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Are all 5-HT_3 Receptor Antagonists the Same?

Paul L. R. Andrews, Pritiranjan Bhandari, Philip T. Davey, Sharon Bingham, Helen E. Marr and Peter R. Blower

A number of 5-HT_3 receptor antagonists are currently in clinical development as antiemetics. In this paper we focus on two of these antagonists, granisetron and ondansetron, and compare their antiemetic activity against cisplatin (10 mg/kg i.v.)- or whole body X-irradiation (200 rads)-induced emesis in the conscious ferret. The results presented here have been discussed in the light of the recently published literature. Our data suggest that in comparison to ondansetron, granisetron is a more potent, longer acting and pharmacologically "cleaner" compound with a more conventional dose-response profile. The possible impact of these features upon the performance of these compounds in the clinic is discussed particularly with respect to dosing regimens and clinical efficacy. Differences appear to be emerging between granisetron and ondansetron in both these respects, although a direct head-to-head clinical comparison has yet to be carried out. This would involve studies monitoring a sufficiently high number of patients receiving severely emetogenic regimes to allow real clinical differences to be detected with the appropriate statistical power.
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INTRODUCTION

THE DISCOVERY, published in 1986 by Miner and Sanger of Beecham Pharmaceuticals [1], that an antagonist acting at the 5-hydroxytryptamine$_3$ receptor (5-HT_3 receptor) could block emesis induced by the highly emetic cytotoxic drug cisplatin, has had a dramatic impact upon the treatment of this unpleasant side effect of anticancer therapy. Subsequent animal and clinical studies have demonstrated that this antiemetic effect is not confined to cisplatin but applies to emesis induced by a wide range of other cytotoxic chemotherapeutic agents (e.g. cyclophosphamide, trimelamol, doxorubicin) and X-irradiation (total body and regional).

The site of action is still under investigation but the weight of evidence from animal studies favours a peripheral site on vagal afferent neurones supplying the upper gut, preventing their activation (or sensitisation) by 5-HT released from enterochromaffin cells as a consequence of exposure to the cytotoxic drug or X-irradiation [2]. This does not exclude additional central site(s), e.g. in the nucleus tractus solitarius in the brain stem or the area postrema, but at present their contribution appears minor. The major afferent and efferent pathways involved in emesis induced by anticancer therapy are summarised in Fig. 1.

Since the original identification of the antiemetic effects of 5-HT_3 receptor antagonists, a wide range of compounds have been developed for this purpose or transferred from other uses e.g. antimigraine. As a number of these compounds are, or soon will be, available for clinical use, it is timely to ask "Are there any differences between them which may influence their clinical performance?". In this paper we focus on granisetron (BRL43694A, KYTRIL, SmithKline Beecham) and ondansetron (GR38032F, ZOFRAN, Glaxo) but the issues raised may also be relevant to other compounds. Data for the comparison of the two compounds are drawn from two sources, (a) the published literature; and (b) experimental studies in the ferret directly comparing the antiemetic activity of the two compounds. The experimental studies will be described first and then these results discussed together with those from the literature to provide a more comprehensive assessment of their differences which may have an impact upon clinical performance.

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EXPERIMENTAL STUDY

Materials and methods

Animals. The choice of animal species for studies of emesis is complicated by the fact that the common laboratory species such as the rat, mouse, guinea-pig and rabbit do not vomit. Studies can therefore only be performed on either primates or carnivores e.g. cats and dogs. The present study was performed using the ferret, a small carnivore which has become widely used in studies of the mechanism of emesis and for the identification of antiemetic agents as an alternative to cats and

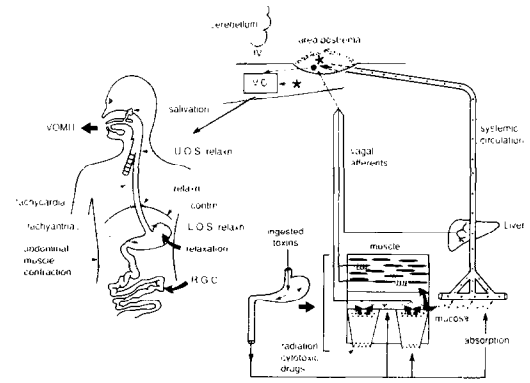


Fig. 1. Diagram of basic pathways involved in the emetic response to radiation and cytotoxic drugs - asterisks indicate most probable site(s) of action of granisetron (redrawn from Andrews *et al.* 1988) [2]. L.O.S. = lower oesophageal sphincter, R.G.C. = retrograde giant contraction, V.C. = vomiting centre, U.O.S. = upper oesophogeal sphincter.

Table 1. Effective emetic agents in the ferret

Subcutaneous	Intravenous or intraperitoneal	Per oral	Other
Apomorphine	Adriamycin	Calcium glucoronide	X-irradiation
Lisuride	Apomorphine	Choline chloride	Vagal stimulation
Loperamide	Cisplatin	Copper sulphate	
Morphine	Cisplatin analogues	Emetine	
Morphine-6-glucuronide	Cyclophosphamide	Glucose	
	Cycloheximide	Ipecacuanha	
	Diacetoxyscirpinol	Mannitol	
	Emetine	Potassium chloride	
	Enkephalin	Sodium chloride	
	Hyperammonemia	Syrup B.P.	
	Mustine	Veratridine	
	Para-chlorophenylalanine		
	Trimelamol		
	Urethane		

Table taken from Andrews *et al.* (1990) [3].

dogs [3]. Previous studies have shown that the ferret has an emetic response to all the anticancer therapies that induce emesis in man (Table 1) and to date it has been an accurate predictor of the range of antiemetic efficacy in man and in the case of granisetron, a predictor of the human dose.

Methods. Ferrets (*Mustela putorius furo* L) of either sex or strain (albino/fitch), weighing between 600 and 2000 g, housed in a temperature- and humidity-controlled environment with a 12-h light and dark cycle and fed standard pelleted carnivore diet, were used for the study. All experiments were performed on conscious animals.

Emetic stimulus. Two different emetic stimuli were used - X-irradiation or cisplatin. Whole body X-irradiation was administered as X-rays at 250 KV, 15 mA to achieve a dose of 200 rads. This dose is just above that which induces emesis in all ferrets tested [3, 4]. Cisplatin (Lederle) at a dose of 10 mg/kg i.v. was administered 3-4 days after an indwelling jugular vein catheter was implanted according to the method of Bermudez *et al.* [5]. This dose has previously been shown to induce emesis reliably and consistently in ferrets [6].

Antiemetics. Granisetron (BRL43694A, SmithKline Beecham Pharmaceuticals) or ondansetron (GR38032F, Glaxo) were used as antiemetics at doses between 1 and 5000 μg/kg given intravenously 15-20 min before the emetic challenge or for the subcutaneous route of administration, 30 min prior to challenge. Doses are expressed in terms of pure free base.

Observation of emesis. The ferrets were observed continuously in a large pen for at least 2 h after X-irradiation and 4 h after cisplatin administration. Records were kept manually as the actual time of each episode of retching, vomiting or other behaviour e.g. defecation. From these observations the following could be derived: (a) latency to first retch; (b) latency to first vomit (actual oral expulsion of material); (c) total number of retches in observation of period; (d) total number of vomits in observation period; (e) number of animals that showed retching out of the total number tested in that group, and (f) number of animals that showed vomiting out of the total number of animals tested in that group.

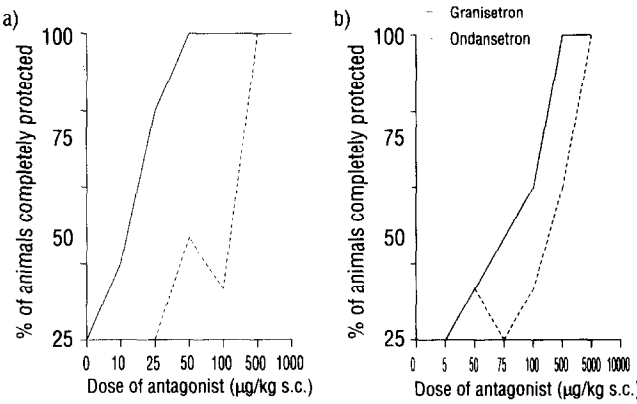


Fig. 2. Comparison of the degree of protection against emesis induced by (a) total body radiation (200 rads 250 k.v.) or (b) cisplatin (10 mg/kg i.v.) in the ferret. Results expressed as % of animals in group totally protected (no retches or vomits). Group size = 4-6 at each dose.

Statistics. Results are expressed as mean ± SEM. Differences between means were compared using Student's t test and were considered significant at $P \leq 0.05$.

Results

For convenience the effect of the two drugs on each parameter of the emetic response will be described separately.

Incidence. All control (no drug treatment) animals responded to X-irradiation (n = 15) or cisplatin (n = 6). The percentage number of animals completely protected with either drug is shown in Fig. 2. Granisetron completely abolished X-irradiation-induced emesis at 50 μg/kg s.c. whereas a dose of ondansetron of 500 μg/kg s.c. was required to achieve a similar effect. For cisplatin-induced emesis again a higher dose of ondansetron (5000 μg/kg i.v.) was required to achieve complete protection from emesis compared with granisetron (500 μg/kg i.v.).

Effect on total emetic episodes. Figs. 3 and 4 illustrate the effect of the two antiemetic drugs on the total number of vomits observed, as a percentage of control (Fig. 3) and total vomits observed (Fig. 4). For X-irradiation, granisetron showed the expected dose-related decrease in vomits but for ondansetron, after an initial decrease reaching a nadir at 50 μg/kg s.c. there was an increase in emesis peaking at 100 μg/kg s.c. Vomiting was abolished at a dose of 500 μg/kg. Complete protection was achieved by granisetron at 50 μg/kg s.c. and there was no evidence of a reappearance of emesis with higher doses. The pattern for cisplatin-induced emesis was similar. With ondansetron after an initial decline there was a sharp increase in vomiting at 75 μg/kg i.v. but as with X-irradiation-induced emesis this was not seen with granisetron. When the incidence of retching was monitored, both compounds showed the same profile of antiemetic activity that had been demonstrated against vomiting.

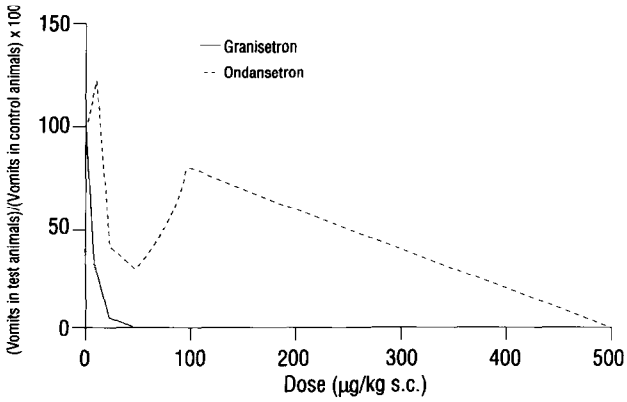


Fig. 3. Diagram illustrating the dose-response curves for ondansetron and granisetron against whole body X-irradiation (200 rads) induced emesis in the ferret. Figures drawn from a minimum of 36 animals for each compound.

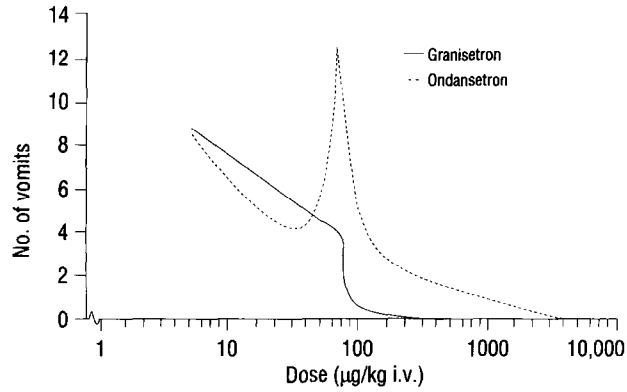


Fig. 4. The antiemetic effect of ondansetron or granisetron (0.005 - 5.0 mg/kg i.v.) against emesis induced by cisplatin (10 mg/kg i.v.) in the ferret. Figures drawn from a minimum of 36 animals for each compound.

Duration of action. The studies of cisplatin-induced emesis indicate that the duration of action of granisetron may be about twice as long as that of ondansetron. First, using the same microgram/kg dose of 500 μg/kg i.v. granisetron completely protected 2 out of 4 ferrets when given 6 h before cisplatin whereas when ondansetron was given 2 h before cisplatin all 4 animals were vomiting before the end of the observation period (Davey and Marr, unpublished observations).

As results from the above study could be attributed to differences in compound potency as well as duration of action, a further study was undertaken using approximately equieffective doses of the two compounds; these were 5000 μg/kg i.v. of ondansetron and 500 μg/kg i.v. of granisetron both administered 3 h prior to cisplatin (10 mg/kg i.v.). As Fig. 5 shows, within 4 h of cisplatin administration, 3 of the 4 animals given ondansetron were retching and vomiting whilst only one of the animals in the granisetron group showed any emesis.

Differences in potency. Whilst there are a number of different ways in which the relative potencies of the two compounds can be assessed, because the complete control of emesis is clinically the most important measurement we report only that comparison in this paper. Using the results from the study of X-irradiation-induced emesis, which has more data points than the cisplatin study, the dose of drug required to completely protect 50% of the animals totally from retching and vomiting (PD_{50}) was calculated using Probit analysis. This analysis gives a value of 20.7 ± 6.0 μg/kg for granisetron and a value of 221.0 ± 78.3 μg/kg for ondansetron. Interestingly a study of another 5-HT_3 receptor antagonist tropisetron (ICS-205-930, Navoban, Sandoz) shows that this is also more potent than ondansetron with a PD_{50} value of 47.3 ± 22.4 μg/kg (Bhandari and Andrews, unpublished observations).

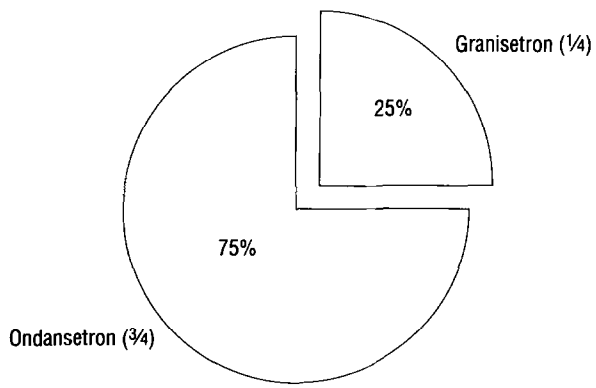


Fig. 5. The percentage of animals vomiting following administration of granisetron (0.5 mg/kg i.v.) or ondansetron (5.0 mg/kg i.v.) given 3 h before cisplatin (10 mg/kg i.v.). n/n = number of animals vomiting.

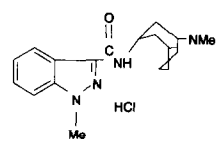
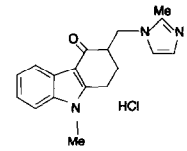
DISCUSSION OF EXPERIMENTAL STUDY AND LITERATURE SURVEY

The published literature on the two compounds was surveyed with particular attention focusing on pharmacological differences between the two drugs. Taking these observations together with those from the experimental study reported above we can now make a preliminary comparison of the two drugs and provide an insight into how such differences observed in preclinical studies may impact on clinical performance. The comparison detailed below is summarised in Table 2.

Structure

Granisetron and ondansetron differ in their structures with the former being an indazole and the latter a carbazole.

Table 2. A comparison between granisetron and ondansetron

Parameter	Granisetron	Ondansetron
Structure		
	indazole	carbazole
Racemate	no	yes
Potency*	5-10 x	1
Dose response	linear	non-linear
Duration of action	2 x	1
Receptor specificity**	5-HT_3	5-HT_3, 5-HT_{1B}, 5-HT_{1C}, α_3, μ

* Calculated from data in Figs. 3, 4.
** From Van Wijngaarden *et al.* 1990 [12].

Interestingly whilst granisetron is a single isomer, ondansetron is a racemic mixture of R- and S-stereoisomers. Pharmacologically, the R- and S-isomers were found to be approximately equipotent on the rat vagus nerve, while the R-isomer was 10-fold more potent than the S-isomer in blocking contractions mediated by 2-methyl-5-HT (a selective 5-HT$_3$ receptor agonist) in the guinea-pig isolated ileum [7]. However, no data have been published on the antiemetic properties of each isomer and their relative contributions to the clinical effects of the racemate.

Dose response, potency and duration characteristics

A direct comparison of granisetron and ondansetron has not yet been published from clinical trials and therefore, to provide some basis for such a comparison, we undertook the experiments described above. These studies revealed three major differences. First, granisetron is more potent than ondansetron. Granisetron was at least 5 times more potent in achieving complete protection against either X-irradiation, or cisplatin-induced emesis. In a clinical setting it is more important to achieve a complete block rather than a partial decrease in the number of episodes, as even a few emetic episodes may be sufficient to cause psychological aversion to anticancer therapy resulting in the difficult to treat problem of anticipatory nausea and vomiting. Second, the preliminary data presented here suggest that granisetron acts for at least twice as long as ondansetron. Third, an unexpected finding was that instead of the expected curvi-linear decrease in emesis with increasing dose of drug as seen with granisetron, that for ondansetron was complex: an initial decrease in emesis was followed by an increase and then as the dose was further increased full control of emesis was achieved (Fig. 2). This type of "non-monotonic" dose-response curve seen with ondansetron has also been reported for metoclopramide [8] and the novel 5-HT$_3$ receptor antagonist L-683,877 [9]. The precise reason for these effects is not known at present but, by analogy with other compounds (e.g. zacopride), it suggests that as the dose is increased, some other pharmacological action of the compound is recruited which interferes with the primary antiemetic action mediated by 5-HT$_3$ receptor antagonism. Zacopride is a 5-HT$_3$ receptor antagonist but has a benzamide structure derived from the parent compound of the metoclopramide series. Zacopride is also a racemic mixture with the stereoisomers having different properties, i.e. in addition to antagonist properties of the racemate the S-isomer is an agonist at the 5-HT$_3$ receptor whilst both enantiomers are weak agonists at the 5-HT$_4$ receptor which results in stimulation of gut motility [10, 11]. There is also emerging evidence for an involvement of 5-HT$_4$ receptor activation in zacopride and copper sulphate-induced emesis in the ferret [4]. Further studies of the pharmacological characteristics of ondansetron, its isomers and metabolites are required to resolve this potentially important issue.

Receptor-binding profile

A comparison of the binding characteristics of granisetron and ondansetron [12] showed that, whilst as expected both bind to 5-HT$_3$ receptors, ondansetron had detectable binding (pKi>5) at 5-HT$_{1B}$, 5-HT$_{1C}$, alpha-1 adrenergic and opioid μ sites. These non 5-HT$_3$ binding sites accounted for 20% of the total binding. Whilst these studies have yet to be confirmed and their functional significance (if any) investigated they do suggest

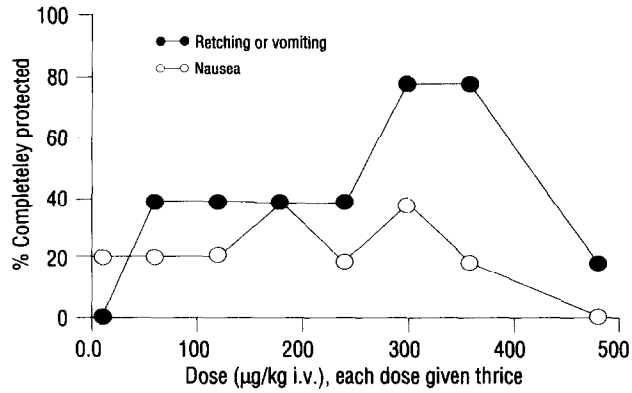


Fig. 6. The effect of increasing doses of ondansetron on the emetic response to cisplatin (≥ 60 mg/m²) containing regimes in man. Plotted from the data of Grunberg *et al.* (1989) (Tables 2 and 3 [16]).

that ondansetron does not have such a "clean" profile as granisetron. Such binding studies must be interpreted with caution as they give little information about the functional effects of a drug binding to such sites *in vivo*. In addition, it is important that binding studies are undertaken on the tissue where it is assumed that the drug may be having its therapeutic or "side effect" because of regional differences in receptor characteristics. In the context of emesis the most relevant regions would be the abdominal vagal afferents and the gut mucosa, the nucleus tractus solitarius, and perhaps the area postrema.

Clinical differences

Direct comparison trials of the two compounds have not yet been published. At present, granisetron is used as a single intravenous injection of 3 mg (equivalent to 40 μg/kg in a 75 kg patient) for all chemotherapeutic regimens whereas ondansetron is given by a multiple dosing schedule which varies across the different chemotherapeutic regimens and which appears to be under review [13]. However, while they may have similar efficacy against moderately emetogenic chemotherapy (74% of patients were complete responders for both), it would appear that, overall, granisetron is more effective than ondansetron against harsher regimes such as high-dose cisplatin (57% vs. 44% of patients were completely protected when given granisetron or ondansetron respectively) [14, 15]. In a dose-ranging study Grunberg *et al.* [16, 17] have reported a decrease in antiemetic efficacy with higher doses of ondansetron (0.36 mg/kg i.v. and above) (Fig. 6). This may limit its clinical use in patients refractory to conventional therapeutic doses. Such a phenomenon has not been reported for granisetron.

CONCLUSION

Differences are emerging from preclinical studies that indicate important differences between ondansetron and granisetron. Our data suggest that in comparison to ondansetron, granisetron is a more potent, longer acting and pharmacologically "cleaner" compound with a more conventional dose-response profile.

The combination of potency and duration of action can influence the dosing regimens and clinical efficacy of novel compounds in the clinic. Differences are emerging between

granisetron and ondansetron in both these respects, although a direct head-to-head comparison has yet to be carried out.

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Intravenous Granisetron - Establishing the Optimal Dose

Darab Kamanabrou
on behalf of the Granisetron Study Group

Three double-blind, dose-ranging studies, involving 996 chemotherapy-naive patients, were conducted to determine the optimal prophylactic dose of intravenous (i.v.) granisetron for prevention of cytotoxic-induced emesis. The antiemetic efficacy of prophylactic i.v. granisetron doses ranging from 2-40 μg/kg (study 1) and 40-160 μg/kg (study 2) were examined in patients receiving high-dose cisplatin regimens. In study 3, i.v. doses of 40 and 160 μg/kg were compared in patients receiving other emetogenic cytotoxic therapies. In study 1, 67.9% (36/53) of patients were complete responders at 24 h following the 40 μg/kg dose compared with 61.5% (32/52) and 30.8% (16/52) in the 10 and 2 μg/kg groups, respectively (40 vs. 2 μg/kg; $P < 0.001$). There were no significant differences between doses of 40 and 160 μg/kg in any efficacy parameter in Studies 2 and 3. Granisetron was well tolerated across the dose range examined and no dose-related toxicity was observed. In conclusion, a single 40 μg/kg prophylactic dose provides optimal control of cytotoxic-induced nausea and vomiting. A simple 3 mg single-dose i.v. regimen (equivalent to 40 μg/kg in a 75 kg person) is recommended for prevention of acute emesis associated with all cytotoxic regimens.
Eur J Cancer, Vol. 28A, Suppl.1, pp. S6-S11, 1992.

INTRODUCTION

ONE of the most unpleasant side effects of many chemotherapeutic regimens is acute nausea and vomiting occurring in the 24 h after initiation of treatment. Inadequate management of these symptoms can lead to poor compliance with further potentially curative treatment, as well as many other distressing and undesirable effects such as weakness, malnutrition, dehydration and so on. All these factors may severely interfere with daily activities, result in prolonged hospitalisation and adversely affect quality of life.

The importance of achieving maximal control of acute emesis at the patient's first cycle has been recently highlighted by studies showing that incomplete protection predisposes to

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