Urocanic acid in photodermatology

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PART I
BIOKINETICS OF THE UROCANIC ACID ISOMERS

CHAPTER 2

Photoisomerization spectrum of urocanic acid in human skin and in vitro: effects of simulated solar and artificial ultraviolet radiation

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Abstract

Ultraviolet (UV) irradiation of trans-urocanic acid (trans-UCA), a major UV-absorbing component of the epidermis, leads to the formation of cis-UCA, which mediates immunosuppressive effects. In this study, the net yield of cis-UCA was measured after the photoisomerization of urocanic acid by narrow UV-wavebands (spectral range 295-405 nm), with the irradiation doses related to solar irradiance at sea level. The formation of cis-UCA in Caucasian skin (in vivo), as well as in aqueous solution (in vitro) was determined by HPLC analysis. The same irradiation conditions were met in both components of the study. The in vivo experiments showed high efficiency of cis-UCA formation in the spectral region of 305-341 nm, whereas high efficiency in vitro was found at 305 and 326 nm. Remarkably, at 350 and 363 nm cis-UCA was only formed in vivo, but not in vitro. At longer test wavelengths up to 405 nm, no significant formation of cis-UCA was detectable. The established partition between UV-B and UV-A at 320 nm is not relevant for the isomerization pattern of UCA. Additional studies revealed a substantial cis-UCA formation in the human skin by UV-A phototherapy lamps. Furthermore, raised levels of 295 nm irradiation dos-
es, a possible effect of stratospheric ozone reduction, were found to increase the *cis*-UCA yield. Our results demonstrate that the formation of *cis*-UCA in the skin with common exposures takes place over a broad spectral range of UV-B and UV-A up to at least 363 nm. These findings emphasize the potency of UV-A to isomerize UCA and they may contribute to further elucidation of the effects of phototherapy and sunbathing.

1. Introduction

The skin is exposed to solar-and artificial UV-radiation on innumerable occasions. At the earth's surface solar UV-radiation intensities increase from approximately 290 nm towards longer wavelengths, reaching a maximum in the visible range. Stratospheric ozone filters out the radiation below 290 nm (UV-C). This property may be impaired by the gradual breakdown of the ozone layer, resulting in an increased intensity of UV-B and UV-C radiation reaching the earth's surface. This environmental change in solar radiation is expected to cause a larger immunosuppressive effect (1) in the skin or elsewhere in the body.

Epidermal layers are rich in UCA which is formed by deamination of histidine by histidase. UCA is initially formed in the *trans*-form, which can be photoisomerized to *cis*-UCA by UV-radiation (2,3). Continued UV-exposures could lead to the photo stationary state with approximately 70% *cis*-UCA and 30% *trans*-UCA. Evidence has been provided supporting the view that the UV-induced immunosuppressive effect is mediated by the formation of *cis*-UCA in the epidermis (4,5). Exogenous *cis*-UCA was shown to act suppressively in certain immunological test models, such as allograft rejection (6), delayed type hypersensitivity (7,8), and antigen presenting functions (9). In their pioneer work, De Fabo and Noonan (10) constructed an action spectrum from 250-320 nm of the UV-induced suppression of contact hypersensitivity in mouse skin. The lowest irradiation dose required to produce 50% suppression was at 260-270 nm, which also corresponds to the absorption maximum of UCA. Towards longer wavelengths (UV-B, UV-A) higher irradiation levels were required to produce 50% suppression, although UV-radiation up to at least 340 nm caused isomerization of UCA (11).

Solar exposures of the skin comprise a much larger flux of the longer UV-A wavelengths than those of UV-B. In this respect, the contribution of longer wavelengths to *cis*-UCA formation has not been clearly defined. This lack of information prompted us to determine the spectrum of *cis*-UCA formation that would occur in commonly encountered solar UV-exposures in the range of 0.25 to 1 minimum erythema dose(s)(MED). The net yield of *cis*-UCA was measured for each test wavelength, in the human skin, and in
Photoisomerization spectrum of UCA solutions \textit{in vitro}. The results provide a perspective of the distribution of cis-UCA yield across the UV-B and UV-A spectrum. In addition, we studied the yield of cis-UCA after an increase of 295 nm irradiation, as a simulation of the effect of stratospheric ozone reduction. We also examined the yield of cis-UCA after exposures to a UV-A lamp, from which the UV-B emission was blocked or transmitted by appropriate cut-off filters. Such lamps are widely used in phototherapy and for tanning purposes. Our results may have practical implications for the interpretation of the effects of solar or artificial UV-exposures in relation to photoimmunosuppression.

2. Methods

2.1 Urocanic acid

\textit{Trans-3-[4-Imidazolyl]-acrylic acid} (\textit{trans-UCA}), was supplied by Aldrich Chemie (Steinheim, Germany). This compound exhibited a single peak after injection in our liquid chromatograph. Melting point determination was in accordance with supplier’s data (226-228 °C). Chromatographically purified cis-UCA was kindly donated by dr. M. Norval, University of Edinburgh, Scotland.

2.2 High performance liquid chromatography (HPLC)-analysis of trans- and cis-urocanic acid

Both isomers could be determined separately in one run at ambient temperature with an isocratic HPLC-system, equipped with a 25 x 0.4 cm C18 reversed phase column and a UV-detector set at 280 nm (both: Pharmacia, Uppsala, Sweden). Peak areas were assessed by a Spectra Physics integrator model SP 4010. The detector response ratio of equimolar amounts of \textit{trans-UCA} to \textit{cis-UCA} was 1.52. The eluent was a phosphate buffer (50 mM, pH 3.6) with 4% methanol and sodium octanesulphonate (1.0 mM). The \textit{trans-} and \textit{cis-}isomers were eluted after 17 and 23 minutes respectively with a flow rate of 1.0 ml/min. The detection limits of \textit{trans-} and \textit{cis-UCA} were 18 and 29 pmol, respectively.

2.3 UV-irradiation conditions

The narrow band irradiations were performed with a 1000 Watt xenon arc lamp (Oriel, CT, USA). The set-up is a modification of the solar simulator design (12) (Fig. 1). In order to minimize infra-red (heat) radiation, the exit beam was passed through a water filter of 7 cm pathway and the short-wave part (< 500 nm) of the beam was reflected by a dichroic mirror. The UV-emission spectrum was a smooth continuum, from which the desired narrow band was selected by an interference filter (Oriel, CT,
Fig. 1. The equipment for narrow band irradiations. After passage through a 7 cm waterfilter, the xenon arc beam hit a dichroic mirror (a), from which a large part of the UV-spectrum was reflected. Then, the beam passed a narrow band interference filter (b) and a long pass glass filter (c). A positive quartz lens (d) focussed the radiation beam into the entrance aperture of the liquid light guide for transfer to detector or skin target.

USA) and a glass filter (Schott-Jena, Mainz, Germany). Nine narrow wavebands were selected from 295 nm to 405 nm. The half bandwidth of the filter combinations was 5-8 nm. The use of narrow band filters in combination with long pass glass filters provided blocking (transmittance < 10⁻³), 11-15 nm from the transmission maximum at short-wave side. This spectral property is particularly important for the determination of the ultimate active wavelength of photoisomerization. The blocking at the long-wave side was provided by the narrow band filter itself, at a distance of 17 to 23 nm from the transmission maximum. Transmission spectra of the narrow band filters, long pass glass filters, and their combinations, were recorded on a Perkin Elmer 550-S UV/VIS spectrophotometer.

UV-radiation output was measured by a silicon probe of the EG&G (Salem, Mass., USA) type 550-1 radiometer, fitted with a neutral density filter. The probe sensitivity for 5 nm wavelength intervals was calibrated by the manufacturer using standards traceable to the National Bureau of Standards. The readings were corrected for the probe sensitivity and for the attenuation of the neutral density filter, in order to obtain an output in terms of W/m². The irradiation dose of 305 nm was adjusted to 716 J/m².
Table 1. Irradiation data based on solar dosimetry.

<table>
<thead>
<tr>
<th>Center wavelength (nm)</th>
<th>Relative irradiation doses series (MED)</th>
</tr>
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<tbody>
<tr>
<td>295</td>
<td>0.009</td>
</tr>
<tr>
<td>305</td>
<td>1</td>
</tr>
<tr>
<td>326</td>
<td>6.56</td>
</tr>
<tr>
<td>341</td>
<td>8.47</td>
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<td>350</td>
<td>9.12</td>
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<tr>
<td>379</td>
<td>11.9</td>
</tr>
<tr>
<td>391</td>
<td>13.0</td>
</tr>
<tr>
<td>405</td>
<td>13.9</td>
</tr>
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* The doses were related to the one of 305 nm with a value of 716 J/m².

which corresponded to 1-2 MED under our conditions. This wave band has virtually the shape of the erythemal action spectrum, adapted to solar flux [13]. This radiation should cause the same degree of erythema as that produced by the complete spectrum of solar UV radiation.

The irradiation doses at other narrow UV-bands were related to the dose of 305 nm by a factor derived from the solar irradiance at sea level [Table 1] and did not appear to be eryhemogenic. A clear sky, and the sun at 0° zenith angle were selected as the reference conditions [14]. In additional series the irradiation doses were reduced to a half and a quarter to study dose-response behaviour. The series were marked as 0.5 MED and 0.25 MED [Table 1]. The range of increased 295 nm UV-doses has been derived from literature [15,16] and the maximum dose included is comparable with approximately 16% stratospheric ozone reduction. Six TL-10R UV-A lamps (Philips, Eindhoven, The Netherlands), commonly used for phototherapy and tanning, were used in this study, after filtering their emission with WG 295 and WG 335 cut-off filters of 3 mm thickness. The latter showed 1% and 0.1% transmission at 316 and 311 nm, respectively. The WG 295 filter did not block the UV-B emission from the lamp. The manufacturer stated that 0.1% of the total emission is UV-B radiation. UV-A levels were monitored with an IL442A radiometer of International Light Newburyport, MA, USA) fitted with a cosine receiving probe.
2.4 Irradiation of human skin and sampling of UCA

Narrow band irradiations were performed with a liquid light guide (Fig. 1; Oriel, Stratford, CT, USA) to ensure fixation of the irradiation spot on the skin during the exposure time. This spot was 7 cm from the exit aperture of the light guide. In this way a circular irradiation spot of 2.54 cm² was formed. Three volunteers (white Caucasians) were used for each test wavelength and irradiation dose. Irradiations and analyses were performed on duplicate samples derived from both upper arms. The skin had not been exposed to a UV-source for at least two weeks prior to the experiment.

The non-invasive sampling method used was a modification of that of Jansen (17), called chamber sampling. It is based on alkaline extraction of the epidermis. A filter paper of a patch tester (Silverpatch™, van der Bend, Holland) was moistened with 20 μl of 0.1 M potassium hydroxide and attached on the skin test area by elastic adhesive tape. After one hour, the patch testers were removed and the filter papers soaked in 470 μl 0.1 M potassium hydroxide. They were vigorously stirred on a Vortex mixer for one minute and acidified with 10 ml phosphoric acid 87%; followed by stirring for one minute. The epidermal extract was passed through 0.22 μm membrane filter prior to injecting 40-100 μl into the HPLC system. Negative control samples were taken from unirradiated skin in close proximity of the irradiation spots.

2.5. Irradiation of UCA-solutions

Trans-UCA was dissolved in ultrapure water at concentrations of 2, 10 and 50 μM. One and a half ml of these solutions was transferred to quartz cuvettes prior to irradiation at room temperature. The cuvette, with an optical path length of 1 cm, was positioned in the filtered xenon arc beam in front of the light monitoring silicon probe. The UCA-solutions were either magnetically stirred or not, and were uniformly irradiated with the same doses at the same wavelengths as those employed in the in vivo experiments. The cuvette of the non-stirred UCA-solution was then turned 180°, and a similar irradiation dose was given to the other side. The quartz window of the cuvette transmitted 93% of the incident UV-radiation.

The applied UV-doses were corrected for this value. Each point on the graphs shown in Fig. 5-7 is an average of four analyzed samples. Negative control values were obtained in a similar way, except that the light path was blocked by a black metal plate of the same dimensions as the optical filters. Photoisomerization was also studied at elevated temperatures (40-45 °C) and in two buffer media with different acidity. One buffer consisted of 0.2 M acetate/acetic acid pH 4.0 and the other one of 0.2 M phosphate pH 5.8.
2.6 Calculations of the net yield of cis-UCA

The relative amounts of both UCA isomers were derived from the peak areas of the HPLC chromatograms. Corrections were made for detector response (trans/cis = 1.52) at 280 nm. In the figures the outcome is marked as % cis-UCA (corrected). This value was calculated according the following formula, and it represents the relative net yield of cis-UCA formed upon irradiation: in which:

\[
\frac{\% \text{ cis irradiated} - \% \text{ cis control}}{\% \text{ trans control}} \times 100 = \% \text{ cis-UCA (corrected)}
\]

- \% cis_{irradiated} = relative amount of cis-UCA found in irradiated skin,
- \% cis_{control} = relative amount of cis-UCA found in control (non-irradiated) skin,
- \% trans_{control} = relative amount of trans-UCA found in control (non-irradiated) skin.

The relative amount of trans-UCA in control skin was considered to be the amount available for photoisomerization.

3. Results

3.1. In vivo experiments

HPLC-analyses of extracts from irradiated human skin showed that net formation of cis-UCA was detectable in the wavelength range 295-363 nm (Fig. 2 and 2a) after single exposures of the 1 and 0.5 MED series (Table 1). High ratios were found at 305, 326 and 341 nm, demonstrating a broad spectral range of cis-UCA formation (Fig. 2). However, if irradiation doses were reduced four times (0.25 MED), significant net formation of cis-UCA occurred in the range 305-350 nm. With the reduction of the maximum irradiation dose at 305 and 326 nm, the photostationary state in the isomerization behaviour became apparent, as cis-UCA yield showed a reduction less than proportional. Cis-UCA was not significantly formed at 379 nm, nor at longer wavelengths of 391 and 405 nm (data not shown). Each error bar shows the standard deviation of a set of six data derived from the left and right upper arms of the three volunteers. Duplicate samples of non-irradiated skin showed a mean value of 1.4% cis-UCA (S.D. ± 0.4%). The total quantity of UCA, in absolute terms, extracted from the epidermis was 23.3 nmol/cm² (S.D. 6.6 nmol/cm²; n = 18). This value is close to previously reported levels (17,20).
Fig. 2. The relative net yield of cis-UCA in human skin in three series of irradiation doses. The correction on the percentage of cis-UCA has been explained in the Materials & Methods section. The curve of solar irradiation doses (dashed line) has been included in Fig. 2, 5 and 6 for reference purposes and it is congruent with solar irradiance at sea level. Upper line: irradiations normalized to 1 MED; center line: irradiations normalized to 0.5 MED; lower line: irradiations normalized to 0.25 MED.

Fig. 2a. Significant cis-UCA formation of the 1 and 0.5 MED series at 363 nm.
Fig. 3. The relative net yield of cis-UCA in the human skin after UV-A doses from Philips phototherapy/tanning lamps type TL-10R. Upper line: the relative net yield of cis-UCA after filtering by WG 295. Lower line: the relative net yield of cis-UCA after filtering by WG 335.

These in vivo findings revealed the potency of UV-A radiation to cause cis-UCA formation. Therefore, we studied the effect of a UV-A lamp on UCA isomerization. Fig. 3 shows the relative net yields of cis-UCA by two series of graded irradiation doses from UV-A tanning/phototherapy lamps. The applied doses did not exceed commonly employed tanning regimens, and did not evoke any perceptible tan or erythema on the skins of the volunteers. The UV-A radiation was filtered by a WG 295 cut-off filter, which permitted transmission of UV-B radiation emitted from the UV-A lamp. A WG 335 cut-off filter was used to block UV-B and a part of the UV-A-II (320-340 nm) radiation. This model revealed (Fig. 3) that roughly one-third of the cis-UCA yield was due to pure UV-A radiation, whereas the majority of the cis-UCA yield was caused by the short waves of the UV-A lamp, including UV-A-II and traces of UV-B radiation.

Another experiment was set up to study the effect of ozone layer reduction on cis-UCA formation. Solar UV-B radiation at sea level is largely influenced by the amount of ozone in the earth’s atmosphere, and the most pronounced effects of ozone reduction are related to the shortest UV-B wave
Fig. 4. Increased net yield of cis-UCA in the human skin by elevated 295 nm UV-doses. The error bars represent the difference between duplicate measurements.

lengths reaching terrestrial level. Therefore, our shortest test wavelength (295 nm) was chosen to simulate the effect of stratospheric ozone reduction. Increased cis-UCA ratios associated with the elevated 295 nm UV-doses are shown in Fig. 4. An eightfold increase of the 295 nm UV-dose, equivalent to approximately 16% stratospheric ozone reduction [15,16], caused the formation of 19.4% cis-UCA, which is considerably higher than with the original dose of 6.4 J/m$^2$ [no ozone reduction]. The data showed an almost linear relationship increasing by 0.4% cis-UCA per J/m$^2$.

3.2 In vitro experiments

UCA-solutions of two, ten and fifty micromol per liter were irradiated using the same conditions at every test wavelength as the in vivo component of this study. The dose-response appeared to be linear at every test wavelength from 295 to 341 nm, without a tendency to the photostationary state. The relative net yields of cis-UCA appeared to be virtually independent of the UCA concentration, when the solutions were stirred (Fig. 5a,b). However, in another experiment without stirring the amount of the cis-UCA yield was dependent on the initial trans-UCA concentration. The cuvette was irradiated on both sides with the same dose which
Fig. 5a. The relative net yield of cis-UCA in vitro in three sets of irradiation doses based on solar irradiance. Initial trans-UCA concentration is 50 μM. This solution was stirred during exposure. Upper solid line: irradiations normalized to 1 MED; center solid line: to 0.5 MED; lower solid line: to 0.25 MED. At some data-points the error bars were not larger than the symbols.

Fig 5b. The same as in (a), except the initial trans-UCA concentration is 2 μM.
was used throughout this study, so the number of photons entering the trans-UCA solution was twice that in the stirred solutions. The 50 μM UCA solution showed a similar ratio of cis-UCA yield to that shown by the stirred solution (compare Fig. 5a with Fig. 6), but the 2 μM UCA solution showed an almost twice greater ratio of cis-UCA yield (compare Fig. 5b with Fig. 6). This finding indicated the virtual absence of any shielding effect of UCA molecules against incoming photons at this low concentration.

In all in vitro experiments, a maximum net yield of cis-UCA occurred at 305 nm (Fig. 5a,b and 6), whereas the in vivo curves (Fig. 2) showed their maximum at 326 nm. Although the irradiation dose at this wavelength was increased more than sixfold (Table 1) compared with the dose at 305 nm, the net yield in vitro was substantially decreased across this interval. The longer test wavelength of 341 nm was less efficient at isomerization of trans-UCA, and no significant net yield of cis-UCA was detected at 350 nm. This finding is in marked contrast with the net yield of cis-UCA in the human skin, which was clearly appreciable. Narrow band irradiations of 350 nm up to at least 405 nm did not cause a significant net yield of cis-UCA in vitro (Fig. 5a,b and 6).

The absorbance of a trans-UCA solution of 50 μM with 1 cm path length amounted to 0.82 at 268 nm. This level is similar to that \( A = 0.71 \) of stratum corneum at an average thickness of 15 μm (18). UCA is its main UV-absorbing material and is present in a concentration of approximately 0.5% of the dry weight (19) (0.2% of wet weight; own estimation). In comparison, a UCA solution of 0.2% in a layer of 15 μm should exhibit an absorption level equal to that of 22 μM trans-UCA solution in a 1 cm cuvette, as could be predicted according to Beer's law. Thus, a 50 μM UCA solution with 1 cm path length reflects fairly well the upper limits of UCA absorption in the stratum corneum.

In order to elucidate the observed discrepancy between the in vitro and in vivo findings, in the spectral region 350-363 nm, we studied the effect of temperature and acidity on photoisomerization behaviour. An elevation of the temperature (40-45 °C) of the irradiated UCA solution (5 μM) or a change in acidity (pH 5.8 and pH 4.0) did not significantly alter the UCA photoisomerization behaviour (data not shown).

The Philips TL 10R UV-A source was also used for studying the in vitro photoisomerization. The irradiation conditions, including the use of the WG 295 and WG 335 cut-off filters, were the same as in the in vivo experiment. The trans-UCA solutions of 2 and 50 μM were stirred during the UV-A irradiations and the cis-UCA ratio appeared to be concentration independent, as was shown above in the results of the narrow band irradiations.
Fig. 6. In vitro photoisomerization at different UCA concentrations without stirring during irradiation. The one MED series of irradiation doses (Table 1), was applied. Both sides of the cuvette were exposed. Upper line: 2 μM. Center line: 10 μM. Lower line: 50 μM.

Fig. 7. The relative net yield of cis-UCA in vitro after UV-A doses from Philips phototherapy/tanning lamps type TL-10R. Each line has been made up of the data of two stirred and irradiated UCA solutions of 2 and 50 μM. Upper line: after filtering by WG 295. Lower line: after filtering by WG 335. No significant cis-UCA was formed at the data point marked with *.

PART I: Biokinetics of the UCA isomers
Chapter 2

The relative net yield of cis-UCA \textit{in vitro} was lower than \textit{in vivo} under the same irradiation conditions. Each line of the graph in Fig. 7 represents averaged data from the 2 and 50 $\mu$M solutions. An even greater effect (Fig. 7) of the WG 335 cut-off filter was found than in the \textit{in vivo} situation (Fig. 3). Roughly one-sixth of the cis-UCA net yield \textit{in vitro} was due to the UV-A radiation transmitted by the WG 335 filter.

4. Discussion

It is now well established that cis-UCA is an important mediator of UV-induced immunosuppression [1,4,5,10]. Our findings regarding cis-UCA formation indicate the possibility of a broad spectrum of immunosuppression, from 295 nm up to at least 350 nm, by daily sun exposures. Exposures commonly experienced from the sun comprise wavelengths from 290 nm to the infra-red waves (over 2400 nm). Pronounced biological effects of solar exposures can be observed when erythema formation occurs. In view of this, we chose a dose of narrow band irradiation at 305 nm, which caused 1-2 MED. The other narrow band irradiation doses did not cause erythema formation in our dosimetric setting.

There is a discrepancy between the \textit{in vitro} and \textit{in vivo} behaviour at the long-wave end of the isomerization spectrum. One explanation may be the difference in temperature between \textit{in vivo} and \textit{in vitro} photoisomerization; another may be the acidity of the skin's surface. These possibilities were excluded, however, as no differences were observed in additional experiments. Another possibility could be the absorption of UV-B radiation in the skin by superficially located biomolecules. This phenomenon should render UV-B radiation less active in photoisomerization of UCA in deeper located epidermal layers in relative favour of UV-A radiation effects. However, there is evidence that UCA occurs predominantly in the superficial stratum corneum, because removal of this layer by tape stripping results in loss of immunosuppression [4,10]. Further elucidation of this unsolved phenomenon might be derived from the UV-A exposures with the WG 295 and WG 335 cut-off filters [Fig. 3,7]. The results of the \textit{in vitro} study show a lower relative net yield of cis-UCA than those obtained \textit{in vivo}. The difference in cis-UCA yield between the two cut-off filters \textit{in vivo} was roughly by a factor of 3 (Fig. 3), whereas the difference \textit{in vitro} (Fig. 7) was by a factor of 6. This indicates a weak \textit{in vitro} effect of radiation distributed around 340 nm. The short-wave end of solar UV-A spectrum (mainly UV-A-II, 320 -340 nm) contributed strongly to photoisomerization. This observation is in accordance with the results of Schwarz et al. [20], who found elevated skin levels of cis-UCA after UVASUN 3000 exposure. Thus, common exposures to UV-A radiation, such as during sunbathing and UV-
Photoisomerization spectrum of UCA

A phototherapy, may cause immunosuppression via substantial cis-UCA formation. Application to the skin of sunscreens containing only UV-B filters (18), may not prevent immunosuppression via cis-UCA formation, because solar or artificial UV-A radiation is not effectively absorbed. The UCA solutions under study were stirred during irradiation and a concentration independent photoisomerization behaviour was demonstrated. However, the circumstances in which UCA exists in the skin should be associated with 'non-stirring' conditions. The incident photons encounter a gradient concentration of trans-UCA. Non-stirred, in vitro photoisomerization conditions revealed a substantial effect on the relative net yield of cis-UCA with UCA solutions of 2 and 50 μM. This factor should be taken into account, when transposing in vitro results to photochemical skin events.

Recently, Gibbs et al. (11) published action spectra about the unweighed photo-isomerization of UCA, showing that maximal effectiveness of isomerization was found in the spectral range 300-315 nm in mouse skin as well as in vitro. However, we found that the range 305-341 nm was maximally active in human skin, with a dosimetry based on solar irradiance. This extension to the long-wave end of the spectrum is probably due to the relatively high levels of UV-A radiation, inherent in solar exposure. The 340nm (11) and 341 nm test wavelengths were found to be active in photoisomerization in both studies, but the application of the ultimate longer wavelengths of photoisomerization were demonstrated in the present study. The spectral limits of photoisomerization should be considered together with the applied irradiation dose. In the present study, the limits were related to common daily solar exposure, and excessive exposure was avoided.

We conclude that the cis-UCA formation by photoisomerization extends well into the UV-A range under daily solar irradiation circumstances. Our experiments show that appreciable cis-UCA formation can easily be generated in the skin after a single UV-A exposure. Furthermore, an increment of UV-B radiation, which might be caused by stratospheric ozone reduction, caused an elevated net yield of cis-UCA. The claim that every induction of cis-UCA formation causes increased immunosuppression is a topic under current investigation.

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References


CHAPTER 3

PART I: Biokinetics of the UCA isomers


