PROPHYLAXIS OF ORAL MUCOSITIS ASSOCIATED WITH CHEMORADIOTHERAPY FOR ORAL CARCINOMA BY AZELASTINE HYDROCHLORIDE (AZELASTINE) WITH OTHER ANTIOXIDANTS

Tokio Osaki, DDS, DMD, Eisaku Ueta, DDS, Kazunori Yoneda, DDS, Jusui Hirota, DDS, DMD, and Tetsuya Yamamoto, DDS

Background. One of the dose-limiting adverse effects of chemoradiotherapy is mucositis, especially oral mucositis. Prophylaxis of severe mucosal reaction would allow application of aggressive chemoradiotherapy to malignancies.

Methods. Sixty-three patients who received inductive concomitant chemoradiotherapy with cobalt 60 (^{60}Co, ~30 Gy), peplomycin (PLM, ~38 mg), and 5-fluorouracil (5-FU, ~3,500 mg) were included in this study. From the start of therapy to the disappearance of oral erosion, 37 patients received daily doses of Azelastine (2 mg) + vitamin C (500 mg) + vitamin E (200) + glutathione (200 mg) (azelastine group), whereas the other 26 patients received the same regimen without azelastine (control group). The severity of oral mucositis in both groups was evaluated periodically.

Results. At 10 Gy with 15 mg PLM and 1,250 mg 5-FU, grade 1 mucositis (redness of the oral mucosa) was induced in 14 patients in the control group and five patients in the Azelastine group. At 20 Gy with 30 mg PLM and 2,500 mg 5-FU, grade 2 (erosion with mild irritation) and grade 3 (extensive erosion with marked irritation) stomatitis were observed in 9 and 3 of the control patients and 5 and 1 in the Azelastine group, respectively. At the completion of treatment, mucositis in 21 patients in the Azelastine group remained at grade 1 or grade 2, whereas grades 3 and 4 (ulceration with severe contact pain) mucositis were observed in 6 and 10 patients, respectively. However, in the control group, grades 1 and 2 were observed in only 2 and 3 cases, whereas grades 3 and 4 stomatitis were induced in 6 and 15, respectively. Azelastine suppressed neutrophil respiratory burst both in vivo and in vitro, and also suppressed cytokine release from lymphocytes. However, neutrophil superoxide dismutase (SOD) activity was negligibly suppressed.

Conclusion. A regimen including Azelastine, which suppresses reactive oxygen production and stabilizes cell membranes, may be useful for the prophylaxis of mucositis due to chemoradiotherapy.

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Ulcerative mucositis is one of the most serious and dose-limiting adverse effects of chemo- and radiotherapy. Severe contact pain markedly reduces the patient's quality of life. Even very soft food cannot be ingested when the oral mucosa is severely ulcerated, and the pain and irritation caused by this condition may lead the patient to abandon treatment.[1]

Among antineoplastic agents, bleomycin (BLM) and its derivative peplomycin (PLM), as well as 5-fluorouracil (5-FU), are well known causes of severe oral mucositis.[2–6] At the optimal concentration, PLM stimulates neutrophils and macrophages to produce reactive oxygen species,[7,8] and also enhances lymphocyte cytokine production. Highly reactive radicals are also pro-

From the Department of Oral Surgery, Kochi Medical School, Kochi, Japan.

Address reprint requests to Dr. Osaki at the Department of Oral Surgery, Kochi Medical School, Kohasu, Oko-cho, Nankoku-city, Kochi 783, Japan.

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duced by 5-bromouracil, a DNA base analogue, in the presence of hydrated electrons.[9] A large amount of reactive oxygen species and lipoperoxides are produced in irradiated tissues.[10] Therefore, maximal cell impairment is induced by the combination of these drugs and ratiotherapy.

Some reactive oxygen-scavenging and suppressive agents have been tried clinically in attempts to control mucositis due to chemoradiotherapy.[11–13] In these trials, vitamin C and vitamin E have been used due to their antiradical activities. Glutathione has also been used because –SH base reduces oxidants. Despite these efforts, any effective method of preventing mucositis has not yet been developed. Aggressive induction chemoradiotherapy, which allows surgery and preservation of oral function, can be applied to head and neck cancer if mucositis is alleviated.

Azelastine hydrochloride (Azelastine) has widely been used in many allergic diseases. Based on its cell membrane stabilizing and leukocyte-suppressing actions,[14–17] clinical improvement of the diseased conditions including aphthous ulcers in Behcet's disease has been obtained. This study investigated the protective effect of azelastine on the oral mucosa in oral cancer patients who received inductive chemoradiotherapy.

MATERIALS AND METHODS

Patients and Treatments. Sixty-three patients with oral squamous cell carcinoma (except for one case of maxillary sinus carcinoma) were included in this study. To avoid interference of γ-ray and radio-ostitis after radiotherapy (and because of periodontal disease and large cavities), the teeth corresponding to the tongue and floor of the mouth lesions were extracted before cancer therapy. Metal crowns were removed if possible with the aim of decreasing irritation to the mucous membrane.

All patients received inductive chemoradiotherapy with cobalt 60 (^{60}Co) + PLM + 5-FU. Irradiation was fractionated (2 Gy/day) and delivered five times a week. PLM (5 mg) was generally administered subcutaneously three times a week, but 12 patients received intraarterial PLM three times a week. Immediately after each irradiation session, 5-FU (250 mg) was injected intravenously. When complete response was obtained, the patient was followed without surgical treatment. In cases of partial or minor response, 31 and 22 tumors in the Azelastine and the control group, respectively, were surgically removed. In the first (1988–1989) and second (1990–1992) periods of the study, 26 patients received regimen 1 (control group) and 37 patients received regimen 2 (azelastine group) for mucositis prophylaxis. Regimen 1 was composed of oral administration of vitamin C (500 mg/day), vitamin E (200 mg/day), and glutathione (200 mg/day), whereas regimen 2 included Azelastine (2 mg/day; Eisai Co., Tokyo, Japan) as well. These drugs were administered about 3 hours after irradiation or injection of PLM and 5-FU. The clinical profiles of the subjects (age, gender, tumor site, tumor stage, and mean doses of ^{60}Co, PLM, and 5-FU) in both groups (control and azelastine group) are shown in Table 1. There was no difference in the distribution of TNM stages between the two groups.

Grading of Oral Mucositis. The severity of oral mucositis was graded as follows: grade 1, no inflammation or the oral mucosa became reddish without any irritation; grade 2, the oral mucosa showed erosion with mild irritation, but solid food could be eaten; grade 3, the oral mucosa showed extensive erosion in association with marked irritation, and only soft food could be taken; grade 4, the oral mucosa was so extensively ulcerated that oral intake of food was impossible due to severe contact pain. There were no cases of suspected recurrent herpetic infection during the ex-

Table 1. Clinical profiles of the two groups examined.

Group	Male/ female	Mean age ± SD	Tumor location					Radiotherapy* (Gy)	PLM* (mg)	5-FU* (mg)
			Gingiva	Tongue	Floor	Cheek	Sinus			
Control (n = 26)†	19/7	64.7 ± 11.3	11(2)‡	9(1)‡	4	2	0	28.5 ± 4.9	38.8 ± 11.2	3,640 ± 1,330
Azelastine (n = 37)†	26/11	64.6 ± 13.5	20(7)‡	14(1)‡	2	0	1(1)‡	28.5 ± 5.3	35.5 ± 12.1	2,970 ± 1,070

*There were no statistically significant differences in the doses of ^{60}Co, PLM, or 5-FU between the control and Azelastine groups.
†In 8 control and 4 Azelastine-treated patients, the planned treatment could not be accomplished because of severe mucositis.
‡Numbers in parentheses represent chemotherapy via intraarterial infusion.

amination. Only mouthwash with an antifungal reagent solution was ordered, and no special treatment for oral hyperesthesia was applied before or after the inductive cancer therapy.

Preparation of Peripheral Blood Polymorphonuclear Leukocytes (PMN) and Peripheral Blood Mononuclear Cells (PBMC). Leukocytes were collected from heparinized peripheral blood using Ficoll-Hypaque gradients (Pharmacia Fine Chemicals, Piscataway, NJ). After centrifugation at 400 *g* for 30 minutes, the white blood cells lying just above the red blood cell layer were collected and resuspended in phosphate-buffered saline (PBS). Following dextran sedimentation, contaminating red blood cells were lysed by hypotonic shock with sterile-distilled water for 30 seconds. The remaining cells were then washed twice with PBS and adjusted to the desired concentration.

PBMC were collected from the top layer, washed three times with PBS, and suspended in RPMI 1640 medium supplemented with 10% autologous serum.

Prepared PMN were stained with Giemsa solution, and more than 96% purity was morphologically ascertained. More than a 98% viability was proven by trypan blue exclusion.

Assay of O_2^- Generation. O_2^- generation was spectrophotometrically assayed by reduction of ferricytochrome C (Sigma, St. Louis, MO) using a Shimadzu UV-300 double-wavelength spectrophotometer (Shimadzu Seisakusho Ltd., Kyoto, Japan) equipped with a thermostatted cuvette holder. The reaction mixture of PMN (1×10^7 cells/mL) and 100 μM ferricytochrome C was preincubated at 37°C for 1 minute, the stimulating agent (50 ng/mL phorbol myristate acetate [PMA], Sigma: 10^{-7} M *N*-formyl-methionyl-leucyl-phenylalanine [FMLP], Sigma) was added, and the change in absorbance at 550–540 nm was measured. The O_2^- concentration was then calculated from the linear portion of the cytochrome c reduction curve.

Assay of Superoxide Dismutase (SOD) Activity. Japanese white rabbits were treated with intramuscular injection of saline or azelastine (0.04–1.0 mg/kg/day) for 5 days. Five hours after the fifth injection, peripheral PMNL, alveolar macrophages,[18] and the lung tissue were obtained, and total SOD activity was assayed by Ōyanagi's method[19] measuring optical absorption at 550 nm.

Titration of Cytokines. PBMC (2×10^6 cells) obtained from five healthy volunteers were incubated in RPMI 1640 medium containing 10% autologous serum with or without Azelastine (3 ng/mL to 3 μg/mL) and 100 U/mL of interleukin-2 (IL-2) for 72 hours. After incubation, the culture supernatants were stored at −80°C until cytokine titration. IL-1β, tumor necrosis factor-α (TNF-α), and GM-CSF levels were determined using enzyme-amplified sensitivity immunoassay (EASIA) kits, and IL-6 and IL-8 levels were measured with an enzyme-linked immunosorbent assay (ELISA) kit according to the manufacturers' directions (IL-β, TNF-α, and GM-CSF, MEDGENIX Diagnostic, Fleurus, Belgium; IL-6, Research and Diagnostic Systems, Minneapolis, MN; IL-8, Tore Fuji Bionics Co., Tokyo, Japan). Each kit use for measuring cytokines in the supernatants was standardized against WHO standards.

Hypotonic Shock and H_2O_2 Treatment. The effect of Azelastine on cell membrane stability was examined in vitro by assessing red cell hemolysis and viability after hypotonic shock and H_2O_2 treatment. Sheep red blood cells (SRBC; 5×10^6/mL) were preincubated at 37°C for 1 or 2 hours in saline containing various concentrations of Azelastine. The incubation solution was then diluted twofold with distilled water, and hemolysis was measured by the change in absorbance at 600 nm after 1 hour of incubation in the hypotonic solution.

Two squamous cell carcinoma cell lines (SCC-1 and SCC-2) were used for the membrane stability test. The tumor cells were suspended in DMEM supplemented with 10% FBS and Azelastine (0–30 μg/mL), and then preincubated in a 5% CO_2 incubator at 37°C for 1 or 24 hours. Subsequently, H_2O_2 was added to the medium at a final concentration of 30 mM, the cells were incubated for the indicated time and cell viability was examined by trypan blue exclusion.

Tumor Remission Rate. Two to three weeks after induction therapy, clinical response in each case underwent final evaluation, and the tumor remission rate was calculated by the following formula:

$$\frac{(A \times B - A' \times B') \times 100}{\text{longitudinal (A)} \times \text{transversal tumor diameter (B) before treatment}} (\%)$$

where, A′ and B′ indicate tumor diameters after treatment.

RESULTS

Inflammation of the oral mucosa occurred later and was less severe in the Azelastine group. Although redness of the mucosa was noted only in a few patients of the Azelastine group during the first week, the oral mucosa in nearly half of the control patients became reddish and slightly irritated at 10 Gy of irradiation with 15 mg PLM plus 1,250 mg 5-FU (about 1 week after treatment started). Ulceration of the mucosa occurred in almost half of the controls at approximately 16 Gy with 25 mg PLM and 2,000 mg 5-FU (Figure 1). Erosion was observed in about half of the Azelastine group 2 weeks after treatment (20 Gy + 30 mg PLM + 2,500 mg 5-FU), when most patients in the control group had oral erosion and ulcers.

The severity of mucositis differed greatly between the control and Azelastine groups as therapy advanced (Table 2). At 16 Gy of radiotherapy + 20 mg PLM + 2,000 mg 5-FU, the control group exhibited grade 1 mucositis in 13 cases, grade 2 in 9 cases, grade 3 in 3 cases, and grade 4 in 1 case. At the same point in therapy, the severity of mucositis in the Azelastine group was grade 1 in 20 cases, grade 2 in 5 cases, grade 3 in 1 case, with no cases of grade 4 mucositis.

As treatment continued, mucositis was aggravated in both groups, but the azelastine group had less severe mucositis compared with the control group. Even at 24 Gy + 30 mg PLM + 2,500 mg 5-FU, 14 patients in the Azelastine group remained in grade 1, and 11 patients in grade 2. For about 3 weeks after treatment, grade 1, grade 2, grade 3, and grade 4 mucositis were observed in 2, 3, 6, and 15 patients in the control group, and 8, 13, 6, and 10 in the Azelastine group, respectively. Significant differences of the rates of grade 1 and grade 3 mucositis were observed between the Azelastine group and the control group ($p < 0.02$, χ^2-test). Compared to the azelastine group, the control group needed a longer time for grade 4 mucositis to improve. Duration from the completion of therapy to the disappearance of oral ulcers was 14.9 ± 4.8 and 19.0 ± 5.8 days, respectively ($p < 0.05$, t test).

PMN superoxide production in both groups was about 86 pmol/min/10^4 cells with PMA stimulation and about 73 pmol with FMLP stimulation before treatment (Figure 2). After treatment, O_2^- production was lower in the Azelastine group. In this group, PMA- and FMLP-induced O_2^- production was reduced to 60.3 ± 12.7 pmol and 52.5 ± 12.4 pmol, respectively, whereas these levels in the control group were 69.8 ± 15.1 pmol and 57.3 ± 15.2 pmol, respectively, being significantly superior to the Azelastine group in PMA-induced O_2^- volume. However, no correlation was found between the O_2^- generation level and the severity of mucositis.

SOD activity was only slightly decreased in vivo by treatment with Azelastine (Table 3). When a low (0.04 mg/kg/day) or median dose (0.2 mg/kg/day) of Azelastine was injected, SOD activities in the peripheral PMN, alveolar macrophages, and rabbit lung tissue were negligibly decreased, and the decrease of SOD activities was

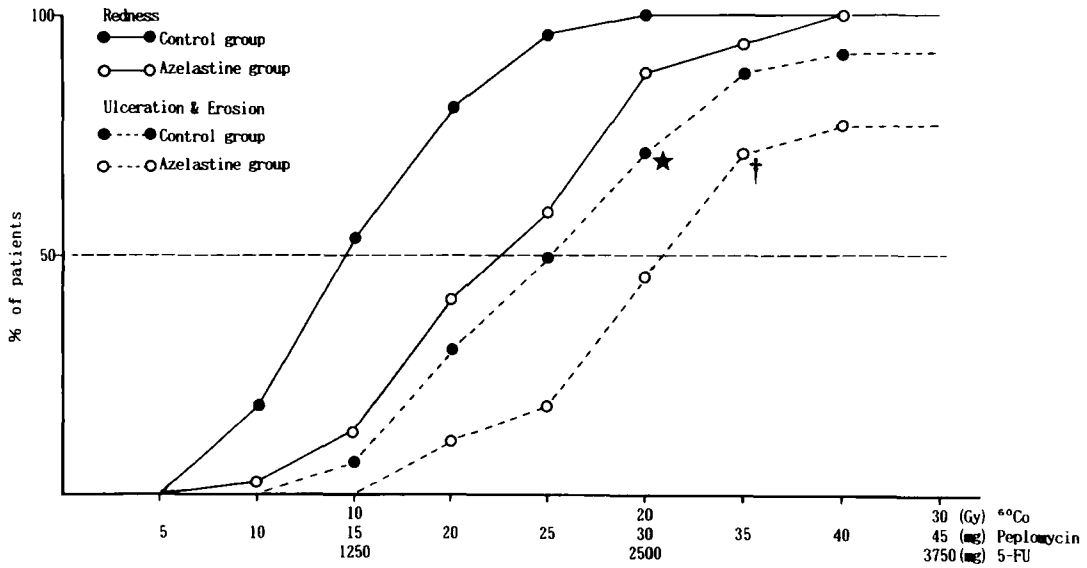

FIGURE 1. Chemoradiotherapy-induced mucositis in oral cancer patients with (★) or without (†) Azelastine therapy. The planned treatment was finished in 2 and 4 cases, respectively.

Table 2. Severity of mucositis during the course of chemoradiotherapy.

Dose	Azelastine	Severity of mucositis			
		Grade 1	Grade 2	Grade 3	Grade 4
Replomycin 20 mg	–	13(2)(50.0%)	9(1)(34.6%)‡	3 (11.5%)	1 (3.8%)
^{60}Co 16 ± 1.2 Gy	+	20(4)(76.9%)	5(4)(19.2%)‡	1(1)(3.8%)	0 (0%)
Replomycin 30 mg	– (*n* = 22)*	3(1)(13.6%)‡	9(1)(40.9%)	7(1)(31.8%)‡	3 (13.6%)
^{60}Co 24 ± 1.9 Gy	+ (*n* = 33)*	14(1)(42.4%)‡	11(4)(33.3%)	5(2)(15.2%)‡	3(2)(9.1%)
Completion of treatment†	–	2 (11.5%)‡	3(2)(15.4%)‡	6 (23.1%)	15(1)(57.7%)‡
	+	8 (21.6%)‡	13(2)(35.1%)‡	6(4)(16.2%)	10(3)(27.0%)‡

PLM, peplomycin; ^{60}Co, cobalt^{60}.
Numbers in parentheses represent intraarterial infusion.
**Four patients whose treatment finished less than 30 mg of PLM were excluded.*
†When the scheduled treatment was abandoned because of severe mucositis, time of cessation was regarded as the end-point of the treatment.
‡$p < 0.02$ (χ^2 test).

not significant even after a high Azelastine dose (1.0 mg/kg/day).

Azelastine suppressed lymphocyte production of IL-6, TNF-α, and GM-CSF in a dose-dependent manner, but negligibly suppressed IL-1β or IL-8 generation (Table 4). When 300 mg/mL or more of Azelastine was added to the culture medium, lymphocytes produced significantly less TNF-α and GM-CSF compared with the control levels (1,680 ± 164 pg/mL vs 2,220 ± 245 pg/mL for TNF-α and 170 ± 34.6 pg/mL vs 252 ± 46.5 pg/mL or GM-CSF).

Azelastine dose-dependently protected SRBC and tumor cells against hypotonic shock, and also protected both SRBC and tumor cell lines from damage by H_2O_2 (Figure 3). Cell viability after H_2O_2 treatment was increased by 10% to 20% in the presence of 30 to 300 ng/mL Azelastine, but at concentrations greater than 3 μg/mL azelastine reduced cell viability.

Good tumor remission was observed in both groups (Figure 4). In 26 tumors of the control group, 9 demonstrated a complete response (CR) to inductive chemoradiotherapy, 12 a partial re-

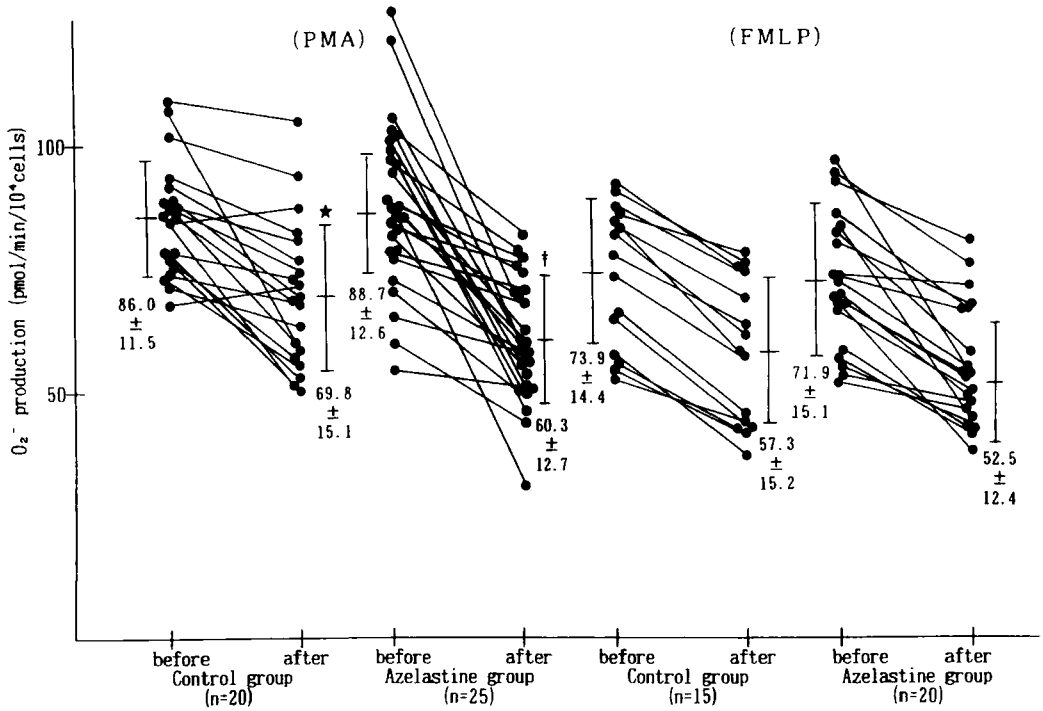

FIGURE 2. PMN superoxide production in patients with or without Azelastine therapy. In PMA-induced O_2^- generation after treatment, a statistically significant difference between the control group (★) and the Azelastine group (†) was detected by *t* test ($p < 0.05$).

Table 3. Influence of in vivo Azelastine on superoxide dismutase (SOD).

	Saline (control)	Azelastine		
		0.04 mg/kg	0.2 mg/kg	1.0 mg/kg
Peripheral PMN	3.97 ± 0.38*	3.92 ± 0.51	3.60 ± 0.42	3.10 ± 0.45
Alveolar Mø	9.60 ± 0.82	9.28 ± 0.82	9.09 ± 0.76	8.38 ± 0.93
Lung tissue	9.02 ± 1.37	8.43 ± 1.69	8.30 ± 1.62	8.16 ± 1.72

Three rabbits were treated with intramuscular injection of saline and each indicated dose of Azelastine for 5 days. Five hours after the fifth injection, peripheral PMN, alveolar macrophages, and lung tissue were obtained, and total SOD activity (units) was assayed.
**U/mg protein.*

sponse (PR) (>50% of regression rate), and 5 a minor response (MR) (<50% of regression rate). In the Azelastine group, CR, PR, and MR were obtained in 15, 19, 3 tumors, respectively.

DISCUSSION

Combining radiotherapy with chemotherapy increases tumor remission,[20,21] but also induces more severe adverse effects, especially mucositis. Mucositis is more frequent and more hazardous than bone marrow suppression in patients with head and neck tumors. Permanent atrophy of the tongue papillae with hyperirritability and even aspiration pneumonia may develop if the ulcerative mucositis is not adequately controlled.

BLM, a PLM analogue, binds iron (Fe) to form many salts. These BLM–Fe complexes activate oxygen and cause denaturation of DNA.[22–24] PLM is also considered to produce this reaction. The optimal dose of PLM enhances leukocyte cytokine production and release of active oxygen species from neutrophils and macrophages,[8] and this seems to contribute not only to pulmonary fibrosis[7] but also to mucositis. In addition to suppressing DNA synthesis, 5-FU seems to damage cells by producing radioactive uracilylradicals.[25] Recently, continuous tegafur–uracil (UFT) administration has frequently been used in patients with large bowel tumors, and severe mucositis has been associated with such therapy.[3] The recent increase in the use of PLM and 5-FU has increased the need to find agents that prevent mucositis.

Gamma rays are potent radical inducers. Thus, the combination of PLM and 5-FU with ionizing radiotherapy can cause very severe tissue damage and severe ulcerative mucositis in the early stages of combined therapy.

All the drugs used in the present study to prevent mucositis have a radical scavenging ability. Vitamin C scavenges O_2^-, hydroxy radicals (·OH), and peroxide radicals (HOO·), but it cannot trap the radicals in membrane lipids.[26–28] Vitamin E is a fat-soluble vitamin and reacts with the membrane radicals.[29] Vitamin C and vitamin E cooperate synergistically by reducing each other.[29] Glutathione also reduces ascorbic acid and vitamin E.[30] Reduction of hydrogen peroxide to water occurs in the presence of glutathione peroxidase and its substrate glutathione. Thus, the combined administration of these radical scavengers was hoped to be effective, but satisfactory prevention of mucositis was not obtained.

It is well known that Azelastine suppresses

Table 4. Influence of Azelastine on cytokine generation in lymphocytes.

Azelastine	IL-1β	IL-6	IL-8	TNF-α	GM-CSF
0	28.7 ± 14.6	1,110 ± 53	1,540 ± 170	2,220 ± 245	252 ± 46.5
3 ng/mL	26.9 ± 7.2	ND	1,540 ± 210	2,170 ± 217	207 ± 28.9
30 ng/mL	26.9 ± 10.4	933 ± 79*	1,520 ± 190	1,850 ± 240	193 ± 37.9
300 ng/mL	26.0 ± 14.9	880 ± 73*	1,520 ± 180	1,830 ± 201*	188 ± 20.2*
3 μg/mL	25.3 ± 14.5	ND	1,510 ± 210	1,680 ± 164*	170 ± 34.6*

ND, not determined; IL, interleukin; TNF-α, tumor necrosis factor-α.
Lymphocytes (10^6 cells/mL) were incubated for 72 hours in the presence of 100 U/mL IL-2 and each indicated concentration of Azelastine. Cytokines produced in the supernatants were measured as described in Materials and Methods. Each value indicates mean ± standard deviation (pg/mL) of duplicate in four leukocyte donors. Values indicate mean ± standard deviation.
**$p < 0.05$, t test.*

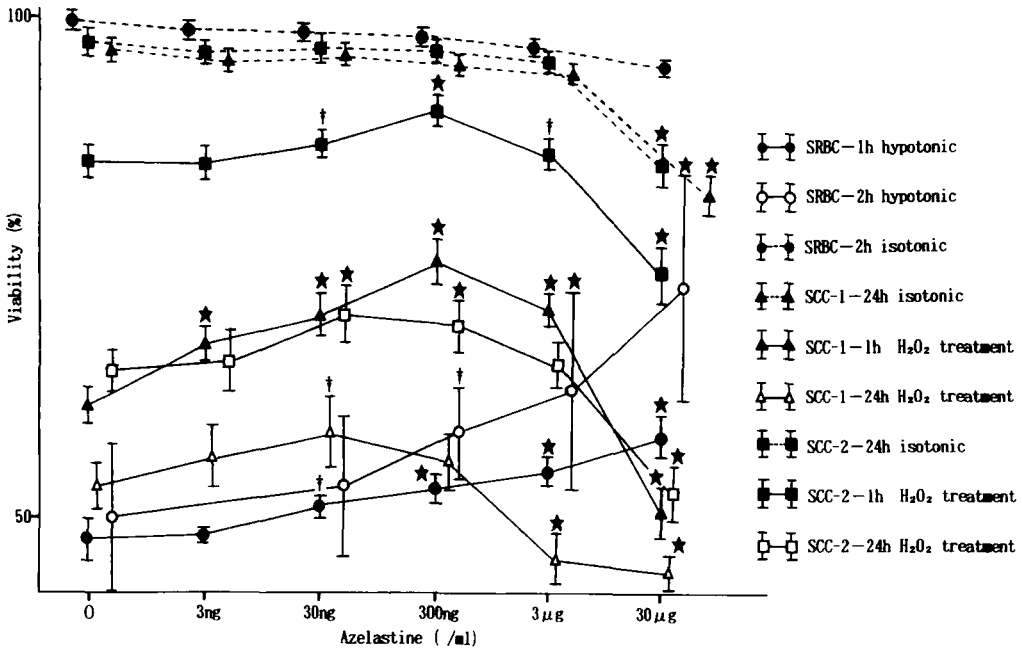

FIGURE 3. Protection of SRBC and tumor cells from hypotonic shock and H_2O_2 by Azelastine. SRBC and tumor cell lines were incubated in the presence of the indicated volume of Azelastine for a hour, and then suspended in hypotonic medium (half saline) or in the culture medium containing 30 μM H_2O_2. After incubation for 1, 2, or 24 hours, cell viability was determined by trypan blue exclusion. A protective effect was observed at Azelastine concentrations of ≥30 ng/mL, but 3–30 μg/mL Azelastine actually damaged the cells (★), $p < 0.0002$; †, $p < 0.05$ by t test [vs controls]).

neutrophil reactive oxygen production[14–17] and mast cell histamine release.[31,32] However, it negligibly affects SOD. SOD activity of rabbit pulmonary macrophages was almost constant even after rabbits were treated with high doses of Azelastine. Furthermore, the ability of Azelastine to protect the cell membrane was demonstrated in the present study. Taken together, it is likely that combining this drug with other radical scavengers is advantageous for cancer therapy-induced mucositis. In the Azelastine group, oral ulceration developed much later during treatment, and the severity of mucositis was milder. Azelastine allowed chemoradiotherapy up to doses of approximately 20 Gy, with 25 mg PLM and 2,500 mg 5-FU, without severe (grades 3 and 4) mucositis. Therefore, the combined anti-mucositis regimen that included Azelastine seemed to be satisfactory.

Although it is likely that the main effective pharmacologic action of Azelastine is the suppression of reactive oxygen generation, this action was shown to be weak at low serum concentrations. Along with the direct suppression of reactive oxygen generation, Azelastine may indirectly suppress generation of reactive oxygen species by down-regulating cytokine production. In fact, the production of IL-6, TNF-α, and GM-CSF, which enhance reactive oxygen production[33–35] of neutrophils and macrophages and also aggravate inflammation,[36,37] were lowered in the presence of Azelastine. Thus, the clinical

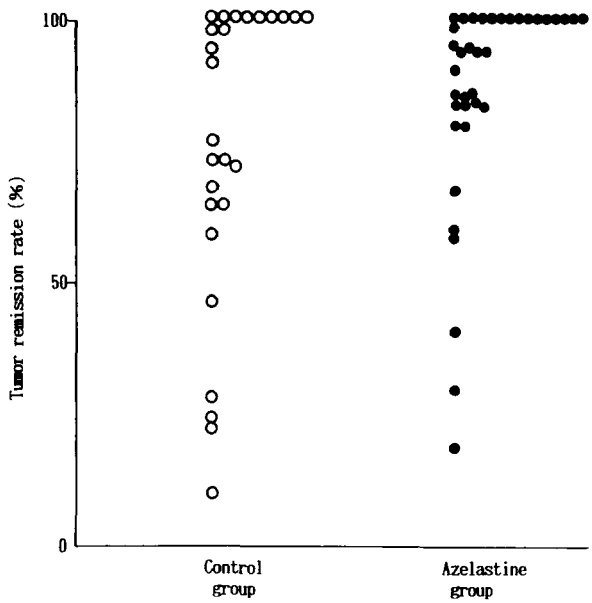

FIGURE 4. Tumor remission rate in the control and Azelastine groups. Two to 3 weeks after induction therapy, tumor remission rate was calculated by the formula described in Materials and Methods. A slightly higher remission rate was obtained in the Azelastine group.

effect of Azelastine seems to depend largely on suppression of reactive oxygen generation either directly or via modulating cytokine production, as well as a membrane-protective effect.

Some investigators consider that the life of reactive oxygens is extremely short, and they rapidly disappear. Therefore, no remarkable therapeutic effects can be expected if the scavenging agents are administered some hours after reactive oxygen are generated. However, there are long-lasting reactive oxygen species, such as lipoperoxide and oxygenated–SH residues. To reduce the reactive oxygen species, vitamins E and C are used and decreased in the tissue. Therefore, a supply of these vitamins seems to be needed for maintenance of good reactive oxygen-scavenging ability. Furthermore, down-regulation of reactive oxygen generation seems to be maintained during administration of Azelastine. Considering this, the mechanism of the present result appears to be explained.

Azelastine suppressed in vitro tumor cell proliferation and DNA synthesis, and also inhibited induction of lymphokine-activated killer cells (data not shown). Therefore, this agent does not seem to be disadvantageous for cancer therapy, although contradictive for immunotherapy. In fact, even a slightly improved tumor response was observed in the Azelastine group compared to that in the control group. To obtain greater prevention of cancer therapy-induced mucositis, further studies including the timing of anti-oxidant administration are recommended.

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