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Cis-urocanic Acid Does Not Inhibit Mitogen Induced Lymphocyte Transformation in Man

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Urocanic acid (UCA) is a photoreceptor in the epidermis which absorbs UVB. To elucidate the role of UCA as an immunomodulator in the mechanism of immune suppression which occurs after UV irradiation in human, we studied the effect trans-UCA as well as that of its UV induced cis-isomer (cis-UCA) on mitogen induced lymphocyte transformation and on delayed type responses elicited by intradermal testing of a standardized battery of microbial antigens. No inhibitory effect was seen either in vitro or in vivo. Our negative results, both in vivo and in vitro, suggest that if UCA and UCA photoproducts exert an immunomodulatory effect, their effect should be analysed by a more subtle evaluation of the immune system. *Key words: Urocanic acid (cis and trans); Immune responses; Intradermal skin tests; Topical urocanic acid.* (Received January 3, 1986.)

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The possibility that exposure to UV might influence the immune system in man and in experimental animals is now widely accepted. The mechanism of the immunosuppression after UV irradiation has been mainly investigated on antigen specific contact hypersensitivity. On the other hand, in vivo UV irradiation leads to a decrease in PHA stimulated lymphocyte proliferation in healthy individuals (1). A question is how UV irradiation that is mainly absorbed in the epidermis may lead to a decrease in PHA induced proliferation of lymphocytes and in the expression of contact hypersensitivity. Normal human skin contains a large amount (0.4% of the wet weight) of trans-urocanic acid (trans-UCA) (2). The function of this accumulation has not been established, although the fact that the

absorption spectrum of UCA, 240–310 nm, max. 267 nm in water/HCl, covers in part the UVB range (280–320 nm) has led to the widespread belief that UCA acts as a natural sunscreen. The possible role of UCA as an immuno-modulator was considered when De Fabo et al. (3) found (i) that contact hypersensitivity could be suppressed and tumor rejection delayed in mice by UV irradiation (ii) that the immune suppressive effect of UV could be abolished by removal of stratum corneum and (iii) that the wavelength of UV inducing the immune suppressive effect was in the range of the absorbtion spectrum of UCA. Furthermore, Swartz described a small molecular weight factor in the serum of UV-treated mice which could transfer inhibition of contact sensitivity (4). These observations support the hypothesis that trans-UCA or its photoproduct(s) may participate in a UV-induced immunosuppressive mechanism. Irradiation of trans-UCA, the natural form, by UV or day light produces the geometrical isomer cis-UCA (5, 6). We therefore wondered if the isomer cis-UCA could have an immune modulatory effect in the human and studied (i) in vitro, the effect of both cis- and trans-UCA on mitogen induced lymphocyte transformation and (ii) in vivo the effect of a cream containing 5% cis-UCA on delayed type skin responses.

MATERIAL AND METHODS

Lymphocyte transformation test

Cis-UCA was prepared by irradiation of an alcoholic solution of 2% trans-UCA under N_2 with a low pressure mercury lamp for 10 h (7). Cis-UCA was isolated and purified by recrystallization in water and alcohol. Thin layer chromatography on paper (8) gave one spot whose melting point corresponded to the cis form. Both cis- and trans-UCA were freshly diluted to the appropriate concentrations in culture medium (RPMI 1640, 10% fetal calf serum, Penicillin 100 U/ml, Streptomycin 100 µg/ml, 2 mM Glutamine) for each experiment. Heparinized venous blood was obtained from 6 normal healthy volunteers. Peripheral blood mononuclear cells (PBMC) were isolated on a Ficoll-Hypaque gradient, washed three times in phosphate buffered saline, then resuspended in fresh culture medium. Phytohemagglutinin (PHA) and Concanavalin A (Con A) were used at previously determined optimal and suboptimal concentrations. Cultures were carried out in microtiter plates, incubated with PHA or Con A and cis- or trans-UCA which was added directly to the microtiter plates, and cultured for 4 days at 37°C and 5% CO_2 in a humidified atmosphere. For the last 6 h of culture they were pulsed with tritiated thymidine. The cells were harvested on filter paper and incorporation of tritiated thymidine was assessed by liquid scintillation. Percent inhibition was counted as follows:

$$\% \text{ Inhibition: } \left(1 - \frac{\text{Sample with UCA (cpm)}}{\text{Sample without UCA (cpm)}}\right) \times 100\,(\%)$$

Intradermal test

Six healthy volunteers, all from the medical staff, were prick tested twice at 4 week intervals with the multitest Mérieux, a standardized battery including the following antigens: Tetanus toxoid, Diphteria, Streptococcus, Tuberculin, Proteus, Trichophyton, and Candida albicans. Cis-UCA cristals were finely powdered and dispersed in an anionic hydrophilic cream up to a concentration of 5%. This cream or the vehicle alone was applied on the anterior aspect of the forearm, double blind, three times per day for two days. The prick test was performed 3 h after the first application and evaluated according to the standard design of the multitest Mérieux (the mean of horizontal and vertical diameters (mm) of induration). Each subject served as his own control.

RESULTS

Lymphocyte transformation test

The effect of cis- and trans-UCA on the lymphocyte transformation induced by suboptimal doses of PHA and Con A is shown in Fig. 1. No significant effect was observed in the

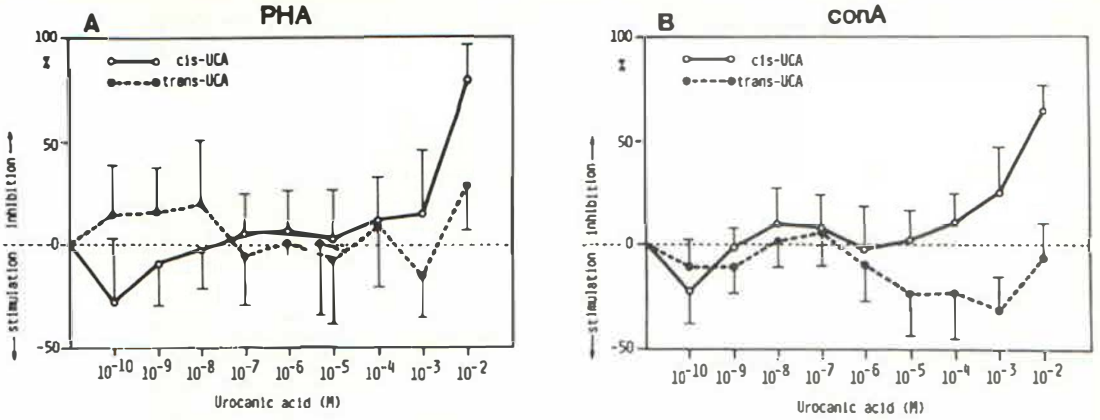

Fig. 1. Percent inhibition of different concentrations of cis- and trans-UCA on ^{3}H-thymidine incorporation of 4-day cultured PBMC stimulated suboptimally by PHA (mean of 6 experiments +/−1 SD, Fig. 1 A) and by Con A (mean of 7 experiments +/−1 SD, Fig. 1 B).

concentration range of 10^{-3}–10^{-10} M for either isomer. Only cis- UCA at a concentration of 10^{-2} M showed a significant inhibition whereas trans- UCA at 10^{-2} and 10^{-3} M did not influence the lymphocyte proliferation response. The inhibitory effect seen at 10^{-2} M and 10^{-3} M cis- UCA might be due to a toxic effect but was not further investigated since the occurrence of such high levels in vivo seems improbable. Likewise, we did not observe any effect of both cis- and trans- UCA on optimally stimulated lymphocytes except the mentioned high dose inhibition (data not shown). Furthermore, harvesting lymphocytes between days 3 and 7 of culture did not reveal any additional effect (data not shown). Finally, when PBMC were pretreated for 48 h with either isomer and then stimulated with PHA for 4 days, no significant inhibition or enhancement occurred.

Intradermal test

In the prick tests, no differences were seen either at 24 h, 48 h or 72 h in the areas treated by cis-UCA as compared to the vehicle treated areas. The random design of the study excluded either the booster or inhibitory effect of previous testing in each subject. We conclude therefore that cis-UCA 5% does not inhibit delayed type skin test.

DISCUSSION

In this study we have been unable to demonstrate any inhibitory effect of either trans- or cis-urocanic acid on mitogen induced lymphocyte proliferation.

Morrison et al. (1) found a decrease in the response of circulating cells to PHA after a single whole-body exposure to UVB radiation which produced a mild or marked erythema. Subjects who developed only a mild erythema did not show this decrease in reactivity to PHA, suggesting that this effect might be related to the dose of UV radiation. The mechanism of these changes in lymphocyte reactivity following exposure to UV radiation is unknown. Several studies about UV induced immune suppression suggested that UV irradiation is likely to affect the induction phase of contact hypersensitivity (9), and to generate antigen specific suppressor cells (10). There might be a possibility of the participation of a photoreceptor acting in this mechanism. We could speculate that a photopro-

duct after UV absorption by the photoreceptor may alter antigen presentation and consequently suppressor cell formation (3), or that the release of mediators in the skin may also influence the function of lymphocytes (1). Our results suggest that, as far as the mitogen induced lymphocyte proliferative response is concerned, the inhibition observed by Morrison et al. (1) after a single whole body exposure to UVB radiation is not likely to be due to a direct effect of either trans- UCA or its main photoproduct cis-UCA. The concentrations of UCA used in vitro, up to 10 mM, are probably exceeding the levels that could be reached in the serum after a single whole body exposure to UVB, although the exact levels or blood trans- UCA and cis-UCA in such situations are not known. At any rate, the highest concentrations of cis-UCA in vitro seem to exert a toxic effect on the cells.

Topical application of 5% cis-UCA did not modify the expression of delayed hypersensitivity response to common intradermally injected antigens. We have chosen this type of skin test instead of epicutaneous application of an allergen in previously sensitive subjects in order to avoid any quenching effect of cis-UCA on the allergen. That topical cis-UCA did not inhibit the delayed hypersensitivity response to intradermally injected antigens may be due to several factors which are difficult to take apart, such as: lack of penetration, concentration, time of exposure, and finally absence of an immunomodulatory effect of cis-UCA in this particular situation.

The concept that immunomodulatory molecules might derive from photoproducts of UCA is an interesting one. In this preliminary study in humans, we have looked only at two parameters of the immune function. Our negative results, both in vivo and in vitro, suggest that if UCA and UCA photoproducts exert an immunomodulatory effect, their effect should be analysed by a more subtle evaluation of the immune system.

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