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Hydrolysis of RRR-α-tocopheryl acetate (vitamin E acetate) in the skin and its UV protecting activity (an in vivo study with the rat)

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Abstract

Vitamin E acetate is often used rather than vitamin E as an ingredient of skin care products and dermatological preparations, because it lacks the free phenolic OH group. However, because of this the acetate as such is biologically inactive. In spite of this intrinsic inactivity, the skin is protected against the harmful effects of sunlight after topical application of vitamin E acetate. Therefore it is supposed that hydrolysis takes place in the skin and that the reaction product, the radical scavenger vitamin E, is responsible for the protection observed.

In this in vivo study with the rat, we have investigated the hydrolysis of RRR-α-tocopheryl acetate (vitamin E acetate) in the epidermis in relation to UV radiation protection. (As a measure of protection, we used the UV-induced binding of 8-methoxypsoralen to epidermal biomacromolecules.)

After a period of 5 h from a single application of vitamin E acetate, hydrolysis into free vitamin E was not observed. No protection was found at this time point, corresponding with the absence of vitamin E.

After treatment for 5 days, consisting of one topical application daily, the percentage of acetate present in the stratum corneum which was hydrolysed into free vitamin E was less than 1%, whereas the corresponding value for the viable layer of the epidermis was about 5%.

The hydrolysis of vitamin E acetate in the epidermis proceeded very slowly. As a result, the absolute amount of free vitamin E, found in the total epidermis after treatment for 5 days with the acetate, was only a few times higher than the normal level. Yet, this very small amount of free vitamin E proved to be sufficient for maximal protection in this animal model.

The results show that vitamin E acetate acts as a prodrug, which very slowly releases minute amounts of active vitamin E.

Keywords: Vitamin E; RRR-α-tocopherol; RRR-α-tocopheryl acetate; Vitamin E acetate; Sun protector; 8-Methoxypsoralen

1. Introduction

Reactive intermediates formed in sunlight exposed skin, e.g. singlet oxygen and free radicals, whether or not coupled to oxygen, are an important cause of deleterious effects on this organ. This holds for direct effects, caused by sunlight alone [1–6], as well as for those which result from the combination of a phototoxic xenobiotic and light [7]. These reactive intermediates damage cell membranes by lipid peroxidation and react with proteins, e.g. enzymes and with DNA/RNA.

In addition to sunscreens, which decrease the penetration into the skin of UVB (290–320 nm) and UVA (320–400 nm) light, scavengers of reactive intermediates appear to be promising with regard to the suppression or retardation of the occurrence of deleterious effects, e.g. skin aging and cancer.

RRR-α-tocopherol (vitamin E) has an important function with regard to the protection of the skin [8,9]. The protective activity of vitamin E is based on its radical [10] and singlet oxygen [11–15] scavenging activity. Tocopherols are therefore currently receiving attention as constituents of cosmetic preparations to prevent damage by overexposure to sunlight.

Because they only absorb a small part of the UVB and are transparent to UVA, it is probable that the absorption of radiation is only marginally responsible for their protective effect against sunlight. The scavenging of reactive intermediates formed by UV radiation, seems to be more probable [16]; the free phenolic OH group in the tocopherols plays an essential role in this.

Vitamin E acetate is often used rather than vitamin E as an ingredient of skin care products and dermatological preparations. One reason may be that oxidation is not a problem with the acetate, because it lacks the free phenolic OH group. However, because of this the acetate as such is biologically

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inactive. In spite of this intrinsic inactivity, the skin is protected against the harmful effects of sunlight after topical application of vitamin E acetate. Therefore it is supposed that hydrolysis into free tocopherol occurs in the skin. To what extent hydrolysis takes place in vivo is the subject of this study. Besides, it was investigated whether the amount of free vitamin E found could account for the protection observed.

2. Materials and methods

2.1. Materials

8-[methyl-^{3}H]methoxypsoralen (8-[Methyl-^{3}H]MOP), specific activity 81 Ci mmol^{-1} (Amersham International plc, Amersham, UK) was dissolved in ethanol (50 μCi mmol^{-1}). Ethanol and unlabelled 8-MOP (Aldrich-Chemical Co., Inc., Milwaukee, USA) were added to obtain a 2.27×10^{-3} M 8-MOP solution with a specific activity of 1.33 μCi ml^{-1}.

RRR-α-tocopherol (αTocOH), RRR-α-tocopheryl acetate (αTocOAc) and RRR-γ-tocopheryl acetate (γTocOAc) were obtained from Henkel KGaA (Düsseldorf, Germany); γTocOAc was used as an internal standard for high performance liquid chromatography (HPLC) analysis.

Demineralized water was distilled in an all-glass apparatus before use. Organic solvents were "chemically pure"; their quality was verified by refractive index measurement. Other chemicals were used as purchased.

2.2. Animals

Male Wistar derived rats (200–220 g; TNO, Netherlands), whose backs were shaven under brief ether anaesthesia, were used. (If necessary shaving was repeated during the 5 day experiment.)

2.3. HPLC analysis

For the quantitative determination of αTocOAc and α-TocOH, alone or in the presence of each other, HPLC analysis was used. By means of a Promiss autosampler, samples were taken from glass vials and injected into an HPLC apparatus. The latter consisted of an LKB 2150 solvent delivery system, connected to a column (200 mm × 3.8 mm; Lichrospher RP8, Chrompack, Middelburg, Netherlands) and an LKB 215 variable wavelength detector set at 290 nm. Chromatographic data were processed by an HP3390A integrator (Hewlett Packard).

With acetonitrile–water (90:10) as the mobile phase (flow speed, 0.8 ml min^{-1}), the retention times were as follows: 14.4 min (γTocOAc, internal standard), 12.5 min (α-TocOH) and 17.3 min (αTocOAc). The detection limits were 0.5 μg ml^{-1} (αTocOAc) and 0.25 μg ml^{-1} (α-TocOH).

2.4. Determination of αTocOH and αTocOAc in epidermal material

2.4.1. Total epidermis: stratum corneum plus viable part

The epidermis was separated from the dermis by placing a piece of isolated skin (approximately 10 cm^2) in 2 M KI for 1 h at room temperature [17]. The epidermis was then removed with a scalpel. The internal standard, 25 μl γ-TocOAc (1–10 μg γTocOAc per millilitre of acetonitrile), was added to the isolated epidermal material and the tissue was extracted with 10 ml of acetonitrile for 20 h at room temperature. Thereafter, the sample was centrifuged (100g) and the supernatant was collected; the latter contained more than 99% of αTocOAc or αTocOH. The residue, the epidermal material, was dried at 80 °C and weighed.

The amounts of αTocOAc and αTocOH in the extract were determined by HPLC and expressed in nanograms per milligram of epidermis.

2.4.2. Viable part only

Immediately after the rat was sacrificed, the stratum corneum was stripped off with adhesive tape. (The efficacy of this procedure was verified by attenuated total reflectance IR spectroscopy [18].) Isolation of the remainder of the epidermis and the procedure followed thereafter are described in Section 2.4.1.

2.5. UVA exposure

Philips TL 80/10R lamps (spectral region 345–410 nm; peak at 370 nm; Philips, Eindhoven, Netherlands) were used. The overall light intensity was 60 W m^{-2}, as measured in close proximity to the rats with a UVX radiometer equipped with a UVX-36 sensor (Ultraviolet Products Ltd., Cambridge, UK). The sensitivity range of the UVX-36 sensor was between 300 and 400 nm with a maximum at 360 nm.

2.6. Photobinding of 8-MOP to skin biomacromolecules

Shortly before the 8-MOP application, rats were anaesthetized with pentobarbitone sodium (intraperitoneal (i.p.) injection of a solution of 15 mg pentobarbitone sodium in 250 μl water–ethanol–propyleneglycol (7:1:2)) and placed on a thermostatically controlled plate (37 °C). 8-[Methyl-^{3}H]MOP (0.7 ml) (2.27×10^{-3} M, 1.33 μCi ml^{-1}) in ethanol was evenly applied (approximately 0.1 μmol cm^{-2}) to two areas of the shaven back of each rat. (The position of the two areas was symmetrical with respect to the backbone.) One of the areas had been pretreated with a solution of either αTocOAc or αTocOH in ethanol and the other with the pure solvent only. After a period of 15 min after 8-MOP application, the rats were UVA exposed for 1 h (216 kJ m^{-2}). Immediately thereafter they were sacrificed and the following procedure was followed with each rat.

The two areas of dorsal skin mentioned above were isolated. With each of these two pieces of skin, the epidermis

was separated from the dermis (Section 2.4.1). Unbound radioactivity was removed from the two samples of epidermal material by extracting with methanol–water (4:1) for 16 h, followed by washing once with the same mixture and centrifugation [19]. The two pellets of extracted epidermis contained irreversibly bound 8-MOP only. Each of the two samples of extracted epidermis was separated into two fractions, one containing proteins (together with nucleic acids) and the other lipids [19]. The amount of bound 8-MOP in each fraction was determined by radioactivity measurements with a Packard Tri-Carb 4640 scintillation counter. The amount of lipids was determined by the method of Zollner and Kirsch [20] and the protein content according to Lowry et al. [21]. 8-MOP photobinding in the αTocOAc (or αTocOH) treated part of the skin was expressed as the percentage of that found in the control side to which only ethanol was applied.

2.7. Experiments

2.7.1. Transport in (horizontal) and through (penetration) the epidermis; αTocOAc vs. αTocOH

Ten rats were taken with shaven dorsal skin. To approximately 12 cm^2 of the left side of the back of each rat, ethanol (35 $\mu l\ cm^{-2}$) was applied containing both 2.5% αTocOAc and 2.5% αTocOH. An equal surface on the right side was treated with ethanol only (35 $\mu l\ cm^{-2}$). After 5 h, two rats were killed and from the left and right sides of their dorsal skin a piece was taken. (With each rat, the pieces of skin taken had an equal surface; their position was symmetrical with respect to the backbone and at the same distance from the latter; mirror image.) The determination of αTocOAc and αTocOH in the total epidermis was performed according to Section 2.4.1. During the following 4 days the whole procedure was repeated with the remaining 8, 6, 4 and 2 rats.

2.7.2. Amount of αTocOAc and αTocOH in the epidermis after a single application of 0.25% αTocOAc

To the shaven back (approximately 20 cm^2) of five rats, 0.25% αTocOAc in ethanol was applied evenly (700 μml each; 35 $\mu l\ cm^{-2}$). After 1, 2, 3, 4 and 5 h, one rat was sacrificed and from both the left and right sides of the dorsal skin two equal pieces were isolated. Each piece formed a symmetrical pair (mirror image) with one from the opposite side. In one pair of symmetrical parts, the amounts of αTocOAc and αTocOH were determined according to Section 2.4.1; with the other pair, the procedure in Section 2.4.2 was followed.

2.7.3. Amount of αTocOAc and αTocOH in the epidermis after daily application of 0.25% αTocOAc

To approximately 12 cm^2 of the left side of the shaven dorsal skin of ten rats, 0.25% αTocOAc in ethanol (35 $\mu l\ cm^{-2}$) was applied and to approximately 12 cm^2 of the right side the same amount of ethanol only. After 5 h, two rats were sacrificed and the left and right sides of their dorsal skin were isolated separately. The left was divided into two parts. In one part of the left side, the amounts of αTocOAc and αTocOH were determined according to Section 2.4.1 (concentration in total epidermis); with the other part, the procedure in Section 2.4.2. was followed (concentration in viable layer). In the right part, treated with ethanol only, the amounts of αTocOAc and αTocOH were determined according to Section 2.4.1 (concentration in total epidermis). Over the next 4 days the whole procedure was repeated with the remaining 8, 6, 4 and 2 rats.

2.7.4. Inhibition of 8-MOP photobinding

(1) On each of 5 consecutive days, 0.25% αTocOAc (or αTocOH) in ethanol was applied to approximately 12 cm^2 of the left side of the shaven back (35 $\mu l\ cm^{-2}$) of six Wistar rats. An equal surface of the right side received pure ethanol (35 $\mu l\ cm^{-2}$) only. After 5 h from the last application, the extent of photobinding in both skin sides was determined by following the procedure described in Section 2.6. The amount of αTocOH present in the total epidermis, 5 h after the last application to the left side, was also determined.

(2) The same as (1), but 5 h after a single application of αTocOAc (or αTocOH). With αTocOH, not only 0.25%, but also 0.01%, 0.005% and 0.002% were investigated in this way.

3. Results

At the beginning of each day, ethanol containing an equal amount of αTocOAc and αTocOH was applied to the left side of the dorsal skin (Section 2.7.1). The isolation of αTocOAC and αTocOH from the side of application at the end of days 1, 2, 3, 4 and 5 showed that the recovery of both compounds was equal. For example, if the cumulative amount of αTocOAc (and of αTocOH) applied to the skin is 100%, approximately 10% was found at the end of day 1 and approximately 20% after 5 days. From this, it was concluded that αTocOAc and αTocOH behave similarly with regard to transport through the epidermis (i.e. penetration).

Also their horizontal migration in the epidermis is the same. This follows from the results presented in Table 1; at the end of each of the 5 consecutive days, almost the same amount of αTocOAc and αTocOH had migrated to the right side, opposite to the side of the skin where both compounds were applied.

Therefore, it can be concluded that, in the skin of the right side, the amount of αTocOAc remains equal to that of αTocOH during the experiment. This, added to the fact that the concentration of both compounds in this part of the skin, where the mixture of the two compounds was not applied, is 20–40 times lower than that in the left side, has another consequence. It means that, even at a much lower concentration, αTocOAc and αTocOH do not differ from each other with regard to their migration in the epidermis. This holds for horizontal transport as well as for penetration. This result

Table 1
Horizontal transport in the epidermis: 35 μl cm^{-2} of ethanol, containing both 2.5% αTocOAc and 2.5% αTocOH, was applied to the left side of the shaven back skin of rats at the beginning of each day; 5 h after application, the concentrations of αTocOAc and αTocOH were determined in the epidermis from both the left and right sides of the back of two rats; for both compounds, the concentration found in the right side was expressed as a percentage of that in the left side (Section 2.7.1; for each day, $n=2$ rats; mean ± standard deviation (SD))

Day	αTocOAc (%)	αTocOH (%)
1	2.7±2.31	2.6±2.21
2	3.3±2.48	3.2±1.82
3	4.7±2.23	4.8±1.93
4	5.0±4.39	5.4±3.58
5	5.8±1.91	6.0±1.57

Table 2
Concentration (nanograms per milligram of tissue) of αTocOAc and α-TocOH in the epidermis (both viable layer only and total epidermis (stratum corneum + viable layer), 1, 2, 3, 4 and 5 h after a single application of 0.25% αTocOAc in ethanol (35 μl cm^{-2}) to the shaven back of rats. After each hour, one animal was sacrificed and two spino-symmetrical skin samples from the back were taken (one was tape stripped first to remove the stratum corneum before the amounts of αTocOAc and αTocOH were determined) (Section 2.7.2; each value is the mean of duplicate samples from one animal ± range)

Total epidermis		Hour	Viable layer	
αTocOAc	αTocOH		αTocOAc	αTocOH
1160±100	14±1	1	290±30	16±1
830±290	15±4	2	230±25	22±8
1290±110	16±3	3	240±30	16±1
1230±220	17±5	4	320±40	17±1
1920±330	16±2	5	540±300	24±6

justifies the application, in the experiments described in Sections 2.7.2–2.7.4, of a solution of much lower concentration of αTocOAc or αTocOH than that used in Section 2.7.1.

In Table 2, the amounts of αTocOAc and αTocOH, present 1, 2, 3, 4 and 5 h after a single application of αTocOAc, are given. Hydrolysis of αTocOAc may account for part of the αTocOH. However, to determine the extent to which the hydrolysis of αTocOAc contributes to the amount of α-TocOH given in Table 2, correction should be made for the amount of endogenous αTocOH. The amount of endogenous αTocOH was estimated as follows.

After 5 h from pretreatment with pure ethanol only (700 μl on about 15 cm^2 shaven dorsal skin), the amounts of αTocOH in both the stratum corneum and the viable layer were determined. The value obtained in both cases was 17±4.9 ng mg^{-1} ($n=6$±SD). If this amount already present in the epidermis is taken into account, it can be concluded that αTocOH is not formed to a measurable extent by hydrolysis of αTocOAc, 5 h after a single application of the latter (Table 2).

Because the mass of the stratum corneum exceeds by far that of the viable layer, values concerning the latter do not contribute substantially to those of the total epidermis. As a consequence of this, values concerning the total epidermis reflect the situation in the stratum corneum.

As can be seen in Table 3 (panel A), the amount of α-TocOAc gradually increases in both the stratum corneum and the viable part of the epidermis. After 5 days, 3–4% of the amount of αTocOAc present in the total epidermis (panel A) is found on the side to which ethanol only was applied (panel B).

Although the amount of αTocOH formed by hydrolysis of αTocOAc is small, even after application on several consecutive days, there is a steady increase during the 5 day treatment (Table 3, panel A). After correction for the αTocOH already present without any application of αTocOAc (panel B), hydrolysis amounts to less than 1% of the αTocOAc in the stratum corneum and about 5% of the αTocOAc in the viable layer after 5 days (Table 3, panel A).

Furthermore, it was investigated whether the minute amount of free vitamin E formed by hydrolysis of the acetate could account for the protection observed. The inhibition of UVA-induced photobinding of 8-MOP to epidermal bioma-

Table 3
Concentration (nanograms per milligram of tissue) of αTocOAc and αTocOH in the epidermis. Panel A (both total epidermis (stratum corneum + viable layer) and viable layer only) concerns the left side of the shaven back after daily application of 0.25% αTocOAc in ethanol (35 μl cm^{-2}) and panel B concerns the total epidermis of the right side which received each day ethanol (35 μl cm^{-2}) only. On days 1, 2, 3, 4 and 5, two rats were sacrificed 5 h after application. From each rat, two spino-symmetrical samples of the skin of the back were taken. The sample from the left side was divided into two equal parts, one of which was tape stripped to remove the stratum corneum (Section 2.7.3; for each day, $n=2$ rats; mean ± SD)

Panel A					Panel B		
Total epidermis		Day	Viable layer		Day	Total epidermis (ethanol)	
αTocOAc	αTocOH		αTocOAc	αTocOH		αTocOAc	αTocOH
1240±180	16±2.1	1	380±70	33±6.3	1	18±5.8	12±0.8
2510±50	22±5.2	2	640±125	47±8.1	2	25±7.9	19±4.6
3330±110	28±1.9	3	1400±130	66±8.9	3	47±3.1	18±1.7
3930±830	35±5.8	4	1000±250	80±12.3	4	67±5.4	19±3.3
4040±600	42±4.3	5	1510±160	102±19.7	5	149±7.5	20±2.6

Table 4
Relation between αTocOH concentration and inhibition of 8-MOP photobinding. (A) On each of 5 consecutive days, 35 μl cm^{-2} ethanol containing 0.25% αTocOAc or αTocOH was applied to the left side of the shaven back of rats, whereas the right side was treated with ethanol (35 μl cm^{-2}) only. On day 5, 5 h after the last application, the extent of photobinding of 8-MOP to proteins and lipids of the total epidermis was determined. The values found for the left (αTocOAc or αTocOH treated) side are expressed as a percentage of those for the right (ethanol treated) side. In addition, the αTocOH concentration of the epidermis on the left side was determined. (B) This experiment differs from (A) in that there was only a single application of αTocOH (Section 2.7.4; for each experiment ((A) or (B)), $n=6$ rats; mean ± SD)

Experiment	Proteins	Lipids	αTocOH (ng mg^{-1})
(A) αTocOAc (0.25%)	79 ± 5.2	102 ± 9.8	42 ± 3.8
(A) αTocOH (0.25%)	88 ± 7.9	65 ± 2.1	3900 ± 340
(B) αTocOH (0.01%)	81 ± 9.3	72 ± 3.2	52 ± 8.2
(B) αTocOH (0.005%)	82 ± 12.6	n.d.	32 ± 4.8
(B) αTocOH (0.002%)	105 ± 27.9	n.d.	19 ± 2.9

n.d., not done.

cromolecules was taken as a measure of the UV-protecting efficacy.

After a single application of αTocOAc (Section 2.7.4, (2)), no inhibition of photobinding was found (results not presented). Pretreatment with 0.25% αTocOAc on each of 5 consecutive days (Section 2.7.4, (1)) did afford protection as can be seen in Table 4. However, it is remarkable that an extremely small amount of αTocOH is responsible for the protection observed (only about twofold higher than that which is already present without any treatment with tocopherol or its acetate).

To address this remarkable fact, some experiments were performed with pure αTocOH. One single application of 0.01%, 0.005% and 0.002% αTocOH in ethanol produced a very small amount of αTocOH in the total epidermis after 5 h. (No additional αTocOH was found in the viable part.) The results presented in Table 4 show that, with 0.01%, almost three times the normal amount of αTocOH is reached, with 0.005% about two times, and with 0.002% there is no significant extra αTocOH found. It was concluded from the inhibition of photobinding found with 0.01% and 0.005% αTocOH that such a minute amount (as was also found after 5 days treatment with αTocOAc) is indeed responsible for protection (Table 4).

4. Discussion

That αTocOAc and αTocOH behave similarly with regard to the penetration of and the horizontal migration through the epidermis is comprehensible if we take into account that the pH is estimated to be approximately 5 in the stratum corneum and 7.4 in the viable part. The aromatic hydroxyl group in αTocOH ($pK \approx 10$) is not dissociated under these conditions. (The pK value of αTocOH is estimated to be about 10. This estimation is based on the pK value of various phenol derivatives with comparable molecular structures.) As αTocOH is not dissociated, the difference between αTocOAc and αTocOH with regard to the physicochemical parameters which determine skin transport is negligible. Furthermore, the fact that the amounts of αTocOAc and αTocOH found in the epidermis do not differ significantly, even after 1 week, is an indication that the hydrolysis of αTocOAc only occurs to a minor extent.

Although in skin care products a higher amount of vitamin E (acetate) is normally used, the highest percentage in most experiments of this study was 0.25%. These solutions are found to be sufficient to give a maximal protective effect in the animal model used.

The hydrolysis of αTocOAc proceeds very slowly and to a very low extent. After a period of 5 h after a single application of αTocOAc, the amount of αTocOH found does not significantly differ from that already present (Table 2). After 5 days, the amount of αTocOH is only a few times higher than the endogenous quantity (Table 3). Hydrolysis in the viable part of the epidermis is more efficacious, e.g. after 5 days, about 5% of the total αTocOAc and αTocOH is present as αTocOH (Table 3). This is important, because serious damage to this cell layer by sunlight can cause skin cancer. (Recently, Norkus et al. [22] reported the bioconversion of αTocOAc in the skin of hairless mice. These investigators found that about 10% of total material, αTocOAc and αTocOH together, was present as αTocOH. This result nicely corresponds with the 5% found here for the viable part of the epidermis. This is more evident if it is taken into account that the 10% was found after 25 not 5 days of daily topical application. In addition, their study concerned the whole skin, i.e. epidermis plus dermis. The enzymatic activity in the dermis, with its system of capillary blood vessels, may be higher than in the epidermis; this would result in a higher production of αTocOH.)

The following line of argument was the reason why the inhibition of the photobinding of 8-MOP to epidermal biomacromolecules was taken as a measure of the possible UV-protecting efficacy of αTocOAc.

On exposure to a combination of 8-MOP plus UVA or UVB only, molecules in their triplet excited state (singlet oxygen and free radicals), whether or not coupled to oxygen, are assumed to play an important role in photodamage [23–25]. Since the photosensitizing properties of 8-MOP are based on a variety of reactive intermediates, such as singlet oxygen, hydroxyl radicals and triplets, it is particularly suitable as a model compound to simulate the deleterious effects of sunlight alone [16]. Moreover, the dose-dependent biological damage in vivo, caused by over exposure to UV radiation alone, shows striking similarities to that found by a combination of 8-MOP plus UVA, such as the production of sunburn cells [26–28], oedema [29,30], erythema [31,32] and the induction of ornithine-decarboxylase activity [33–35]. In addition, all the 8-MOP plus UVA induced effects mentioned have already been shown to be useful parameters

for quantifying the protecting efficacy of topically applied compounds against the damaging effects of UV radiation alone [36–41].

From a mechanistic point of view, the phototoxic effects of 8-MOP are mainly due to photoreaction with lipids, proteins and DNA. Of the different kinds of photoreactions with these biomacromolecules, photobinding is considered to be essential for the photobiological effects of 8-MOP [23,42,43]. Photobinding of 8-MOP to epidermal biomacromolecules in vivo has already been extensively investigated [19,24].

With regard to the problem investigated in this study, the use of UVA has the following important consequences.

(1) The protection found is not caused by the absorption of radiation by αTocOAc or αTocOH, because both do not absorb any UVA.

(2) The fact that protection against UVA-induced photobinding of 8-MOP has been found proves that αTocOH acts as a scavenger of radicals and other reactive intermediates from photoexcited 8-MOP. (Because αTocOAc without the free aromatic hydroxyl group is not a radical scavenger, the protecting effects only result from interference with αTocOH formed by hydrolysis of αTocOAc.)

(3) The correlation between UVA-induced photobinding of 8-MOP and UVB, with regard to the photochemical and photobiological effects described above, indicates that the scavenging of radicals and excited states plays a role in the protection by αTocOH against UVB-induced damage.

After a single application of αTocOAc (Section 2.7.4, (2)), no inhibition of photobinding was found (results not presented). This corresponds with the fact that no extra α-TocOH was observed (Table 2) at the time that protection was measured. Schoonderwoerd et al. [44] also found no protection against UVA-induced photobinding of 8-MOP shortly after a single application of vitamin E acetate, even at a concentration ten times higher than that used in the present study.

Another remarkable finding is that maximal protection is reached with an amount of αTocOH in the epidermis which is only a few times higher than the normal endogenous concentration. This can be concluded from Table 4 by a comparison of the values for 0.25% αTocOH and 0.01% αTocOH; the values for the inhibition of photobinding are almost the same, whereas the amount of αTocOH in the total epidermis is 3900 ± 340 ng mg^{-1} vs. 52 ± 8.2 ng mg^{-1}.

This conclusion is supported by Schoonderwoerd et al. [44]; after a single application of a 4.3% solution of DL-αTocOH in ethanol, these workers found the same protection as in this study.

For other compounds, the maximum effect is different from that for αTocOH. For example, under the same experimental conditions, values for di-butyl-hydroxytoluene (BHT) are: proteins, $66\% \pm 2.1\%$; lipids, $58\% \pm 5.9\%$; for *N*-acetylcysteine the values are: proteins, $26\% \pm 3.2\%$; lipids, $14\% \pm 2.3\%$ [45,46].

5. Conclusions

From this study in the rat, it can be concluded that α-TocOAc is available in the epidermis several hours after a single application, not only in the stratum corneum but also in the viable part. However, protection against UVA-induced photobinding of 8-MOP is only reached after several applications and not after a single application of αTocOAc. This conclusion is supported by an investigation of protection against UV-induced oedema [47]. A solution of 1% αTocOH in ethanol applied to the skin of hairless mice resulted in oedema reduction of 25% to 30%, whereas a single application of αTocOAc (up to 5% in ethanol) did not show any significant effect.

Another conclusion from our investigation with this in vivo model is that the hydrolysis of αTocOAc into αTocOH is essential for protection against UV-induced damage. An interesting aspect of this is that the hydrolysis of αTocOAc is very slow and inefficient and that a minute amount of αTocOH provides maximal protection. Indeed, vitamin E acetate can be considered as a prodrug, which very slowly releases minute amounts of the protector vitamin E.

Human skin contains a variety of metabolic enzymes, e.g. esterases, which can convert prodrug esters to bioactive drugs [48]. The rat is often used as a model for human skin metabolism. However, although the hydrolysis of diflucortolonvaleriate is slower in human skin than in rat skin [49], it is not yet known whether this is also the case with regard to vitamin E acetate.

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