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Short Communication

Involvement of Histamine Released From Mast Cells in Acute Radiation Dermatitis in Mice

Saiko Moriyasu[1], Kouichi Yamamoto[1,*], Naoko Kureyama[1], Keita Okamura[1], Toshiji Ikeda[2], and Atsushi Yamatodani[1]

[1]*Department of Medical Physics and Engineering, Division of Health Sciences, Graduate School of Medicine, Osaka University, Yamadaoka 1-7, Suita, Osaka 565-0871, Japan*
[2]*Department of Accelerator Science, The Institute of Scientific and Industrial Research, Osaka University, Mihogaoka 8-1, Ibaraki, Osaka 567-0047, Japan*

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Abstract. A possible involvement of histamine in acute radiation dermatitis in mice was investigated. The dose of 40 Gy of gamma irradiation induced erythema and edema in C57BL/6 mice treated with vehicle. However, in C57BL/6 mice treated with chlorpheniramine and WBB6F1-W/W^v mice, erythema and edema were not observed. In all of these mice, epilation and dry desquamation were induced, but bepotastine significantly reduced the extent of these areas. These results suggest that gamma irradiation-induced erythema and edema were caused by histamine released from mast cells via histamine H_1 receptor, and epilation was induced by other inflammatory mediators.

Keywords: radiation dermatitis, histamine, mast cell

Radiation dermatitis induced by a large skin exposure dose can be a limiting factor of radiotherapy. In humans, the acute skin changes usually occur within 90 days after radiotherapy. The erythema is apparent by 10 to 14 days after irradiation and is followed by edema, epilation, dry and moist desquamation, and erosion (1). Recently, topical application of anti-inflammatory drugs such as corticosteroid is the most common treatment for radiation dermatitis (1, 2), but the results are not always satisfactory in terms of response.

A previous study demonstrated that histamine released from mast cells induced erythema and edema (3). Hirabayashi et al. reported that X-ray irradiation increased degranulation of mast cells in mice (4). Moreover, Murakami et al. reported that azelastine, which has both anti-histaminic and anti-allergic actions, reduced radiation dermatitis in mice (5). However, the precise etiology is uncertain.

In this study, we investigated the effects of gamma irradiation on the skin of mice and the effects of chlorpheniramine, a classical and selective histamine H_1-receptor antagonist, and bepotastine, a second generation anti-histaminic and anti-allergic drug, on the skin reactions. Furthermore, in order to study the involvement of mast cells, we investigated the effects of gamma irradiation on the skin of mast cell deficient mice (WBB6F1-W/W^v) (6).

Seven-week-old male C57BL/6, WBB6F1-W/W^v, and WBB6F1-+/+ mice, weighing about 25 g at the start of experiment, were obtained from Japan SLC (Shizuoka). The mice were housed in individual cages in a room with a 12-h light / 12-h dark cycle (lights on 5:00 – 17:00) at a constant temperature (23 ± 1°C) and humidity (50 ± 5%). They were fed standard laboratory chow pellets (MF; Oriental Yeast, Osaka) and water ad libitum. Experiments were approved by the Animal Care Committee of the School of Allied Health Sciences, Faculty of Medicine, Osaka University and conducted in accordance with the Animal Experiment Guideline of Osaka University.

Prior to irradiation, mice were anesthetized with sodium pentobarbital (50 mg/kg, i.p.; Dainippon Sumitomo Pharma, Osaka) and taped on an acrylic plate with one hind leg extended and covered with a 1-cm-thick water equivalent material. The hind leg received gamma irradiation by cobalt-60 (source-to-surface distance of

*Corresponding author. kouichi@sahs.med.osaka-u.ac.jp
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25 cm, dose rate of 1.94 Gy/min) as a single dose. The bodies were protected from irradiation by lead shield blocks.

The C57BL/6 mice received sham irradiation (n = 2) or 20 and 40 Gy of irradiation (n = 6, each group). Acute skin reactions were assessed every three days until the 60th day after irradiation using a visual skin score, based on the scoring method described by Abe et al. (7) with slight modification: 0 = normal; 0.25 = transient erythema, edema, or dry desquamation on dorsum or sole of the foot; 0.5 = slight epilation in <50% of exposure area; 1.0 = epilation in about 50% of exposure area; 1.5 = epilation in >50% of exposure area or some signs of dry desquamation on the leg; 2.0 = complete epilation, red foot, or dry desquamation in ≤50% of exposure area with epilation in ≥50% of exposure area; 2.5 = complete epilation with definite edema or dry desquamation in >50% area; 3.0 = moist desquamation in a small area; and 3.5 = moist desquamation in most of the area.

Another group of C57BL/6 mice received 40 Gy of gamma irradiation and were administered orally chlorpheniramine maleate (0.3, 1 mg/kg per day, n = 5 for each dose; Sigma, St Louis, MO, USA) and bepotastine besilate (1, 10 mg/kg per day, n = 5 for each dose; Tanabe Seiyaku, Osaka). Since we found that daily water intake of mice was about 5 ml, we provided the drinking water added chlorpheniramine (1.5 and 5 μg/ml) and bepotastine (5 and 50 μg/ml) during the whole observation period. Acute skin reactions were determined as described above.

Finally, to investigate the involvement of mast cells, WBB6F1-W/W^v and +/+ (wild type) mice (n = 6, each strain) received 40 Gy of gamma irradiation and acute skin reactions were determined as described above.

The skin score data were represented as the mean ± S.E.M. and compared using a two-way repeated-measures analysis of variance (ANOVA) followed by Dunnett multiple comparison tests. Differences were considered statistically significant when the value of P was less than 0.05.

In C57BL/6 mice, 40 Gy of gamma irradiation induced transient erythema and edema on the 9th day after irradiation and induced epilation and slight dry desquamation on the 15th day in all mice. At the 30th day, four of six mice showed epilation developing on 50% of the exposure area with dry desquamation, and moist desquamation developed subsequently in two of six mice. Although 20 Gy of gamma irradiation induced slight epilation on the 40th day after irradiation, other skin reactions were not observed. Sham irradiation did not cause any changes in the skin throughout the observation period (Fig. 1).

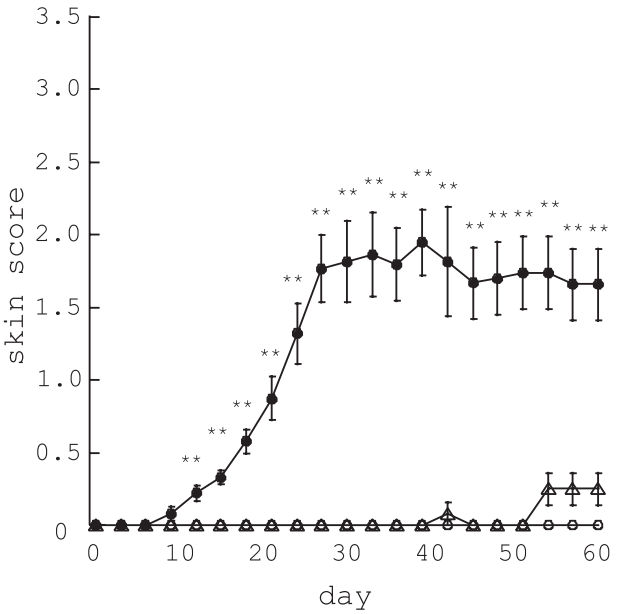

Fig. 1. The skin score of C57BL/6 mice after sham (open circle, n = 2), 20 Gy (open triangle, n = 6), and 40 Gy (closed circle, n = 6) of gamma irradiation. The points and bars represent the mean value ± S.E.M. of the skin score. **P<0.01 *vs* sham irradiated group.

Chlorpheniramine at a dose of 0.3 mg/kg per day did not affect the skin reactions induced by 40 Gy of gamma irradiation (data not shown). At a dose of 1 mg/kg per day, the drug significantly inhibited transient erythema in all mice and transient edema in three of five mice. Epilation and dry desquamation appeared in all of the chlorpheniramine-treated mice after irradiation from the 18th day (Fig. 2a).

In mice treated with bepotastine at doses of 1 and 10 mg/kg per day, erythema appeared in three of five and three of four mice, respectively, and edema was observed in all mice. However, the epilation area was significantly suppressed from the 20th to the 42nd day after the irradiation (Fig. 2b).

In WBB6F1-+/+ (wild type) mice, gamma irradiation induced transient erythema and edema on the 9th day after irradiation in four of six mice and induced epilation and dry desquamation on the 15th day in all mice. In the mast-cell deficient WBB6F1-W/W^v mice, gamma irradiation did not induce erythema and edema, but epilation was observed on the 15th day in all mice. Dry desquamation was found on the 15th day in five of six WBB6F1-W/W^v mice (Fig. 3).

The skin is an external organ that has the most frequent opportunities for exposure to radiation. In addition, the skin is a radiation-sensitive organ and easily develops dermatitis even by a relatively low dose. The skin reactions to radiation have a certain threshold and latency. In mice, Xiao et al. reported that a single

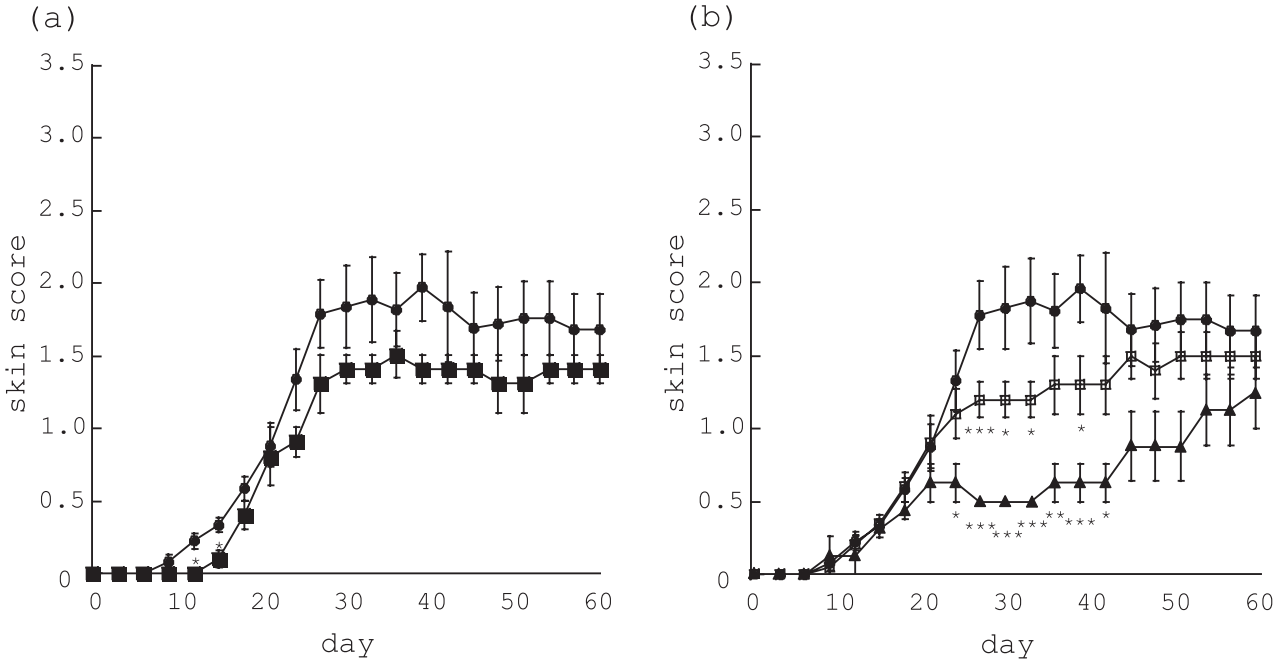

Fig. 2. The effect of chlorpheniramine (a) and bepotastine (b) on the skin reaction after 40 Gy of gamma irradiation. a: vehicle (closed circle, n = 6) and chlorpheniramine, 1 mg/kg per day (closed square, n = 5). b: vehicle (closed circle, n = 6); bepotastine, 1 mg/kg per day (open square, n = 5); and bepotastine, 10 mg/kg per day (closed triangle, n = 4). The points and bars represent the mean value ± S.E.M. of the skin score. **P*<0.05, ***P*<0.01, ****P*<0.001 *vs* vehicle group.

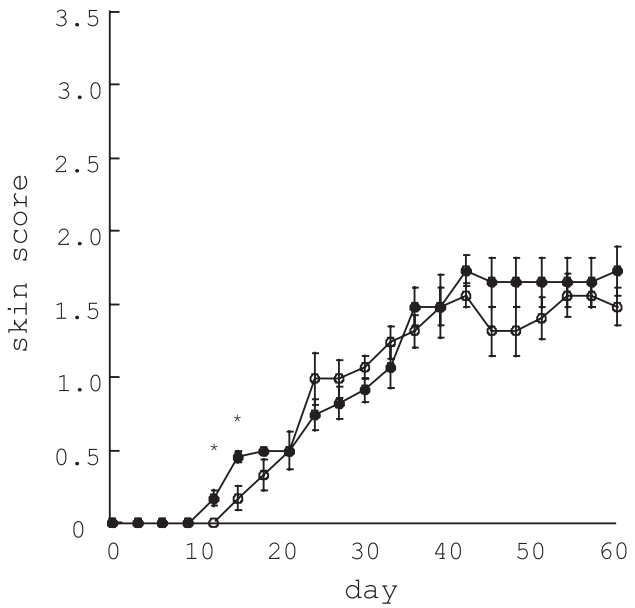

Fig. 3. The skin score of WBB6F1-wild type mice (closed circle, n = 6) and WBB6F1-W/W^v mice (open circle, n = 6) after 40 Gy of gamma irradiation. The points and bars represent the mean value ± S.E.M. of the skin score. **P*<0.05 *vs* W/W^v.

dose of 30 Gy of cesium-137 gamma-ray induced significant skin changes such as erythema, epilation, dry desquamation, and moist desquamation (8). Maeng et al. reported that dermatitis was induced in the hairless mice-1 by 40 Gy of X-irradiation, and erythema was detectable 10 days after the irradiation (9). In our study, 40 Gy of gamma irradiation induced erythema and edema on about the 9th day after irradiation and epilation and dry desquamation on about the 15th day after irradiation. In contrast, 20 Gy of gamma irradiation induced only slight epilation (Fig. 1). Our results are almost consistent with the previous results, and we thus selected the dose of 40 Gy for further experiments.

Histamine plays a pivotal role in inflammation caused by allergy and chemical or physical irritation. Chlorpheniramine, a classical and selective H_1-receptor antagonist, has been used for treatment of erythema and edema in various forms of dermatitis (3). In this study, chlorpheniramine inhibited erythema and edema induced by gamma irradiation (Fig. 2a). Moreover, erythema and edema were never observed in the skin of WBB6F1-W/W^v mice (Fig. 3). Previous reports suggested that chlorpheniramine inhibited the compound 48/80-induced skin reaction and scratching behavior via H_1-receptor antagonistic action (10, 11). These results suggested that histamine released from mast cells and H_1 receptors are involved in the development of radiation-induced erythema and edema.

There were no significant differences between the control and chlorpheniramine treated or WBB6F1-

W/W^v mice in the gamma irradiation-induced epilation. On the other hand, bepotastine, which has anti-inflammatory effects additional to H_1-receptor antagonistic action, significantly suppressed the epilation from the 24th to the 42nd day (Fig. 2b). Previous studies reported that substance P, a neuropeptide, manipulated murine hair follicle cycling and inhibited hair growth (12, 13). Andoh et al. (14) reported that bepotastine suppressed the substance P-induced itch-associated response. These results suggest that substance P might be concerned with the development of radiation-induced epilation and bepotastine may block its action. However, bepotastine had no affect on erythema and edema (Fig. 2b). The half-life of bepotastine is estimated to be about 3 h in humans (Drug Package Insert) and is shorter than that of chlorpheniramine (about 29 h in humans) (3). In this study, it is possible that the effective blood concentration of bepotastine was not maintained throughout the day by this administration method. Further investigations are required in order to decide the effective dose and administration methods for the treatment of erythema and edema.

In conclusion, in early acute radiation dermatitis, erythema and edema are caused by histamine release from mast cells via H_1 receptors and the later epilation is induced by inflammatory mediators other than histamine.

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