Interventional radiology, which is defined as the delivery of minimally invasive treatments performed using image guidance, is used in the treatment of various diseases worldwide, but clinical reports have revealed that this method often induces radiation dermatitis such as erythema, edema, desquamation, and hair loss (1 – 3). Corticosteroids are normally used as the first choice treatment for radiation dermatitis (3, 4), but the results are not always satisfactory. Previous reports have suggested that histamine, an important inflammatory chemical mediator, which is released from mast cells and the histamine H₁ receptor (H₁R) are involved in the development of dermatitis (5). Actually, we reported that gamma-irradiation induced erythema and edema were inhibited by pretreatment with the H₁R antagonists chlorpheniramine and bepotastine in mice. However, radiation-induced hair loss was not inhibited by H₁R antagonists in normal mice or in mast cell deficient W/W⁺ mice (6), suggesting that gamma irradiation-induced hair loss is induced by inflammatory mediators other than histamine. Previous reports have demonstrated that hair loss was induced by substance P (SP), a product of preprotachykinin-A (PPT-A) mRNA, and its receptor, neurokinin-1 receptor (NK₁R) (7), and that no hair loss was observed in the skin of NK₁R-knockout mice (8). From the finding mentioned above, we hypothesized that SP and NK₁R are involved in radiation dermatitis.

In this study, we investigated the involvement of substance P (SP) and the neurokinin-1 receptor (NK₁R) in the development of radiation-induced hair loss in mice. A dose of 40 Gy of gamma irradiation induced hair loss from the 10th to at least the 60th day after irradiation. A specific NK₁R antagonist, CP-99,994, significantly delayed radiation-induced hair loss and reduced its severity. Furthermore, gamma irradiation induced the expression of preprotachykinin-A, a precursor protein of SP, mRNA in irradiated murine skin on the 10th and 30th days after irradiation. These results indicated that gamma irradiation-induced hair loss was mediated by SP via NK₁R.
left hind leg, covered with a 1-cm-thick water equivalent material, received a single dose of 40 Gy (a source-to-surface distance of 25 cm, dose rate: 1.33 Gy/min) with a $^{60}$Co gamma ray source (The Institute of Scientific and Industrial Research, Osaka University). The right hind leg was used as a non-irradiated control. After irradiation, the mice were returned to their home cages and administered CP-99,994 (Pfizer, Groton, CT, USA) at an s.c. dose of 5 mg/kg (n = 4) every 12 h for 60 days. The control animals received saline injections (n = 3). Hair loss was assessed every day until 60 days after irradiation using a hair loss score as mentioned in Table 1.

On the 10th, 20th, and 30th day after irradiation, the hind leg skin of the mice was collected, and total RNA was extracted by using RNeasy Fibrous Tissue Mini Kit (Qiagen, Hilden, Germany) according to the manufacturer’s instructions. The RNA was converted to first-stranded cDNA (SuperScript III First-Strand Synthesis System for reverse transcription (RT); Invitrogen, Carlsbad, CA, USA), and the cDNA was then used as a template for the RT polymerase chain reaction (PCR) with PPT-A– and glyceraldehyde 3-phosphate dehydrogenase (GAPDH)–specific primers and a Takara PCR Thermal Cycler. The primers for PPT-A and GAPDH were 5′-ACC AGA TCA AGG AGG CAA TG-3′ (sense) and 5′-GCC CAT TAG TCC AAC AAA GG-3′ (antisense), and those for GAPDH were 5′-GGG TGT GAA CCA CGA GAA AT-3′ (sense) and 5′-TTA CTC CTT GGA GGC CAT GT-3′ (antisense). The temperature conditions for RT-PCR were as follows: Step 1: 95°C for 3 min, Step 2: 95°C for 30 s; 55°C for 30 s, and 72°C for 60 s (PPT-A: 45 cycles, GAPDH: 25 cycles), and Step 3: 72°C for 5 min. The PCR products (PPT-A: 220 bp and GAPDH: 610 bp) were separated on 3.0% agarose gels (Nacalai Tesque, Kyoto) and stained with a 1/10,000 dilution of SYBR Safe (Invitrogen). The gels were captured with E-graph (AE-900, ATTO, Tokyo), and the band density was quantified by the ATTO CS Analyzer ver3.0 (ATTO). All data were represented as the mean ± S.E.M. and were compared using a two-way repeated-measures analysis of variance (ANOVA) followed by Dunnett’s multiple comparison test and the non-parametric Mann-Whitney U test, as appropriate. Differences were considered statistically significant when the value of $P$ was less than 0.05.

As shown in Fig. 1, gamma irradiation at a dose of 40 Gy induced hair loss on the 10th day in all mice, and the hair loss extended to more than 50% of the irradiated area during the observation period. On the 30th day, some mice showed hair loss in all of the irradiated area with dry and/or moist desquamation. The hair loss was slightly improved on the 40th day after irradiation (Fig. 1). The NK,R antagonist CP-99,994 significantly prolonged the latent period of hair loss, and the extent of hair loss area was also smaller compared with that of the control mice. Furthermore, in the CP-99,994–treated mice, the hair loss was recovered by the 50th day after irradiation (Fig. 1).

Ionizing radiation causes hair loss only in the area being exposed because radiation stops the growth of hair follicles as they are radiosensitive. Obviously, hair loss does not result in death, and the hair will regrow after treatment ends, but it can impair the quality of life of patients. Patients often purchase wigs before treatment since there are almost no drugs that prevent hair loss from occurring.

Recently, SP is considered as an important regulator of the hair growth cycle. Siebenhaar et al. demonstrated that the numbers of SP-immunoreactive nerves and SP levels in the skin tissue of mice was increased during early stages of alopecia areata (AA) development and decreased during advanced stages of AA (7). In this study, we demonstrated that PPT-A mRNA expression in the skin of gamma-irradiated mice was detected on the 10th and 30th days after irradiation and that radiation-
induced hair loss was inhibited by CP-99,994 in response to the elevation of PPT-A mRNA expression. We have previously reported that bepotasine besilate, which has anti-inflammatory effects in addition to its histamine H₁-receptor antagonistic action, significantly suppressed radiation-induced hair loss (6). Andoh et al. reported that bepotasine suppressed substance P–induced skin reactions in mice (9). From these observations, the present results indicated that SP via NK₁R is involved in the development of radiation-induced hair loss. We could not elucidate the precise cells expressing PPT-A mRNA in the skin. However, Bae et al. and Katayama et al. reported that PPT-A mRNA was expressed in dermal fibroblasts and epidermal keratinocytes in cutaneous inflammatory conditions (10–12). Furthermore, previous studies reported that PPT-A mRNA was found in monocytes, lymphocytes, and macrophages (13, 14). Therefore, it is speculated that PPT-A mRNA expression may be induced in the dermal or epidermal cells or inflammatory cells infiltrated into dermis around the hair follicle in the irradiated skin tissue.

We found that severe hair loss was observed in the mice treated with an NK₁R antagonist around 20 days after irradiation and that PPT-A mRNA was not detected in the irradiated skin on the 20th day. Xiao et al. reported that inflammatory cytokines such as tumor necrosis factor-α, interleukin-1α/β, and nitric oxide were involved in the development of gamma irradiation-induced hair loss, indicating that severe hair loss is induced by inflammatory mediators other than SP and NK₁R (15).

In summary, our studies revealed that SP and NK₁R may play important roles in the development of radiation-induced hair loss and that pharmacotherapy with NK₁R antagonist is a promising method for preventing such symptoms in humans.

**Fig. 2.** Time course of the expression of preprotachykinin-A (PPT-A) mRNA in the skin of sham-irradiated (n = 3) and 40 Gy of gamma irradiated (n = 3) mice. Columns and bars are represent the means value ± S.E.M. of values expressed as the ratio of PPT-A to GAPDH mRNA expression. *P < 0.05 vs. sham-irradiated group.

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**References**