Urocanic acid in photodermatology
Kammeyer, A.

Citation for published version (APA):

General rights
It is not permitted to download or to forward/distribute the text or part of it without the consent of the author(s) and/or copyright holder(s), other than for strictly personal, individual use, unless the work is under an open content license (like Creative Commons).

Disclaimer/Complaints regulations
If you believe that digital publication of certain material infringes any of your rights or (privacy) interests, please let the Library know, stating your reasons. In case of a legitimate complaint, the Library will make the material inaccessible and/or remove it from the website. Please Ask the Library: http://uba.uva.nl/en/contact, or a letter to: Library of the University of Amsterdam, Secretariat, Singel 425, 1012 WP Amsterdam, The Netherlands. You will be contacted as soon as possible.
PART II
OXIDATION OF UROCANIC ACID BY HYDROXYL RADICALS

CHAPTER 5
Urocanic acid isomers are good hydroxyl radical scavengers: a comparative study with structural analogs and with uric acid

Arthur Kammeyer\textsuperscript{a}, Teunis A. Eggelte\textsuperscript{b}, Jan D. Bos\textsuperscript{a},
Marcel B.M. Teunissen\textsuperscript{a}

\textsuperscript{a}Department of Dermatology Academic Medical Center, P.O. Box 22660, 1100 DD Amsterdam, Netherlands
\textsuperscript{b}Department of Clinical Pharmacology Academic Medical Center, P.O. Box 22660, 1100 DD Amsterdam, Netherlands

Biochimica et Biophysica Acta 1428 (1999) 117-120

Abstract

UV-exposure of the epidermis leads to the isomerization of \textit{trans}-UCA into \textit{cis}-UCA as well as to the generation of hydroxyl radicals. It was shown by the deoxyribose degradation test that UCA isomers are more powerful hydroxyl radical scavengers than the other 4-(5-) substituted imidazole derivatives, such as histidine, though less powerful than uric acid. UCA, present in relatively high concentrations in the epidermis, may well be a major natural hydroxyl radical scavenger.

\textit{Trans}-urocanic acid (\textit{trans}-UCA) is a major ultraviolet (UV) absorbing component of the human epidermis. Absorption of UV radiation from the UV-C region (200-290 nm) into the UV-A-I region (340-400 nm) causes photoisomerization of \textit{trans}-UCA into \textit{cis}-UCA \textit{in vivo} as well as \textit{in vitro
Because of this property, trans-UCA had been used as natural sunscreen agent [4]. This use had later been minimized since it became clear that photoproducut cis-UCA can mimic some of the effects of UV on immunity, suggesting that this compound is an important mediator of UV-induced immunosuppression [5]. UV exposure of the skin causes an increased level of oxidative stress with the inherent formation of reactive hydroxyl radicals [6]. These species can be generated from hydrogen peroxide upon UV irradiation and upon contact with metal ions (e.g. Fe$^{2+}$ and Cu$^{2+}$), the Fenton reaction. Both types of reaction can occur in the epidermis [7]. Under these conditions, UCA isomers may interact with the randomly produced hydroxyl radicals in situ.

In this study we tested in vitro the hydroxyl-radical scavenging ability of both UCA isomers, of chemically related compounds, and of known scavenger uric acid. The results of this comparative study point to certain molecular structures required for good hydroxyl radical scavenging ability and provides a ranking of trans-UCA and cis-UCA among other (known) scavengers.
UCA isomers are good hydroxyl radical scavengers

Table 1. The hydroxyl radical scavenging ability of urocanic acid isomers and related compounds

<table>
<thead>
<tr>
<th>Hydroxyl radical scavenger</th>
<th>Second order rate constant x 10^9 M^{-1}.s^{-1}</th>
<th>S.D.</th>
<th>n</th>
<th>Inhibition of deoxyribose degradation [scavenger]=[deoxyribose]=3 mM</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>[deoxyribose]=3 mM</td>
<td></td>
</tr>
<tr>
<td>Imidazoles</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>trans-Urocanic acid</td>
<td>8.0</td>
<td>0.9</td>
<td>8</td>
<td>67</td>
<td></td>
</tr>
<tr>
<td>cis-Urocanic acid</td>
<td>7.1</td>
<td>0.6</td>
<td>6</td>
<td>64</td>
<td></td>
</tr>
<tr>
<td>L-Histidine</td>
<td>2.6[c]</td>
<td>0.9</td>
<td>4</td>
<td>34</td>
<td></td>
</tr>
<tr>
<td>Dihydrourocanic acid</td>
<td>2.7</td>
<td>0.9</td>
<td>3</td>
<td>34</td>
<td></td>
</tr>
<tr>
<td>Imidazole-4-acetic acid</td>
<td>2.2</td>
<td>0.1</td>
<td>3</td>
<td>30</td>
<td></td>
</tr>
<tr>
<td>Imidazole</td>
<td>13.0</td>
<td>0.9</td>
<td>5</td>
<td>78</td>
<td></td>
</tr>
<tr>
<td>2-Methylimidazole</td>
<td>11.7</td>
<td>2.6</td>
<td>5</td>
<td>76</td>
<td></td>
</tr>
<tr>
<td>Other compounds</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>L-Alanine</td>
<td>0.1</td>
<td>0.0</td>
<td>3</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>trans-2-furylacrylic acid[a]</td>
<td>&lt; 0.1</td>
<td>-</td>
<td>3</td>
<td>&lt; 2</td>
<td></td>
</tr>
<tr>
<td>Uric acid</td>
<td>27.8</td>
<td>3.0</td>
<td>4</td>
<td>91</td>
<td></td>
</tr>
</tbody>
</table>

a. trans-2-furylacrylic acid was not tested in concentrations > 8 mM because of poor solubility.
b. n represents the number of slopes from which the rate constant was calculated.
c. 2.3 - 3.0 x 10^9 M^{-1}.s^{-1} in literature [8].

Part II: Oxidation of UCA by hydroxyl radicals
concentrations. The reaction was started by the addition of premixed di-
sodium EDTA and ferrous iron solutions (final concentrations 0.5 mM
and 0.2 mM, respectively).
Ferrous ammonium sulphate served as source for ferrous ions (Fe\(^{2+}\)). Fe\(^{2+}\)
solutions were freshly prepared each time and were purged with nitrogen.
The mixture was left for 15 minutes at room temperature. After addition of
1.0 mL 1% thiobarbituric acid in 50 mM NaOH and 0.75 ml 2.8%
trichloroacetic acid, the tubes were heated for 20 minutes in a boiling
water bath. The pink color was read at 532 nm and reciprocal absorption
values were plotted against the concentration of the test compound after
subtraction of appropriate blanks.
A series of six duplicate determinations from test compound dilutions
was employed to construct a graph slope for the calculation of a rate con-
stant value. A typical graph with slopes to derive rate constants from is
shown in Fig. 2 for both UCA isomers. The mean, SD, number of rate
constants and the percentage of inhibition of deoxyribose degradation at
equimolar concentrations of scavenger (3 mM) is calculated for each test
compound and summarized in Table 1.

Trans-UCA and cis-UCA are substantially stronger in scavenging hydroxyl
radicals (8.0 and 7.1 \(\times 10^9\) M\(^{-1}\).s\(^{-1}\), respectively), than the other 4-{5-}-substitu-
ted imidazoles, including L-histidine (2.6 \(\times 10^9\) M\(^{-1}\).s\(^{-1}\)). L-histidine, the pre-
cursor of UCA, was included as a known scavenger [8-10] with structural sim-
ilarities to UCA. L-alanine was used as a known poor scavenger [10].
Trans-FAA was tested as a non-imidazole acrylic acid derivative, having a
furan ring instead. This substitution yielded a very poor scavenging ability.
Other 4-{5-} substituted imidazole analogues, dihydrourocanic acid or 3-
(imidazol-4-yl)-propionic acid and imidazole-4-acetic acid, showed mod-
erate scavenging ability, comparable to histidine. However, unsubstituted
imidazole and its 2-methyl derivative appeared to be stronger scavengers
than the UCA isomers.
The known strong hydroxyl radical scavenger uric acid [11] showed an
excellent scavenging ability (27.8 \(\times 10^9\) M\(^{-1}\).s\(^{-1}\)). To summarize, trans-UCA
and cis-UCA, two epidermal compounds, are good hydroxyl radical scav-
engers. Their scavenging ability is weaker than that of uric acid, but
larger than the other 4-(5-) substituted imidazoles, e.g. histidine.

Trans-UCA and cis-UCA occur in substantial concentrations in the epider-
mis, the latter in the UV-exposed skin. There is strong evidence for the
occurrence of hydroxyl radicals in the epidermis, especially upon UV irra-
diation [7]. Normal human skin contains approximately 200 \(\mu\)M iron [12,13],
predominantly complexed to ferritin. The release of free ferrous ions by
UV irradiation [14] and the presence of hydrogen peroxide [15,16] are pre-
UCA isomers are good hydroxyl radical scavengers

Fig. 2. A determination of the second order rate constants of trans-UCA (○) and of cis-UCA (■) with hydroxyl radicals. The rate constant was derived from the slope of the line ($k = \text{slope} \times k_{dR} \times [dR] \times A_0$), where $A_0$ is the absorbance, measured in the absence of hydroxyl radical scavenger. $k_{dR}$ was taken as $3.1 \times 10^9$ M$^{-1}$s$^{-1}$, derived from pulse radiolysis studies [8], and $[dR] = 3$ mM. The rate constants in this particular set were 8.49 and $7.33 \times 10^9$ M$^{-1}$s$^{-1}$ for trans-UCA and cis-UCA, respectively. The other scavengers were studied similarly.

requisites for the generation of hydroxyl radicals. Other reports indicate the UV-induced presence of hydroxyl radicals indirectly since their effects on epidermal constituents could be neutralized with antioxidants [17,18]. UCA is an imidazole compound and several other imidazole derivatives have already been shown to be good hydroxyl radical scavengers, e.g. histidine [8-10], histamine [19], histidine containing dipeptides [10,20], cimetidine and other histamine (H2) receptor antagonists [21]. This study reveals that several other imidazoles show similar properties (Table 1). Hydroxyl radicals can react with the imidazole ring to form imidazolone derivatives. Their formation has led to the proposal to use the imidazolones of histidine and histamine as markers for oxidative stress [9,19]. The importance of the imidazole ring in UCA molecules was also demonstrated in our experiments. The poor scavenging ability of trans-FAA, having a furan ring instead, was a remarkable contrast. Furthermore, the presence of the

Part II: Oxidation of UCA by hydroxyl radicals
acrylic acid moiety in UCA molecules conjugated with the imidazole ring may account for its increased scavenging ability towards hydroxyl radicals as compared to the other 4-(5-) substituted imidazoles. Unsubstituted imidazole and its 2-methyl derivative are stronger hydroxyl radical scavengers, accentuating that the presence of an imidazole ring is a prerequisite for sufficient hydroxyl radical scavenging ability. However, these compounds do not occur physiologically and are harmful (LD50 oral rat 220 mg/kg for imidazole and 1500 mg/kg for 2-methylimidazole).

Two explanations for the relatively high concentration of UCA in the epidermis have already been put forward: 1. for trans-UCA as natural sunscreen agent and 2. for cis-UCA as immunosuppressant. Our findings point to another physiological role for the UCA isomers. Trans-UCA and cis-UCA may be major natural hydroxyl radical scavengers, providing a new view on the antioxidant status of the skin.

We would like to thank dr. W.M.P.B. Menge (Free University, Department of Pharmacochemistry, Amsterdam, The Netherlands) for the supply of cis-UCA.

References.
UCA isomers are good hydroxyl radical scavengers


Part II: Oxidation of UCA by hydroxyl radicals