Oxidative Stress-Independent Depletion of Epidermal Vitamin A by UVA

Olivier Sorg,* Christian Tran,*† Pierre Carraux,* Liliane Didierjean,* Francoise Falson,† and Jean-Hilaire Saurat*

*Department of Dermatology, University Hospital, Geneva, Switzerland; †Pharmacy Department, University of Lyon, France

In hairless mice, epidermal vitamin A (retinol and retinyl esters) is strongly decreased following a single exposure to UVB. Here, using the same mouse model, we studied the effects of UVA on epidermal vitamin A content, lipid peroxidation, and CRBP-I expression, as well as the putative prevention of vitamin A depletion or lipid peroxidation by topical α-tocopherol. An acute exposure to UVA completely depleted epidermal vitamin A with EC_{50} of 0.25 and 0.5 J per cm^2 for retinyl esters and retinol, respectively; these values were 0.1 J per cm^2 for both retinoids under UVB exposure. CRBP-I expression was increased 2-fold 8 h following UVA exposure (10 J per cm^2), and this increase persisted for at least 16 h. A single UVA exposure induced a concentration-dependent epidermal lipid peroxidation (EC_{50} = 3.5 J per cm^2) giving rise to 55.4 ± 4.2 nmol lipid peroxides per g at 20 J per cm^2, whereas UVB, up to 1 J per cm^2, did not increase the basal concentration of 6.7 ± 0.9 nmol lipid peroxides per g. On the other hand, topical menadione induced a concentration-dependent lipid peroxidation, but did not affect vitamin A content. Pretreatment with α-tocopherol (i) did not inhibit UV-induced vitamin A depletion, (ii) completely inhibited the increased lipid peroxidation induced by UVA or menadione, and (iii) accelerated reconstitution of epidermal vitamin A after UVB but not UVA induced depletion. Thus acute UVA induced both epidermal vitamin A depletion and lipid peroxidation, UVB induced only vitamin A depletion, and menadione induced only a lipid peroxidation; topical α-tocopherol prevented lipid peroxidation but not vitamin A depletion. These observations indicate (i) that CRBP-I neither provides protection to UVB- and UVA-induced epidermal vitamin A depletion, nor interferes significantly with reconstitution, and (ii) that the UV-induced vitamin A depletion and lipid peroxidation in mouse epidermis are unrelated processes. UV light does not destroy epidermal vitamin A through an oxidative stress but probably by a photochemical reaction in which UV radiations at about 325 nm give the corresponding activation energy. ***Key words: CRBP/epidermis/hairless mice/lipid peroxidation/ultraviolet/vitamin A. J Invest Dermatol 118:513–518, 2002***

Vitamin A is present in mouse epidermis as free and esterified retinol (Vahlquist, 1982; Vahlquist *et al*, 1985; Törmä and Vahlquist, 1987; Sorg *et al*, 1999). We previously showed that both ROL and RE were depleted by a single UVB exposure, and that a pretreatment with antioxidants (a mixture of ascorbic acid, α-tocopherol, and melatonine) did not prevent this depletion (Sorg *et al*, 1999). UVB (280–320 nm) are much more potent than UVA (320–400 nm) in inducing biologic effects (Bissett *et al*, 1989; Morlière *et al*, 1995), but they account for only 3%–5% of UV radiations from the sun at the earth's surface (Schaefer *et al*, 2000). Moreover, most commercially available sunscreens provide a good protection only in the UVB range, whereas only the most recent ones absorb the short part of UVA (UVA_2, 320–340 nm). There was thus a need to assess the effect of UVA on epidermal vitamin A (retinol) and its storage form (RE). Although the physiologic implications of a low content of epidermal vitamin A are still unclear, a low epidermal vitamin A content was reported in actinic keratoses (Rollman and Vahlquist, 1981), a condition than can lead to squamous cell carcinomas, and, in chronically UVB irradiated hairless mice (Berne *et al*, 1985), photocarcinogenesis (Ananthaswamy and Pierceall, 1990; Nomura *et al*, 1997; Meunier *et al*, 1998). Törmä and colleagues reported several years ago a decrease of epidermal vitamin A, assayed as the sum of ROL and RE, after a single UVA exposure, in hairless mice (Törmä *et al*, 1988). Whereas DNA is a major cellular target of UVB radiations (Mitchell and Nairn, 1989; Kripke *et al*, 1992; Tzung and Rünger, 1998), UVA interact with other chromophores such as NAD(P)(H), melanins, or flavins, leading to the production of free radicals and reactive oxygen species (Linetsky and Ortwerth, 1996; Kvam and Tyrrell, 1997; Yasui and Sakurai, 2000). Primary reactive intermediates such as superoxide (O_2^-) and hydrogen peroxide (H_2O_2) can give rise to the highly oxidant hydroxyl radical (·OH), especially in the presence of transition metals (Cu^+, Fe^{2+}) (Halliwell and Gutteridge, 1999). When hydroxyl radical comes in contact with biological membranes, it initialises the peroxidation of lipids promoted by molecular oxygen. Thus UVA

Manuscript received August 27, 2001; revised November 7, 2001; accepted for publication November 14, 2001.

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

Abbreviations: BHT, butylated hydroxytoluene; RA, all-*trans*-retinoic acid; RAL, all-*trans*-retinaldehyde; RE, all-*trans*-retinyl esters; ROL, all-*trans*-retinol; TPP, triphenylphosphine.

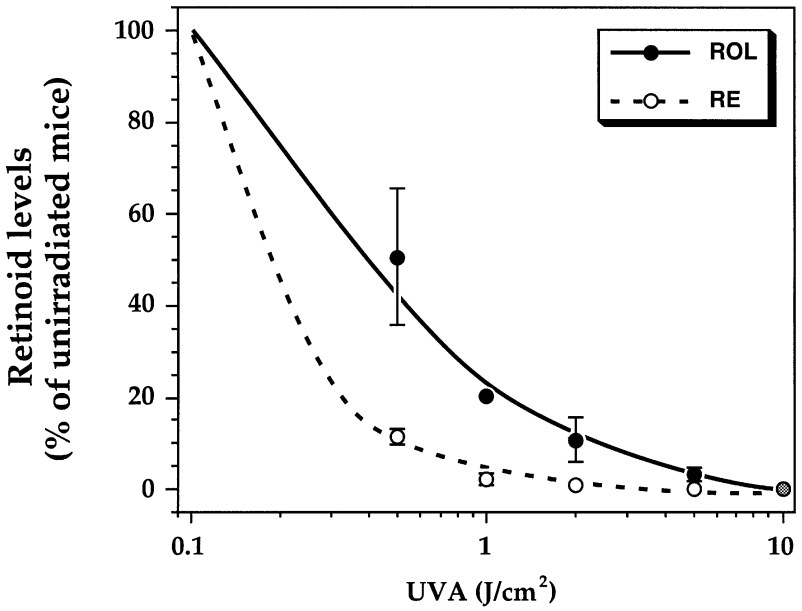

Figure 1. Dose–response curve of epidermal ROL and RE depletion induced by UVA. Hairless mice received a single UVA dose, and were sacrificed 10 min later. ROL and RE contents were determined in the epidermis. Results, which represent the means ± SE of four mice, are expressed as the percentages of ROL and RE that remained in the epidermis after UVA, as compared with unirradiated mice, whose epidermal ROL and RE contents were 188 ± 4 and 2250 ± 594 pmol per g, respectively.

should be able to induce a peroxidation of epidermal lipids via reactive oxygen intermediates (Ogura *et al*, 1991). In such a situation, topical antioxidants that are lipophilic enough to penetrate into the skin, and that present free radical scavenging properties, should prevent UVA-induced lipid peroxidation, as well as UVA-induced biologic effects mediated by lipid peroxidation.

In this study, we examined the effects of a single UVA exposure on epidermal vitamin A (ROL and RE) and lipid peroxidation of hairless mice, and evaluated the link between these two phenomena by blocking epidermal lipid peroxidation with topical α-tocopherol (vitamin E), an endogenous epidermal antioxidant with radical scavenging properties.

MATERIALS AND METHODS

Chemicals Retinaldehyde (RAL) was applied as a 0.05% oil-in-water cream (Didierjean *et al*, 1996); α-tocopherol 0.25% (6 mM) and antioxidants (a mixture of α-tocopherol 0.25% (6 mM) and ascorbic acid 0.5% (28 mM)), all from Sigma (Saint Louis, MO), were dissolved in ethanol:water:polyethylene glycol 400 (3:1:1) (Sorg *et al*, 1999). Reference retinoids and common chemicals were purchased from Sigma and organic solvents from Merck (Darmstadt, Germany).

Topical treatment and UV irradiation of mice For topical treatment, RAL or antioxidants were applied once a day for 3 d on the back of adult SKH1 (h/ h) albino hairless mice, then the mice were washed for tissue harvesting or UV irradiation as previously described (Sorg *et al*, 1999; Tran *et al*, 2001). UVB source was constituted by a series of three Philips TL 20 W tubes providing an irradiance of 1.67 mW per cm^2 in the UVB range (280–320 nm), and 0.2 mW per cm^2 in the UVA range (320–400 nm), as measured by a Waldmann UV-meter; UVA source was a series of three Philips UVA Cleo Performance tubes (40 W) whose radiations were filtered by a glass plate of 3 mm thickness: this source provided an irradiance of 4 mW per cm^2 in the UVA range and was almost devoid of UVB. The sacrifice of mice and the separation of epidermis from dermis by a short heat treatment (30 s; 56°C) for retinoids, tocopherols, and lipid peroxides analysis or dispase digestion for CRBP-I expression were performed as previously described (Sorg *et al*, 1999). Epidermis was frozen in liquid nitrogen and kept at –80°C until retinoid extraction.

Analysis of retinoids Retinoid extraction from mouse epidermis was performed in a mixture of aqueous and organic solvents, then the retinoids were analyzed by reversed-phase HPLC with UV detection as previously described (Sorg *et al*, 1999).

Lipid peroxidation Lipid peroxidation was assessed by determining the concentration of epidermal lipid peroxides by the method of ferrous oxidation version 2 described by Nourooz-Zadeh *et al* (1994) for plasma LDL. Lipid peroxides oxide ferrous ions (Fe^{2+}) into ferric ones (Fe^{3+}), then ferric ions form a complex with xylenol orange, and the optical density is read at 585 nm; in order to distinguish between ferric ions oxidized by lipid peroxides and those from another source, samples are split into two parts, and one of them is incubated with triphenylphosphine (TPP) prior to xylenol orange addition in order to remove lipid peroxides; thus the difference between total and nonspecific signals gives the signal from lipid peroxides. Epidermal samples (~150 mg) were minced with scissors and homogenized in ice-cold methanol containing 5 mM butylated hydroxytoluene (BHT), using a Polytron PT 3100 homogeniser. Sample homogenates were then sonicated and centrifuged, and supernatant was harvested and separated into two 500 μl aliquots; one aliquot was incubated with 50 μl TPP 10 mM in methanol (30 min, room temperature), while 50 μl methanol was added in the other aliquot; 500 μl of a mixture containing 25 mM sulfuric acid, 250 μM ammonium ferrous sulfate (Mohr salt), 100 μM xylenol orange, and 4 mM BHT in 90% methanol, were then added in both aliquots (room temperature), and optical density was read at 585 nm 1 h later. Lipid peroxide concentration was determined using cumene peroxide as a standard (0.5–8 nmol).

Extraction of cellular retinol-binding protein-I To assess epidermal CRBP-I, epidermal samples were homogenized as mentioned for enzymatic activity determination, then proteins were extracted from the cytosolic fraction, and those binding [^{3}H]-retinol were separated by nondenaturing polyacrylamid gel electrophoresis and visualized by autoradiography, as described by Siegenthaler (1990).

Analysis of data Results represent the means ± SE of 3–5 experimental values. The effects of UV irradiation and retinoid pretreatment on retinoid content were assessed by performing an analysis of variance (ANOVA).

RESULTS

Degradation of epidermal vitamin A by UVA A single exposure of mice to UVA induced in 10 min a concentration-dependent decrease of epidermal ROL and RE with EC_{50} of 0.5 J per cm^2 and 0.25 J per cm^2, respectively (**Fig 1**). Thus UVA are slightly less potent in depleting epidermal vitamin A than UVB, whose EC_{50} for both ROL and RE are about 0.1 J per cm^2 (Sorg *et al*, 1999); however, UVA are more efficient than UVB, as UVA-induced vitamin A depletion was total (**Fig 1**), whereas it reached only 90% of RE and 67% of ROL content for UVB (Sorg *et al*, 1999).

Lack of protection by topical antioxidants of UVA-induced retinoid depletion In order to assess the potential protection by topical retinoids or antioxidants on UVA-induced epidermal

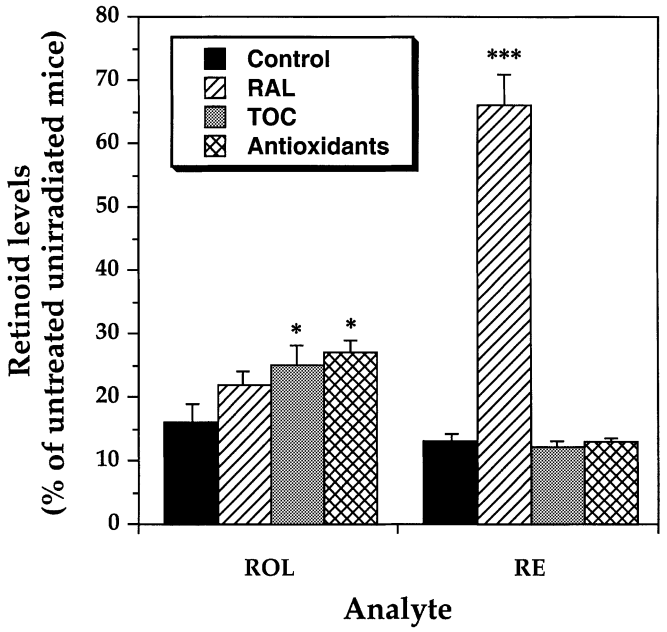

Figure 2. Effects of topical RAL and antioxidants on ROL and RE depletion induced by UVA. Hairless mice were pretreated once a day for 3 d with topical RAL, α-tocopherol (TOC), or antioxidants. Twenty-four hours following the last treatment, they received 5 J per cm^2 UVA, and were sacrificed 10 min later. ROL and RE contents were determined in the epidermis. Results, which represent the means ± SE of four mice, are expressed as the percentages of ROL and RE that remained in the epidermis after UVA, as compared with untreated and unirradiated mice, whose epidermal ROL and RE contents were 314 ± 24 and 1491 ± 291 pmol per g, respectively.

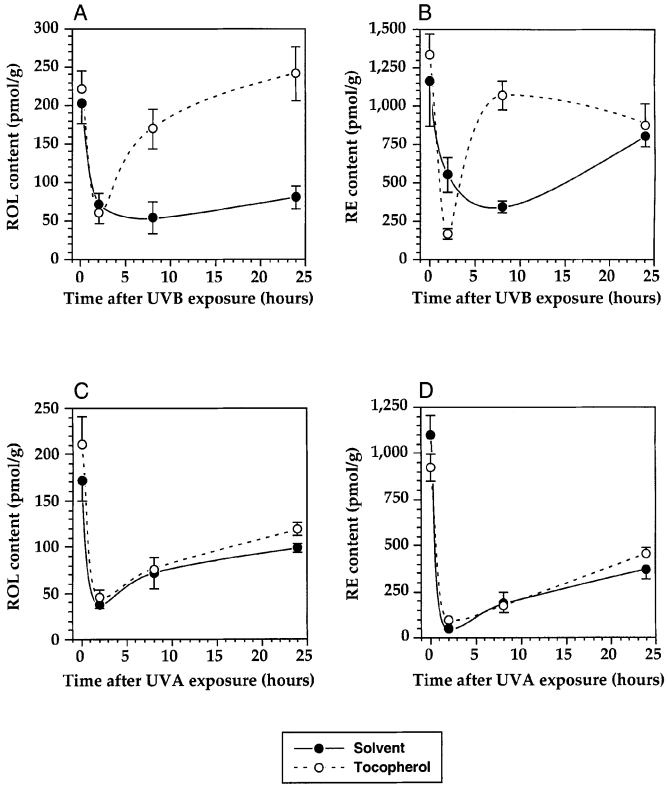

Figure 3. Effect of topical α-tocopherol on the reconstitution of ROL and RE after UV-induced depletion. Hairless mice were pretreated once a day for 3 d with topical α-tocopherol. Twenty-four hours following the last treatment, they received either 1 J per cm^2 UVB (*A*, *B*) or 20 J per cm^2 UVA (*C*, *D*), and were sacrificed 2–24 h later. ROL (*A*, *C*) and RE (*B*, *D*) contents were determined in the epidermis. Results represent the means ± SE of three mice.

vitamin A depletion, the mice were pretreated for 3 d with topical RAL 0.05%, α-tocopherol 0.25%, a mixture of antioxidants (α-tocopherol 0.25% + ascorbic acid 0.5% + melatonine 0.25%), or their respective vehicle, and then exposed to 5 J per cm^2 UVA. These pretreatments did not prevent epidermal vitamin A depletion (**Fig 2**). The apparent protection of RE provided by RAL can be explained by the fact that topical RAL promotes a manifold increase of epidermal RE (Sorg *et al*, 1999): thus, although a high proportion of epidermal RE is destroyed by UVA, the final RE concentration is not very different from that of untreated and nonirradiated mice.

Accelerated reconstitution of epidermal ROL and RE promoted by topical α-tocopherol Although topical α-tocopherol did not protect epidermal ROL and RE from UVA (**Fig 2**) or UVB (Sorg *et al*, 1999) action, we wondered if α-tocopherol could affect the kinetics of epidermal retinoid reconstitution following retinoid depletion induced either by UVA or by UVB. As shown in **Fig 3**(***A–D***), epidermal ROL and RE levels from untreated mice were, 24 h after UVA or UVB irradiation, still much lower than those from nonirradiated mice, indicating a slow spontaneous reconstitution process. Reconstitution was accelerated by pretreatment with α-tocopherol when depletion was induced by UVB (**Fig 3*A***, ***B***). This effect of α-tocopherol on the kinetics of reconstitution was not observed when depletion was induced by UVA (**Fig 3*C***, ***D***).

Effects of UVA on functional CRBP-I expression In previous studies, we showed that epidermal cellular retinol-binding protein I (CRBP-I) expression was slightly and slowly decreased by UVB exposure, whereas ROL and RE were highly and quickly decreased by the same UVB dose. On the other hand, CRBP-I was induced by topical retinoids, and was not decreased by UVB at a time where retinoids were almost completely depleted. We concluded that CRBP-I did not protect epidermal vitamin A from UVB. Here we analyzed the expression of functional epidermal CRBP-I in mice exposed to UVA. As shown in **Fig 4**, CRBP-I expression was increased 8 h and 24 h after UVA exposure (**Fig 1**), whereas vitamin A was depleted.

Effects of UVA and UVB on epidermal lipid peroxidation Because UV irradiation is able to induce an oxidative stress (Axelrod *et al*, 1990; Packer *et al*, 1990; Steenvoorden and van Henegouwen, 1997; Halliwell and Gutteridge, 1999), we wondered if the depletion of vitamin A following UVA and UVB exposure was related to an oxidative stress. We used the peroxidation of epidermal lipids as an index of oxidative stress. Mice exposed to UVB did not show any increase of epidermal lipid peroxides, whose levels were about 7–15 nmol per g, up to UVB doses of 1 J per cm^2 (**Fig 5*A***). In contrast, a single UVA exposure induced a concentration-dependent increase of epidermal lipid peroxides with an EC_{50} of 3.5 J per cm^2, giving rise to an epidermal lipid peroxide concentration of 55 nmol per g, which corresponded to a 5.5-fold increase from basal levels (**Fig 5*B***).

Induction of epidermal lipid peroxidation by menadione Thus we found a stimulus, acute UVA exposure, that leads to vitamin A depletion and lipid peroxidation of mouse epidermis, and another one, acute UVB exposure, that affects only epidermal vitamin A. In order to provide further evidence on the absence of a link between oxidative stress and vitamin A degradation, we looked for a condition that would induce epidermal lipid peroxidation without affecting epidermal vitamin A. Menadione (vitamin K3) is a quinone that penetrates into the skin and possesses oxidant properties. It was a good candidate to induce a lipid peroxidation of

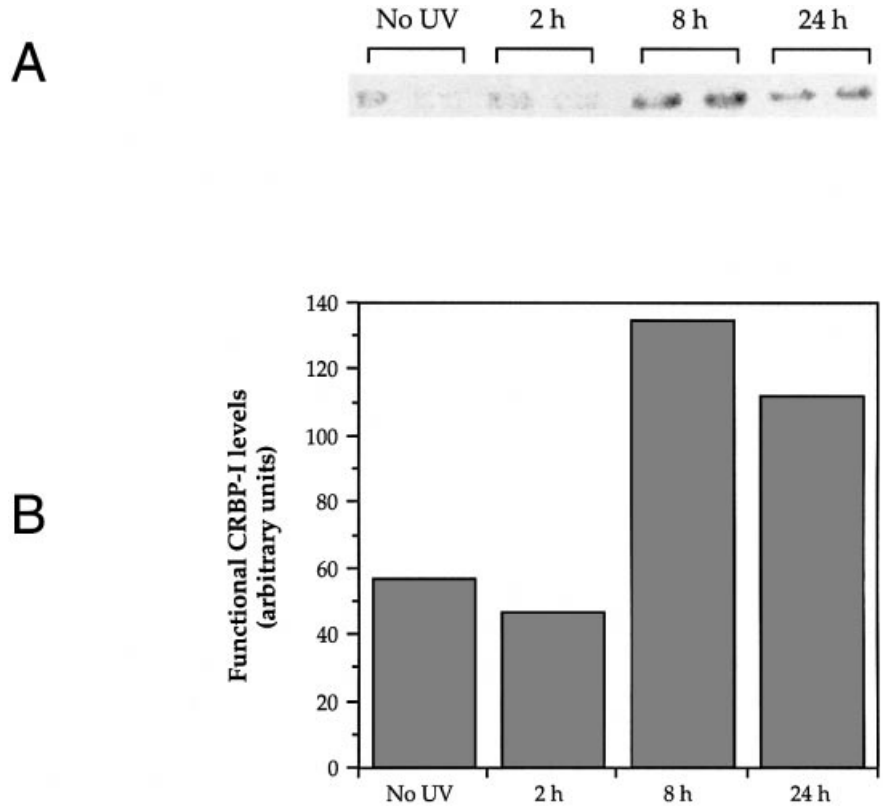

Figure 4. Effect of UVA on CRBP-I. (*A*) Hairless mice were exposed to 10 J per cm^2 UVA and sacrificed 3, 8, or 24 h later. Epidermal CRBP-I was extracted and separated in nondenaturing conditions, and identified by referring to the study reported by Vettermann *et al* (1997) and Sorg *et al* (1999), in which retinoid-binding proteins were assayed in the same conditions. (*B*) Densitometry analysis of blots A performed with a densitometer from Molecular Dynamics and the software ImageQuant.

mouse epidermis. Indeed, when menadione was applied once a day for 3 d on the back of hairless mice, and epidermis harvested 30 min following the last topical treatment, epidermal lipid peroxide levels were increased in a concentration-dependent manner (**Fig 6**). The concentration-dependent curve showed a first plateau at about 50 mM menadione, possibly corresponding to a direct effect due to lipid oxidation by menadione, followed by a second phase that could be due to an inflammation induced by menadione (**Fig 6**). Epidermal vitamin A levels (ROL and RE) were assayed in the same conditions with 50 mM menadione; as shown in **Table I**, epidermal ROL and RE were not affected by menadione.

Prevention of epidermal lipid peroxidation by topical α-tocopherol The lipid peroxidation induced by UVA is probably due to a free radical chain reaction initiated by the removal of a proton and an electron from a lipid, and propagated by the addition of molecular oxygen (a biradical) to the lipid radical (Ogura *et al*, 1991; Jurkiewicz and Buettner, 1996; Halliwell and Gutteridge, 1999). Thus hairless mice were pretreated with topical α-tocopherol, a classical endogenous free radical scavenger, before inducing the peroxidation of epidermal lipid by UVA or topical menadione. At the time of UVA irradiation, i.e., 4 h following the last topical treatment, epidermal content of α-tocopherol was increased from 5.0 ± 0.4 nmol per g to 245 ± 22 nmol per g. As shown in **Fig 7**, topical α-tocopherol completely suppressed the increase of epidermal lipid peroxides induced either by a single UVA dose of 20 J per cm^2 or by a 3 d topical treatment with 50 mM menadione.

DISCUSSION

In previous studies, we have analyzed the depletion of epidermal vitamin A induced by UVB; in particular, we observed that neither topical antioxidants (Sorg *et al*, 1999) nor a several-fold induction of the cellular retinol-binding protein by topical retinaldehyde (Tran *et al*, 2001) could protect epidermal vitamin A from UVB-induced depletion (Sorg *et al*, 1999). The mechanism of this depletion has not been established and current belief rather implicates a direct effect of UVB on ROL and RE via their chain containing poly conjugated dienes. Although the most deleterious consequences of UV exposure, i.e., skin tumor induction, is mainly attributed to wavelengths between 280 nm and 320 nm, corresponding to UVB (Wulf *et al*, 1989), vitamin A absorbs UV light with a maximum at 325 nm, corresponding to short UVB or long UVA. Thus, UVA, which represent 95%–97% of UV radiations from the sun at the earth's surface (Schaefer *et al*, 2000), could greatly contribute to epidermal vitamin A depletion under acute sun exposure. In fact we have found that UVA were almost as potent as, and even more efficient than, UVB in depleting epidermal vitamin A. Because UVA were shown to induce an oxidative stress in skin (Gonzalez and Pathak, 1996; Steenvoorden and van Henegouwen, 1997; Podda *et al*, 1998), this suggests that vitamin A depletion might be in part an indirect consequence of UV-induced oxidative stress. The purpose of this study was to examine the link between oxidative stress and epidermal vitamin A depletion. During UV-induced oxidative stress in biologic tissues, lipid molecules from phospholipid membranes lose protons and free electrons, giving rise to lipid radicals. These radicals can react with molecular oxygen, a biradical, to produce lipid peroxyl radicals that react with other lipids to give lipid hydroperoxides, which are relatively stable, as well as other lipid radicals. Thus the chain reaction can continue as long as there is enough oxygen in the system, and lipid hydroperoxides accumulate (Halliwell and Gutteridge, 1999). For this reason we used lipid hydroperoxides as an index of oxidative stress upon UV exposure. Our results clearly show an absence of correlation between oxidative stress and vitamin A depletion in mouse epidermis. Thus (i) acute UVA exposure induced both vitamin A depletion and lipid peroxidation; (ii) acute UVB induced vitamin A depletion, but did not increase lipid peroxidation; (iii) topical menadione increased lipid peroxidation without affecting vitamin A; and (iv) topical antioxidants prevented efficiently epidermal oxidative stress induced either by UVA or by menadione, but did not provide any protection against vitamin A depletion induced either by UVA or by UVB.

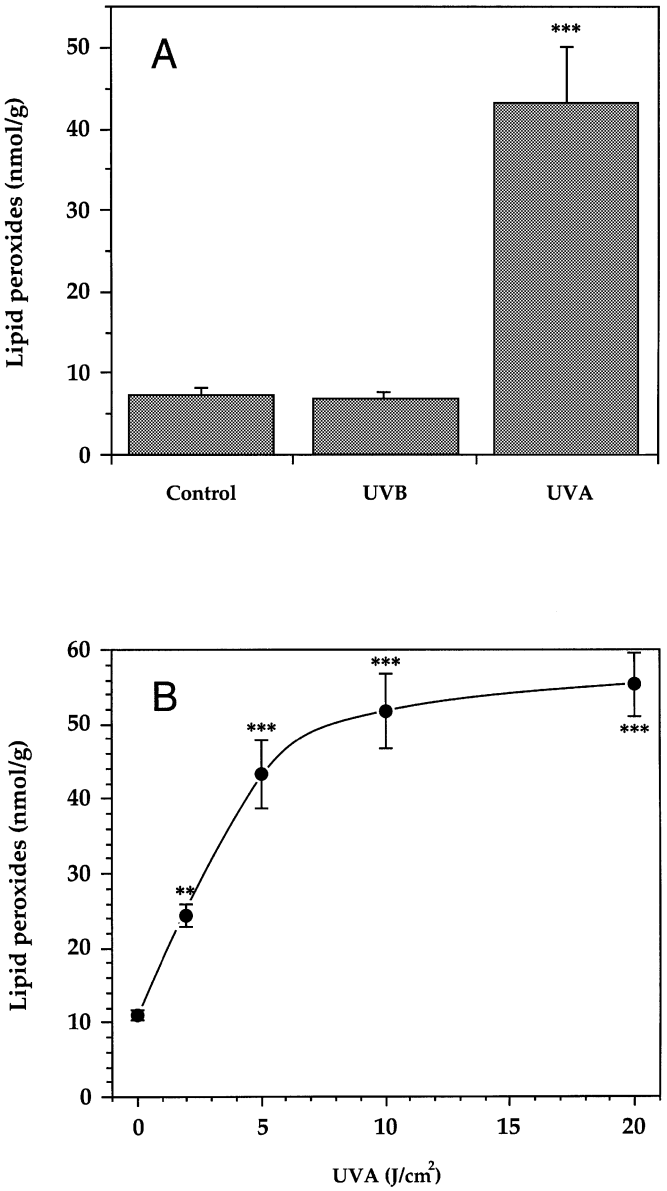

Figure 5. Lipid peroxidation induced by UVA and UVB. (*A*) Hairless mice received either 1 J per cm^2 UVB or 20 J per cm^2 UVA, and were sacrificed 10 min later. (*B*) Hairless mice received a single UVA dose comprised between 2 and 20 J per cm^2, and were sacrificed 10 min later. Lipid peroxides levels were determined in the epidermis (*A*, *B*). Results are expressed as means ± SE of five (*A*) or three (*B*) mice. Values significantly higher than unirradiated controls were indicated (**p > 0.01; ***p < 0.001).

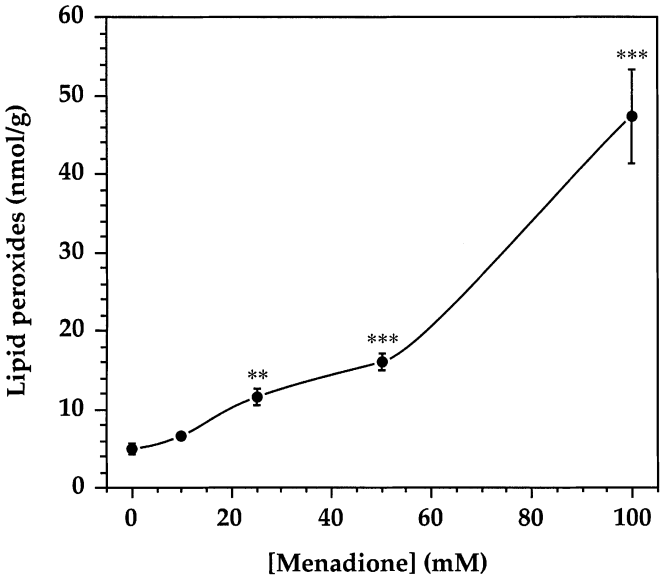

Figure 6. Lipid peroxidation induced by menadione. Topical menadione (10–100 mM) was applied on the back of hairless mice once a day for 3 d, and the mice were sacrificed 30 min later. Lipid peroxides levels were determined in the epidermis. Results are expressed as means ± SE of three mice. Values significantly higher than unirradiated controls were indicated (**p > 0.01; ***p < 0.001).

Table I. Effect of topical menadione on epidermal vitamins A and E[a]

Treatment	ROL	RE	TOC
Solvent	116 ± 10	1294 ± 103	1889 ± 122
Menadione	141 ± 7	1471 ± 141	1787 ± 107

[a]Topical menadione (50 mM) or its solvent were applied once a day for 3 d on the back of hairless mice. The mice were sacrificed 30 min after the last treatment, and epidermal content of vitamin A (ROL and RE) and α-tocopherol (vitamin E) were determined. Results, which are the means ± SE of six mice, are expressed in pmol per g.

The reconstitution of epidermal vitamin A was accelerated by topical α-tocopherol following depletion induced by UVB; in contrast, α-tocopherol did not accelerate reconstitution after UVA-induced depletion. The reconstitution process requires the uptake of circulating vitamin A (mainly as ROL bound to serum retinol-binding protein) by the dermis, and its diffusion to the epidermis (which is devoid of blood vessels), and then esterification by the epidermis of most part of retinol with free fatty acids (Sorg *et al*, 1999). The uptake of vitamin A by epidermal cells is not well understood, but might require CRBP-I; thus we wondered if this binding protein is affected by UV exposure in this model. We did not found a clear relationship between the level of CRBP-I and either the depletion or the kinetics of vitamin A reconstitution; thus, in UVB-irradiated epidermis, there was a decrease in CRBP-I (Sorg *et al*, 1999), whereas the levels of this protein were increased in UVA-irradiated epidermis. These observations indicate that CRBP-I neither provides protection to UVB- and UVA-induced epidermal vitamin A depletion, nor interferes significantly with reconstitution.

Reconstitution was accelerated by pretreatment with α-tocopherol, which suggests that vitamin A uptake by epidermal cells is sensitive to oxidative stress; this effect was demonstrated only after UVB-induced depletion. We explain this observation as follows: UVA, but not UVB, increased epidermal lipid peroxides in our model, which indicates a stronger induction of oxidative stress. It is thus possible that topical α-tocopherol was unable to counterbalance the oxidative stress induced by UVA and therefore rescue the biochemical pathway involved in the reconstitution of vitamin A; this phenomenon warrants further studies.

Altogether, our results, summarized in **Fig 8**, indicate that the acute vitamin A depletion and lipid peroxidation in mouse epidermis are unrelated processes. Vitamin A is probably destroyed by UV light in a photochemical reaction in which UV radiations at about 325 nm give the corresponding activation energy. On the other hand, the peroxidation of epidermal lipids could be mediated, in our model, by a chromophore absorbing in relatively long UVA, i.e., quite different from 325 nm. This would explain why in our model, the prevention of UVA-induced lipid peroxidation did not prevent UVA-induced vitamin A destruction. The biologic consequences of the depletion of epidermal vitamin A induced

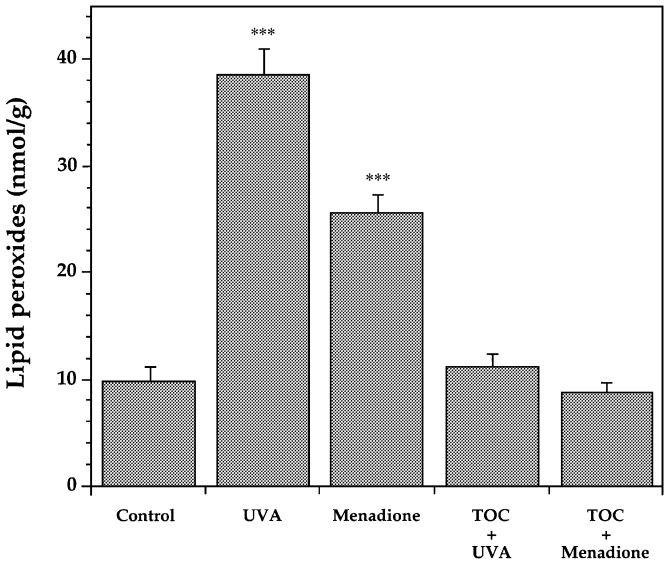

Figure 7. Protection by α-tocopherol of lipid peroxidation. Epidermal lipid peroxidation was induced in hairless mice either by acute UVA irradiation (10 J per cm^2) or by topical menadione 50 mM (once a day for 3 d). Half of the mice that were exposed to UVA or menadione were treated with topical α-tocopherol once a day for 3 d. In the case of UVA irradiation, mice received 10 J per cm^2 UVA 4 h after the last topical treatment; in the case of menadione, α-tocopherol was applied 30 min before menadione. Mice were sacrificed 10 min after UVA exposure or 30 min after the last treatment with menadione, then lipid peroxide levels were determined in the epidermis. Results are expressed as means ± SE of four mice. Values significantly higher than unirradiated and untreated controls were indicated (***$p < 0.001$).

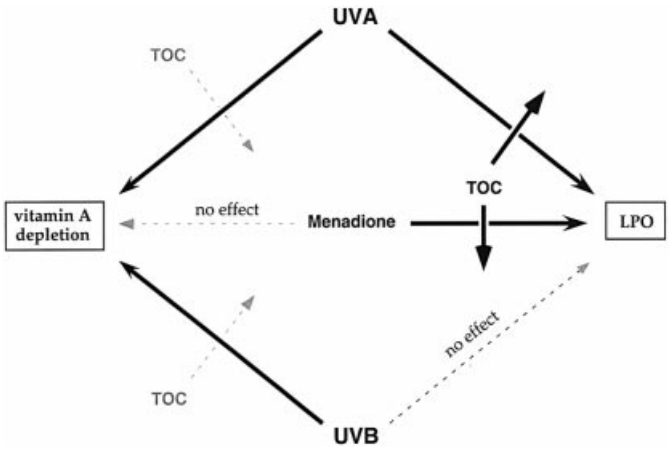

Figure 8. Scheme summarizing the results obtained with UV irradiation or topical menadione on vitamin A (ROL and RE) and lipid peroxides in mouse epidermis. The black arrows indicate an effect (UVA, UVB, menadione) or a protection (tocopherol). The gray arrows in dotted lines indicate an absence of effect (UVB, menadione) or an absence of protection (tocopherol).

by UVA and UVB are not well understood. In particular, the role of vitamin A in the upper layers of the epidermis (if any) is unknown. Because vitamin A absorbs UV light, its destruction under UV exposure prevents the energy used for this process to activate other cellular targets whose consequences could be deleterious to the cells. In this case, in a skin pretreated with topical retinoids in order to induced an accumulation of epidermal retinoids (Tran *et al*, 2001), vitamin A (especially RE, which represent about 85% of epidermal vitamin A (Sorg *et al*, 1999)) could act as a UV filter by loading the epidermis with a high amount of RE (Tran *et al*, 2001), and thus would be beneficial to the skin in the case of a moderate UV exposure.

REFERENCES

Ananthaswamy HN, Pierceall WE: Molecular mechanisms of ultraviolet radiation carcinogenesis. *Photochem Photobiol* 52:1119–1136, 1990

Axelrod M, Serafin D, Klitzman B: Ultraviolet light and free radicals: An immunologic theory of epidermal carcinogenesis. *Plast Reconstr Surg* 86:582–893, 1990

Berne B, Staberg B, Vahlquist A: Skin cancer and epidermal vitamin A levels in UV-irradiated hairless mice. In: Saurat JH, ed. *Retinoid Symposium, Geneva, 1984.* Basel: Karger, 1985:pp. 252–255

Bissett DL, Hannon DP, Orr TV: Wavelength dependence of histologic, physical and visible changes in chronically UV-irradiated hairless mice skin. *Photochem Photobiol* 50:763–769, 1989

Didierjean L, Carraux P, Grand D, Sass JO, Nau H, Saurat JH: Topical retinaldehyde increases skin content of retinoic acid and exerts biological activity in mouse skin. *J Invest Dermatol* 107:714–719, 1996

Gonzalez S, Pathak MA: Inhibition of ultraviolet-induced formation of reactive oxygen species, lipid peroxidation, erythema and skin photosensitization by polypodium leucotomos. *Photodermatol Photoimmunol Photomed* 12:45–56, 1996

Halliwell B, Gutteridge JMC: *Free Radicals in Biology and Medicine*. Oxford: Oxford University Press, 1999

Jurkiewicz BA, Buettner GR: EPR detection of free radicals in UV-irradiated skin: mouse versus human. *Photochem Photobiol* 64:918–922, 1996

Kripke ML, Cox PA, Alas LG, Yarosh DB: Pyrimidine dimers in DNA initiate systemic immunosuppression in UV- irradiated mice. *Proc Natl Acad Sci USA* 89:7516–7520, 1992

Kvam E, Tyrrell RM: Induction of oxidative DNA base damage in human skin cells by UV and near visible radiation. *Carcinogenesis* 18:2379–2384, 1997

Linetsky M, Ortwerth BJ: Quantitation of the reactive oxygen species generated by the UVA irradiation of ascorbic acid glycated lens proteins. *Photochem Photobiol* 63:649–655, 1996

Meunier L, Raison-Peyron N, Meynadier J: Immunosuppression photo-induite et cancers cutanès. *Rev Med Interne* 19:247–254, 1998

Mitchell DL, Nairn RS: The biology of the (6–4) photoproduct. *Photochem Photobiol* 49:805–819, 1989

Morlière P, Moysan A, Tirache I: Action spectrum for UV-induced lipid peroxidation in cultured human skin fibroblasts. *Free Radic Biol Med* 19:365–371, 1995

Nomura T, Nakajima H, Hongyo T, *et al*: Induction of cancer, actinic keratosis, and specific p53 mutations by UVB light in human skin maintained in severe combined immunodeficient mice. *Cancer Res* 57:2081–2084, 1997

Nourooz-Zadeh J, Tajaddini-Sarmadi J, Wolff SP: Measurement of plasma hydroperoxide concentrations by the ferrous oxidation-xylenol orange assay in conjunction with triphenylphosphine. *Anal Biochem* 220:403–409, 1994

Ogura R, Sugiyama M, Nishi J, Haramaki N: Mechanism of lipid radical formation following exposure of epidermal homogenate to ultraviolet light. *J Invest Dermatol* 97:1044–1047, 1991

Packer L, Fuchs J, Leipala J, Witt E, Marenus K, Maes D, Smith WP: UV effects on skin antioxidants. *Clin Res* 38:672A, 1990

Podda M, Traber MG, Weber C, Yan LJ, Packer L: UV-irradiation depletes antioxidants and causes oxidative damage in a model of human skin. *Free Radic Biol Med* 24:55–65, 1998

Rollman O, Vahlquist A: Cutaneous vitamin A levels in seborrheic keratosis, actinic keratosis, and basal cell carcinoma. *Arch Dermatol Res* 270:193–196, 1981

Schaefer H, Chardon A, Moyal D: Photoprotection of skin against ultraviolet damage. *Meth Enzymol* 319:445–465, 2000

Siegenthaler G: Gel electrophoresis of cellular retinoic acid-binding protein, cellular retinol-binding protein, and serum retinol-binding protein. *Meth Enzymol* 189:299–307, 1990

Sorg O, Tran C, Carraux P, Didierjean L, Saurat JH: Retinol and retinyl ester epidermal pools are not identically sensitive to UVB irradiation and antioxidant protective effect. *Dermatology* 199:302–307, 1999

Steenvoorden DP, van Henegouwen GM: The use of endogenous antioxidants to improve photoprotection. *J Photochem Photobiol B* 41:1–10, 1997

Törmä H, Vahlquist A: Retinol esterification by mouse epidermal microsomes: evidence for acyl-CoA: retinol acyltransferase activity. *J Invest Dermatol* 88:398–402, 1987

Törmä H, Berne B, Vahlquist A: UV irradiation and topical vitamin A modulate retinol esterification in hairless mice epidermis. *Acta Derm Venereol* 68:291–299, 1988

Tran C, Sorg O, Carraux P, Didierjean L, Saurat JH: Topical delivery of retinoids counteracts the UVB-induced epidermal vitamin A depletion in hairless mouse. *Photochem Photobiol* 73:425–431, 2001

Tzung TY, Rünger TM: Assessment of DNA damage induced by broadband and narrowband UVB in cultured lymphoblasts and keratinocytes using the comet assay. *Photochem Photobiol* 67:647–650, 1998

Vahlquist A: Vitamin A in human skin. I. Detection and identification of retinoids in normal epidermis. *J Invest Dermatol* 79:89–93, 1982

Vahlquist A, Törmä H, Rollman O, Berne B: Distribution of natural and synthetic retinoids in the skin. In: Saurat JH, ed. *Retinoids New Trends in Research and Therapy*. Basel: Karger, 1985: pp. 159–167

Vettermann O, Siegenthaler G, Winter H, Schweizer J: Retinoic acid signaling cascade in differentiating murine epidermal keratinocytes. Alterations in papilloma- and carcinoma-derived cell lines. *Mol Carcinog* 20:58–67, 1997

Wulf HC, Poulsen T, Davies RE, Urbach F: Narrow-band UV radiation and induction of dermal elastosis and skin cancer. *Photodermatol* 6:44–51, 1989

Yasui H, Sakurai H: Chemiluminescent detection and imaging of reactive oxygen species in live mouse skin exposed to UVA. *Biochem Biophys Res Commun* 269:131–136, 2000