Rétinoïdes et caroténoïdes : métabolisme dans la peau humaine après application topique et rôle dans la photoprotection

Antille, Christophe

Université de Genève

Faculté de Médecine
Section de médecine clinique
Département des Neurosciences Cliniques et Dermatologie
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Monsieur Christophe ANTILLE

originaire de Chalais (VS)

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RETINOIDES ET CAROTENOIDES : METABOLISME DANS LA PEAU HUMAINE APRES APPLICATION TOPIQUE ET ROLE DANS LA PHOTOPROTECTION

La Faculté de médecine, sur le préavis de Monsieur Jean-Hilaire SAURAT, professeur ordinaire au Département des Neurosciences Cliniques et Dermatologie, autorise l'impression de la présente thèse, sans prétendre par là émettre d'opinion sur les propositions qui y sont énoncées.

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A. Résumé

L'épiderme humain contient des rétinoides endogènes et des caroténoïdes. Nous présentons une méthode d'évaluation de la pénétration et du métabolisme des rétinoïdes (acide rétinoïque, rétinaldéhyde, rétinol, palmitate-de-rétinyle) dans la peau humaine *ex-vivo* à partir de peau totale incubée dans des cellules de Franz. Les résultats montrent qu'il n'y a pas de métabolisme pour l'acide rétinoïque, forme active de la vitamine A qui lie les récepteurs nucléaires, alors qu'on observe un métabolisme d'oxydation et de réduction pour le rétinaldehyde, ainsi qu'une augmentation des rétinoïdes endogènes pour le rétinol et ses esters. Nous mettons en évidence pour la première fois que le β-carotène est un précurseur de la vitamine A dans l'épiderme humain. L'exposition de la peau aux UV diminue son contenu en vitamine A. Nous montrons un effet filtre de la vitamine A (palmitate-de-rétinyle) *in vivo* chez l'homme qui contribue potentiellement à la protection de l'ADN contre les effets des UVB.

B. Introduction

Les rétinoïdes

La peau contient des quantités significatives de rétinoïdes (environ 1 nmol/g), ainsi que les enzymes impliquées dans leur métabolisme [1] [2-5]. Les rétinoïdes sont des dérivés naturels ou synthétiques de la vitamine A. Ces composés exercent des effets biologiques, via l'activation de récepteurs nucléaires (RAR et RXR).

On les classe en 2 groupes :

- Les rétinoïdes naturels, dont fait partie le rétinol (ROL, vitamine A) et tous ses dérivés métaboliques : le rétinaldéhyde (RAL), l'acide tout trans-rétinoïque (RA, trétinoïne), l'acide 9-cis rétinoique (alitrétinoïne), l'acide 13-cis rétinoïque (13-cis RA, isotrétinoïne), ainsi que ses esters (RE) tels que le palmitate de rétinyle (ROL-Palm).

- Les rétinoïdes de synthèse : ce sont des molécules dont une portion a été modifiée, dans le but d'avoir des composés ayant un seul effet relatif à la vitamine A. Les principales molécules sont l'étrétinate (Ro 10-9359), l'acitrétine (Ro 10-1670) [6], le tazarotène, l'adapalène, le bexarotène, le motrétinide.

Les rétinoïdes participent à un grand nombre de processus biologiques tels que l'embryogenèse, la reproduction, la vision, la croissance, l'inflammation, la différenciation, la prolifération et l'apoptose [7]. Ils agissent en activant des récepteurs cellulaires : les récepteurs de l'acide rétinoïque (RAR) et les récepteurs des rétinoïdes X (RXR). Il existe trois isoformes de chaque récepteur (α, β, γ). L'activation du récepteur par le ligand déclenche la fixation du complexe [récepteur-ligand] à des sites spécifiques de l'ADN, les *retinoic acid response elements* (RARE) et les *retinoid X response elements* (RXRE). Les RXR peuvent former des hétérodimères avec d'autres récepteurs nucléaires incluant les récepteurs de la vitamine D, des hormones thyroïdiennes et les *peroxisome*

proliferator activated receptors (PPARs) [8]. L'épiderme humain contient deux formes principales de vitamine A (rétinol et esters rétinyliques) ainsi que des caroténoïdes (bêta-carotène) [9, 10]. La vitamine A est stockée dans les kératinocytes par l'estérification du rétinol en esters rétinyliques. Cette étape est catalysée par deux enzymes ; l'acyl CoA acyltransférase (ARAT) et la lécitine:rétinol acyltransférase (LRAT). Leur expression est modulée par les radiations UV et l'état de différenciation des kératinocytes [11, 12]. L'hydrolyse des esters rétinyliques en rétinol est catalysée par la rétinyl ester hydrolase. D'un autre coté le rétinol est une pro-hormone de l'acide rétinoïque. Il est oxydé en rétinal (rétinol deshydrogénase) puis le rétinal est oxydé en acide rétinoïque (rétinal deshydrogénase) [1], forme biologiquement active de la vitamine A [7].

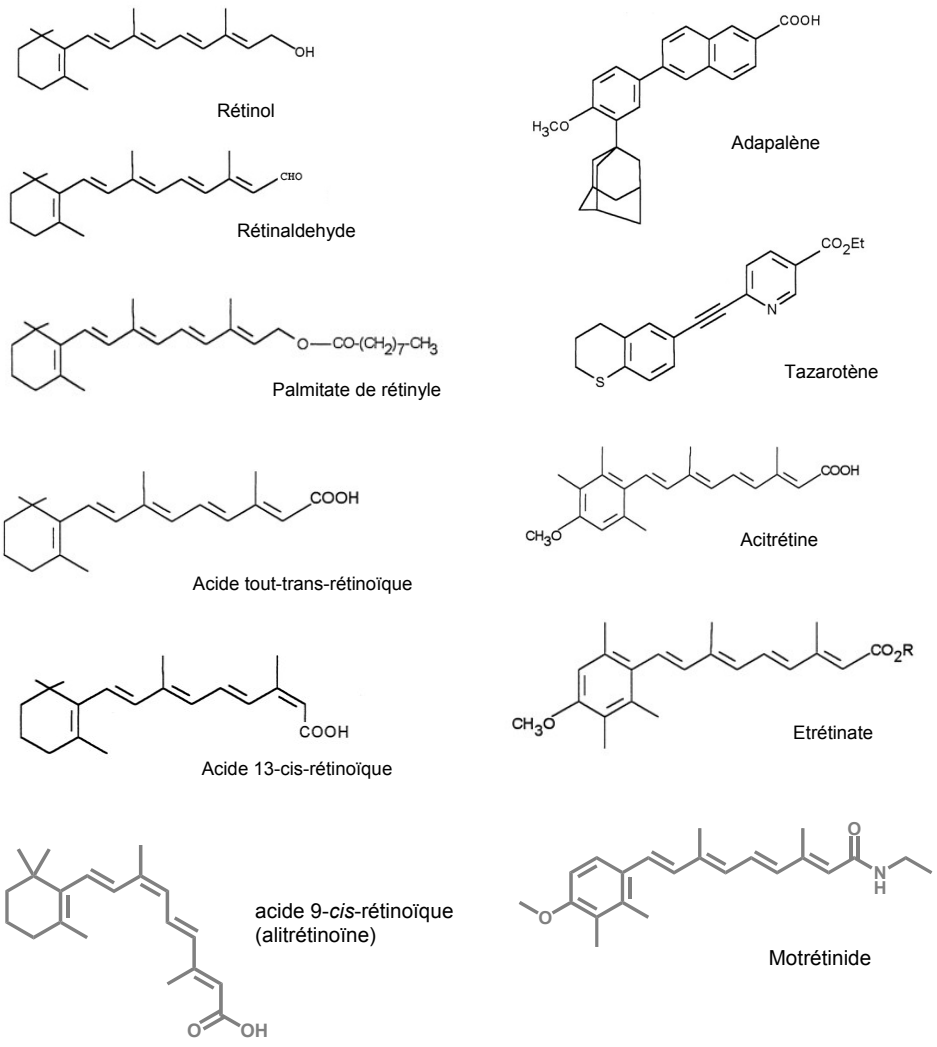

Figure 2 : Rétinoïdes naturels et rétinoïdes synthétiques

Les RE sont considérés comme la forme de stockage de la vitamine A, étant donné : i) que les concentrations dans l'épiderme sont plus élevées que dans le derme ou encore dans le sang, et ii) qu'ils sont les précurseurs des autres formes actives de vitamine A [13, 14].

La nature chimique des rétinoïdes, comprenant une chaîne lipidique polyinsaturée (figure 2), de même que leur propriétés physiques leur conférant un maximum d'absorption compris entre 320 et 390 nm, les rend capables

d'interagir avec les UV, ou avec l'oxygène pour produire des formes réactives d'oxygène ou des radicaux libres [15, 16]. Ils peuvent être isomérisés par les UV, l'isomérisation du 11-*cis*-RAL en tout-*trans* déclenche des réactions nécessaires au mécanisme de la vision [17]. En solution, le tout-*trans*-RA s'isomérise en 9-*cis*, 11-*cis*, 13-*cis* et 9,13-di-*cis*, cette réaction pouvant être d'autant plus importante que les concentrations de RA au départ sont faibles [18]. Cela serait dû à une grande flexibilité moléculaire, plus importante que celle des caroténoïdes par exemple [19]. *In vitro* et *in vivo*, Berne et al ont montré que les rétinoïdes tels que le 13-*cis*-RA et des analogues synthétiques, étrétinate et acitrétine, s'isomérisaient aussi sous l'action des UV [20]. Mais dans le plus grand nombre de cas, les rétinoïdes sont purement et simplement détruits par l'action des UV [21] en solution et *in vivo* [11, 22]. Il semblerait que les RE soit plus sensibles aux UV que le ROL [23]. L'épiderme exposé au soleil contient moins de RE que la peau non exposée, indiquant que les UV induisent une déficience en vitamine A [22]. Ceci, ainsi que d'autres observations, supportent l'hypothèse selon laquelle la déplétion en vitamine A, induite par le soleil, est impliquée dans la pathogenèse de certains cancers de la peau ainsi que de son vieillissement prématuré [24-28]. Cette déplétion pourrait être compensée par l'apport externe de vitamine A.

L'application topique de rétinoïdes est connue pour apporter de la vitamine A à la peau [29-31]. Le ROL topique exerce des effets biologiques similaires (mais moins intenses) à ceux du RA topique, avec cependant moins d'irritation : ces réponses sont médiées par la conversion de ROL en RA, la forme biologiquement active de la vitamine A [32]. Le ROL, ainsi que ses esters, doivent impérativement être convertis en RA pour induire des effets biologiques dans les kératinocytes humains [3, 33] (figure 1). Voorhees & coll. [32, 34] suggèrent que le ROL topique soit une voie plus efficace pour induire des effets rétinoïdes dans la peau que le RA lui-même, étant donné que le ROL induit des changements comparables à ceux provoqués par le RA, mais avec moins d'irritation. De la même manière, le RAL est converti à la fois en forme de stockage (RE) et en forme active (RA) [35], exerce des activités biologiques

significatives [30, 31, 36] et est mieux toléré que le RA [37]. Il est aussi un bon candidat pour l'apport de vitamine A dans l'épiderme [37-39].

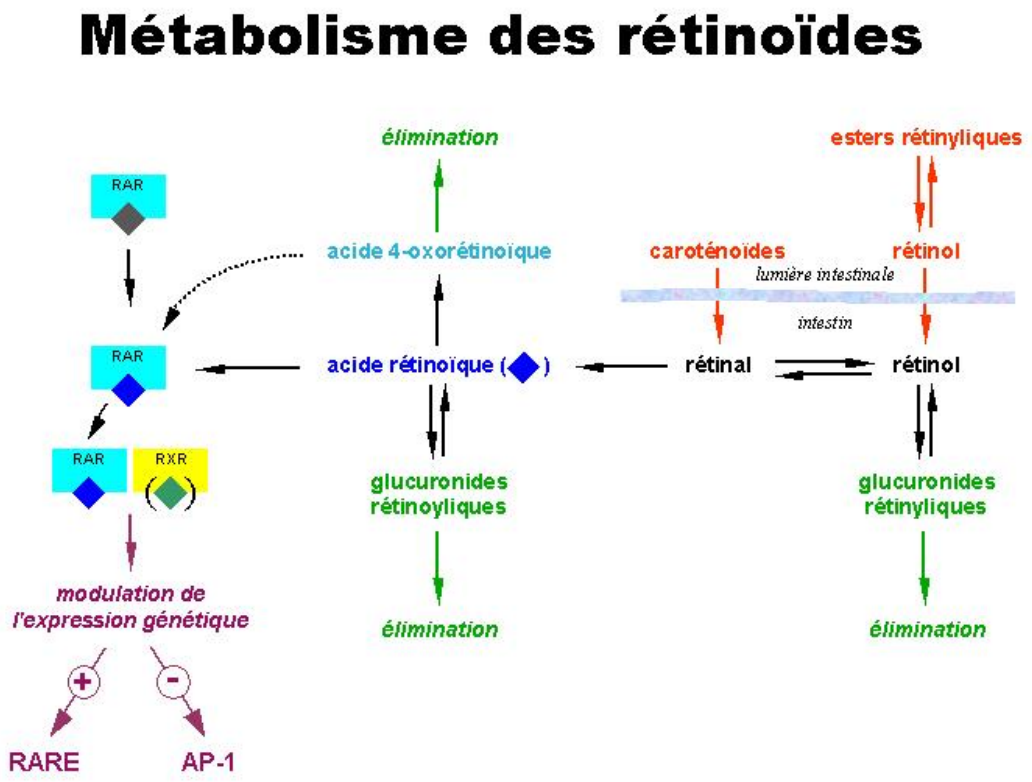

Figure 3. Métabolisme de la vitamine A.

L'application des rétinoïdes a lieu selon deux stratégies en dermatologie:

i) à des doses pharmacologiques dans le cas de traitement de pathologies cutanées : l'application de trétinoïne (RA) (gels ou crèmes de 0,025% à 0,1%) a pour indication principale le traitement de l'acné. Elle s'utilise aussi dans le traitement du photovieillissement (héliodermie) et des kératoses actiniques. L'alitrétinoïne topique est utilisée pour le traitement du sarcome de Kaposi [40]. L'application d'isotrétinoïne (crèmes à 0,05%) se fait dans le cas d'acné nodulo-kystique, acné rosacée, et dans certains cas dans la prévention de cancers cutanés (kératoses actiniques carcinomes spinocellulaires et

basocellulaires). Le tazarotène est indiqué dans le traitement du psoriasis (gels à 0,05% 0,1%) et l'adapalène (gels 0,1%) dans celui de l'acné. Le motrétinide (crème ou lotion à 0,1 %) est quant à lui indiqué dans le traitement de l'acné légère [7] [41].

ii) à des fins cosmétiques : le ROL-Palm (gels de 0,5% à 5%), le ROL (0,1 % à 0,6 %) et le RAL (0,05 %) sont utilisés pour les traitements anti-âge et soins réparateurs.

Pour les rétinoïdes naturels, les études sur les lapins n'indiquent pas d'augmentation de la concentration sanguine de RA après application topique de RA. Il semble en effet que la peau stocke ce dernier et ainsi régule sa distribution dans le système sanguin [42] . Il n'y aurait donc pas d'effets tératogènes observables.

Chez l'homme, l'application de RA et de son isomère le 13-*cis*-RA n'affectent pas les concentrations sanguines. Et ce que quelle que soit la concentration de la crème initiale (0.01% à 0.05%) et quelle que soit la durée du traitement (dose unique ou 28 jours). Les quantités retrouvées dans le sang sont de l'ordre de 2 % de la quantité initiale (soit ≈ 10 nM), ce qui n'est pas plus élevé que les valeurs endogènes. Il y a peu d'absorption dans le sang, ce qui peut laisser présager un effet tératogène limité [43, 44] [45]. Ces résultats sont corroborés par Willhite *et al.* chez le hamster [46]. Barua *et al.* remarquent que l'application de RA et de rétinoyl glucuronide (RAG) radiomarqués chez le rat *in vivo* donne lieu à une rapide absorption de ces composés par la peau [47]. Ils se retrouvent rapidement dans le sang, avec un maximum à 2 heures pour le RA et 4 heures pour le RAG. Mais on y retrouve moins de 1% de la quantité appliquée. Dans le foie, ces molécules ne représentent que 1,2 % de la quantité initiale après 6 heures pour le RA et 12 heures pour le RAG, et environ 1,5% et 3% des quantités initiales respectives se retrouvent dans les excréments après 24 heures. Toutefois, il semblerait que l'absorption soit plus rapide que chez l'homme [47]. Bronaugh compare l'absorption du ROL-Palm marqué chez le cobaye et chez l'homme, 24 heures après application. Il montre qu'elle est plus importante chez le cobaye

(33,4 % de la quantité appliquée contre 17,9 % chez l'homme). Il remarque de plus que 30,2 % du ROL-Palm absorbé est transformé en ROL chez le cobaye et 43,9 % chez l'homme. Dans le perfusat, il ne retrouve respectivement que 0,6 % et 0,2 % de ROL, ramené à la quantité initiale de ROL-Palm. Il ne trouve nulle part des traces de RA. Il en déduit que les effets sur la structure de la peau dus à l'application de ROL-Palm sont liés à la formation du ROL pendant l'absorption percutanée [48]. Duell *et al.* ont comparé les effets biologiques obtenus chez l'homme après application de ROL, RAL et ROL-Palm, par rapport à l'application de RA, et suggèrent que le ROL puisse remplacer le RA, car pour de mêmes effets biologiques induits (quoique moins intenses), le ROL ne provoque pas d'irritation [49].

Les analogues de synthèse sont des molécules de structure modifiée mais qui ont les mêmes effets biologiques bien qu'ils soient supposés avoir moins d'effets secondaires (irritation, photosensibilité) [50].

La distribution chez l'homme de trétinoïne (RA) après application topique est de 97 % en surface, 1,8 % dans l'épiderme et 0,67 % dans le derme, après 24 heures. Lehman remarque la formation d'isotrétinoïne 24 heures après l'application de trétinoïne. Les quantités d'isotrétinoïne détectées dans les mêmes conditions, mais après exposition à la lumière, sont égales aux quantités de trétinoïne encore présentes [51].

En fait l'absorption, sur de la peau humaine ou de singe, de ces molécules mais aussi d'acitrétine et d'étrétinate dépend de l'excipient employé. Et pour un même excipient chacune de ces molécules a une absorption différente. Le RA est celui qui a la meilleure absorption dans le cas d'un excipient à base d'isopropanol [52].

Chez l'homme, lorsque la peau a été prétraitée 24 heures auparavant avec du sulfate de lauryle et de sodium (SLS), elle laisse passer plus de RA : au bout de 4 heures, on y retrouve 0,37 % de la quantité appliquée contre 0,22 % chez les contrôles [53].

Par contre chez les cobayes hairless, il ne semble pas qu'il y ait plus d'acitrétine, après application topique dans la peau avec ou sans traitement préalable au SLS [54]. L'absorption percutanée n'est pas affectée non plus [54], indiquant que la

pénétration de ces molécules n'est pas influencée par l'irritation induite par le SLS.

Depuis les premiers travaux sur la présence de vitamine A cutanée dans les années 1970 [55] très peu d'études ont concerné la pénétration trans-cutanée des rétinoïdes topiques et leur métabolisme par la peau humaine [9, 10, 49, 56, 57]. On ne sait donc pas grand-chose sur ce qu'il advient des rétinoïdes que des millions de personnes s'appliquent régulièrement sur la peau mis à part leur inaptitude à augmenter de manière significative les rétinoïdes circulants [58-60]. Dans ce travail nous avons étudié le métabolisme des différents rétinoïdes naturels après leur application topique sur la peau humaine *ex-vivo*, ainsi que sur la peau de souris *in vivo* et *ex-vivo* [61] (§ C.1).

Les Caroténoïdes

Les caroténoïdes sont des composés polyisoprénoïdes synthétisés par les végétaux. Une partie d'entre eux sont des précurseurs de la vitamine A. En raison de leurs nombreuses double liaisons conjuguées, ils absorbent fortement la lumière visible et agissent comme antioxydants [62] en désactivant des molécules d'oxygène activées par des substances photosensibilisantes et en réduisant des radicaux libres produits lors d'un stress oxydant. Il est possible que l'effet photoprotecteur résulte de ces propriétés. Ils n'ont pas d'effet écran et très peu d'effet filtre pour les UV et ne diminuent donc pas ou modestement [63] le seuil érythémal aux UVB, aux UVA ou à la PUVA (= Psoralènes + exposition aux UVA) et ne préviennent pas les lésions de l'ADN [64].

La peau humaine contient des caroténoides dont la forme principale est le bêtacarotène; celui-ci se concentre principalement dans l'épiderme et l'hypoderme [10], le lycopène se concentre principalement dans le plasma, la peau et le tissus adipeux. Les caroténoïdes ne sont pas synthétisés par l'organisme. Leur unique source est alimentaire (fruits et légumes), leur biodisponibilité est supérieure dans les formes galéniques. Le clivage

enzymatique du bêtacarotène par la bêtacarotène 15,15'-dioxygénase donne dans la muqueuse intestinale deux molécules de rétinaldéhyde qui peuvent être transformées en rétinol (vitamine A) puis stockées en esters de rétinol. La régulation de ce métabolisme (une grande partie de bêtacarotène ingéré n'est pas métabolisé en vitamine A) explique que la prise orale de doses importantes de bêtacarotène n'induise pas d'hypervitaminose A. On a longtemps pensé que le clivage du bêtacarotène se faisait seulement dans les entérocytes. Des expériences récentes montrent que des kératinocytes humains en cultures transforment le bêtacarotene en rétinol [65]. Nous avons aussi observé ce phénomène au niveau tissulaire dans l'épiderme humain en démontrant un métabolise du bêtacarotène en esters de rétinol qui sont la forme de stockage de la vitamine A.Dans ce travail nous démontrons ce processus *in vivo* chez la souris et *ex-vivo* chez l'homme : l'épiderme humain métabolise le bêta-carotène en esters de rétinol qui sont la forme de stockage de la vitamine A. Le bêta-carotène (en utilisation topique) est donc un précurseur de la vitamine A dans la peau humaine [66] (§ C.2 pages 25-27). La *canthaxanthine* ne peut pas se transformer en vitamine A.

L'administration orale de bêtacarotène à haute dose est un outil thérapeutique efficace dans la protoporphyrie érythropoïétique. Par analogie on a pensé que le bêtacarotène pouvait avoir un effet photoprotecteur sur la peau humaine. En pratique il est modérément ou peu actif dans les autres photodermatoses. La concentration de bêtacarotène est diminuée dans les kératoses actiniques et les carcinomes basocellulaires comparativement à la peau saine [67]. Par contre la supplémentation en caroténoides dans les programmes de chimioprophylaxie des cancers cutanés, de prévention/traitement de la sénescence, voire de traitement des précancéroses n'a pas apporté de résultats probants [68]. Certains utilisent les caroténoïdes pour leur effet colorant de la peau dans un but de camouflage des dépigmentations (vitiligo), ou seulement cosmétique. En conclusion, les observations actuelles ne permettent pas de recommander la

supplémentation orale en bêtacarotène du point de vue de la photoprotection ou de la chimioprophylaxie des cancers cutanés.

Peau, rétinoïdes et rayonnement UV

Les rayonnements solaires incluent la lumière visible, les ultraviolets (UV), les infrarouges et d'autres radiations électromagnétiques. Ces émissions sont caractérisées par leur longueur d'onde exprimée en nanomètres (1 nm=10^{-9} m). De même que la lumière visible peut être divisée en plusieurs couleurs, les UV peuvent être classifiés en 3 subdivisions :

UVA (315-400nm) représentent la part la plus importante d'UV qui atteint la surface de la terre. Ils provoquent sur la peau le bronzage et peuvent l'endommager en profondeur à cause de leur facilité à pénétrer les tissus.

UVB (280-315 nm) représentent une plus petite proportion de rayons atteignant le sol, mais ont une contribution importante aux effets biologiques consécutifs aux expositions solaires. Ils peuvent provoquer rougeurs, brûlures, bronzage, lésions oculaires. Leur pénétration dans la peau est plus faible.

UVC (200-280 nm) sont pratiquement entièrement absorbés par la couche d'ozone de l'atmosphère. Ils ne pénètrent que peu dans la peau mais peuvent provoquer des lésions oculaires.

Les principaux effets des UV sur la peau sont : l'érythème, le bronzage, le vieillissement (photosénescence), le cancer de la peau. Chez l'homme, l'épiderme, dont l'épaisseur varie entre 100 et 150 µm, forme une barrière optique par absorption des radiations, et très peu par dispersion des rayonnements [69]On considère que 4 % à 7 % d'un rayonnement incident est réfléchi par l'épiderme sur l'ensemble du spectre de 250 nm à 3000 nm [69]. .

Les principaux chromophores responsables de ces réponses biologiques sont : les acides aminés, l'ADN et l'acide urocanique, dans le domaine des UVC , mais qui ont aussi une légère sensibilité aux UVB, et les protéines et la mélanine dans

les UVB . L'épiderme ne possède pratiquement pas de molécules absorbant dans les UVA [70], à part la mélanine [69]Cette absorption peut être augmentée par hyperplasie et/ou par mélanogénèse. Le derme, épais de 2 à 4 mm, peut être assimilé à une matrice de tissus turbide, qui disperse les radiations, cette dispersion optique étant inversement proportionnelle à la longueur d'onde [69] (figure 1).

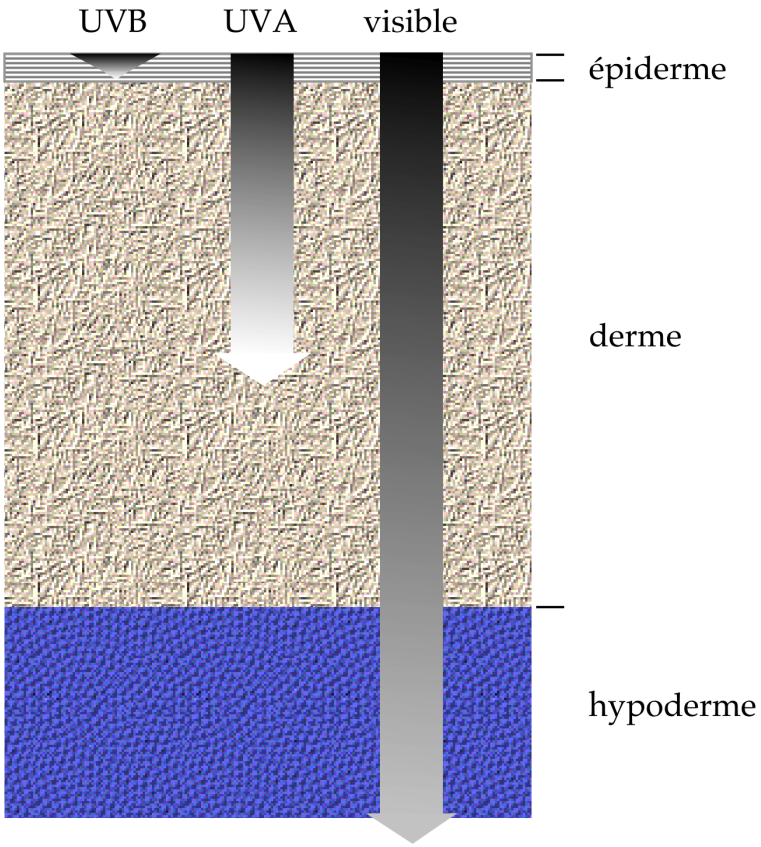

Figure 1 . Pénétration dans la peau humaine des différentes composantes de la lumière visible et ultraviolette.

Après exposition aux UV, Fisher et al. montrent qu'il y a augmentation de l'expression des metalloprotéinases, dans l'épiderme et le derme. Ces enzymes qui altèrent la production de collagène dans le derme contribuent à la photosénescence (apparition de rides). L'induction de ces enzymes est maintenue par des expositions à répétition [71]. L'épaississement du derme est dû à l'accumulation de matériel élastique. La production d'interleukines par l'épiderme, suite à une irradiation UV, contribue à la destruction du tissu

conjonctif. Il semblerait que les effets observés dans le derme soient attribuables aux UVA, qui pénètrent jusque-là [72], contrairement aux UVB.
Chez la souris hairless, comme chez l'homme, on observe l'apparition d'hyperplasie et de rides après irradiation à long terme par les UVB, et la peau devient flasque sous l'action des UVA. Les effets observés dépendent de la longueur d'onde des irradiations. Le derme s'épaissit, après irradiation avec des longueurs d'ondes comprises entre 295 et 300 nm, les fibres de collagène sont endommagées, et il y a production de fibres élastiques anormales (élastose) [73]. On observe aussi des lésions des microvaisseaux et une augmentation de la concentration en glycosaminoglycanes. Ces derniers, contrairement à la peau saine, vont se trouver dans la partie supérieure du derme et non entre les fibres élastiques [74-77]. Il y a aussi doublement de la membrane basale avec augmentation du nombre de cellules inflammatoires, du nombre de fibroblastes et du nombre de fibres élastiques. Après 10 semaines d'irradiations avec des UVB, il n'y a plus de fibres élastiques normales [78].

De nombreuses études ont permis d'établir un lien entre exposition solaire chronique et prédisposition à développer des cancers de la peau [25, 79-81]. Le développement de cancer est dû à des changements génétiques causés par une réplication altérée d'ADN. Ces altérations peuvent être le résultat direct (formation de cyclobutane pyrimidine dimères) ou indirect (formation de 8-oxodésoxyguanosine) des UV [27, 82]. De plus, les UV modifient certains gènes tels que le p53 responsable de l'élimination ou de la réparation des lésions au niveau de l'ADN [83].
Les UVB et les UVA n'ont pas le même effet sur l'immunité cellulaire chez l'homme. Les UVA sont moins carcinogènes [84]. De Gruijl et al. montrent que la carcinogenèse dépend de la longueur d'onde des radiations. L'efficacité est maximale à 293 nm, et au-dessus de 340 nm, elle est dix mille fois moindre [85]. Les UVB jouent donc un rôle dominant dans l'effet carcinogène de l'exposition solaire, les UVA représentent seulement 10% à 20% de ces effets [86]. Les UVA induisent l'apparition de formes réactives d'oxygène responsables de

l'immunosuppression [87]. Les UVB jouent un rôle majeur dans l'altération de l'ADN. Les UVA n'ont qu'un impact mineur sur la mutation de p53 [88]. Cette mutation est un point important dans le développement des tumeurs, elle en augmente significativement le nombre [89]. En outre, l'épaisseur du *stratum corneum* (SC) n'est pas un facteur intervenant dans la sensibilité constitutive aux UV, ce serait plutôt la pigmentation [90]. La mélanine intra-mélanocytaire peut être comparée à une épée à deux tranchants : l'absorption des UV dans les mélanocytes génère des produits qui augmentent la carcinogenèse tandis que la mélanine extra-mélanocytaire a plutôt un rôle protecteur. Les UVA absorbés par la mélanine des mélanocytes seraient donc responsables des cancers cutanés [91]. En fait les UVA sont responsables de l'initiation du mélanome, mais les UVB sont aussi responsables de son développement jusqu'à la métastase [92].

La principale indication du rétinal et de l'acide rétinoïque est le traitement de l'héliodermie [93-95]; les résultats d'études récentes et anciennes sont contradictoires concernant le rôle des rétinoïdes dans la photocarcinogenèse cutanée. On sait que l'exposition de la peau aux UV diminue son contenu en vitamine A (rétinol et rétinyl esters) [22, 96]. Le traitement topique par les rétinoïdes contrecarre cette déplétion induite par les UV [5, 97]. Des observations sur les souris hairless ont confirmé que la déplétion cutanée en vitamine A est impliquée dans la photocarcinogenèse [5, 98]. Les expériences sur les souris hairless décrivent une absence d'effet [99], une augmentation [100, 101] ou une inhibition [102, 103] de la photocarcinogenèse cutanée par l'acide rétinoïque. Des expériences *in vitro* ont montré que le rétinol et le rétinal pouvaient être les médiateurs de dommages à l'ADN via le stress oxydant [104].

Les rétinoïdes absorbent fortement le rayonnement UV (par exemple ε_{325} = 50'000 $[(mol/l)^{-1}\ cm^{-1}]$ pour le rétinol) : on peut ainsi envisager qu'une application topique de rétinoïdes diminue la dose de rayonnement UV reçue par la peau. Ce concept a été mis en évidence dans ce travail lors d'une étude chez l'homme sur la photoprotection réalisée par le palmitate de rétinyle topique [105] (§ C.3), puis confirmée dans une étude ultérieure [106].

C. Publications

1. **Les rétinoïdes : étude du métabolisme de la vitamine A dans la peau humaine après application topique de rétinoïdes naturels et de bêta-carotène. Les modèles humain et murin.**

C. Antille, C. Tran, O. Sorg & J.H. Saurat (2004), Penetration and metabolism of topical retinoids in ex vivo organ cultured full-thickness human skin explants Skin Pharmacol. Appl. Physiol. 17 124-128.

2. **Les caroténoïdes : étude du métabolisme des caroténoïdes dans la peau humaine après application topique de bêta-carotène. Les modèles humain et murin.**

C. Antille, C. Tran, O. Sorg & J.H. Saurat (2004), Topical β-carotene is converted to retinyl esters in human skin ex vivo and mouse skin in vivo Exp. Dermatol. 13 558-561.

3. **La vitamine A topique exerce un effet filtre aux UVB dans la peau de souris et la peau humaine.**

C. Antille, C. Tran, O. Sorg,, P. Carraux, L. Didierjean, & J.H. Saurat (2003), Vitamin A exerts a photoprotective action in skin by absorbing UVB radiations J. Invest. Dermatol.121 1163-1167.

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Penetration and Metabolism of Topical Retinoids in ex vivo Organ-Cultured Full-Thickness Human Skin Explants

C. Antille C. Tran O. Sorg J.-H. Saurat

Department of Dermatology, University Hospital, Geneva, Switzerland

Key Words
Vitamin A · Retinoids · Skin · Topical application

Abstract
The human epidermis contains endogenous retinoids [retinol (vitamin A) and retinyl esters] and carotenoids (mostly β-carotene). Previous studies in the mouse have shown that the enzymes involved in retinoid metabolism are present in the epidermis. In this study, we wanted to assess the skin penetration and metabolism of topical retinoids in the human. To do this, fresh surgically excised human abdominal skin was mounted on Franz perfusion cells. Topical retinoic acid, retinal, retinol and retinyl palmitate were applied at 2.5 mg/cm^2 in oil-in-water creams containing 0.05% retinoids on the donor compartment, while the receptor compartment was filled with culture medium. The skin was incubated for 24 h at 37 °C, then epidermal retinoid concentrations were determined by HPLC. The same experiment was performed with mouse back skin mounted on Franz cells. Finally, topical retinoids were applied on the back of hairless mice for 24 h; then the mice were sacrificed and retinoid concentrations were assayed in the epidermis. In all three models, retinol and its esters were found to be endogenous, as was the case in previous studies in the mouse in vivo. The four applied retinoids penetrated well into the epidermis. Topical retinoic acid did not increase endogenous retinoids, whereas the latter were greatly increased following topical retinal in the mouse. Retinal was also metabolized into retinoic acid, unlike topical retinol and retinyl palmitate, which only increased endogenous retinoids. Topical retinal and retinol did undergo a higher metabolism in both mouse models than in human skin. In summary, the penetration and metabolism patterns of topical retinoids were quite similar in the two mouse models used, indicating that the Franz cells appear to be a good model to predict in vivo metabolism of topical retinoids. When applying this concept to our results obtained in Franz cells with human skin, we conclude that topical retinol and retinal load human skin with both storage and functional vitamin A.

Introduction

The retinoid family comprises vitamin A (retinol) and its natural derivatives such as retinal, retinoic acid and retinyl esters, as well as a large number of synthetic analogs. Retinoids are required for a vast number of biological processes. In particular, they are involved in embryogenesis, reproduction, vision, growth, inflammation, differentiation, proliferation and apoptosis [1]. Human epi-

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Dr. Olivier Sorg
Clinique de Dermatologie, Hôpital Cantonal Universitaire
24, rue Micheli-du-Crest
CH–1211 Genève 14 (Switzerland)
Tel. +41 22 372 8994/9691, Fax +41 22 372 9693, E-Mail olivier.sorg@hcuge.ch

dermis contains two major retinoids, retinol and retinyl esters [2, 3]. The mechanisms of retinol uptake by keratinocytes are not fully understood. Vitamin A can be stored in keratinocytes through esterification of retinol into retinyl esters. This step is catalyzed by two enzymes, acyl CoA:retinol acyltransferase and lecithin:retinol acyltransferase; their expression is modulated by the differentiation state of the keratinocyte [4–6]. The hydrolysis of retinyl esters to retinol is catalyzed by a retinyl ester hydrolase [7]. Retinol, via its oxidation to retinal, is a prohormone of retinoic acid [8], the biologically active form of vitamin A, that modulates gene expression following its binding to nuclear receptors [1, 9]. Retinoic acid and retinal have been extensively used topically for the treatment of photoaging [10–13]. Retinal and retinoic acid also showed other biological activities on human skin from the point of view of metaplastic and hyperplastic parameters [11]. However, in vivo human studies aimed at assessing the pharmacokinetics of topical retinoids are still lacking; thus our aim was to assess the in vivo human patterns of cutaneous penetration and metabolism of topical retinoids from studies performed on ex vivo human skin mounted on Franz perfusion cells. To confirm the usefulness of this predictive model of in vivo human metabolism, we performed a similar study on both in vivo and ex vivo mouse skin.

Methods

Materials

Retinoids used for topical treatments (retinoic acid, retinal, retinol, retinyl palmitate) were applied in oil-in-water creams containing 0.05% retinoids [14]. The purity of the applied retinoid creams was checked by HPLC as described below. Chemicals were purchased from Sigma Chemicals (Saint Louis, Mo., USA), organic solvents from Merck (Darmstadt, Germany) and culture media from Gibco BRL (Life Technologies, Paisley, UK).

Franz Perfusion Cells

Our Franz perfusion cells were handmade and especially designed for the experiment. They consisted of donor and recipient compartments [15] (fig. 1). The donor compartment was secured to the receptor compartment using a clamp. It was opened to air and exposed to the epidermis, and it was covered with an air-permeable filter to exclude bacterial colonization from the laboratory environment. 2.5 mg/cm² (~ 5 nmol/cm²) retinol, retinal, retinoic acid or retinyl palmitate cream were applied in the donor chamber with the tip of a stirring rod. The skin was clamped between the ground glass of the two chambers. The receptor compartment was filled with 50 ml RPMI 1640 medium containing penicillin-streptomycin (100 units/ml; Gibco BRL, Life Technologies). The temperature was maintained at 37 °C during the incubation period. Air bubbles that accumulated in the skin culture medium interface were periodically removed. In one experiment with three pieces of skin from different patients, the culture medium was checked for the presence of bacteria (cultures in aerobic and anaerobic conditions), and the viability of the skin samples was assessed using the MTT assay as described by Gélis et al. [16] in hairless rat skin; briefly, 6-mm punches from skin samples were rinsed for 15 min in PBS followed by a 2-hour incubation in 2 ml of 1 mg/ml MTT solution in PBS, then the formazan salt resulting from succinate dehydrogenase activity was extracted overnight in 2 ml of 2-methoxyethanol, and the absorbance was read at 570 nm.

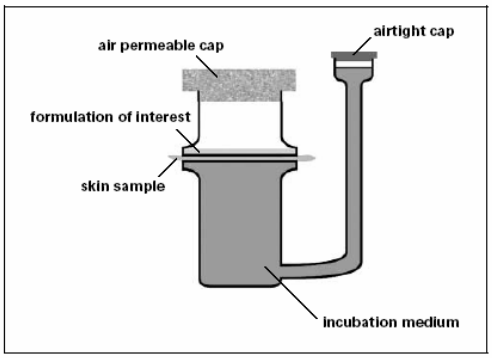

Fig. 1. The Franz cell model. The skin sample is placed between the two glass parts and tightly sealed. The formulation of interest is applied on the skin surface, and the upper glass part is closed with a cap permeable to air, but not to microorganisms. The recipient compartment is filled with cultured medium (DMEM), and tightly sealed.

Patients

A total of 5 female subjects, aged between 26 and 66 years (mean age 42), participated in the study. Twenty-five abdominal skin explants were obtained from these obese patients undergoing an abdominoplasty. Immediately after removal the skin specimens were wrapped in saline-moistened gauze and transported on ice. The tissue was carefully trimmed of subcutaneous fat using a surgical blade and the skin was mounted in Franz perfusion cells within 1 h. Only skin that appeared normal was used.

Analysis of Retinoids

Epidermis was separated from dermis by heat (PBS 56 °C, 45 s). The mean weight of epidermal sheets was 76 ± 4 mg for a 35-mm diameter; epidermal sheets were free of dermis (histological check). The whole processing of extraction was performed at a cool temperature (4 °C) and under yellow dim light, as described previously for retinoid assay [17]. Epidermis was minced with scissors in 1.92 ml extraction buffer, then it was homogenized by using a Polytron PT 3100 homogenizer in 1.92 ml extraction buffer. The latter consisted of 400 µl acetate buffer 50 m*M* pH 4, 1.5 ml isopropanol:tetrahydrofuran (1:1) with 200 µ*M* butylated hydroxytoluene and 20 µl retinyl acetate 10 µ*M* as internal standard. Homogenate was sonicated for

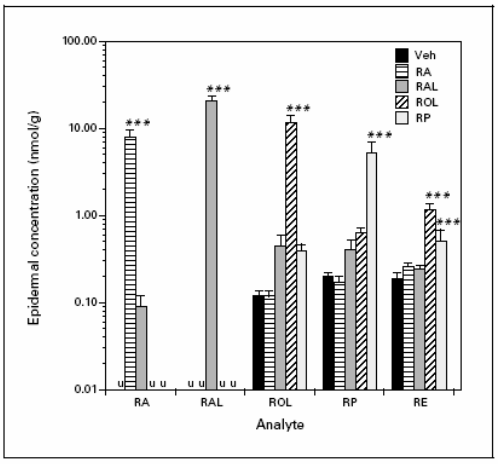

Fig. 2. Epidermal retinoid profile in ex vivo human skin explants. Topical retinoic acid, retinaldehyde, retinol or retinyl palmitate creams were applied on the epidermal side of human skin explants mounted on Franz cells at a concentration of 2.5 mg/cm² for 24 h, then epidermis was separated from dermis, and epidermal retinoids were assayed. Values under the detection limit are indicated by 'u'. Results are the means ± SE of 5 cells per treatment. Veh = Vehicle; RA = retinoic acid; RAL = retinaldehyde; ROL = retinol; RP = retinyl palmitate; RE = retinyl esters. *** $p < 0.001$.

10 s at a low power (50 W) and centrifuged for 10 min at 12,000 *g*, then the supernatant was extracted with 4 ml hexane. Hexane fraction was evaporated to dryness under nitrogen flux, then a sample was reconstituted in 100 µl HPLC mobile phase, before being injected into the HPLC [17]. Retinol, retinyl esters and retinyl acetate (internal standard) were detected by UV absorption at 325 nm, retinal at 385 nm, and retinoic acid at 340 nm. The limit of detection was 10 pmol/g epidermis.

Analysis of Data

Results represent the means ± SE of the experimental values. Analysis of variance was performed to compare topical retinoids with their vehicle; differences with $p < 0.05$ and $p < 0.001$ are shown.

Results

Viability of the Skin Samples after a 24-Hour Incubation Period

To test the viability of the skin during incubation on Franz cells, skin samples were analyzed for MTT assay before and at the end of a 24-hour incubation period: the reduction of MTT to the formazan derivative was decreased by 40% in samples incubated for 24 h on Franz cells, as compared to fresh ones (data not shown), a result very similar to that reported by Gélis et al. [16] on hairless rat skin. Thus, the moderate decrease of cellular energy metabolism of skin samples during incubation on Franz cells indicates that these skin samples are still able to metabolize topical agents which penetrate into the epidermis in a way similar to fresh samples, although at a slightly lower rate.

Epidermal Retinoids in ex vivo Human Skin Explants

Untreated human epidermis contains sizable amounts of retinoids. We measured 0.12 ± 0.02 nmol/g of retinol and 0.12 ± 0.01 nmol/g of retinyl esters in the freshly excised abdominal epidermis of 15 healthy subjects. No other retinoid was detected in these conditions. The pattern of epidermal retinoids following topical retinoic acid was very similar, except for the presence of high amounts of retinoic acid, indicating that the latter penetrates well into the epidermis and does not undergo reduction metabolism. Topical retinal penetrated well into the epidermis, induced a slight increase of endogenous retinoids (retinol and retinyl esters), and a small part was oxidized into retinoic acid; topical retinol and its palmitic ester highly increased endogenous retinoids, whereas no oxidized retinoid (retinal, retinoic acid) was found (fig. 2).

Epidermal Retinoids in both ex vivo and in vivo Mouse Skin Models

As for ex vivo human skin, only retinol (0.2 ± 0.02 nmol/g) and its esters (1 ± 0.12 nmol/g) were found in freshly excised mouse skin. In both ex vivo and in vivo mouse models, topical retinal, retinol and retinyl pamitate penetrated well into the epidermis, and underwent a higher metabolism than in ex vivo human skin, whereas topical retinoic acid, as for human skin, did not produce any reduction metabolite (fig. 3, 4).

Discussion

Due to their actions on growth and differentiation, retinoids are widely used as treatments for various cutaneous diseases [18–21], as well as in cosmetic preparations [22–24]. Only severe conditions require the use of oral retinoids, in order to induce high systemic concentrations, with the problems of side effects and teratogenic properties that preclude their use for pregnant women [25]. For both cosmetic use and treatments of mild to

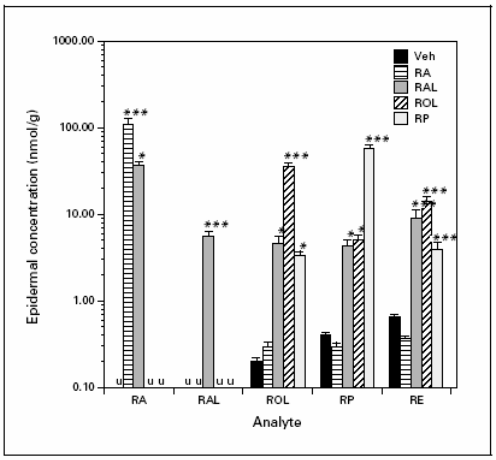

Fig. 3. Epidermal retinoid profile in ex vivo mouse skin explants. Topical retinoids were applied on the epidermal side of hairless mouse skin explants as described in the legend of figure 2. Values under the detection limit are indicated by 'u'. Results are the means ± SE of 3 cells per treatment. Abbreviations are explained in the legend of figure 2. * $p < 0.05$; *** $p < 0.001$.

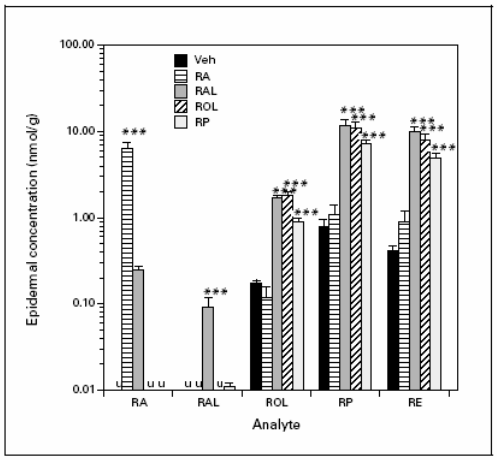

Fig. 4. Epidermal retinoid profile in hairless mice in vivo. Topical retinoids were applied on the back of hairless mice at a concentration of 2.5 mg/cm^2 for 24 h, then the mice were sacrificed, and epidermis was separated from dermis, and epidermal retinoids were assayed. Values under the detection limit are indicated by 'u'. Results are the means ± SE of 3 mice per treatment. Abbreviations are explained in the legend of figure 2. *** $p < 0.001$.

moderate cutaneous diseases, topical application of retinoids represent a good approach to deliver retinoids directly to the skin; in such cases, systemic concentrations seem to be unaffected [26, 27]. However, very few in vivo human studies examining the epidermal concentrations of retinoids following topical application were performed to date [28, 29].

Our previous studies in hairless mice demonstrated that topical retinol and retinal load the epidermis with both functional (retinoic acid) and storage (retinol and retinyl esters) forms of vitamin A [14, 30, 31]. Since it is difficult to perform such in vivo experiments in humans, we addressed the question regarding the ability of the Franz diffusion cell model to reflects the penetration and metabolism profiles of topical retinoids in vivo. Thus, we applied topical retinoids at the same concentrations on the back of living hairless mice, as well as on skin samples from hairless mice or humans mounted on Franz diffusion cells; then epidermal retinoid concentrations were determined. The epidermal retinoid patterns following topical retinoids were very similar in both mouse models, indicating that fresh mouse skin mounted on Franz diffusion cells is a suitable model to study the penetration and the metabolism of topical retinoids for an incubation time of at least 24 h. In this study human and mouse skin samples were shown to survive well for 24 h at 37 °C in RPMI culture medium, and other authors reported a survival of human skin explants at 3–4 days, based on histological evaluation [32] or lactate dehydrogenase activity in culture fluid [33].

The epidermal retinoid profiles following a 24-hour incubation in mouse and human skin mounted on Franz cells were quite similar; all topical retinoids penetrated wells into the epidermis and gave the same retinoid metabolites: topical retinoic acid did not produce reduction metabolites, topical retinal gave both oxidation and reduction metabolites, and topical retinol and its palmitic ester increased endogenous retinoids. The main difference between ex vivo mouse and human skin was the higher metabolism of topical retinal, retinol and retinyl palmitate in mouse, as compared to human epidermis.

Although the only way to study the transcutaneous profile of topical retinoids in humans is to perform in vivo experiments on healthy volunteers, our study demonstrated that freshly excised skin mounted on Franz diffusion cells the receptor compartment of which is filled with

a culture medium allows the skin sample to survive at least for 24 h, while maintaining an activity of the enzymes involved in retinoid metabolism. Thus Franz cells represent an interesting alternative method to study the epidermal profile of topical retinoids in human skin.

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Topical β-carotene is converted to retinyl esters in human skin *ex vivo* and mouse skin *in vivo*

Antille C, Tran C, Sorg O, Saurat J-H. Topical β-carotene is converted to retinyl esters in human skin *ex vivo* and mouse skin *in vivo*.
Exp Dermatol 2004: 13: 558–561. © Blackwell Munksgaard, 2004

Abstract: Human epidermis contains endogenous retinoids (retinol and retinyl esters) and carotenoids (mostly β-carotene). Previous studies have shown that the enzymes involved in retinoid metabolism are present in human epidermis. There is still a controversy about the presence in the skin of the enzymes able to convert β-carotene into vitamin A (retinol), although a recent study demonstrated the conversion of β-carotene into retinol in human cultured epidermal cells. In this study, we addressed the question of the possible bioconversion of topical β-carotene into vitamin A or derivatives by human and mouse skin. Surgically excised human abdominal skin was mounted on Franz perfusion chambers to assess the cutaneous penetration of topical β-carotene as well as its metabolism, after a 24-h incubation period, whereas hairless mice received topical β-carotene 24 h before assaying epidermal β-carotene and retinoid concentrations. Epidermal retinoid and β-carotene concentrations were determined by high-pressure liquid chromatography. Topical β-carotene penetrated well into human and mouse epidermis and induced a 10-fold (human) and a threefold (mouse) increase of epidermal retinyl esters, which demonstrates that topical β-carotene is converted into retinyl esters by human and mouse epidermis and thus appears as a precursor of epidermal vitamin A.

Christophe Antille, Christian Tran, Olivier Sorg and Jean-Hilaire Saurat

Department of Dermatology, University Hospital, Geneva, Switzerland

Key words: epidermis – human – mouse – retinoids – retinyl esters – vitamin A – β-carotene

Dr Olivier Sorg, Clinique de Dermatologie, Hôpital Cantonal Universitaire, 24 rue Micheli-du-Crest, 1211 Genève 14,Switzerland
Tel.: +41 22 372 9457/9691
Fax: +41 22 372 9693
e-mail: olivier.sorg@hcuge.ch

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Introduction

Vitamin A occurs in food of animal origin mainly as retinyl esters and in fruit and vegetables as carotenoids (mainly β-carotene). In human, absorbed β-carotene is converted into retinal in enterocytes and the liver by a specific enzyme (15,15′-dioxygenase), which generates retinal by central cleavage (1–3). Another metabolic pathway is eccentric cleavage of β-carotene via β-apocarotenals to retinal (2). Retinal is then converted into retinol by dehydrogenases, and retinol is transported by retinol-binding protein, a specific plasma protein, to target tissues. Human epidermis contains two major retinoids (retinol and retinyl esters) and carotenoids (mainly β-carotene) (4,5). Vitamin A can be stored in keratinocytes through esterification of retinol into retinyl esters. This step is catalyzed by two enzymes, acyl-CoA : retinol acyltransferase (ARAT) and lecithin : retinol acyltransferase (LRAT); their expression is modulated by the differentiation state of the keratinocytes (6–8). Hydrolysis of retinyl esters into retinol is catalyzed by retinyl ester hydrolases. Retinol, via its oxidation into retinal, is a pro-hormone of retinoic acid (9), the biologically active form of vitamin A that modulates gene expression following its binding to nuclear receptors (1,10). Thus retinal, retinol, and its esters are endogenous precursors of the biologically active form of vitamin A.

Although the presence of a transcript for a 15,15′-dioxygenase protein has been found in mouse skin (11), there is still a controversy concerning the possible bioconversion of carotenoids into vitamin A outside the intestine and the liver. A recent study demonstrated the bioconversion of [^{14}C] β-carotene into [^{14}C] retinol by cultured human melanocytes and keratinocytes (12). In

this study, we addressed the question of the possible bioconversion of β-carotene into vitamin A by human skin, using *ex vivo* human skin samples mounted onto Franz diffusion chambers, as well as in hairless mice *in vivo*.

Methods

Materials

β-carotene was applied in isopropanol : CH_2Cl_2 : PEG400 (5 : 4 : 1) at a concentration of 2 mM. The purity of the β-carotene solution was checked by high-performance liquid chromatography (HPLC) as described below. Chemicals were purchased from Sigma Chemicals (Saint Louis, MO, USA), organic solvents from Merck (Darmstadt, Germany) and culture media from Gibco BRL (Life technologies Ltd, Paisley, Scotland).

Franz diffusion cells

Our Franz diffusion cells were handmade and especially designed for the experiment. They consisted in donor and recipient compartments (13). The donor compartment was secured to the receptor compartment using a clamp. The donor compartment was opened to air and exposed to the epidermis; it was covered with an air permeable filter to exclude bacterial colonization from the laboratory environment. A 2 mM β-carotene solution (200 nmol/cm^2) was applied in the donor chamber. The skin was clamped between the ground glass of the two chambers with a 35 mm inner diameter. The receptor compartment was filled with 50 ml RPMI 1640 medium containing penicillin-streptomycin (100 units/ml; Gibco BRL, Life technologies Ltd, Paisley, Scotland). The temperature was maintained at 37°C during the incubation period, and the cells were continuously agitated. Air bubbles that accumulated in the skin culture medium interface were periodically removed. After a 24-h incubation period with the skin samples, the culture medium was still free of bacteria (cultures in aerobic and anaerobic conditions), and the viability of the skin samples was assessed using the 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazoliumbromide (MTT) assay as described by Gélis and colleagues in hairless rat skin (14); briefly, 6 mm punches from skin samples were rinsed for 15 min in phosphate-buffered saline (PBS) followed by 2-h incubation in 2 ml of 1 mg/ml MTT solution in PBS, then the formazan salt resulting from succinate dehydrogenase activity was extracted overnight in 2 ml of 2-methoxyethanol, and the absorbance was read at 570 nm.

Animals

Female SKH1 (h/h) albino hairless mice were maintained on a standard laboratory diet (Provimi Kliba, Kaiseraugst, Switzerland) containing 14 000 IU vitamin A (14 μmol) per kg (but no carotene) and had a free access to food and water. The animals were under veterinary control in the animal housing facility of the Faculty of Medicine. Groups of three adult mice (5 months old) per dose point were tested. After application of the β-carotene solution (100 μl), the mice were maintained in the hands until complete absorption into the stratum corneum (1–2 min), before returning to their cage. Because of the organic solvents used to dissolve β-carotene, this ensured a rapid absorption into the stratum corneum.

Human

The human skin specimens came from patients who underwent abdominoplasty. For the β-carotene group, skin samples came from one male and two female patients (54–60 years old), and those for the control groups came from two male and 13 female patients (age range 22–65 years with a median age of 44). Immediately after removal, the skin specimens were wrapped in saline-moistened gauze and transported on ice. The tissue was carefully trimmed of subcutaneous fat using a surgical blade, and the skin was mounted in Franz diffusion cells within 1 h. Only skin that appeared normal was used.

Analysis of retinoids and β-carotene

Skin samples were rinsed in ice-cold PBS buffer, before being immersed for 30 s in PBS buffer heated to 56°C, and put back to ice-cold PBS, then epidermis was separated from dermis using forceps. Epidermal sheets were carefully rinsed in PBS and dried using a tissue before being frozen in liquid nitrogen and stored at −80°C. Epidermal sheets were free of dermis (histological check), and their mean weight was 75 mg for a 35 mm diameter. The whole processing of extraction was performed at a cool temperature (4°C) as described previously for retinoid assay (15). Epidermis was minced with scissors in 1.92 ml extraction buffer, then epidermis was homogenized by using a Polytron PT 3100 homogenizer. Extraction buffer consisted in 400 μl acetate buffer 50 mM pH4, 1.5 ml isopropanol : tetrahydrofuran (1 : 1) containing 200 μM butylated hydroxytoluene (BHT) and 20 μl retinyl acetate 10 μM as internal standard. Homogenate was sonicated for 10 s at a low power (50 W) and centrifuged 10 min at 12 000 × *g*, then supernatant was extracted with 4 ml hexane. Hexane fraction was evaporated to dryness under nitrogen flux, then sample was reconstituted in 100 μl HPLC mobile phase, before being injected into the HPLC (15). Retinol and retinyl esters were detected by UV absorption at 325 nm and β-carotene at 450 nm. The limit of detection was 10 pmol/g epidermis.

Analysis of data

Results represent the means ± SE of the experimental values. Student's *t*-test analysis was performed to compare results for treated and non-treated skin.

Results

Human epidermis contains sizable amounts of retinoids and β-carotene. We measured 0.12 ± 0.02 nmol/g of retinol, 0.22 ± 0.03 nmol/g of retinyl esters, and 0.36 ± 0.03 nmol/g of β-carotene in the abdominal epidermis of 15 healthy subjects. Untreated mouse epidermis contains 127 ± 5 pmol/g of retinol and 1263 ± 117 pmol/g retinyl esters, but less than 10 pmol/g β-carotene ($n = 6$).

Because Vahlquist and colleagues found that human cultured keratinocytes and melanocytes were able to convert β-carotene into retinol, we applied 200 nmol/cm^2 β-carotene on seven human skin explants in Franz cells for 24 h. Topical β-carotene penetrated easily the epidermis; it induced a 160-fold increase (57 ± 2 nmol/g) of epidermal β-carotene and a 10-fold increase (2.24 ± 0.41 nmol/g) of epidermal retinyl esters, whereas epidermal retinol was unchanged (Fig. 1). In order to test the bioconversion of epidermal β-carotene into vitamin A in an *in vivo* model, we applied topical β-carotene (100 μl) on the back of

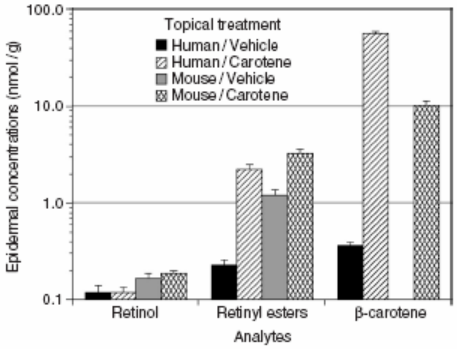

Figure 1. Conversion of topical β-carotene to epidermal retinyl esters. Topical β-carotene was applied on human skin explants mounted on Franz cells or on the back of hairless mice for 24 h at a concentration of 200 nmol/cm^2, then human skin was harvested and the mice sacrificed, epidermis was separated from dermis, and epidermal retinoids and β-carotene were assayed by high-performance liquid chromatography (HPLC). Results are the means ± SE of seven human skin samples from three patients, and six mice. Significant differences between topical β-carotene and vehicle, for a given model, were indicated (***, $P < 0.001$).

hairless mice for 24 h; as for *ex vivo* human skin, topical β-carotene penetrated well into the epidermis and was increased from less than 10 pmol/g to 10.1 ± 1.1 nmol/g. In the same time, epidermal retinyl esters were increased by threefold, whereas epidermal retinol was unchanged (Fig. 1).

Discussion

Carotenoids are produced by plants and are precursors of vitamin A and other retinoids for animals and humans. Until recently, carotenoids, and in particular β-carotene, the predominant vitamin A precursor in humans, were believed to be converted into vitamin A and other retinoids exclusively in enterocytes and the liver. This would indicate that topical application of carotenoids would not increase cutaneous vitamin A concentration. The discovery of a transcript encoding a protein with 15,15′-carotenoid dioxygenase activity from mouse skin (11), as well as the bioconversion of [^{14}C] β-carotene to [^{14}C] retinol by cultured human melanocytes and keratinocytes (12) revived the debate concerning the ability of topical carotenoids to act as cutaneous vitamin A precursors.

In human skin, β-carotene is mainly located in the epidermis (5). In this study, we found 0.360 ± 0.03 nmol/g β-carotene per gram of human epidermis, a value in accordance with that reported by other authors (5,16–18). Twenty-four hours following a single application in human skin explants, this value rose to 160-fold, indicating a good penetration of β-carotene in the epidermis, as compared to the 17-fold increase in skin carotenoid concentrations 12 weeks following a daily ingestion of 24 mg β-carotene (17); during the same time, epidermal retinyl esters rose to 10-fold. This demonstrates that *ex vivo* human epidermis converts β-carotene into retinal, which is reduced to retinol, then retinol is esterified with fatty acids; the end products of β-carotene bioconversion by human skin into retinoids are thus retinyl esters, as it is the case for ingested carotenoids, an observation that suggested to Van Vliet and coworkers to use the ratio of retinyl esters to β-carotene as an index of β-carotene bioconversion into vitamin A (19). In order to reproduce this bioconversion of topical β-carotene in an *in vivo* model, we applied topical β-carotene on the back of hairless mice. Unlike human skin samples, no β-carotene was detected in the epidermis of untreated mice; this is not surprising, because animals and man cannot synthesize carotenoids, and the presence in their body of any carotenoid is due to the presence of carotenoids in their food; our mice were not fed β-carotene and thus no carotene was detected in their skin. On the other hand, the conversion of topical β-carotene to epidermal retinyl esters was confirmed in this *in vivo* model.

These results demonstrate that topical β-carotene can load human and mouse epidermis with high amounts of β-carotene and that the latter can be converted into retinyl esters, the storage form of vitamin A (2,20); such a bioconversion of β-carotene to retinyl palmitate was also observed in the liver of mice fed β-carotene (21). Because retinyl esters are precursors of all endogenous retinoids (2,22), including retinoic acid, the biologically active form of vitamin A, topical β-carotene appears as a precursor of both storage and functional epidermal vitamin A. β-carotene is a scavenger of free radicals and singlet oxygen, and, as shown in this study, a precursor of cutaneous vitamin A when applied topically; thus, because acute UV exposure induces both an oxidative stress (23–25) and a depletion of vitamin A (20) in the skin, topical β-carotene could be used to prevent these deleterious actions of UV.

Acknowledgements

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Vitamin A Exerts a Photoprotective Action in Skin by Absorbing Ultraviolet B Radiation

Christophe Antille, Christian Tran, Olivier Sorg, Pierre Carraux, Liliane Didierjean, and Jean-Hilaire Saurat
Department of Dermatology, University Hospital, Geneva, Switzerland

Retinyl esters, a storage form of vitamin A, concentrate in the epidermis, and absorb ultraviolet radiation with a maximum at 325 nm. We wondered whether these absorbing properties of retinyl esters might have a biologically relevant filter activity. We first used an *in vitro* model to assess the photoprotective properties of retinyl palmitate. We then applied topical retinyl palmitate on the back of hairless mice before exposing them to 1 J per cm^2 ultraviolet B, and assayed the levels of thymine dimers produced in epidermal DNA 2 h following ultraviolet B exposure. Finally, we applied topical retinyl palmitate or a sunscreen on the buttocks of human volunteers before exposing them to four minimal erythema doses of ultraviolet B; we assayed the levels of thymine dimers produced 2 h following ultraviolet B exposure, and determined the intensity of erythema 24 h after ultraviolet B. *In vitro*, retinyl palmitate was shown to be as efficient as the commercial filter octylmethoxycinnamate in preventing ultraviolet-induced fluorescence or photobleaching of fluorescent markers. The formation of thymine dimers in mouse epidermis was significantly inhibited by topical retinyl palmitate. In human subjects, topical retinyl palmitate was as efficient as a sun protection factor 20 sunscreen in preventing sunburn erythema as well as the formation of thymine dimers. These results demonstrate that epidermal retinyl esters have a biologically relevant filter activity and suggest, besides their pleomorphic biologic actions, a new role for vitamin A that concentrates in the epidermis. *Key words: DNA damage/retinoids/sunscreening agents/ultraviolet rays. J Invest Dermatol 121:1163–1167, 2003*

The skin contains sizable amounts of vitamin A (retinol). Retinol can be esterified by free fatty acids. Free and esterified retinol reach together about 1 nmol per g in both epidermis and dermis (Vahlquist, 1982; Vahlquist *et al*, 1982). Studies in mouse have shown that, in epidermis, esters of retinol account for approximately 90% of total vitamin A, the remaining 10% being retinol (Törmä *et al*, 1987; Sorg *et al*, 1999). In mouse, retinol concentrations decrease from the serum to the dermis, then to the epidermis, whereas the opposite is observed for retinyl esters (Sorg *et al*, 1999). Thus epidermis, like the liver, which stores most of body vitamin A, concentrates vitamin A in the form of retinyl esters.

Vitamin A (retinol and retinyl esters) strongly absorbs ultraviolet (UV) radiation between 300 and 350 nm, with a maximum at 325 nm, a wavelength range received from the sun at earth level. Thus it is not surprising that human or mouse epidermal vitamin A is destroyed by sun or UV exposure (Berne *et al*, 1984; Tang *et al*, 1994; Andersson *et al*, 1999; Sorg *et al*, 1999; 2002; Tran *et al*, 2001). The mouse epidermis can be loaded with large amounts of vitamin A following a topical application of retinol or retinaldehyde. This epidermal vitamin A originating from topical application is also very sensitive to the destructive action of UVA and UVB (Tran *et al*, 2001; Sorg *et al*, 2002).

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Reprint requests to: Dr Olivier Sorg, Clinique de Dermatologie, Hôpital Cantonal Universitaire, 24 rue Micheli-du-Crest, 1211 Genève 14, Switzerland. Email: olivier.sorg@hcuge.ch

Abbreviations: DCFH, 2′,7′-dichlorofluorescein; OMC, octylmethoxycinnamate.

Besides vitamin A, UV radiation can be absorbed by many other chromophores in the skin; thus the major manifestations of the skin after acute UV irradiation are erythema, sunburn cell formation, and cyclobutane pyrimidine dimers, the latter being a direct index of DNA damage. DNA not only represents an important target for UV, but any damage to DNA can have deleterious consequences for the cells and the organ to which they belong. The cells can avoid the consequences of the destructive actions of UV by (1) preventing the interactions between UV and DNA and (2) detecting DNA modifications and repairing them before they can have biologic consequences (Zhou and Elledge, 2000; Rouse and Jackson, 2002).

The main strategies to prevent skin DNA damage consist in (1) limiting direct skin exposure to sunlight, (2) wearing protective clothes, and (3) using topical molecules that either act as UV filters or interfere with the biochemical reactions induced by UV. Among these molecules, sunscreens (Black *et al*, 1997; Ananthaswamy *et al*, 1998; Young *et al*, 2000), green tea polyphenols (Katiyar *et al*, 2000; 2001), and α-tocopherol (Chen *et al*, 1997; McVean and Liebler, 1997; 1999) were shown to reduce erythema, sunburn cell formation, and pyrimidine dimers in mouse and human skin. DNA lesions can still be induced at a very high rate even in sunscreen-protected skin, however (Liardet *et al*, 2001), and some studies suggest that sunscreen use could be associated with an increase in melanoma incidence (Bigby, 1999; Autier, 2000). The fact that this is only due to increased exposure times by people who believe that they are well protected is still controversial (Autier *et al*, 1999; 2000), and toxic photoreactions involving sunscreen molecules are not excluded. Therefore the use of topical natural molecules that have photoprotective properties would represent a safe alternative to artificial sunscreens in the prevention of photocarcinogenesis.

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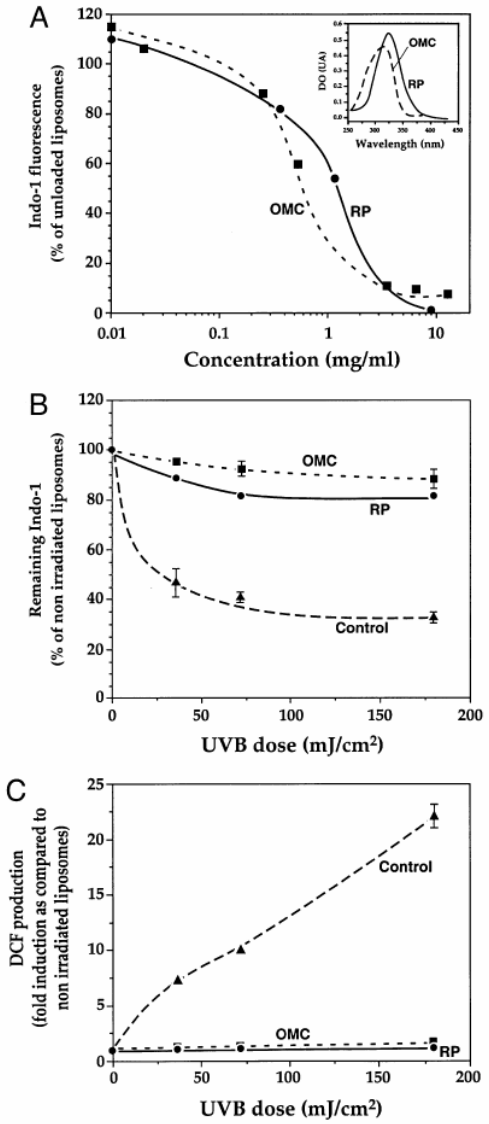

Figure 1. Retinyl palmitate has photoprotective properties in an *in vitro* model. (*A*) Indo-1-encapsulated liposomes, the membranes of which contained increasing concentrations of OMC or retinyl palmitate (R.P), were analyzed for Indo-1 fluorescence (excitation 330 nm; emission 405 nm). Insert shows the absorption spectrum of R.P. (*B*) Indo-1-encapsulated liposomes, the membranes of which contained 6.5 mg per ml OMC or 10 mg per ml R.P, were exposed to increasing UVB doses, and then Indo-1 was extracted and assayed by fluorometry. The graph shows the percentage of intact Indo-1 as a function of UVB dose. (*C*) DCFH-encapsulated liposomes, the membranes of which contained 6.5 mg per ml OMC or 10 mg per ml R.P, were exposed to increasing UVB doses, and then DCF fluorescence was analyzed (excitation 485 nm; emission 530 nm). The results for OMC are reproduced from a previous publication (Tran *et al*, 2002).

The contribution of natural endogenous molecules in preventing the interactions between UV and DNA is therefore of major importance. In this study, we used an *in vitro* model (Tran *et al*, 2002) to assess the ability of retinyl palmitate – the predominant form of epidermal vitamin A – to prevent DNA damage and erythema induced by a single UVB exposure, due to its absorption properties. In this model, fluorescent markers (Indo-1 or 2′,7′-dichlorofluorescein (DCFH)) are encapsulated into liposomes. Indo-1 becomes fluorescent when liposomes are exposed to a low intensity excitation wavelength, and when the liposome membranes contain molecules able to absorb the excitation wavelength the fluorescence is decreased in a concentration-dependent manner. If the intensity of the excitation beam is high enough, it can destroy Indo-1 by photobleaching. In this case, it is possible to assess the photoprotective action of absorbing molecules dispersed in liposome membranes. Finally, when DCFH is encapsulated into liposomes, it can be oxidized to DCF upon UVB excitation, and it is possible to assess the prevention of this photooxidation by absorbing molecules dispersed into liposome membranes. In this study, using this model, the photoprotective properties of retinyl palmitate were compared to those of octylmethoxycinnamate (OMC), a well-known UV filter contained in many sunscreens, which was shown to be efficient in the mentioned liposome model (Tran *et al*, 2002). We then applied topical retinyl palmitate on the skin of hairless mice or human subjects, before exposing them to UVB, and determined the amounts of thymine dimers in epidermal DNA. In human subjects, we also observed the UVB-induced erythema 24 h after UV exposure.

MATERIALS AND METHODS

Chemicals Egg yolk dried L-α phosphatidylcholine 60% (lecithin), retinyl palmitate, and DCFH diacetate were purchased from Sigma Chemical (St Louis, MO). DCFH was produced by hydrolysis of DCFH diacetate (NaOH 0.1 mol per L, 30 min at room temperature) (LeBel and Bondy, 1990). Indo-1 was purchased from Molecular Probes (Leiden, The Netherlands). All solvents were from Merck (Darmstadt, Germany). OMC was a gift from Hoffmann-La Roche (Basel, Switzerland). Retinyl palmitate, as a 2% oil-in-water cream, and its vehicle were provided by Pierre Fabre Dermo-cosmetique. The sunscreen All Day 20+ (Louis Widmer, Rheinfelden, Switzerland) was bought in a drugstore; it contains the filter ethylhexylmethoxycinnamate and the sunscreen titanium dioxide, and has a sun protection factor (SPF) of 20.

***In vitro* experiments** Liposomes were prepared as previously described (Tran *et al*, 2002). Briefly, 30 mg lecithin and 15 mg cholesterol were dissolved in 2 ml chloroform in a glass tube, and then chloroform was evaporated under nitrogen flux. The film of lipids was then resuspended in phosphate buffer containing the fluorescent marker (Indo-1 6 μmol per L, or DCFH 370 μmol per L); the suspension was sonicated 30 s at 50 W and filtered through 0.45 μm pores, and finally dialyzed through a 6000–8000 Da membrane in order to remove excess fluorescent markers. Irradiation of liposomes was performed using six TL 20 W/12 Philips tubes. The UVB and UVA fluxes were 3.7 mW per cm^2 and 0.5 mW per cm^2, as determined by a digital radiometer Waldmann 585.100 recently calibrated, and the distance from the liposome suspension was 30 cm. The photoprotective properties of lipophilic agents were assessed as previously described (Tran *et al*, 2002).

Treatments and irradiation of mice Six adult female hairless mice were used. Three mice were treated on the back with retinyl palmitate 2%, and three others with its vehicle, once a day for 3 d, as previously described (Tran *et al*, 2001). Twenty-four hours after the last topical treatment, the mice received a single dose of 1 J per cm^2 UVB. This dose was chosen in accordance with a previous study showing the dose-dependent action of UVB on the photodestruction of mouse epidermal retinoids (Sorg *et al*, 1999). Two hours later, the mice were sacrificed, the skin was harvested, and epidermis was separated from dermis by heat (Tran *et al*, 2001).

Human subjects Six adult males participated in the study; their age ranged from 22 to 26 y (mean age 24±1). One subject was phototype I, one phototype II, and four were phototype III. All subjects were in good

health with no evidence of acute, chronic illness or cutaneous disease. They had no history of abnormal response to sunlight and did not take medication with known photosensitizing properties.

Human protocol The human protocol was approved by the Ethic Committee of the Hôpitaux Universitaires de Genève. Written consent was obtained from all participants in the study. Three visits per person were required. During the first visit the individual minimal erythema dose (MED) was determined by exposing the buttock skin to graded doses of UVB from a medical UVB lamp (TL 20 W/12; Phillips). The lowest dose resulting in uniform erythema over the irradiation site 24 h after irradiation was considered the MED. The five subjects with phototypes II–III had an MED of 74 mJ per cm^2; the subject with phototype I had an MED of 55 mJ per cm^2. On the first visit 20 mg per cm^2 vehicle, retinyl palmitate 2%, or the commercial sunscreen All Day with SPF 20 were applied to a 2×2 cm area of the buttock on an occlusive dressing to prevent dispersion and mixing of the creams. In previous studies on the metabolism of topical retinoids, this dose of 20 mg per cm^2 was shown to load the epidermis with high amounts of retinyl esters (Tran *et al*, 2001; Sorg *et al*, 2002). The occlusive dressing was left 3–4 h. Patients returned 24 h later for a second application. Thirty minutes later, excess product was eliminated using a compress, and the treated zones were exposed to 2 or 4 MED UVB (these UVB doses were absolute doses, and were not adjusted according to an SPF value). Skin punch biopsies 2 mm in diameter were taken at each site 30 min after the irradiation and snap-frozen immediately in order to assess DNA damage. Erythema was measured with a Minolta chromameter CR-300 (Minolta, Osaka, Japan) 24 h after the irradiation as described (Park *et al*, 2002).

Pyrimidine dimers The spatial distribution of thymine dimers was visualized in mouse histologic slices using an antibody that binds to thymine dimers (clone KTM53, Kamiya, Seattle, WA). For quantitation of thymine dimers, DNA was extracted from mouse epidermis or the human punch biopsies (Gemmell and Akiyama, 1996), and then thymine dimers were detected by immuno dot blots using the mentioned antibody, as described by Smit *et al* (2001). The quantitation of dot blots was performed by a densitometer from Molecular Dynamics (Redwood City, CA) and the software ImageQuant.

Statistical analysis Student's *t* test and ANOVA were performed to compare two or three series of data, respectively. Values significantly different from vehicle-treated skin were indicated: *, $p < 0.05$; ***, $p < 0.001$.

RESULTS

Retinyl palmitate has photoprotective properties in an *in vitro* model Using an *in vitro* model (Tran *et al*, 2002), we evaluated the photoprotective properties of retinyl palmitate; OMC, which was shown to be an efficient UV filter in this model (Tran *et al*, 2002), was used as a positive control. This model assesses the filter capacity of a lipophilic molecule contained in the membranes of liposomes, as well as its ability to prevent the photobleaching or the photooxidation of hydrophilic molecules encapsulated in the aqueous compartment of the liposomes (Tran *et al*, 2002). In this model, retinyl palmitate decreased the fluorescence of Indo-1 in a concentration-dependent manner, indicating a filter capacity similar to that of the sunscreen OMC (**Fig 1*A***). When liposomes containing Indo-1 or DCFH were exposed to increasing UVB doses, retinyl palmitate was as efficient as OMC in preventing Indo-1 photobleaching (**Fig 1*B***) or DCFH oxidation (**Fig 1*C***).

Topical retinyl palmitate inhibits thymine dimer formation in hairless mice Thymine dimer formation is a primary marker of DNA damage and represents a risk factor for the development of skin cancers. In order to assess the potential ability of retinyl palmitate to prevent a deleterious biologic action of UVB, we measured the amount of thymine dimers in DNA from the epidermis of mice that had been exposed to UVB following a 3 d topical treatment with retinyl palmitate 2% or its vehicle. Two hours after UVB, the density of epidermal nuclei stained for thymine dimers was lower in mice treated with retinyl palmitate 2% compared to those treated with vehicle (**Fig 2*A***). This was confirmed by assessing the density of thymine dimers per mass unit of DNA using immuno dot blots followed by densitometric analysis: the amounts of thymine dimers in 10 ng DNA was 43% lower in mice treated with retinyl palmitate 2% compared to its vehicle (**Fig 2*B, C***), indicating that retinyl palmitate inhibited the formation of thymine dimers by filtering UVB.

Topical retinyl palmitate inhibits thymine dimer formation in human skin We then wondered whether these results could be reproduced in human. We measured the amount of thymine dimers in the punch biopsies of volunteers who had been exposed to 2 or 4 MED UVB following a topical treatment with 20 mg per cm^2 retinyl palmitate 2%, commercial sunscreen with SPF 20, or retinyl palmitate vehicle. Thirty minutes after UV exposure, the density of epidermal nuclei stained for thymine dimers was lower in zones treated with either sunscreen or retinyl palmitate, compared to those treated with vehicle (**Fig 3*A***). In DNA from zones treated with vehicle and exposed to 2 and 4 MED, two spots were clearly apparent, the one from the zone exposed to 4 MED being darker; this indicates that thymine dimers are produced in cutaneous DNA from zones exposed to 2 and 4 MED in a dose-dependent manner. In zones pretreated with SPF 20 sunscreen or retinyl palmitate, no spot was visible, although the densitometric analysis revealed a low amount of darker areas (**Fig 3*B, C***). In other words, topical retinyl palmitate and the sunscreen used prevented the formation of thymine dimers in zones exposed to up to 4 MED.

Topical retinyl palmitate inhibits UVB-induced erythema in human skin The erythema observed 24 h after a single UVB

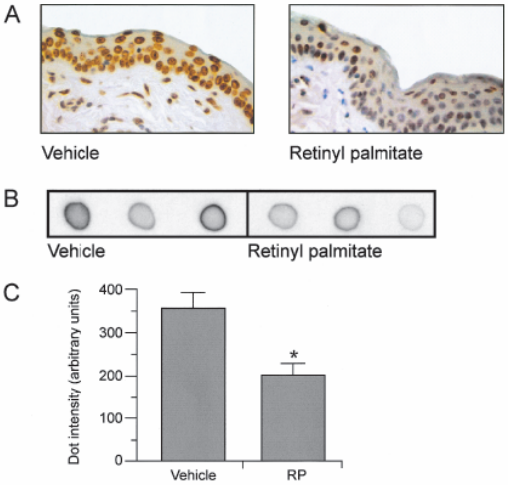

Figure 2. Topical retinyl palmitate inhibits thymine dimer formation in hairless mice. Hairless mice were treated with topical retinyl palmitate 2% or vehicle for 3 d before being exposed to 1 J per cm^2 UVB. Thymine dimers were assessed in epidermal sections by immunohistochemistry (*A*) and immuno dot blots (*B*) followed by densitometric analysis (*C*). (*A*) Histologic slices stained for thymine dimers from one mouse; positive nuclei are stained brown. (*B*) Dot blots showing the amounts of thymine dimers for each mouse. (*C*) Densitometric analysis of the dot blots, showing the mean pixel density ± SE of the three blots shown in (*B*). This experiment was repeated with another group of three mice, with similar results.

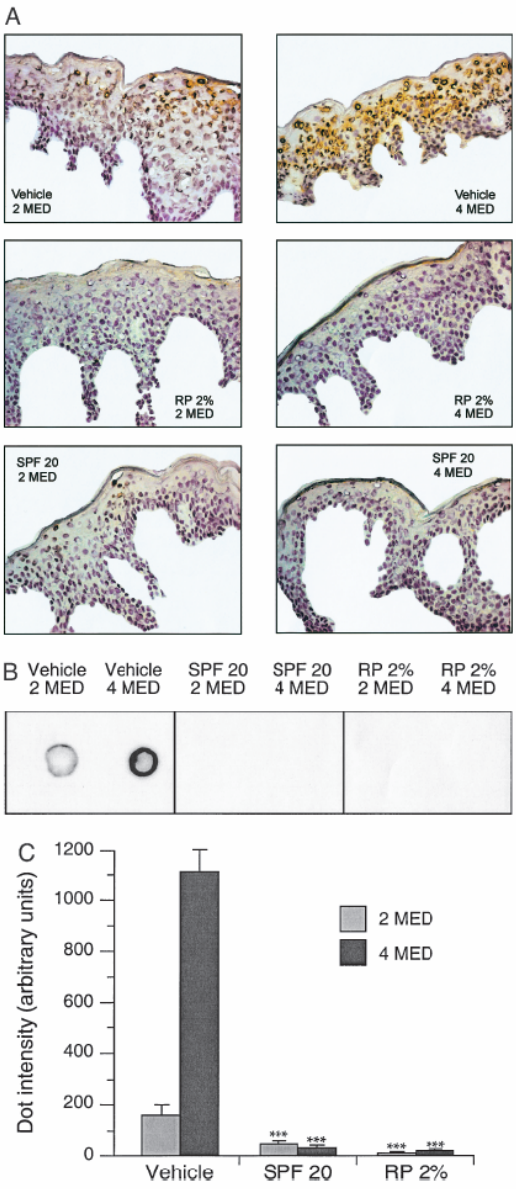

Figure 3. Topical retinyl palmitate inhibits thymine dimer formation in human skin. Delimited zones of the buttocks of human volunteers were treated with either retinyl palmitate (RP), its vehicle, or the sunscreen All Day (SPF 20); the same zones were treated again 24 h later with the same products, and then exposed 30 min later to 2 or 4 MED UVB. Two millimeter punch biopsies were removed 30 min after UVB irradiation and analyzed for thymine dimers. (*A*) Histologic slices stained for thymine dimers from one subject, showing positive nuclei in brown. (*B*) Immuno dot blots showing thymine dimers in DNA from skin biopsies. (*C*) Densitometric analysis of the dot blots, showing the mean pixel density ± SE of the three blots shown in (*B*).

exposure is considered to be a marker of cutaneous injury and inflammation. This marker is used to determine the SPF of sunscreens. The skin of human volunteers was pretreated with 20 mg per cm^2 retinyl palmitate 2%, commercial sunscreen with SPF 20, or retinyl palmitate vehicle, and then exposed to either 2 or 4 MED UVB. Twenty-four hours following UVB exposure the intensity of erythema was assessed *in vivo* using a chromameter, and a picture of the erythema appearing on each exposed zone was taken. Both topical retinyl palmitate 2% and SPF 20 sunscreen strongly inhibited the intensity of erythema that developed following 4 MED UVB. In zones exposed to 2 MED UVB, due to the great variability of results, the downward trend is not statistically significant (**Fig 4**). We did not expose human subjects to higher UVB doses than 4 MED for ethical reasons. Although the retinyl palmitate concentration applied on the skin was higher than 2 mg per cm^2 – the concentration used for SPF determinations – **Fig 4*(B)*** suggests an approximate SPF value of 4 for topical retinyl palmitate.

DISCUSSION

Epidermal retinyl esters are considered as the storage form of vitamin A, as (1) this is the case in the liver where 99% of total vitamin A and derivatives are retinyl esters; (2) they account for 85%–90% of total epidermal vitamin A; (3) there is a retinyl ester gradient from the blood to the epidermis; and (4) they are precursors of the other endogenous vitamin A derivatives (Blomhoff *et al*, 1992; Napoli, 1996). Most of the epidermal retinyl esters are depleted by a single exposure to UVB (Sorg *et al*, 1999), indicating that retinyl esters strongly absorb radiation in the UVB range, which is in accordance with their absorption spectrum and extinction coefficient. This destruction is thus triggered by a direct effect of UVB on the retinyl ester molecules and not an indirect effect mediated by UVB-induced oxidative stress (Sorg *et al*, 2002). The energy absorbed by retinyl esters is no longer available to damage other chromophores such as DNA, flavins, or

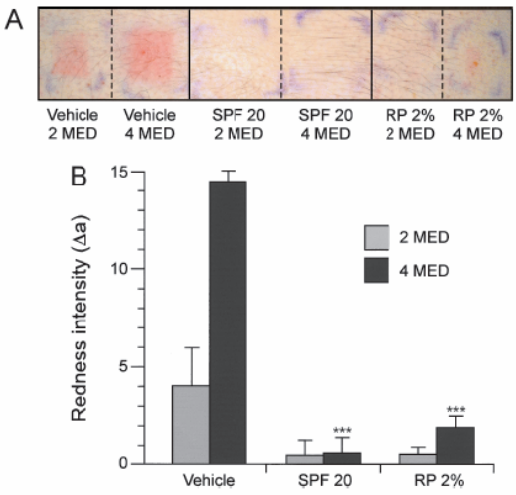

Figure 4. Topical retinyl palmitate inhibits UVB-induced erythema in human skin. Delimited zones of the buttocks of human volunteers were treated and exposed to UVB as described in the legend to **Fig 3**. Twenty-four hours later, pictures were taken from the zones exposed to UVB for one subject (*A*), and the intensity of the observed erythema was analyzed with a chromameter for all six subjects (*B*).

NAD(P)H. We therefore wondered whether these absorbing properties of retinyl esters might have a biologically relevant filter activity. The action spectrum for the formation of thymine dimers – an index of DNA photodamage – has a maximum at 260 nm, corresponding to the maximum of the absorption spectrum of nucleic acids (Matsunaga *et al*, 1991). This is in the UVC range, a wavelength range with no biologic relevance, as UVC is filtered out by the atmosphere. Thymine dimers can still be produced at 300 nm, however, a wavelength received from the sun at the earth's surface. This explains why exposure of the skin to the sun or solar simulators produces epidermal thymine dimers (Clingen *et al*, 1995; Bykov *et al*, 1998). Similarly, the action spectrum for sun-induced erythema has a maximum at 300 nm (Anders *et al*, 1995). On the other hand, retinol and retinyl esters have an absorption maximum at 325 nm, but still absorb UV radiation at 300 nm. Thus it is conceivable that cutaneous retinyl esters, which concentrate in the epidermis, can absorb enough energy from the sun to decrease the formation of epidermal thymine dimers and erythema. The aim of this study was to assess the ability of epidermal retinyl palmitate, loaded in the skin after topical application, to decrease the formation of epidermal thymine dimers and erythema induced by UV radiation. The UV source we used consisted of TL 20 W/12 Phillips tubes (medical UVB), and was shown to be adequate to produce epidermal thymine dimers.

Before applying topical retinyl palmitate *in vivo*, we assessed its physical absorbing properties using an *in vitro* model (Tran *et al*, 2002). According to this model, retinyl palmitate was shown to be as efficient as OMC – a well-known molecule used in sunscreens – in preventing the photobleaching or the photooxidation of molecules encapsulated into liposomes. This indicates that retinyl palmitate, incorporated into phospholipid membranes, acts as a filter able to protect hydrophilic molecules from the destructive action of UVB. This *in vitro* filter effect was subsequently shown to have a biologic relevance *in vivo* both in mice and in human healthy volunteers, as demonstrated by the inhibition by topical retinyl palmitate of thymine dimer formation and erythema appearance following UVB exposure. As the endogenous cutaneous retinyl ester concentration ($\approx$1 μM) is much lower than that obtained atfer topical application ($\approx$100 μM), it is unlikely that endogenous cutaneous vitamin A could significantly prevent DNA damage by its filter property.

In conclusion, these data reveal a new potential role of epidermal retinyl esters, the storage form of epidermal vitamin A: thus, besides serving as precursors of the biologically active forms of epidermal vitamin A, which have antiphotocarcinogenic properties, epidermal retinyl esters also contribute to protecting DNA from UV damage. Retinyl esters are endogenous substances that can easily be loaded in high amounts in the epidermis by topical application; that they possess such an efficient filter action towards solar UV radiation compared to synthetic solar filters is of great interest, due to their lower toxicity, physiologic regulation, and other biologic properties relevant to the prevention of photocarcinogenesis.

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D. Conclusion

De nombreuses études ont permis d'établir un lien entre exposition solaire chronique et prédisposition à développer des cancers de la peau [25, 79-81]. De plus, on a montré que l'épiderme exposé au soleil contient moins d'esters de rétinol (RE) que la peau non exposée, indiquant que les UV induisent une déficience en vitamine A [22], étant donné que les RE constituent l'essentiel de la vitamine A cutanée. Ceci, ainsi que d'autres observations supportent l'hypothèse selon laquelle la déplétion en vitamine A, induite par le soleil, est impliquée dans la pathogenèse de certains cancers de la peau ainsi que de son vieillissement prématuré [24-27]. Plusieurs travaux de C. Tran et O.Sorg montrent que chez la souris hairless, le rétinol (ROL) et ses esters (RE) représentent à eux seuls plus de 99 % du contenu total en rétinoïdes de l'épiderme, dont 90 % sont des RE [5, 22]. Des souris ont été irradiées avec des UVB, étant donné que la longueur d'onde d'absorption maximale du ROL et de RE est de 325 nm. On observe que dix minutes après une dose unique de 1 J/cm^2 d'UVB, les concentrations épidermiques et dermiques en ROL et RE sont fortement diminuées.

La diminution du ROL de l'épiderme atteint un plateau pour des doses d'UVB supérieures à 200 mJ/cm^2, suggérant l'existence de 2 réservoirs de ROL avec des sensibilités distinctes vis-à-vis des UVB. Les mesures des activités des enzymes qui estérifient le ROL ou qui hydrolysent les RE en ROL ne sont pas affectées par une telle irradiation aiguë d'UVB. Cela suggère que la déplétion UVB-induite des rétinoïdes est due à une interaction directe des UVB avec les rétinoïdes. Cette observation est d'autant plus intéressante que la forme majoritaire des rétinoïdes de l'épiderme, les RE, est aussi celle qui est la plus sensible aux UVB.

Après l'exposition aux UVB, la diminution en ROL et RE de l'épiderme de souris hairless persiste pendant au moins 8 heures, puis les niveaux en rétinoïdes commencent à augmenter. Après la destruction due aux UVB, le stock de ROL est reconstitué plus rapidement que les RE, ce qui est logique du fait que ce

processus requiert un apport de ROL provenant de la circulation sanguine, ROL qui est la forme de vitamine A la plus importante du sérum de souris hairless.

Un des moyens pour contrecarrer cette déplétion est le prétraitement avec des rétinoïdes naturels (acide rétinoïque, rétinol et rétinaldehyde) qui induit une augmentation importante en ROL et RE dans l'épiderme de souris hairless [5]. Les rétinol et rétinaldehyde (RAL) topiques augmentent à la fois le ROL et les RE, alors que seuls ces derniers sont augmentés par application d'acide rétinoïque (RA) [5]. Ceci peut s'expliquer par la nature hydrophobe des rétinoïdes qui leur permet de pénétrer dans l'épiderme. Le RAL peut alors être réduit en ROL, dont la majeure partie est ensuite estérifiée par des acides gras. Le RA ne peut être réduit en ROL mais peut induire l'expression d'enzymes telles que la LRAT, laquelle catalyse l'estérification de ROL en RE. Le RAL topique régule de manière plus efficace les ROL et RE de l'épiderme par rapport au ROL, malgré le fait que le RAL doive être d'abord réduit en ROL. Cela est dû au fait que la forte activité de réduction du RAL dans le cytosol n'est pas une étape limitante dans la production de ROL et RE. Cela est aussi probablement dû au fait que le RAL pénètre mieux que le ROL.
A coté de leurs actions sur le contenu en rétinoïdes de l'épiderme, les RA, ROL et RAL topiques induisent une augmentation de 6 à 7 fois de la *cellular retinolbinding protein-1* (CRBP-1), une action médiée par l'oxydation du ROL en RAL puis RA, ce dernier étant connu pour induire cette protéine dans la peau humaine. Chez les souris prétraitées avec des rétinoïdes naturels (RA, ROL, et RAL), 10 minutes après irradiation, la CRBP-1 fonctionnelle reste très induite, alors que les niveaux de ROL ont diminué de 98 % et ont atteint des niveaux inférieurs à ceux des animaux non traités. Ceci montre, que dans ces conditions expérimentales, au moins, la CRBP-1 ne protège pas le ROL de la dégradation induite par les UVB. Le fait que la CRBP-1 constitutive et celle induite par le traitement par le RAL commencent toutes les deux à diminuer plusieurs heures après irradiation UVB ne joue pas en faveur d'une interaction directe entre le rayonnement et la protéine, mais serait plutôt compatible avec une diminution de

l'expression génétique ou une séquestration de la protéine par un complexe multienzymatique impliqué dans la reconstitution du ROL.

Etant donné que le ROL et les RE constituent les précurseurs des formes biologiquement actives de la vitamine A, leur reconstitution après UVB représente un processus physiologique important. Chez les souris non traitées, cette reconstitution prend plus de 24 heures, alors qu'il est accéléré, en particulier pour les RE, si les souris ont été prétraitées avec du RAL. Ceci indique que le prétraitement induit des réponses métaboliques qui persistent après l'irradiation et qui peuvent promouvoir la reconstitution en vitamine A de l'épiderme.

Le métabolisme des rétinoïdes dans l'épiderme humain après application de rétinoïdes topiques a été peu étudié, probablement parce qu'il nécessite le prélèvement de grandes quantités d'épiderme (2 à 4 cm^2) pour le dosage des rétinoïdes, ce qui constitue un acte invasif. Dans ce travail, nous décrivons une méthode de culture ex vivo de peau totale qui offre l'avantage d'utiliser de la peau excisée lors d'opérations de chirurgie plastique. Afin de comparer les valeurs obtenues ex vivo et in vivo, nous avons analysé la pénétration et le métabolisme des rétinoïdes topiques sur ces deux modèles chez la souris hairless. La comparaison entre les résultats obtenus sur des souris in vivo et sur des cellules de Franz (ex vivo) sont similaires, ce qui tendrait à montrer que les résultats que nous obtenons chez l'homme sur le modèle des cellules de Franz sont probablement proches de ceux que l'on obtiendrait sur l'épiderme humain in vivo. Grâce à ce modèle nous démontrons une pénétration et un métabolisme des rétinoïdes dans l'épiderme humain : il n'y a pas de métabolisme pour l'acide rétinoïque qui lie les récepteurs nucléaires, tandis que l'on observe un métabolisme d'oxydation et de réduction pour le rétinal et une augmentation des rétinoïdes endogènes pour le rétinol et les esters rétinyliques. La même méthode a été utilisée afin de démontrer un métabolisme du β-carotène dans l'épiderme humain. Ce métabolisme a été démontré par un autre groupe sur des kératinocytes et des mélanocytes en culture [65]. Dans ce travail nous avons étudié chez la souris hairless in vivo et chez l'homme sur le modèle des cellules

de Franz la conversion du β-carotène en rétinoïdes. Nous avons montré dans ces deux modèles que le β-carotène topique est converti par l'épiderme humain et murin en esters rétinyliques, lesquels constituent la forme de stockage de la vitamine A. Ces observations sont les premières à démontrer un tel métabolisme dans l'épiderme humain. Le β-carotène topique est donc un précurseur de la vitamine A cutanée.

Chez l'homme les esters rétinyliques sont la forme majoritaire des rétinoïdes de l'épiderme, ils sont considérés comme une forme de stockage de la vitamine A. Ils absorbent fortement les UVB (ε_{325} = 50'000 [$(mol/l)^{-1}$ cm^{-1}]), sont photosensibles [22] et donc leur concentration diminue après irradiation par les UVB. Un des moyens de contrecarrer cette déplétion photoinduite de vitamine A cutanée consiste en le prétraitement avec des rétinoïdes naturels. Etant donné que le rétinol et ses esters constituent les précurseurs des formes biologiquement actives de la vitamine A, leur reconstitution après UVB représente probablement aussi chez l'homme un processus physiologique important. Les conséquences biologiques de la déplétion en vitamine A cutanée induite par les UV ne sont pas encore totalement connues, en particulier le rôle de la vitamine A dans les couches supérieures de l'épiderme. On peut imaginer que la vitamine A, et particulièrement les esters rétinyliques qui représentent près de 90 % des rétinoïdes totaux de l'épiderme, pourraient jouer un rôle de filtre UV, d'autant plus efficace après application topique que l'épiderme est chargé en rétinoïdes après traitement par un rétinoïde naturel topique [5]. En absorbant ainsi une partie de l'énergie du rayonnement UV, on diminue leur interaction avec d'autres cibles cellulaires, dont les conséquences pourraient être néfastes pour les cellules.

Afin de tester in vitro l'effet filtre potentiel de molécules lipophiles telles que les rétinoïdes, nous avons utilisé une méthode mise au point dans une étude antérieure [107]. Des liposomes sont utilisés comme modèles de membrane cellulaire et de leur compartiment cytoplasmique interne. Les molécules filtres lipophiles sont intégrées dans les couches de phospholipides et les marqueurs fluorescents Indo-1 et dichlorofluorescine (DCFH) représentent quant à eux les

cibles intracellulaires des UVB. On peut alors analyser la corrélation entre les propriétés spectrales d'un filtre chimique et sa capacité à prévenir la dégradation induite par les UV de molécules intracellulaires. Appliqué au palmitate de rétinyle, les résultats laissent à penser que les esters du rétinol jouent un rôle de filtres endogènes. En effet, in vivo, la différence de formation de thymidine dimère - un marqueur de photodommages de l'ADN - observée 2 heures après exposition aux UVB chez des sujets sains ne peut vraisemblablement être due qu'à un effet filtre, un mécanisme de réparation enzymatique de l'ADN étant difficilement envisageable sur une si courte durée. Ces résultats mettent en évidence un nouveau rôle potentiel des esters rétinyliques dans l'épiderme : en-dehors de leur rôle de stockage de la vitamine A cutanée et de précurseurs des formes biologiquement actives de la vitamine A cutanée, ils contribueraient à la protection de l'ADN contre les effets des UVB. Si les concentrations endogènes de vitamine A et ses esters sont un peu faibles pour réaliser un effet filtre notable, elles peuvent aisément être augmentées par application topique de rétinoïdes naturels tels que le rétinal, le rétinol ou le palmitate de rétinyle [5]. Ceci constitue un réel intérêt vu la bonne tolérance et le rôle préventif potentiel de la vitamine A dans la photocarcinogenèse [108, 109].

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