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● *Biology Contribution*

THE EFFECT OF AZELASTINE ON ACUTE RADIATION DERMATITIS IN MICE MODELS

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Purpose: In our previous report we described the clinical value of azelastine, an oral antiallergic agent, as an inhibitor of radiation dermatitis. Here we studied the effect of azelastine on normal skin and tumor size after irradiation in a mouse model.

Methods and Materials: The modifying effects of azelastine on both the degree of radiation dermatitis and antitumoral effect of radiation therapy were investigated in the normal skin as well as in SCC VII tumors of C3H/He mice. The right hind legs, with or without tumors, were irradiated with 20–60 Gy at 0.62 Gy/min. Azelastine was administered via the mouse chow, and acute skin reactions and tumor growth curves were compared between the azelastine and control groups.

Results: The acute skin reactions of the azelastine group were significantly less prominent than those of the control group ($p < 0.01$). At a dose of 40 Gy the dose modification factors were 1.19–1.25. The tumor growth curves of the azelastine and control groups were almost identical, indicating that the treatment response of irradiation was not affected by administration of azelastine.

Conclusions: Application of azelastine reduces the degree of acute radiation dermatitis without affecting the antitumoral effect of radiation therapy. © 1997 Elsevier Science Inc.

Azelastine, Radiation injury, Radiation dermatitis, Mouse skin, Mouse tumor.

INTRODUCTION

Radiation injury of the skin is a common adverse effect of radiation therapy. The acute skin reaction causes itching, pain and ulceration, and can be a limiting factor of radiation therapy. Experimental and clinical studies have suggested that anti-inflammatory agents can reduce radiation injury of the skin (11, 12, 14) Topical application of steroidal or nonsteroidal anti-inflammatories is the most common treatment for radiation injury of the skin, but the results are not always satisfactory in terms of response, local toxicity, and patient compliance (4).

Azelastine is a long-acting and orally active antiallergic agent, which has been clinically effective and safe in the management of bronchial asthma, allergic rhinitis, urticaria, and atopic dermatitis (3, 10). Although azelastine has anti-inflammatory action, the mechanisms of this drug differ from those of steroidal and nonsteroidal anti-inflammatories and histamine receptor antagonists. Pharmacologically, azelastine inhibits the release of chemical mediators such as histamine, serotonin and leukotriene. The interference in the influx of Ca^{2+} into the cells, stabilization of the cell membrane, increase in cAMP level, and inhibition of free radical generation are suggested as the inhibitory effects of azelastine (10, 15, 16).

In our previous report we described the clinical effects of azelastine as an inhibitor of acute radiation dermatitis (5). If azelastine can reduce radiation injury without affecting antitumoral effect of radiation therapy, it may be an effective means of preventing and treating radiation induced normal tissue complications. In this experimental study, azelastine was studied in mice with experimental acute radiation dermatitis and tumor models.

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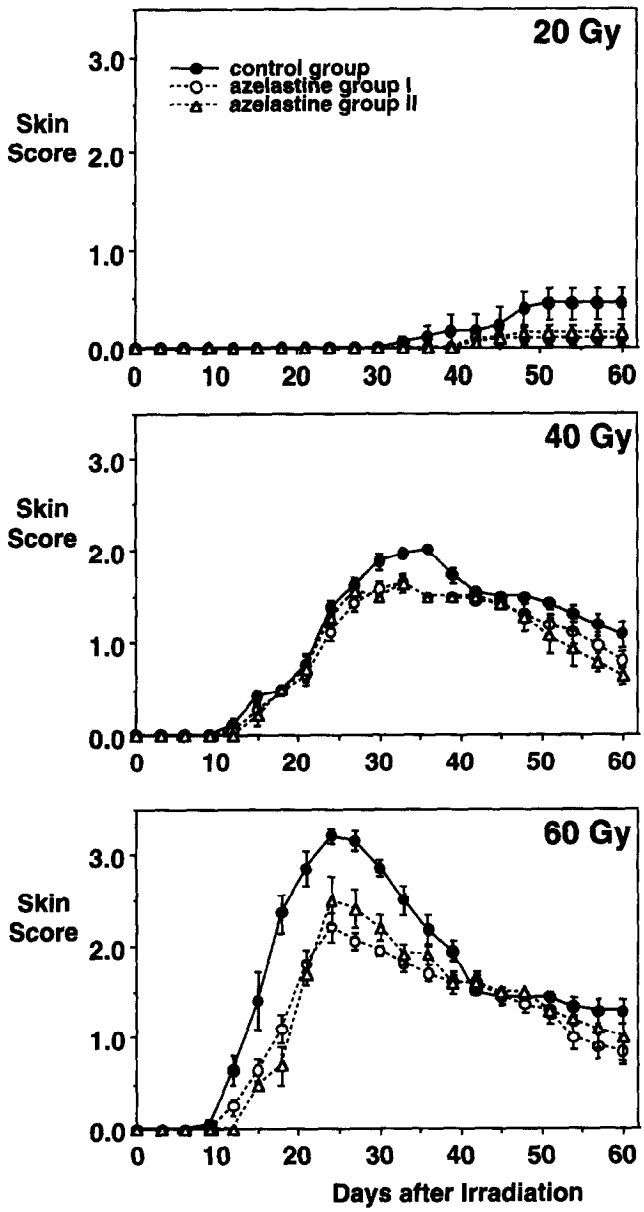

Fig. 1. The time courses of the average skin score after 20, 40, and 60 Gy irradiations. Error bars indicate ± SE.

MATERIALS AND METHODS

Mice, drug, and irradiation

Male C3H/He mice, 8–10 weeks of age and weighing 25–28 g at the time of irradiation, were used in these experiments. Mice were caged in groups of five and fed chow and water ad libitum. Azelastine [4 (p-chlorobenzyl)-2-(hexahydro-1-methyl-1H-azepine-4yl)-1(2H)-phthalazinone hydrochloride].[1] was added to the chow at a concentration of 10 mg/Kg. The mean rate of the chow consumption was 1.3×10^2 g/Kg body weight/day, and the mean dose of administration was 1.3 mg azelastine/Kg body weight/day. X rays at 230 kV with a dose rate of 0.62 Gy/min were irradiated to the right hind legs of mice without anesthesia.

Normal skin studies

Administration of azelastine added to the chow was initiated one week before irradiation (azelastine group I) or immediately after irradiation (azelastine group II). Mice in the control group were fed chow without drug. Mice were irradiated with a single dose of 0, 20, 40 or 60 Gy. Ten mice were used for each dose group. Acute skin reactions were evaluated and scored every three days until the 60th day after irradiation using the modification of the skin score system proposed by Abe *et al.* (1); 0 = normal, 0.5 = slight epilation, 1.0 = epilation in an about 50% area, 1.5 = epilation in a more than 50% area, 2.0 = complete epilation, 2.5 = complete epilation with definite edema or dry desquamation in a more than 50% area, 3.0 = moist desquamation in a small area, and 3.5 = moist desquamation in most of the area. Skin reactions of the azelastine group I and II were compared with those of the control group. The potential difference in the three groups was evaluated using MANOVA, and statistical significance of differences between the azelastine and control groups was tested with the Wilcoxon two-sample test. A value of $p < 0.01$ was considered significantly different.

For the histologic study, specimens of the right hind leg were obtained immediately after sacrifice every five days until 40 days following 40 Gy irradiation. Three mice were sacrificed for each histologic evaluation. Microscopic findings were compared between the azelastine group initiated one week before irradiation and the control group without the drug.

Tumor studies

We used SCC VII tumors to evaluate the modifying effects of azelastine on radiation therapy. Tumors were implanted in the right hind leg by inoculating 1×10^6 tumor cells in 0.05 ml of the suspension fluid. Administration of azelastine added to the chow was initiated immediately after inoculation in the azelastine group. Mice in the control group were fed the chow without the drug. Seven to 10 days after tumor cell inoculation, tumors in the volume range of 500–700 mm^3 were selected and irradiated with a single dose of 0, 10, 20 or 40 Gy. Seven to 10 mice were used for each treatment group. The size of the tumors was measured every three days, and the day at which its volume reached three times ($T_{300\%}$) was determined. The difference in tumor growth between the two groups were analyzed. Skin reactions were evaluated using the same methods as the normal skin studies.

[1] Eisai Co., Ltd. Tokyo, Japan.

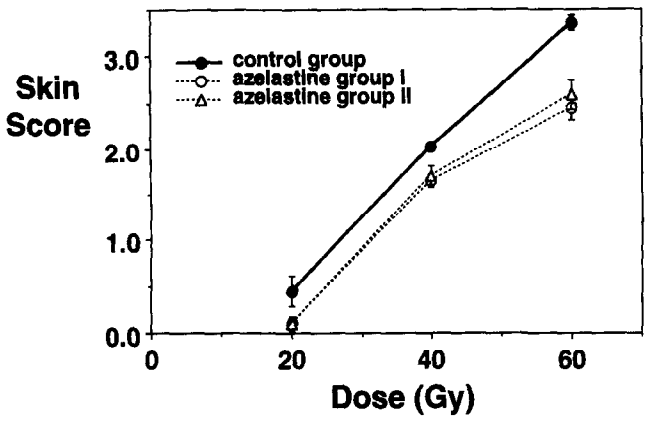

Fig. 2. Dose-response relationship for peak skin reaction. Error bars indicate ± SE.

RESULTS

The drug was well tolerated with no toxic effects. All of the mice gained weight during the course of the experiment, and there was no difference in the average weights between the control and azelastine groups.

The progress of the average skin score after irradiation of each group is shown in Fig. 1. In all three irradiation doses, skin reactions of the azelastine groups I and II were less prominent than those of the control group. In mice exposed to 20 Gy, epilation (scores of 0.5 or greater) appeared in six of 10 mice (60%) of the control group, and five of 20 mice (25%) of the azelastine groups I and II. In mice exposed to 40 Gy, complete epilation (scores of 2.0 or greater) appeared in all 10 mice (100%) of the control group, and eight of 20 mice (40%) of the azelastine groups I and II. In mice exposed to 60 Gy, moist desquamation (scores of 3.0 or greater) appeared in all 10 mice (100%) of the control group, and eight of 20 mice (40%) of the azelastine groups I and II. The mice exposed to 40 and 60 Gy irradiation in both the azelastine groups I and II showed significantly lower peak skin scores compared with that of respective irradiation doses in the control group. Fig. 2 shows dose-response relationship for peak skin reaction. At a dose of 40 Gy the dose modification factors [isoeffect dose with drug/isoeffect dose without drug(8)] of the azelastine groups I and II were 1.25 and 1.19, respectively.

There were no significant differences in the histologic findings and its progress between the azelastine and control groups. The epidermis declined with infiltration of inflammatory cells for the first 20 days, followed by a rapid hyperplasia over the next 10 days, and returned to the initial level within 40 days; however, infiltration of inflammatory cells in the azelastine group tended to be less prominent than that in the control group.

The tumor growth curves of the azelastine and control groups are shown in Fig. 3. $T_{300\%}$ (mean ± standard deviation) of the control group in a dose of 0, 10, 20 and 40 Gy were 3.5 ± 0.8, 6.0 ± 1.1, 18.7 ± 5.8, and 39.9 ± 19.8 days, respectively; that of the azelastine group in 0, 10, 20 and 40 Gy were 4.1 ± 1.2, 7.5 ± 1.9, 17.4 ± 5.7, and 37.1 ± 12.3 days, respectively. There were no significant differences between the azelastine and control groups. With regard to the acute skin reaction by 20 Gy irradiation, epilation appeared in six of eight mice (75%) of the control group, and two of seven mice (29%) of the azelastine group. With 40 Gy irradiation, complete epilation developed in all nine mice (100%) of the control group, and five of nine mice (56%) of the azelastine group.

DISCUSSION

Radiation injury of the skin is caused by epidermal basal cell damage with a secondary inflammatory reaction, and late injury occurs due to vascular damage (2, 7). The increase in vascular permeability associated with inflammation following various injuries to skin is believed to be initiated by the release of histamine, and a continuance or later increase is maintained by the release of other vasoactive substances (14). Although the mechanism of radiation dermatitis which may have a multi-step process with cellular and humoral events is uncertain, some agents modifying the radiation dermatitis may be useful for clarifying the mechanism of radiation injury. Anti-inflammatory agents have been used to reduce radiation damage in experimental and clinical studies (11, 12, 14). Glucocorticoids inhibit eicosanoid synthesis primarily by interfering with phospholipase A_2, while nonsteroidal anti-inflammatory drugs prevent prostaglandin/thromboxane synthesis by inhibiting cyclooxygenase. Eicosanoids are short-lived, local acting mediators of inflammatory reactions, and are not stored, but rapidly synthesized from cell membrane phospholipids on the arachidonic acid cascade. Previous reports indicate that radiation effects are accompanied by excessive production of eicosanoids such as prostaglandins, thromboxanes, and leukotrienes (11, 12).

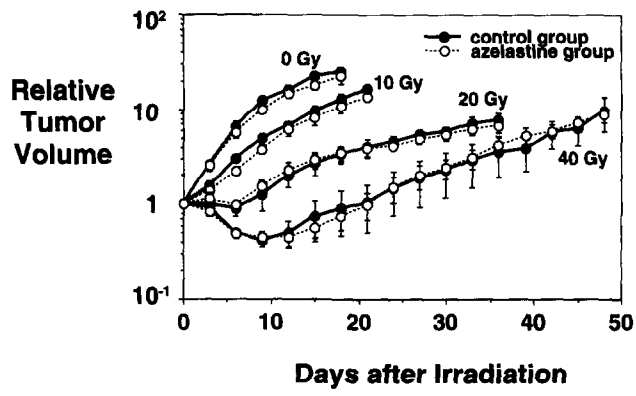

Fig. 3. The tumor growth curves after irradiation. Error bars indicate ± SE.

Anti-inflammatory agents decrease the endogenous eicosanoid levels and reduce a rise in cutaneous vascular permeability observed after irradiation. In contrast to normal tissue protection by agents that block endogenous eicosanoids, an apparent paradox is presented by data showing that certain exogenous eicosanoids can be radioprotective, but the mechanism of the action associated with this protection is unknown (6, 8, 12).

In our preliminary clinical report (5), azelastine was effective for both the prevention and treatment of acute radiation dermatitis. The results of this experimental study indicate that application of azelastine, administered either before or after irradiation, reduces the degree of acute radiation dermatitis without affecting antitumoral effects of irradiation in a mouse model. The exact mechanism of the preventative effect of azelastine on acute radiation dermatitis is unclear at present; however, azelastine is known as a histamine release inhibitor, and inhibits the increased vascular permeability in allergic reaction (10, 15). This drug may also inhibit the first step of the arachidonic acid cascade producing eicosanoids (16).

It is suggested that the total radiation period is an important factor in determining radiation effects (13). Azelastine may increase the tolerance of skin in patients who receive radiation therapy, leading to improvement in tumor control rate. The inhibition effects of free radical formation by azelastine may have radioprotective effects (16); therefore, we used a mouse tumor model to study whether azelastine may have some modifying effects on radiation therapy.

In order to prevent and treat clinical radiation dermatitis, topical treatments should be applied cautiously to avoid local infection, and build-up and scattered rays that can act to enhance the skin dose. Topical steroids are not recommended for moist desquamation (4). Azelastine is an orally-administered drug, and is more convenient to use compared with topical treatments or intravenously-administrated agents. The most frequent reported adverse effects in clinical studies with azelastine are altered taste perception (2-26%) and drowsiness or fatigue (3-18%); these adverse effects are generally mild and transient (10). On short or long term azelastine application, no clinically significant changes in blood pressure, heart rate, or hematological and serological tests were observed (10).

In this experiment, the drug dose may be approximate and may vary from mouse to mouse, since azelastine was delivered in the chow. The mean dose of administration was 1.3 mg azelastine/Kg body weight/day. This dose is 30–40 times higher than the clinical dermatological dose of 2 mg/body/day. Although the drug was effective on this study, the optimum dose and method of drug administration should be selected with experimental and clinical evaluations.

Our tumor studies to evaluate the modifying effects of azelastine on radiation therapy were performed using implanted tumors; the vascularization of the tumors created in the artificial manner does not necessarily reflect the vascularization of naturally occurring tumors. We cannot ignore that the condition of blood supply may affect the radiosensitivity.

Another limitation of this study was that the significant differences in the histologic findings and its progress between the azelastine and control groups could not be observed. Further histologic studies to observe the microscopic and subcellular findings should be performed.

Clinical effects of azelastine on reducing the radiation induced normal tissue damage were reported not only on dermatitis of our preliminary report (5) but also on stomatitis (17) and pneumonitis (9). Further studies will be required to evaluate the effects for various tissues and to examine the subcellular changes. If the benefits of azelastine are confirmed by further experimental and clinical evaluations, routine use of the drug will be recommended for patients who have risks of radiation dermatitis, because the safety of this drug is already confirmed (3, 10).

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