ELSEVIER

European Journal of Pharmacology 305 (1996) 181–185

ejp

Short communication

Broad spectrum antiemetic effects of CP-122,721, a tachykinin NK_1 receptor antagonist, in ferrets

Susan Gonsalves [a,*], John Watson [b], Cynthia Ashton [a]

[a] *Department of General Pharmacology, Box 384, Central Research Division, Pfizer Inc., Eastern Point Road, Groton, CT 06340, USA*
[b] *Department of Immunology, Central Research Division, Pfizer Inc., Groton, CT 06340, USA*

Received 11 January 1996; revised 6 March 1996; accepted 12 March 1996

Abstract

The potent, selective, tachykinin NK_1 receptor antagonist, CP-122,721 ([(+)-(2*S*,3*S*)-3-(2-methoxy-5-trifluoromethoxybenzyl)amino-2-phenylpiperidine]), at 0.01–1 mg/kg, s.c. reduced retching and vomiting elicited by loperamide, copper sulfate, ipecac syrup and cisplatin in a dose-dependent manner. ID_{50} values after subcutaneous administration ranged from 0.02 mg/kg (loperamide) to 0.08 mg/kg (ipecac). Oral CP-122,721 reduced cisplatin-induced emesis with an ID_{50} of approximately 0.08 mg/kg. The less active (2*R*,3*R*)-enantiomer, CP-132,687, did not significantly suppress retching or vomiting induced by any of the emetogens. These data support the hypothesis that CP-122,721 blocks emesis by a specific action at tachykinin NK_1 receptors. Its broad spectrum of antiemetic activity suggests a central site of action.

Keywords: Emesis; Tachykinin NK_1 receptor antagonist; CP-122,721; (Ferret)

1. Introduction

The hypothesis that substance P plays a role in mediating the emetic reflex is supported by a growing body of immunocytochemical, behavioral and electrophysiological evidence. For example, investigators (see Leslie, 1985) have detected substantial amounts of substance P-like immunoreactivity in vagal fibers and in brain regions (e.g., the area postrema and the underlying dorsal vagal complex) thought to be involved in the emetic response. Other studies have demonstrated that substance P elicits emesis in dogs after systemic administration, albeit on an inconsistent basis (Carpenter et al., 1984), and induces retching in urethane-anesthetized ferrets after topical application to the area postrema (Andrews, 1994). Substance P resembles a variety of other emetogens in that it increases the firing rate of cells in the area postrema and nucleus tractus solitarius after iontophoretic application in vivo (Carpenter et al., 1983). More recently, a number of investigators have independently demonstrated that the selective, tachykinin NK_1 receptor antagonist, CP-99,994 (McLean et al., 1993), blocks emesis induced by a variety of emetogens when given s.c. to ferrets (cisplatin, morphine, loperamide, apomorphine, ipecac syrup, X-irradiation and copper sulfate; Bountra et al., 1993; Tattersall et al., 1993; Watson et al., 1995), i.v. to dogs (apomorphine, copper sulfate; Watson et al., 1995) or i.p. to the house musk shrew *Suncus murinus* (nicotine; Tattersall et al., 1995).

The present study profiles the antiemetic activity of CP-122,721 ([(+)-(2*S*,3*S*)-3-(2-methoxy-5-trifluoromethoxybenzyl)amino-2-phenylpiperidine]), a potent, selective, apparently non-competitive, orally active, tachykinin NK_1 receptor antagonist (McLean et al., 1996). The results suggest that CP-122,721 is about three times more potent than CP-99,994 in blocking the retching and vomiting responses to central (loperamide), peripheral (copper sulfate, cisplatin) and mixed site (ipecac syrup) emetogens.

2. Materials and methods

2.1. Animals

Adult male ferrets (Marshall Farms, 750–1445 g) were housed individually in a temperature-, light- and humidity-controlled environment for 1–2 weeks prior to

* Corresponding author. Tel.: (1) (860) 441-5315; fax: (1) (860) 441-8367.

PII S0014-2999(96)00216-6

experiments. They were given a standard pelleted diet and water ad libitum. They were fasted overnight prior to administration of intragastric copper sulfate and ipecac syrup.

2.2. Procedures

CP-122,721 (0.01–1.0 mg/kg) or its less active (2*R*,3*R*)-enantiomer, CP-132,687 (1 mg/kg) was injected s.c. 30 min prior to intragastric administration of copper sulfate (0.5 mg/ml, 12.5 mg/kg) or ipecac syrup (1 mg/kg of ether soluble alkaloids of ipecac), subcutaneous administration of loperamide (250 μg/kg), or intraperitoneal administration of cisplatin (10 mg/kg). The dose of each emetogen was selected based on previous data showing that it elicited emesis in at least 90% of ferrets tested.

After administration of an emetogen, ferrets were observed continuously in individual polycarbonate cages for 60 (ipecac syrup, copper sulfate), 90 (loperamide) or 120 (cisplatin) min. Previous studies have demonstrated that these intervals are adequate for characterization of the emetic response to the above-mentioned stimuli (see Watson et al., 1995). The latency to first retch and vomit and the number of retches and vomits were recorded for each animal. Retching was quantified by counting rhythmic abdominal contractions, while vomiting was scored as oral expulsion of liquid or solid stomach contents. Behaviors characteristically observed before and between bouts of retching and vomiting were additionally quantified in ferrets given copper sulfate or loperamide. Instances of gagging (i.e., a single, forced abdominal contraction against a closed glottis with the mouth held wide open and culminating in an explosive release of the glottis), lip-licking and backing were recorded in aggregate and expressed as the mean number of behaviors per animal ± S.E.M. An individual animal was tested no more than three times with 5–7 days between experiments. Ferrets given cisplatin were killed at the end of the experiment.

2.3. Statistics

Results were expressed as mean ± S.E.M., n = number of animals per group. ID_{50} values for suppression of retching and vomiting by CP-122,721 were estimated from

Table 1
Effect of s.c. CP-122,721 and CP-132,687 on emesis induced by copper sulfate (12.5 mg/kg p.o.), loperamide (250 μg/kg s.c.), ipecac syrup (1 mg/kg p.o.) and cisplatin (10 mg/kg i.p.) in ferret

Emetogen	Treatment	Dose (mg/kg)	R/T [a]	Retches Mean ± S.E.M. (range)	V/T [b]	Vomits Mean ± S.E.M. (range)
Copper sulfate [c]	Saline		21/21	29.3 ± 4.1 (8–68)	20/21	4.1 ± 0.5 (0–8)
	CP-122,721	0.03	6/6	14.0 ± 4.5 * (4–34)	6/6	3.2 ± 0.5 (2–5)
		0.1	3/12	2.0 ± 1.2 * (0–12)	4/12	0.9 ± 0.5 * (0–5)
		0.3	1/9	0.3 ± 0.3 * (0–3)	1/9	0.2 ± 0.2 * (0–2)
		1.0	1/7	0.1 ± 0.1 * (0–1)	0/7	0 ± 0 *
	CP-132,687	1.0	6/6	20.7 ± 3.2 (9–29)	6/6	3.5 ± 0.6 (2-6)
Loperamide [d]	Saline		19/19	56.2 ± 6.9 (22–144)	18/19	4.6 ± 0.6 (0–10)
	CP-122,721	0.01	6/6	33.5 ± 11.0 (3–82)	4/6	3.0 ± 1.3 (0–8)
		0.03	6/6	12.0 ± 3.3 * (2–22)	2/6	0.5 ± 0.3 * (0–2)
		0.1	0/9	0 ± 0 *	1/9	0.1 ± 0.1 * (0–1)
		0.3	0/7	0 ± 0 *	0/7	0 ± 0 *
		1.0	0/7	0 ± 0 *	0/7	0 ± 0 *
	CP-132,687	1.0	6/6	48.3 ± 15.1 (10–100)	4/6	4.0 ± 1.8 (0–11)
Ipecac syrup [e]	Saline		24/24	30.8 ± 3.9 (10–82)	24/24	4.3 ± 0.4 (1–9)
	CP-122,721	0.03	5/5	21.8 ± 7.5 (2–40)	5/5	4.6 ± 1.1 (1–7)
		0.1	3/5	2.6 ± 1.5 * (0–8)	3/5	0.8 ± 0.4 * (0–2)
		0.3	0/10	0 ± 0 *	0/10	0 ± 0 *
		1.0	0/5	0 ± 0 *	0/5	0 ± 0 *
	CP-132,687	1.0	11/11	28.5 ± 6.2 (5–73)	10/11	2.8 ± 0.6 (0–7)
Cisplatin [f]	Saline		6/6	109 ± 11.2 (91–137)	6/6	9.7 ± 1.1 (6–12)
	CP-122,721	0.03	6/6	54.3 ± 14.3 * (5–100)	6/6	5.0 ± 1.2 * (1–8)
		0.1	4/6	9.8 ± 2.2 * (0–15)	4/6	1.2 ± 0.4 * (0–2)
		0.3	0/6	0 ± 0 *	0/6	0 ± 0 *
		1.0	0/6	0 ± 0 *	0/6	0 ± 0 *
	CP-132,687	1.0	6/6	106.2 ± 9.4 (78–135)	6/6	8.2 ± 1.1 (4–12)

[a] R/T, number of animals retching/number tested.
[b] V/T, number vomiting/number tested.
[c] Copper sulfate: $F_{retch}(4,50) = 15.2$, $F_{vomit}(4,50) = 16.5$; CP-132,687: $t_{retch}(25) = 0.11$, $t_{vomit}(25) = 1.02$.
[d] Loperamide: $F_{retch}(5,48) = 16.1$, $F_{vomit}(5,48) = 12.2$; CP-132,687: $t_{retch}(23) = 0.50$, $t_{vomit}(23) = 0.88$.
[e] Ipecac syrup: $F_{retch}(4,44) = 11.3$, $F_{vomit}(4,44) = 18.8$; CP-132,687: $t_{retch}(33) = 0.76$, $t_{vomit}(33) = 1.32$.
[f] Cisplatin: $F_{retch}(4,25) = 32.7$, $F_{vomit}(4,25) = 33.4$; CP-132,687: $t_{retch}(10) = 0.10$, $t_{vomit}(10) = 0.81$.
* $P < 0.05$ vs. saline (Dunnett's multiple comparison test).

dose-response data by interpolation. Dose-response data were analyzed by a one-way analysis of variance followed by Dunnett's multiple comparison test. CP-132,687 emesis data and other behavioral measures were analyzed by a robust *t*-test (R/S 1 Release 4 Software, BBN Software Products Corp.) that uses medians to generate the *t*-statistic. Where variances were unequal, statistical results were verified using censored survival analysis for response latency measurements and Poisson regression for retch and vomit counts. A $P < 0.05$ was considered statistically significant.

2.4. Reagents

A commercial brand of ipecac syrup (Barre-National, Baltimore, MD) containing 1.5 mg/ml of ether-soluble alkaloids of ipecac, 1.5% alcohol and unspecified concentrations of deionized water, glycerin, sucrose syrup and methylparaben and propylparaben preservatives was used. Copper sulfate pentahydrate (copper sulfate), loperamide HCl (loperamide) and cis-platinum(II) diamine dichloride (cisplatin) were purchased from Sigma Chemical Company. Copper sulfate was dissolved in water while loperamide was dissolved in distilled water containing 7% propyleneglycol. Cisplatin was dissolved in 154 mM NaCl. CP-122,721 ([(+)-(2*S*,3*S*)-3-(2-methoxy-5-trifluoromethoxybenzyl)amino-2-phenylpiperidine]) and CP-132,687, the (2*R*,3*R*)-enantiomer of CP-122,721, were synthesized by the Medicinal Chemistry Department at Pfizer. Both CP-122,721 and CP-132,687 were dissolved in saline.

3. Results

CP-122,721 (0.01–1 mg/kg, s.c.) suppressed retching and vomiting elicited by copper sulfate, loperamide, ipecac syrup and cisplatin in a dose-dependent manner (Table 1). CP-122,721 inhibited loperamide-induced retching with an ID_{50} of approximately 0.02 mg/kg vs. ID_{50} values of approximately 0.03, 0.03 and 0.05 mg/kg for copper sulfate, cisplatin and ipecac, respectively. The corresponding ID_{50} values for inhibition of vomiting ranged from 0.02 mg/kg for loperamide to 0.08 mg/kg for ipecac. CP-122,721 provided complete protection against loperamide-induced emesis at 0.1 mg/kg and against the other three emetogens at 0.3 mg/kg. On the other hand, the less active (2*R*,3*R*)-enantiomer, CP-132,687, at 1 mg/kg, s.c., did not significantly reduce the frequency of emetic episodes induced by any of the emetogens tested compared to vehicle-treated animals.

In contrast to its effects on the number of retches, CP-122,721 did not significantly increase the latency to retching. A trend towards increased latency was, however, noted after both ipecac (mean ± S.E.M.: ipecac vehicle = 27.4 ± 1.8 min [$n = 24$]; 0.03 mg/kg = 25.6 ± 2.4 min [$n = 5$]; 0.1 mg/kg = 36.0 ± 8.6 min [$n = 3$]) and cisplatin (mean ± S.E.M.: cisplatin vehicle = 26.7 ± 4.5 min [$n = 6$]; 0.03 mg/kg = 37.3 ± 14.6 min [$n = 6$]; 0.1 mg/kg = 40 ± 5.8 min [$n = 5$]). Although the latency to retch in the cisplatin experiments was much shorter than observed in previous studies (Watson et al., 1995), statistical comparisons were made with vehicle-treated animals tested concurrently.

A variety of prodromal signs of emesis (e.g., gagging, lip-licking and backing) were evident in both CP-122,721- and vehicle-treated animals. Quantification of preemetic events in two groups showed that doses of CP-122,721 sufficient to reduce retching by at least 50% did not significantly suppress the gagging, lip-licking and backing elicited by either copper sulfate (mean number of behaviors ± S.E.M.: saline = 9.4 ± 1.6 [$n = 21$]; CP-122,721 (0.03 mg/kg) = 14.0 ± 2.6 [$n = 6$]) or loperamide (mean number of behaviors ± S.E.M.: saline = 31.4 ± 3.1 [$n = 19$]; CP-122,721 (0.03 mg/kg) = 27.0 ± 3.1 [$n = 6$]).

CP-122,721 was also effective as an antiemetic when given orally. Against cisplatin, oral CP-122,721 inhibited retching and vomiting with an ID_{50} of approximately 0.08 mg/kg (Table 2). A 1 mg/kg oral dose blocked emesis completely.

Table 2
Effect of oral CP-122,721 on cisplatin-induced (10 mg/kg i.p.) emesis in ferret

Emetogen	Treatment	Dose (mg/kg)	R/T [a]	Retches Mean ± S.E.M. (range)	V/T [b]	Vomits Mean ± S.E.M. (range)
Cisplatin [c]	Saline		5/5	80.4 ± 13.5 (36–121)	5/5	9.6 ± 1.7 (3–13)
	CP-122,721	0.03	5/5	68.4 ± 17.6 (31–104)	5/5	8.0 ± 1.4 (4–12)
		0.1	4/5	32.0 ± 10.0 * (0–61)	4/5	3.4 ± 1.3 * (0–7)
		0.3	0/5	0 ± 0 *	1/5	0.4 ± 0.4 * (0–2)
		1.0	0/5	0 ± 0 *	0/5	0 ± 0 *

[a] R/T, number of animals retching/number tested.
[b] V/T, number vomiting/number tested.
[c] Cisplatin: $F_{retch}(4,20) = 11.8$, $F_{vomit}(4,20) = 13.5$.
* $P < 0.05$ vs. saline (Dunnett's multiple comparison test).

4. Discussion

A member of the 2-phenylpiperidine class of tachykinin NK_1 receptor antagonists, CP-122,721 is an analog of CP-99,994. It was selected for evaluation because it has a high affinity for the human tachykinin NK_1 receptor ($pIC_{50} = 9.8$ ($IC_{50} = 0.14$ nM), determined by measuring the displacement of [^{125}I]-Bolton Hunter-substance P binding from IM-9 cells); has excellent oral activity; and, in contrast to CP-99,994, interacts with the receptor in a non-competitive manner (McLean et al., 1996). The present study confirms previous work with CP-99,994 (Bountra et al., 1993; Tattersall et al., 1993, 1995; Watson et al., 1995) that demonstrated the broad spectrum of antiemetic activity of this class of compounds. This study also highlights the potency of CP-122,721. When dosed either subcutaneously or orally in ferrets, it inhibited vomiting and retching induced by both centrally and peripherally acting emetogens with ID_{50} values between 0.02 and 0.08 mg/kg. In contrast, the (2*R*,3*R*)-enantiomer, CP-132,687, which has a pIC_{50} of 6.0 ($IC_{50} = 897$ nM) at the human tachykinin NK_1 receptor expressed in IM-9 cells (McLean et al., 1996), failed to reduce significantly the incidence of emetic events when given at 1 mg/kg, s.c. This finding supports the hypothesis that CP-122,721 acts through specific blockade of tachykinin NK_1 receptors.

In contrast to its effects on retching and vomiting, CP-122,721 failed to block the gagging, lip-licking and backing that consistently preceded the mechanical acts of retching and vomiting. In contrast, 5-HT_3 receptor antagonists (Bermudez et al., 1988) suppress both prodromal events and retching/vomiting in a dose-dependent manner. Some investigators have suggested that animal behaviors such as lip-licking and backing may be analogous to self-reported nausea in humans (Bermudez et al., 1988). However, the relationship between the two phenomena remains unclear; thus, the present data cannot be used to predict whether or not the tachykinin NK_1 receptor antagonists will block nausea in clinical trials.

The emetogens used in this study exercise their effects through a variety of pathways, i.e., centrally at the area postrema (loperamide; Bhandari et al., 1992), peripherally by activation of predominantly vagal (abdominal visceral) afferents (copper sulfate and cisplatin; Andrews et al., 1990) and both centrally and peripherally (ipecac syrup; Leslie and Reynolds, 1992). The ability of CP-122,721 to block the action of both central and peripheral emetic stimuli suggests that it acts at a central convergence site in the emetic pathway, possibly some portion of the nucleus tractus solitarius (Gardner et al., 1994; Watson et al., 1995). The failure of CP-122,721 to block gagging, lip-licking and backing at doses that significantly reduce retching and vomiting suggests that these prodromal behaviors may not be NK_1-mediated and that they may involve neural pathways distinct from those responsible for the mechanical acts of retching and vomiting.

Finally, data showing complete blockade of retching and vomiting after the 0.3 mg/kg dose of CP-122,721 compared to the 1 mg/kg dose of CP-99,994 for three of the four emetogens suggest that CP-122,721 is at least three times more potent than CP-99,994 as an antiemetic (Watson et al., 1995). The present data do not explain the differences in antiemetic potency between the two receptor antagonists; however, the non-competitive binding profile of CP-122,721 and its three times greater potency for the tachykinin NK_1 receptor ($IC_{50} = 0.14$ vs. 0.48 nM for CP-99,994) may be contributing factors. Thus, based on its pharmacologic and pharmacodynamic profile, including its failure to block prodromal behaviors, CP-122,721 represents an additional tool for elucidating both the pathways involved in the emetic reflex and the role of the tachykinin NK_1 receptor in emetic responses.

Acknowledgements

The authors gratefully acknowledge Gerald Antognoli for technical assistance and Craig Trost, Ph.D. for statistical support.

References

Andrews, P.L.R., 1994, 5-HT_3 receptor antagonists and antiemesis, in: 5-HT_3 Receptor Antagonists, eds. F.D. King, B. Jones and G.J. Sanger (CRC Press, Boca Raton, FL) p. 255.

Andrews, P.L.R., C.J. Davis, S. Bingham, H.I.M. Davidson, J. Hawthorn and L. Maskell, 1990, The abdominal visceral innervation and the emetic reflex: pathways, pharmacology and plasticity, Can. J. Physiol. Pharmacol. 68, 325.

Bermudez, J., E.A. Boyle, W.D. Miner and G.J. Sanger, 1988, The anti-emetic potential of the 5-hydroxytryptamine$_3$ receptor antagonist BRL 43694, Br. J. Cancer 58, 644.

Bhandari, P., S. Bingham and P.L.R. Andrews, 1992, The neuropharmacology of loperamide-induced emesis in the ferret: the role of the area postrema, vagus, opiate and 5-HT_3 receptors, Neuropharmacology 31, 735.

Bountra, C., K. Bunce, T. Dale, C. Gardner, C. Jordan, D. Twissel and P. Ward, 1993, Anti-emetic profile of a non-peptide neurokinin NK_1 receptor antagonist, CP-99,994, in ferrets, Eur. J. Pharmacol. 249, R3.

Carpenter, D.O., D.B. Briggs and N.L. Strominger, 1983, Response of neurons of canine area postrema to neurotransmitters and peptides, Cell. Mol. Neurobiol. 3, 113.

Carpenter, D.O., D.B. Briggs and N. Strominger, 1984, Behavioral and electrophysiological studies of peptide-induced emesis in dogs, Fed. Proc. 43, 2952.

Gardner, C.J., C. Bountra, K.T. Bunce, T.J. Dale, C.C. Jordan, D.J. Twissel and P. Ward, 1994, Anti-emetic activity of neurokinin NK-1 receptor antagonist is mediated centrally in the ferret, Br. J. Pharmacol. 112, 516P.

Leslie, R.A., 1985, Neuroactive substances in the dorsal vagal complex of the medulla oblongata: nucleus of the tractus solitarius, area postrema, and dorsal motor nucleus of the vagus, Neurochem. Int. 7, 191.

Leslie, R.A. and J.A. Reynolds, 1992, Functional anatomy of the emetic circuitry in the brainstem, in: Mechanisms and Control of Emesis, Vol. 223, eds. A.L. Bianchi, L. Grelot, A.D. Miller and G.L. King (Colloque INSERM/John Libbey Eurotext) p. 19.

McLean, S., A. Ganong, P.A. Seymour, R.M. Snider, M.C. Desai, T. Rosen, D.K. Bryce, K.P. Longo, L.S. Reynolds, G. Robinson, A.W. Schmidt, C. Siok and J. Heym, 1993, Pharmacology of CP-99,994; a nonpeptide antagonist of the tachykinin neurokinin-1 receptor, J. Pharmacol. Exp. Ther. 267, 472.

McLean, S., A. Ganong, P.A. Seymour, D.K. Bryce, R.T. Crawford, J. Morrone, L.S. Reynolds, A.W. Schmidt, S. Zorn, J. Watson, A. Fossa, M. DePasquale, J. Heym and T. Rosen, 1996, Characterization of CP-122,721; a non-peptide antagonist of the neurokinin NK_1 receptor, J. Pharmacol. Exp. Ther. (in press).

Tattersall, F.W., W. Rycroft, R.J. Hargreaves and R.G. Hill, 1993, The tachykinin NK_1 receptor antagonist CP-99,994 attenuates cisplatin induced emesis in the ferret, Eur. J. Pharmacol. 250, R5.

Tattersall, F.W., W. Rycroft, N. Marmont, M. Cascieri, R.G. Hill and R.J. Hargreaves, 1995, Enantiospecific inhibition of emesis induced by nicotine in the house musk shrew (*Suncus murinus*) by the neurokinin$_1$ (NK_1) receptor antagonist CP-99,994, Neuropharmacology 34, 1697.

Watson, J.W., S.F. Gonsalves, A.A. Fossa, S. McLean, T. Seeger, S. Obach and P.L.R. Andrews, 1995, The anti-emetic effects of CP-99,994 in the ferret and the dog: role of the NK_1 receptor, Br. J. Pharmacol. 115, 84.