Novel Submicronized Rebamipide Liquid with Moderate Viscosity: Significant Effects on Oral Mucositis in Animal Models

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This study aimed at developing a novel rebamipide liquid for an effective treatment of oral mucositis. The healing effects of a variety of liquids comprising submicronized rebamipide crystals were investigated using a rat cauterization-induced oral ulcer model. Whereas 2% rebamipide liquid comprising micro-crystals did not exhibit significant curative effect, 2% rebamipide liquids comprising submicronized crystals with moderate viscosities exhibited healing effects following intra-oral administration. The 2% and 4% optimized rebamipide liquids showed significant healing effects in the rat oral ulcer model (p<0.01). In addition, in the rat radiation-induced glossitis model, whereby the injury was caused to the tongue by exposing only around the rat’s snout to a 15 Gy of X-irradiation, the 2% optimized rebamipide liquid significantly reduced the percent area of ulcerated injury (p<0.05). In conclusion, the submicronized rebamipide liquid with moderate viscosity following intra-oral administration showed better both healing effect in the rat oral ulcer model and preventive effect in the rat irradiation-induced glossitis model.

Key words rebamipide; mucositis; submicronized; X-irradiation; liquid

Cancer therapies frequently cause oral mucositis, an important adverse event for which no effective preventive or therapeutic medications have been established to date. Oral mucositis is often painful and causes chewing and swallowing difficulties (dysphagia). In particular, for the patients with the head and neck cancer, the radiochemotherapy causes oral mucositis at almost 100%. Oral mucositis is one of the major causes lowering quality of life of patients and interfere with subsequent cycles of treatment, which is regarded as an important issue. Thus, cancer therapy-related oral mucositis is frequently a dose-limiting complication and underlines the need to establish a new medical treatment for its prevention and cure.25

Rebamipide was developed to promote the healing of acetic acid induced gastric ulcers and also to prevent ulcer recurrence in rats by Otsuka Pharmaceutical Co., Ltd. (Tokyo, Japan) and has a chemical name of 2-(4-chlorobenzoylamino)-3-(2-oxo-1,2-dihydroquinolin-4-yl) propanoic acid.29 This agent is commercially available in Japan and other Asian countries for the treatment of gastric mucosal lesions such as peptic ulcer and gastritis. In addition, the ophthalmic suspension of rebamipide micro-crystals has been approved for the treatment of dry eye in Japan.

The main pharmacological effects of rebamipide have been reported to enhance endogenous prostaglandin production in the gastric mucosa5,5 and to suppress the generation of oxygen free radicals,5,7 a key mechanism contributing to gastric mucosal injury. Rebamipide has also been shown to inhibit neutrophil activation8,9 production of inflammatory cytokines from mononuclear cells, gastric mucus, and vascular endothelial cells,10,11 and other inflammatory reactions.22 These effects required 10−8 to 10−7M (0.37 to 37 µg/g tissue) of rebamipide in vitro.4,6,9,10 Healthy volunteers administered a single oral dose of 100 to 300 mg rebamipide showed local gastric level of rebamipide ≥10−4 M (37 µg/g tissue) within 1 to 2 h postdose indicating that this agent highly distributes to gastric mucosa.13 Pharmacokinetic studies in animals suggested that rebamipide acts directly on peptic ulcer and gastritis.14

The effects of rebamipide on oral mucositis have also been investigated. Pilots studies demonstrated that gargle with rebamipide before radiotherapy effectively prevented the development of radiation-induced oral mucositis.15–17 In these clinical studies, gargle formulations were prepared by suspending rebamipide tablets in a mixture of an aqueous solution of polyethylene oxide (Alkox6, Meisei Chemical Works, Ltd., Kyoto, Japan) and a gelling agent (Inagel7, Ina Food Industry Co., Ltd., Nagano, Japan) or by suspending in water or in an aqueous solution of carboxymethylcellulose sodium (CMCNa).17 In addition, the concentrations of rebamipide in these formulations were entirely low (0.1% or 0.2%).

Considering that the gastric mucosal levels of rebamipide were an important factor influencing its effect on peptic ulcer and gastritis, we hypothesized that the oral mucosal concentration of rebamipide was key to the prevention and cure of oral mucositis. However, no detailed information exists on the conditions or parameters (such as the rebamipide concentration, particle size, formulation viscosity, types of excipients, and the route of administration) that might enhance the direct distribution of rebamipide to the oral mucosa.

Here, we tried to develop a new rebamipide liquid which can higher distribute to the oral mucosa directly. The oral mucositis healing and prevention effects of the formulation were investigated using a rat cauterization-induced oral ulcer model and a rat radiation-induced glossitis model as a more clinically relevant experimental system. We controlled particle sizes of rebamipide, liquid viscosity and rebamipide concentration in...
the formulation, which is thought to contribute the distribution of rebamipide to the oral mucosa, in order to develop more effective rebamipide liquid.

MATERIALS AND METHODS

Materials A drug substance of rebamipide, manufactured by Otsuka Pharmaceutical Co., Ltd., was used as an active ingredient in this study. As dispersants, hydroxypropylmethylcellulose (HPMC, TC-5E grade, Shin-Etsu Chemical Co., Ltd., Tokyo, Japan) and polyvinylpyrrolidone K25 or K30 (PVP K25 or PVP K30, BASF AG, Land Rheinland-Pfalz, Germany) were used. As viscosity enhancing agents, hydroxypropylcellulose (HPC, HPC-L grade, Nippon Soda Co., Ltd., Tokyo, Japan), HPMC (60SH4000 grade, Shin-Etsu), PVP K90 (BASF), and pullulan (Hayashibara Co., Ltd., Okayama, Tokyo, Japan) were used. CMCMNa (Wako Pure Chemical Industries, Ltd., Osaka Japan) was used to disperse the suspensions of micro-crystals. D-Sorbitol (Wako) was used as an isotonic agent, stevia (Steviron\textsuperscript{®} C, Morita Kagaku Kogyo Co., Ltd., Osaka, Japan) was used as a sweetening agent, and methyl parahydroxybenzoate (Wako) as a preservative. A commercial strawberry flavoring agent (San-Ei Gen F. F. I., Inc., Osaka, Japan) was also employed. Hydrochloric acid and sodium hydroxide were purchased commercially (Wako).

Animals Specific pathogen-free, male Crl:CD Sprague-Dawley rats (Charles River Laboratories Japan, Inc., Hino, Japan) were used. Rats were housed at a temperature of 23±2°C and a humidity of 60±10%, with a 12-h light-dark cycle, and were allowed access to food (CRF-1 pellets, Oriental Yeast Co., Ltd., Tokyo, Japan) and tap water ad libitum. All experiments were carried out in accordance with the “Otsuka Pharmaceutical Guidelines on Animal Experiments,” which complied with the “Principles of Laboratory Animal Care” (NIH publication No. 85-23, revised in 1985).

Preparation of Rebamipide Liquids Suspensions of submicronized rebamipide crystal were prepared on a per-liter basis as described below.\textsuperscript{18} The amounts of the components were calculated in relation to the desired total volume.

A predetermined amount of the dispersant was dissolved in 200 g of purified water and mixed with 14.2 g of concentrated hydrochloric acid. Purified water was then added to make a 275 g dispersant-containing hydrochloric acid solution. Separately, a sodium hydroxide solution was prepared by dissolving 4.4 g of sodium hydroxide in 650 g of purified water, and 20 g of rebamipide was added to the solution and dissolved by heating. Purified water was then added to make a 735 g rebamipide-containing sodium hydroxide solution. Preliminary analyses showed that HPMC and PVP provided stable liquids of submicronized crystals, and these dispersants were used in subsequent experiments.\textsuperscript{18} The predetermined amounts of dispersant were 5, 10, and 20 g when the rebamipide-to-dispersant ratios were 1:4, 1:2, and 1:1, respectively.

The dispersant-containing hydrochloric acid solution was placed in a rotary homogenizer and stirred at a speed of 3000 to 5500 rpm. The rebamipide-containing sodium hydroxide solution, warmed at 50°C, was slowly added to the dispersant-containing hydrochloric acid solution to allow precipitation of rebamipide crystals. After the entire amount of the rebamipide-containing sodium hydroxide solution was added, the mixture was stirred for 20 min. The mixture was left to stand overnight, and the pH was adjusted to 6 using a 5 n sodium hydroxide solution.

To obtain rebamipide submicronized crystals, the resulting aqueous rebamipide suspension was subjected to dispersion for 40 min to primary particles on a high-speed homogenizer that employed liquid-liquid shearing force at blade and screen rotor speeds of 18000 and 16000 rpm, respectively (Clearmix\textsuperscript{®} W-Motion, M Technique Co., Ltd., Osaka, Japan). The fine dispersion was then subjected to concentration and demineralization using ultrafiltration equipment (Pellicon 2 Mini, Merck Millipore, Massachusetts, U.S.A.).

The rebamipide concentration in the resulting dispersion was measured by high performance liquid chromatography and then adjusted to the desired values by adding purified water.

HPLC operating conditions for the assay of rebamipide concentration are shown below.\textsuperscript{19}

Detector: An ultraviolet absorption photometer (wave length: 254 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilsilanized silica gel for liquid chromatography.

Column temperature: A constant temperature of about 25°C.

Mobile phase: to 300 mL of phosphate buffer solution, pH 6.2, add 750 mL of water. To 830 mL of this solution add 170 mL of acetonitrile.

A variety of test rebamipide liquids comprising submicronized crystals (Liquids C to I) were prepared by adding viscosity enhancers, an isotonic agent (D-sorbitol), a sweetener (stevia), a preservative (methyl parahydroxybenzoate), and a strawberry flavoring agent. Liquids comprising submicronized rebamipide crystals easily aggregated and the addition of common viscosity enhancers resulted in particle aggregation and sedimentation. In order to increase the viscosities without particle aggregation, HPC for TC-5E, PVP K90 and pullulan for PVP K25 or K30 were chosen as specific viscosity enhancers for use in combination with the dispersants.

As control, 2% rebamipide liquids comprising micro-crys
tals were prepared (Liquids A and B). Predetermined amounts of CMCMNa and D-sorbitol were dissolved in purified water, and the pH was adjusted to 6. Rebamipide powder was dispersed in this solution to prepare a 2% rebamipide liquid comprising micro-cystals (Liquid A). For Liquid B, PVP K30, PVP K90, D-sorbitol, stevia, methyl parahydroxybenzoate, and the strawberry flavoring agent were dissolved in purified water, and the pH was adjusted to 6. Rebamipide powder was dispersed in this solution to prepare a 2% rebamipide liquid comprising micro-cystals (Liquid B).

The compositions of Liquids A to I are presented in Table 1.

The compositions of each vehicle control for Liquids A to I are the same as those of Liquids with the exception of no addition of Rebamipide. These vehicle controls were prepared by dissolving excipients in purified water, and the pH was adjusted to 6.

The viscosities of the test preparations were measured using a rotational viscometer (RC-100 A, Toki Sangyo Co., Ltd., Tokyo, Japan). The mean particle sizes were determined by dispersing the rebamipide liquid in water with refractive index: 1.70–0.20 i using a laser diffractometer (SALD-3000J,
Slowly dripped onto the tongue surface using a feeding tube of rebamipide in such a manner that the test preparations were consumed. 9-week-old male rats were administered a single dose following Intra-oral and Intra-gastric Administration. Differences were considered significant when the p-value was less than 0.05. Plasma and Mucosal Concentrations of Rebamipide Following Intra-oral and Intra-gastric Administration. Consciously 9-week-old male rats were administered a single dose of rebamipide in such a manner that the test preparations were slowly dripped onto the tongue surface using a feeding tube for intra-oral administration. Intra-gastric administration of rebamipide was carried out using a feeding tube according to a conventional procedure. Blood samples were withdrawn into heparinized tubes from the abdominal vena cava of isoflurane-anesthetized rats at 5, 15, 30, 60, 120, 240, and 480 min postdose. After sacrificed by exsanguination, left buccal and tongue tissue samples were prepared. Plasma, and homogenates (10%) of buccal tissue and tongue tissue were prepared and subjected to rebamipide concentration measurements by high performance liquid chromatography-tandem mass spectrometry. Rat Oral Ulcer Model. Oral ulcer was induced in rats using cauterization. First, 7-week-old male rats were anesthetized with isoflurane. After the rats were positioned in a supine position, a 2-mm tip monopolar electrode was placed on the center of the left buccal mucosa to effect cauterization, while the mouth was held open using a rib retractor. The cauterized animals were returned to their cages and allowed to spontaneously recover from anesthesia. After cauterization, lidocaine ointment was applied to the cauterized area to alleviate pain.

The day on which cauterization was performed was defined as the start of the experiment (Day 0). Two days after cauterization (Day 2), the rats were divided into groups by stratified random sampling based on body weight. The test rebamipide liquids and their vehicle controls were applied at doses of 0.5 mL/kg 4 times daily for 5 d, starting on Day 3 after cauterization. For each application, the rats were anesthetized with isoflurane and placed in a left-lateral position. The ulcers on the left buccal mucosa were treated with their mouths held open using a pair of tweezers or a rib retractor. Digital images of the ulcers were taken on Day 8, and the ulcerated area were measured using image processing software (WinROOF, version 5.7.2, Mitani Corporation, Tokyo, Japan). Differences in the ulcerated area between the test rebamipide liquids and their vehicle controls were compared using a two-tailed t-test, except for the experiments to test the dose-dependent profiles of the responses. Dose dependency was tested by comparing responses to the vehicle control and different doses of rebamipide liquids using Dunnett’s test. Differences were considered significant when the p-value was less than 0.05.

Rat Model of Radiation-Induced Glossitis. We recently developed an experimental model of glossitis whereby male rats were exposed to a single dose of X-irradiation to induce glossitis, and this model was used to evaluate the oral mucositis preventive effect of rebamipide liquid. Briefly, 7-week-old male rats were anesthetized with intraperitoneal 45 mg/kg dose of pentobarbital sodium, and two lead plates (each 0.5 mm thick) were used to shield the animal from exposure to irradiation except for the snout (the region of the head anterior to the eyes), which was irradiated with a single 15 Gy dose using an X-irradiation system (CP160, Faxitron X-Ray Corporation, Illinois, U.S.A.). The day on which irradiation was performed was defined as the start of the experiment (Day 0).

Rats were divided into groups by stratified random sampling based on body weights had measured 8 d before irradiation (Day −8). A 2% rebamipide liquid (Liquid I) and its vehicle control were applied at dose of 0.5 mL/kg 6 times daily for 14 d from 7 d before (Day −7) to 6 d after irradiation (Day 6).

Seven days after irradiation (Day 7), rats were anesthetized with isoflurane and euthanized by exsanguination from the abdominal aorta and vena cava after opening of the abdomen. Then, tongue specimens were collected and digital images were taken. Image processing software (WinROOF, version 5.7.2) was used to determine the tongue total surface area (A), the total injured surface area consisting of leukoplakia and ulcers (B), and the ulcerated surface area (C). The preventive effect of rebamipide liquid was evaluated by comparing the

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### Table 1. The Compositions (mg/mL), Mean Particle Sizes (µm), and Viscosities (mPa·s) for Rebamipide Liquids

<table>
<thead>
<tr>
<th>Sample</th>
<th>Micro-crystals</th>
<th>Submicronized crystals</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>A</td>
<td>B</td>
</tr>
<tr>
<td>Active ingredient</td>
<td>Rebamipide</td>
<td>20</td>
</tr>
<tr>
<td>Dispersing agent</td>
<td>CMCNa</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>HPNC (TC-5E)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>PVP K-25</td>
<td></td>
</tr>
<tr>
<td></td>
<td>PVP K-30</td>
<td></td>
</tr>
<tr>
<td>Viscosity enhancing agent</td>
<td>HPC</td>
<td></td>
</tr>
<tr>
<td></td>
<td>HPMC (60SH4000)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>PVP K-90</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Pullulan</td>
<td></td>
</tr>
<tr>
<td>Isotonic agent</td>
<td>d-Sorbitol</td>
<td>40</td>
</tr>
<tr>
<td>Sweetening agent</td>
<td>Stevia</td>
<td></td>
</tr>
<tr>
<td>Preserving agent</td>
<td>Methylparaben</td>
<td></td>
</tr>
<tr>
<td>Flavor</td>
<td>Strawberry flavor</td>
<td></td>
</tr>
<tr>
<td>Solvent</td>
<td>Purified water</td>
<td>q.s.</td>
</tr>
<tr>
<td>Particle size (µm)</td>
<td>Mean</td>
<td>13.9</td>
</tr>
<tr>
<td></td>
<td>S.D.</td>
<td>0.5</td>
</tr>
<tr>
<td>Viscosity (mPa·s)</td>
<td>12</td>
<td>23</td>
</tr>
</tbody>
</table>

The rebamipide concentrations are 0 mg/mL in the vehicle controls. q.s.: quantum sufficient, S.D.: standard deviation, N.T.: not tested.
total injury area ratio and the ulcer-like area ratio with those of the corresponding control group, calculated by the following formula:

Total injury area ratio (%) = \( \frac{\text{total injured surface area (B)}}{\text{tongue total surface area (A)}} \times 100 \)

Ulcer-like area ratio (%) = \( \frac{\text{ulcer-like area (C)}}{\text{tongue total surface area (A)}} \times 100 \)

The differences in the percent areas of total injury (B) and ulcerated injury (C) between treatments were evaluated using a two-tailed t-test at a significance level of less than 0.05.

RESULTS

Rat Oral Ulcer Model

Rat was employed as a convenient screening method to evaluate the healing effect of rebamipide liquid on oral mucositis. In this model, the ulcerated area reached the largest level on Day 5 after cauterization (Fig. 1b) compared with normal condition (Fig. 1a), and key histological features characterizing oral mucositis were confirmed with submucosal infiltration of inflammatory cells (Fig. 1c). The ulcerated surface area then gradually decreased thereafter with spontaneous recovery. We defined the ulcerated surface area on Day 8, at which stable and reproducible measurements could be obtained, as the indicator of healing effect.

Healing Effects of Rebamipide Liquids Comprising Micro-crystals or Submicronized Crystals in the Rat Oral Ulcer Model

Liquid A, a 2% rebamipide liquid (suspension) comprising micro-crystals, which is similar to the formulation used in the pilot clinical study\(^{17}\) except for rebamipide concentration, and its vehicle control were applied to the cauterization-induced oral ulcer model in rats by intra-orally, and the ulcerated surface areas were quantitatively determined. The result is shown in Table 2. Compared to the vehicle control, the treatment with Liquid A did not show any significant improvement in oral ulcer model.

We then investigated the effect of submicronized rebamipide crystals on the oral ulcer. The compositions, viscosities and mean particle sizes for each Liquids are presented in Table 1. Liquids C to E (suspension), comprising 2% rebamipide submicronized crystals prepared using HPMC (TC-5E) as a dispersant. Liquid C, comprising no viscosity agent with low viscosity, Liquid D, comprising HPC with moderate viscosity, and Liquid E, comprising HPMC with high viscosity (gelling formulation) and their vehicle controls were applied to
the cauterization-induced oral ulcer rat model by intra-oral administration of Liquid I (2%) to awake rats; either applied to the left buccal mucosa (intra-oral) or placed directly into the stomach via a feeding tube (intra-gastric).

The results are graphically depicted in Fig. 3. Intra-oral administration of Liquid I achieved the higher rebamipide concentration in the left buccal tissue than in the plasma. Furthermore, in order to evaluate the effect of micro-crystals or submicronized crystals from the standpoint of absorption and distribution, the rebamipide concentration in the plasma, buccal tissue and tongue tissue were determined after administering single 0.5 mL/kg doses of Liquid B, comprising 2% micro-crystals in the same composition as Liquid I, to the left buccal mucosa (intra-oral).

The results are also graphically depicted in Fig. 3. Plasma concentration profile of Liquid B (intra-oral of micro-crystals) is slightly lower than that of Liquid I (intra-oral of submicronized crystals) (Fig. 3a). Intra-oral administration of Liquid I achieved the higher rebamipide concentrations of the left buccal tissue at 5 min after dosing than intra-oral administration of Liquid B (Fig. 3b, c).

Preventive Effect of Rebamipide Liquids in the Irradiation-Induced Glossitis Model in Rats Irradiation induced glossitis was induced by exposing only around the rat’s snout to a single dose of X-irradiation (15 Gy). Liquid I was intra-orally administered into rat six times a day at 0.5 mL/kg doses daily for 14 d, starting 7 d before irradiation. The injured surface areas were measured on Day 7 after irradiation (Fig. 4). The total injury area ratios were 51.0% and 36.9% for rats treated with the vehicle control and rebamipide Liquid I, respectively. Rebamipide Liquid I significantly reduced the ulcer-like area: 17.0% and 7.2% for rats treated with the vehicle control and rebamipide Liquid I, respectively (p<0.05, t-test). This suggests that rebamipide Liquid I exhibited the preventive effect of on irradiation-induced glossitis.

### Table 2. Ulcer Healing Effects of 2% Rebamipide Liquids in the Rat Oral Ulcer Model

<table>
<thead>
<tr>
<th>Sample</th>
<th>Reduction ratio (%)</th>
<th>p Value vs. vehicle control group</th>
</tr>
</thead>
<tbody>
<tr>
<td>Liquid comprising micro-crystals</td>
<td>A 8.7</td>
<td>N.S.</td>
</tr>
<tr>
<td>HPMC based liquids comprising submicronized crystals</td>
<td>C 10.2</td>
<td>N.S.</td>
</tr>
<tr>
<td>D 18.1</td>
<td>p&lt;0.05</td>
<td></td>
</tr>
<tr>
<td>E 13.7</td>
<td>N.S.</td>
<td></td>
</tr>
<tr>
<td>PVP based liquids comprising submicronized crystals</td>
<td>F 11.9</td>
<td>N.S.</td>
</tr>
<tr>
<td>G 25.1</td>
<td>p&lt;0.01</td>
<td></td>
</tr>
<tr>
<td>H 24.8</td>
<td>p&lt;0.01</td>
<td></td>
</tr>
</tbody>
</table>

*The reduction ratios of ulcerated area were calculated as compared to the ulcerated area of vehicle control. N.S.: not significant*
DISCUSSION

Severe oral mucositis, induced by radiotherapy and chemotherapy in patients with cancer of the head and neck, often causes interruption or discontinuation of the therapy. This suggests that there are unmet needs for developing new preventive and therapeutic treatments. In the current study, for treating oral mucositis, we successfully achieved the development of novel and more effective rebamipide liquid formulation, which comprised submicronized rebamipide crystals with enhanced viscosities, termed as Liquid I in this study. Liquid I showed better oral mucositis healing effect in rat oral ulcer model and preventive effect in the rat irradiation-induced glossitis model (Figs. 2, 4). This formulation we proposed here may be a better option or alternative for treating and preventing oral mucositis, resulting in increasing quality of life of patients with severe oral mucositis.

Rebamipide moderately viscous liquid comprising submicronized crystals induced better healing effect than micro-crystals of rebamipide in rat oral ulcer model (Table 2), indicating the importance of both size of rebamipide particles and viscosity of liquid in the healing of ulcers. In particular, the addition of viscosity enhances improved the healing effect of submicronized rebamipide liquid: the liquids with viscosities ranging from 20 to 200 mPa·s demonstrated significant healing effects, whereas liquids with lower viscosities did not (Table 2). Interestingly, gel preparations (Liquid E), which had high viscosities but high degrees of particle aggregation, did not exhibit a significant therapeutic effect (Table 2). High viscosity of liquid or particle aggregation may inhibit the distribution of rebamipide to the oral mucosa. In addition, experiments using different rebamipide concentrations up to 4% showed that the higher concentrations with slightly higher viscosity achieved better results (Fig. 2). Taken together, these results suggest that fine particle size without aggregation, as well as appropriate range of viscosity is a physical factor predominating the ulcer healing effect of rebamipide liquid. Specifically, we developed the optimized Liquid I, which com-
posed of submicronized rebamipide crystals, PVP K30 as the dispersant, PVP K90 and pullulan as the viscosity enhancers, δ-sorbitol, stevia, methyl parahydroxybenzoate, and a flavoring agent with consideration for not only the healing effect but also the stability, safety and compatibility of the excipients and suppression of bitterness.

Our preliminary studies showed that intra-oral administration of submicronized rebamipide liquid induced much better healing effect than intra-gastric administration (data not shown). In this paper, intra-oral administration induced higher rebamipide concentrations in the left buccal and tongue tissues than intra-gastric administration (Fig. 3). The higher healing effect obtained by intra-oral administration might reflect the higher rebamipide concentration in buccal tissue. Whereas the absorption rate of rebamipide by oral administration is low, highly distribution of rebamipide to gastric mucosa is known. Absorption rate of rebamipide by oral administration is low, whereas the higher rebamipide concentration in buccal tissue. Whereas the absorption rate of rebamipide by oral administration is low, highly distribution of rebamipide to gastric mucosa is known to act directly on peptic ulcer and gastritis. In this study, intra-oral administration also resulted in higher rebamipide concentrations in the buccal tissue than in the plasma. These findings indicate that the direct administration to oral cavity to allow direct contact between the buccal mucosa and rebamipide liquid is efficient way to treat oral mucositis.

Regarding the impact of particle size, in the intra-oral administration, submicronized crystal liquids achieved somewhat higher levels of rebamipide in plasma and the buccal and tongue tissues compared to micro-crystals. This finding supports that the submicronized particles more elevate the distributions of rebamipide into the oral mucosa than micro-crystal particles following intra-oral administration. Deep distribution and penetration of rebamipide in the buccal mucosa would lead good healing effect against ulcers. Of note is the fact that the rebamipide concentrations were determined using non-inflamed left buccal and tongue tissues in this experiment. No specific data were available on the rebamipide concentrations of the mucosal epithelium or the ulcerated areas. The absence of such information warrants further study on the penetration of rebamipide through the inflamed mucosal layer.

Although pilot clinical studies reported that the lower concentration (0.1% or 0.2%) of gargle suspending rebamipide micro-crystals before radiotherapy showed the preventive effect for the radiation-induced oral mucositis, the healing effect is also considered clinically important with respect to the duration and degree of oral mucositis. This study demonstrated the better healing effect of Liquid I, which was suspending higher concentration submicronized rebamipide crystals with moderately viscosity, by intra-oral administration in animal oral ulcer model (Fig. 2). The formulation may extend the better healing effect to the patients already had oral mucositis.

Sonis ST have proposed that radiotherapy and/or chemotherapy-induced mucositis develops via five pathophysiological phases: initiation, up-regulation and generation of messenger signals, signaling and amplification, ulceration, and healing. This is an example of the efforts to understand the complicated mechanisms underlying cancer therapy-induced oral mucositis, which have not yet been completely elucidated. For complicated mechanisms of radiotherapy-induced oral mucositis, any potential healing agents for oral mucositis should be evaluated before clinical studies in the experimental animal models that can simulate the pathology of radiotherapy-induced oral mucositis. We recently developed a new rat model of irradiation-induced glossitis as a more clinically relevant experimental system, whereby the rat’s snout was irradiated with a single dose of X-ray. The pathogenesis of radiochemotherapy-induced oral mucositis is known to involve the production of inflammatory cytokines and chemokines. Our experimental model shows transient biphasic elevations of expression of these inflammatory modulators, suggesting its advantage in simulating the pathological process. Six-time daily administration of the 2% rebamipide Liquid I significantly reduced the ulcer-like area ratio in the radiation-induced rats’ glossitis (Fig. 4). The rebamipide liquid we proposed in this research would provide a new option for treating and preventing oral mucositis commonly observed in patients undergoing radiotherapy.

Acknowledgments The authors wish to thank Ms. Sachiko Tsujimi, Otsuka Pharmaceutical Co., Ltd., for her cooperation in rebamipide concentration measurements. The authors also acknowledge the technical assistance of Mr. Toshihiro Osaka, Otsuka Pharmaceutical Co., Ltd.

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