Cis-Urocanic Acid Down-regulates Histamine-mediated Activation of Adenyl Cyclase in the Pig Epidermis

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Urocanic acid (UCA), one of the skin’s major components for absorbing UV radiation, is present naturally in the stratum corneum as a trans-isomer. On absorption of UVB radiation either in vitro or in the skin, UCA undergoes trans- to cis-isomerization in a dose-dependent manner. Although the mechanism by which cis-UCA suppresses immunity remains unclear, recent studies have indicated that cis-UCA appears to inhibit the induction of cyclic AMP in fibroblasts, which suggests that this molecule plays an active role in modifications to the skin. Here, we report that although neither trans-UCA nor cis-UCA increases cyclic AMP in the pig epidermis, cis-UCA actively down-regulates the increase of cyclic AMP induced by histamine. The effects of cis-UCA on the pig epidermis are revealed through the modulation of the effects caused by histamine. These findings suggest that in the pig epidermis, the initial biochemical and cellular event for UVB-induced immune suppression – that is, the step immediately following the isomerization of trans-UCA to cis-UCA – is down-regulation of cyclic AMP brought about by the activity of cis-UCA. Key words: urocanic acid; cyclic AMP; pig epidermis; histamine.

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Urocanic acid (UCA) is present in the stratum corneum, predominantly as a trans-isomer (deaminated histidine) (1). On ultraviolet B (UVB) irradiation, it undergoes isomerization to a cis-isomer (2). UVB-induced immune suppression in vivo has been shown to be associated with modification of antigen-presenting cell function and the induction of T-suppressor cells. It has also been shown that cis-UCA administered to mice can induce similar modifications of antigen-presenting cells, thereby replacing the UVB component (3, 4). It is generally believed that cis-UCA mediates impairment of the induction of contact hypersensitivity to UVB (5). Although we have recently demonstrated that cis-UCA promotes tolerance and induces suppressor cells in mice (6), the precise mechanism by which cis-UCA alters the immune system is currently unknown. A study involving UCA analogues and histamine receptor antagonists in the modulation of the delayed hypersensitivity response to the herpes simplex virus has indicated that UCA may act through histamine-like receptors (7). UCA is structurally homologous to histamine, and a recent report has shown that both histamine and trans-UCA up-regulate intracellular cyclic AMP, and that such induction is down-regulated by cis-UCA in dermal fibroblasts (8). Keratinocytes express H1 and H2 receptors, and both receptor types play a role in mediating the responses of keratinocytes to cis-UCA and histamine (9). It has also been reported that in cultured human keratinocytes, cis-UCA synergizes production of PGE2 in response to histamine, and that this is linked to indomethacin-inhibitable UVB-induced immunosuppression (9). In this report, we here examine the effects of trans- and cis-UCA on adenyl cyclase, as measured by cyclic AMP formation in the pig epidermis.

MATERIALS AND METHODS

Reagents
Histamine, the trans-isomer of urocanic acid (trans-UCA) (4-imidaseocacrylic acid) and 3-isobutyl-1-methyl-xanthine (IBMX) were all purchased from Sigma (St. Louis, MO, USA). The cis-isomer of UCA was prepared by UVB irradiation (FL20SE30 fluorescent lamp; Clinical Supply Co., Tokyo, Japan). Briefly, a thinly spread solution of trans-UCA in a phosphate-buffered saline (PBS) solution was irradiated for 8 h at a distance of 46 cm. The irradiated solution was then diluted in PBS to reach a final concentration of 1 mg/ml (6). Analysis via high-performance liquid chromatography (HPLC) revealed approximately 50.5% of the irradiated UCA present as the cis-isomer. The cis-UCA was then purified by HPLC, using the method of Caron et al. (10) with some modifications. The solution of irradiated UCA was subjected to HPLC on an analytical reversed-phase column (10) with KH2PO4 (0.05 M)-acetonitrile (1:1, v/v) as the mobile phase. The cis-UCA fraction was collected, and this collection procedure was repeated. Contamination by the trans-form was not detectable in the purified cis-UCA fraction. All other chemicals were of reagent grade.

Preparation of the pig epidermis
Pig skin slices were obtained using a keratome adjusted to a depth of 0.2 mm. The skin slices were then treated with 1.000 U/ml of dispase in RPMI 1640 medium for 30 min at 37°C. After the dispase treatment, the pure epidermal sheets were peeled off with sharp forceps. The slices were cut into 5 × 5-mm squares and floated on RPMI 1640 medium at 37°C with the keratin layer up.

The pig epidermis for cyclic AMP measurements
After 20-min preincubation at 37°C in the presence of 1 mm IBMX (8) to standardize the initial cyclic AMP levels in the epidermal pieces (11), the specimens were incubated for 5 min with either 5 × 10⁻⁵M histamine (12), or various doses of UCA (trans or cis form). To examine the effect of the trans-UCA or cis-UCA concentration on the histamine adenylate cyclase response, we stimulated preincubated epidermal slices for 20 min with various concentrations of trans- or cis-UCA plus 1 mm IBMX, and for a further 5 min with 5 × 10⁻⁵M histamine. The generation of cyclic AMP was terminated by quickly freezing the skin pieces between two plates of dry ice.

Radioimmunoassay for cyclic AMP
After the frozen skin pieces were boiled and homogenized, the supernatant was succinylated and the cyclic AMP content measured by radioimmunoassay using a Yamasa cyclic AMP assay kit (Yamasa Shoyu Co., Tokyo, Japan) as previously described (13). The protein concentration was measured by the method of Lowry et al. (14).

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There is increasing evidence that cis-UCA may be a mediator of at least some of the effects of UVB radiation, including immunosuppression. Using a murine model of the herpes simplex virus infection, Ross et al. have shown that cis-UCA is highly effective in suppressing delayed hypersensitivity responses to the virus (15). We have recently shown that cis-UCA induces tolerance and suppressor cells in mice (6). Other studies suggest that the immunosuppressive effects of cis-UCA occur through histamine or histamine-like receptor pathways (16, 17).

Although in the present study cis-UCA was found to inhibit histamine-mediated adenylate cyclase activation in the pig epidermis, neither trans- nor cis-UCA affected the transmembrane signals. Our results agree with a previous report that cis-UCA down-regulates the histamine response in cultured dermal fibroblasts (8). Those experiments, however, demonstrated that trans-UCA is biologically active in fibroblasts, and induces adenyl cyclase, as measured by cyclic AMP (8). The relation between cis-UCA and histamine is not simple. These discrepancies of induction of cyclic AMP by UCA may indicate that the trans- and cis-isomers of UCA differ in their effects on various cell types in the skin. Such differential effects may also occur in vivo after exposure to UV irradiation.

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Fig. 1. The concentration effects of trans-UCA or cis-UCA on the histamine adenylate cyclase response in the pig epidermis. Epidermal slices preincubated for 20 min with various concentrations of trans- ( ) or cis- (●) UCA plus 1 mm IBMX, were stimulated with 5 x 10^-5 M histamine for 5 min. Data represent the mean ± SEM of three independent experiments. Results for trans- and cis-UCA are from separate experiments. For any results of cis- (●) UCA marked with an asterisk, a significant difference (p < 0.05) was detected between it and the control (5 x 10^-5 M histamine stimulated without cis-UCA).

The pig epidermis trans- or cis-UCA contents
Homogenates of the pig epidermis were subjected to HPLC by the analytical method of Caron et al. (10).

RESULTS AND DISCUSSION
We first examined whether trans-UCA and cis-UCA could induce adenylate cyclase activation. Decreasing concentrations of purified trans- or cis-UCA were added to the pig epidermis, and the cyclic AMP levels were determined. Neither trans-UCA nor cis-UCA induced cyclic AMP at concentrations from 1 x 10^-6 M to 1 x 10^-3 M, but 5 x 10^-5 M histamine did induce cAMP (data not shown).

Given that neither trans- nor cis-UCA induced cyclic AMP, we next attempted to determine whether UCA could down-regulate the induction of cyclic AMP brought about by histamine. Decreasing amounts of trans- or cis-UCA, together with a fixed amount of histamine (5 x 10^-5 M), were added to the pig epidermis, and cyclic AMP induction was measured. Figure 1 shows that cis-UCA, at concentrations from 1 x 10^-6 M to 1 x 10^-3 M, inhibited the cyclic AMP-inducing effects of histamine, while trans-UCA did not. The effect of cis-UCA on the induction of the inhibition of cyclic AMP by histamine was not dose-dependent. A possible explanation for this result is that cis-UCA may have caused the uncoupling of the receptor from its signalling system, as Palaszynski et al. have reported (8). The original contents of trans-UCA and cis-UCA were 313 ± 33.3 and 198 ± 18.4 pmol/mg tissue, respectively.


