Effects of HSP-117, a Novel Tachykinin NK<sub>1</sub>-Receptor Antagonist, on Cisplatin-Induced Pica as a New Evaluation of Delayed Emesis in Rats

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ABSTRACT—The effects of a novel tachykinin NK<sub>1</sub>-receptor antagonist HSP-117 {(2S,3S)-3-[(5-isopropyl-2,3-dihydrobenzofuran-7-yl)methyl]amino-2-phenylpiperidine dihydrochloride} on cisplatin-induced pica, i.e., the eating of nonnutritive substances such as kaolin were examined in rats. HSP-117 inhibited kaolin intake in a dose-dependent manner for 2 days. The 5-HT<sub>3</sub>-receptor antagonist ondansetron inhibited only on the first day, but not on the second day. These results indicate that the cisplatin-induced kaolin intake on the first day is related to both 5-HT<sub>3</sub>- and NK<sub>1</sub> receptors, while only the NK<sub>1</sub> receptor is involved on the second day. Thus, cisplatin-induced continuous pica in rats represents a useful model of not only acute but also delayed emesis.

Keywords: Cisplatin-induced pica, Tachykinin NK<sub>1</sub>-receptor antagonist, Delayed emesis

Although rats are commonly used as laboratory animals in many studies, they have not been commonly used for studies on emesis, because rats as well as mice and guinea pigs are not susceptible to vomiting. Takeda et al. (1 – 3) reported that pica, i.e., the ingestion of nonnutritive substances such as kaolin, in rats was induced by various emetic stimuli such as copper sulfate, apomorphine, cisplatin, an anticancer drug, and motion. Based on their findings, they concluded that pica in rats is analogous to vomiting in other species and suggested that it may be a useful animal model for research on emesis.

Bountra et al. (4) reported that the tachykinin NK<sub>1</sub>-receptor antagonist CP-99,994 had anti-emetic activity in ferrets, and this was followed by a number of pharmacological studies demonstrating that selective NK<sub>1</sub>-receptor antagonists such as CP-122,721 (5), GR203040 (6) or GR205171 (7) exhibited potent anti-emetic properties against a wide variety of emetogens, which act centrally, peripherally or at a mixed site. The anti-emetic activity and effect of these NK<sub>1</sub>-receptor antagonists were potent and broad. Furthermore, the characteristics of these drugs have been shown to be dependent on penetration into the brain (8, 9), which are consistent with their acting centrally. Hence, the NK<sub>1</sub> antagonists are represented as a new class of antiemetics (10).

We previously reported that a novel and selective NK<sub>1</sub>-receptor antagonist, HSP-117 {(2S,3S)-3-[(5-isopropyl-2,3-dihydrobenzofuran-7-yl)methyl]amino-2-phenylpiperidine dihydrochloride}, inhibited morphine- and copper sulfate-induced emesis in ferrets, suggesting that HSP-117 may act centrally via the nucleus tractus solitarius and/or the area postrema (11, 12).

However, no reports have mentioned the effects of NK<sub>1</sub>-receptor antagonists on pica in rats as an analogue to emesis in other species, especially the evaluation of a cisplatin-induced delayed emesis, which occurs 24 h or later after cisplatin injection. The use of pica to evaluate the delayed emesis in rats would be useful in further examining the involvement of NK<sub>1</sub> receptor in the developmental mechanism of emesis, because rats have been extensively researched morphologically, electrophysiologically and neuroanatomically. In the present study, we have examined the effects of HSP-117 on cisplatin-induced pica in rats.

Male Wistar rats weighing about 150 g (Kyudo Co., Kumamoto) were used. The rats were housed in a room at 20 – 23°C with a 12:12-h light-dark cycle (light on at
07:00 h) and were given free access to commercial food (Kyudo Co.) and tap water. A kaolin clod was prepared by the method of Takeda et al. (1) with slight modifications. Kaolin was mixed with 1% gum arabic in adequate distilled water to form golf ball size clods weighing about 50 g, which were dried at room temperature. The glass container of kaolin clods was placed before the experiment, and the animals were allowed to adapt to the presence of both containers. Each kaolin clod and standard food container was removed, weighed to the nearest 0.1 g refilled, and replaced at 18:00 h each day. Kaolin splinters were collected, dried, and weighed, in order to arrive at a correct value of intake.

After a 3-day adaptation period, animals were given cisplatin via an intraperitoneal (i.p.) injection. Each kaolin and standard food intake was then measured at 24-h intervals for a maximum of 7 days. HSP-117, its inactive enantiomer (2R,3R)-3-[(5-isopropyl-2,3-dihydrobenzofuran-7-yl)methyl]amino-2-phenylpiperidine dihydrochloride ((R)-HSP-117) and CP-99,994 ((+)-(25,35)-3-(2-methoxybenzylamino)-2-phenylpiperidine) were administered i.p. or intracerebroventricularly (i.c.v.) by a single administration 10 min prior to the cisplatin injection. Ondansetron (2 mg/kg) and its combination with dexamethasone (1 mg/kg) were given i.p. at 10 min before, 24 and 48 h after the cisplatin injection.

For i.c.v. injection of drugs, a 21-gauge stainless guide cannula (length 13 mm) was inserted into the right lateral brain ventricle (coordination site: anterial, −0.8 mm; lateral, 1.3 mm from the bregma). The tip of the guide cannula was located vertically 3.3 mm below the skull surface and the cannula was fixed to the skull with dental cement and two holding screws. The animals were then allowed to recover for 7 days before experiments.

CP-99,994, HSP-117 and (R)-HSP-117 synthesized at Hisamitsu Pharmaceutical Co., Inc. (Tsukuba). Ondansetron and dexamethasone were purchased from Sankyo (Tokyo). Cisplatin was purchased from Sigma (St. Louis, MO, USA). These compounds were dissolved in saline or artificial cerebrospinal fluid. Kaolin and gum arabic were purchased from Katayama Chemical (Osaka).

Data are expressed as the mean ± S.E.M. of each treatment group. Significant differences between the control and treatment groups were determined by one-way analysis of variance (ANOVA) by Dunnett’s test.

To elucidate the characteristics of cisplatin-induced pica in rats, kaolin intake was observed for 7 days after the injection of cisplatin at doses of 5 and 10 mg/kg. The intake on the first day and on the second day after cisplatin (10 mg/kg) increased significantly during the observation period (Table 1).

| Table 1. Time course for kaolin intake induced by cisplatin in rats |
|------------------------|--------|--------|--------|--------|--------|--------|--------|
| Days after cisplatin injection | 0     | 1      | 2      | 3      | 4      | 5      | 6      | 7      |
| Control                | 0.15 ± 0.05 | 0.10 ± 0.00 | 0.10 ± 0.04 | 0.28 ± 0.18 | 0.15 ± 0.03 | 0.15 ± 0.07 | 0.15 ± 0.09 | 0.13 ± 0.06 |
| Cisplatin, 5 mg/kg     | 0.10 ± 0.03 | 0.34 ± 0.16 | 0.20 ± 0.08 | 0.46 ± 0.20 | 0.30 ± 0.16 | 0.20 ± 0.06 | 0.14 ± 0.05 | 0.26 ± 0.10 |
| Cisplatin, 10 mg/kg    | 0.26 ± 0.08 | 0.90 ± 0.06 | 0.71 ± 0.17 | 0.30 ± 0.03 | 0.17 ± 0.05 | 0.34 ± 0.08 | 0.36 ± 0.11 | 0.27 ± 0.12 |

Cisplatin was dissolved in saline and administered intraperitoneally. Each value represents the mean ± S.E.M. of 4–9 animals. Asterisks * and ** indicate a value significantly different from the control at P<0.05 and P<0.01, respectively.
I.p. injection of cisplatin at doses of 5 and 10 mg/kg induced an increase of kaolin intake, especially significantly at 10 mg/kg, for 2 days afterward in a dose-dependent manner (Table 1). I.p. single administration of selective NK₁-receptor antagonists, HSP-117 and CP-99,994, markedly inhibited the cisplatin-induced increase of kaolin intake both on the first day and the second day (Fig. 1A). Recently, the potent and broad-spectrum anti-emetic activity of NK₁-receptor antagonists have been demonstrated in several emetic models (5–7), and they have been regarded as potential novel antiemetics (10). In particular, NK₁-receptor antagonists inhibited cisplatin-induced delayed emesis in ferrets (13). In addition, HSP-117 also resulted in inhibition of cisplatin-induced delayed emesis in ferrets (data not shown), which suggested that it may act centrally via the area postrema (11, 12). These findings suggested that inhibition of pica in rats on the second day by NK₁-receptor antagonists corresponds to suppression in delayed emesis in ferrets by them. Furthermore, a single i.c.v. injection of HSP-117 was very effective, but (R)-HSP-117 had no effect (Fig. 1B). Therefore, central NK₁ receptors appear to play an important role in pica, which is induced by cisplatin in rats.

Ondansetron, a 5-HT₃-receptor antagonist, significantly inhibited the cisplatin-induced increase of kaolin intake on the first day, but not on the second day (Fig. 2). The result here of the first day after cisplatin was consistent with that of Takeda et al. (1), which was measured by kaolin intake on the first day alone after administration of ondansetron (1 and 2 mg/kg, i.p.). The finding that ondansetron did not affect the cisplatin-induced increase of kaolin intake on the second day provides additional new evidence for emesis research.

A combination of ondansetron (2 mg/kg) with dexamethasone (1 mg/kg) tended to inhibit the increase of kaolin intake on the second day (Fig. 2). The 5-HT₃-receptor antagonist ondansetron has been shown to substantially improve cisplatin-induced acute emesis (14), and the interaction between ondansetron and dexamethasone have been investi-
gated with respect to delayed emesis. Rudd and Naylor reported that the co-administration of ondansetron and dexamethasone had an additive effect in abolishing cisplatin-induced delayed emesis in ferrets (15). It is interesting because their report is consistent with clinical findings and our present results. Thus, we concluded that the cisplatin-induced increase in kaolin intake on the second day is analogous to delayed emesis in other species.

In conclusion, cisplatin-induced pica in rats was observed by the continuous increase of kaolin intake, the increase of which was not biphasic. However, the reactivity against HSP-117 and ondansetron was different with respect to the first and the second increase in kaolin intake. The present results indicate that the first increase is related to both 5-HT\(_1\) and NK\(_1\) receptors, while the second increase is related to NK\(_1\) but not to 5-HT\(_3\) receptors. These findings suggest that cisplatin-induced continuous pica in rats represents a useful animal model of not only acute but delayed emesis. In addition, the NK\(_1\) antagonist HSP-117 will become a novel and worthy antiemetic for acute and delayed emesis.

REFERENCES


5 Gonsalves S, Watson J and Ashton C: Broad spectrum anti-emetic effects of CP-122,721, a tachykinin NK\(_1\) receptor antag-