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Short communication

Effects of a 5-HT_{1A} receptor agonist on acute and delayed cyclophosphamide-induced vomiting

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Abstract

LY228729 [(−)-4(dipropylamino)-1,3,4,5-tetrahydrobenz-{c,d}indole-6-carboxamide}], an agonist at the 5-HT_{1A} subtype of 5-HT receptor, was studied as an antiemetic in pigeons dosed with a highly emetic oncolytic agent, cyclophosphamide. An intramuscular injection of 0.32 mg/kg of LY228729 administered 15 min prior to the intravenous injection of 200 mg/kg of cyclophosphamide totally prevented the acute emetic response induced by cyclophosphamide. When used as a rescue therapy in a separate group of pigeons, LY228729 (0.32 mg/kg, i.m.) prevented further emetic episodes when it was administered after vomiting had already been induced by cyclophosphamide. Injections of LY228729 given at intervals over the next 2 d also attenuated the delayed emetic response induced by cyclophosphamide. LY228729 appears to be a broad spectrum antiemetic agent that is effective against the anticipatory, the acute and the delayed stages of emesis induced by oncolytic agents. © 1997 Elsevier Science B.V.

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1. Introduction

Serotonin (5-hydroxytryptamine, 5-HT) plays a critical role in chemotherapy-induced nausea and vomiting. Both clinical and preclinical data (Kamoto et al., 1993) indicate that antagonists at the 5-HT_3 receptor block the acute phase of emesis which occurs within the first 8 h after the infusion of chemotherapy agents such as cisplatin. However, these 5-HT_3 receptor antagonists (e.g., ondansetron, tropisetron, granisetron) do not prevent either the delayed emesis which occurs 1–3 d following chemotherapy (Kris et al., 1994), or the anticipatory nausea and vomiting which may occur prior to subsequent treatments. The lack of efficacy of both traditional anti-emetics and the newer 5-HT_3 receptor antagonists in the treatment of anticipatory nausea and vomiting and delayed emesis remains a significant problem for cancer patients.

In pigeons, 5-HT_{1A} receptor agonists, as well as 5-HT_3 receptor antagonists, prevent emesis induced by a variety of emetogenic agents such as cisplatin, syrup of ipecac, emetine and a 5-HT_3 receptor agonist, *m*CPBG (*m*-(chlorophenyl)-biguanide) (Wolff and Leander, 1995). However, the 5-HT_{1A} receptor agonists have a much broader range of antiemetic activity in that they also prevent vomiting that has been conditioned to occur in response to specific environmental stimuli (an animal model of anticipatory nausea and vomiting) as well as vomiting induced by DTG (ditolyguanidine). In contrast, 5-HT_3 receptor antagonists are ineffective in these situations (Wolff and Leander, 1994). Moreover, vomiting induced by motion both in the cat (Lucot and Crampton, 1987) and in *Suncus murinus* (Okada et al., 1994), by apomorphine in the ferret (Rudd et al., 1992), by nicotine and veratrine in *Suncus murinus* (Okada et al., 1994), or by xylazine in the cat (Lucot and Crampton, 1987) are also prevented by 5-HT_{1A} receptor selective agonists but not by 5-HT_3 receptor antagonists.

Cyclophosphamide, a commonly used potent anti-cancer drug, induces nausea and vomiting in the clinic (Beck, 1995). As the pigeon responds to a wide range of emetic stimuli (Wolff and Leander, 1994, 1995), it is a useful species for the study of clinical problems related to therapy-induced emesis. The present study was conducted to determine if cyclophosphamide would induce the acute and delayed vomiting in the pigeon that is associated with its clinical use in humans. LY228729 [(−)-4(dipropylamino)-1,3,4,5-tetrahydrobenz{c,d}indole-6-carboxamide}], a potent 5-HT_{1A} receptor agonist (Foreman et al., 1993),

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was subsequently tested as an antiemetic at a dose of 0.32 mg/kg which is known to eliminate vomiting to a variety of emetic stimuli in the pigeon (Wolff and Leander, 1994, 1995).

2. Materials and methods

2.1. Animals

Fourteen male White Carneaux pigeons that weighed approximately 550–650 g were purchased from the Palmetto Pigeon Plant, Sumter, SC. The birds were housed in individual stainless steel cages and were given free access to Purina ProGrains For Pigeons, water and oyster shell grit, except during the acute emetic test session. The colony room was illuminated from 6 a.m. to 6 p.m. daily and was kept at a constant temperature and humidity.

2.2. Drugs

LY228729 (synthesized by Eli Lilly, Indianapolis, IN) was dissolved in distilled water with the aid of a drop of lactic acid. Cyclophosphamide (purchased from Sigma Chemical, St. Louis, MO) was dissolved in a small volume of alcohol and then diluted with distilled water. All drugs were injected in a volume of 1 ml/kg of body weight. The LY228729 (0.32 mg/kg) and associated vehicle were injected into the breast muscle (i.m.), whereas the cyclophosphamide (200 mg/kg) and associated vehicle were injected into a vein (i.v.) on the underside of the wing.

2.3. Acute emetic effects of cyclophosphamide

Three pigeons were injected with 200 mg/kg of cyclophosphamide and 2 birds were injected with an equivalent volume of the cyclophosphamide vehicle. The birds were then placed in individual plexiglas observation cages and were observed continuously for the next 4 h (approximately 1 h after the occurrence of the last emetic episode). The latency for the onset of emesis, the number and time of vomiting episodes, and the weight of the pigeons at the end of the observation period were recorded. The 3 pigeons that were injected with cyclophosphamide were euthanized immediately following the experiment.

2.4. Antiemetic effects of LY228729 on cyclophosphamide-induced emesis

Nine pigeons were divided into 3 treatment groups ($n = 3$/group). The 3 birds assigned to the control group were injected with saline. The 3 pigeons in the prophylactic group were injected with 0.32 mg/kg of LY228729, whereas the 3 pigeons in the rescue group were not given an injection at this time. Fifteen min later, all 9 pigeons were injected with 200 mg/kg of cyclophosphamide and then were placed into individual plexiglas observation cages for the next 5 h. Once vomiting had been established in the 3 pigeons in the rescue group (they had not been given the first injection of saline or LY228729), they also were administered 0.32 mg/kg LY228729.

The pigeons were observed continuously for the first $\frac{1}{2}$ h after the administration of cyclophosphamide. After that, they were checked at 1 h intervals for the presence or absence of vomitus in the bottom of the observation box. The birds were returned to their home cages 5 h after the cyclophosphamide injection. Members of the rescue group were injected (i.m.) with an additional dose of 0.32 mg/kg of LY228729 at 3 and again at 6 h after the first administration of LY228729. Thereafter, these 3 birds (rescue group) were injected with 0.32 mg/kg of LY228729 at 8 a.m. and again at 4 p.m. for the next 2 d. Members of the other 2 groups (control and prophylactic) were injected with saline at the same times. The cages of all the birds were checked for the presence or absence of vomitus at this same time.

3. Results

3.1. Emetic effects of cyclophosphamide

All 3 pigeons injected with cyclophosphamide vomited after an average latency of 10.3 ($\pm$3.7 S.E.M.) min. The birds averaged 9.3 ($\pm$1.3) vomits and lost an average of 77 ($\pm$4) g of body weight during the 4 h observation period. All of the vomits occurred within the first 3 h after cyclophosphamide administration. None of the pigeons injected with the cyclophosphamide vehicle vomited. The vehicle treated birds lost an average of 11 g of body weight during the observation period.

3.2. Anti-emetic effects of LY228729 on acute and delayed emesis induced by cyclophosphamide

The data are summarized in Table 1. The saline (control) pretreated birds vomited within the first 30 min of cyclophosphamide administration and continued to show additional signs of vomiting for the first 3 h of the observation period. Weight lost was 72 ($\pm$5) g at the end of the 3 h time period. In contrast, LY228729 administered 15 min prior to cyclophosphamide (prophylactic group) totally prevented the acute emetic response to cyclophosphamide and the weight loss was only 20 ($\pm$8 S.E.M.) g. When injected after vomiting had begun (rescue group), LY228729 prevented further vomiting within 30 min of its administration. By the end of the acute 3 h observation period, these birds had lost an average of 42 ($\pm$4) g. During the next 2 d, the saline treated birds and the birds in the prophylactic group (that were given only one injection of LY228729) continued to exhibit evidence of delayed vomiting. However, only one instance of vomiting

Table 1
The emetic response in pigeons following i.v. administration of 200 mg/kg of cyclophosphamide and the effect of 0.32 mg/kg of LY228729 (i.m.) on both the acute (within 5 h of emetic administration) and delayed (within 10–72 h of emetic administration) cyclophosphamide-induced emesis

Condition	1/2 h	5 h	6–24 h	24–34 h	34–48 h	48–58 h	58–72 h
Saline	3/3	3/3	2/3	2/3	1/3	2/3	3/3
LY228729 (prophylactic)	0/3	0/3	1/3	2/3	2/3	1/3	3/3
LY228729 (rescue)	3/3	0/3	0/3[a]	0/2	1/2	0/2	0/2

Data are shown as the number of pigeons vomiting/number tested. Evidence of vomit was recorded at the end of each observation period. Pigeons were euthanized after 72 h.
[a]One of the 3 pigeons died approximately 24 h after the cyclophosphamide injection.

was noted in the birds that were injected twice daily with LY228729. At the end of the 3 d experiment, the birds that had received saline had lost an average of 53 (±5) g (control group) and 43 (±5) g (prophylactic group) since the beginning of the experiment, whereas the 2 birds remaining in the LY228729 rescue group lost an average of 25 g.

4. Discussion

Cyclophosphamide (200 mg/kg) reliably induced emesis in 100% of the pigeons that were not administered the anti-emetic LY228729. The pigeon's response to cyclophosphamide is similar to that found in other species. For instance, 200 mg/kg of cyclophosphamide induces a short latency emesis in the ferret (Hawthorn et al., 1988) and in *Suncus murinus*, a house musk shrew indigenous to Japan (Torii et al., 1991), as well as in domestic pigs (Szelenyi et al., 1994). Pigeons (this study), domestic pigs (Szelenyi et al., 1994) and man (Beck, 1995) all experience vomiting up to 72 h after the administration of cyclophosphamide.

Due to the toxic effects of chemotherapy agents, the development of a suitable animal model for the study of delayed emesis has continued to be a problem. The dose of cyclophosphamide used in the present study was well tolerated by the birds during the acute emetic period and, with the exception of one animal that died within 24 h of its administration, the pigeons appeared to remain in good health for the next 72 h. After this time, the pigeons became increasingly lethargic and were euthanized. As unprotected birds continued to vomit for an extended period of time after cyclophosphamide administration, the pigeon appears to be a reasonable model in which to study the mechanisms of delayed emesis produced by oncolytics.

LY228729, administered either as a prophylactic or as a rescue therapy, blocked the emetic response to cyclophosphamide in the pigeon. Furthermore, LY228729 also prevented the delayed vomiting which occurred up to 72 h after the injection of cyclophosphamide. We have previously shown that, in the pigeon, LY228729 blocked the emetic response induced by conditioning to environmental stimuli (an animal model of anticipatory nausea and vomiting: Wolff and Leander, 1994) as well as the acute retching and vomiting induced by an i.v. injection of 10 mg/kg of cisplatin (Wolff and Leander, 1995). In the present study, LY228729 also promptly stopped vomiting once it had been established. Consequently, LY228729 shows promise as a broad spectrum antiemetic capable of preventing all 3 stages of chemotherapy-induced emesis — i.e., anticipatory, acute, and delayed vomiting.

Rudd and Naylor (1994) found that, although ondansetron blocked the vomiting which normally occurs up to 18 h following cisplatin administration in the ferret, additional doses of ondansetron did not protect these animals from vomiting on days 2 and 3 following cisplatin administration. The mechanism responsible for delayed emesis has not yet been determined but it is quite likely that its cause is different from that which gives rise to the acute emetic response and may well involve various emetogenic factors. The fact that 5-HT_3 receptor antagonists are effective anti-emetics against the acute effects of cyclophosphamide (Torii et al., 1991), and other cytotoxic agents such as cisplatin, but that they are ineffective during the delayed emetic phase (Kris et al., 1994) is evidence for such a hypothesis. Furthermore, although the acute phase of vomiting is accompanied by an increased release of serotonin (as indicated by urinary 5-hydroxyindoleacetic acid (5-HIAA) concentrations), serotonin release is not increased during the delayed emetic phase (Wilder-Smith et al., 1993). In order to completely protect the patient from the emetic side effects of chemotherapy, anti-emetics with a much broader spectrum of activity than the 5-HT_3 receptor antagonists would appear to be required. The use of a 5-HT_{1A} receptor agonist, such as LY228729, might well be considered in this context.

In summary, the present data extend the diversity of emetic stimuli against which LY228729 has been shown to be effective and suggest that LY228729 will have a broader spectrum of anti-emetic activity than do the 5-HT_3 receptor antagonists.

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