Physiol. Res. 51: 285-290, 2002

Protective Effects of Topical Alpha-Tocopherol Acetate on UVB Irradiation in Guinea Pigs: Importance of Free Radicals

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Received April 14, 2001
Accepted October 5, 2001

Summary

Reactive oxygen species can be generated by daily exposure of the skin to ultraviolet light and may cause some subchronic and chronic skin disorders. The aim of this study was to investigate a possible preventive role of α-tocopherol acetate (ATA) on ultraviolet B (UVB) induced peroxidation by assessing lipid peroxide (LPO) levels and activity of reactive oxygen scavenging enzymes including glutathione peroxidase and superoxide dismutase (SOD) in guinea pigs. ATA was topically applied to the skin for three weeks before a single dose of 0.9 J/cm^2 UVB irradiation on the skin and lipid peroxide levels and antioxidants in plasma, skin and liver and erythrocytes were determined after decapitation. Topical application of ATA prevented the UVB irradiation-induced reduction of scavenging enzyme activities in skin and erythrocytes. In conclusion, we suggest that topical applications of ATA before UVB irradiation is effective in protecting the skin from unwanted effects of UVB irradiation.

Key words

Alpha-tocopherol • Antioxidants • Guinea pig • Lipid peroxide • Skin • Ultraviolet B

Introduction

With increasing solar ultraviolet B (UVB) radiation reaching the Earth's surface, and changes in lifestyle and development of holiday habits, the incidence of UVB related skin problems and interest in the capacity of sun protection products against UVB is rising steadily (Young 1997, Araki *et al.* 1999). Formation of free radicals and subsequent lipid peroxidation is considered to be the major mechanism of UV irradiation-induced cutaneous photodamage (Cohen *et al.* 1998). UVB irradiation as well as exposure to toxic or allergic chemical noxes can cause production of reactive oxygen species (ROS). The endogenous antioxidant system is able to deal with this ROS under normal conditions, however, when this system cannot handle ROS oxidative stress arises. Oxidative stress induces a variety of cellular insults including skin aging, DNA mutations leading to skin cancer and immunosuppression and pathogenesis of other skin diseases (Chan *et al.* 1986, Hurks *et al.* 1995, Fuchs *et al.* 1998). The endogenous antioxidant system contains catalase, superoxide dismutases, glutathione peroxidase and glutathione reductase as enzymatic antioxidants in skin as well as nonenzymatic antioxidants including glutathione, uric acid and α-lipoic acid (Shindo *et al.* 1994, Fuch 1998, Ichihashi *et al.* 2000). The latest group of antioxidants can be synthesized endogenously or taken up with food.

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Among several photoprotective agents, antioxidants are currently receiving considerable attention. Alpha-tocopherol (AT) is the major lipid-soluble chain-breaking antioxidant in the blood plasma and membranes (Kagan and Packer 1994). Previously, several studies have indicated that topical ATA applications have photoprotective effects on the epidermis in different models of photodamage (Beijersbergen van Henegouwen *et al.* 1995, McVean and Liebler 1997, Quevedo *et al.* 2000). However, in vivo antioxidant efficacy of topically applied antioxidants still remains to be fully determined.

We have investigated the effects of topical ATA application on UVB-induced peroxidative damages (LPO levels in skin, liver, and erythrocytes; GSH levels in skin and erythrocytes, the activities of scavenging enzymes on the skin and erythrocytes) in guinea pigs. We also attempted to test whether the possible photoprotective effects of topically applied ATA were restricted to early phase of antioxidative stress response in the skin or to a more systemic endogenous redox system.

Methods

The guinea pig was chosen as the experimental animal for this study not only because its skin histologically and biochemically is similar to the human skin. Furthermore, it also has the same natural history as human nevi and previously being described as robust small laboratory animal model for solar-simulated light experimentation (Menzies *et al.* 1998). The protocol of this study was approved by the Firat University Medical School Ethics Committee for Animal Experimentation. Forty albino guinea pigs weighing 350- 430 g obtained from the Veterinary Research and Control Institute of Elazig, Turkey, were used in this study. The animals were disease-free and had unrestricted access to a standard diet and water during the experiments. Animals were anesthetized with ketamine hydrochloride (50 mg/kg, i.m.; Ketalar, Eczacibasi, Istanbul, Turkey). The dorsal skin of the guinea pigs was washed and about 35 cm^2 area was shaved before either the application of ATA, the vehicle or exposure to UVB.

A single dose of UVB (290-320 nm) from a Phototherapy and PUVA cabinet (Dermalight 6000. Dr. Hönle AG, UV-technology, Planegg, Germany) was used so that the total energy exposure of the guinea pig was 0.9 J/cm^2. The irradiation time was approximately 30 sec. UV radiation exposure was performed by placing cages containing a maximum of five guinea pigs on the floor of the cabinet. Food, water, and microisolator lids were removed from the cages during the UVB exposure.

Daily single topical applications of ATA (Ephynal: 300 gelatine capsule, Roche, Istanbul, Turkey) at a concentration of approximately 8 mg/cm^2 along the shaved skin area of the guinea pigs for three weeks were applied before UVB irradiation. The other group of animals received only comparable volumes of vehicle of Ephynal. Guinea pigs were killed by decapitation 24 and 48 h after the irradiation. 6 ml blood were obtained from each animal. The samples were left for 30 min at room temperature, and then centrifuged at 3500-4000 x *g* for 20 min. The samples were kept frozen (-70 °C) until analysis. All samples were analysed within one month.

The shaved dorsal skin was carefully dissected free from the underlying panniculus. One g skin and 1 g liver obtained from each animal. Thereafter, each sample was thoroughly rinsed with physiological saline, diluted ten-times with distilled water and stored at -70 °C. The frozen tissue samples were homogenized in a homogenizer (Ultra-Turrax T25, Janke and Kunkel-IKA Labortechnik, Staufen, Germany).

The end-product of polyunsaturated fatty acid peroxidation, malondialdehyde (MDA) reacting with thiobarbituric acid (TBA), was determined by the method of Placer *et al.* (1966) modified by Matkovics *et al.* (1998). The values of MDA reactive material were expressed in terms of MDA (nmol/ml plasma).

The reduced glutathione (GSH) content of the plasma was measured using the method of Sedlak and Lindsay (1968). Glutathione peroxidase (GSHPx) activities were measured spectrophotometrically (Schimadzu 2R/UV, Kyoto, Japan) at 37 °C and 412 nm according to methods of Lawrence and Burk (1976) and Matkovics *et al.* (1998). The GSHPx values were expressed as IU/g protein. The protein content of the plasma was measured by the method of Lowry *et al.* (1951).

The SOD activity was assayed by the method described by Sun *et al* (1988) for estimation of SOD activities in blood cells. This method is based on the reduction of superoxide, which is produced by xanthine oxidase enzyme system, by nitroblue tetrazolium. One unit of SOD was determined as amount reduces nitroblue tetrazolium's reduction by 50 %. Results are expressed as U/g hemoglobin.

The results are given as means ± S.E.M. ANOVA and the least significant difference test was used for comparison between groups. Data were analyzed

statistically with the SPSS for Windows statistical program (SPSS; Chicago, Illinois). Results were considered to be significant when $p < 0.05$.

Results

The results of this study are summarized in Tables 1, 2, and 3.

UVB irradiation caused time-dependent changes in LPO levels among skin, liver, erythrocyte and plasma. If comparisons were made 24 h after UVB irradiation, LPO levels were higher in the skin but lower in the liver, erythrocytes and plasma but none of these changes was statistically significant. 48 h after UVB irradiation LPO levels were higher in all tissues or cells mentioned above compared to control group being only significant in the skin and the liver. Topical ATA application effectively prevented increases in LPO levels in the skin but was not marked in liver, erythrocytes or plasma as much as in the skin (Table 1).

Table 1. The skin, liver, erythrocyte and plasma lipid peroxide (LPO) levels and indices obtained from statistical analysis in group of animals involved in this study.

Groups	Skin LPO (μmol/g)	Liver LPO (μmol/g)	Erythrocytes LPO (nmol/ml)	Plasma LPO (nmol MDA/ml)
Control (Vehicle) group	5.63 ± 2.95 [c, e]	14.71 ± 3.54 [c, d]	25.33 ± 1.14	1.27 ± 0.08 [b, d ,e]
Group 1 (Vehicle + UVB at 24 h)	6.76 ± 1.55 [d]	12.45 ± 2.38 [c]	23.51 ± 2.21 [c]	1.17 ± 0.06 [c, d]
Group 2 (Vehicle + UVB at 48 h)	7.76 ± 2.19 [a, e]	17.67 ± 2.42 [a, b, e]	26.62 ± 3.53 [b]	1.46 ± 0.17 [b, e]
Group 3 (ATA + UVB at 24 h)	3.95 ± 1.03 [b]	10.31 ± 1.99 [a, e]	24.16 ± 2.44 [e]	1.06 ± 0.08 [a, e]
Group 4 (ATA + UVB at 48 h)	3.63 ± 0.35 [a, c]	14.43 ± 1.46 [c, d]	27.21 ± 1.71 [d]	1.59 ± 0.20 [a, c, d]

[a] Significantly different from control group ($p<0.05$), [b] Significantly different from group 1 ($p<0.05$), [c] Significantly different from group 2 ($p<0.05$), [d] Significantly different from group 3 ($p<0.05$), [e] Significantly different from group 4 ($p<0.05$). The values are presented as mean ± S.E.M.; each group consisted of 10 guinea pigs.

Table 2. Skin and erythrocyte GSHPx, activities and GSH levels of each group of guinea pigs.

Groups	Skin GSHPx (IU/g protein)	Erythrocytes GSHPx (IU/g protein)	Erythrocytes GSH (mmol/L)
Control (Vehicle) group	0.31 ± 0.03 [b, d, e]	63.05 ± 3.65 [b c d e]	0.73 ± 0.09 [b, c, d, e]
Group 1 (Vehicle + UVB at 24h)	0.05 ± 0 .01 [a, c]	46.57 ± 9.50 [a, c]	1.12 ± 0.25 [a]
Group 2 (Vehicle + UVB at 48h)	0.21 ± 0.05 [a, b, d, e]	27.49 ± 8.38 [a, b, e]	1.14 ± 0.34 [a]
Group 3 (ATA + UVB at 24h)	0.09 ± 0.03 [a, c]	48.73 ± 12.05 [a, e]	1.35 ± 0.50 [a]
Group 4 (ATA +UVB at 48 h)	0.12 ± 0.01 [a]	13.83 ± 3.42 [a, c, d]	1.24 ± 0.30 [a]

[a] Significantly different from control group ($p<0.05$), [b] Significantly different from group 1 ($p<0.05$), [c] Significantly different from group 2 ($p<0.05$), [d] Significantly different from group 3 ($p<0.05$), [e] Significantly different from group 4 ($p<0.05$). The values are presented as Mean ± S.E.M.; each group consisted of 10 guinea pigs.

Erythrocyte GSH levels in irradiated animals 24 and 48 h after UVB irradiation were higher than in the control group ($p<0.05$), but topical application of ATA had no effect on UVB irradiation-induced changes in erythrocytes GSH levels (Table 2). Twenty-four hours after UVB irradiation, the GSHPx activities in skin and erythrocyte of the UVB-irradiated animals were significantly lower than in the control group ($P<0.05$). The ATA applied and irradiated guinea pigs skin and erythrocyte GSHPx activities were lower than the only irradiated group at 48 h after UVB-irradiation ($p<0.05$). Topical applications of ATA fail to return GSHPx levels to the control levels in skin or erythrocytes (Table 2).

After UVB irradiation, SOD activities of the skin and the content of hemoglobin were significantly lower than those of control group ($P<0.05$). Topical application of ATA prevented this UVB irradiation-induced decrease in skin but not that of hemoglobin (Table 3).

Table 3. Skin and erythrocyte SOD activities of each group of guinea pigs.

Group	Skin SOD (IU/g protein)	Erythrocyte SOD (IU/g hemoglobin)
Control (Vehicle) group	6.24 ± 0.48 [b, c]	355.00 ± 11.71 [b, c, d, e]
Group 1 (Vehicle + UVB at 24h)	1.34 ± 0.18 [a, c, d]	58.37 ± 5.13 [a, c]
Group 2 (Vehicle + UVB at 48h)	3.26 ± 0.38 [a, b, e]	194.75 ± 6.03 [a, b]
Group 3 (ATA + UVB at 24h)	6.40 ± 1.00 [b, c]	63.11 ± 10.22 [a, e]
Group 4 (ATA + UVB at 48 h)	6.80 ± 1.22 [b, c]	186.53 ± 8.05 [a, d]

[a] Significantly different from control group ($p<0.05$), [b] Significantly different from group 1 ($p<0.05$), [c] Significantly different from group 2 ($p<0.05$), [d] Significantly different from group 3 ($p<0.05$), [e] Significantly different from group 4 ($p<0.05$). The values are presented as mean ± S.E.M., each group consisted of 10 guinea pigs.

Discussion

The results suggest that topical application of ATA significantly reduced UVB irradiation-induced lipid peroxide production and decreased SOD activities in the skin compared to the application of its vehicle formula**.** The initial elevation of LPO levels seems to be due to the ROS-producing effect of UVB radiation and the resultant formation of lipid peroxides from unsaturated fatty acids. The lower enzyme activities observed after irradiation were most likely due to the enzyme inactivating activity of ROS induced by UVB radiation (Record *et al.* 1991). These findings are consistent with the results of previous studies (Miyachi *et al.* 1987, Record *et al.* 1991, Iizawa *et al.* 1994). It is more likely that the effect of topically applied ATA is related to its UV absorption capacity and restricted to the early phase of the antioxidative system in the skin rather than to the endogenous redox system as only limited protection was observed in the liver, erythrocytes and plasma.

Topical application of ATA has different effects on level and activities of LPO and antioxidants in the skin, liver, erythrocyte, and plasma. It is possible that topical ATA only affected the skin but not other organs or cells. The decrease of LPO in the liver by ATA might be due to the fact that the level of liver LPO mostly reflects carried LPO from the skin and the LPO production in the skin was suppressed by topical ATA.

Antioxidants such as ascorbic acid and d-alpha-tocopherol have been found to be photoprotective in some *in vitro* studies and animal experiments. Alpha tocopherol can act as a scavenger of free radicals or singlet oxygen. Previous studies have indicated that topically applied vitamin E has photoprotective effects in mouse epidermis (McVean *et al.* 1997). Systemic administration of various antioxidants, including α-

tocopherol, has been found to be photoprotective in some *in vivo* studies and animal experiments against sunburn-induced photodamage (Eberlein-Konig *et al.* 1998). Trevithick *et al.* (1992) found that the use of topical ATA reduced erythema, edema, and sensitivity when applied immediately after UVB exposure. Another study showed that vitamin E inhibited the UVB mediated production of malonyl dialdehyde, which is the end product of lipid peroxidation and causes a significant reduction in polyamine biosynthesis (Khettab *et al.* 1988).

In vitro studies have also been employed to investigate antioxidant effects against UV radiation**.** In an *in vitro* model system using human squamous cell carcinoma line and human newborn keratinocytes, alpha-tocopherol has been shown to protect against UV-mediated cell death or growth arrest (Werninghaus *et al.* 1991)

Skin and erythrocyte GSHPx levels were suppressed by UVB irradiation and application of ATA before UVB irradiation did not help to prevent this suppression except the erythrocyte GSHPx levels, which were significantly decreased at 48 h and were not influenced by ATA treatment.

The increase in erythrocyte GSH levels by UVB irradiation may be due to the decreased activity of the GSHPx, which catalyses the conversion of the GSH to the oxidized glutathione. Application of ATA did not change the erythrocyte GSH levels.

There were only negligible changes in erythrocyte LPO levels after UVB irradiation and application of ATA before UVB irradiation had no effect on UVB induced changes in erythrocyte LPO levels. Although 24 h after irradiation the plasma LPO levels showed a significant decrease, it returned to its basal levels by 48 h. Plasma LPO levels at 48 h were significantly increased by topical application of the vitamin E in guinea pigs. It was difficult to interpret the mechanisms underlying the decrease of the plasma LPO levels at 24 h after UVB irradiation and the increase at 48 h after treatment with ATA.

In conclusion, the topical application of ATA for three weeks before UVB irradiation can significantly protect the skin against UVB-induced damage by enhancing the antioxidant capacity and also by reducing the lipid peroxidation products. But these parameters cannot reflect the overall damage induced by UVB irradiation and therefore further study is required including observations of human skin in order to investigate systemic effects of topically applied antioxidants.

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