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

TSUKASA TANII[1], TAKESHI KONO[1], NOBUYUKI MIZUNO[1], MASAYOSHI FURUKAWA[1], KUMIE NAGAHAMA[1], YUKIKO SOMEDA[1], TOSHIO HAMADA[1], TADAYOSHI SHIBATA[2] and TADASHI OKADA[3]

[1]Department of Dermatology, Osaka City University Medical School, [2]Osaka Prefectural Institute of Public Health, Osaka and [3]Department of Physiology, Aichi Medical University, Aichi, Japan

We have devised a new *in vitro* model of type I cutaneous anaphylaxis. Male albino rats were sensitized with DNP-Ascaris. 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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T. Tanii, Department of Dermatology, Osaka City University Medical School, 1–5–7, Asahimachi, Abeno-ku, Osaka 545, Japan.

It is still not certain whether UV radiation has any effect on type I anaphylactic reactions. PUVA has been used for the treatment of solar urticaria (1, 2), and its therapeutic effect has been attributed to elevation of the threshold of mast cell degranulation (1).

PUVA has also been reported to inhibit erythema and flare induced by mast cell degranulating agents in the human skin (3–5). Moreover, the percentage degranulation of mouse skin mast cells after injection of compound 48/80 has been reported to be reduced by a single PUVA irradiation (6). However, no experimental model for evaluation of the effects of PUVA on type I anaphylactic reactions has yet been established.

We studied the effects of PUVA in an *in vitro* model of type I anaphylactic reaction, prepared from rat dermis by removing the epidermis with a dermatome.

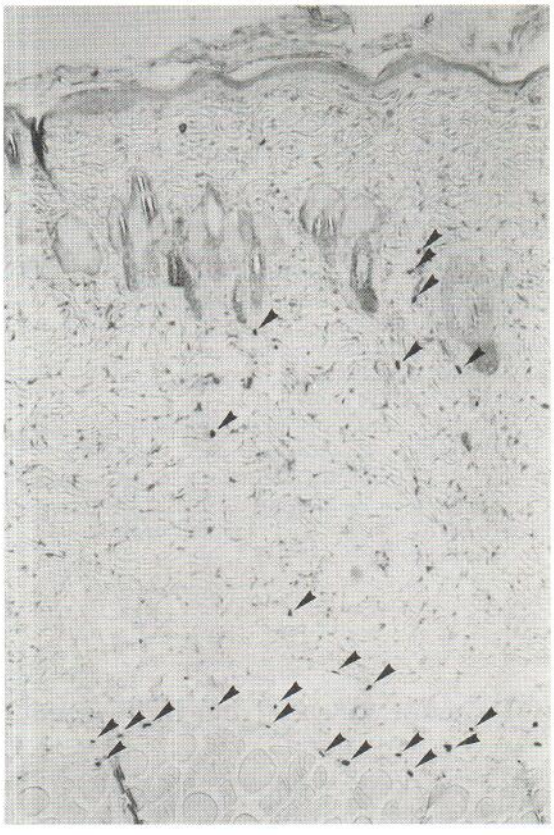

Fig. 1. Histological findings in the rat abdominal skin. Arrow: mast cells. Toluidine blue stain, ×135.

MATERIALS AND METHODS

Animals

Male albino rats of Wistar strain weighing 100–200 g were used.

Mast cells in rat skin

Tissue specimens 7 μm thick were collected from the rat abdominal skin and stained with toluidine blue. Mast cells were counted microscopically in 1 mm^2 areas of the upper and lower dermis (7).

Preparation of antigen

Ascaris suum extract, obtained *ad modum* Strejan & Campbell (8), was bound to 2,4-dinitro-phenylsulfate (DNP, Tokyo Kasei) by the method of Eisen et al. (9).

Sensitization of rat skin

Ten rats were sensitized by the method of Tada & Okumura (10,11). First, 10^{10} killed *Bordetella pertussis* bacteria were injected intraperitoneally, and the Ascaris extract (1 mg) was injected into the foot pad. After 5 days, DNP-Ascaris (0.5 mg) was injected in the dorsal muscle. The titre of anti-DNP antibodies of class IgE was determined by the passive cutaneous anaphylaxis (PCA) reaction (12). A blue 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 was allowed to react with the dermis at 37°C for 30 min. The reaction system was cooled to 4°C, the supernatant and the 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:

$$\%\,HR = \frac{\text{Histamine concentration of supernatant}}{\text{Histamine concentration of supernatant} + \text{Histamine concentration of tissue}} \times 100$$

Light source

A Toshiba Dermaray, 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/cm^2.

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/cm^2 (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 I. *Effects of PUVA irradiation on antigen-specific histamine release from dermal tissue* (mean±S.D.).

Rat no.	%histamine release	
	Non-irradiated	Irradiated
1	49.6±5.0	24.9±5.1
2	26.4±7.2	16.3±6.5
3	39.8±5.4	24.7±6.1
4	34.9±1.6	27.3±5.8
5	29.8±5.8	25.3±3.1
6	32.8±5.4	29.1±3.8
7	24.1±1.4	22.6±4.8
8	24.3±2.3	22.8±1.5
9	24.0±7.5	24.7±12.3
10	16.4±4.1	17.3±3.1
Mean±S.D.	30.2±9.5	23.5±4.0

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/mm^2) than in the upper dermis (33 ± 12/mm^2). 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 I).

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.

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