

# Mucoadhesive suppositories of ramosetron hydrochloride utilizing Carbopol®

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## Abstract

Suppositories are the preferable dosage form for patients at home or experiencing nausea. Serotonin (5-HT<sub>3</sub>)-receptor antagonists are used to treat vomiting in intravenous or oral administration but not suppository form. Ramosetron hydrochloride (RAM) is a new 5-HT<sub>3</sub> antagonist which effectively inhibits vomiting, and we prepared RAM suppositories using Witepsol® H-15 (H-15) containing Carbopol® 934P (CP). The viscosity of suppository base and RAM release properties from suppositories were examined. Plasma RAM concentrations after administration of suppositories to rabbits were estimated and irritation of rectal tissues were observed. Antiemetic effects of suppositories were studied using ferrets. The base viscosity increased with addition of CP. Suppositories containing CP exhibited better absorption in rabbits compared to H-15 suppositories, correlated with release behavior. Suppositories containing 2% CP had 2.5 times larger AUC<sub>0–24 h</sub> than H-15 suppositories, and the MRT was prolonged by 5.8 h compared with i.v. administration. 10% CP suppositories administered to rabbits for 5 days did not irritate the tissues. Antiemetic studies indicated that 2% CP suppository of RAM might have the same effect as i.v. administration. These results suggest that RAM suppositories containing CP are safe and useful in once-a-day dosage form for treatment of chemotherapy-induced nausea. © 2000 Elsevier Science B.V. All rights reserved.

**Keywords:** Mucoadhesive suppository; Carbopol®; Serotonin receptor antagonist; Antiemetic; Ramosetron

## 1. Introduction

Nausea and vomiting are severe side-effects of cancer chemotherapy and may lead to patient refusal of therapy (Triozi and Laszlo, 1987; Tortorice and O'Connell, 1990). Furthermore, many problems exist such as change of drug absorption

ratio by oral routes and nutritive control. It has recently become clear that serotonin (5-HT<sub>3</sub>)-receptor antagonists can inhibit vomiting evoked by cytotoxic drugs like cisplatin and cyclophosphamide (Andrews et al., 1990; Andrews and Bhandari, 1993). Ramosetron hydrochloride, (-)-(R)-5-[(1-methyl-1*H*-indol-3-yl)carbonyl]-4,5,6,7-tetrahydro-1*H*-benzimidazole monohydrochloride (RAM, Fig. 1), is a new 5-HT<sub>3</sub>-receptor antagonist with approximately 242 and 956 times larger

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ED<sub>50(6 h)</sub> antiemetic activity in ferrets than granisetron and ondansetron respectively (Fujihara et al., 1994).

Suppositories are preferable to i.v. injection or oral preparations for patients at home or experiencing nausea, but are not on the market. From the viewpoint of improving the patient's quality of life, a suppository formulation of a 5-HT<sub>3</sub> receptor antagonist is significant (Hsyu et al., 1994; Kobayashi et al., 1996; Moriyama et al., 1997). Then, RAM suppositories were prepared using Witepsol® H-15 (H-15) as base and Carbopol® 934P (CP) as additive. CP is a carboxyvinyl polymer having mucoadhesive properties (Machida et al., 1979; Iwata et al., 1987) which is used to improve drug absorption (Morimoto et al., 1980, 1983). In this study, we investigated the viscosities of suppository bases and the release and plasma concentration profiles of RAM from CP suppositories compared with non-additived H-15 suppositories. Furthermore, we evaluated rectal tissue irritation and antiemetic effects of suppositories.

## 2. Materials and methods

### 2.1. Materials

Ramosetron hydrochloride was supplied by Yamanouchi Pharmaceutical (Japan). Witepsol® H-15 and Carbopol® 934P were supplied by Mitsuba Trading (Japan) and B.F. Goodrich (USA), respectively. White beeswax and cisplatin were purchased from Wako Pure Chemical Industries (Japan). All other chemicals used were of reagent grade.

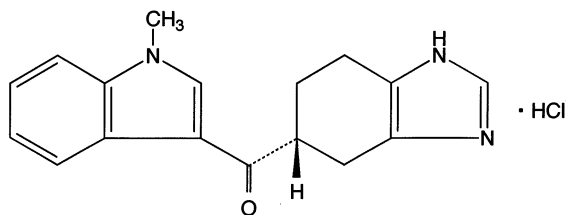


Fig. 1. Chemical structure of ramosetron {(–)-(R)-5-[(1-methyl-1H-indol-3-yl)carbonyl]-4,5,6,7-tetrahydro-1H-benzimidazole monohydrochloride}.

### 2.2. Preparation of suppositories

Stock solution of RAM was prepared by dissolving 10 mg of RAM in 5 ml of distilled water, and stored at 10°C. Aliquots of stock solution were transferred to a beaker and evaporated to dryness under a stream of dry nitrogen. After dispersing the residue in H-15 with ultrasonication for 15 min at 60°C, CP was dispersed in the mixture using ultrasonication for 10 min. Then the mixture was poured into a suppository mold (for 2 g, Erbo, Germany) and cooled at room temperature. Suppositories were stored at 10°C and used within 1 week.

### 2.3. Measurement of the viscosity of base

Viscosities of suppository bases were measured using Ubbelohde viscosimeter (SU-8914430, Sibata, Japan) according to JPXIII. Viscosimeter was fixed in a container kept at  $37 \pm 0.1^\circ\text{C}$  during the experiments.

### 2.4. In vitro drug release tests

The release tests were carried out according to the method reported by Iwata et al. (1995). Membrane filter (SS, pore size 3.0  $\mu\text{m}$ , Millipore, USA) was fixed at the bottom of a stainless steel cell (40 mm i.d., 20 mm high). A suppository containing 0.3 mg RAM was put into the cell and attached to a JP XIII dissolution tester (Toyama Sangyo, Japan). A hole (2 mm diameter) in the cell cover was firmly filled with cotton. The cell was rotated at 120 rpm in 500 ml of JP XIII second fluid (pH 6.8,  $37 \pm 0.5^\circ\text{C}$ ). At predetermined times, 5 ml of dissolution test fluid was collected and passed through a membrane filter (pore size 0.45  $\mu\text{m}$ , Gelman Sciences, USA). RAM in sample fluid were measured by high-performance liquid chromatography (HPLC). 100  $\mu\text{l}$  of solution was injected into HPLC column (TSK-gel ODS-80Tm,  $4.6 \times 250$  mm, Tosoh, Japan) at room temperature. Sampling was compensated immediately by the dissolution test fluid of same temperature and same amount. The mobile phase was 0.1 M potas-

sium biphosphate/ 0.1 M phosphoric acid/ acetonitrile (3:3:2, v/v/v). The HPLC system was equipped with a pump (LC-6AD, Shimadzu, Japan) which was set at a flow rate of 1 ml/min and a UV detector (SPD10-AV, Shimadzu, Japan). UV absorption at 311 nm was monitored.

### 2.5. Absorption studies using rabbits

Japanese White male rabbits (2.79–3.24 kg) were used after fasting for 24 h but allowed free access to water. At predetermined intervals after rectal administration of 0.5 mg RAM suppositories to rabbits, 2 ml blood samples were withdrawn from ear veins using syringes with heparin, centrifuged at 3000 rpm for 10 min. The plasma samples were frozen and stored at  $-15^{\circ}\text{C}$  until assayed. Plasma RAM concentrations were measured by HPLC according to the method reported by Miura et al. (1994).

First, 1 ml plasma was mixed with 1  $\mu\text{g}$  prazocine hydrochloride as an internal standard (Kelly et al., 1993). After addition of 1 ml of saturated sodium bicarbonate solution and 5 ml of ethyl acetate, the resulting mixture was shaken for 15 min (1600 rpm, same after this. IKA-VI-BRAX VXR shaker, Germany), centrifuged for 10 min at  $1200 \times g$ , and the organic layer was transferred to another centrifuge tube. To the organic layer, 2.5 ml of 0.4 M hydrochloric acid was added and the resulting mixture shaken for 15 min. Then centrifugation for 5 min at  $800 \times g$ , the organic layer was discarded. To the aqueous layer, 2 ml of the saturated sodium bicarbonate solution was added and the resulting mixture stirred for 5 s using a vortex mixer. Allowing to stand for 20 min at room temperature, 4.5 ml of ethyl acetate was added to the mixture followed by shaking for 15 min. After centrifugation for 5 min at  $800 \times g$ , the organic layer was transferred to a centrifuge tube and evaporated to dryness under a stream of dry nitrogen under reduced pressure. The residue was dissolved in 100  $\mu\text{l}$  of mobile phase, passed through a membrane filter (pore size 0.45  $\mu\text{m}$ , Gelman Sciences, USA), and 60  $\mu\text{l}$  of solution was injected into the HPLC system. The HPLC system was equipped with a column (TSK-gel ODS-80-Tm,  $4.6 \times 250$  mm, To-

soh, Japan), a pump (LC-6AD, Shimadzu, Japan) at a flow rate of 1 ml/min and a UV detector (SPD10-AV, Shimadzu, Japan). UV absorption at 311 nm was monitored and plasma concentrations of RAM were estimated using Chromatopac C-R7A Plus (Shimadzu, Japan) by the internal standard method.

### 2.6. Pharmacokinetic data analysis

Following pharmacokinetic parameters were obtained using Program MULTI (moment calculation, PRG5-1): area under the plasma concentration-time curve ( $\text{AUC}_{0-24 \text{ h}}$ ), mean residence time (MRT).

### 2.7. Statistical data analysis

Statistical analysis was performed using Student's *t*-test. The data were considered to be significantly different when the *P* value was less than 0.05.

### 2.8. Morphological test of rectal tissues

Each suppository was administered to Japanese White male rabbit (3.18–4.18 kg) once a day for 5 days. At 24 h after the fifth administration, the rectum (3–10 cm from anus) was isolated, rinsed with a saline solution, fixed in 10% formaldehyde/saturated calcium carbonate solution, cut into slices in order to observe serous membrane, and embedded in paraffin. The slices were stained with hematoxylin–eosin and observed under a light microscope ( $\times 100$ ).

### 2.9. Evaluation of antiemetic effects

The antiemetic effects of RAM suppositories were studied against retching and vomiting induced by cisplatin according to the method reported by Stables et al. (1987). Cisplatin was used by dissolving in saline solution (1 mg/ml) before administration.

Male ferrets (0.98–1.38 kg, Saitama Experimental Animals Supply, Japan) were used after fasting for 24 h but allowed free access to water. Suppositories consisting of H-15 alone were used

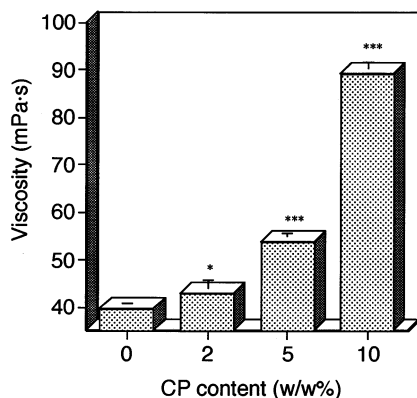


Fig. 2. Viscosity of Witepsol H-15 containing Carbopol® 934P (\*  $P < 0.05$ , \*\*\*  $P < 0.001$  vs. H-15 alone). Each column represents the mean  $\pm$  S.D. of five experiments.

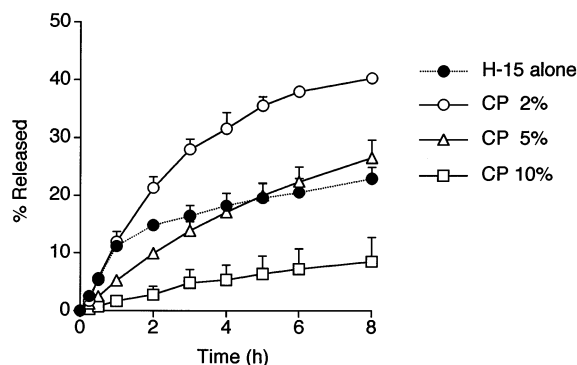


Fig. 3. Release profiles of ramosetron from suppositories in second fluid of JP XIII at 37°C. Each point represents the mean  $\pm$  S.E. of three experiments.

for control. 2% CP suppositories containing 0.1, 3, 30  $\mu\text{g/kg}$  RAM were tested. Each suppository was administered to ferret 30 min before a single intraperitoneal dose of cisplatin (10 mg/kg), then the number of retches, number of vomits, latency to the first retch and duration of retching were recorded, as were any behavioral changes for 6 h after administration of cisplatin.

### 3. Results and discussion

#### 3.1. The viscosity of base

As shown in Fig. 2, the viscosities of supposi-

tory bases at 37°C increased with increasing CP concentrations in the suppository, 10% CP had a 2.2 times larger viscosity than H-15 alone. It is thought that approximately 3 ml of rectal solution is present in human rectum, so the viscosity of the suppository base may get larger by forming a gel with rectal solution.

There are various grades of CP having different abilities for forming gels. CP 934P, used in this study, has a middle viscosity of 29 400–39 400 mPa s at 0.2%, 20 rpm, 25°C.

#### 3.2. Drug release properties of suppositories

As shown in Fig. 3, the release rate of RAM was larger in 2% CP suppositories than in 10% CP suppositories. We hypothesized that addition of small amounts of CP improved the water absorbability of the base and facilitated the release of RAM, but large amounts of CP formed a highly viscous gel layer and suppressed the release of RAM.

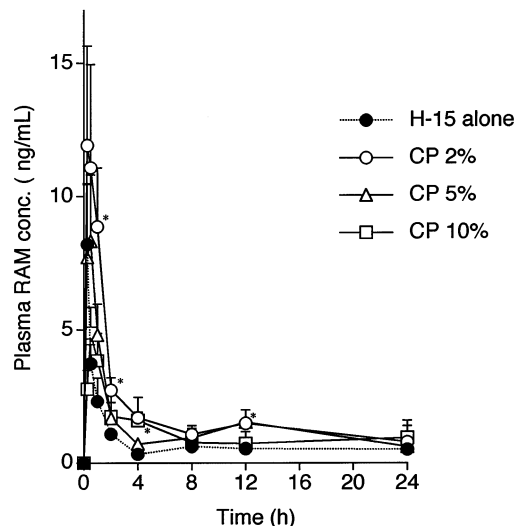


Fig. 4. Plasma concentration profiles of ramosetron after rectal administration of suppositories to rabbits (\*  $P < 0.05$  vs. H-15 alone). Each point represents the mean  $\pm$  S.E. of 3–4 rabbits.

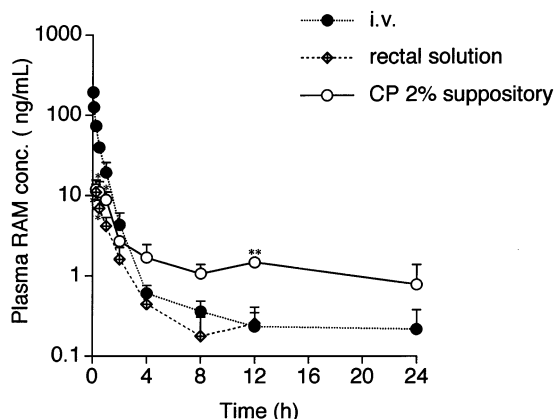


Fig. 5. Plasma concentration profiles of ramosetron after i.v. or rectal administration to rabbits (\*  $P < 0.05$ , \*\*  $P < 0.01$  vs. H-15 alone). Each point represents the mean  $\pm$  S.E. of 3–4 rabbits.

### 3.3. Animal studies

Fig. 4 shows the plasma concentration profiles of RAM after rectal administration of each suppository. Suppositories containing CP showed larger absorption compared with H-15 suppositories, and 2% CP suppositories had the highest  $C_{\max}$ . Further, 2% CP suppositories had remarkably prolonged plasma concentration profiles compared with H-15 suppositories with a RAM concentration of 1.7 ng/ml at 4 h after administration.

Fig. 5 shows logarithmic plot of plasma RAM concentrations after administration of 2% CP sup-

positories and i.v. injection (0.5 mg/3 kg, an identical dose as suppositories). At 4 h after administration, the plasma RAM concentrations supplied by 2% CP suppositories were larger than caused by i.v. of RAM, suggesting clinical usefulness.

### 3.4. Pharmacokinetic data analysis

Table 1 shows the pharmacokinetic parameters of RAM for each administration route. As it was reported that optimum adding amount of CP exists at diclofenac-Na suppository (Iwamoto et al., 1987), 2% CP suppositories had the highest AUC, 2.5 times larger than H-15 suppositories. The AUC decreased with increasing concentrations of CP, correlating with in vitro release behavior.

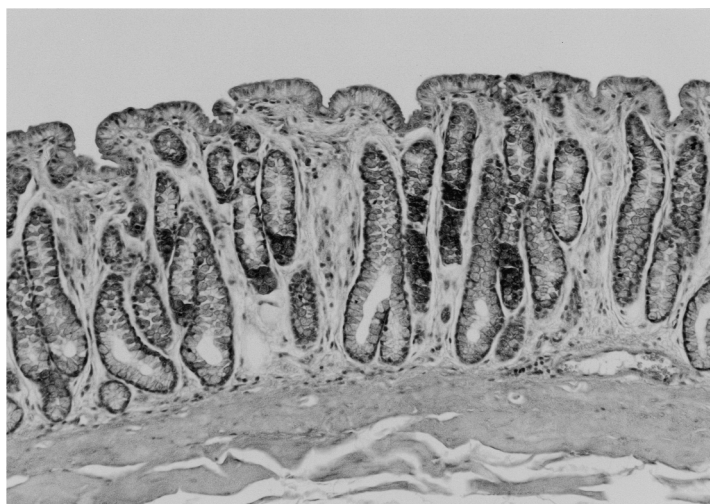
It also might be intended that absorption area of RAM should be limited in some extent and absorption content was restricted by much addition of CP. Drugs absorbed to the lower region of the rectum enter directly into the systemic circulation, therefore rectal absorption of high clearance drugs in the lower region of the rectum may result in increased systemic availability due to avoidance of hepatic first-pass elimination (De Boer et al., 1982). RAM however, may not be accepted first-pass effect so much, and its AUC was influenced by absorption area rather than absorption region. By addition of 2% CP, the increase of base viscosity was small and release rate improved as already described, therefore might bring a highest AUC.

Table 1  
Pharmacokinetic parameters following administration of ramosetron to rabbits (0.5 mg/kg)<sup>a</sup>

Route of administration	$C_{\max}$ (ng/ml)	$T_{\max}$ (h)	$AUC_{0-24\text{ h}}$ (ng h/ml)	MRT (h)	BA (%)
Intravenous	$211.5 \pm 5.7$	–	$83.7 \pm 14.7$	$1.3 \pm 0.3$	100.0
Rectal (solution)	$11.3 \pm 2.6^{***}$	$0.3 \pm 0.1$	$14.2 \pm 4.0^*$	$2.1 \pm 0.8$	16.9
Rectal (suppository)					
H-15 alone	$8.5 \pm 2.2$	$0.4 \pm 0.2$	$17.8 \pm 6.0^*$	$6.5 \pm 2.4$	21.3
CP 2%	$14.9 \pm 2.5^{***}$	$0.6 \pm 0.2$	$43.9 \pm 8.6$	$7.0 \pm 1.5^*$	52.4
CP 5%	$9.7 \pm 2.8^{***}$	$0.6 \pm 0.2$	$32.6 \pm 4.3^*$	$8.4 \pm 0.5^{***}$	38.9
CP 10%	$5.3 \pm 0.6^{***}$	$0.6 \pm 0.2$	$27.4 \pm 9.4^*$	$8.7 \pm 2.8$	32.8

<sup>a</sup> Each point represents the mean  $\pm$  S.E. of four experiments (\*  $P < 0.05$ , \*\*\*  $P < 0.001$  vs. i.v.).

H-15 alone



CP 10%



Fig. 6. Microphotographs of recta from rabbits at 24 h after administration of each suppositories for 5 days. H&E stain ( $\times 100$ ).

It was reported that bioavailability (BA;  $AUC/AUC_{i.v.}$  (%)) of ondansetron after rectal administration of its solution is 58% (Hsyu et al., 1994). In this study, BA for rectal administration of RAM solution was 16.9%, suggesting that RAM is highly water soluble (574 mg/ml) and may be difficult to be absorbed by rectal membranes.

MRT values at all suppositories increased compared by i.v. injection, became larger with increasing amounts of CP added in suppository correlating with viscosity of suppository base.

### 3.5. Morphological test of rectal tissues

Rectal irritation tests were performed and visual examinations did not detect erythema, edema, dots of bleeding, or any other inflammations. Shown in Fig. 6, the morphology of rectal tissues also indicated that suppository containing 10% CP did not irritate or damage rectal tissues. Whereas the maximum content of CP for external use is 4 w/w % in Japan, this study indicates 10% CP suppositories are safe.

### 3.6. Antiemetic effects

The antiemetic effects of 2% CP suppositories of RAM were examined. Each suppository was administered to ferret 30 min before i.p. administration of cisplatin (10 mg/kg) and the ferrets were observed for 6 h. As a control, injection of cisplatin was followed after a latency period of 98 min by 53 retches and 10 vomits over a duration of 183 min. At 0.1 µg/kg of RAM, cisplatin was followed after latency of 221 min by 47 retches and 6 vomits over a duration of 117 min, suggesting a significant delay in the onset of retching as well as a marked reduction in the number of retches and vomits. At 3 and 30 µg/kg of RAM, emetic responses to cisplatin were completely abolished.

Fujihara et al. (1994) i.v. administered a RAM solution to five ferrets 30 min before i.v. administration of cisplatin (10 mg/kg) and emesis was recorded for 6 h after administration of RAM. Emesis was defined as rhythmic abdominal contractions, either with (vomiting) or without (retching) associated expulsion of solid or liquid material in their study. As a control, cisplatin was followed after a latency period of  $74 (\pm 4)$  min by  $11.6 \pm (2.0)$  emesis. At 0.1 µg/kg of RAM, cisplatin was followed after a latency period of  $197 (\pm 44)$  min by  $7.8 (\pm 3.0)$  emesis, and emetic responses to cisplatin were completely abolished at 3 µg/kg of RAM. Even though each suppository was examined in one ferret in our studies, these results indicate that 2% CP suppository might have the same antiemetic effect as conventional i.v. administration.

## 4. Conclusion

In absorption studies, AUC with RAM suppository containing 2% CP were 2.5 times larger than conventional H-15 suppository because of an increased release rate and a MRT prolonged by 5.8 h over the MRT of i.v. injection. Further, our antiemetic studies indicated that RAM suppository containing 2% CP might have the same antiemetic effects as i.v. injections. Onset and duration of nausea induced by cytotoxic agents

like cisplatin has been shown to be about 1–48 h (Triozi and Laszlo, 1987), suggesting that 2% CP suppositories of RAM may be suitable as safe and effective antiemetic drugs, having clinical significance from a viewpoint of the patient's quality of life.

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