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Further investigations on the role of ascorbic acid in stratum corneum lipid models after UV exposure

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Abstract

This study is the continuation of our research into vitamin C and its possible effects on human skin after topical administration. The effects of ascorbic acid, iron ions and UV irradiation on stratum corneum lipid models were investigated. The lipid models used were: a simple system (linolenic acid dispersion), a complex system (liposomes consisting of dipalmitoylphosphatidylcholine, cholesterol and linolenic acid) and complex systems with additionally incorporated ceramides (types III and IV). The lipid peroxidation was quantified by the thiobarbituric acid assay. A human adult low-calcium high-temperature (HaCaT) keratinocytes cell culture was used as a second in-vitro model. The amount of intracellular peroxides was determined by measuring the fluorescence intensity using the dihydrorhodamine 123 assay. Electron paramagnetic resonance spectroscopy was used to study the influence of ascorbic acid and iron ions on the signal intensity of 5-doxylstearic acid during UV exposure. Ascorbic acid showed prooxidative properties in the thiobarbituric acid assay whereas cell protection was measured in the HaCaT keratinocytes experiments. Electron paramagnetic resonance investigations revealed different extents of free radical production generated by iron ions, ascorbic acid and UV irradiation. In evaluating the results from this study new aspects of the mechanism of lipid damage caused by these three factors were suggested, transcending the simple redox behaviour of ascorbic acid.

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Introduction

Ascorbic acid, or vitamin C, is an important water-soluble vitamin and part of the so-called non-enzymatic antioxidative defence system of humans (Niki 1991). Vitamin C is involved in many physiological functions of living organisms, for example the regulation of collagen synthesis, the synthesis of muscle carnitine and enzyme stimulation in tyrosin metabolism (Barja 1996; Tsao 1997). However, many controversies regarding the health benefits of ascorbic acid have been discussed in the literature (Hirschelmann & Peter 1990; Halliwell 1996; Naidu 2003). There are two questions frequently asked in regard to this topic: (i) is ascorbic acid a pro- or an antioxidative compound in vivo (Carr & Frei 1999; May 1999) and (ii) does this vitamin prevent or induce cancer in vivo (Halliwell 2001; Lee et al 2003)?

Many investigations exist that show the beneficial effects of ascorbic acid on the condition of oxidative stress tested in various studies by the use of many different experimental designs, including both in-vitro and in-vivo scrutinies (Colven & Pinnell 1996; Chen et al 2000; Jurkovic et al 2003; Suh et al 2003; Tauler et al 2003; Polidori et al 2004). However, there are just as many publications demonstrating the opposite effects of vitamin C (Almaas et al 1997; Podmore et al 1998; Proteggente et al 2000; Song et al 2001; Brown et al 2002; Meves et al 2002). Often the redox interaction of ascorbic acid with transition metal ions is regarded as a reason for its prooxidant potential when examining in-vitro systems (Asplund et al 2002). In 1954 Udenfriend et al used a mixture of ascorbic acid, transition metals and hydrogen peroxide for the hydroxylation of aromatic compounds. Since then this combination has been called the Udenfriend system.

In 2001 Lee et al showed that the damage of biomolecules by ascorbic acid is not stringently bound to the redox interplay between vitamin C and transition metals. Ascorbic acid was shown to degrade lipid peroxides, which results in damaging aldehydic species. This then raises the question of the effects of vitamin C on human skin when topically administered. Is ascorbic acid acting as a pro- or an antioxidant for the skin?

Under physiological conditions there is the chance for an interplay between the small molecule antioxidants and the enzymatic antioxidant system of the skin (Fuchs 1998; Thiele et al 2001). Therefore topically administered vitamin C in in-vivo studies mostly offers modest protective effects with little or no harmful response (Thiele et al 2000).

Our previous studies have shown that the prooxidative effect of ascorbic acid in stratum corneum lipid model systems demonstrates concentration dependency and that iron ions as transition metal catalysts are required (Trommer et al 2002). Contrary to these results, electron paramagnetic resonance (EPR) experiments showed the ability of ascorbic acid to lower both the signal of spin-trapped hydroxyl radicals generated in the Fenton system and the signal of the stable organic 2,2-diphenyl-1-picrylhydrazyl hydrate radical. The interplay between iron ions and UV irradiation was discussed and it was concluded that the administration of ascorbic acid in formulations for topical use should be carefully considered (Trommer et al 2002).

Continuing this research work on ascorbic acid, vitamin C (100 μM) was tested in four stratum corneum lipid model systems of different complexity for its effects on the level of UV-induced iron ion catalysed lipid peroxidation. To examine the influences of vitamin C on the cellular components of the skin, human adult low-calcium high-temperature (HaCaT) keratinocytes were used. These determined ascorbic acid influences on the amount of intracellular peroxides generated by UV irradiation. The EPR spectroscopy was applied to the differentiation of the influences of ascorbic acid and iron ions on stratum corneum lipids stressed by UV irradiation.

Materials and Methods

Materials

L-Ascorbic acid, α-linolenic acid (LLA), L-α-dipalmitoylphosphatidylcholine (DPPC), cholesterol (Chol), ceramide IV (C IV; N-2-hydroxyacyl-sphingosine from bovine brain), ferrous sulfate, malondialdehyde-bis-(dimethylacetal), 2-thiobarbituric acid and trichloroacetic acid for the thiobarbituric acid (TBA) assay (all of analytical grade) were obtained from Sigma (Deisenhofen, Germany) as well as 5-doxylstearic acid (5-DSA) for the EPR investigations and dihydrorhodamine 123 (DHR) for the determinations of the intracellular peroxide content. Analytical grade ceramide III (C III; N-stearyl-phytosphingosine) was provided by Cosmoferm (Delft, The Netherlands). Methanol of gradient grade and chloroform (LiChrosolv) were purchased from Merck (Darmstadt, Germany).

HaCaT keratinocytes were kindly provided by N. E. Fusenig, Deutsches Krebsforschungszentrum – DKFZ (Heidelberg, Germany). Serum-free medium, epidermal growth factor (EGF) and bovine pituitary extract were obtained from Gibco Life Technologies (Eggenstein, Germany).

Lipid sample preparation

The lipids for the experiments were chosen according to the lipid composition of the human horny layer lipid matrix. LLA, Chol, C III and C IV as sphingolipid models and DPPC as a liposome generator were used.

The simple system was an oil and water dispersion of LLA obtained by shaking the system for 120 min using a laboratory flask shaker (GFL 3006, Gesellschaft für Labortechnik, Burgwedel, Germany).

The complex systems were prepared as liposomes using the thin-layer method. The lipids were dissolved in 0.5 mL chloroform. The solvent was removed using a rotation vaporiser (Labo-Rota C-311, resona technics, Switzerland) and a vacuum pump (PIZ 100 Mini-Tower-MPC 050-Z, Saskia Hochvakuum- und Labortechnik, Ilmenau, Germany). To obtain a thin and homogeneous lipid film a fast rotation was chosen (150 rpm). The remaining lipid film was dispersed in 20.0 mL of double-distilled water by shaking the system intensively for 360 min. The remaining double-distilled water was added to achieve the final concentration. The concentrations of the ingredients in the liposome stock solutions were: DPPC 1.0 mM; Chol, LLA, C III, C IV and 5-DSA (present only in the liposomes examined by EPR spectroscopy) 0.5 mM.

To obtain vesicles of a uniform size the stock solution was passed through a 400-nm polycarbonate filter (Costar, Cambridge, UK) at 20°C under nitrogen pressure using an extruder device (Lipex Biomembranes, Vancouver, BC). This step was repeated five times. To control the vesicle size and the success of extrusion, the particle diameters were determined via photone correlation spectroscopy using a Malvern Instruments Autosizer 2c equipped with a series 7032 Multi-8 Correlator (Malvern, Worcester, UK).

To initiate the creation of reactive oxygen species (ROS) via a Fenton-type reaction ferrous sulfate (10 μM) was added to the samples as an electron donor and catalyst of the Haber–Weiss reaction. Previous investigations have shown that there is no effect when irradiating lipid model systems without transition metal catalysts (Trommer et al 2001).

All the liposome suspensions and fatty acid dispersions were freshly prepared just before use.

HaCaT keratinocytes

For the cell culture experiments HaCaT keratinocytes from the passages 20–30 were used. The cells were maintained and subcultured in serum-free medium supplemented with 0.1 ng mL^{-1} EGF and 25 μg mL^{-1} bovine pituitary extract. The keratinocytes were grown in a humidified incubator at 37°C, 5% CO_2 and 95% air and seeded at a density of

5×10^3 cells per well in 96-well microtitre plates with 200 μL of medium. Four days after seeding, the cells were incubated for 180 min with ascorbic acid. Subsequently, the HaCaT keratinocytes were damaged as described in the following section.

UV irradiation

UV irradiation experiments with the stratum corneum lipid model systems and the HaCaT keratinocytes were carried out using special irradiation equipment (Dr Gröbel UV-Elektronik, Ettlingen, Germany) enabling selective exposure to UV-A and UV-B. For UV-A irradiation the special lamp TL-DK 30W/05 (Philips, Hamburg, Germany) was used. This lamp emits in the range of 320–420 nm with a peak emission at 370 nm. UV-A irradiation was monitored with a UV-A sensor RM-11 (Dr Gröbel UV-Elektronik, Ettlingen, Germany). For the UV-B irradiation experiments a F15/T8 15 W lamp with a main emission range of 290–320 nm and a peak emission at 309 nm (Sankyo Co., Tokyo, Japan) was employed.

Prior to irradiation, the stratum corneum lipid model samples were transferred to open glass dishes of 55.0 mm diameter. The optical pathlength was 2.1 mm and a homogeneous exposure was assumed in spite of light scattering. The samples were treated with a UV-B dose of 0.25 J cm^{-2}, which corresponds approximately to the 10-fold of the minimal erythemal dose of humans. This high dosage was required to provoke stress conditions.

HaCaT keratinocytes were irradiated as follows. Prior to the UV radiation treatment, the incubation medium was removed. The keratinocytes were washed once with phosphate buffered saline (PBS) and afterwards buffer was added. The untreated control cells were covered with aluminium foil. The temperature during the irradiation was monitored and kept stable at 30°C by means of the irradiation chamber cooling system. After UV treatment, the evaporated buffer was added again and incubation was continued for 20 h in the humidified incubator.

As an irradiation device for continuous irradiation during the EPR experiments the high-pressure mercury vapour lamp HBO 200 W (Mess- und Regelungstechnik Dessau, Werk Wetron, Weida, Germany) was used. The lamp was focused onto the slits of the EPR resonator after having the protective cover removed. This lamp has got silica optics and emits a line spectrum with several lines in the UV and visible regions. It was used without optical filtering. The peak of its emission spectrum is in the UV-A range at approximately 360 nm.

TBA assay

The TBA test is a quantitative assay for the detection of malondialdehyde (MDA), and is the most widely used technique to determine lipid peroxidation products (Irat et al 2003; Sreekanth et al 2003).

In this study, the Buege–Aust method was used (Buege & Aust 1978). Briefly, 2.0 mL of a stock TBA reagent containing 15% (w/v) trichloroacetic acid in 0.25 M HCl and 0.37% (w/v) thiobarbituric acid in 0.25 M HCl was added to 1.0 mL of the UV-B treated sample. After incubating at 90°C for 15 min and cooling down, the red TBA:MDA complex (2:1) appears, allowing fluorescence measurement. An HPLC system (Merck-Hitachi, Darmstadt, Germany) equipped with an auto sampler AS-4000A, interface D-6000A, pump L-6200A, UV-VIS-Detector L-4250, fluorescence detector F-1080 was used to quantify the pigment. A reversed-phase column (LiChrospher 100, RP 8, particle size 5 μm) was used with a mobile phase methanol:water 30:70 for the HPLC procedure (Merck, Darmstadt, Germany). The excitation wavelength was 515 nm and the emission measurement was performed at 555 nm.

A calibration curve was generated using malondialdehyde, which was formed from malondialdehyde-bis-(dimethylacetal) under acidic conditions (Trommer et al 2003).

DHR 123 assay

The examination of the UV-induced formation of intracellular peroxides was performed by the DHR 123 assay. The oxidation of dehydrorhodamine to red fluorescent rhodamine 123 was measured following a procedure described by Peus et al (1998).

The confluent HaCaT keratinocytes in 96-well plates were incubated with DHR (5 μM) for 45 min. After washing the cells twice with PBS, irradiation treatment took place as described in the corresponding section. During UV irradiation the medium was completely replaced by PBS to avoid side-effects due to culture medium ingredients.

Previous HaCaT keratinocytes investigations have shown higher fluorescence signals in the DHR 123 assay after UV-A irradiation compared to UV-B radiation treatment. This means that the influence of ROS on cellular damage is more important at UV-A irradiation in cultured HaCaT cells (Podhaisky 2001). A UV-A dose of 8 J cm^{-2} was therefore used for the HaCaT keratinocytes experiments. Forty-five minutes after the end of UV irradiation, fluorescence intensity was measured at 538 nm using the microtitre plate reader Fluoroskan Ascent (Labsystems, Helsinki, Finland). The excitation wavelength was 485 nm.

EPR spectroscopy

EPR studies were carried out in the X-band using a Bruker ESP 380 E FT-EPR spectrometer (Bruker, Rheinstetten, Germany). The investigations were performed using the cylindric cavity ER 4103 TM (Q = 12 000) with corresponding aqueous sample cells ER 166 FCTMVT (Bruker, Rheinstetten, Germany). The resonator settings were: microwave frequency 9.76 GHz; microwave power 3.7 mW; modulation frequency 100 kHz; modulation amplitude 0.5 mT; sweep width 15 mT; conversion time 40.96 ms; time constant 10.24 ms; sweep time 41.94 s.

Before transferring the specimen into the cavity the liposomal stock solution was diluted with double-distilled water or mixed with ascorbic acid or ferrous sulfate according to the kind of experiment. The final concentrations in a 5.0 mL sample were: DPPC 200 μM, LLA

100 μM, Chol 100 μM, 5-DSA 100 μM, ascorbic acid (if present) 100 μM, ferrous sulfate (if present) 10 μM. The spectrum recording was started exactly 120 s after the mixing of the sample ingredients. After having recorded the spectrum at t = 0 the high-pressure mercury vapour lamp (switched on 10 min before the continuous irradiation experiments were started to reach its final irradiation strength) was focused on the slits of the cavity. Spectrums were recorded every 5 min. All signals are the results of three time accumulations.

The evaluation of the EPR raw spectra was carried out using WinEPR software (Bruker, Rheinstetten, Germany).

Statistical analysis

All quantitative data shown represent the mean values ± s.d. of sextuple measurements (n = 6). Statistical analysis of the effects of ascorbic acid on the TBA reaction productions concentration after UV-B irradiation and on the fluorescence intensity after UV-A exposure in the DHR 123 assay were performed using a one-way ANOVA. In all cases, post-hoc comparisons of the means of individual groups were performed using Dunnett's multiple comparison test. A significance level of $P < 0.05$ between groups was accepted as being statistically significant. All calculations were performed using GraphPad Prism 2.0 (GraphPad Software Inc., San Diego, CA).

Results

TBA assay

Figure 1 shows the effects of ascorbic acid on UV irradiation-induced lipid peroxidation of stratum corneum lipid model systems in the presence of iron ions as transition metal catalysts.

Ascorbic acid (100 μM) was able to increase the lipid peroxidation level, measured as MDA content, significantly in all systems tested. The concentration dependency of this prooxidative effect of vitamin C on stratum corneum lipids was previously demonstrated by our group (Trommer et al 2002). Figure 1A illustrates the results of the MDA determinations in the simple and complex systems. 100 μM ascorbic acid increased the TBA levels after UV exposure from 220 ng mL^{-1} up to 1100 ng mL^{-1} in the simple system and from 460 ng mL^{-1} to 1300 ng mL^{-1} in the complex system. In each case ferrous sulfate (10 μM) was present. Figure 1B presents the TBA assay results obtained by examining the complex liposomal systems with C III and C IV. Ascorbic acid also acted prooxidatively in the complex systems with the ceramides. 100 μM vitamin C increased the MDA level from 460 ng mL^{-1} to 1220 ng mL^{-1} in the complex system with C III and from 420 ng mL^{-1} to 1230 ng mL^{-1} in the complex system with additionally incorporated C IV. Again, 10 μM iron ions were present in each sample.

Cell culture investigations

A more complex system was additionally tested to assist in a better evaluation of the damaging effects of ascorbic acid, iron ions and UV irradiation in stratum corneum lipid models. HaCaT keratinocytes were used for modelling the cellular species of the epidermis. Figure 2 shows the influences of different vitamin C amounts on the fluorescence intensity in the DHR 123 assay. This method was applied for the determination of intracellular peroxides. The cell culture was stressed by a UV-A dose of 8 J cm^{-2}.

In contrast to the results of the TBA assay in the cell culture investigations, a decrease of intracellular peroxides was measured after adding ascorbic acid. This decrease showed concentration dependency. The highest vitamin C concentration used in this study, 1 mM ascorbic acid, was able to decrease the amount of intracellular peroxides from 330% for the samples irradiated without ascorbic acid down to the peroxide content of the non-irradiated sample. This may be due to the increased complexity of

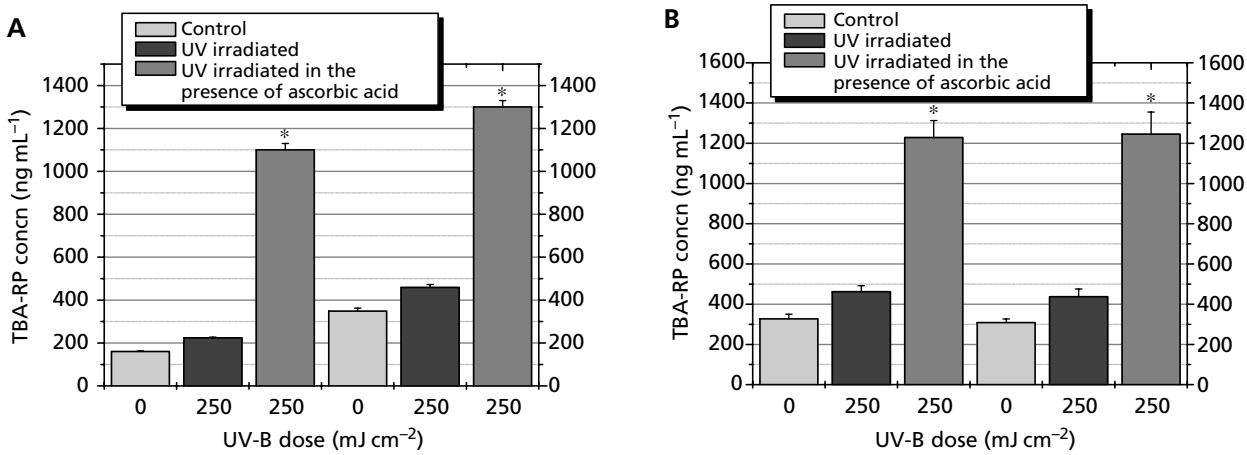

Figure 1 Concentration of the TBA reaction products (TBA-RP) and influence of UV-B irradiation and 100 μM ascorbic acid in the systems used. (A) Simple (columns 1, 2, 3) and complex (columns 4, 5, 6) stratum corneum lipid model system. (B) Complex systems including the ceramides III (columns 1, 2, 3) and IV (columns 4, 5, 6). *$P < 0.05$, UV exposed in the presence of ascorbic acid vs irradiated without additives.

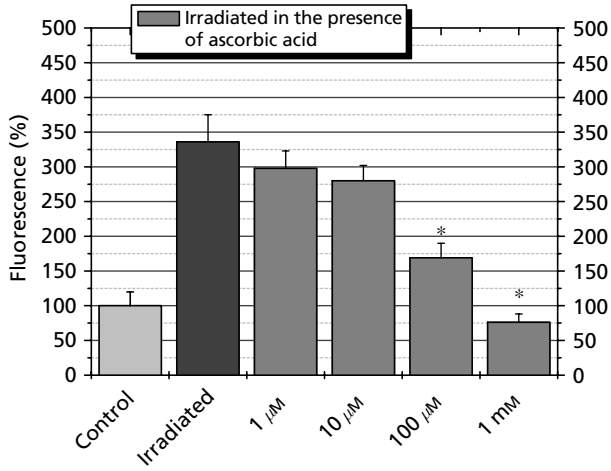

Figure 2 Influence of UV-A irradiation ($8\,J\,cm^{-2}$) and ascorbic acid on the fluorescence intensity of the DHR 123 assay using an HaCaT keratinocytes cell culture. $*P < 0.05$, UV exposed in the presence of ascorbic acid vs irradiated without additives.

the keratinocytes compared to the quite artificial lipid model systems. Cultured cells have their own antioxidative defence network. Ascorbic acid acts antioxidatively when added to the cell culture because of its redox cycling abilities. The results obtained in our HaCaT keratinocytes experiments are comparable with the results of Rosenblat et al (2001). The Rosenblat group used dermal and foreskin fibroblasts for studying ascorbic acid and palmitoyl ascorbate. In long-time cultured cells (72 h) a reduction of intracellular peroxides was measured. Rosenblat et al also determined the effects of vitamin C on newly plated cells (24 h). Here the results indicated that ascorbic acid and palmitoyl ascorbate increased the intracellular content of ROS. In both cases the lipid peroxidation in the cytoplasm membrane was stimulated. It was shown in this study that ascorbic acid is also able to act prooxidatively in cellular systems.

EPR spectroscopy

For a better interpretation of the results obtained by the TBA acid assay, EPR spectroscopy studies, using a spin-labelled fatty acid incorporated into liposomes, were performed. The EPR signal of the spin label should decrease after time-dependent irradiation of the 5-DSA-containing liposomes. The reason is the generation of lipid radicals by UV exposure. These carbon-centred radicals are able to recombine with the spin label radical, causing the reduction of the EPR signal. Figure 3A confirms this presumption.

Which effect may be expected when adding vitamin C to the liposomes? Considering the prooxidative behaviour of ascorbic acid measured in the TBA assay, vitamin C should decrease the EPR signal before and after irradiation. The effect should be a large one and a concentration dependency should be visible. However, this prediction will only be true when the mechanism of lipid peroxidation by ascorbic acid includes free radical intermediates. Figure 3B shows that in general the anticipated effects were determined but the amount of damaging effect of ascorbic acid and UV light combined was much less than expected. The figure illustrates that the signal intensity without irradiation ($t = 0$ min) is lower when ascorbic acid is present. The change in the relative signal intensity between $t = 0$ min and $t = 10$ min is smaller when vitamin C is present.

Figure 4 shows EPR quantifications of the irradiated spin-labelled liposomes in the presence of iron ions and in the presence of both iron ions and vitamin C. The ascorbic acid again exhibits only a moderate effect on the EPR signal intensity of 5-DSA compared to the large influence of iron ions.

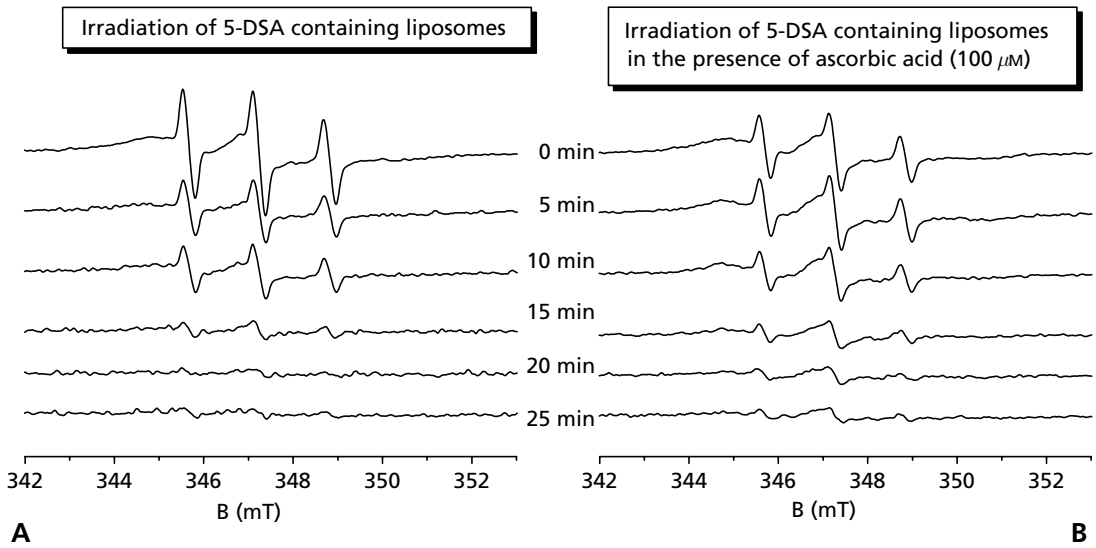

Figure 3 X-Band EPR spectra of liposomes containing spin-labelled 5-DSA. (A) Influence of irradiation time using the HBO 200 W lamp. (B) Influence of 100 μM ascorbic acid and irradiation time using the HBO 200 W lamp.

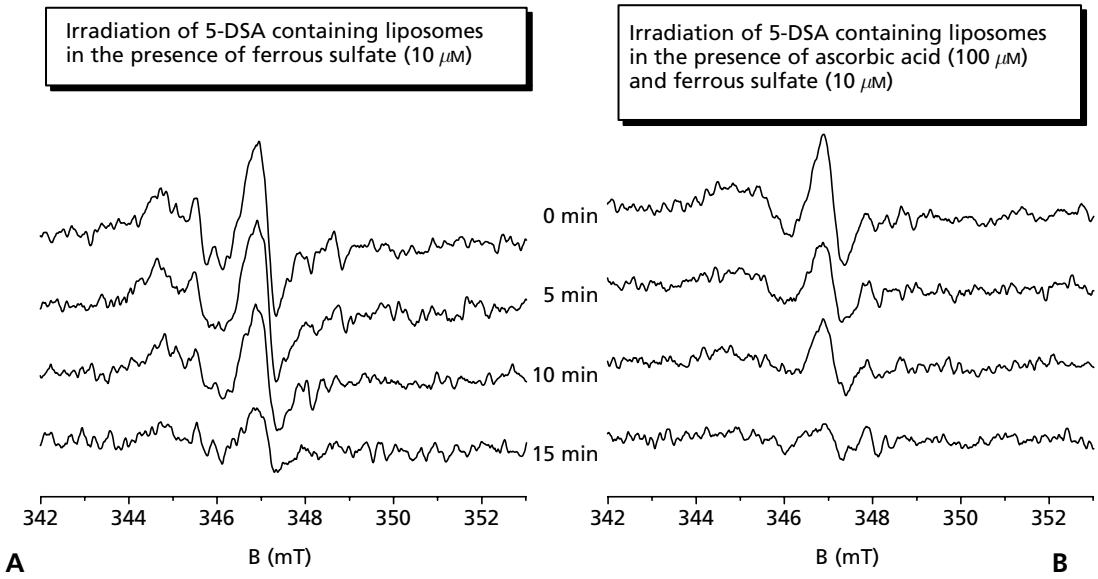

Figure 4 X-Band EPR spectra of liposomes containing spin-labelled 5-DSA. (A) Influence of 10 μM ferrous sulfate and irradiation time using the HBO 200 W lamp. (B) Influence of 100 μM ascorbic acid, 10 μM ferrous sulfate and irradiation time using the HBO 200 W lamp.

A better comparison of this effect is shown in Figure 5. This figure shows the results measured with all four model systems in one diagram at the irradiation points $t = 0$ min and $t = 10$ min. Once again it is the strong effect of iron ions causing the high reduction of the EPR signal intensity after UV irradiation while the presence of ascorbic acid alone is only able to slightly reduce the EPR signal intensity. At first glance this is a strong result because the TBA assay experiments showed the opposite. Ascorbic acid together with iron ions had a much more lipid-damaging influence than irradiation in the presence of iron ions only. An explanation is given in the following section.

Discussion

The TBA assay results, the results of the experiments with the spin-labelled fatty acid and the results of Lee et al (2001) conclude in a new interpretation of action of the triad ascorbic acid/iron ions/UV irradiation. Figure 6 explains this new view in a two-step scheme.

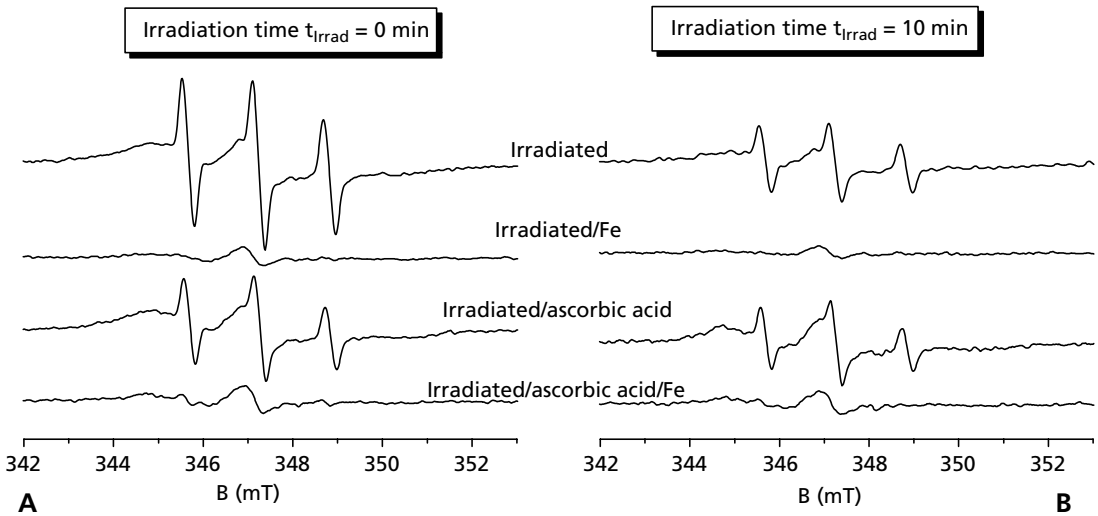

Figure 5 X-Band EPR spectra of liposomes containing spin-labelled 5-DSA. (A) Influence of 100 μM ascorbic acid and 10 μM ferrous sulfate. (B) Influence of 100 μM ascorbic acid, 10 μM ferrous sulfate and irradiation time using the HBO 200 W lamp.

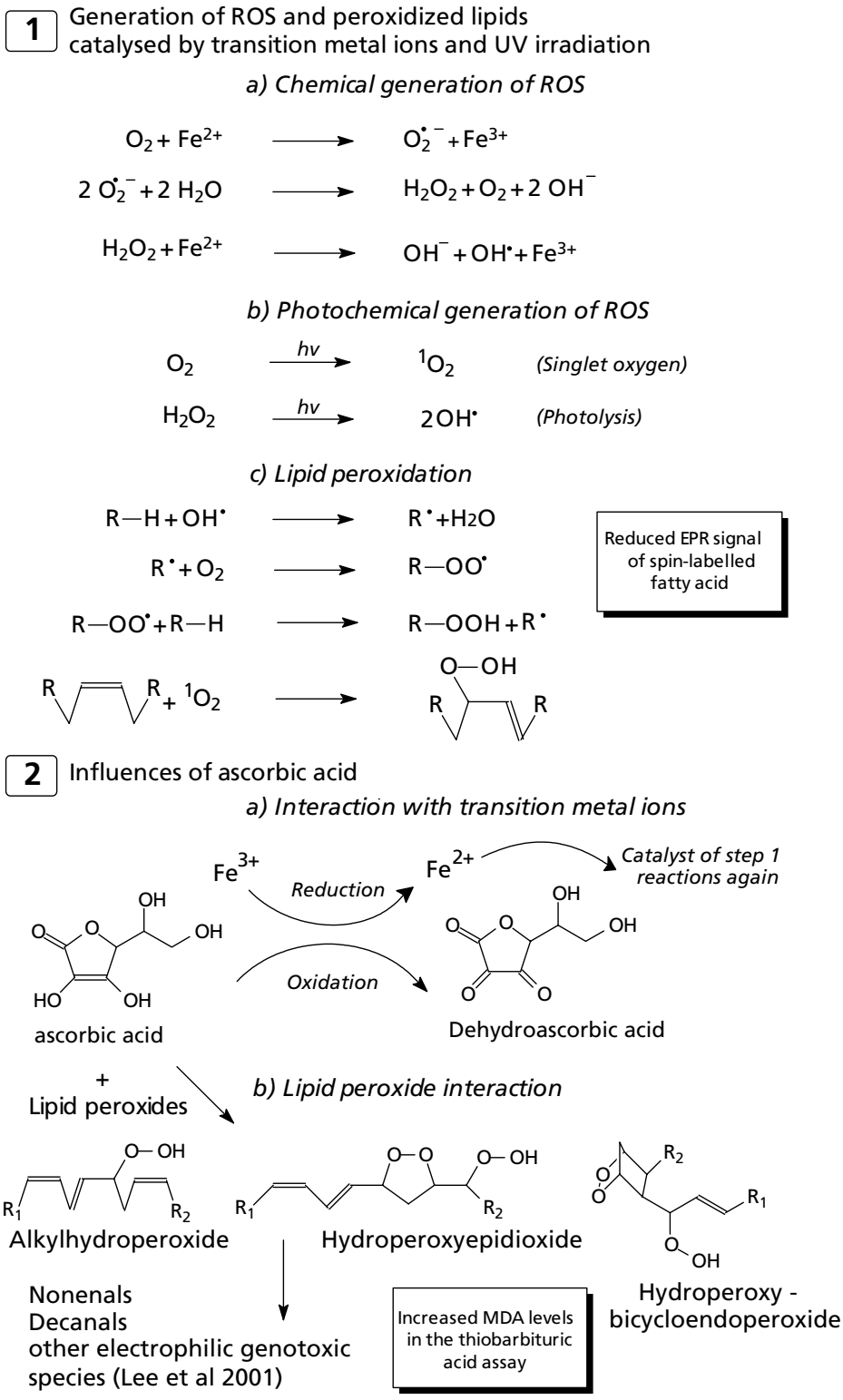

Figure 6 Two-step mechanism of the damaging effects of ascorbic acid and iron ions on skin lipids after UV exposure of skin lipid model systems.

In the first step the iron ions catalyse the Haber–Weiss reaction and generate hydroxyl radicals via a Fenton-type mechanism. Later on alkyl and peroxyl radicals are formed. The EPR results obtained by use of the spin-labelled fatty acid incorporated into liposomes support this theory.

UV radiation is also able to catalyse the generation of ROS. Singlet oxygen may be generated by triplet oxygen activation. This species has also got the ability to react with unsaturated lipids, resulting in more lipid peroxides. The UV-induced photolysis of hydrogen peroxide, which is formed by the stepwise reduction of oxygen, contributes to the generation of hydroxyl radicals.

According to the results of Lee et al (2001), ascorbic acid is able to decompose the resulting lipid peroxides to genotoxic electrophilic species. The generation of nonenals and decanals (as demonstrated by Lee et al) and probably also of malondialdehyde explains the increased TBA results measured after the addition of vitamin C. This is the second step in the two-step scheme shown in Figure 6. Another part of the ascorbic acid molecules available in the mixture is used for the reduction of oxidized iron ions, maintaining the ROS generation.

By this redox-active metal activation the vitamin C probably contributes to the inflammation process in vivo via radical generation, as recently hypothesised by Fisher & Naughton (Fisher & Naughton 2003). This is where the vitamin C molecules are decomposed as demonstrated in our previous study on that topic by mass spectrometry (Trommer et al 2002). Lavoie et al recently suggested that the light-induced by-products of vitamin C could be of biological importance as they themselves have the ability to induce organic peroxide generation (Lavoie et al 2004).

Our results suggest that there are new aspects for the explanation of the damaging effects of ascorbic acid, iron ions and UV irradiation transcending the mechanism of damage by simple redox behaviour. The increase in the concentration of lipid radicals as determined by EPR spectroscopy may be explained by the catalytic effects of transition metal ions and UV light. In a Fenton-type reaction and by photochemical reactions ROS are generated and damage lipids by lipid peroxidation. This is via a free radical mechanism, leading to EPR signal reduction. Ascorbic acid exhibits only a moderate EPR signal-decreasing effect. This very low effect is probably due to spin-label reduction by the reductant ascorbic acid rather than lipid damage via free radical generation. The increased MDA levels in the TBA assay are due to iron ion reduction and lipid peroxide decomposition by ascorbic acid.

However, of what relevance are the effects measured in this study in vivo? As shown above, vitamin C acted cell protective when tested in the HaCaT keratinocytes cell culture. McArdle et al (2002) demonstrated a decreased skin MDA content after oral vitamin C supplementation. In contrast, Childs and co-workers (2001) measured increased oxidative stress after vitamin C supplementation in humans after an acute muscle injury. There has also been report of DNA damage in healthy volunteers induced by iron and vitamin C co-supplementation (Rehman et al 1998).

Normally, in the human body there is an antioxidative network where several antioxidative systems influence and renew themselves (Sander et al 2004). Ascorbic acid plays an important role in the recycling process of these physiologically present co-antioxidants (Heller et al 2004). Damage of biomolecules only occurs when all antioxidative systems, such as the vitamin E redox system, are exhausted or reduced in function. The whole reductive potential of vitamin C would then be available for the reduction of Fe^{III} ions and the degradation of lipid hydroperoxides. Firstly this would lead to ascorbyl radicals, dihydroascorbate and Fe^{II} ions as catalysts of the Haber–Weiss reaction, generating more damaging hydroxyl radicals, and secondly to aldehydic lipid peroxide decomposition products as determined by the TBA assay.

Conclusion

In this study the influence of vitamin C on different in-vitro model systems was investigated, continuing our research work on the redox behaviour of ascorbic acid and UV irradiation-induced oxidative stress. The evaluation of the experiments with the stratum corneum lipid models of differing complexity showed prooxidative properties of ascorbic acid when added to the samples at a concentration of 100 μM.

Examinations of HaCaT keratinocytes as a model system for the cellular stratum corneum species displayed opposing results. The ascorbic acid was able to lower the intracellular peroxide content in a concentration-dependent manner.

EPR experiments detecting a spin label incorporated into stratum corneum lipid liposomes revealed differences in the free radical generating potential of the investigated factors of influence, ascorbic acid, iron ions and UV irradiation.

Using the results, gathered from the different analytical methods, a new mechanism of lipid damage by ascorbic acid and transition metal ions after UV exposure was determined, transcending simple redox behaviour and transition metal interaction.

The topical administration of ascorbic acid in semi-solid pharmaceutical or cosmetic formulations for skin protection therefore needs to be further evaluated. The results suggest that there may be an adverse effect from ascorbic acid when topically administered on human skin and no co-antioxidants are present. This factor would need to be seriously considered when using vitamin C under these conditions.

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