INHIBITION OF ERYTHEMA OF THE SKIN PHOTOSENSITIZED WITH 8-METHOXYPSORALENE BY α-TOCOPHEROL

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8-Methoxypsoralene (8-MOP) and other psoralene derivatives are effective photosensitizers of the animal and human skin to near ultraviolet radiation (320-400 nm), and under these circumstances erythema (PUVA erythema) [5] and pigmentation of the skin [8] develop. In clinical practice the psoralenes have found widespread application for the phototherapy of vitiligo, psoriasis [5], and certain other skin diseases. The photochemical reaction of the psoralenes which has received the most study is their covalent photoaddition to DNA with the formation of cyclobutane adducts [3]. Many workers consider that this photochemical reaction of the psoralenes lies at the basis of induction of photobiological effects observed in the skin, for the ability of different psoralene derivatives to photosensitize the skin correlates closely with their ability to undergo photoaddition to DNA [4, 14]. However, the psoralenes can also take part in other photochemical reactions. For example, in the presence of oxygen 8-MOP photosensitizes inactivation of lysozyme [13] and for ribosomes from *Escherichia coli* [15], uncoupling of oxidative phosphorylation in mitochondria [11], and accumulation of oxidation products of lipids in suspensions of mitochondria [11] and liposomes [7]. It is considered that these processes take place through the participation of singlet oxygen generated by 8-MOP under the influence of near UV radiation [2]. The psoralene can thus take part in a wide variety of photochemical reactions. As yet no attempt has been made to evaluate the effect of photo-oxidative reactions involving psoralenes on the induction of erythema of the skin. One way of studying this problem is to use antioxidants. In the investigation described below the role of the antioxidant α-tocopherol in the induction and development of erythema of the human and rabbit skin, photosensitized with 8-MOP, was investigated.

EXPERIMENTAL METHOD

α-Tocopherol and α-tocopheryl acetate (synthetic D,L-forms) were synthesized and generously provided by I. K. Sarycheva (M. V. Lomonoscov Moscow Institute of Fine Chemical Technology), and 8-MOP was obtained from J. Barth (Dermatological Clinic, Karl Marx University, Leipzig, East Germany) and from G. B. Zavil'gel'skii (Institute of Molecular Biology, Academy of Sciences of the USSR, Moscow).

Experiments were carried out on human skin and skin of albino rabbits. By means of a microsyringe 10 μl of a solution of 8-MOP in ethanol (2.5×10^{-3}M) or 10 μl of a mixture of 8-MOP and α-tocopherol (2.5×10^{-3}M and 5×10^{-3}M, respectively) was applied to human skin on an area of 2.3 cm^2; the surface concentration of 8-MOP and α-tocopherol were approximately 2.2×10^{-8} and 4.4×10^{-8} mole/cm^2, respectively. Ethanol was applied to control areas for 30 min before irradiation. The solvent evaporated in the course of a few seconds.

The hair on the rabbits' back was shaved with a safety razor 24 h before irradiation. Forty minutes before irradiation 0.23 ml of a solution of 8-MOP in ethanol was applied to an

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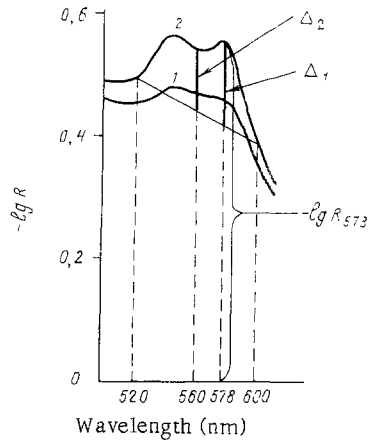

Fig. 1. Spectra of diffuse reflection of light from human skin before irradiation (1) and after development of erythema induced by UV radiation in the region 285-315 nm (2). Top curve shows method of measurement of log R_{578} and log R_{600} and also of Δ_1 and Δ_2 used for calculating factor C ($C = \Delta_1/\Delta_2$).

area of skin bounded by a polyethylene ring 17 mm in diameter (the surface concentration varied from 5×10^{-10} to 5×10^{-9} mole/cm^2). Twenty minutes before or immediately after irradiation 0.23 ml of a solution of α-tocopherol or α-tocopheryl acetate in ethanol (10^{-10}-10^{-7} mole/cm^2) was applied. Ethanol alone was applied in the same order to control areas of skin. The solvent was evaporated by a current of air in the course of 2-3 min.

Irradiation was given by means of a very high pressure mercury-quartz lamp (SVD-120A), the light from which, focused by a quartz lens, was passed through an SS-1 filter with a transmission band in the long-wave region above 320 nm. To determine the sensitivity of the skin 8-MOP was applied to a series of different areas, which were then irradiated with doses of UV light increasing by 30% from one area to the next. The degree of erythema was determined visually 2 days later. The dose causing hardly distinguishable erythema was taken as the minimal erythema dose (MED).

The development of erythema of the skin was measured by recording diffuse light reflection spectra. These spectra were measured on the SF-14 spectrophotometer. Spectra of reflection of light from the human skin before irradiation and after the development of UVB-erythema, induced (without sensitizers) by UV radiation in the region of wavelengths of 285-315 nm (UVB) are shown in Fig. 1. In erythema the spectrum as a whole was raised and resolution of the maxima at about 540-578 nm, evidently due to oxyhemoglobin, was increased. The reflective power (R) was calculated by the following equation:

$$R = \frac{I}{I_0},$$

where I is the intensity of light reflected from the skin and I_0 its intensity reflected from a white standard background, usually from a magnesium oxide plate. Reflective power measured at 579 nm can be used to assess the degree of erythema of the skin and pigmentation, whereas the same parameter at 600 nm can be used to measure only pigmentation [10]. In the present experiments the value of log R at 578 and 600 nm was recorded. In addition a parameter (the so-called factor C) was introduced, whereby the degree of resolution of the maxima at 540 and 578 nm can be assessed. The method of calculating factor C is shown in Fig. 1. It is shown previously that in UVB-erythema values of factor C increase in a straight line with an increase in the hemoglobin concentration in the skin [9].

Changes in the mechanicoelectrical properties of the skin photosenitized by 8-MOP also were studied by the method of electroelastometry [12]. An electrode consisting of an outer hollow circular electrode and an inner spherical electrode was applied in the skin. An alternating current with a frequency of 10 kHz was applied to the electrodes. Inside the

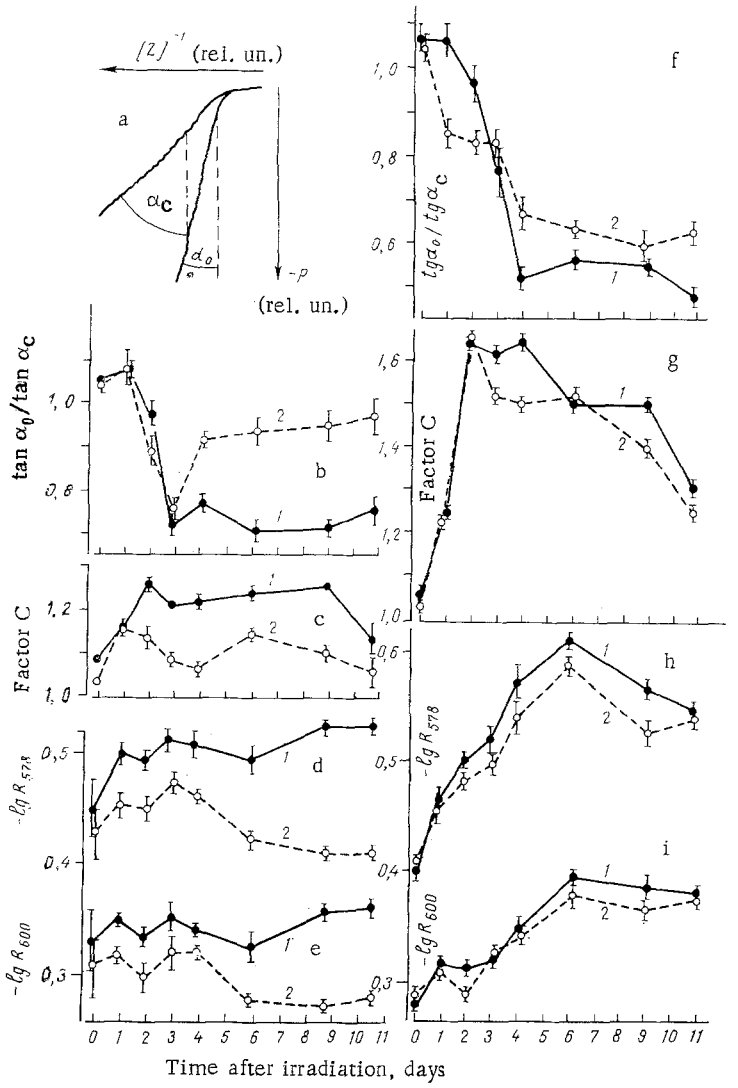

Fig. 2. Effect of α-tocopherol on development of erythema (c, d, g, h), pigmentation (e, i), and changes in mechano-electrical properties (b, f) of human skin under the influence of UV radiation with wavelengths of over 320 nm, in the presence of 8-MOP, with doses of 3 MED (b, c, d, e) and 10 MED (f, g, h, i): 1) 8-MOP, 2) 8-MOP + α-tocopherol. a) Method of recording tan P_0 and tan α_c.

circular electrode a reduced pressure was created and the skin was drawn into the electrode, thereby increasing the area of contact with the spherical electrode, with a consequent increase in electrical conduction. To prevent mechanical injury to the skin a pressure of not more than 0.02 atm was used. The dependence [12] was satisfied:

$$|Z|^{-1} = \frac{A}{E \cdot \rho \cdot d} \cdot P,$$

where Z is the impedance, A the area of skin inside the circular electrode, E the modulus of electricity, ρ the electrical conductivity, d the thickness of the skin, and P the pressure Values of tan $\alpha = |Z|^{-1}/P$, the method of calculation of which is shown in Fig. 2a, were recorded. Values of tan α_0 of the irradiated area of skin at different times after irradiation were com-

pared with tan α_c of the neighboring unirradiated area. The value of tan α falls if the product $E \cdot \rho \cdot d$. rises; this method thus records a parameter which depends on the elastic and electrical properties of the skin simultaneously.

EXPERIMENTAL RESULTS AND DISCUSSION

The effect of α-tocopherol on PUVA-erythema was determined visually on the skin of 39 rabbits 24 h after irradiation. In 58 experiments the antioxidant was applied to the skin before irradiation and in 49 of them considerable or total inhibition of PUVA-erythema was observed (the dose was less than 5 MED). In nine experiments no protective effect was observed, and in six of them the dose of irradiation was considerable and exceeded 5 MED. In 33 of 46 experiments of which α-tocopherol was applied immediately after irradiation, no protective effect whatsoever was observed, and in nine experiments the effect was weaker than that observed when the antioxidant was applied before irradiation. In four experiments the protective effect was the same whether α-tocopherol was applied after or before irradiation. α-Tocopheryl acetate did not affect PUVA-erythema, whatever the method of its application.

The results show that α-tocopherol protects the skin against PUVA-erythema during irradiation; if it is applied after irradiation, its inhibitory effect in most cases either disappears or is greatly weakened. This is evidence of a significant difference between the mechanisms of PUVA- and UVB-erythema. An important role in the development of UVB-erythema is played by photochemical after-reactions, most probably the development of processes of lipid peroxidation in the skin under the influence of UV radiation. These peroxidation processes were inhibited equally by α-tocopherol irrespective of whether it was applied before or immediately after UVB-irradiation, whereas in PUVA-erythema the photochemical after-reactions dependent on α-tocopherol were evidently absent. α-Tocopherol can take part in two types of reactions: a) it inhibits free-radical processes by interacting directly with free radicals [1]; b) it is an effective quencher of singlet oxygen generated by many dyes during irradiation [6]. The possibility cannot be ruled out that both types of reactions of α-tocopherol play an important role in the inhibition of PUVA-erythema, for 8-MOP, in the presence of UV-irradiation, can generate both singlet oxygen and free-radical products [2].

The development of PUVA-erythema of the human skin was recorded as diffuse reflection spectra and changes in mechanoelectrical properties after the use of doses of 3 and 10 MED (Fig. 2). α-Tocopherol was applied before irradiation. It will be seen that a marked protective effect was observed for all parameters recorded after a dose of 3 MED, but after a dose of 10 MED the antioxidant had a very weak or virtually no inhibitory action.

Differences in the character of development of erythema assessed on the basis of factor C and of reflective power of 578 nm will be noted. This difference is particularly clear if Fig. 2, g and h are compared. Factor C reaches a maximum sooner and begins to fall sooner than $-\log R_{578}$. Pigmentation of the skin begins to develop 3-4 days after irradiation ($-\log R_{600}$ rises). If α-tocopherol was present during irradiation (3 MED) pigment-formation was inhibited (Fig. 2e). Comparison of Fig. 2d with Fig. 2e, h, i, shows that pigmentation had a considerable effect on the values of reflective power at 578 nm, and for that reason estimation of the degree of erythema on the basis of this parameter is highly distorted. Pigmentation has much less or no effect whatever on the value of factor C (Fig. 2c and Fig. 2e, g, i). Evidently the two parameters of the reflection spectra used to assess the degree of erythema carry different information.

Judging from the results obtained by electroelastometry, an increase in the value of the product $E \cdot \rho \cdot d$. increases with time in the irradiated skin. It is not known exactly which of the multiplicands make the greatest contribution to this effect. It was noted visually that parallel with the decrease in the ratio $\tan \alpha / \tan \alpha_c$ swelling of the skin took place and evidently led to an increase in the thickness (d) and modulus of elasticity (E). In a dose of 3 MED α-tocopherol (Fig. 2b) considerably weakened the change in mechanoelectrical properties of human skin photosensitized by 8-MOP. If α-tocopherol was present during irradiation, it also reduced visible swelling of rabbit skin photosensitized by 8-MOP.

In doses of irradiation of about 5 MED, α-tocopherol thus considerably inhibits PUVA-erythema and pigmentation and prevents changes in the mechanoelectrical properties of human and rabbit skin. An inhibitory effect was manifested only when the antioxidant was applied before irradiation, and no such effect appeared when it was applied after irradiation.

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EFFECT OF DISSEMINATED NECROSIS OF THE MYOCARDIUM ON ATPase ACTIVITY, Ca^{++} TRANSPORT, AND LIPID PEROXIDATION OF MITOCHONDRIAL AND MICROSOMAL MEMBRANES OF THE HEART

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The sympathetic nervous system plays an important role in the development of myocardial infarction. Injection of adrenalin or sympathomimetic drugs into animals in doses much higher than normal leads to ischemia followed by the development of foci of necrosis.

One possible mechanism of the action of catecholamines in producing necrosis is an increase in permeability of cell membranes, together with activation of lipases. Changes in these factors may arise as a result of structural changes in protein and lipid components of subcellular membranes. It is particularly important that in the presence of a disturbance of membrane structures of the myocardial cell Ca++ transport is affected and, as a result, an excess of intracellular calcium arises [8]. Together with an excess of fatty acids in the cell, this may be the cause of depression of ATP resynthesis in the mitochondria [5, 6]. An increase in the fatty acid content in the myocardium during hypoxia combined with an increase in the content of promotors of free-radical peroxidation of lipids (PL) [1] also suggests that damage to the cell membranes takes place as a result of PL [3].

Against this background it was decided to study Ca^{++} transport processes through mitochondrial membranes and fragments of the sarcoplasmic reticulum (SR) and the intensity of PL in them, using a model reproducing the development of foci of necrosis by injection of an excess of the β-adrenomimetic isoproterenol.

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