Reduced Levels of Histidine and Urocanic Acid in Suction Blister Fluids from Patients with Psoriasis

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Metabolites of histidine were determined by high performance liquid chromatography in suction blister fluids from lesions and normal appearing skin of patients with psoriasis and from healthy subjects. There was a significant decrease in the levels of histidine and urocanic acid in the samples obtained from patients with psoriasis as compared to healthy subjects. Virtually only the E-isomer of urocanic acid was detected. (Received February 10, 1986.)

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Normal human epidermis contains about 0.5% urocanic acid dry weight which is mainly localized in the stratum corneum (1, 2, 3). It has been proposed to function as a natural sunscreen in the UV-B region (2, 4) and to be a mediator of immunological changes after exposure to UV irradiation (5). Ultraviolet irradiation of the skin produces an E(trans) to Z(cis) isomerisation of urocanic acid, which is proposed to be an effective mechanism in the skin for the absorption of energy (2).

Urocanic acid is derived from histidine (Fig. 1) by the action of histidase (histidine ammonia-lyase; E.C. 4.3.1.3.) and is degraded by urocanase which is present in the liver and in bacteria. The relatively high amount of urocanic acid in the stratum corneum seems to be due to the fact that no degrading enzymes have been found in the skin (1, 6, 7, 8). Psoriatic scales have low levels of urocanic acid although histidase activity is slightly increased (9). These seemingly contradictory results prompted us to study further the disturbed histidine metabolism in psoriasis. Histidine and urocanic acid were determined in plasma, and suction blister fluid from lesions and normal appearing skin of patients with psoriasis and healthy subjects. Histamine was also assayed in some of the blister fluids.

MATERIALS AND METHODS

Patients
Suction blisters were raised on uninvolved skin of 15 men and 4 women (age 23 to 75 years) with typical psoriatic plaque lesions. Ten of them had recently been treated with UV-B irradiation and anthralin or PUVA bath whereas 10 patients had had no treatment at all the previous month. Seven patients had extensive and increasing lesions covering most areas of the body and 2 had minor lesions limited to the elbows and knees. The others had plaque lesions in a stationary phase covering 15-30% of the body. In 10 of the patients blisters were also raised on untreated lesions and in 8 patients treated with UV-B or PUVA-bath blisters were produced on cleared or nearly cleared lesions. Healthy volunteers (37 men and 11 women, age 21-56 years) served as controls.

Plasma samples
Blood from the antecubital vein, was collected into heparinized Vacutainer tubes (10 ml), centrifuged and the separated plasma was stored at -40°C until use.
Suction blisters

Suction blisters were raised on the volar side of the forearm using continuous suction (200 mmHg below atmospheric pressure) as described by Kästilä & Mustakallio (10). The blister fluid was sampled and measured with a microsyringe. The roofs were then gently removed with forceps and fixed in neutralized formalin, embedded in paraffin and stained with Pauly's reagent to reveal the histidine rich layer as described by Reaven & Cox (9).

Protein levels

The level of albumin in the blister fluid was determined by high performance size exclusion chromatography (HPSEC). Column TSK 3000 SW (600 x 7.5 mm). Eluent: KH$_2$PO$_4$ 0.1 M + NaCl 0.15 M, pH 7.1, flow rate: 1 ml min$^{-1}$, UV-detection: 280 nm, room temperature. 40 µl of blister fluid was diluted with 60 µl of the eluent buffer and then submitted to HPLC. The blister albumin (MW 66 KD) level is taken as an index of protein content.

HPLC analysis

The HPLC apparatus used consisted of a Waters 6000A pump, a Rheodyne 7125 injection valve, either a Waters M441 UV detector (urocanic acid) or a Kratos URS 051 Post-Column Reaction System fitted with a Waters M420 Fluorescence Detector (histidine) and a Metrohm E586 strip chart recorder.

Fig. 1. Biochemical pathway for histidine metabolism.

Fig. 2. Typical chromatogram of (a) blister fluid, (b) artificial mixture of E and Z urocanic acid.
Quantification of E-urocanic acid

The extraction and HPLC procedure has been previously described (11) and was used with the following minor modifications: blister fluids (10 µl) were diluted with 40 µl of distilled water and deproteinized with 100 µl of acetonitrile. After Vortex mixing for 15 s, the samples were centrifuged (2 min, 10 000 g at 4°C) and 50 µl of the resulting supernatant were injected. A typical HPLC chromogram of a blister-fluid extract is shown in Fig. 2a, an artificial mixture of E and Z urocanic acid in Fig. 2b.

Quantification of L-histidine

Blister fluids (20 µl) were diluted with 40 µl of distilled water and deproteinized with 20 µl of HClO₄ 0.4 M. Plasma samples (20 µl) were deproteinized with 80 µl of HClO₄ 0.2 M. After 15 s Vortex mixing, the samples were centrifuged (2 min, 10 000 g, 4°C) and 50 µl of the resulting supernatant were injected.

Free L-histidine levels were determined using the HPLC separation and post column detection technique described below:

Eluent: 97% of KH₂PO₄ 0.075 + octane sulfonic acid sodium salt 0.005 M adjusted at pH 3.9 with H₃PO₄; 3% acetonitrile.

Column: Du Pont Zorbax ODS - 25 cm x 4.6 mm i.d. 7 µm particle size. Flow rate: 1 ml/min.

Detection reagent: Borate buffer 12 g/l adjusted at pH 10.5 with KOH (2 N), orthophthalaldehyde (0.8 g/l), 2-mercaptoethanol (0.2 g/l). Reagent flow rate: 0.3 ml/min. Room temperature. Fluorescence detection: Excitation: 338 nm. Emission 445 nm.

Quantification of histamine

The histamine levels were determined by radioenzymatic assay (12).

Table 1. Histidine and urocanic acid in suction blister fluid from involved and normal appearing skin of patients with psoriasis and healthy subjects

<table>
<thead>
<tr>
<th></th>
<th>Number of patients</th>
<th>E-Urocanic acid, µg/ml (mean ± SE)</th>
<th>L-Histidine, µg/ml (mean ± SE)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Healthy subjects</td>
<td>41</td>
<td>11.6±0.5</td>
<td>26.2±1.1</td>
</tr>
<tr>
<td>Patients with psoriasis</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Uninvolved skin</td>
<td>19</td>
<td>1.5±0.3**</td>
<td>15.7±1.8***</td>
</tr>
<tr>
<td>Non-treated lesions</td>
<td>8</td>
<td>3.0±1.0***</td>
<td>17.8±2.0**</td>
</tr>
<tr>
<td>UV-treated lesions</td>
<td>7</td>
<td>4.9±1.6***</td>
<td>17.4±1.9**</td>
</tr>
</tbody>
</table>

** p<0.01, *** p<0.001 compared to healthy subjects
RESULTS
Both E-urocanic acid and histidine were significantly reduced in blister fluids from involved and uninvolved skin of patients with psoriasis compared to healthy controls (Fig. 3 and Table I). Only low levels of Z-(cis) urocanic acid were detected under these conditions. We were not able to detect any correlation between the severity of the disease and the histidine or urocanic acid levels, although the patient with the highest histidine and urocanic acid levels in normal appearing skin had lesions limited to the elbows. Patients who had been UV irradiated showed similar low levels of urocanic acid and histidine as the non-treated patients. Three patients who responded poorly to UV treatment did not differ in their histidine or urocanic acid levels from 7 patients receiving the same treatment but cleared rapidly.

No significant difference was seen between the histamine levels in blister fluids obtained from 8 patients with psoriasis and 8 healthy subjects (mean ± SE 3.7±3.0 and 6.0±8.7 ng/ml). In blister fluid no difference was seen in the albumin level between 16 patients with psoriasis and 6 healthy subjects (mean ± SE 15.2±3.3 mg/ml and 17.0±4.1 mg/ml respectively). The mean ± SE level of histidine in plasma from 13 patients with psoriasis was 11.0±1.7 µg/ml as compared to 11.5±1.0 in healthy subjects. Non-involved skin from psoriatics and healthy controls both showed positive Pauly staining reactions of the granular layer whereas in the granular layer of psoriatic lesions less intense staining was observed.

DISCUSSION
The low levels of urocanic acid in blister fluid from patients with psoriasis could theoretically be a result of a decreased formation from histidine or be due to a breakdown of urocanic acid by urocanase (Fig. 1). Urocanase is not present in normal skin and we have not been able to detect it by allowing urocanic acid to interact with blister fluid from our patients. An increased degradation of urocanic acid in the skin of patients with psoriasis therefore seems unlikely.

A decreased formation of urocanic acid could occur if the enzyme, histidase, is not functioning or if there is no histidine available for the enzyme. Histidase activity has been reported to be slightly increased in psoriatic lesions but decreased in the normal appearing skin of patients with psoriasis (9). Scott (13) found that there was only little histidase in the normal living epidermis and that the enzyme was almost completely restricted to the stratum corneum. Here, however, it was inactivated at low pH and the normal conversion of 75% of available free histidine occurred in alkaline solution. A defect in the histidase activity in patients with psoriasis linked to local pH variations can therefore not be excluded.

We do not know the reason for the low histidine levels in the blister fluid of psoriatics. Plasma levels of histidine are reduced in some disorders such as rheumatoid arthritis, uremia and Hodgkin's disease (14). In our patients the plasma levels of histidine were normal. The albumin levels in the blister fluid were the same in psoriatics and healthy subjects indicating that there is no change in the passage of serum proteins into the blister fluid in psoriasis.

A decreased synthesis of the histidine rich protein in the granular layer was demonstrated in psoriatic lesions but not in the normal appearing skin (19). The Pauly staining, which reflects the presence of this protein, was not changed from normal in the non-lesional skin. It can therefore not explain the low levels of histidine.

An altered metabolism of histidine in psoriasis therefore seems more plausible.
increased excretion of methyl histidine is known to occur in conjunction with catabolic responses after stress and trauma (15). An alternative pathway would be an increased formation of histamine metabolites. We found no significant increase of histamine in the blister fluid of patients with psoriasis, compared to normal controls, but an increase of histamine in the basal cell layer and the papillae has been described (16). An increased histamine forming capacity in psoriatic lesions and an increased urinary excretion of histamine has also been detected in patients with psoriasis (17). In this context it is interesting to note that high levels of histamine and histidine decarboxylase have recently been found in growing experimental tumours (18).

The preceding discussion leads us to propose that in psoriasis there is a shift in the metabolic conversion of histidine towards the production of histamine rather than urocanic acid.

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REFERENCES