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The role of *cis*-urocanic acid in UVB-induced suppression of contact hypersensitivity

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Abstract

Ultraviolet light B (UVB) is well recognized to suppress the contact hypersensitivity (CHS) response and it has been postulated that *cis*-urocanic acid (UCA) is a mediator of the immunosuppression. This study was designed to examine the effect of UCA on CHS and to clarify its role in UVB-induced immunosuppression in C57BL/6 mice. Intradermal injection of 0.5–50 μg *cis*-UCA into the ear 2 h before DNFB sensitization resulted in a 60–70% reduction of CHS assessed by ear swelling, whereas *trans*-UCA did not have a significant effect on CHS except at a high dose (50 μg) which showed a 20–40% suppression. Intraperitoneal injection of anti-*cis*-UCA antibody before administration of *cis*-UCA abrogated the suppression. To examine the effect of UCA on UVB-induced immunosuppression, some mice were pre-treated with anti-*cis*-UCA antibody and then exposed to UVB (960 J/m^2). After 3 days the mice were sensitized either on the irradiated abdominal skin or on the unirradiated dorsal surface of the right ear followed by the challenge on the left ear. The CHS response was significantly suppressed in UVB-irradiated mice both locally (abdominal sensitization, suppression was 45%, $P < 0.001$) and systemically (ear sensitization, suppression was 53%, $P < 0.0025$). The CHS response was partially restored in both abdominal sensitized mice and ear sensitized mice by pre-treatment with anti-*cis*-UCA antibody. These results confirmed the immunosuppressive effects of *cis*-UCA on CHS and suggest that *cis*-UCA plays a role in UVB-induced local and systemic immunosuppression.

Keywords: Delayed type hypersensitivity; Immunosuppression; Urocanic acid

1. Introduction

Urocanic acid (UCA) is found in the upper layer of the epidermis as the *trans*-isomer. UCA is the deaminated form of histidine and is produced enzymatically in the stratum corneum [1]. Although UCA is not an end metabolite of histidine, metabolism of UCA does not occur due to the absence of urocanase in the epidermis, resulting in the accumulation of UCA in the epidermis [1]. It has been suggested that UCA may function as a natural photoprotecting agent for DNA as it absorbs strongly at 277 nm and is located predominantly in the stratum corneum [2]. On irradiation of the skin with UVB, the *trans*-isomer converts to the *cis*-isomer [3]. It is well known that UVB induces suppression of selected immune responses to various antigens, including viruses [4], bacteria [5], tumours [6], parasitic protozoa [7], skin-sensitizing agents [8] and histocompatibility antigens [9] (reviewed in Ref. [10]).

In 1983 De Fabo and Noonan [11] found that the absorption spectrum of UCA matched the action spectrum for UVB-induced suppression of contact hypersensitivity (CHS), and postulated that *trans*-UCA may act as a photoreceptor for UVB with the resulting *cis*-isomer then mediating immunosuppression. UVB-irradiated UCA, administered to mice by skin painting, intravenously or subcutaneously, has been shown to cause a suppression of the delayed type hypersensitivity (DTH) response to herpes simplex virus (HSV) [12]. Antigen presentation by epidermal cells is altered fol-

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lowing skin painting with UVB-irradiated UCA resulting in suppression of DTH to HSV [13]. Intravenous injection of *cis*-UCA has been reported to induce a defect in the ability of dendritic cells to present antigen [14]. A suppressor factor in the serum of mice following injection of UVB-irradiated UCA has been demonstrated [15]. UCA has also been shown to decrease interleukin-1 production by human epidermal cells and to reduce HLA DR expression on monocytes as well as in UVB-irradiated epidermis [16].

It is not clear how *cis*-UCA might affect immune responses. Streilein and colleagues [17] have proposed that, on UVB irradiation, *trans*-UCA is converted to *cis*-UCA in the epidermis which, in turn, causes the release of tumour necrosis factor-α (TNF-α); TNF-α then inhibits Langerhans cell (LC) migration from the skin to the regional lymph nodes, thereby impairing antigen-specific T cell activation [18]. On the other hand, Cumberbatch and Kimber [19] have shown an obligatory requirement for TNF-α in accumulation of dendritic cells in the draining lymph nodes. Moodycliffe et al. have shown that *cis*-UCA does not have any effect on dendritic cell numbers in draining lymph nodes [20], and evidence has been published to indicate that the mechanism of action of *cis*-UCA is different from TNF-α, with *cis*-UCA perhaps acting through histamine-like receptors [21,22]. In the present study we confirmed previous reports that *cis*-UCA administered intradermally mediates suppression of CHS in a murine system [18]. In addition, by using a monoclonal antibody with specificity for *cis*-UCA, we were able to demonstrate the relative contribution of *cis*-UCA to the suppression of CHS induced by UVB exposure in C57BL/6 mice.

2. Materials and methods

2.1. Animals

Female C57BL/6 mice were obtained from the Charles River Breeding Laboratories (Quebec, Canada) and used at 8–12 weeks of age. Mice were housed in a specific pathogen-free facility at Sunnybrook Health Science Centre and fed a standard diet and water ad libitum. Five to eight mice were employed within each experimental group.

2.2. Chemicals

Dinitrofluorobenzene (DNFB) was purchased from Sigma Chemical Co. (St. Louis, MO) and dissolved in acetone/olive oil, 4:1 (vol/vol). *Trans*-UCA (4-imidazoleacrylic acid) was also purchased from the Sigma Chemical Co. *Cis*-UCA was purified from an irradiated solution of *trans*-UCA following irradiation, and the purity of *cis*-UCA was 97% assessed by high-performance liquid chromotography [23]. Both of the isomers were dissolved in dimethylsulfoxide (DMSO) at a concentration of 10 mg/ml, then diluted appropriately (0.5–50 μg/40 μl) using phosphate-buffered saline (PBS) just before use.

2.3. Monoclonal antibody to cis-UCA (anti-cis-UCA Ab)

The production of the monoclonal antibody specific to *cis*-UCA has been described elsewhere [24]. Ascitic fluid was produced in female Balb/c mice (8 weeks old, pre-treated intraperitoneally with 0.5 ml of pristane). It had a titre of 1:32000 to *cis*-UCA by ELISA and contained approximately 0.15 mg/ml IgG_1. An isotype matched irrelevant monoclonal antibody hybridoma to Border Disease Virus was kindly supplied by Dr. P.F. Nettleton (Moredun Research Institute, Edinburgh) and was used to produce a negative control ascitic fluid containing an equivalent amount of IgG_1.

2.4. Assay for contact hypersensitivity (CHS)

Induction of CHS was carried out by the method described previously with minor modifications [25]. Either abdominal skin or a dorsal surface of the ear was used for sensitization. Abdominal skin of mice was shaved and painted with 25 μl 0.5% DNFB. In case of the ear, 15 μl 0.5% DNFB was applied onto the dorsal surface of the right ear. Five days later, mice were challenged on the dorsal surface of the left ears with 10 μl 0.2% DNFB. Ear thickness was measured with a Peacock spring-loaded micrometer (Ozaki Co., Tokyo, Japan) immediately prior to challenge and at 24 h after challenge. The extent of ear swelling was used as a measure of CHS. The results were expressed as the change (from pre-challenge levels) in ear thickness and represent the mean increase at 24 h after challenge. Percentage suppression of CHS was calculated according to the formula:

$$\%\text{Suppression} = \left(1 - \frac{\text{net ear swelling in experimental mice}}{\text{net ear swelling in control mice}}\right) \times 100$$

The statistical significance of the differences of the mean ear thickness between treated ears and control ears was determined by Student's *t*-test.

2.5. Cis-Urocanic acid treatment

To examine the effect of local administration of *cis*-UCA on the sensitization phase of CHS, *cis*-UCA

(0.5–50 μg in 40 μl of vehicle) was injected intradermally into the right ear 2 h before sensitization. An equal volume of vehicle was injected into right ears of the control group. *Trans*-UCA (0.5–50 μg) was also examined using other groups of mice. To determine whether anti-*cis*-UCA specific antibody can block the effects of *cis*-UCA in our model, mice were injected intraperitoneally with 300 μl of a 1/500 dilution of anti-*cis*-UCA Ab (equivalent to 0.1 μg IgG) 2 h before *cis*-UCA treatment. Furthermore, in order to examine whether anti-*cis*-UCA Ab affect UVB-induced immunosuppression, mice were injected intraperitoneally with 300 μl of a 1/500 dilution of anti-*cis*-UCA Ab 2 h before UVB irradiation. This dose of antibody has been shown to abrogate suppressive effects induced by UVB [26]. Furthermore, this was confirmed by pilot studies. During the sensitization, challenge and UCA treatment, mice were anaesthetized with pentobarbital (50 mg/kg, i.p.) (Nembutal, Abbott Lab., Ontario, Canada).

2.6. UVB radiation

UVB radiation was delivered by a bank of four unfiltered polychromatic fluorescent sun lamps (FS20T12-UVB, National Biological Corporation, Twinsburg, OH), which emits wavelengths mainly between 280–320 nm, peaking at 313 nm. The irradiation was 0.36 mW/cm^2 at a target distance at 15 cm, as measured by an IL-1400A radiometer, equipped with a SEL240/UVB 1/TD UVB detector with a spectral sensitivity in the range of 280–320 nm (International Light Inc., Montreal, Que). Prior to exposure to 960 J/m^2, mice were razor-shaved on the abdominal area and were then exposed to 960 J/m^2 UVB (267-s exposure). This dose has been shown to induce suppression of CHS to 1-chloro-2,4,6-trinitrobenzene (TNCB) [4]. During exposure, mice were anesthetized with pentobarbital (50 mg/kg, i.p.), each extremity was extended gently with masking tape and then fastened to a wooden board, and the ears were shielded with black electrical tape. UVB irradiation was carried out 3 days prior to sensitization.

3. Results

3.1. Suppression of contact hypersensitivity response in cis-UCA-treated mice

To study the effect of UCA on the development of CHS, either *cis*-UCA, *trans*-UCA or vehicle was injected intradermally into the right ear and 2 h later the injected ear was painted with 15 μl 0.5% DNFB for sensitization. Five days after the sensitization, mice were challenged with 10 μl 0.2% DNFB on the contralateral left ears, and ear thickness was measured at 24 h after challenge. Significant suppression was observed in the *cis*-UCA (0.5–50 μg)-treated mice (Fig. 1). Percent suppression of ear swelling in ears injected with 50 μg of *cis*-UCA was about 70%. The highest concentration of *trans*-UCA (50 μg) injection also resulted in a slight but significant suppression in ear swelling (20%, $P < 0.05$), whereas low concentration of *trans*-UCA (0.5 or 5 μg)- or vehicle-treated mice showed no suppression of ear swelling. In a dose-response study, we determined that significant suppression (63%, $P < 0.001$) was induced in C57BL/6 mice with as little as 0.5 μg *cis*-UCA applied intradermally to the sensitized area. Anti-*cis*-UCA Ab was injected 2 h prior to the *cis*-UCA treatment to see if the suppressive effect of *cis*-UCA could be abrogated in the mouse model. Anti-*cis*-UCA Ab (300 μl of 1/500 dilution) was injected intraperitoneally 2 h before intradermal injection of *cis*-UCA (5 μg) into the right ear. Two hours later, sensitization was carried out by painting 15 μl 0.5% DNFB on the same ear. More than 90% of the suppressive effect of *cis*-UCA on CHS was abrogated by anti-*cis*-UCA Ab pre-treatment (Fig. 2).

3.2. Anti-cis-UCA Ab effect on UVB-induced immunosuppression

As *cis*-UCA has been proposed as one of the important mediators for UVB-induced immunosuppression, we attempted to block UVB-induced immunosuppression with anti-*cis*-UCA Ab. Mice were pre-treated with anti-*cis*-UCA Ab by intraperitoneal injection 2 h prior to UVB irradiation. Control mice were pre-treated with the same volume of irrelevant antibody. Shaved abdominal skin of those mice was exposed to 960 J/m^2 UVB. The mice were then sensitized either on the

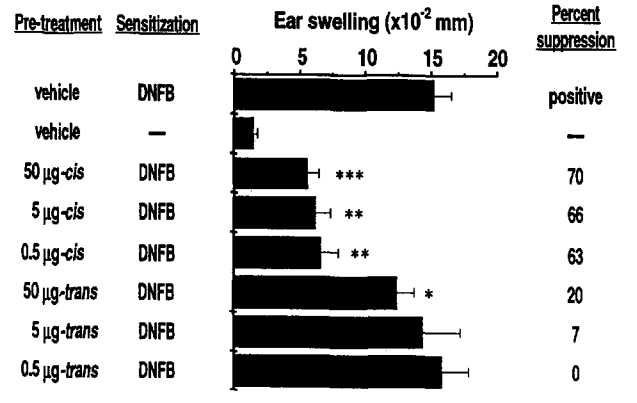

Fig. 1. Suppressive effects of *cis*-UCA on CHS. *Cis*-UCA or *trans*-UCA was injected intradermally into the right ear at various concentrations. Sensitization was carried out by applying 15 μl 0.5% DNFB to the injected right ear 2 h later. Ear challenge was performed on the left ear 5 days after sensitization. Ear swelling was measured at 24 h after challenge and expressed as mean ± SD of 12 mice per group. Percent suppression was calculated by the formula in the Methods. *$P < 0.05$; **$P < 0.001$; ***$P < 0.0005$.

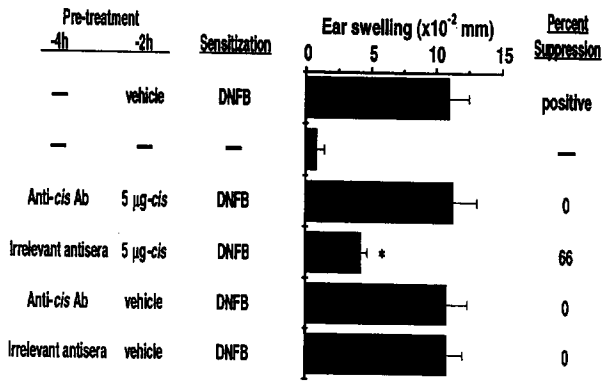

Fig. 2. Anti-*cis*-UCA antibody abrogates the effect of *cis*-UCA on CHS. Anti-*cis*-UCA antibody or irrelevant antisera was injected intraperitoneally 4 h before sensitization and 5 μg *cis*-UCA was injected intradermally into the right ear 2 h before sensitization. Ear swelling was measured at 24 h after challenge and expressed as mean $\pm$ SD of eight mice per group. $^*P < 0.0005$.

irradiated abdominal site (local immunosuppression) or on the unirradiated dorsal surface of ear (systemic immunosuppression) 3 days after the irradiation. No significant differences in the CHS response were found between mice treated with the irrelevant antisera plus UVB and mice treated with UVB alone. Therefore, only the mice treated with the irrelevant antisera plus UVB are included as a control for clarity.

UVB exposure on the abdominal skin before sensitization significantly suppressed the CHS response (local immunosuppression) (Fig. 3). The degree of suppression averaged about 45% in irradiated mice compared with sham-irradiated control mice ($P <$

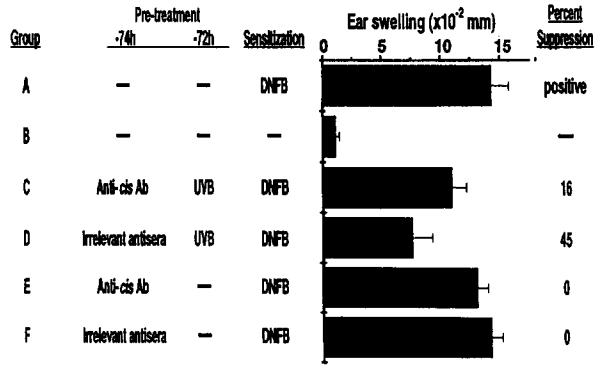

Fig. 3. Effects of anti-*cis*-urocanic acid antibody on UVB-induced local immunosuppression of CHS. Abdominal skin of mice were shaved and exposed to a single dose of UVB (960 J/m^2) 72 h (3 days) bdfore sensitization. Prior to UVB exposure (2h), anti-*cis*-urocanic acid antibody was injected intraperitoneally. Sensitization was performed by applying 25 μl 0.5% DNFB to the irradiated abdominal skin followed 5 days later with 10 μl 0.2% DNFB to left ear. Ear swelling was measured at 24 h after challenge and expressed as mean $\pm$ S.D. of 10 mice per group. CHS response was suppressed by UVB exposure ($p < 0.001$, D vs A), but pre-treatment with anti-*cis*-urocanic acid antibody partially restored the UVB-induced local suppression ($p < 0.01$, C vs D; $p < 0.025$, C vs A).

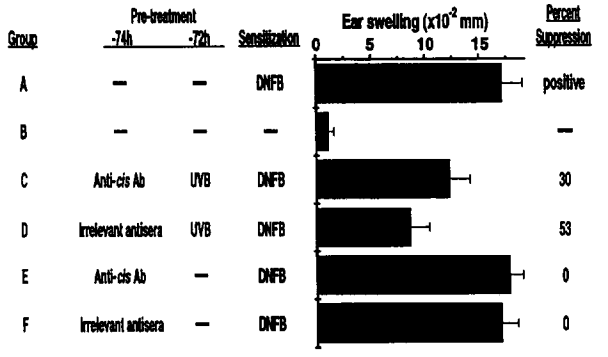

Fig. 4. Effects of anti-*cis*-UCA antibody on UVB-induced systemic immunosuppression of CHS. Abdominal skin of mice were shaved and exposed to a single dose of UVB (960 J/m^2) 72 h (3 days) before sensitization. Prior to UVB exposure (2 h), anti-*cis*-UCA antibody was injected intraperitoneally. Sensitization was performed by applying 15 μl 0.5% DNFB to an unirradiated dorsal surface of the right ear followed by the challenge to the left ear 5 days later with 10 μl 0.2% DNFB. Ear swelling was measured at 24 h after challenge and expressed as mean $\pm$ SD of 10 mice per group. CHS response was suppressed by UVB exposure ($P < 0.0025$, D vs. A), but pre-treatment with anti-*cis*-UCA antibody partially restored the UVB-induced systemic suppression ($P < 0.05$, C vs. D; $P < 0.05$, C vs. A).

0.001). This UVB-induced local immunosuppressive effect was markedly, but not completely, restored by anti-*cis*-UCA Ab pre-treatment. The percentage of recovery induced by Ab treatment was 65% (45% suppression compared with 16% suppression, $P < 0.01$).

CHS response was also impaired by UVB exposure in the mice which were sensitized on the unirradiated dorsal surface of the ear (systemic immunosuppression). The systemic immunosuppression induced by the same UVB dose was slightly larger than local immunosuppression. The suppression rate was 53% in those mice. This systemic suppression was partially but significantly restored by anti-*cis*-UCA Ab treatment before sensitization (Fig. 4). The restoration induced by anti-*cis*-UCA Ab was 43% (53% suppression compared with 30% suppression, $P < 0.05$).

4. Discussion

In this study, we have confirmed previous work by Kurimoto and Streilein [18] that exogenous *cis*-UCA applied intradermally before sensitization with DNFB suppressed the elicitation of CHS in C57BL/6 mice (Fig. 1). The suppression could be totally abrogated by pre-treatment of the mice with a monoclonal antibody specific for *cis*-UCA (Fig. 2). In addition, we have shown that pre-treatment of mice with the antibody partially restored the suppression in both local and systemic CHS induced by UVB irradiation (Figs. 3 and 4). Thus, a significant role for *cis*-UCA in regulating the extent of CHS following UVB exposure has been demonstrated.

The mechanism by which *cis*-UCA affects the immune system has not yet been elucidated. Following intradermal injection of *cis*-UCA, it has been observed that epidermal LC were reduced in number by 75% with the loss of dendritic processes [18]. Mice, pretreated with antibodies to TNF-α before *cis*-UCA administration, showed only a small loss in LC numbers with partial restoration of dendrites [18]. Previously, it had been demonstrated that TNF-α injected intradermally caused similar changes to a proportion of LC [17]. In addition, pre-treatment of mice with antibodies to TNF-α abrogated the suppression in CHS induced by *cis*-UCA [27]. On the basis of these results, it was speculated that *cis*-UCA could bind to receptors on keratinocytes, this process activating their TNF-α genes and leading to the changes in LC described [18].

However, the implied induction of TNF-α by *cis*-UCA in the epidermis has not been confirmed as another study showed that epicutaneous application of *cis*-UCA did not cause accumulation of dendritic cells in draining lymph nodes [20]. This was in contrast to UVB irradiation which resulted in the migration of dendritic cells to lymph nodes draining the site of exposure; TNF-α was shown to be a critical mediator of this event [20,28]. Thus, it is possible that *cis*-UCA may exert its principal effect by a mechanism other than TNF-α release, and several reports describing a variety of approaches indicate that it may act through histamine-like receptors [29–31]. An interesting finding demonstrated the synergistic effect of *cis*-UCA and TNF-α on upregulation of ICAM-1 expression on keratinocytes cultured in vitro [32]. Furthermore, recently, we demonstrated that TNF-receptor (p55) gene-targeted mutant mice are immunosuppressed by UVB exposure [33], suggesting that TNF-α is not required for the induction of UVB-induced immunosuppression of CHS. Our preliminary results showing that TNF-receptor (p55) gene-targeted mutant mice can be suppressed in CHS by *cis*-UCA (Kondo et al., unpublished observations) also suggest that the immunosuppressive effects of *cis*-UCA is not mediated by TNF-α.

Several lines of evidence suggest that keratinocyte-derived cytokines induced by UVB are mediators of UVB-induced effects on the immune system. Rivas and Ullrich [34] have demonstrated the role of IL-10 in the induction of systemic immunosuppression of delayed type hypersensitivity. They also observed that neutralization of IL-10 by monoclonal antibody is able to partially block the UVB-induced immunosuppression suggesting the involvement of other factors in the final immunosuppression. Support of their speculation is found from our results that pre-treatment with the monoclonal antibody to *cis*-UCA, at a dose sufficient to completely reverse the suppression in CHS induced by *cis*-UCA, did not totally abrogate the suppression following UVB exposure. Thus, it is likely that, in addition to *cis*-UCA, other mediators may contribute to the immunomodulation, such as interleukin-10 [34,35] or prostaglandins [36].

In conclusion, we demonstrate that *cis*-UCA blocks the sensitization of CHS. Anti-*cis*-UCA Ab abrogates the immunosuppressive effects of *cis*-UCA on CHS, but partially blocks UVB-induced immunosuppression. Our results indicate that *cis*-UCA plays a role in UVB induced local and systemic immunosuppression and reinforce the importance of studies where the mediators induced by UVB irradiation are examined in parallel.

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