Effect of PUVA Radiation on Anaphylactic Histamine Release from Rat Dermal Tissues

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We have devised a new in vitro model of type I cutaneous anaphylaxis. Male albino rats were sensitized with DNP-AscAc. Abdominal skin was shaved, and thin, split-thickness slices of skin were cut with a dermatome. The dermis was excised and cut into 100 mg pieces. The dermal tissue was incubated with antigen in Tyrode's solution for 30 min at 37°C. Antigen-induced histamine release from dermal tissue was measured fluorimetrically. Using this system, we measured histamine release from PUVA-irradiated and non-irradiated dermal tissues. A single PUVA irradiation inhibited type I cutaneous anaphylaxis, but did not affect spontaneous histamine release or total dermal histamine. Our model is considered to be useful for investigation of the mechanism of suppression of type I cutaneous anaphylaxis by PUVA.

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spot measuring more than 5 mm in diameter was considered positive.

Preparation of dermal tissue
Rats were killed by exsanguination under ether anesthesia, and the abdominal skin was depilated. A 700 μm thick section of skin containing the upper dermis was removed with a Padgett-Hood dermatome. The remaining lower dermis, approximately 1.5 mm thick, was detached from the subcutaneous adipose tissue, placed in Tyrode's solution, cooled at 4°C, and using scissors, cut into fragments with wet weight 100 mg.

Determination of percentage histamine release (% HR) from dermal tissue
A 100 mg portion of sensitized dermis was placed in 1.8 ml of Tyrode's solution (containing 2% fetal bovine serum) and preincubated at 37°C for 10 min. DNP-Ascaris (0.2 ml), prewarmed to 37°C was added and allowed to react with the dermis at 37°C for 30 min. The reaction system was cooled to 4°C, the supernatant and tissue were immediately separated, and histamine released into the supernatant and histamine remaining in the tissue were assayed using the method of Shore et al. (13). The % HR from the dermis was calculated by the formula:

\[
\frac{\text{Histamine concentration of supernatant}}{\text{Histamine concentration of supernatant} + \text{Histamine concentration of tissue}} \times 100
\]

Light source
A Toshiba Dermasay, which incorporated 10–20 W fluorescent black lights (Toshiba FL20S BLB), was used as the UVA light source. These black lights emitted rays with wavelengths of 300–400 nm, with a peak at 360 nm (mainly UVA). The total irradiance of this instrument measured at a target distance of 20 cm was 7.4 mW/cm².

PUVA radiation
The sensitized rat abdominal region was shaved. One hour after a 0.5% solution of 8-methoxypsoralen (8-MOP, Sigma) in 99% ethanol was applied on the right half of the abdomen, the rats were placed in an immobilizer, and the left half of the abdomen shielded with a black cloth. UVA was directed to the right half of the abdomen. PUVA irradiation with total energy of 2 J/cm² (below MED) was performed. The PUVA-irradiated dermal tissue (right side) and non-irradiated dermal tissue (left side) were prepared, and the % HR in dermal tissue was measured.

Statistical evaluation
For statistical evaluation, Student’s t-test was applied to pairs of values (mean ± S.D.); the value in a PUVA-irradiated group was compared with that in a non-irradiated group.
Table 1. Effects of PUVA irradiation on antigen-specific histamine release from dermal tissue (mean±S.D.).

<table>
<thead>
<tr>
<th>Rat no.</th>
<th>% histamine release</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Non-irradiated</td>
</tr>
<tr>
<td>1</td>
<td>49.6±5.0</td>
</tr>
<tr>
<td>2</td>
<td>26.4±7.2</td>
</tr>
<tr>
<td>3</td>
<td>39.8±5.4</td>
</tr>
<tr>
<td>4</td>
<td>34.9±1.6</td>
</tr>
<tr>
<td>5</td>
<td>29.8±5.8</td>
</tr>
<tr>
<td>6</td>
<td>32.8±5.4</td>
</tr>
<tr>
<td>7</td>
<td>24.1±1.4</td>
</tr>
<tr>
<td>8</td>
<td>24.3±2.3</td>
</tr>
<tr>
<td>9</td>
<td>24.0±2.5</td>
</tr>
<tr>
<td>10</td>
<td>16.4±4.1</td>
</tr>
<tr>
<td>Mean±S.D.</td>
<td>30.2±9.5</td>
</tr>
</tbody>
</table>

RESULTS

Mast cell count in rat cutaneous tissue

Mast cells were observed in the upper and lower dermis, but not in the epidermis (Fig. 1). The mast cell count was greater in the lower dermis (68 ± 13/mm²) than in the upper dermis (33 ± 12/mm²). Hence the mast cell count per unit tissue weight is considered to be greater in the lower dermis than in the whole-skin thickness.

%HR of rat dermal tissue

The %HR was greatest at an antigen concentration of 100 µg/ml, in rats with PCA ≥ 200 and 10 ≤ PCA < 200. The %HR used was slightly lower at 500 µg/ml. Therefore, the antigen concentration used for the subsequent studies was 100 µg/ml.

Correlation between %HR of rat dermal tissue and PCA titre

The %HR increased with increase in PCA titre (PCA ≥ 400, 40.4% ± 6.3 %HR; 200 ≤ PCA < 400, 31.0% ± 3.0 %HR; 10 ≤ PCA < 200, 25.0% ± 4.1 %HR). The %HR was greater in the dermis (31.0% ± 3.0%) than in whole skin (22.3% ± 1.0%). Therefore, the type I anaphylactic reaction occurring in the dermis is considered to be more intense in rats with a higher PCA titre.

Effects of PUVA radiation on %HR of rat dermis

Ten rats were studied, and measurements were made in quadruplicate. Despite some individual variation, the mean %HR of irradiated dermal tissue was slightly lower than that of non-irradiated dermal tissue (p < 0.1) (Table 1).

Effects of PUVA radiation on spontaneous histamine release from rat dermal tissue

Spontaneous %HR from irradiated dermal tissue was 9.3% ± 3.5%, and from non-irradiated dermal tissue, 7.1 ± 2.4%. The difference between the two groups was not statistically significant.

Effects of PUVA radiation on total dermal histamine

Dermal histamine concentration of irradiated dermal tissue was 26.1 ± 7.7 ng/mg dermis, and of non-irradiated dermal tissue, 24.7 ± 8.0 ng/mg. There was no significant difference between the two groups.

DISCUSSION

Greaves et al have reported an in vitro model of type I anaphylactic reaction, using rat skin (14). We used the system to measure the %HR of slices of DNP-Ascaris sensitized rat skin. The antigen-specific HR was 22.3%, and the spontaneous HR was 10.6%.

In this study, we established an in vitro system for evaluation of type I anaphylactic reaction, based on histamine release from dermal tissue. The usefulness of this system was suggested by the antigen concentration-dependent increase in %HR, low spontaneous histamine release (less than 10%), correlation of histamine release with PCA reaction, and higher mast cell count in the lower dermis (Fig. 1).

In this experimental system, single PUVA irradiation was shown to inhibit type I anaphylactic reactions in spite of some individual variation between animals (Table I). It did not affect spontaneous histamine release or total dermal histamine.

PUVA therapy has been prescribed for patients with diseases in which mast cells are involved; e.g. urticaria pigmentosa (15-17) and diseases due to type I anaphylactic reaction such as solar urticaria (1,2). Our model of type I anaphylactic reaction, although prepared from rats, is considered to be useful for investigation of the mechanism of suppression of type I anaphylactic reaction by PUVA.

REFERENCES

1. Kealcy TM, Lavker RM, Kaidbey KH, Atkins PC, Zweiman B. Studies on the mechanism of clinical toler-
A statistical analysis of the relationship between otitis externa and various clinical and etiological variables was carried out in 64 patients. Between 1988 and 1989, true eczema of the auditory canal was found in 43 of the 64 patients seen sequentially. 23.5% of all the patients found to have dermatitis could be regarded as having allergic contact dermatitis and the allergen identified. This incidence is less than the 40% and the 55% found in previous studies. We did not find any specific difference in sex and age between the allergic and non-allergic groups. In the allergic group, topical drugs were the commonest sensitizing agents, followed by chemicals and resins found in the ear prosthesis. Twenty-one patients with negative patch tests were classified as seborrhoeic dermatitis and 11 as atopic dermatitis. The other 19 patients, who were discharged before patch testing, were diagnosed as having psoriasis (8) or chronic bacterial (6) or fungal infections (5), without true blister reaction. We think that accurately selected series must be used for these studies because of the low incidence of allergic contact dermatitis.

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External otitis can be caused by endogenous (constitutional, familial), exogenous (physical, chemical, bacterial, mycotic) or unknown factors. Occasion-