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Diphenidol Has No Actual Broad Antiemetic Activity in Dogs and Ferrets

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Abstract. Previous studies showed that diphenidol was effective on emetogens-induced pica, eating of non-nutritive substances, in rats, a model analogous to emesis in other species. We evaluated the actual antiemetic activity of diphenidol against four emetic stimuli in the dog and ferret, animals that possess an emetic reflex. In dogs, emetic responses to apomorphine were significantly prevented by diphenidol (3.2 mg/kg, i.v.), whereas diphenidol (3.2 mg/kg, i.v. × 2) showed a weak inhibition to the vomiting evoked by cisplatin. In ferrets, diphenidol (10 mg/kg, i.p.) exhibited a weak antiemetic activity on the emesis induced by copper sulfate and had no activity on emesis by loperamide. On the other hand, CP-122,721, a NK_1-receptor antagonist, significantly reduced the emetic episodes to all four stimuli. These results suggest that the prediction of antiemetic activity of compounds in animals lacking an emetic reflex does not always correspond with actual antiemetic activity.

Keywords: cisplatin, copper sulfate, apomorphine, loperamide, pica

Introduction

The study of emesis is complicated by the fact that not all animals have a vomiting reflex. A majority of studies have used dogs and ferrets. In small animals, *Suncus murinus* (house musk shrew) has become established as a model for the study of emesis (1, 2). In contrast, common small laboratory rodents such as the rat and guinea pig lack the emetic reflex. Administration of poison or motion stimulus in rats induced a response called 'pica' which means the increased intake of a non-nutritional diet such as kaolin (3). It has been hypothesized that pica in rats is analogous to emesis in other species and that this model would be useful for pharmacological research of the emetic reflex (4). Conditioned taste aversion induced by some toxins in the rat is also considered to reflect the activation of pathways that are analogous to those involved in nausea and/or vomiting in other species (5, 6).

Diphenidol, having antiemetic activity against apomorphine (7), is a drug for the treatment of vertigo in Japan. Takeda et al. (8, 9) showed that diphenidol prevented pica induced by motion stimulus, apomorphine, and cisplatin in rats, suggesting that diphenidol might inhibit a common locus of emesis in the brain. NK_1-receptor antagonists that penetrate the blood-brain barrier exhibited potent antiemetic properties against a wide variety of emetic stimuli in ferrets and dogs (10 – 12). CP-122,721 is a potent NK_1-receptor antagonist that suppresses vomiting caused by various emetic stimuli in ferrets and dogs (13, 14).

To date, there have been no reports on the effect of diphenidol on experimental emesis models, except an original report on apomorphine-induced emesis in dogs (7). Therefore, we evaluated the actual antiemetic activity of diphenidol on peripherally or centrally acting drug-induced emesis in dogs and ferrets that possess an emetic reflex and compared the results with CP-122,721.

Materials and Methods

Animals

Beagle dogs of either sex weighing 8.0 – 14.5 kg (Kitayama Labes, Yamaguchi or Yakken Farm, Hyogo)

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and adult male ferrets weighing 1.0 – 2.3 kg (Marshall Research Animal, North Rose, NY, USA) were used in this study. Both dogs and ferrets were individually housed in an animal room at 23 ± 1°C with light on between 07:00 and 19:00, routinely fed a dry pellet, TC-1 (Maruha Pet Food, Tokyo) and Ferret Diet (PMI Feeds, St. Louis, MO, USA), respectively; water was available ad libitum. In all experiments, animals were removed from their home cages and transferred to observation cages in a quiet room. All animal experimental procedures were performed under the guidelines of the Animal Experiment Committee of Fujisawa Pharmaceutical Co., Ltd.

Dosage of diphenidol

In a preliminary experiment, since intravenous injection of diphenidol at 10 mg/kg caused non-lethal convulsions in dogs, doses of diphenidol were used at less than 3.2 mg/kg for dogs. Systemic administration of diphenidol has not previously been studied in the ferret and therefore the dose to be used was selected on the basis of preliminary studies in dogs.

Experiments in dogs

Apomorphine-induced emetic responses in dogs: Apomorphine at 0.1 mg/kg was subcutaneously administered in dogs. The animals were observed continuously for 60 min for the emetic responses, the time of onset to vomiting, and the number of both retches and vomits. According to a method described previously (15), animals were retested 2 weeks later with apomorphine, 10 min after treatment with intravenous injection of diphenidol (0.32 – 3.2 mg/kg), CP-122,721 (0.1 mg/kg), or vehicle.

Cisplatin-induced acute emesis in dogs: After the administration of cisplatin (3.2 mg/kg), animals were observed continuously for 5 h, and the numbers of incidences of emesis were counted. The presence of vomiting separated from the next bout by at least 1 min was considered as a single emetic episode. Diphenidol (3.2 mg/kg), CP-122,721 (0.1 mg/kg), or vehicle was intravenously administered 10 min before and 90 min after the injection of cisplatin.

Experiments in ferrets

Copper sulfate-induced emesis in ferrets: Ferrets were deprived of food overnight. Copper sulfate solution (40 mg/kg) was rapidly flushed into the stomach via an orogastric tube. Following administration of copper sulfate animals were observed continuously for 30 min and the number of incidences of emetic responses consisting of retches plus vomits were counted. Diphenidol (3.2 and 10 mg/kg), CP-122,721 (0.32 and 1 mg/kg), or vehicle was intraperitoneally administered 10 min before the administration of copper sulfate.

Loperamide-induced emesis in ferrets: Ferrets were subcutaneously injected with loperamide (0.5 mg/kg). The animals were observed continuously for 90 min for the emetic responses, the time of onset to retching, and the number of both retches and vomits. Diphenidol (3.2 and 10 mg/kg), CP-122,721 (1 mg/kg), or vehicle was intraperitoneally administered 10 min before loperamide-treatment.

NK_1-receptor binding

Professor S. Nakanishi (Kyoto University, Kyoto) kindly provided Chinese hamster ovary (CHO) cells stably transfected with the human tachykinin NK_1 receptor. The crude CHO cell membranes were prepared as previously described (14). Cell membrane (6 μg/ml) were incubated with ^{125}I-BH-substance P (0.1 nM; New England Nuclear, Boston, MA, USA) in the absence and presence of test compounds in 0.25 ml of medium (50 mM Tris-HCl, pH 7.4, 5 mM $MnCl_2$, 20 μg/ml chymostatin, 40 μg/ml bacitracin, 4 μg/ml leupeptin, 5 μg/ml *p*-APMSF, and 200 μg/ml BSA) for 90 min at room temperature. Reactions were terminated by rapid vacuum filtration through filters presoaked in 0.1% polyethyleneimine. The filters were then washed with buffer (50 mM Tris-HCl, pH 7.4, and 5 μM $MnCl_2$). The radioactivity was counted by using an auto γ-counter. Nonspecific binding was determined using excess unlabeled substance P (3 μM). Experiments were carried out in duplicate.

Drugs

Cisplatin, apomorphine, loperamide, and diphenidol were purchased from Sigma-Aldrich (St. Louis, MO, USA). Copper sulfate pentahydrate was purchased from Wako Pure Chemicals (Osaka). and CP-122,721 [(+)-(2*S*,3*S*)-3-(2-methoxy-5-trifluoro-methoxybenzyl)amino-2-phenylpiperidine] were synthesized at the Medicinal Chemistry Laboratories of Fujisawa Pharmaceutical Co. (Osaka). Cisplatin was prepared in saline at 70°C followed by gradual cooling to 40°C and administered immediately in a volume of 1 ml/kg. Apomorphine was dissolved in saline (0.2 mg/ml). Copper sulfate was dissolved in distilled water (20 mg/ml). Loperamide was dissolved in distilled water containing 7% propylene glycol (0.25 mg/ml). Diphenidol and CP-122,721 were dissolved in 5% glucose solution and administered in volume of 0.5 ml/kg for dogs or 2 ml/kg for ferrets. For in vitro experiments, diphenidol was dissolved in dimethyl sulfoxide.

Statistical analysis

Group results are expressed as the mean ± S.E.M. Dunnett's test or a paired Student's *t*-test was used as a measure of significance. Values of $P<0.05$ were regarded as statistically significant.

Results

Experiments in dogs

Apomorphine-induced emetic responses in dogs: As shown in Table 1, pretreatment with diphenidol (0.32 – 3.2 mg/kg, i.v.) reduced the emetic responses induced by apomorphine in a dose-dependent manner. Diphenidol at 3.2 mg/kg markedly reduced the number of retches + vomits, increased the latency to vomit, and completely prevented emesis in 3 of 4 dogs. CP-122,721 (0.1 mg/kg, i.v.) completely inhibited apomorphine-induced emesis (Table 1).

Cisplatin-induced acute emesis in dogs: As shown in Table 2, diphenidol (3.2 mg/kg, i.v. × 2) administered 10 min before and 90 min after cisplatin weakly reduced the emesis caused by cisplatin, whereas CP-122,721 (0.1 mg/kg, i.v. × 2) significantly inhibited the emesis and completely prevented emesis in 1 of 3 dogs.

Experiments in ferrets

Copper sulfate-induced emesis in ferrets: As shown in Table 3, the numbers of retches + vomits were weakly inhibited by diphenidol (3.2 and 10 mg/kg, i.p.). CP-122,721 (0.32 and 1 mg/kg, i.p.) reduced the emetic episodes caused by copper sulfate and increased, but not significantly, the time for onset of emesis. CP-122,721 at both doses completely prevented emesis in one of three ferrets (Table 3).

Loperamide-induced emesis in ferrets: Intraperitoneal injection of diphenidol at 3.2 mg/kg showed a weak inhibition of loperamide-induced emesis, whereas diphenidol at 10 mg/kg was ineffective (Table 4). On the other hand, CP-122,721 (1 mg/kg, i.p.) produced a significant increase in the latency to the first emesis, markedly reduced the number of retches + vomits, and completely prevented emesis in 2 of 3 ferrets.

NK_1-receptor binding

Diphenidol at 1 and 10 μM inhibited ^{125}I-BH-Substance P binding to membranes prepared from CHO cells expressing human tachykinin NK_1 receptor by –3.4% and 8.7%, respectively.

Table 1. Effects of diphenidol and CP-122,721 on apomorphine-induced emesis in dogs

Treatment[a]	Animals (vomits/total)	Latency[b] (min)	Retches + Vomits	Inhibition (%)
Control	3/3	3.7 ± 1.2	112.7 ± 1.2	—
Diphenidol (0.32 mg/kg)	3/3	4.6 ± 0.6	75.3 ± 1.7	29.7
Control	3/3	2.7 ± 1.2	111.7 ± 16.8	—
Diphenidol (1 mg/kg)	2/3	24.1 ± 12.3	26.3 ± 13.4	71.8
Control	4/4	6.2 ± 2.2	95.8 ± 19.2	—
Diphenidol (3.2 mg/kg)	1/4	51.2 ± 8.8[c]	3.8 ± 3.8[c]	97.3
Control	3/3	5.8 ± 0.8	116.0 ± 3.1	—
CP-122,721 (0.1 mg/kg)	0/3	60.0 ± 0.0[d]	0.0 ± 0.0[d]	100

[a]Drugs were intravenously administered 5 min before the injection of apomorphine. [b]If a dog did not vomit, the latency period was taken to be equal to the observation time (60 min). Compared with the control (paired *t*-test), [c]$P<0.05$, [d]$P<0.01$.

Table 2. Effects of diphenidol and CP-122,721 on cisplatin-induced acute emesis in dogs

Treatment[a]	Animals (vomits/total)	Latency[b] (min)	Vomits	Inhibition (%)
Control	3/3	99 ± 2	16.7 ± 2.3	—
Diphenidol 3.2 mg/kg × 2	3/3	118 ± 3	11.0 ± 1.5	34.1
CP-122,721 0.1 mg/kg × 2	2/3	186 ± 58[c]	2.0 ± 1.5[c]	88.0

[a]Drugs were intravenously administered 10 min before and 90 min after the injection of cisplatin. [b]If a dog did not vomit, the latency period was taken to be equal to the observation time (300 min). Compared with the control, [c]$P<0.01$.

Table 3. Effects of diphenidol and CP-122,721 on copper sulfate-induced emesis in ferrets

Treatment[a]	Animals (vomits/total)	Latency[b] (min)	Retches + Vomits	Inhibition (%)
Control	4/4	2.6 ± 0.4	91.3 ± 11.5	—
Diphenidol (3.2 mg/kg)	4/4	3.1 ± 1.1	64.3 ± 8.4	29.6
Control	3/3	2.9 ± 0.5	79.7 ± 18.3	—
Diphenidol (10 mg/kg)	4/4	2.8 ± 1.0	57.8 ± 17.2	27.5
Control	3/3	1.8 ± 0.6	79.0 ± 12.5	—
CP-122,721 (0.32 mg/kg)	2/3	11.2 ± 9.4	23.7 ± 13.3[c]	70.0
CP-122,721 (1 mg/kg)	2/3	12.2 ± 8.9	12.3 ± 8.6[c]	84.4

[a]Drugs were intraperitoneally injected 10 min before the administration of copper sulfate. [b]If a ferret did not vomit, the latency period was taken to be equal to the observation time (30 min). Compared with the control, [c]$P<0.05$.

Table 4. Effects of diphenidol and CP-122,721 on loperamide-induced emesis in ferrets

Treatment[a]	Animals (vomits/total)	Latency[b] (min)	Retches + Vomits	Inhibition (%)
Control	4/4	13.0 ± 0.9	95.5 ± 13.6	—
Diphenidol (3.2 mg/kg)	4/4	13.7 ± 1.6	58.3 ± 18.0	39.0
Control	3/3	13.6 ± 2.0	77.3 ± 11.8	—
Diphenidol (10 mg/kg)	4/4	8.2 ± 2.0	69.5 ± 18.5	10.1
Control	3/3	11.7 ± 2.6	126.0 ± 18.3	—
CP-122,721 (1 mg/kg)	1/3	74.6 ± 15.4[c]	0.3 ± 0.3[d]	99.8

[a]Drugs were intraperitoneally injected 10 min before the administration of loperamide. [b]If a ferret did not vomit, the latency period was taken to be equal to the observation time (90 min). Compared with the control, [c]$P<0.05$, [d]$P<0.01$.

Discussion

In the present study, diphenidol failed to exhibit the practical antiemetic activity against cisplatin-, copper sulfate-, and loperamide-induced emesis, but not that induced by apomorphine, in ferrets and dogs, although it was suggested that diphenidol might have broad antiemetic activity from the results of the rat pica model (8, 9). On the other hand, CP-122,721, a potent NK_1-receptor antagonist, significantly prevented emesis evoked by all four of the above stimuli, as expected. Several studies suggest that the site of the antiemetic action of NK_1-receptor antagonists is located in the brainstem known to be associated with the emetic reflex (11, 12, 16). Because diphenidol had negligible affinity for the NK_1 receptor, it is suggested that the hypothetical site of the anti-pica action of diphenidol by Takeda et al. (8, 9) is different from the site of NK_1-receptor antagonists.

A previous study demonstrated that oral, rectal, or intramuscular administration of diphenidol was effective on apomorphine-induced emesis in dogs, similar to chlorpromazine (7). In the present study, we confirmed that the intravenous injection of diphenidol at 3.2 mg/kg, a maximum safety dose for dogs from our preliminary experiment, markedly reduced emesis caused by apomorphine. On the other hand, diphenidol (3.2 mg/kg × 2) had weak antiemetic action against cisplatin-induced emesis in dogs, compared with CP-122,721. Animal models, especially dogs or ferrets, of chemotherapy-induced acute emesis successfully predicted the clinical efficacy of the antiemetics, such as 5-HT_3-receptor antagonists, for the controlling of vomiting (17). We previously showed that ondansetron, a 5-HT_3-receptor antagonist, significantly prevented cisplatin-induced emesis in dogs (14). The action of ondansetron on cisplatin-induced pica in rats is controversial. Recently it was shown that ondansetron potentiated cisplatin-induced acute kaolin ingestion (18), while the previous study has shown that ondansetron can reduce the kaolin ingestion induced by cisplatin (9). The mechanisms of pica induced by cisplatin are essentially unknown and not necessarily related to a potential to induce emesis. Therefore, the inhibitory activity of diphenidol on cisplatin-induced kaolin ingestion in rats is considerably different from the actual antiemetic activity of cisplatin in dogs.

There is no data of diphenidol to evaluate the anti-

emetic activity against copper sulfate-induced pica in rodents. In this study, diphenidol only showed a weak inhibition, no more than 30%, in actual emesis caused by copper sulfate in ferrets, whereas CP-122,721 inhibited the emetic responses. It has been hypothesized that cytotoxic chemotherapeutic agents (i.e., cisplatin) and ingested chemical irritants (i.e., copper sulfate) either directly or indirectly activate vagal afferent nerves that trigger the central emetic pathway (19). Indeed, vomiting induced by cisplatin and copper sulfate was inhibited markedly by abdominal visceral nerve section (20, 21). Further studies are required to elucidate the effect of visceral nerve section on cisplatin- or copper sulfate-induced pica in rats.

Loperamide, an anti-diarrheal drug, reliably induces emesis in the ferret when given subcutaneously (22). The emetic response to loperamide is unaffected by abdominal vagotomy but is abolished by ablation of the area postrema (22). Thus, the probable mechanism of emesis by loperamide is activation of opioid receptors in the area postrema (22). The present study showed that CP-122,721 markedly reduced loperamide-induced emetic responses, while diphenidol produced mild inhibition at 3.2 mg/kg and lack of the antiemetic activity at 10 mg/kg. If diphenidol activity is related to inhibition of a common locus of emesis in the brain (8, 9), diphenidol should exhibit antiemetic activities mediated by both peripheral and central pathways, like NK_1-receptor antagonists.

A recent report suggests that the pharmacological sensitivity of pica in rats is different from the clinically observed nausea and vomiting (18). The present study also demonstrated that diphenidol, despite suppressing emetics- or motion-induced pica in rats (8, 9), failed to exhibit uniformly antiemetic activity on each four drugs-induced emesis in animals to vomit. In the experimental protocol of pica in rats, kaolin and food intake were measured for 24 h after treatment with emetogens (8, 9). In the present experimental protocol, animals were observed continuously for 30 min to 4 h after administration of emetic stimuli (see Materials and Methods) because animals usually do not vomit after these observation periods. Thus, it is reasonable that the inhibitory effects on pica in the rodent do not always correspond with the actual antiemetic activity.

In conclusion, the failure of diphenidol to prevent cisplatin-, copper sulfate-, and loperamide-induced emesis in dogs and ferrets may suggest that the assessment of antiemetic activity by pica in rats is unlikely to reflect the actual antiemetic activity against vomiting in animals.

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