Fluorescent light activates the immunomodulator cis-urocanic acid in vitro: implications for patients with systemic lupus erythematosus

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Abstract

Objectives—Erythemagenic (295–305 nm) ultraviolet-B (UVB) radiation is toxic to patients with systemic lupus erythematosus (SLE). Cool white fluorescent lamp emissions produce a similar toxicity even though the UVB radiation emitted is primarily at the relatively non-erythemagenic wavelength of 313 nm. The purpose of this study was to determine if fluorescent light, presumably acting predominantly along the 313 nm wavelength, exhibits photochemical activity sufficient to account for toxicity.

Methods—The photochemical activity of fluorescent light was assessed by testing its capacity to activate urocanic acid, a plentiful and potent epidermal immunomodulatory mediator normally activated by polychromatic UVB radiation but activated maximally at 313 nm. Irradiation-induced isomerisation of trans-urocanic to cis-urocanic acid was quantitated by UV spectroscopy after separation of the isomers by high performance liquid chromatography.

Results—Fluorescent light irradiation of solutions containing the photoreceptor trans-urocanic acid produced a cumulative conversion of trans- to cis-urocanic acid. This photochemical activity was compared with that of erythemagenic sunlamps, high in polychromatic UVB emissions. When normalised for UVB irradiance, the accumulation of cis-urocanic acid produced by both light sources was essentially equivalent. Conventional acrylic diffusers that absorb UVB emissions eliminated the fluorescent light-induced reaction.

Conclusion—The results indicate that radiation from fluorescent lamps possesses substantial photoimmunological capability, sufficient to activate a potent, potentially dangerous, disease-modifying, immunomodulatory pathway and that poorly erythemagenic, primarily monochromatic UVB photons are responsible.

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Cool white fluorescent lamp emissions produce in patients with systemic lupus erythematosus (SLE) a toxicity similar to that triggered by erythemagenic (295–305 nm) ultraviolet-B (UVB; 280–320 nm) wavelengths, even though the UVB emitted by fluorescent lamps is primarily at the relatively non-erythemagenic wavelength of 313 nm. The purpose of this study was to determine if fluorescent light, presumably acting predominantly along the 313 nm wavelength, exhibits photochemical activity sufficient to generate toxicity. We assessed the photochemical activity of fluorescent light by testing its capacity to activate urocanic acid, a plentiful and potent epidermal immunomodulatory mediator, normally activated by polychromatic UVB radiation but activated maximally at 313 nm. Although used here for testing photochemical potential, activation of the urocanic acid pathway in vivo would itself have ample potential for affecting SLE, a disease of immune dysregulation.

The trans isomer of urocanic acid is a naturally occurring photoreceptor in epidermis and comprises up to 1% of human epidermis dry weight. Interaction of trans-urocanic acid with UVB and ultraviolet-A2 (320–340 nm) photons results in its isomerisation in vivo to cis-urocanic acid:

\[\text{HN} \quad \text{N} \quad \text{HN} \quad \text{N} \quad \text{CO}_2\text{H} \quad \text{hv} \quad \text{HN} \quad \text{N} \quad \text{N} \quad \text{CO}_2\text{H}\]

cis-urocanic acid

cis-urocanic acid is a physiological immunomodulator that mimics in vivo many of the immunosuppressive activities of UVB, downregulating local and systemic cell-mediated immune responses to antigens.

UVB radiation, whether delivered at high intensity from polychromatic sunlamps or at low intensity from cool white fluorescent lamps, produced an equivalent conversion of trans- to cis urocanic acid in vitro. Commercial acrylic diffusers, used widely for primarily aesthetic purposes, were found to virtually eliminate the isomerisation of urocanic acid by fluorescent lamps.

Methods

PHOTOMETRY

UVB radiation was measured with an SEE 240 UVB probe and a wide-eyed diffuser (International Light, Newburyport, MA) con-
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sisting of an SCS sharp-cut UVB-1 filter (response from 245 to 320 nm and 10% points at 240 and 320 nm) atop an SEE 240 solar blind vacuum photodiode. Ultraviolet A (UVA; 320–400 nm) radiation was measured with an SEE 015 photodetector and a wide-eyed quartz diffuser fitted with a UVA filter (320–400 nm; 360 nm peak response). Visible light was determined by a Gossen Luna Pro lightmeter (Calumet, Bensenville, IL).

LIGHT SOURCES
The cool white fluorescent light source was a panel of four F40 lamps (American Philips Lighting, Highstown, NJ). At a distance of 12 cm, this source delivered 4·1 μW/cm² of UVB radiation and 30 μW/cm² of UVA radiation. The calculated UVB dose was 0·12 J/cm² at 8 hours, 0·35 J/cm² at 24 hours, and 0·71 J/cm² at 48 hours. [Note: The minimal erythematous dose of UVB irradiance, calculated from wavelengths in the peak erythemal range of 295–305 nm, is 0·05 J/cm² for lightly to moderately pigmented white, or type II, skin.] High-intensity UVB radiation was provided by a panel of seven FS20 sunlamps (White-Westinghouse, Dublin, OH). At a distance of 25 cm this source delivered 430 μW/cm² of UVB and 380 μW/cm² of UVA radiation. The calculated UVB dose was 0·026 J/cm² at one minute, 0·26 J/cm² at 10 minutes and 0·77 J/cm² at 30 minutes.

UROCANIC ACID SOLUTIONS
A stock solution of trans-urocanic acid (Sigma Chemical Co; 5 mg/ml in dimethylsulfoxide) was made at the beginning of every experiment. Mixture of cis- and trans-urocanic acid were prepared by irradiating 5 ml of trans-urocanic acid in a 60 mm diameter dish (Falcon 3002, Becton Dickinson, Lincoln Park, NJ) with the sunlamps at a distance of 25 cm. Irradiation for 15 and 90 minutes resulted in mixtures containing 22% and 61% cis-urocanic acid, respectively.

QUANTITATION OF UROCANIC ACID ISOMERS
The urocanic acid isomers were separated by high performance liquid chromatography. Samples were applied to a C18 column (4·7 × 150 mm, Vydac TP218-5415) equilibrated in 0·1% trifluoroacetic acid and eluted with the same solution at 1 ml/minute. Absorbance at 277 nm was monitored on-line with a Gilson Holochrome detector, and integrals of the absorbance peaks were computed (Radio
tamous Flo-One/Beta software). Separation of the urocanic acid isomers is illustrated in fig 1.

Samples were diluted sufficiently to ensure that absorbances were linear with respect to concentration (verified by dilution and standard curves). The validity of OD₂₇₇ as a measure of both cis- and trans-urocanic acid is supported by the observation that (cis + trans) values were equal to the value for trans-

IRRADIATION
Samples of urocanic acid (5 mg/ml) in uncovered petri dishes containing 0%, 22% and 61% cis-urocanic acid (see above) were exposed to the panel of fluorescent lamps at a distance of 12 cm for 0, 8, 24 and 48 hours at room temperature (26°C). Samples of trans-urocanic acid (5 mg/ml) were exposed to the panel of sunlamps at a distance of 25 cm for 10, 30, 60 and 120 minutes. Controls included (1) samples of the urocanic acid mixtures placed adjacent to the lamps but kept in the dark and (2) irradiated and non-irradiated dimethyl sulfoxide (no urocanic acid).

In one series of experiments, the fluorescent lamps were covered with a fine-mesh acrylic diffuser, a coarse-mesh acrylic diffuser or a sheet of polyester to decrease UVB emissions, and samples were irradiated for 48 hours as above.

Results
ISOMERISATION OF TRANS- TO CIS- UROCANIC ACID
Irradiation of trans-urocanic acid solutions with the panel of fluorescent lamps produced a time-dependent isomerisation of trans- to cis-urocanic acid (fig 2A, solid circles). After 48 hours of exposure to the fluorescent lamps, cis-urocanic acid accounted for 34% of total urocanic acid. During this period no spontaneous isomerisation was observed (open circles), and the total amount of urocanic

Figure 1 Separation of urocanic acid isomers by reverse-phase high-performance liquid chromatography. Aliquots from a solution of trans-urocanic acid were taken before (left panel) and after (right panel) fluorescent lamp irradiation and then chromatographed, as described under Methods. Chromatograms were developed isocratically with 0·1% trifluoroacetic acid at 1 ml/minute, and the UV absorbance at 277 nm was monitored on-line. The peak retention times of the isomers differed by 1·6 minutes.

urocanic acid in non-irradiated samples (fig 1)
Figure 2  Isomerisation of trans-urocanic acid by fluorescent light. (A) trans-urocanic acid solutions were exposed to the panel of F40 fluorescent lamps at a distance of 12 cm for up to 48 hours (solid circles). Matched control samples (open circles) were kept in the dark. Levels of the urocanic acid isomers were quantitated spectrophotometrically after chromatographic separation, as shown in fig 1. Values represent the average of three independent determinations. Standard deviations were less than 15% of the mean. (B) trans-urocanic acid solutions were exposed at a distance of 25 cm from the panel of FS20 sunlamps for up to 120 minutes (solid circles). Matched control samples (open circles) were kept in the dark. Other details were as in A. (C) trans-urocanic acid solutions were pre-irradiated as described under Methods to produce samples having different initial cis:trans ratios (open circles, 81%; solid circles; 22%) and then irradiated with fluorescent lamps as in A. (D) values of cis-urocanic acid production from A (open circles; fluorescent lamp irradiation) and B (solid circles; sunlamp irradiation) have been replotted as a function of the calculated UVB irradiance of the exposure.

Effects of filters on the isomerisation of trans- to cis-urocanic acid produced by 48 hours of fluorescent light irradiation.

<table>
<thead>
<tr>
<th>Filter</th>
<th>UV Light transmitted</th>
<th>Isomerisation of cis-urocanic acid ( percentage of total)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>UVB J/cm²</td>
<td>%</td>
</tr>
<tr>
<td>None</td>
<td>0-71</td>
<td>100</td>
</tr>
<tr>
<td>Acrylic diffuser (fine)</td>
<td>0-04</td>
<td>5</td>
</tr>
<tr>
<td>Acrylic diffuser (coarse)</td>
<td>0-04</td>
<td>5</td>
</tr>
<tr>
<td>Polyester sheet</td>
<td>0-06</td>
<td>8</td>
</tr>
</tbody>
</table>

fluorescent lamp irradiation is well within the range of values (15–40%) reported for normal human epidermis in vivo. An additional 48 hours of fluorescent light increased cis-urocanic acid in previously irradiated samples from 22% to 46%, demonstrating that normal in vivo levels could be exceeded, and from 61% to 65%, indicating that the same apparent steady-state cis to trans ratios were reached by fluorescent lamp and high-intensity UVB irradiation (fig 2C).

UVB-DEPENDENCE OF TRANS- TO CIS- UROCANIC ACID ISOMERISATION

Despite a difference of over two orders of magnitude in the intensities of UVB emissions from the fluorescent lamps versus sunlamps, a UVB-dependent mechanism appeared to underlie the isomerisation of trans- to cis- urocanic acid produced by either light source. As illustrated in fig 2D, trans- to cis-urocanic acid isomerisation was essentially identical when expressed as a function of UVB irradiance, irrespective of the light source. Moreover, filters that decreased UVB fluences by >90% decreased isomerisation of trans- to cis-urocanic acid almost equivalently (table). A decrease in the ultraviolet-A2 wavelengths may have contributed marginally to the decrease in isomerisation since wavelengths up to 330 nm are absorbed by urocanic acid.5

Discussion

While sunlight, which contains all the UVB wavelengths, is obviously capable of activating the urocanic acid pathway, the present results indicate that prolonged exposure to the discontinuous spectrum of emissions from fluorescent lamps does so as well. Cool white fluorescent lamp emissions produced a cumulative trans/cis isomerisation of urocanic acid in vitro, resulting in cis to trans ratios exceeding the normal in vivo range and, when expressed as a function of UVB irradiance, quantitatively equivalent to that produced by the high-intensity polychromatic spectrum of UVB wavelengths emitted by sunlamps. Standard commercial filters that absorbed UVB blocked the isomerisation, indicating that UVB wavelengths were responsible.

The capacity of fluorescent light, which is essentially non-erythemagenic, to isomerise urocanic acid results from the unique spectrum of emissions of white fluorescent lamps and from the action spectrum of cis-urocanic acid production. Approximately 90% of the UVB emissions from fluorescent lamps come from the mercury line at 313 nm, while 10% comes from the line at 302 nm.8 Whereas the action spectrum for trans to cis isomerisation of urocanic acid is maximal at 313 nm,9 that for erythemenagiecy, DNA absorption, (qualities previously considered critical to toxicity in SLE),10 and tanning, peaks at 300 nm which represents only a minor portion of the fluorescent lamp emission.11 The present results not only indicate that fluorescent light has telling photochemical activity, but point to
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a pathway along which this light source may be eliciting toxicity in SLE and suggest that UVB toxicity need not be intrinsically linked with the propensity of UVB photons to induce erythema or be absorbed by DNA. A dissociation between erythema and other UV-mediated dermal events is supported by the observation that sunscreens, although protective against the erythematic and carcinogenic effects of UVB, fail to retard the suppression of contact hypersensitivity.

As to a possible role for cis urocanic acid itself in fluorescent light-induced toxicity, it would seem that suppression by cis urocanic acid of cell mediated immunity may well be harmful to patients with SLE in whom cell mediated immunity is already impaired.11 Moreover, recent studies disclose that cis-urocanic acid mediates this suppression by increasing levels of tumour necrosis factor-α,12 a cytokine shown to produce tissue damage in animals and humans with SLE.13 Also of possible harm is the potent suppression of natural killer cells in vivo by ultraviolet wavelengths of 313–315 nm, an action that may be mediated by urocanic acid.14 Natural killer cells downregulate immunoglobulin secretion and are already functionally impaired in SLE.15

The potential relevance of the present in vitro findings to in vivo events is underscored by the fact that isomerisation of trans- to cis-urocanic acid in vivo actually requires less photon energy than isomerisation in vitro.7 For example, 600 mJ of UVB radiation was required from either light source to increase the content of cis-urocanic acid from 0% to 30%, and even greater amounts were required to increase the amount from 30% to 60%. By contrast, 32 mJ of UVB elevated cis-urocanic acid levels to 60% in human skin and 96 mJ of UV increased dermal levels in mice from 4% to 31%.6 Thus the levels of fluorescent lamp UVB emissions shown to be capable of isomerising urocanic acid in the present in vitro studies are predicted to be at least as, if not more, effective in activating urocanic acid in vivo.

In summary, non-ionising radiation from fluorescent lamps possesses substantial photo-immunological activity, sufficient to activate a potent in vivo immunomodulatory pathway, potentially dangerous to patients with SLE.

Essentially monochromatic poorly erythematic 313 nm UVB photons appear to be responsible. The findings offer some basis for the reported noxiousness of fluorescent light in SLE.1 The failure of fluorescent lamp emissions to elicit the erythema that typically signals toxicity in SLE makes exposure to this light source somewhat insidious. Fortunately, acrylic diffusers, commonly used for aesthetic purposes, effectively eliminate these UVB emissions and should offer protection from whatever noxious actions these emissions and the trans/cis isomerisation of urocanic acid produce.

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