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Is all radiation-induced emesis ameliorated by 5-HT_3 receptor antagonists?

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SUMMARY

Exposing ferrets to gamma rays or X-rays produces vomiting that can be attenuated by 5-HT_3 receptor antagonists and by subdiaphragmatic vagotomy. The present experiments evaluated the effectiveness of these treatments on emesis evoked by exposure to other types of radiation, fast neutrons from a nuclear reactor and high-energy protons (200 MeV), which differ in the relative effectiveness with which they produce vomiting. The results indicated that higher doses of 5-HT_3 receptor antagonists Eusatron (0.03 and 0.30 mg/kg, s.c.) and Ondansetron (0.10 and 0.30 mg/kg, s.c.) prevented emesis following neutron irradiation. Lower doses of these 5-HT_3 receptor antagonists and subdiaphragmatic vagotomy attenuated neutron-induced emesis, increasing the latency and decreasing the severity of the emetic episodes. Ondansetron (0.50 and 1.00 mg/kg, s.c.) completely prevented vomiting following exposure to high-energy protons. The results are interpreted as indicating that similar 5-HT_3-dependent mechanisms mediate emesis produced by exposure to different types of radiation, despite differences in their relative effectiveness.

Tout vomissement radio-induit est-il amélioré par les antagonistes des récepteurs 5-HT_3?

Résumé: *L'exposition de furets aux rayons gamma ou aux rayons X produit des vomissements qui peuvent être atténués par les antagonistes des récepteurs 5-HT3 et par la vagotomie sous-diapragmatique. Les expériences présentées ont évalué l'efficacité de ces traitements sur la réponse émétique évoquée par l'exposition à d'autres types de radiation, comme les neutrons rapides provenant d'un réacteur nucléaire et les protons à haute énergie (200 MeV), qui diffèrent dans leur efficacité relative à produire des vomissements. Les résultats ont montré que de fortes doses d'antagonistes des récepteurs 5-HT3 Eusatron (0,03 et 0,30 mg/Kg, s.c.) et d'Ondansetron (0,10 et 0,30 mg/Kg, s.c.) empêchent la réponse émétique qui suit l'irradiation neutronique. Des doses plus faibles de ces antagonistes et la vagotomie sous-diaphragmatique atténuent la réponse émétique induite par les neutrons, en augmentant leur latence et en diminuant leur sévérité. L'Ondansetron (0,50 et 1,00 mg/Kg s.c.) bloque le vomissement induit par l'exposition aux protons à haute énergie. Les résultats sont interprétés en indiquant que des mécanismes semblables à ceux dépendant des récepteurs 5-HT3 provoquent la réponse émétique produite par l'exposition aux différents types de radiation, en dépit de différences dans leur efficacité relative.*

INTRODUCTION

Exposure to sublethal doses of ionizing radiation can produce nausea and emesis. For the most part, studies of radiation-induced emesis have utilized exposure to X-rays (Andrews & Hawthorn, 1987; Miner *et al.*, 1987) or gamma rays (King, 1988). Pretreating ferrets with serotonin type-3 (5-HT_3) receptor antagonists or performing bilateral subdiaphragmatic vagotomy reliably attenuates the emetic response to these types of radiation, causing significant increases in latency to the first response and ameliorating the severity of the episode (Andrews & Hawthorn, 1987; Miner *et al.*, 1987; King & Landauer, 1990).

In addition to gamma rays and X-rays, emesis can be produced by exposure to other types of ionizing radiation, including neutrons and protons. However, the relative effectiveness of these types of radiation in producing emesis differs from that of gamma rays (Fig. 1). Working with a suprathreshold dose of fast neutrons from a nuclear reactor, Young (1986) reported that increasing the proportion of neutrons in a mixed neutron/gamma field increased the number of

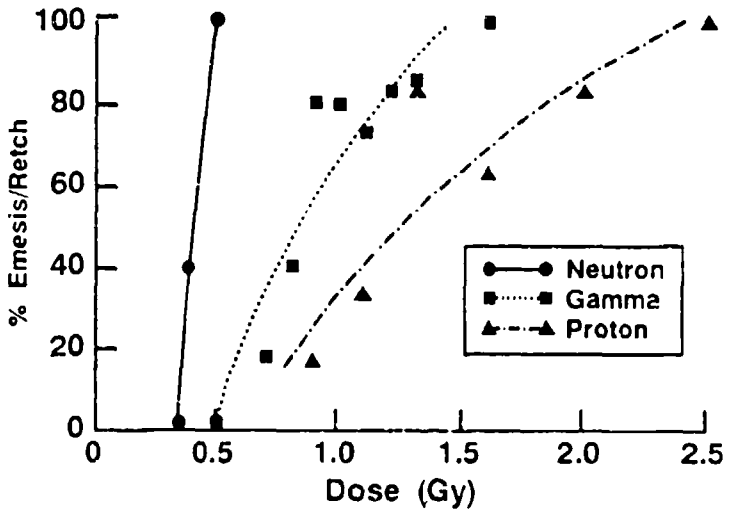

Fig. 1. Dose-response curves for emesis in ferrets following exposure to mixed neutron/gamma radiation (midline neutron dose:total dose ratio = 0.86), gamma rays (cobalt-60), and protons (200 MeV). The ED_{50}s and 95% confidence limits were: neutrons, 40 cGy (confidence limits could not be calculated because there was only a single dose with other than a 0% or 100% response); gamma rays, 85 cGy, 59/98 cGy; protons, 123 cGy, 86/153 cGy. Data redrawn from Rabin *et al.* (1992a; 1992b) and King (1988)

bouts of vomiting in an individual monkey, but did not increase in the total number of monkeys that vomited. Similarly, Rabin *et al.* (1992a) have shown that exposure to mixed fast neutron/gamma radiation from a nuclear reactor (midline neutron dose:dose ratio, 0.86) evokes emesis at lower doses than exposure to cobalt-60 gamma rays. In contrast, exposure to protons is significantly less effective in producing emesis than is exposure to gamma rays (Rabin *et al.*, 1992b). Both the threshold dose and the ED_{100} for proton-induced emesis are greater than required for gamma ray-induced emesis.

Because neutron irradiation can cause significantly greater tissue damage and gastrointestinal effects than gamma rays, Young (1986) has suggested that different mechanisms may mediate neutron-induced emesis than those that mediate emesis following exposure to gamma rays. Similarly, the observation that protons are significantly less effective in eliciting emesis than gamma rays may also imply the involvement of different mechanisms. Because 5-HT_3 receptor antagonists are not equally effective in disrupting emesis produced by all types of emetic stimuli (Andrews & Hawthorn, 1987; Costall *et al.*, 1990; Lucot, 1989), it is possible that 5-HT_3 receptor antagonists may not be effective in preventing neutron- or proton-induced emesis.

The present experiments were undertaken to evaluate the effectiveness of 5-HT_3 receptor antagonists in preventing emesis in ferrets produced by exposure to two different types of radiation: fast neutrons from a nuclear reactor and high-energy protons. For the studies of neutron-induced emesis, the relative effectiveness of two different 5-HT_3 receptor antagonists, Ondansetron and Eusatron, was evaluated using procedures designed to determine the ED_{50} of each compound. For proton-induced emesis, only Ondansetron was tested using doses that had been previously established to be effective against cisplatin-induced emesis (Higgins *et al.*, 1989). In addition, the effect of bilateral abdominal vagotomy was also tested against emesis evoked by exposure to neutrons in order to provide a comparison with previous research using gamma rays (Andrews & Hawthorn, 1987; Miner *et al.*, 1987; King & Landauer, 1990).

METHODS

Subjects: The subjects for all experiments were male fitch ferrets weighing 1.0 to 1.5 kg obtained from Marshall Farms (North Rose, NY). They were castrated and descented by the supplier. The ferrets were maintained in AAALAC-accredited animal facilities at the Armed Forces Radiobiology Research Institute (AFRRI) and at Brookhaven National Laboratory (BNL). Food and water were continually available.

Drugs: Both Ondansetron (Glaxo Res., Inc., Research Triangle Park, NC) and Eusatron (Rhone-Poulenc Rorer, King of Prussia, PA) were made fresh using 0.9% NaCl as the vehicle and given in a final volume of less than 1 cc. Both were gifts intended for research use.

Neutron Experiments:

Surgery: For laparotomy (n = 4) or bilateral abdominal vagotomy (n = 4), all animals were medicated, anesthetized, and surgically

manipulated as previously described (King and Landauer, 1990). In order for the results to be comparable with those of other experiments (Andrews and Hawthorn, 1987), animals were allowed a 7-10 day postoperative recovery period prior to irradiation.

Procedure: Twenty min prior to irradiation, ferrets were given an s.c. injection of either the compounds or vehicle. They were individually placed in a well-ventilated plastic tube and mechanically transferred to and from the exposure room through an extractor tube. The transfer took approximately 2 min in each direction. Following exposure, the ferrets were placed in a large, well-ventilated clear plastic box and behaviors observed and recorded for 2 hr, as previously described (King and Landauer, 1990). Behaviors were recorded on a personal computer. For those animals receiving 5-HT_3 receptor antagonists, the doses of Ondansetron ranged between 0.003 and 0.30 mg/kg (n = 18), and for Eusatron, between 0.001 and 0.30 mg/kg (n = 17). Test doses of these compounds were based on the method of Golub and Grubbs (1956) that can provide an ED_{50}.

Radiation and Dosimetry: Exposure to a mixed field of neutron and gamma radiation was carried out by placing the ferrets in shielded containers and exposing them to a fixed total dose of 2.0 Gy in the AFRRI TRIGA reactor. Prior to animal irradiation, the dose rate at the midline was established in a lucite phantom (which approximated the size and shape of the ferret) by using the paired-ion chamber technique (Goodman, 1985). All experiments were carried out unilaterally and the doses reported as mid-tissue dose (MTD). Corresponding values of dose-rates used in the neutron-to-total dose ratios were 1 Gy/min and 0.86 (neutron/gamma ≈ 6), respectively. Along the length of the ferret the MTD values were found to vary: the head and tail ends of the animal recorded MTDs of 93% and 85% of the midpoint MTD, respectively. Representative free-in-air photon and neutron spectra information is contained in Verbinski *et al.* (1981).

Data Analysis: For these experiments, latency to the first event was measured from the time that the irradiation ended. The individual values for the various emetic parameters obtained for each group were compared by ANOVA or the Kruskall-Wallis Rank Sum test, as appropriate. Further comparison among the groups was done with the Dunn's test or the Newman-Keul's multiple range test.

Proton Experiments:

Procedure: Thirty min prior to exposure, ferrets were given an s.c. injection of Ondansetron. The doses were 0.5 mg/kg (n = 4) and 1.0 mg/kg (n = 7). These doses were selected because previous research showed that they produced a relatively complete disruption of emesis evoked by exposure to X-rays or gamma rays (Higgins *et al.*, 1989). For exposure, the ferrets were placed in a well-ventilated plastic tube. Following exposure, the ferrets were placed in large, well-ventilated clear plastic cages where their behavior was monitored for 1.5-2.0 hr.

Radiation and Dosimetry: Exposure to protons was performed using the linear accelerator at BNL. Ferrets were placed in the center of a 35 cm diameter field perpendicular to the proton beam and given whole-

body exposures of 2.5 Gy of 200 MeV protons using 50 μsec pulses with a pulse rate of 0.333 pulses/sec. At this energy, there is a uniform dose-depth distribution through the ferret. This dose was selected because it was the lowest dose that produced emesis in 100% of the untreated subjects. The dose rate was 1.0-2.0 Gy/min. Dosimetry was performed using a 0.05 cc ionization chamber located in an acrylic phantom placed in the center of the proton field. The midline dose to the phantom was determined according to a standard protocol (Vinckier *et al.*, 1991).

RESULTS

Neutron Exposure:

The preliminary results (Table 1) show that all neutron-irradiated animals (intact, laparotomized, and vagotomized) retched and or vomited postirradiation. As reported in the table, however, the vagotomized animals responded significantly later and with significantly fewer retches than did the intact or laparotomized animals. The overall duration of the episode (from first to last retching event) was significantly less for the vagotomized animals than for those receiving laparotomy.

TABLE 1

Effect of Subdiaphragmatic Vagotomy on Neutron-Induced* Emesis

Pretreatment	n	Latency to 1st Episode (min)**	Episode Duration (min)	No. Retches
Intact	4	15.6 ± 6.6	59.4 ± 3.9	97.8 ± 34.8
Surgery				
Laparotomy	4	10.1 ± 1.5	83.8 ± 15.2[b]	133.3 ± 32.7
Vagotomy	4	51.4 ± 4.9[a]	12.8 ± 7.7[b]	17.0 ± 8.8[a]

* 2.0 Gy, mixed neutron/gamma radiation; neutron:gamma ratio ≈ 6:1
** All values are mean ± S.E.M.
[a] Significantly different from intact and laparotomy, $p < 0.05$.
[b] Significantly different from one another, $p < 0.05$.

As shown in Table 2, pretreating ferrets with Eusatron (0.03 and 0.30 mg/kg) and Ondansetron (0.1 and 0.3 mg/kg) was generally effective in preventing vomiting following exposure to a suprathreshold dose of reactor neutrons. Preliminary data (data not shown; n = 2/compound) using lower doses of Eusatron (0.01 mg/kg) and Ondansetron (0.056 mg/kg) indicate that at these doses the 5-HT_3 receptor antagonists did not prevent the emetic response to 2.0 Gy of neutron irradiation, although there were clear reductions in the values of several emetic parameters for the treated animals compared with untreated controls. Both 5-HT_3 receptor antagonists increased the latency to vomiting

following exposure and ameliorated the severity of the episode, decreasing both the duration of the episode and the number of retches observed during the episode.

Proton Exposure:

The effects of pretreatment with the 5-HT_3 receptor antagonist Ondansetron on proton-evoked emesis are summarized in Table 2. Both doses of Ondansetron completely prevented emesis following exposure to 2.5 Gy of high-energy protons for the entire observation period of 1.5-2.0 hr.

Table 2

Effect of 5-HT_3 Receptor Antagonists on Neutron*- and Proton**-Induced Emesis

	Dose (mg/kg)	Number Vomiting	Number Tested
Neutron			
Eusatron	0.03	0	3
	0.30	0	2
Ondansetron	0.10	1	4
	0.30	0	2
Proton			
Ondansetron	0.00	6	6
	0.50	0	4
	1.00	0	7

*2.0 Gy, mixed neutron/gamma radiation
**2.5 Gy, 200 MeV protons

DISCUSSION

The present results indicate that 5-HT_3 receptor antagonists were effective treatments against the emesis evoked by exposure to fast neutrons from a nuclear reactor and high-energy protons in ferrets. These preliminary experiments suggest that pretreating ferrets with low doses of the 5-HT_3 receptor antagonists Ondansetron and Eusatron delayed the onset of emesis and ameliorated the severity of emesis following exposure to fast neutrons. However, from these preliminary data we have not yet established ED_{50} values for the two compounds in order to compare their efficacy. The higher doses of both Eusatron and Ondansetron more completely disrupted neutron-evoked emesis, with only 1 of 11 ferrets vomiting. Similarly, pretreatment with higher dose Ondansetron was equally effective in preventing emesis following exposure to high-energy protons within the 1.5- to 2-hr observation period. In addition, bilateral subdiaphragmatic vagotomy caused a

significant attenuation of the emesis evoked by exposure to fast neutrons, both delaying the onset of the response and attenuating the severity of the episodes.

Despite the fact that fast neutrons from a nuclear reactor are more effective in evoking emesis in ferrets than are gamma rays while exposure to high-energy protons are significantly less effective (Rabin *et al.*, 1992a; 1992b), pretreatment with 5-HT_3 receptor antagonists was effective in preventing the vomiting produced by exposure to either type of radiation. The present results parallel the results obtained following exposure to X-rays and gamma rays (Andrews & Hawthorn, 1987; Miner *et al.*, 1987; King & Landauer, 1990). With these types of radiation, lower doses of 5-HT_3 receptor antagonists and bilateral subdiaphragmatic vagotomy increase the latency of the emetic response following irradiation and attenuate the severity and duration of vomiting. Higher doses of 5-HT_3 receptor antagonists, on the other hand, are more effective in preventing radiation-induced emesis. Because the present results fast neutrons and high-energy protons show the same pattern of effects, these results do not support the hypothesis that different mechanisms mediate emesis produced by exposure to different types of radiation (Young, 1986). Rather, the present results are consistent with the hypothesis that similar mechanisms mediate the emetic response to the different types of radiation.

While these results indicate that similar 5-HT_3-dependent and vagal mechanisms mediate emesis following exposure to different types of ionizing radiation, the nature of those mechanisms needs further clarification. Andrews and Hawthorn (1987) have proposed a dual mechanism for radiation-induced emesis: an early phase that is dependent upon vagal innervation and a later phase that is dependent upon a circulating emetic agent, possibly 5-HT, which may act upon 5-HT receptors in the area postrema, the chemoreceptive trigger zone. Previous research using X-rays (Andrews & Hawthorn, 1987) and gamma rays (King & Landauer, 1990) as well as the present results with fast neutrons supports that hypothesis, showing that administration of low doses of 5-HT_3 receptor antagonists or bilateral subdiaphragmatic vagotomy disrupts the early emetic response to ionizing radiation without producing an equivalent disruption of the later emetic response. Also supporting the hypothesis of dual mechanisms mediating radiation-induced emesis in ferrets is the observation by King & Landauer (1990) that the combined treatment with the 5-HT_3 receptor antagonist zacopride and subdiaphragmatic vagotomy is more effective in disrupting emesis following irradiation than is either procedure by itself.

In the ferret, serotonergic neurons are found in the gut and in abdominal visceral afferents (Andrews *et al.*, 1988) as well as in a variety of brain stem structures, including the area postrema, dorsal vagal complex and nucleus of solitary tract (Barnes *et al.*, 1988; Higgins *et al.*, 1989; Kilpatrick, *et al.*, 1989; Leslie *et al.*, 1990; Pinkus *et al.*, 1989). Bilateral subdiaphragmatic vagotomy eliminates the binding of 5-HT_3 receptor antagonists in the dorsal vagal complex, where the majority of afferent vagal fibers terminate (Leslie *et al.*, 1990). Thus, exposing ferrets to ionizing radiation may produce immediate effects on the gut and on vagal afferents to produce short-latency vomiting. The longer latency emetic response following irradiation may result from the excitation of brain stem structures

by radiation-released peripheral serotonin or an emetic toxin that affects brain stem serotonergic systems, either in the area postrema or elsewhere. Higher doses of 5-HT_3 receptor antagonists may disrupt serotonergic transmission at these brain stem structures, in addition to affecting the peripheral release of serotonin from the gut, thereby causing a more complete blockage of radiation-induced vomiting.

In summary, the present results show that administration of 5-HT_3 receptor antagonists are as effective in disrupting emesis evoked by exposure to fast neutrons from a nuclear reactor and to protons as they are with X-rays and gamma rays, despite the difference in the effectiveness with which the different types of radiation elicit vomiting. Also, the results on the effects of subdiaphragmatic vagotomy on emesis evoked by exposure to fast neutrons are similar to those obtained following exposure to X-rays and to gamma rays. These results, therefore, support the hypothesis that similar mechanisms mediate the emetic response to different types of radiation.

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