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Biphasic Emetic Response of Cyclophosphamide in the Ferret

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WONG, R. H., L. LAO, B. M. BERMAN, A. K. CARTER, AND R. L. WYNN. *Biphasic emetic response of cyclophosphamide in the ferret.* PHARMACOL BIOCHEM BEHAV **58**(1) 179–182, 1997.—Cyclophosphamide (177 mg/kg, IV; $n = 8$) produced a biphasic emetic response in the ferret with a mean ± SE of 23.3 ± 4.0 emetic episodes during a 4-h observation period. The emetic profile of cyclophosphamide showed a first phase with 18.6 ± 3.9 episodes and a second phase with 4.7 ± 1.2 episodes. Ondansetron (0.07 and 0.13 mg/kg, IV) and droperidol (0.25 and 0.79 mg/kg, IV) significantly reduced the number of emetic episodes in the first phase. Metoclopramide (2.24, 4.08, and 7.07 mg/kg, IV) also significantly reduced the number of emetic episodes in the first phase, and the dose of 7.07 mg/kg completely prevented emetic episodes in the second phase. In addition, ondansetron-treated ferrets (0.04, 0.07, and 0.13 mg/kg, IV) had a significant increase in the number of emetic episodes in the second phase. © 1997 Elsevier Science Inc.

Cyclophosphamide Emesis Ondansetron Metoclopramide Droperidol Ferret

BREAST CANCER patients are often treated with an adjuvant chemotherapy regimen that includes cyclophosphamide (2,3,9). This alkylating agent has a high potential for inducing nausea and vomiting, which adds to the difficult nature of cancer treatment (6,9). Unlike many chemotherapy agents, emesis associated with cyclophosphamide may often extend up to 72 h (2). Cyclophosphamide induces emesis in a ferret model (1,7), possibly through release of serotonin to stimulate the 5-HT_3 receptor in the gastrointestinal tract and the chemoreceptor trigger zone (6,7). The serotonin (5-HT_3) receptor antagonists are effective antiemetics for cyclophosphamide-induced emesis in ferrets (1) and humans (2,3,6,9). Side effects have included headache, light-headedness and transient elevations of hepatic transaminases (2–4,6,8,9). Metoclopramide, a dopamine (D_2) receptor/5-HT_3 receptor antagonist, has been moderately effective in reducing cyclophosphamide-induced emesis in humans (2). However, metoclopramide can produce adverse extrapyramidal reactions in humans (10). The dopamine (D_2) antagonist droperidol has not been tested against cyclophosphamide-induced emesis. The purpose of this study was to examine further the characteristics of the emetic effects of intravenous cyclophosphamide in the ferret and to evaluate the antiemetic efficacy of ondansetron, a selective 5-HT_3 antagonist, metoclopramide and droperidol.

MATERIAL AND METHODS

Castrated male ferrets (fitch or albino), 1.0–2.0 kg in weight (Triple F Farm, Sayre, PA), were housed three to a cage on a 12-h light cycle. Food (Lab Diet) and water were given ad libitum. Each ferret was used only once. For testing, ferrets were placed under general anesthesia (isoflurane 5%-O_2 mixture) delivered from a vaporizer (Fortec) calibrated for isoflurane through polyethylene tubing into an anesthesia chamber. The anesthetic was scavenged out by using a vacuum tubing vented to the outside air. Each ferret was removed after loss of righting (2–5 min) and immediately weighed. For the intravenous (IV) injections, each animal was maintained under general anesthesia (isoflurane 2.5%–O_2) with a second vaporizer (Fortec) through a small nose cone. Both forepaws were shaved for ease of vein location. Cyclophosphamide monohydrate (Sigma) was dissolved in a small amount of absolute alcohol (200 mg/400 μl) immediately prior to injection. Saline (154 mM) was used to dilute the cyclo-

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TABLE 1
EMETOGENIC EFFECT OF CYCLOPHOSPHAMIDE BY DOSE IN FERRETS

Dose (mg/kg)	No. Vomiting/*n*	Emetic Episodes (Mean ± SE)	Retches (Mean ± SE)
Cyclophosphamide			
56	4/6	2.2 ± 0.9	2.8 ± 1.9
100	5/6	7.3 ± 3.2	30.5 ± 17.5
177	8/8	23.3 ± 4.0	85.3 ± 20.4
237	2/2	23.5 ± 7.5	62.5 ± 38.5

phosphamide to a final concentration of 100 mg/ml. Cyclophosphamide injections were made into the cephalic vein on the dorsal aspect of the front paw by using a rubber tourniquet and a 3- or 5-ml syringe with a 25-gauge needle. Intravenous puncture was confirmed by aspiration of a small volume of blood into the syringe, and the injections were confirmed by the lack of resistance to the syringe plunger. The following log doses of cyclophosphamide were tested: 56 (n = 6), 100 (n = 6), 177 (n = 8) and 237 (n = 2) mg/kg. For administration of the antiemetic drugs (n = 5/dose), a second IV injection was made into the opposite forepaw within 1 min after cyclophosphamide (177 mg/kg) injection. This dose of IV cy-

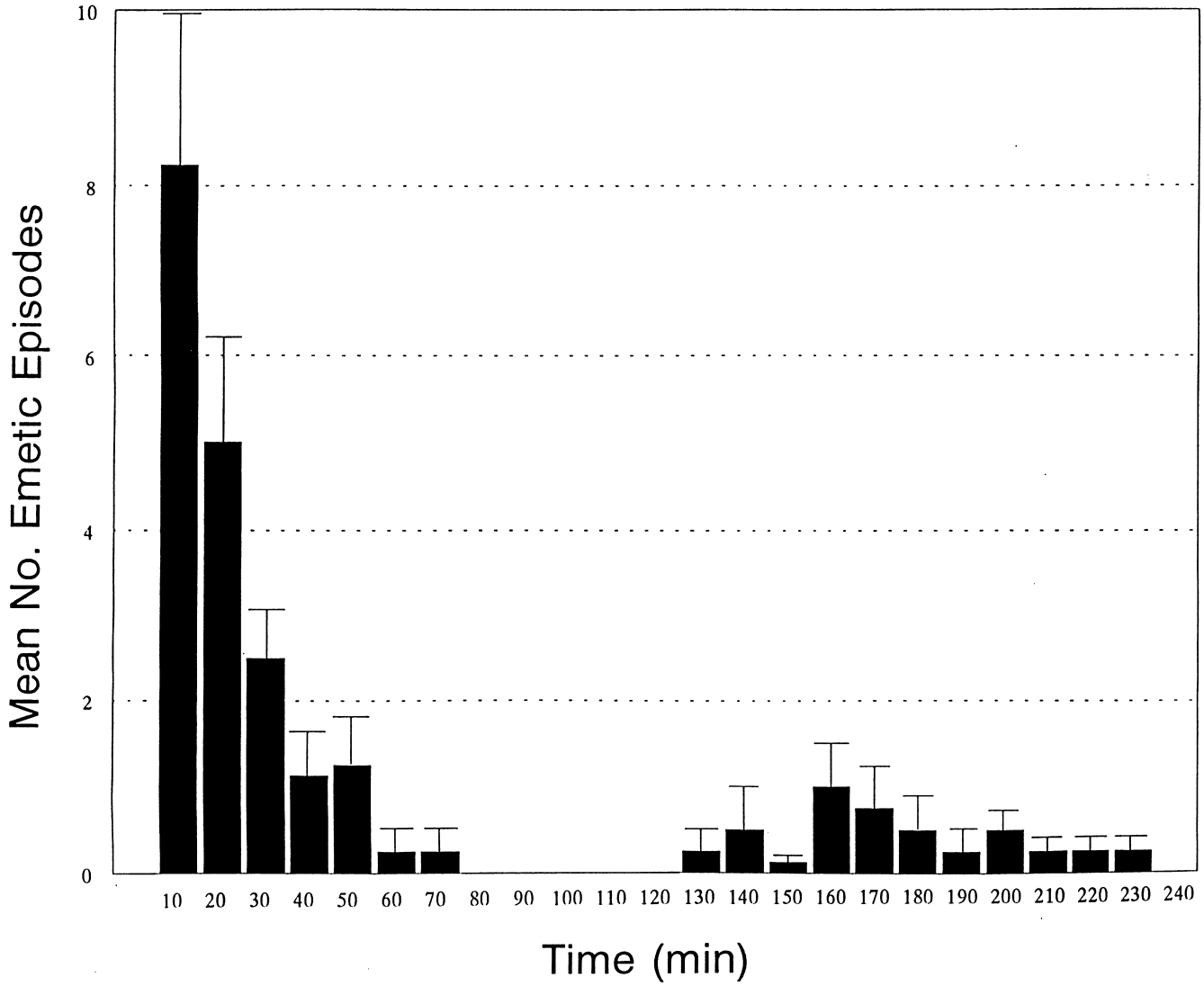

FIG. 1. Emetic profile of IV cyclophosphamide at 177 mg/kg. Two distinct phases of emesis are present.

clophosphamide was chosen for the subsequent antiemetic experiments because it produces the maximal number of emetic episodes without toxicity. Ondansetron (Glaxo, 2 mg/ml), metoclopramide (A.H. Robbins, 5 mg/ml) and droperidol (American Regent, 2.5 mg/ml) were obtained from the University of Maryland Hospital pharmacy as commercial preparations. Logarithmic doses of the antiemetic drugs were given as follows: ondansetron, 0.04, 0.07 and 0.13 mg/kg; metoclopramide, 2.24, 4.08 and 7.07 mg/kg; and droperidol, 0.25, 0.45 and 0.79 mg/kg.

After injection, each ferret was placed into an individual compartment ($60 \times 60 \times 38$ cm^2) of a six-compartment cage rack, with wire mesh floors elevated to the height of the door threshold (modified with a plexiglass front door) for observation. Each ferret was observed for 4 h after recovering from anesthesia (3–10 min). The time and number of each episode of retching and vomiting were recorded. Retching was counted as the rhythmic contraction of the abdomen without expulsion of material and emesis as a contraction with the expulsion of solid or liquid. Total emetic episodes were averaged for each group (± SE), and the effect of treatment was calculated as the percentage of reduction of emetic episodes, as previously described (11). Differences between the mean number of emetic episodes for the treatment groups and the cyclophosphamide group (177 mg/kg) were compared by Student's two-tailed *t*-test, with $p \leq 0.05$ considered significant. This study was approved by the Institutional Animal Care and Use Committees at the School of Medicine and the Dental School, University of Maryland at Baltimore.

RESULTS

The dose effect profile of cyclophosphamide-induced emesis in the ferret after intravenous injection is shown in Table 1. The dose of 237 mg/kg produced edema around the eyes and erythema in the facial area, which was interpreted as toxicity, and only two animals were tested at this dose. Evaluation of the duration of emetic episodes using time bins of 10 min revealed two distinct phases over the entire duration of effect for the doses of 100 and 177 mg/kg. These two emetic phases for the dose of 177 mg/kg are shown in Fig. 1. The first phase had a mean onset time of 1.1 ± 0.5 min and duration of 70 min. The second phase occurred 60 min after the termination of the first phase.

Based on the total number of emetic episodes, ondansetron reduced cyclophosphamide-induced emesis (177 mg/kg) by 0, 42 and 9% (0.04, 0.07, 0.13 mg/kg), metoclopramide by 48, 65 and 98% (2.24, 4.08, 7.07 mg/kg) and droperidol by 24, 16 and 38% (0.25, 0.45, 0.79 mg/kg). Significant differences in the mean number of total emetic episodes were found for metoclopramide at doses of 4.08 mg/kg ($p \leq 0.05$) and 7.07 mg/kg ($p \leq 0.005$) as compared with vehicle control. All three antiemetic drugs (ondansetron at 0.07 and 0.13 mg/kg, metoclopramide at 2.24, 4.08 and 7.07 mg/kg and droperidol at 0.25 and 0.79 mg/kg) significantly reduced the number of emetic episodes in the first phase (Table 2). Metoclopramide at 7.07 mg/kg completely prevented the second emetic phase, whereas both ondansetron- and droperidol-treated ferrets had a significant increase in the number of emetic episodes in this phase (Table 2). During the time interval of 80–120 min, ondansetron-treated ferrets had a mean ± SE of 0.4 ± 0.2, 0.4 ± 0.2 and 0.04 ± 0.04 emetic episodes (0.04, 0.07, 0.13 mg/kg); metoclopramide-treated ferrets had 0.12 ± 0.12, 0.16 ± 0.16 and 0.0 ± 0.0 emetic episodes (2.24, 4.08, 7.07 mg/kg); and droperidol-treated ferrets had 0.12 ± 0.12, 0.16 ± 0.16 and 0.0 ± 0.0 emetic episodes (0.25, 0.45, 0.79 mg/kg) during this time period.

DISCUSSION

A previous literature report evaluating cyclophosphamide at two dosages and different routes of administration showed that this chemotherapeutic drug produced emesis in the ferret (7). At an IV dose of 100 mg/kg, this study reported a mean number ± SE of 0.4 ± 0.2 emetic episodes and 4.6 ± 2.4 retches during a 4-h observation period (7). However, in our study, we observed 7.3 ± 3.2 emetic episodes and 30.5 ± 17.5 retches at 100 mg/kg (IV) in a 4-h observation period (Table 1). The only methodological differences were in our use of direct IV injection and in our preparation of the cyclophosphamide, using a final concentration of 100 mg/ml, which is commonly used in chemotherapy patients. In our study, a dose of 177 mg/kg produced two phases of emesis (Fig. 1).

The early-onset emetic phase induced by cyclophosphamide may be due to the direct central effects involving the 5-HT_3 or D_2 receptor. The antiemetic drugs ondansetron (5-HT_3 receptor antagonist) and droperidol (D_2 receptor antagonist) were only able to reduce significantly the number of emetic episodes in this phase while increasing the second phase (Table 1). Furthermore, the emetic profile of cyclophosphamide for the first phase (Fig. 1) showed a decline in episodes over time, which may indicate the clearance of the drug from the site of action.

The second emetic phase induced by cyclophosphamide may involve a different mechanism. Metoclopramide, a D_2/5-HT_3 antagonist, was the only effective antiemetic drug to reduce the number of emetic episodes in this phase (Table 1). However, the high dose of metoclopramide (7.07 mg/kg) did produce head shakes, which were interpreted as adverse central nervous system effects (mean ± SE = 82.6 ± 16.6 head shakes vs. 19.3 ± 5.9 head shakes in controls). These head shakes were characterized as a rapid, back-and-forth movement of the head occurring over a 1–2-s time period. Each 1–2-s time period was counted as one head shake. The signifi-

TABLE 2

DOSE RESPONSE OF ANTIEMETICS AGAINST CYCLOPHOSPHAMIDE IN FERRETS

		Mean ± SE Emetic Episodes	
Dose (mg/kg)	*N*	First Phase†	Second Phase‡
Vehicle	8***	18.6 ± 3.9	4.7 ± 1.2
Ondansetron			
0.04	5	12.2 ± 3.7	15.4 ± 2.8**
0.07	5	0.4 ± 0.4**	11.0 ± 2.1*
0.13	5	2.8 ± 1.7*	18.2 ± 3.5**
Metoclopramide			
2.24	5	3.2 ± 2.7*	8.2 ± 3.1
4.08	5	3.6 ± 1.9*	3.8 ± 1.3
7.07	5	0.4 ± 0.2**	0.0 ± 0.0
Droperidol			
0.25	5	5.8 ± 2.6*	11.4 ± 2.7*
0.45	5	12.0 ± 2.3	6.8 ± 2.2
0.79	5	5.6 ± 3.1*	8.8 ± 1.7

*$p \leq 0.05$, **$p \leq 0.005$ versus vehicle control values; ***2/8 concurrent controls. †Time 0 to 70 min. ‡Time 130 to 240 min.

cant increase in emetic episodes in the second phase seen with ondansetron and droperidol (Table 1) may be due to the elimination of these drugs in the tissues, thus allowing for the emetogenic effects of cyclophosphamide to predominate. Further studies are necessary to examine the different mechanisms that may be involved in the biphasic emetogenic response of cyclophosphamide. Because peak symptoms of nausea and vomiting occur 12 h after cyclophosphamide and can continue for at least 2 days in patients (5), future studies in the ferret will also need to have longer observation periods to evaluate similarities with cancer patients undergoing drug therapy.

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