Acta Derm Venereol (Stockh) 1994; 74: 266–268

Effects of Cis- and Trans-urocanic Acids on the Secretion of Interleukin-1β and Tumour Necrosis Factor-α by Human Peripheral Blood Monocytes

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In order to investigate the mechanism of urocanic acid (UCA)-mediated immune modulation, we studied the effect of cis- and trans-UCA on interleukin-1β and tumour necrosis factor-α production by human peripheral blood monocytes, using immunospecific ELISA techniques. Trans-UCA augmented the interleukin-1β production and inhibited tumour necrosis factor-α production in a dose-dependent manner, whereas cis-UCA had no effect on the secretion of these cytokines by phorbol myristate acetate or lipopolysaccharide-stimulated monocytes. This is a novel example of trans-UCA mediating a biological effect, a finding earlier reported for cyclic adenosine monophosphate up-regulation in human fibroblasts by Palaszynski and coworkers and for human natural killer cell inhibition by ourselves. Our data suggest an important role for trans-UCA as an immunomodulator in the skin. ***Key words: cytokines; immunomodulation; phorbol myristate acetate; lipopolysaccharide; cyclic AMP.***

(Accepted January 10, 1994.)

Acta Derm Venereol (Stockh) 1994; 74: 266–268.

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The immunosuppressive effect of UV radiation has been unequivocally demonstrated by several research groups (1). Photoisomerization of urocanic acid (UCA), a small molecule in epidermis, acts as an immunological transduction signal (2–4). Generally, cis-UCA has been reported to be the immunologically active principle and trans-UCA less active or inactive, but in the case of human natural killer (NK) cell cytotoxicity modulation, the reverse is true (5).

UCA might influence antigen-presenting cells and other immune cells either directly or via secondary messengers, such as cytokines (6, 7). Here we report data on the effect of UCA isomers on the interleukin-1β (IL-1β) and tumour necrosis factor-α (TNF-α) production by human peripheral blood monocytes (HPBM), a well-established model cell for cytokine secretion.

MATERIAL AND METHODS

Urocanic acid

Trans-UCA was purchased from Sigma Chemical Co. (St. Louis, MO, USA). Cis-UCA was prepared from the trans isomer with UV irradiation in an alkaline solution followed by ion exchange chromatography. The purity of each isomer was more than 98%, as detected by HPLC (8). The isomers were dissolved in DMSO, diluted in the culture medium and sterilized through a 0.22 μm filter before addition to the test.

Isolation of HPBM

Leucocyte-rich buffy coats were obtained from the Finnish Red Cross Blood Transfusion Service (Turku, Finland). Mononuclear cells were isolated by gradient centrifugation on Ficoll-Isopaque (Pharmacia, Uppsala, Sweden). The cells were washed with Hank's balanced salt solution (HBSS), and the remaining erythrocytes were lysed with ammonium sulphate. After further washing with HBSS, mononuclear cells were resuspended, at 10^7 cells/ml, in RPMI-1640 medium (Gibco, Paisley, UK) containing 10% heat-inactivated fetal calf serum (Biological Industries, Kibbutz Beth Haemek, Israel), 2 mM L-glutamine, and 50 mg/l gentamycin (Orion, Espoo, Finland), referred to as complete medium. Fifteen to 20 ml of this cell suspension was incubated in a 250-ml culture flask (Costar, Cambridge, MA, USA) at 37°C, 5% CO_2, for 1 to 2 h. After incubation, the non-adherent cells were removed by washing the flask with warm complete medium. The adherent cells (HPBM) were collected into cold complete medium with a rubber policeman.

Induction of cytokine production by HPBM

Three million HPBM in 1 ml of complete medium were cultured in 24-well cell culture plates (Costar) and stimulated either with 5 μg/ml of LPS (lipopolysaccharide W, E. coli O55: B5; Difco Laboratories, Detroit, MI, USA) or 2.5 ng/ml of PMA (phorbol 12-myristate 13-acetate). Trans-UCA or cis-UCA was added in various concentrations with DMSO as the control. After 24 h, the plates were centrifuged, and the supernatants were collected and stored at −20°C for measurements in IL-1β and TNF-α ELISAs.

Specific ELISA for IL-1β and TNFα

The cytokine content in HPBM supernatants was determined with ELISA kits for IL-1β (Cistron Biotechnology, Pine Brook, NJ, USA) and TNF-α (Endogen, Boston, MA, USA). A standard curve was run for every determination, and sample concentrations above the linear range were disqualified and re-assayed after dilution.

Statistical analysis

Analysis of variance (anova) was used to determine the significance of difference in means.

Table I. *Response of IL-1β production by human monocytes to PMA stimulation*

Data from ELISA determinations of 5 sets of supernatants from different buffy coat batches (A-E) are presented (ND, not determined).

PMA (ng/ml)	Interleukin-1β (pg/ml)				
	A	B	C	D	E
–	600	1000	200	900	700
2.5	650	43,500	2,400	2,000	69,000
25	12,500	49,500	11,000	ND	ND

 ISSN 0001-5555

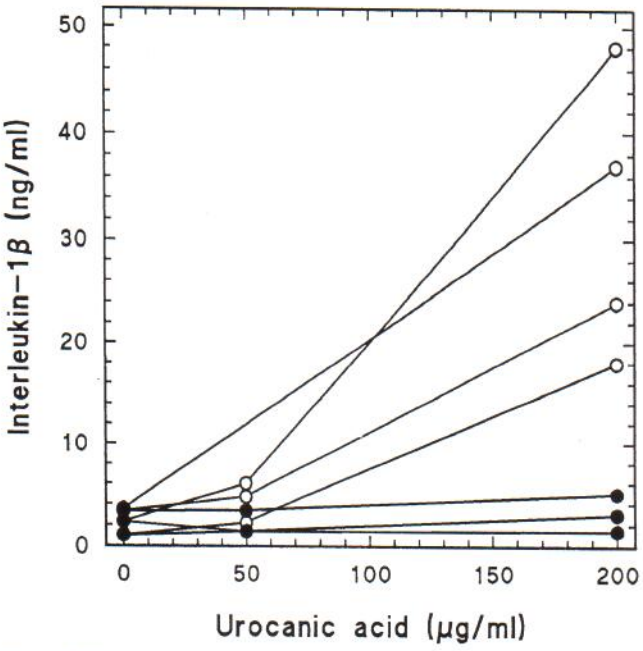

Fig. 1. Effects of trans-UCA (○) and cis-UCA (●) on the IL-1β levels in human monocyte cultures stimulated with LPS (5 μg/ml), as determined by ELISA. Results of 3 parallel experiments with both isomers and 1 experiment with trans-UCA only are shown. At 200 μg/ml of UCA, a significant difference was found between the isomers (p = 0.016) and between trans-UCA and control (p = 0.0049).

RESULTS

In vitro effect of UCA isomers on IL-1β production by human monocytes

As shown in Table I, PMA-induced IL-1β production by HPBM in a dose-dependent manner. LPS was found to have a similar effect (data not shown). PMA and LPS were used in further studies to investigate the role of cis-UCA and trans-UCA on IL-β production by HPBM. The addition of cis-UCA in concentrations up to 200 μg/ml had no significant effect on the IL-1β production (Fig. 1). In contrast, the addition of trans-UCA resulted in a dose-dependent increase in the IL-1β production (Fig. 1). These divergent effects of the UCA isomers were recorded both with LPS (Fig. 1) and PMA stimulation (data not shown).

In vitro effect of UCA isomers on TNF-α production by human monocytes

As shown in Table II, trans-UCA had a dose-dependent inhibitory effect on TNF-α production into supernatants of PMA-stimulated HPBM, while cis-UCA was inactive.

DISCUSSION

A considerable amount of data from different laboratories demonstrate that UCA mediates immune-modulating effects (9). In previously reported systems, the cis isomer has been shown to mediate immunological down-regulation, while trans-UCA has been biologically ineffective. In the present report, we have studied the effects of cis and trans-UCA on cytokine production by human monocytes. Our results clearly indicate that trans-UCA is biologically active, increasing IL-1β secretion and decreasing TNF-α secretion, while cis-UCA is inactive. Interestingly, we have recently observed that also in the case of human NK cell mediated cytolysis, trans-UCA is the biologically effective isomer, causing inhibition, while the cis isomer has no effect (5). These findings suggest that both UCA isomers may possess regulatory activities in immunobiological systems. Because no data are available to indicate whether UCA isomers can be found in the blood in concentrations that can modulate blood cell functions, the significance of the results cannot be directly demonstrated. In human epidermal skin, however, as much as 75 μmol/g (10.4 mg/g) (10) and about 30 μmol/g (4.1 mg/g) (11) have been detected. Therefore, the concentration range 50–200 μg/ml used in our assays in vitro may well compare with the levels of UCA found locally in the epidermis.

Previously, Räsänen et al. reported that cis-UCA decreased cytokine production by HPBM, as measured by a mouse thymocyte comitogenicity assay (6). At that time, this assay was thought to reflect IL-1 activity, but it has later been shown to be a non-specific measure of several cytokines, including IL-1, TNF-α (12) and IL-6 (13). Furthermore, the assays of Räsänen et al. and those of the present study differed in some other important parameters, such as plasma source (autologous vs. fetal calf), stimulus (S. epidermidis vs. LPS or PMA) and in length of incubation (48 h vs. 24 h). The fact that monocytes can also secrete a specific inhibitor of IL-1, the interleukin-1 receptor antagonist (IL-1ra) (14), could also explain the differences in the assays for IL-1 using a test for biological activity or an immunospecific protein detection method (15). One of the tested isomers (trans-UCA) may increase the secretion of both IL-1 and of IL-1ra, while the other isomer (cis-UCA) may only mediate an increase in IL-1ra secretion. This would lead to an apparent decrease in IL-1 by cis-UCA as measured biologically, but to no change in MoAb detectable levels, because IL-1 shares little immunologic cross-reactivity with IL-1ra (16). On the other hand, trans-UCA would appear to have no effect in the

Table II. *Effects of cis-UCA and trans-UCA on TNF-α production by human monocytes stimulated with PMA*

Results of 2 experiments are presented, and statistical significance has been calculated from the combined data (NS, non-significant difference).

	TNF-α (pg/ml)				
		Cis-UCA (μg/ml)		Trans-UCA (μg/ml)	
PMA (ng/ml)	Control	50	200	50	200
–	<12	<12	<12	<12	<12
			*		
2.5	2100	1050	1370	600	220
		NS			
25	2200	1450	2300	600	<12
250	1350	1270	1150	530	<12
			*		
2.5	1200	450	1000	100	<60
		NS			

*, p=0.079.

bioassay, while the MoAb could detect an increased amount of IL-1. At the time of this study, IL-1ra MoAbs were not generally available, and we therefore could not test this hypothesis.

In experiments with human fibroblasts, Palaszynski et al. have shown that trans-UCA (but not cis-UCA) up-regulates intracellular cyclic AMP (cAMP) levels (17) and, as we have pointed out elsewhere (5), a similar mechanism could be operative in NK cell cytotoxicity inhibition by trans-UCA. Interestingly, Hurme has shown that cAMP acts as a secondary messenger in PMA-induced stimulation of IL-1 production by HPBM (18). Other studies have shown that secretion of IL-1 and TNF-α is differentially regulated by the intracellular cAMP level in LPS-stimulated human mononuclear cells (19) and monocytes (20, 21). Elevation in cAMP by specific phosphodiesterase inhibitors leads to a decrease in TNF-α secretion (19, 21, 22) and to an increase in IL-1 secretion (20). In light of this regulatory mechanism, trans-UCA may actually be considered a potential mediator of phosphodiesterase inhibition. Clearly, the role of cAMP in the signal transduction of UCA-induced immunomodulations should be studied, and we are performing such investigations.

ACKNOWLEDGEMENT

The study was supported by the University Foundation of Turku, Orion Corporation Farmos, and Leiras Oy, Turku, Finland.

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