Studies on Development of Dosage Forms for Pediatric Use (V) Oral Mucosal Irritation Study of Gummi Drugs in Hamster Cheek Pouch

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In the present study we investigated irritation of the oral mucosa and the safety of gummi drugs containing acetaminophen (AAP). The oral mucosae of hamsters were macroscopically examined for any evidence of irritation after gummi drugs were inserted into the cheek pouch and left there for 1 h. The cheek pouch tissue was also macroscopically and microscopically examined 24 h after gummi drugs were withdrawn from the cheek pouch.

As a result, no evidence of irritation was found macroscopically 1 h after insertion, or macroscopically and microscopically 24 h after the withdrawal of the gummi drugs or placebos as compared with negative controls (saline). Considering these results, the gummi drugs administered in the present study produced no irritation to the oral mucosa.

Key words acetaminophen; hamster; cheek pouch; irritation; oral mucosa

Recently, in order to increase compliance, a number of studies on the development of new dosage forms have been reported and received clinical attention.^{1–6)} Particularly, new dosage forms for the elderly have been developed in hospital pharmacies and one expected to practical use.^{7–19)} Hopefully, in future, these dosage forms will be come valuable treatment options.

In addition, noncompliance by children is common, so that development of these dosage forms are also needed for pediatric use. To increase compliance with treatment schedules in children, we developed gummi drugs a gel formulation with gelatin, that can easily be taken orally by mastication without water. A gummi drug is defined as a gel preparation made by adding the drug to gummi, a gel confection made of sugars and gelatin. So far, we have presented reports on the preparation of such drugs, release of acetaminophen (AAP), stability of the drug under storage and time courses of plasma concentrations of AAP after oral administration to dogs.^{20–23)} It is anticipated, however, that the drug will remain relatively longer in the oral cavity compared with ordinary oral drugs, as the gummi drug is meant to swell after being masticated in the oral cavity. From a safety point of view, it was considered necessary to perform a study where gummi drug was examined to see if it caused irritation of the oral mucosa. Therefore, we performed an oral mucosa irritation study using the hamster cheek pouch, often used for this purpose.^{24–26)}

MATERIALS AND METHODS

Materials The formula of the thick, viscous solution for gummi (designated as gummi base, hereinafter) to be used in gummi drugs and the formula of the gummi drug containing AAP are shown in Table 1. The AAP powder preparation (sieved to 75–150 μ m particle size, Yamanouchi Pharmaceutical Co., Ltd.), gelatin (S-1204, molecular weight: about 30000–700000, average molecular weight: about 100000, Nippi Gelatin Industries. Ltd.), sugar (HA, Nippon Beet Sugar Mfg. Co., Ltd.), glucose syrup (HI-MAL, Sanmatsu Kogyo Company Ltd.), flavor (peach flavor, T. Hasegawa Co., Ltd.), fruit juice (1/5-concentrated peach juice, Sanyo

Foods Co., Ltd.), coloring matter (San Red[®] RC, San-Ei Gen Foods and Food Ingredients Inc.), sodium citrate (Takasago Corporation) and ethanol (denatured ethanol for food use, government monopoly ethanol, 95%, first grade, No. 36, H-4) were used. For the negative controls in the oral mucosal irritation study, saline (Otsuka saline for injection, Otsuka Pharmaceutical Co., Ltd.) was used. An anesthetic was used, pentobarbital sodium injection (injectable Nembutal[®], Dainabot Co., Ltd.). All other reagents were of analytical grade.

Preparation of Gummi Drug The preparation of gummi drugs was the same as in the previous report.²⁰⁾ AAP was dissolved in ethanol at 60 °C and mixed with gummi base. Bubble formation ceases following mixing by warming at 60 °C in a water-bath. When the smell of ethanol disappeared, water was added to make the final weight (200 g). This mixture was injected using a syringe into a plastic casting plate made of vinyl chloride (35 mm thick) and left in a refrigerator at 10 °C for 24 h to allow formation of the gummi drug. As reported in a previous paper,²⁰⁾ the shape of the gummi drug is rectangular (20×20×10 mm), and the content of AAP was 25 mg/piece. The gummi drug placebo contained all these ingredients except AAP. The preparation method for the placebo was the same as that for gummi drug.

Animals Seventeen hamsters (Std: Syrian, males, 7 weeks of age, Japan SLC.) were quarantined and acclimatized for 7 d, nine of these hamsters were selected according

Table 1. Formulas of Gummi Base and Gummi Drug

Formula of gummi base		Formula of gummi drug	
Component	Content (g)	Component	Content (g)
Gelatin	21.0	Gummi base	195.5
Sugar	120.0	Acetaminophen	0.5
Glucose syrup	141.0	95% Ethanol	2.5
Sodium citrate	3.9	Water	q.s.
Fruit juice	18.0		
Coloring matter	0.12	Total amount	200.0
Flavor	0.18		
Water	q.s.		

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to body weight (body weight: 108–118 g) and used for the present experiment. After the gummi drug was withdrawn from the cheek pouches, animals were given only water for 24 h.

Animal Experiments We performed the animal experiments using the cheek pouch of the hamsters, often used for oral mucosae irritation studies.^{24–26)} Pentobarbital sodium 75 mg/ml/kg was injected into the abdominal cavity in order to anesthetize the animals for about 90 min, from the time of the injection to recover of lighting reflexes. The dose was enough to anesthetize the hamsters for the period of insertion of gummi drug into the cheek pouches and observation of the cheek pouch mucosae in addition to a period of about 60 min for the drug to remain in site. After the injection, the cheek pouches were carefully exposed, rinsed with 10 ml warmed saline and replaced. If a whole piece of gummi drug were in-

serted into the cheek pouch, it might cause physical injury at the time of withdrawal, which could be a source of inaccurate evaluation of irritation. On the other hand, if several pieces of divided gummi drug, the masticated model, are inserted into

Table 2. Criteria for Macroscopic Evaluation of Gummi Drug-Induced Irritation of the Oral Mucosa of Hamsters

Criteria	Score
No reaction	0
Mild erythema	1
Marked erythema (well-demarcated)	2
Very mild furring	3
Mild furring (well-demarcated)	4
Moderate furring (thick fur covering less than 50% of the area)	5
Severe furring (thick fur covering more than 50% of the area)	6

Photo A
Gummi Drug held in the
right cheek pouch of
hamster No. 3

Photo B
Placebo held in the left
cheek pouch of hamster
No. 3

Photo C
Negative control
Saline held in the left
cheek pouch of hamster
No. 7

Fig. 1. Micrographs of the Oral Mucosae of Hamster No. 3 Taken 24 h after Withdrawal of a Gummi Drug or Placebo Held in the Cheek Pouch for 1 h (H-E stain, $\times 170$)

the cheek pouch, it might be impossible to ensure that all pieces of the drug were removed from the cheek pouch because of pieces adhering to the cheek pouch mucosae. Considering these factors, the gummi drug preparation was cut into halves. These halves and the placebo halves were respectively inserted into the right and left pouches of 6 hamsters after being moistened with saline. Other 3 hamsters received only rinsing of the pouches with saline and served as negative controls.

Gummi drug and placebo were left in the cheek pouches for 1 h. After the gummi drug was withdrawn, the cheek pouches were exposed again and the cheek pouch mucosae were macroscopically examined by comparing them with negative controls. At the time of withdrawal, the dissolved gummi drug or placebo adhered to the surface of the cheek pouch mucosae, therefore microscopic examination was not possible. If these adhering pieces are removed intentionally, it might cause physical injury, which could be a source of inaccurate evaluation of irritation. So the animals were given only drinking water for 24 h until these adhering pieces were washed away naturally from the surface of the cheek pouch mucosae by water and saliva. This procedure was based on the result of a preliminary experiment using 3 hamsters showing that these pieces disappeared after 24 h. Then, after the animals were anesthetized, the cheek pouches were excised and the mucosae were macroscopically reexamined. Two pieces of tissue were sampled, about 5 mm apart, from the pouch area that had contact with the gummi drug and fixed in 10% neutral buffered formalin solution. Paraffin sections of the specimens were H-E (hematoxyline-eosin) stained and pathologically examined by microscope.

Criteria for Macroscopic Evaluation of Mucosal Irritation Evaluation of irritation to the oral mucosae by gummi drug or placebo was performed 1 h after insertion or 24 h after withdrawal, based on the criteria shown in Table 2. A score was given according to the degree of erythema or furring.

Criteria for Microscopic Evaluation of Mucosal Irritation Histopathological evaluation of irritation in the cheek pouch focused on hypertrophy, submucosal edema, vasodilation edema, epithelial loss, cell infiltration in the tissues obtained 24 h after gummi drug or placebo was withdrawn.

RESULTS AND DISCUSSION

Macroscopic Evaluation of Irritation in the Oral Mucosa Evaluation of irritation in the oral mucosae was performed based on the criteria presented in Table 2. No distinctive differences were found in the appearance of the mucosae of all hamsters in the gummi drug and placebo groups, compared with the negative control group immediately or 24 h after withdrawal of drug. No evidence of any abnormality was observed, such as erythema or furring. Therefore, we

judged there was no macroscopic irritation.

Microscopic Evaluation of Irritation in the Oral Mucosa Figure 1 shows microscopic photographs selected randomly from those examined histopathologically. No evidence of irritation, such as hypertrophy, submucosal edema, vasodilation edema, epithelial loss and cell infiltration, was found in the mucosal tissues of the cheek pouches microscopically 24 h after the withdrawal of gummi drugs or placebo compared with negative controls (saline). Considering these results, gummi drug and placebo were judged not to cause irritation to the oral mucosae of hamsters.

REFERENCES

- 1) Ansseau M., Doumont A., Cerfontaine J. L., Mantanus H., Rousseau J. C., Berthier M. T., *Neuropsychobiology*, **12**, 255—259 (1984).
- 2) Smith G. B., Hughes D. G., Kumar V., *Anaesthesia*, **40**, 368—371 (1985).
- 3) Brampton W. J., Plantevin O. M., *J. Int. Med. Res.*, **13**, 169—173 (1985).
- 4) Wilson C. G., Washington N., Peach J., Murray G. R., Kennerley J., *Int. J. Pharm.*, **40**, 119—123 (1987).
- 5) Wilson C. G., Washington N., Norman S., Greaves J. L., Peach J. M., Pugh K., *Int. J. Pharm.*, **46**, 241—246 (1988).
- 6) Washington N., Wilson C. G., Greaves J. L., Norman S., Peach J. M., Pugh K., *Int. J. Pharm.*, **57**, 17—22 (1989).
- 7) Watanabe A., Hanawa T., Sugihara M., Yamamoto K., *Chem. Pharm. Bull.*, **42**, 1642—1645 (1994).
- 8) Watanabe A., Hanawa T., Sugihara M., Yamamoto K., *Int. J. Pharm.*, **117**, 23—30 (1995).
- 9) Hanawa T., Watanabe A., Tsuchiya T., Ikoma R., Hidaka M., Sugihara M., *Chem. Pharm. Bull.*, **43**, 284—288 (1995).
- 10) Hanawa T., Watanabe A., Tsuchiya T., Ikoma R., Hidaka M., Sugihara M., *Chem. Pharm. Bull.*, **43**, 872—876 (1995).
- 11) Watanabe A., Sugihara M., *Yakuzaigaku*, **52**, 69—78 (1992).
- 12) Watanabe A., Hanawa T., Sugihara M., *Yakuzaigaku*, **54**, 103—110 (1994).
- 13) Watanabe A., Hanawa T., Sugihara M., *Yakuzaigaku*, **54**, 77—87 (1994).
- 14) Ito A., Tsuchiya T., Obata K., Sugihara M., *Jpn. J. Hosp. Pharm.*, **20**, 41—49 (1994).
- 15) Ito A., Sugihara M., *Jpn. J. Hosp. Pharm.*, **20**, 368—374 (1994).
- 16) Ito A., Sugihara M., *Jpn. J. Hosp. Pharm.*, **21**, 448—453 (1995).
- 17) Ito A., Fukuma M., Wakaizumi Y., Sugihara M., *Jpn. J. Hosp. Pharm.*, **22**, 52—59 (1996).
- 18) Ito A., Sugihara M., *The Pharmaceuticals Monthly*, **37**, 2381—2385 (1995).
- 19) Sugihara M., *FARUAW*, **30**, 1396—1400 (1994).
- 20) Namiki N., Takagi N., Yuasa H., Kanaya Y., *Yakuzaigaku*, **57**, 86—94 (1997).
- 21) Namiki N., Takagi N., Yuasa H., Kanaya Y., *Yakuzaigaku*, **57**, 109—113 (1997).
- 22) Namiki N., Takagi N., Yuasa H., Kanaya Y., *Yakuzaigaku*, **57**, 145—151 (1997).
- 23) Namiki N., Takagi N., Yuasa H., Kanaya Y., *Yakuzaigaku*, **57**, 215—219 (1997).
- 24) Suzuki S., Karasawa N., Yumoto T., Aiuti S., Takahashi M., Tunoda T., Yosibe F., *THE CLINICAL REPORT*, **27**, 5843—5849 (1993).
- 25) Yoshikawa H., *J. Soc. Cosmet. Chem. Japan.*, **14**, 167—171 (1980).
- 26) Futami T., Okamoto K., Ishida A., Amo T., *Journal of Dental Health*, **19**, 1—11 (1969).