

## Healing Effects of Irsogladine Maleate on Acetic Acid-induced Oral Stomatitis in 5-Fluorouracil-treated and -untreated Syrian Golden Hamsters

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### Research Article

#### ABSTRACT

Oral stomatitis is one of the adverse events induced by 5-fluorouracil (5-FU). The healing effects of irsogladine maleate (IM), a therapeutic agent for stomach ulcer, were examined on oral stomatitis with 5-FU. The oral stomatitis model in the cheek pouch were produced by submucosal injection of 25  $\mu$ L of 10% acetic acid solution in male Syrian golden hamsters anesthetized with pentobarbital 30 mg/kg i.p. on day -2. 5-FU was injected at 60 mg/kg/day i.p. on days -4 and -2. In the IM application group, 0.2 mL of IM solution or vehicle was started from day 0 to the oral mucosa of the cheek pouch. Application of 1.2 mg/mL IM solution significantly reduced the areas of injury in 5-FU-untreated animals. Application of 1.2 mg/mL IM suspension in sodium carboxymethyl cellulose (CMC-Na) solution did not reduce the area of oral injury in this model, and no reduction was observed upon p.o. administration of 1.2 and 4.0 mg/mL IM solution. The areas of oral injury in 5-FU-treated animals were significantly larger than those in 5-FU-untreated animals. The period required for the area of oral injury to be reduced to 50% was about 4.9 and 3.9 days in the 5-FU-treated and -untreated animals, respectively. Application of 4.0 mg/mL IM solution significantly reduced the area of injury in the 5-FU-treated animals. In addition, 4.0 mg/mL IM in a solution containing gum ghatti to increase viscosity and retention reduced the area of oral injury significantly. However, p.o. administration of 1.2 or 4.0 mg/mL IM in gum ghatti-containing solution had no reduction effects. These results suggest that application of IM reduces the area of oral injury in acetic acid-induced oral stomatitis developing under both 5-FU-treated and -untreated conditions.

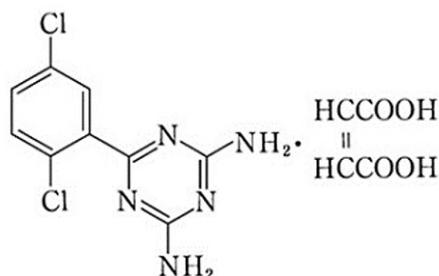
**Keywords:** Oral stomatitis, Pentobarbital, Irsogladine maleate, Hamsters, Chemotherapy, Anticancer drugs

#### INTRODUCTION

Among various agents for chemotherapy of malignant neoplasms, 5-fluorouracil (5-FU) has been widely employed since 1957 [1]. However, 5-FU has side effects such as cell growth inhibition and cytotoxicity, thereby impacting on the quality of life (QOL) of patients. Oral stomatitis is one of the adverse events induced by 5-FU, occurring through direct cell injury and indirectly by decreased resistance to bacterial infections.

It is reported that about 40% of patients receiving standard chemotherapy, and more than 90% of those receiving chemotherapy in combination with localized irradiation develop oral mucositis [2,3]. Serious oral stomatitis not only impairs oral food intake but also hinders verbal communication and disturbs sleep, as well as leading to secondary infections, thus increasing the mortality rate. Therefore, it is important to prevent and treat persistent oral stomatitis [4]. Gargle formulations currently employed to prevent and treat oral stomatitis include allopurinol as eliminator of reactive oxygen species induced by chemotherapy [5], azulene sulfonic acid sodium as oral care [6], lidocaine hydrochloride as symptomatic treatments included in analgesics [7], sodium alginate [8] and sucralfate as mucosal protection [9]. Those are not standardized as the stomatitis therapy treatments.

Recently, irsogladine maleate (IM) (**Figure 1**) has received attention as a drug that has healing effects on oral stomatitis [4], including that associated with rheumatoid disease and Behcet's disease [10,11].



**Figure 1.** Chemical structure of IM.

IM is also sold as a medicinal formulation, "Gaslon N<sup>®</sup>-OD", for stomach ulcer, but is not approval for oral stomatitis. In the present study, to clarify the therapeutic effects of IM on oral stomatitis, occurring because of 5-FU treatment or no treatment, we (1) investigated its healing effects, (2) conducted pharmaceutical studies, and (3) evaluated differences in its therapeutic effects when administered via different routes.

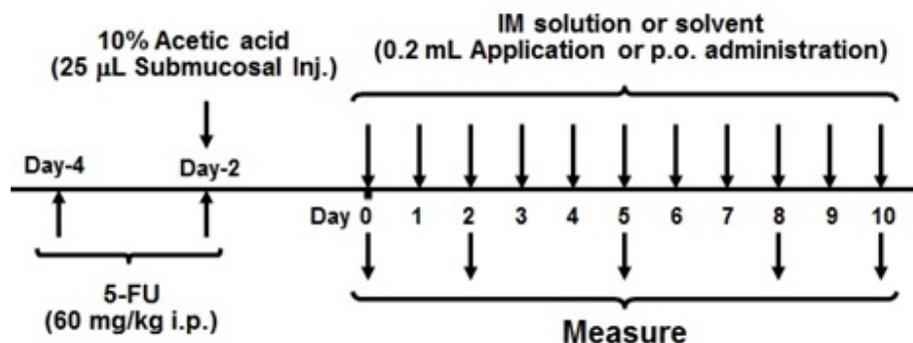
## METHODS

### Animals

Male Syrian golden hamsters weighing 100 g to 150 g were purchased from Sankyo Labo Service Co. (Tokyo, Japan) and given free access to water and commercial food pellets (MF: Oriental Yeast Co. Tokyo, Japan). They were kept in a temperature-(24°C ± 1°C) and humidity-(55% ± 5%) controlled room with a 12 h day-night cycle. All experiments were performed per the guidelines of the animal research committee of Toho university and Meiji Pharmaceutical university.

### Onset of oral stomatitis and drug treatment

The oral stomatitis model was prepared by anesthetizing the hamsters with 30 mg/kg pentobarbital i.p. The center of the cheek pouch was then exteriorized and sandwiched between ring forceps 5 mm in inner diameter, followed by submucosal injection of 25 µL of 10% acetic acid solution through the ring forceps. The day of acetic acid injection was considered the day when oral stomatitis had been induced, and was defined as day -2 of IM application. 5-FU<sup>®</sup> 250 mg (50 mg/mL) was injected at 60 mg/kg/day i.p. on days -4 and -2. In the IM application group, 0.2 mL of IM solution or vehicle was applied on day 0 to the oral mucosa of the cheek pouch, and daily thereafter (**Figure 2**). For p.o. administration, 0.2 mL of IM solution or vehicle was administered directly into the stomach using an oral tube. All IM formulations were stored at 4°C in a dark place. In the control group, only vehicle was administered.



**Figure 2.** Experimental protocol; **Note:** 5-FU: 5-Fluorouracil, IM: Irsogladine maleate.

### Measurement Items and methods

The area of oral injury was determined by measuring the major axis (a) and minor axis (b) of the lesion using a Shinwa Digital Caliper under pentobarbital anesthesia on days 0, 2, 5 and 8 in the 5-FU-untreated group and on days 0, 2, 5, 8 and 10 in the 5-FU-treated group, and calculated as an elliptic area ( $axb\pi/4$ ).

### Preparation of IM solution and suspension

IM solution included IM 80 mg dissolved in refined water and 4 g of hydroxypropyl- $\beta$ -cyclodextrin per 10 mL, and the sprays contained 5 g of gum ghatti in IM solution. IM suspension was prepared 1.2 mg IM suspended in CMC-Na solution.

### Drugs

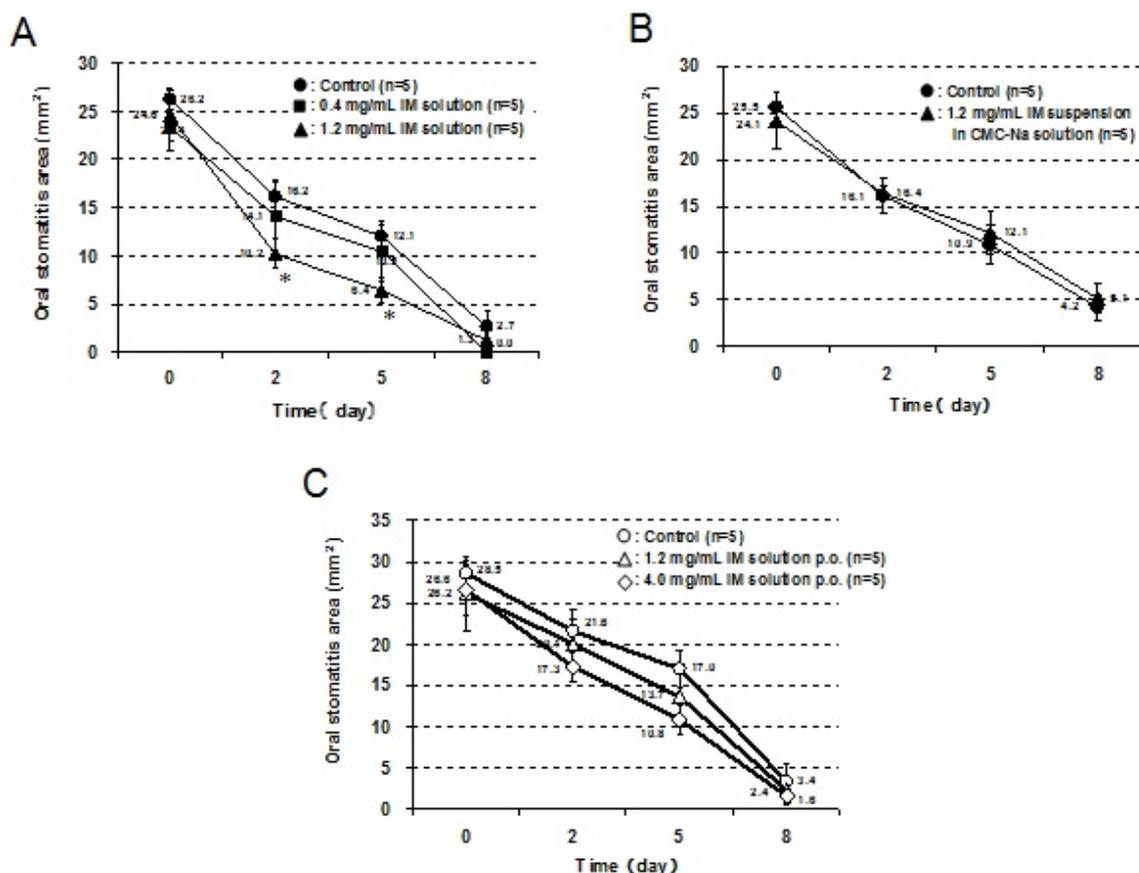
Acetic acid, sodium carboxymethyl cellulose (CMC-Na) (Wako Pure Chemical Industries, Ltd., Osaka, Japan), hydroxypropyl- $\beta$ -cyclodextrin (Nihon Shokuhin Kako Co. Ltd., Tokyo, Japan), 5-FU® Injection 250 mg (50 mg/mL) (Kyowa Hakko Kirin Co., Ltd., Tokyo, Japan), Somunopentyl® (pentobarbital sodium salt 64.8 mg/mL) (Kyoritsu Seiyaku Co., Tokyo, Japan), and gum ghatti (San-ei gen F.F.I., Inc., Osaka, Japan) were obtained commercially. All other chemicals used were of analytical grade.

### Statistical analysis

Numerical results are expressed as means  $\pm$  SEM and statistical significance was calculated with two-way ANOVA for repeated measurements. Differences at  $P < 0.05$  were statistically significant.

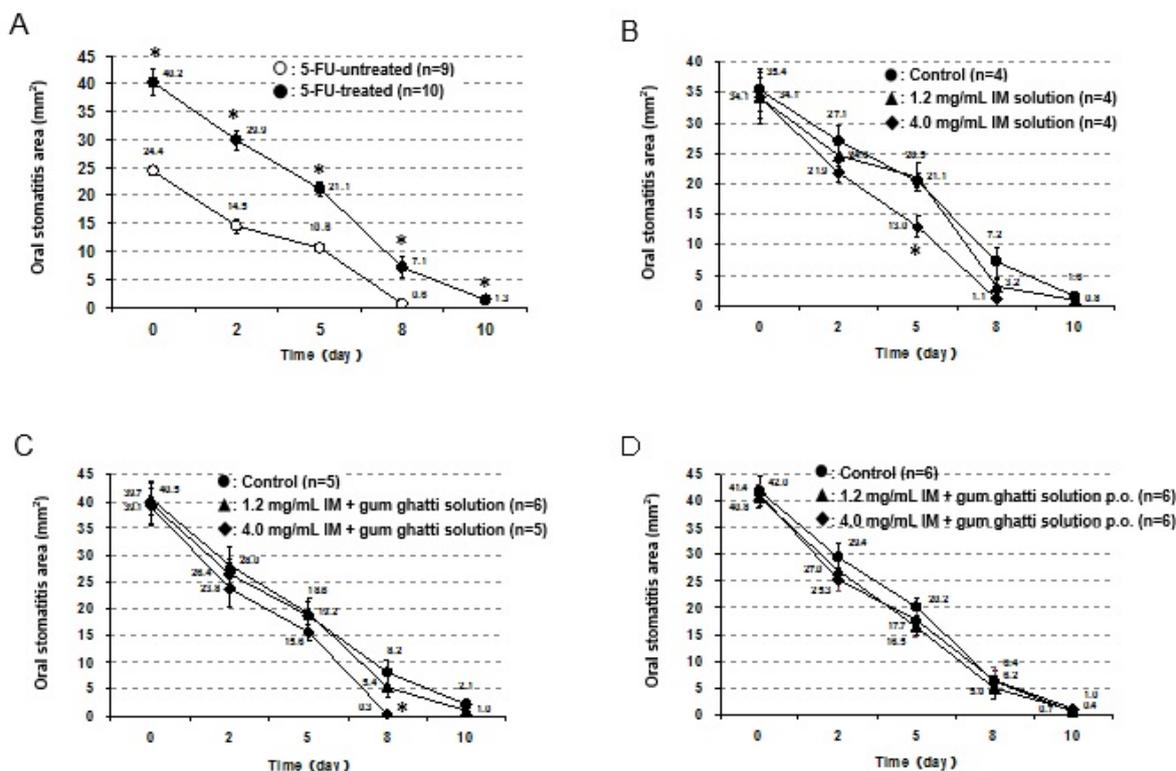
## RESULTS

1. Healing effects of IM solution on acetic acid-induced oral stomatitis in 5-FU-untreated animals. Although 0.4 mg/mL IM solution tended to reduce the area of oral injury in 5-FU-untreated animals, the difference in the reduction relative to IM-untreated animals was not significant. On days 2 and 5, the area of oral injury in the group treated with 1.2 mg/mL IM solution was reduced significantly relative to that in the control group treated with the vehicle (Figure 3A). On the other hand, the area of oral injury in the group treated with a 1.2 mg/mL IM suspension in CMC-Na solution was not significantly different from that in the control group treated with the CMC-Na solution alone (Figure 3B). Changes in the area of oral injury resulting from p.o. administration of 1.2 and 4.0 mg/mL IM solution did not differ significantly from those in the control group administered IM solution p.o. (Figure 3C).



**Figure 3.** Healing effects of IM on acetic acid-induced oral stomatitis in 5-FU-untreated Syrian golden hamster; **Note:** 0.2 mL IM solution (A) and 0.2 mL IM suspension in CMC-Na solution (B) were applied to stomatitis site, and 0.2 mL IM solution was p.o. administration (C). The results represent the mean  $\pm$  SEM. \*:  $P < 0.05$  vs. the values of control group.

2. Healing effects of IM on acetic acid-induced oral stomatitis in 5-FU-treated animals. The area of oral injury in 5-FU-treated animals was significantly greater than that in 5-FU-untreated animals. The period required for the area of injury to be reduced to 50% was about 4.9 and 3.9 days in the 5-FU-treated and -untreated animals, respectively (Figure 4A). Application of 4.0 mg/mL IM solution significantly reduced the area of acetic acid-induced injury in 5-FU-treated animals (Figure 4B). In addition, 4.0 mg/mL IM dissolved in gum ghatti solution to increase viscosity and retention significantly reduced the area of oral injury (Figure 4C). However, p.o. administration of 1.2 or 4.0 mg/mL IM in gum ghatti solution had no lesion-reducing effects (Figure 4D).



**Figure 4.** Healing effects of IM on acetic acid-induced oral stomatitis in 5-FU-treated Syrian golden hamster; (A) Comparison with oral stomatitis in 5-FU-untreated and -treated animals; (B) 0.2 mL IM solution; (C) 0.2 mL IM gum ghatti-containing solution; (D) 0.2 mL IM gum ghatti-containing solutions p.o.; 60 mg/kg 5-FU i.p. was administrated -4 and -2 days before IM application or administration. The results represent the mean±SEM. \*: P<0.05 vs. the values of control group.

## DISCUSSION

The present study showed that a solubilized solution formula of 1.2 mg/mL IM had healing effects on oral stomatitis in 5-FU-untreated Syrian golden hamsters, whereas the therapeutic effects of an IM suspension in CMC-Na solution were less potent. Application of 4.0 mg/mL IM "solution formula" showed accelerated healing effects on acetic acid-induced oral stomatitis in 5-FU-treated animals, and exerted an effect as a "gum ghatti-containing formula". Application of 0.4 mg/mL IM solution had no therapeutic effect on acetic acid-induced oral stomatitis in 5-FU-untreated hamsters, whereas the 1.2 mg/mL IM "solution formula" did show significant therapeutic effects on days 2 and 5. These results suggest that IM solution has therapeutic effects on oral stomatitis when administered at doses of more than 1.2 mg/mL. No significant healing effects of a 1.2 mg/mL IM suspension in CMC-Na solution were observed relative to those of an IM suspension in CMC-Na solution, suggesting that IM needs to be solubilized in "solution formula" to exert therapeutic efficacy. Although no healing effects on oral stomatitis were observed for p.o. administration of 1.2 mg/mL and 4.0 mg/mL IM, there was a tendency for lesion recovery, suggesting that higher IM doses administered p.o. might have healing efficacy. On the other hand, the area of oral injury was significantly decreased by application of 4.0 mg/mL IM "solution formula" and "gum ghatti-containing formula" in comparison with the untreated control. Thus, it is suggested that IM solution with a concentration exceeding 4.0 mg/mL would have therapeutic effects on oral stomatitis in 5-FU-treated animals. In the present study, it was suggested that IM had concentration-dependent healing efficacy against oral stomatitis in this model, and that further experiments to determine the optimal concentration at more than 4.0 mg/mL IM will be necessary in the future.

Both the 4.0 mg/mL IM "solution formula" and "gum ghatti-containing formula" exerted therapeutic effects, and there was a significant difference in the area of oral injury in the groups with and without IM application on days 5 and 8. As a

formula with high viscosity due to addition of gum ghatti ensures a longer-lasting IM retention time in oral stomatitis cavities, we suspect that the healing efficacy of IM "gum ghatti-containing formula" would be stronger than that of the usual "solution formula". However, the therapeutic efficacy of the IM "gum ghatti-containing formula" was similar, and the period until its therapeutic efficacy appeared tended to be delayed in comparison with the IM "solution formula". Gum ghatti is a type of polysaccharide, and it has been reported to support bacterial growth [12], suggesting possible proliferation of bacteria in the "gum ghatti-containing formula". As patients receiving chemotherapy for cancer are sometimes immunocompromised due to treatment with anticancer drugs, the use of the "gum ghatti-containing formula" for such patients would increase the risk of infection, and therefore its clinical application would have to be considered carefully. However, about oral stomatitis induced by a combination of both chemotherapy and radiotherapy, the radiation therapy protocol generally lasts 6-7 weeks [13], and therefore application of a drug to diminish the area of oral injury at an early stage is important for maintenance of QOL in such patients.

In the present study, both the IM "solution formula" and "gum ghatti-containing formula" were effective for therapy of oral stomatitis by buccal application. Hereafter, it will be necessary to confirm the pharmaceutical stability of IM formulas employed for oral stomatitis therapy. Although IM in solution is stable to heat at mildly acidic-neutral-weak alkaline pH, and is also stable to light at neutral pH [14], stability testing of IM-containing formulas will be necessary. In the present study, the IM suspension in CMC-Na solution did not exert any therapeutic effects, suggesting that an IM OD tablet suspended in CMC-Na solution might not be therapeutically effective for oral stomatitis. Ideally, therefore, the IM formula should not be a suspension, but a solution. If an IM suspension in CMC-Na solution has therapeutic effectiveness against oral stomatitis, then the IM OD tablet would be a useful IM formula. Although further study of the stability of the IM formula will be necessary, this study has demonstrated the effectiveness of the IM formula for oral stomatitis, suggesting that it could become useful for patients receiving chemotherapy. For the future, practical use of the IM formula, we think that pharmaceutical testing would be justified.

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