

Topical cis-Urocanic Acid Suppresses both Induction and Elicitation of Contact Hypersensitivity in BALB/C Mice*

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Cis-urocanic acid, converted from trans-urocanic acid in stratum corneum by ultraviolet B irradiation, has been shown to impair contact hypersensitivity induction. To study whether topical cis-urocanic acid also alters contact hypersensitivity elicitation, as well as immediate hypersensitivity and acute irritation, we treated mice with 1% topical cis-urocanic acid or vehicle prior to induction or elicitation of hypersensitivity to contact allergen oxazolone or respiratory allergen trimellitic anhydride or prior to acute irritation from croton oil. Topical cis-urocanic acid suppressed both induction and elicitation of contact hypersensitivity to oxazolone. However, no effect by cis-urocanic acid on induction or elicitation of trimellitic anhydride allergy or croton oil irritation was seen. The possible efficacy of topical cis-urocanic acid as a treatment of inflammatory skin diseases responsive to ultraviolet B irradiation may be worthwhile to investigate. Key words: oxazolone; trimellitic anhydride; croton oil; immunosuppression; ultraviolet light.

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Ultraviolet B irradiation (UVB) has numerous effects on skin, including inflammation due to sunburn. However, it can also be used as a treatment for inflammatory skin disorders such as psoriasis and atopic dermatitis. Trans-urocanic acid, present at large amounts in normal stratum corneum, is transformed to cis-urocanic acid (cUA) by UVB. cUA suppresses, like UVB, induction of contact hypersensitivity (CHS) and has been suggested to act as a mediator of UVB for this purpose (1). cUA has down-regulatory effects on immune responses, including suppression of antigen-presenting cell action (2–5). Treatment with cUA also reduces allograft rejection (6, 7). As cUA may be applied topically, we studied its effects in animal models of skin inflammation to ascertain its potential as a topical anti-inflammatory agent.

MATERIAL AND METHODS

Animals

Female BALB/C mice (Simonsen, Gilrou, CA), purchased at 6–7 weeks age, were housed for at least one week before use. The mice were held in artificial lighting conditions, with no ultraviolet radiation, with 12 h/12 h day/night cycle, and had access to food and water *ad libitum*. Five to 7 animals per group were used.

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Chemicals

cUA was a gift from Ajinomoto Co., Kawasaki, Japan. Oxazolone (4-ethoxymethylene-2-phenyl-2-oxazolin-5-one), trimellitic anhydride (TmA) and croton oil were purchased from Sigma (St. Louis, MO). Diacetamide:acetone:ethanol (4:4:3; all from Sigma) was used as the vehicle for cUA and acetone (Fisher, Fair Lawn, NJ):olive oil (Sigma) (4:1) for other compounds.

Induction of allergy

An approximately 4 cm² area on the trunk of the animals was shaved and stripped with cellophane tape (3M Co., St. Paul, Minnesota) three times immediately before the first topical treatment was received. For treatments with 10 mg/ml cUA or vehicle on shaved trunk 100 µl doses were used. For induction of allergy on the same sites the dosage was 50 µl 10 mg/ml Oxz or 500 mg/ml TmA (8). To enable development of anti-TmA IgE antibodies, as suggested by Dearman et al. (8), induction of TmA allergy was performed again with 50 µl 250 mg/ml TmA 7 days later. The protocols for topical treatments of the animal groups are in Table I.

Elicitation of allergy

Five or 6 days after the last inductions, the animal groups not pretreated with cUA or vehicle during induction were treated with 10 mg/ml cUA or vehicle on dorsums of the ears, 50 µl/ear. The treatment was repeated the next morning. Four hours later (24 h after first cUA or vehicle application) all groups, including groups pretreated with cUA or vehicle only during induction, were challenged with 1 mg/ml oxazolone or 250 mg/ml TmA (8), 25 µl/ear (Table I).

Acute irritant reactions

The mice were treated with 10 mg/ml cUA or vehicle, 50 µl/ear, 24 h and 4 h before applying 25 µl 1 mg/ml croton oil to each ear (Table I).

Ear swelling measurement

The thickness of the animals' ear pinnae was determined with a Digitrix 11 electronic digital micrometer. The thickness immediately before allergen or irritant application was used as baseline, and the ear swelling was calculated as a percentage increase from the thickness of each individual ear. Ear swelling was measured at 24 and 48 h (Oxz, croton oil) and at 1, 2, 4, 24 and 48 h (TmA).

Statistical analysis

The difference between ear swelling of different experimental groups was calculated by using Student's unpaired *t*-test.

RESULTS

Topical cUA suppresses induction of hypersensitivity to contact allergen oxazolone

Topical cUA suppressed induction of contact allergy to oxazolone. At 24 h after elicitation some effect was seen (vehicle 46% vs. cUA 37% swelling; $p=0.115$), which at 48 h became statistically significant (Fig. 1).

Table I. Treatment schedules for study groups

cUA = cis-urocanic acid; TmA = trimellitic anhydride; Oxz = oxazolone; CrO = croton oil; veh = vehicle; Ind. = group treated with cUA during induction of allergy; Elic. = group treated with cUA during elicitation of allergy.

Group	n	Days to challenge:						
		15	14*	8	7*	6*	1	0§
TmA Ind.	6	cUA†	cUA/TmA	cUA	cUA/TmA	-/-	-	-/TmA
Control	6	veh†	veh/TmA	veh	veh/TmA	-/-	-	-/TmA
TmA Elic.	5	-	-/TmA†	-	-/TmA	-/-	cUA	cUA/TmA
Control	5	-	-/TmA†	-	-/TmA	-/-	veh	veh/TmA
Oxz Ind.	6	-	-/-	cUA†	cUA/Oxz	cUA/Oxz	-	-/Oxz
Control	6	-	-/-	veh†	veh/Oxz	veh/Oxz	-	-/Oxz
Oxz Elic.	7	-	-/-	-	-/Oxz†	-/Oxz	cUA	cUA/Oxz
Control	6	-	-/-	-	-/Oxz†	-/Oxz	veh	veh/Oxz
CrO	6	-	-/-	-	-/-	-/-	cUA	cUA/CrO
Control	6	-	-/-	-	-/-	-/-	veh	veh/CrO

*: 5 h interval between cUA (or veh) and allergen (TmA or Oxz) application;

§: 4 h interval between cUA (or veh) and allergen (or CrO) application;

†: Shaving and taping performed immediately before treatment.

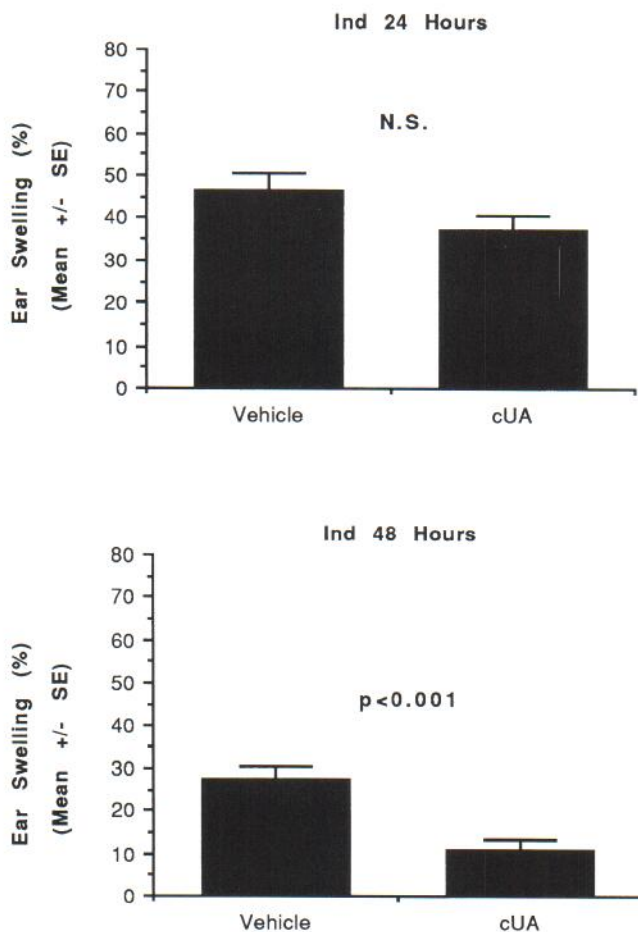


Fig. 1. Effect of topical cUA treatment during CHS induction on CHS elicitation responses: cUA suppressed reactions at 24 h ($p=0.115$) and 48 h ($p<0.001$).

Topical cUA suppresses elicitation of hypersensitivity to oxazolone

As shown in Fig. 2, topical cUA treatment suppressed elicitation of allergy to contact allergen oxazolone.

Topical cUA does not influence induction of hypersensitivity to respiratory allergen trimellitic anhydride

As shown recently, animals sensitized to TmA had a biphasic ear swelling response to topical TmA, a first swelling starting at 1 h and a second swelling peaking at 24 h (8). At all time points, there was no significant difference between ear swelling responses to TmA regardless of treatment with cUA or vehicle during induction of TmA allergy via topical skin application. Trivial enhancement of swelling was seen at 1 h in cUA-treated animals (43% vs. 39%) but the result was not significant ($p=0.406$). At 24 h there was almost no difference (cUA 86%, vehicle 87%; $p=0.865$).

Topical cUA does not suppress elicitation of skin allergy to trimellitic anhydride

There was a very small increase in ear swelling on cUA-pre-treated sites during early reaction (cUA 42%, vehicle 36%; at 2 h), but this effect was not statistically significant ($p=0.156$). At 24 h there was almost no difference (cUA 88%, vehicle 92%, $p=0.699$). There was a statistically non-significant suppression on late TmA reactions (cUA 61%, vehicle 71%, $p=0.084$; at 48 h).

Topical cUA does not suppress acute irritant reactions

There was almost no effect on croton oil ear swelling by cUA application at 24 h (cUA 94%, vehicle 96%, $p=0.882$) or 48 h (cUA 60%, vehicle 56%, $p=0.511$).

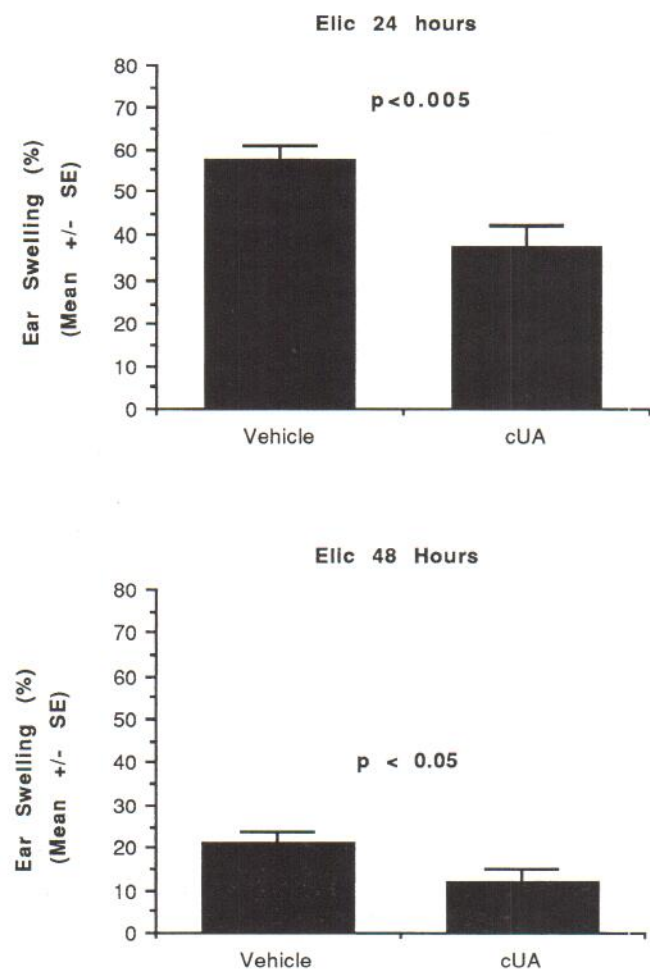


Fig. 2. Effect of topical cUA on CHS elicitation: cUA suppressed reactions at 24 h ($p=0.003$) and 48 h ($p=0.020$).

DISCUSSION

This data shows that topical cUA suppresses induction and elicitation of CHS. However, topical cUA suppressed significantly only CHS to oxazolone. Elicitation of delayed ear swelling to TmA and acute irritant reactions to croton oil were not significantly suppressed by cUA. As the ear thickness increases due to TmA and croton oil at 24 and 48 h were almost two times larger than those to oxazolone, it may be that 1% cUA preapplication could not influence stronger reactions. In earlier studies it has been shown that most of the effect of cUA is related to impairment of antigen-presenting cells (3–5, 9), essential for initiation of CHS elicitation but not for irritation, although some effect on sensitized T cells has also been seen (3, 5). The lack of significant effect in late TmA reactions, presumably due to CHS, may be due to a higher degree of unspecific inflammation after early ear swelling when compared to CHS reactions to oxazolone with late swelling only.

Earlier, a study by Higagi et al. (10) showed that 5% topical cUA cream first applied 3 h before intradermal testing with microbial antigens does not suppress delayed hypersensitivity reactions to these antigens in man. The reasons for the efficacy seen on CHS elicitation in this study may include earlier initial

preapplication (24 h before) and a more permeable mouse ear skin when compared to human forearm skin, enabling earlier tissue penetration and cUA action on epidermal antigen-presenting cells, essential in initiation of CHS elicitation.

TmA is a chemical that is capable of causing, in addition to contact allergy, immediate-type cutaneous hypersensitivity (8), raise in serum IgE levels (11) and asthmatic respiratory symptoms (12). High concentrations are needed for induction and elicitation of TmA skin reactions in mice, though (8, 11), with theoretical possibility of irritancy. Interestingly, cUA seemed to somewhat enhance rather than suppress the early phase of TmA allergy, which is likely to be due to type I immediate allergy (8), although the enhancement was not significant. cUA resembles structurally histamine (1), a mediator of immediate allergy. It has been suggested that cUA binds to histamine-like receptors in skin, as the effects of cUA on CHS induction have been shown to be reversible with antihistamines cimetidine and terfenadine (13, 14).

cUA has been intensely studied, as UVB has been shown to both suppress CHS induction and to cause keratinocyte-derived skin cancer. Suppression of CHS induction may help cancer cells to escape immune surveillance. cUA has been shown to suppress CHS induction like UVB and it may enhance skin tumour yield in animals simultaneously radiated with UVB (15, 16). However, cUA alone, in the absence of UV irradiation, does not seem to be able to cause DNA damage (17).

As a conclusion, results of this preliminary study suggest that it may be appropriate to ascertain whether topical cUA may have a therapeutic effect in inflammatory skin diseases, especially in dermatoses responsive to UVB therapy.

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