour son utilisation pour le traitement des fièvres hémorragiques à virus de Marbourg.



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