Urocanic acid in photodermatology
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CHAPTER 4

Prolonged increase of cis-uropnic acid levels in human skin and urine after single total-body ultraviolet exposures

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Abstract

Cis-uropnic acid (cis-UCA), a mediator of immunosuppression, is formed from trans-UCA upon UV-exposure of the skin. This study describes a liquid chromatographic method for the simultaneous quantification of cis- and trans-UCA in skin, urine and plasma of non-irradiated volunteers. It also describes cis- and trans-UCA kinetics in UV irradiated volunteers. New procedures to remove interfering substances from urine and plasma are reported. Normal levels of cis-UCA in skin, urine and plasma of non-irradiated volunteers were 0.5 nmol/cm², 0.03 μmol/mmol creatinine (median 0.00) and undetectable and those of trans-UCA were 17.1 nmol/cm², 1.36 μmol/mmol creatinine and 0.5 μM, respectively. Upon single total body UV-B (290-320 nm) exposures of 250 J/m², epidermal cis-UCA levels immediately reached a maximum and returned to basic levels 3 weeks later. Cis-UCA levels in urine reached a maximum in 5 to 12 hours post-irradiation and reached baseline values in 8 to 12 days. Additionally, a single total body UV-A (320-400 nm) irradiation of 200 kJ/m² yielded a similar pattern. The kinetics of cis-UCA in plasma could not be followed due to low concentrations, however, that of skin and urine was informative in relation to solar exposures and phototherapy.

PART I: Biokinetics of the UCA isomers
1. Introduction

Trans-urocanic acid (trans-UCA) is a major UV-absorbing component of the skin. It is formed from histidine by the action of histidase. In the epidermis it accumulates predominantly in the stratum corneum, where it cannot be metabolized due to absence of the trans-UCA catabolizing enzyme urocanase [1,2]. One route of trans-UCA removal is sweat excretion and horny layer slough off; a second route may be the inward diffusion from the epidermis into the circulation. Subsequently it passes the liver where it is partially broken down by urocanase [3,4]. Trans-UCA can also be formed by histidase in the liver [5] and the final rate of urinary trans-UCA excretion is influenced by this organ. When ultraviolet (UV) radiation strikes trans-UCA in the epidermis (in vivo), cis-UCA is formed by photoisomerization [6-8]. The most efficient waveband for photoisomerization is 310-320 nm and the longest effective wavelength is 363 nm with a solar exposure of 1 minimum erythema dose (MED) [8]. There are indications that cis-UCA is not catabolized in the internal body [9].

Cis-urocanic acid (cis-UCA) has been shown to mimic the immunosuppressive effects of UV-B radiation in a number of studies, suggesting that this compound is an important mediator of UV-induced immunosuppression [10]. Immunomodulating effects were reported on the induction of contact hypersensitivity [11] and delayed type hypersensitivity [12], allograft rejection [13] and the functions of monocytes and T-lymphocytes [14] as well as natural killer cells [15]. The mechanism of action of cis-UCA is not fully understood, although, its action appears to involve tumor necrosis factor-alpha (TNF-alpha) [16] and histamine-like receptors [17]. It is also not clear as to why UV-B is more potent in suppressing the contact hypersensitivity than UV-A (320-400 nm), although similar levels of cis-UCA were induced by both types of irradiation [18].

The common knowledge that cis-UCA has immunosuppressive properties and that it is easily formed in the epidermis upon solar exposure or upon exposure to artificial UV-radiation, such as during phototherapy, makes it important to obtain information about its formation, persistence and elimination from the human body. This requires a sensitive and reproducible method for the quantification of cis- and trans-UCA in body samples.

So far, a method for the simultaneous quantification of the UCA-isomers in urine or plasma has not been described. Both UCA isomers have been determined simultaneously by liquid chromatography (HPLC) in other skin specimen, such as homogenized horny layers of the human skin.
Prolonged increase of cis-UCA levels (19,20) and homogenized murine skin biopsies (21); other studies reported the assay of UCA-isomers in horny layers collected by tape stripplings (22,23). Human suction blister fluids could be analysed by HPLC without pretreatment (24,25). We adapted a non-invasive sampling method (26) with a slight modification to obtain human skin data.

The above mentioned reports were focussed on cis- and trans-UCA in the epidermis; no correlation was made for UCA isomer levels in internal human body fluids, such as urine and plasma. This report describes the development of an HPLC-analysis for the simultaneous quantification of cis- and trans-UCA in urine and plasma. A newly developed pretreatment (clean-up) procedure for these specimen was found to be essential. The formation, persistence and elimination of cis- and trans-UCA in the epidermis, urine and plasma before and after single UV-B exposures or a UV-A exposure were assessed.

2. Materials and Methods

2.1. Chemicals and buffers

Trans-UCA, and 2-amino-4-thiazole acetic acid (ATAA), were purchased from Aldrich Europe (Bornem, Belgium). ATAA was recrystallized from an acetone/hexane mixture. Cis-urocanic acid (cis-UCA) and cis-2-methyl-urocanic acid (cis-MUCA) were kindly donated by dr M. Norval of the University of Edinburgh. Two types of washing buffers were used in the solid phase extraction procedure. One consisted of a 0.1 M glycine/ HCl buffer pH 2.2, containing 20% methanol (buffer A). The other consisted of 0.1 M sodium citrate/HCl buffer pH 3.5, containing 0.5 M sodium chloride (buffer B). The elution buffer consisted of 0.2 M sodium acetate buffer pH 4.2, containing 0.5 M sodium chloride and 20% methanol.

2.2. Donors and ultraviolet exposures

Healthy males and females aged 12-58 years participated in this study. Four volunteers received a single total body UV-B exposure of 250 J/m² in a cabinet with Philips TL-12 fluorescent tubes (Philips, Eindhoven, The Netherlands). The UV-dose was equivalent to 1.0 to 1.5 MED on skin type II/III volunteers. A single total body UV-A exposure of 200 kJ/m², emitted by Philips TL 10R fluorescent lamps, was received by a skin type III volunteer. Other healthy volunteers were not irradiated and served to obtain more data on normal cis- and trans-UCA levels. None of the donors was exposed to UV-radiation for at least three weeks prior to experimental UV-exposure. Persons that were not exposed to these UV-sources, will be referred to as 'non-irradiated'.
2.3. High Performance Liquid Chromatography (HPLC)

Cis- and trans-UCA, cis-2-methylUCA, trans-2-methylUCA and ATAA were separated on a 4 x 250 mm reversed phase μ-Bondapak column (Waters/Millipore, Milford, MA) with a flow of 1 ml/min, delivered by P-3500 HPLC-pumps (Pharmacia, Uppsala, Sweden). Samples were injected by a Promis II autosampler (Spark Holland, Emmen, The Netherlands) and chromatographic data were recorded on a SP 4270 integrator (Spectra Physics, San Jose, CA). A UV-detector (Applied Biosystems, model 759A, Foster City, CA) was set for 268 nm detection. Isocratic elution was performed with 0.05 M phosphate buffer (pH 3.4-3.6) containing 1 mM sodium octane sulphonate and 4% methanol for skin samples or 3 mM sodium octane sulphonate and 2% methanol for urine and plasma samples.

2.4. Determination of UCA isomers in urine

Urine was collected [see Results] and stored in portions at -20 °C. In contrast to epidermal and plasma sampling, urine was collected from each volunteer at different time-intervals. The creatinine concentration was used to adjust the original urine volume prior to the clean-up procedure following the guideline that a creatinine concentration of approximately 10 mM should correspond with one ml original urine for analysis. This correction was to avoid overload of the solid phase extraction column. The urine samples were cleaned up with strong cation exchange columns of the aromatic sulphonate type using a vacuum manifold SPE 12G (J.T.Baker, Deventer, The Netherlands). One milliliter of buffer A and the internal standard, cis-2-methylUCA (20 nmol) were added to the urine samples, followed by acidification with 20 ml 5 M hydrochloric acid prior to the passage through the solid phase extraction columns. The columns were successively washed with 2 ml buffer A, 2 ml buffer B and 2 x 2 ml pure water. UCA-isomers were then eluted with 2 ml elution buffer. The eluate was filtered through a 0.22 μm membrane filter, prior to HPLC injections of 50-100 microliters under the conditions described above. The urinary cis- and trans-UCA concentrations were either expressed as the percentage of total UCA or as micro-moles per millimoles creatinine. An average urinary creatinine excretion is approximately 12 mmol/24 hours (~ 12 mM).

2.5. Determination of UCA isomers in plasma

Blood was collected in lithium heparin containing tubes at planned time-intervals respective to irradiation. After centrifugation (20 minutes at 2000 g) plasma was collected and stored at -20°C. One milliliter of freshly frozen plasma was thawed and known amounts of internal standards,
ATAA and cis-2-methylUCA, were added. After standing for 10 minutes at room temperature with occasionally stirring, 0.9 ml of a methanol/water 1:5 mixture was added, followed by 0.1 ml 40% trichloroacetic acid under agitation. The tubes were kept on ice for 10 minutes with occasionally stirring followed by centrifugation at 3800 rpm for 20 minutes at 4 °C. The supernatants were then centrifuged at 13.000 rpm for 10 minutes. The supernatants were filtered through 0.22 μm membrane filters. HPLC-analysis was then carried out as described above. The plasma values of cis- and trans-UCA were expressed as micromoles per liter (μM).

2.6. Determination of UCA-isomers in epidermis

Epidermal alkaline extracts or filter samples (originally referred to as 'chamber samples') were collected at planned time-intervals respective to UV-irradiation. Filter samples were taken from the upper arms and from the lumbar spinal region of the volunteers. This non-invasive sampling method was a modification of an earlier reported technique (26), which is based on alkaline extraction of the epidermis. Our modification consisted of the application of patch testers (Silverpatch™, van der Bend, Brielle, The Netherlands), instead of Finn Chambers™. Using the patch testers, we achieved a smaller variation than reported elsewhere (26,27), perhaps because of an improved contact between skin surface and moistened filter paper. A further modification was an enlargement of the filter moistening volume and a reduction of the soaking volume and time. The filter paper (1 cm²) was covered with a plastic sheet (3 cm diameter) and was moistened with 20 μl potassium hydroxide 0.1 M. It was fixed on the skin test area by elastic adhesive tape. After one hour or after 24 hours the filter papers were collected, put in 472 μl 0.1 M potassium hydroxide and shaken for 30 seconds. The medium was acidified with 8 μl phosphoric acid 87%; followed by stirring for 30 seconds. The epidermal extract was passed through 0.22 μm membrane filter prior to injecting 40-100 μl into the HPLC system. The data were expressed as nmol/cm² and as the ratio of cis- or trans-UCA to total-UCA.

3. Results

3.1. The HPLC-analysis of cis-, trans- and total-UCA levels in human skin

The sampling technique, as described earlier (8), was used to quantify epidermal cis- and trans-UCA levels before and after the UV-irradiations (vide infra) as well as the epidermal total-UCA (cis- plus trans-UCA) concentration. In addition, UCA-isomers extracted by 1 hour and 24 hour occlusions were compared, because it has been suggested that the latter method causes the extraction of virtually all UCA (26).
Table 1. *Cis-* and *trans-*urocanic acid levels in skin, urine and plasma in non-irradiated volunteers.

<table>
<thead>
<tr>
<th>Urocanic acid isomer</th>
<th>Skin a</th>
<th>Urine b</th>
<th>plasma c</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Statistical parameter</td>
<td>% of total UCA</td>
<td>µmol/ mmol</td>
</tr>
<tr>
<td></td>
<td>nmol/cm²</td>
<td></td>
<td>% of creatinine</td>
</tr>
<tr>
<td><em>cis</em>-urocanic acid</td>
<td>mean</td>
<td>0.50</td>
<td>2.8</td>
</tr>
<tr>
<td></td>
<td>SD</td>
<td>0.70</td>
<td>2.5</td>
</tr>
<tr>
<td></td>
<td>median</td>
<td>0.27</td>
<td>1.7</td>
</tr>
<tr>
<td><em>trans</em>-urocanic acid</td>
<td>mean</td>
<td>17.1</td>
<td>98</td>
</tr>
<tr>
<td></td>
<td>SD</td>
<td>7.1</td>
<td>2.5</td>
</tr>
<tr>
<td></td>
<td>median</td>
<td>19.0</td>
<td>98</td>
</tr>
</tbody>
</table>

a. Unimodal skew distribution of data. From each individual 2 to 6 filter samples were taken. In total 32 filter samples from 7 individuals were analysed.

b. Unimodal skew distribution of data; *cis*-UCA level was below detection limit in 40 samples (74%) and was taken as zero in statistics. From each individual 1 to 2 urine samples were obtained. In total 53 urine samples from 44 individuals were analysed.

c. In total 12 plasma samples of 12 individuals were assayed, one sample showed a *trans*-UCA level of 1.9 µM and was not included.

Total-UCA extracted with the 1 h and 24 h occlusion methods had a mean value of 16.5 nmol/cm² (± 5.9; 7 individuals, 172 samples) and 50 nmol/cm² (± 18; 2 individuals, 12 samples), respectively. A relatively increased *cis*-UCA/total UCA ratio by a factor 1.37 was found using the 1 hour occlusion method. Because of this finding we chose the 1 h occlusion method to register changes in *cis*-UCA formation by photoisomerization. One hour occlusions also offered more handling convenience. No significant difference was found in total-UCA level between irradiated and non-irradiated skins. Epidermal *cis-* and *trans-*UCA levels of non-irradiated volunteers are summarized in Table 1. Their *cis*-UCA levels in the epidermis were low, though measurable and their *trans*-UCA levels were found comparable to those of total-UCA. The *cis-* and *trans*-UCA detection limits for this analysis were 8 and 5 pmol, respectively.
Table 2. Recoveries of added cis- and trans-uromanic acid and internal standards.

<table>
<thead>
<tr>
<th>Standard compound</th>
<th>Final concentration of added standard compound (µM)</th>
<th>Recovery % of added quantities</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Water</td>
<td>Urine *</td>
</tr>
<tr>
<td></td>
<td></td>
<td>no UV exposure</td>
</tr>
<tr>
<td></td>
<td></td>
<td>UV-exposure</td>
</tr>
<tr>
<td>cis-UCA</td>
<td>0</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>72</td>
</tr>
<tr>
<td></td>
<td>15</td>
<td>75</td>
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<tr>
<td></td>
<td>25</td>
<td>77</td>
</tr>
<tr>
<td></td>
<td>40</td>
<td>75</td>
</tr>
<tr>
<td>trans-UCA</td>
<td>0</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>90</td>
</tr>
<tr>
<td></td>
<td>15</td>
<td>77</td>
</tr>
<tr>
<td></td>
<td>25</td>
<td>77</td>
</tr>
<tr>
<td></td>
<td>40</td>
<td>80</td>
</tr>
<tr>
<td>cis-2-methylUCA</td>
<td>5</td>
<td>72</td>
</tr>
<tr>
<td></td>
<td>15</td>
<td>73</td>
</tr>
<tr>
<td></td>
<td>25</td>
<td>76</td>
</tr>
<tr>
<td></td>
<td>40</td>
<td>74</td>
</tr>
<tr>
<td>ATAA</td>
<td>10</td>
<td>87</td>
</tr>
<tr>
<td></td>
<td>25</td>
<td>87</td>
</tr>
<tr>
<td></td>
<td>40</td>
<td>86</td>
</tr>
<tr>
<td></td>
<td>75</td>
<td>82</td>
</tr>
</tbody>
</table>

* the urine of the non-irradiated volunteer (creatinine: 21.8 mM) contained originally 2.7 µM cis-uromanic acid and 25 µM trans-uromanic acid. The urine of the same, UV-exposed person (creatinine: 9.8 mM) contained originally 10.1 µM cis-uromanic acid and 13.6 µM trans-uromanic acid.

3.2. The HPLC-analysis of cis- and trans-UCA in human urine

Clean-up procedures were required to facilitate the measurements of cis- and trans-UCA in human body fluids, as described in Materials and Methods. To assess the reliability and sensitivity of the HPLC-method, including clean-up procedure, we added our reference compounds cis- and trans-UCA, cis-
2-methylUCA and ATAA in nanomole amounts to the urine samples. The recoveries of added UCA-compounds, corrected for original urinary concentrations of cis- and trans-UCA isomers, are shown in Table 2.

The reproducibility of the method was determined through repeated analysis (18 times) of one ml of urine from a UV-B exposed volunteer supplemented with 20 nmol cis-2-methylUCA and trans-2-methylUCA and 50 nmol ATAA. The coefficients of variation were 3.5%, 4.9%, 7.1%, 3.6% and 7.3% for cis-UCA, trans-UCA, cis-2-methylUCA, trans-2-methylUCA and ATAA, respectively, thus showing an appreciable degree of reproducibility. Cis-2-methylUCA was eluted close to trans-UCA. Based on the above results and on similarities in molecular structure, we selected cis-2-methylUCA in final concentration of 15 µM or more as the internal standard for regular urine analyses. Cis-UCA was eluted considerably faster than its trans-isomer. The HPLC-system, including the UV-detector, provided linear results over a range from 45 pmol to 2.5 nmol cis- and trans-UCA and for cis-2-methylUCA. Detection limits of urine samples were as low as 45 pmol for cis-UCA and 25 pmol for trans-UCA. Cis- and trans-UCA levels in urine of non-irradiated volunteers are summarized in Table 1. Normal cis-UCA levels were extremely low and in a number of analyses its concentration was even below the detection limit. Trans-UCA was measurable in all urine samples. The range of trans-UCA concentrations that were not corrected for creatinine clearance, ranged widely from 2.7 to 44.7 µM.

3.3. The HPLC-analysis of cis- and trans-UCA in human plasma

UCA-levels in plasma might reflect photo-induced changes of epidermal UCA. A part of epidermal cis-UCA may be distributed via the blood circulation across the internal body, which aspect may be important for systemic immunosuppression. Therefore, we had set up an HPLC analysis of cis- and trans-UCA in plasma. To avoid interference by high protein concentration in plasma, a deproteinization step with trichloroacetic acid and methanol was introduced, which allowed the recovery of virtually 100% of the added cis- and trans-UCA and the internal standards cis-2-methylUCA and ATAA. The chromatogram of a plasma sample without any addition is shown in Fig. 1a and the one with additions of cis-, trans-UCA, cis-2-methylUCA and ATAA in final concentrations of 2.0, 2.0, 2.4 and 5.0 µM, respectively, is shown in Fig. 1b. These small additions were readily detected, showing that our method was quite sensitive. However, original UCA levels in plasma were extremely low. Cis-UCA concentration in all plasma samples of non-irradiated volunteers were lower than the detection limit. Normal trans-UCA levels could be quantified in all plasma samples (Table 1). The detection limits in plasma were 25 pmol for cis-UCA and 15 pmol for trans-UCA.
Fig. 1. Chromatograms of cleaned-up plasma samples.

a. (left): a sample of a healthy volunteer without supplements. Peak 2 corresponded to 19 pmol trans-UCA.

b. (right): a sample supplemented with cis- and trans-UCA, cis-2-methylUCA and ATAA, followed by clean-up procedure (see text for final concentrations). In both cases the injected volumes were 100 μl. Peak identity: 1. cis-UCA, 2. trans-UCA, 3. cis-2-methylUCA, 4. ATAA.

When ultrafiltration was used instead of the above mentioned deproteinization procedure, more than 86% binding of these compounds to proteins was observed after mixing of the above standard compounds with 10% bovine serum albumin in phosphate buffered saline. These findings suggest a substantial protein binding of UCA isomers in blood.

PART I: Biokinetics of the UCA isomers
3.4. Cis- and trans-UCA levels in skin, urine and plasma of UV-B-irradiated volunteers

The kinetics of cis-UCA levels were studied in four volunteers that received a single total body UV-B exposure. Skin filter samples, urine and plasma were taken at several time points, having pre-exposure samples as basic level controls. Epidermal cis-UCA levels reached a maximum immediately upon irradiation and a plateau level persisted for 1 to 2 days. The skin levels in absolute terms, derived from two time points in this plateau period, were 8.3 (± 2.9) and 6.3 (± 2.4) nmol/cm$^2$ for cis- and trans-UCA, respectively (4 individuals; 44 samples). In other terms, UV-B irradiation reduces simultaneously epidermal trans-UCA concentration. Five to eight days after irradiation still a considerable amount (half of maximum) of epidermal cis-UCA was measurable and basic levels were reached after 17 -21 days (Fig. 2).

![Image](image_url)

**Fig. 2.** The effect of UV-B irradiation on cis-UCA levels in epidermis (closed; black markers) and in urine (open; white markers). The irradiation dose was 250 J/m$^2$. Urine sampling did not proceed at same timepoints, therefore the excretion patterns are shown separately for each of the four volunteers. The epidermal cis-UCA levels of 4 volunteers are shown as mean value and the error bars represent the standard deviation. Each timepoint of the epidermal pattern represented at least 16 determinations from 4 volunteers. Human epidermis retained increased cis-UCA levels for approximately 3 weeks and human urine showed increased cis-UCA levels for 8 to 12 days.
Fig. 3. Chromatograms of cleaned-up urine samples.

a. (left): from a healthy volunteer before irradiation.
b. (right): from the same person 16 hours after a single total body UV-B irradiation of 1 MED. In both cases 50 μl sample was injected into the HPLC-system. See text for further chromatographic conditions.

Peak identity: 1. cis-UCA, 2. trans-UCA, 3. cis-2-methylUCA.

Urinary cis-UCA level was increased upon UV-B irradiation.

The HPLC-chromatograms of cleaned-up urine samples, obtained before and 16 hours after a UV-B exposure of 1 MED clearly show the marked increase of cis-UCA concentration (Fig. 3a and 3b). The urinary cis-UCA concentrations reached a maximum at 5 to 12 hrs after the UV-B exposure. In absolute terms, maximum cis-UCA excretion was 1.77 (± 1.05) mmol/mmol creatinine and in relative terms 57% (± 9.1) of total UCA (4 individuals; 8 samples, taken around maximum excretion). After one day the cis-UCA level dropped fast, followed by a slow decline. Basic levels were reached
after 8 to 12 days (Fig. 2). The cis-UCA levels in urine consistently followed those in the epidermis, indicating that cis-UCA in urine reflects radiation effects on epidermal UCA. Trans-UCA level in urine was not significantly affected by the UV-B exposure. Its level was 1.38 (± 0.83) mmol/mmol creatinine. When cis-UCA peak excretion was added to trans-UCA excretion, the total-UCA excretion was increased more than twice (3.15 mmol/mmol creatinine), compared to that under non-irradiation conditions (1.36 + 0.03 = 1.39 µmol/mmol creatinine).

A clear rise in photo-induced cis-UCA levels as observed in the skin or in urine could not be observed in plasma, because the levels appeared around the detection limit. Nevertheless, an increase of cis-UCA plasma level in extremely low concentrations could be observed without reliable quantification (0.6 µM, ± 0.4) in samples taken at 6 to 32 hours post-irradiation. In plasma no significant effect of UV-B on trans-UCA levels could be seen. In contrast to urine, the UCA concentrations in plasma were too low to clearly monitor radiation effects on the UCA-isomers in the epidermis. All mean values, expressed as a percentage, show a much smaller standard deviation than the values that were expressed in absolute terms.

3.5. Cis- and trans-UCA levels in skin, urine and plasma of a UV-A-irradiated volunteer

Because UV-A also causes considerable trans- to cis- photoisomerization [8], the effect of UV-A irradiations on UCA-levels of a healthy volunteer subjected to a single total body UV-A exposure of 200 kJ/m² was studied. The results indicated an essentially similar effect as that obtained after a single UV-B irradiation of 250 J/m². The initial epidermal plateau level upon UV-A irradiation was 10.8 (± 1.8) and 7.9 (± 1.1) nmol/cm² for cis- and trans-UCA, respectively (1 individual, 11 samples). In relative terms these levels (58% cis-UCA and 42% trans-UCA) were very close to those obtained after UV-B irradiation. Again, plasma levels of cis-UCA were too low to register a clear irradiation effect. In conclusion, the effect of this UV-A irradiation on cis-UCA levels in skin, urine and plasma is similar to the effect obtained with UV-B irradiations.

4. Discussion

In this study we found low, but significant, amounts of cis-UCA in skin and urine of normal, non-irradiated persons and increased amounts after a single whole body UV-B or UV-A exposure. This increased level of cis-UCA was retained in the body for several days. There was a consistent correlation between urinary and epidermal cis-UCA kinetics. Urine samples are
Prolonged increase of cis-UCA levels

Cis-UCA is expected to be absent in mammalian epidermis and body not exposed to UV, because epidermal histidase only forms the trans isomer of UCA (1) and cis-UCA could only be formed by photoisomerization of trans-UCA. Low basic cis-UCA levels in skin and urine seen in this study could have been formed from trans-UCA (7) by light from many ambient light sources (28) and by solar UV radiation, even filtered through window glass (8). Thus, unprotected skin could contain some cis-UCA. Besides, as shown this study, the effect of one total body UV-exposure on epidermal cis-UCA concentrations lasts for weeks. Thus, accumulation of trace amounts of cis-UCA could take place in the skin that is daily exposed to light sources for relatively long periods. Dramatic accumulation of cis-UCA may be expected after sunbathing or after phototherapy with frequencies higher than 1 time per week. After a single effective UV-exposure, urinary cis-UCA levels return more rapidly (~1.5 weeks) to basic levels than do the skin levels (~3 weeks). This pattern could be based on three events. First, a rapid diffusion of cis-UCA from the deeper epidermal layers into the circulation and then into the urine may take place in the first hours following UV-A or UV-B irradiation. Second, some cis-UCA from the upper epidermal layers may move inwards while the remaining might move upwards followed by horny layer slough off. Third, some cis-UCA may be retained by organs or glands and subsequently released.

Much higher levels of UV-B induced cis-UCA were found by a cis-UCA specific enzyme linked immunosorbent assay (ELISA) in murine serum (29), following the application of UV-B irradiation doses (2160 J/m$^2$ or 2 x 1440 J/m$^2$) to the murine epidermis. Several factors may explain the high cis-UCA levels in murine serum. Because of their small size, the ratio of murine skin surface to body volume is larger, therefore a relatively larger quantity of epidermal cis-UCA may diffuse into the circulation. Second, this effect might be amplified by a more rapid diffusion from the thinner murine epidermis. Moreover, the larger UV-B doses in this murine study may have caused loss of epidermal integrity with subsequent leakage of soluble components, such as cis-UCA, into the circulation. However, the kinetics of UV-induced epidermal cis-UCA were rather similar as a plateau level existed for at least 16 hours post-irradiation and seven days later cis-UCA level was halved (21).
We did not measure a reported urinary condensation product of UCA (mainly the cis-isomer) and L-cysteine, namely 3-[[carboxymethyl]thio]-3-[1H-imidazol-4-yl] propanoic acid, in normal human urine (30) as its urinary excretion was very small (0.04 to 0.07 mM), less than 1% of the maximum urinary cis-UCA excretion found in our investigation. The role of normal and increased systemic cis-UCA levels following UV exposure in relation to immunosuppression remains to be established. Several questions arise that are difficult to answer at this moment; do basic levels of cis-UCA exert immunosuppressive effects or is the immunosuppression only restricted to UV-exposed persons whose cis-UCA levels crossed a certain threshold? If there is no threshold level, do people live with a certain degree of immunosuppression? Alternatively, it may be hypothesized that a cis-UCA derivative mediates the UV-induced immunosuppression.

References.


