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(RESEARCH ARTICLE)

Effects of α-tocopherol on phototoxicity of 8 - methoxypsoralene

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Abstract

Effects of singlet oxygen extinguisher α-tocopherol on combined effects of psoralens and UV on erythema photosensitized with 8-methoxypsoralene were investigated. The leading role of the reactions of photocompounds in induction of PUVA - erythema is considered. A characteristic feature of biologically active psoralens is the high value of quantum output of intercombination conversion, due to which, after absorption of light quants, a significant number of psoralen molecules are formed in a triplet excited state. Psoralen derivatives differ greatly in their ability to photosensitize the skin. In the example of 8-psoralens, a correlation was found between the ability to induce erythema by photocompound to double-spiral DNA in vitro. A clear correlation was identified between the ability to photosensitize erythema and form diadducts. A-tocopherol has been shown to protect the skin from PUVA erythema if presents during irradiation. In the case of application of α-tocopherol after irradiation, the inhibitory effect was not exhibited.

Keywords: 8-methoxypsoralene; Ultraviolet irradiation; Combined exposure of psoralenes; α-tocopherol, α-tocopheryl acetate, Optical density

1. Introduction

Biological objects have sensitivity to light, since light is absorbed by many endogenous chromophores, for example: visual pigments, proteins, nucleic acids. Exogenous chromophores, entering the body, absorb visible or ultraviolet (UV) light, in which case the photosensitivity of tissues increases sharply [1]. Compounds increasing sensitivity of biological objects to light are referred to as photosensitizers. The combined effects of light and photosensitizers cause positive therapeutic effects [2]. On the basis of such combined effects of light and dyes on the human body, photochemotherapy methods are created [3]. Since ancient times, psoralens have been used to treat skin diseases - plant-derived photosensitizers that increase the sensitivity of biological tissues to long-wave UV [4]. Currently, two types of photochemotherapy are best known. In one of them, porphyrins and visible light are used to kill malignancies – this is photodynamic therapy [6.7]. Complex therapy is also used: psoralens and long-wave UV radiation for the treatment of skin diseases (this is PUVA - therapy) [8.9]. The term PUVA is composed of the first letters of the words "psoralen" and the UV range A - UV-A. Separation of UV spectrum region into ranges A (320-400 nm), B (280- 320 nm) and C (wavelengths shorter than 280 nm) is introduced in medicine on the basis of different skin sensitivity to these types of radiation [3.10].

Psoralenes (furocumarins) are a group of substances of plant origin. Some psoralene derivatives are synthesized. In the presence of psoralens, skin sensitivity to near ultraviolet light (UV-315-400 nm) is sharply increased. Psoralens in combination with UV cause a number of photobiological effects. The most important of them are treatment of psoriasis and other skin diseases [8,11]. In addition to therapeutic effects, other photobiological effects are observed in the skin: erythema, oedema, vitiligo. The skin disease of vitiligo (leukoderma) is that in some areas of the skin are degrade and

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melanocytes, the cells that produce the pigment melanin disappear. These areas of the skin are never pigmented by sunlight. For the treatment of vitiligo, photochemotherapy is used using extracts obtained by boiling seeds of plants of the umbrella family *Ammi majus L.* or legumes *Psoralea corylifolia L.* [3]. The molecular mechanisms underlying these photobiological processes have been little studied. Elucidating the causes of PUVA erythema should help in the treatment of skin diseases and avoid unwanted side effects. Researches of the combined effects of psoralens and UV on skin erythema is a actual task.

In the literature, there is a view that the cause of PUVA erythema is the photochemical combination of psoralenes to pyrimidine bases of nucleic acids. This photoreaction is a unique hallmark of psoralens from other photosensitizers and is best studied in model systems. It is the basis of lethal and mutagenic photo effects of psoralens on microorganisms [11,12,13]. Psoralens after absorption of UV light quanta are able to covalently cross-link with pyrimidine bases of nucleic acids. Two regions of the psoralen molecule can participate in the formation of crosslinks with nucleic acids: 3,4 - and 4,5-carbon atoms. Photoadduct by one of these sites results in monoadducts. Monoadducts, absorbing another quantum of light, can be photoadducted by the second region to form diadducts [3]. Diadducts covalently cross-link two strands of DNA.

It has been found that the therapeutic effect of medicinal plants is due to the presence in their extracts of 8-methoxypsoralene molecules (8-MOP) [3]. This compound absorbs in the UV region of the spectrum and is a photosensitizer. The present article examines the effects of the singlet oxygen extinguisher α-tocopherol (vitamin E) on 8-methoxporalene (8-MOP) photosensitized erythema. 8-MOP was chosen because this derivative of psoralen widely used in the clinic.

2. Methods

8-MOP was provided by the Institute of Molecular Biology of the Russian Academy of Sciences and the Dermatology Clinic of the University of Leizzig, Germany.

Timin and highly polymeric DNA were obtained from “Reanal”, Hungary. DNA from calf thymus and DNA were obtained from “Sigma”, USA.

Synthetic preparations of α-tokoferol, α-tocopheryl acetate were obtained from the Moscow Institute of Thin Chemical Technology named after M.V. Lomonosov.

Chloroform, ethanol and methanol were distilled before the test. Acetone was dried in the presence of anhydrous calcium chloride ($CaCl_2$) and distilled.

The source of UV light was a mercury-quartz lamp of ultra-high pressure UHP-120A. When irradiating the skin, the light was focused with a quartz lens through a light filter CC-1, the transmission spectrum of the light filter.

During irradiating solutions, light was focused by a quartz lens and a quartz flask filled with water (heat filter) through a BS-10 light filter. Thermostatting cuvette with the irradiated solution was carried out by the air flow from the fan, the change in the temperature of the solution during irradiation did not exceed 1^0.

The duration of skin irradiation was from a few seconds to 20 minutes. Duration of irradiation of solutions was 30 minutes.

Photoaddition of 8-MOP to DNA was studied by the method of Dall, Akua and co-authors to determine fluorescent cycloadducts of psoralenes: DNA. After DNA hydrolysis, quenching was eliminated and it became possible to measure the fluorescence spectra of cycloadducts.

The effect of tocopherol on photoaddition of 8-MOP to pyrimidine bases has been studied. Irradiation and recording of absorption spectra was carried out in a solution of a mixture of thymine, 8-MOP and α- tocopherol. The solubility of all substances is very different. Thymine is highly soluble in water and poorly in ethanol, tocopherols - vice versa. 8-MOP in the concentrations used, dissolves in a mixture of water : ethanol of any composition. The turbidity of tocopherol and tocopheryl acetate solutions in water : ethanol mixtures was measured on the “Spectrom-201” spectrophotometer at wavelengths where the intrinsic absorption of these compounds is almost zero.

Low turbidity was observed at ethanol concentrations above 60%. 80% ethanol was selected for the experiments, in which all test compounds were dissolved well. A part for comparison was carried out in 2% ethanol, where tocopherol and tocopheryl acetate formed turbid emulsions. Irradiation was carried out under vacuum in a special vacuum quartz cuvette. Remove of oxygen was performed by a vacuum pump. In the case of ethanol 80% to avoid ethanol evaporation, the solution was frozen in liquid nitrogen at -196^{0}C before being pumped out of the air from the cuvette. After 3 minutes of vacuumization, the vacuum valve was closed, the solution thawed and frozen again. After that, the repeated vacuumization was carried out. This operation was repeated 5 times. Some experiments were carried out in an argon atmosphere.

3. Results

The photoaddition of psoralens to DNA differs from the photoaddition to pyrimidine bases in solution, in the case of DNA, fluorescent adducts are formed when irradiated at positive temperatures, while with pyrimidines they are formed only in frozen solutions. Another distinguishing feature of photoaddition of psoralenes to double-spiral nucleic acids is that this photoreaction is weakly inhibited in the presence of oxygen. This is explained that after intercalation, psoralen, firstly, becomes less accessible for oxygen extinguishing and, secondly, intercalated psoralen in the dark is already in contact with the bases which the photoadduct should form. It seems that the dark complex with DNA is sterically very advantageous for photoreaction.

PUVA exposure can be attributed to phototoxic effects. Before discussing the inhibitory effects of tocopherols on PUVA exposure, it is necessary to focus on the features of PUVA toxic reactions of the skin, since little attention is paid to these issues in the literature.

Figure 1 shows the development kinetics of PUVA-erythema measured by -lgR_{578}. Measurements were taken in the first two days after exposure, when pigmentation did not develop (-lgR_{600} did not increase). Under these conditions, the parameter of the reflection spectra lgR_{578} characterizes erythema only and is not distorted by skin pigmentation. However, if the observations are continued for a longer period, it can be seen that already on the 5th day after irradiation, the lgR_{578} values become overestimated due to the development of pigmentation and the resulting general rise of the spectrum (Figure 2, curve I). The kinetics of the development of redness, estimated by the C-factor, look different. Starting from the 4th, 5th day of irradiation (Figure 2), the values of the C-factor began to decrease, although during this period it was the most intense pigment formation took place in the skin. These observations suggest that factor-C in long-term registration of the erythema does not depend on the development of pigmentation and more adequately reflects the development of PUVA-erythema than lgR_{578}. A feature of Figure 1 is that in this experiment the reflection spectra were recorded in detail in the first hours after irradiation. This allowed to find that there was immediately (in the first two hours) a general rise of the spectrum (Figure 1), as well as the resolution of maximums increase at 540 and 578 nm. After 2 hours, these parameters returned to their previous state, after which the slow development of redness and pigmentation began for several days. For further measurements, this rise in spectra was not recorded for methodological reasons. Usually, a large number of spots were recorded, so it was not possible to trace in detail the rapid changes in spectra in the first two hours after irradiation of the each area of the skin. In further experiments, each point on the graph corresponds to 3-5 repeated measurements taken over 2-3 hours and then averaged. By chance, in Figure 5, such multiple measurements coincided with the rapid rise of the spectrum, so the values of the C-factor in the first recording after irradiation, when delayed erythema has not yet begun to develop, have values greater than 1. Typically, the C factor for unreddened skin is close to 1.

A peculiarity of PUVA-erythema is that the latent period at radiation doses close to MED lasts about 2 days, which is much more than for UVB-erythema or UVS-erythema, where the latent period did not exceed 8010 hours. At doses that exceeded the MED several times, the maximum redness developed on the 2-3 day. The latent period of pigment formation lasted 2-3 days, the development of skin pigmentation was completed a week after irradiation.

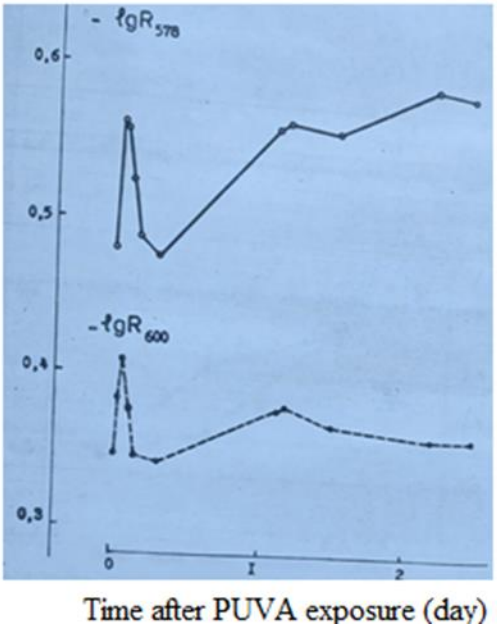

Figure 1 Change -lgR578 and -lgR600 in human skin after PUVA exposure. 8-MOP was applied by the 1st method (see section III.3.1) at a surface concentration of 5 · 10-9 mol/cm2 of the skin. Radiation dose is 2.5 MED. In axes: abscissa - time after irradiation (day); ordinate - lgR, where R is skin reflectivity at 578 (upper curve) and 600 nm (lower curve).

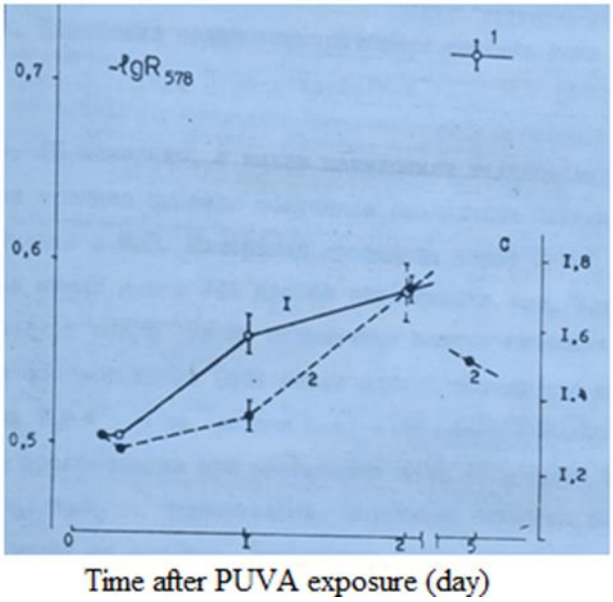

Figure 2 Change in -lgR578 (curve 1) and C-factor (curve 2) of human skin after PUVA exposure. 8-MOP was applied by the 1st method (see section III.3.1) at a surface concentration of 5 · 10-9 mol/cm2 of skin. Radiation dose is 6 MED. In axes: abscissa - time after irradiation (day); ordinate – -lgR578 and C-factor.

Figure 3 shows the change in the mechanoelectric properties of the skin during PUVA exposure. A change in the mechanoelectric properties of rabbit skin was observed by irradiation with various doses of UV light in the presence of 8-MOP. Measurements were carried out a day after irradiation. At small doses of UVA, the curve dependence of tgd_o/tgd_k on dose rises up, then a sharp decrease begins.

When the ratio tgdo/tgdk decreased, the swelling of the skin, an increase in its thickness, was visually observed. The increase in this ratio was not accompanied by any eye-visible skin changes.

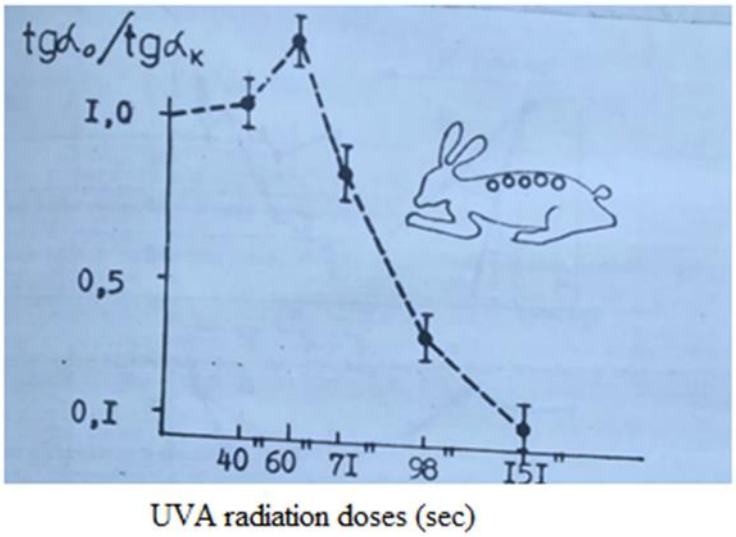

Figure 3 Changes in the mechanoelectric properties of rabbit skin after PUVA exposure. The concentration of 8-MOP in all areas of the skin is constant, doses of UV radiation were varied. Measurements were carried out on the next day after irradiation.

A similar form of dependence was obtained by detailed recording of changes in the mechanoelectric properties of human skin over time after PUVA exposure (Figure 4, A, curve 1). In the initial stages, the curve crawled up without eye-visible skin changes, then there was a decrease accompanied by swelling of the skin (Figure 4, A, B, curves 1).

Based on visual observations, it can be concluded that a certain contribution to the increase in the value of the product of these parameters gives an increase in the thickness (d) and modulus of elasticity (E) of the skin. In order to quantify the contribution of each parameter, independent measurements by other methods are needed.

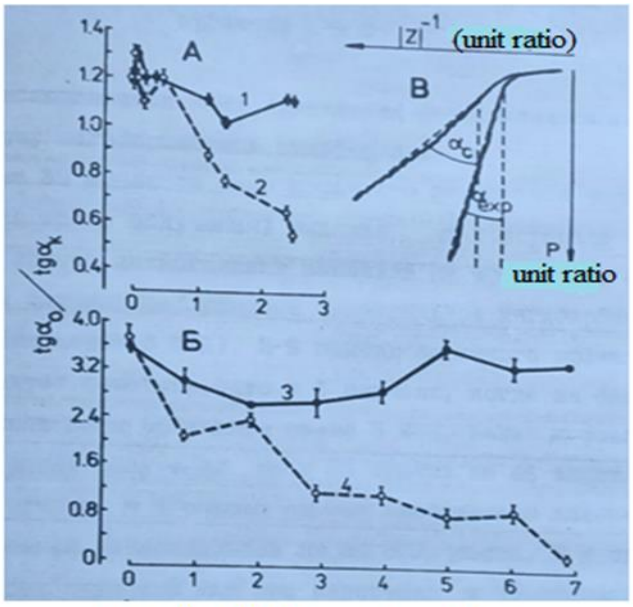

Figure 4 Inhibition of α-tocopherol photosensitized 8-MOP changes in the mechanoelectric properties of the skin.

A- human, 2.5 MED dose, surface concentrations of 8-MOP and α-tocopherol 2.4.10-9 mol/cm2 of skin.

B - rabbit, dose 3 MED, surface concentrations of 8-MOP and α-tocopherol 10-6 and 2.3.10-8 mol/cm2 of skin, respectively. 8-MOP and tocopherol were applied before irradiation. Figure B shows how to calculate tgd.

All the manifestations of the phototoxic action of 8-MOP on the skin that we observed were inhibited by tocopherols. On the skin of 39 rabbits, a visual determination was made (one day after irradiation) of the effect of -tocopherol on PUVA erythema. In 58 tests, the antioxidant was applied to the skin before irradiation, of which 49 showed significant inhibition of PUVA erythema (dose less than 5 MED). No protective effect was observed in 9 tests. It should be noted that in 6 cases where there was no protection, doses of more than 5 MED were used. If the a-tocopherol was applied immediately after irradiation, then in 33 tests out of 46 there was no protective effect at all, in 9 tests it was significantly weaker than in the case of applying an antioxidant before irradiation. In 4 tests, the protective effect was the same both in the application of -tocopherol after and before irradiation. α-tocopheryl acetate did not affect PUVA erythema in any method of its application.

The results of one such tests recorded by the skin diffuse reflection spectrum measurement method are shown in Fig.5. It can be seen that both by -lg R_{578} (Fig. 5, B) and by C-factor (Fig. 5.A) after irradiation of rabbit skin with a dose of 2 MED, the development of redness began (Fig. 5, A, B, curves 2), the application of α-tocopheryl acetate both before and after irradiation (Fig. 5, A.B. curves 5 and 4) or tocopherol after irradiation (Fig.5, A.B, curves 1) had no significant effect on the development of redness.

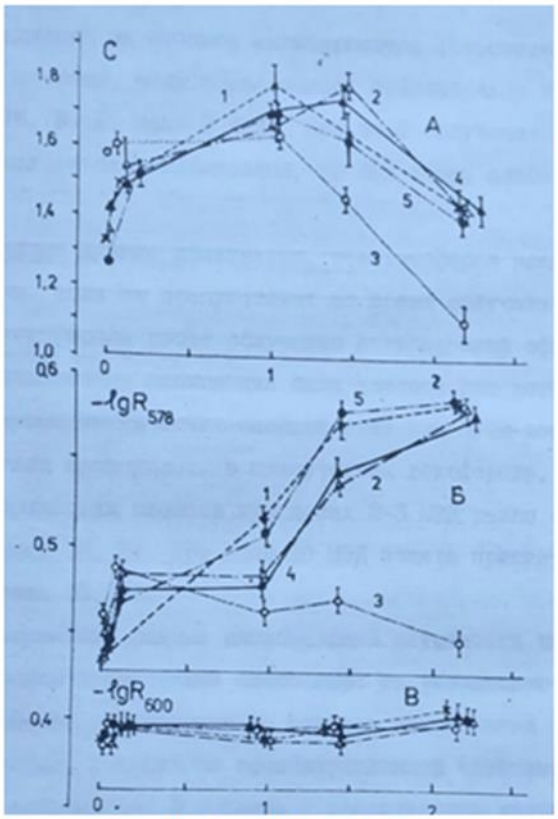

Figure 5 Inhibition by α-tocopherol of skin erythema, photosensitized 8-MOP. Registered: a) C-factor; b) -lgR578; c) -lgR600. The sequence of effects on the skin is as follows: 1) 8-MOP + UVA + tocopherol; 2) 8-MOP + ethanol + UVA4 3) 8-MOP + tocopherol + UVA; 4) 8-MOP + UVA + tocopheryl acetate; 5) 8-MOP + tocopheryl acetate + UVA. Radiation dose 2 MED. 8-MOP, tocopherol and tocopheryl acetate were applied by the 1st method (see section III.3.1) at surface concentrations of 5・10-9, 10-8 and 5・10-8 mol/cm2 of skin, respectively.

The findings show that α-tocopherol protects the skin from PUVA erythema if present during irradiation. In the case of tocopherol application after irradiation, the inhibitory effect was not exhibited. Similar observations were shown when recording changes in mechanoelectric skin properties in PUVA exposures.

4. Discussion

PUVA therapy helps elucidate the molecular mechanisms of photochemical reactions of psoralens. It has been shown that under the action of UV light, psoralenes are able to modify biological molecules in two ways: as a result of oxygen-independent reactions of photoaddition to unsaturated organic molecules and due to oxidative photoreactions [10].

Psoralens are poorly soluble in water. Therefore, when they enter the cell, they bind mainly to hydrophobic structures. Flat psoralen molecules contain three rings and resemble a pair of complementary DNA bases in structure. This spatial organization of psoralens facilitates their binding to double-spiral DNA. In this case, psoralen molecules are inserted (intercalated) between two pairs of DNA bases. Psoralen, having absorbed a quantum of light, goes into an electronically excited state. The excited psoralen molecule covalently attaches to thymine of DNA. Accordingly, after absorption of the photon between psoralene and thymine, a cyclobutane type 3, 4 - or 4 ', 5' -adduct [14] is formed. If such an adduct absorbs a quantum of light, and near the 3, 4-bond in the DNA double helix is thymine, another photoadduct reaction occurs with the occurrence of the 3.4.4 ', 5' -diadduct. The diadduct covalently binds complementary DNA strands. The occurrence of mono- and diadducts has important biological implications for cells and is the main cause of lethal and mutagenic effects in bacteria and viruses. Vitamin E and ionol antioxidants were found to inhibit PUVA erythema without affecting photoaddition of psoralenes to DNA. This was the first direct indication of the important role of photooxidative reactions of psoralens in human skin.

Psoralen derivatives differ greatly in their ability to photosensitize the skin. On the example of 8-psoralens, a close correlation was found between the ability to induce erythema and photoaddition to double-spiral DNA *in vitro*. A clear correlation was identified between the ability to photosensitize erythema and form diadducts. The above facts are the main arguments in favor of the hypothesis about the leading role of photoaddition of psoralens to DNA in induction of PUVA-erythema [15]. However, psoralenes, in addition to the ability to photoaddition to DNA, can participate in other photoreactions [16]. A characteristic feature of biologically active psoralens is the high value of quantum output of intercombination conversion, due to which, after absorption of light quants, a significant number of psoralen molecules are formed in a triplet excited state. Triplet states are easily extinguished with molecular oxygen to form singlet excited oxygen. Singlet oxygen generates only those psoralenes that are capable of inducing PUVA erythema during UV irradiation. The role of photoreactions of psoralens in which singlet oxygen is generated in the induction of PUVA erythema has not been studied. An approach to solve this problem may be to investigate the effect on PUVA erythema of compounds capable of extinguishing singlet oxygen.

5. Conclusion

A-tocopherol has been shown to protect the skin from PUVA erythema during irradiation.

Compliance with ethical standards

Disclosure of conflict of interest

The authors declare no conflict of interest.

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