Cancer Chemother Pharmacol (1991) 28: 470–474
0344570491001362

Cancer Chemotherapy and Pharmacology

Antiemetic activity of the new 5-HT$_3$ antagonist DAU 6215 in animal models of cancer chemotherapy and radiation

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Received 9 March 1991/Accepted 9 July 1991

Summary. The antiemetic activity of DAU 6215, a novel antagonist of 5-HT$_3$ receptors, was investigated in animal models of cytotoxic treatment-evoked emesis and compared with the antiemetic activity of ondansetron and metoclopramide. In dogs, vomiting was induced by i.v. cisplatin; in ferrets, the emetic response was elicited by i.v. doxorubicin or X-ray exposure. Pretreatment with 0.1–1 mg/kg DAU 6215 given i.v. or p.o. prevented the vomiting response to the different emetic agents. In the dog, the antiemetic potency of metoclopramide was 30 times lower than that of DAU 6215. Ondansetron was less potent than DAU 6215 against cisplatin and doxorubicin but was equally effective in the radiotherapy protocol. In this model, lengthening of the pretreatment time to 2 h did not affect the antiemetic efficacy of DAU 6215, whereas it decreased that of ondansetron. The results demonstrate that DAU 6215 is a highly effective and long-lasting inhibitor of cytotoxic treatment-induced emesis in different animal species.

Introduction

Among the therapeutic approaches aimed at counteracting the emetic response associated with chemotherapy or irradiation, the use of metoclopramide at high doses has gained wide acceptance, despite the occurrence of pronounced side effects due to its interaction with dopamine receptors [11]. The antidopaminergic properties of this drug have long been considered to play a crucial role in its antiemetic activity as well; this concept is supported by the knowledge that the dopaminergic system, together with cholinergic and histaminergic pathways, mediates stimuli leading to the emetic response [14, 19]. Recently, the discovery that metoclopramide can block a subclass of serotonin receptors (5-HT$_3$) has provided new insight into the mechanism underlying the antiemetic properties of the drug [8, 9, 12]. In fact, the blockade of 5-HT$_3$ receptors was shown to be fundamental for the occurrence of antiemetic activity, a discovery that became possible due to the development of selective 5-HT$_3$ receptor antagonists [6, 13]. Several of these compounds have been investigated in depth for their antiemetic effect both in animals and in humans, showing high efficacy and producing minimal side effects [5, 7, 20]. In this report we describe the antiemetic activity of a new 5-HT$_3$ receptor antagonist, DAU 6215 [21, 22], as compared with that of ondansetron (GR 38032F) [7, 20] and metoclopramide. We evaluated their ability to protect different animal species from emesis induced by chemotherapeutic agents and irradiation. Some of our preliminary results have been presented elsewhere [17].

Materials and methods

Cisplatin-induced emesis in dogs. Beagle dogs of both sexes (body weight, 8–14 kg; Alserio Allevamento, Castelgabbiano, Italy) were housed individually under controlled environmental conditions and were maintained on a standard pellet diet. They were starved for 24 h prior to the experimental session, with water being available ad libitum. Cisplatin (3 mg/kg) was given i.v. through a cephalic vein in the foreleg. For each experiment, one dose of a test compound was given i.v., s.c. (between the scapulae), or p.o. at 2 min prior to cisplatin injection. Three to four animals were used for each dose given by each administration route. A group of animals receiving cisplatin alone served as controls. Dogs were observed for 5 h after cisplatin administration, and the number of emetic episodes was recorded. The percentage of inhibition of cisplatin-induced emesis was obtained by comparing the number of emetic episodes in each experiment with the mean value obtained for the control group. The dose that produced a 50% reduction in the mean response to cisplatin in controls (ID_{50}, with 95% confidence limits) was estimated by least-squares linear regression analysis. Each dog underwent a maximum of two treatments, and a 2-month washout period was instituted between the two experimental sessions.

Doxorubicin-induced emesis in ferrets. Fitch and albino ferrets of both sexes (body weight, 1.2–1.8 kg; Grayston, Ringwood, UK) were housed

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individually and maintained on cat food (canned meat) and water ad libitum. Animals were anaesthetized with pentobarbitone sodium (30 mg/kg i.p.) and ketamine (10 mg i.m.), and a polyethylene cannula was inserted into the jugular vein and exteriorized behind the neck. A 2-day recovery period was allowed between the surgery and the experimental session; after this time, all of the animals were in good health and could be appropriately exposed to the emetogenic treatment. On the day of the test at 30 min after the end of the meal, ferrets were given one dose of the test compounds either i.v. or p.o., after which they were immediately dosed with 10 mg/kg i.v. doxorubicin. Animals receiving doxorubicin alone served as controls. The number of episodes of vomiting and/or retching was recorded over 4 h post-dosing.

Radiation-induced emesis in ferrets. For irradiation experiments, adult male ferrets (fitch and albino; body weight, 0.8–1.7 kg; Froxfield Farms, Froxfield, UK) were maintained and surgically prepared as described above. On the day of the test at 3 h after the meal, animals were placed in a square Perspex box and then exposed to whole-body radiation via a vertically downward beam (80 cGy/min, 250 kV cp; HVL, 1 mm Cu + 1 mm Al) delivered from a Trimegagil X-ray set (Gilardoni, Italy). In the first set of experiments, animals received 2 ml/kg saline through the cannula and were then immediately exposed to 100 ± 5, 200 ± 10, 400 ± 20 or 800 ± 40 cGy X-ray irradiation. In the second set of experiments, animals received one of the test compounds either i.v. or p.o. immediately or 20 min prior to exposure to 800 ± 40 cGy whole-body radiation, respectively. In other sessions, the test compound was given i.v. at 2 h before irradiation. The number of episodes of vomiting/retching was recorded over 3 h post-dosing.

In both protocols, subsequent episodes of retching only or of retching plus vomiting were considered to be independent events if they were separated by a 30-s interval. The percentage of protection from emesis was calculated in relation to the mean value in the control group. Each ferret was used once.

Drugs. *N*-(Endo-8-methyl-8-azabicyclo[3.2.1]oct-3-yl)-2,3-dihydro-2-oxo-1H-benzimidazol-1-carboxamide HCl (DAU 6215) and ondasetron (both synthesized by Dr. M. Turconi, Istituto De Angeli) were dissolved in sterile saline (1 ml for dogs; 2 ml/kg for ferrets). For oral administration, the solution was either placed in a gelatine capsule (dogs) or given by gastric gavage (ferrets). Metoclopramide was obtained commercially (Plasil, Lepetit; 9.9 mg/ml vials). Cisplatin (*cis*-dichlorodiammineplatinum; Strem Chemicals Inc., Newburyport, Mass., USA) was dissolved at 70° C in sterile saline (6 mg/ml) and then cooled down to 40° C before injection. Doxorubicin (Sigma, St. Louis, Mo., USA) was dissolved in sterile saline (5 mg/ml). All doses refer to the weight of the free base.

Statistical analysis. Student's *t*-test for unpaired data, Neuman-Keuls test and Fischer's exact test (two-tailed) were used as appropriate (see Results). Data represent mean values ± SEM.

Results

Cisplatin-induced emesis in the dog

Cisplatin administration resulted in a series of emetic episodes, which occurred between the 2nd and the 5th h after drug injection. The mean number of emetic episodes in control animals was 23.9 ± 2.1 (n = 14; Fig. 1). The effect of i.v. DAU 6215, ondansetron and metoclopramide is shown in Fig. 2. All compounds displayed the ability to reduce dose-dependently the emetic response observed in controls, with DAU 6215 being slightly more potent than ondansetron and approximately 30 times as active as metoclopramide (see ID_{50} values in Table 1). In addition, 1 mg/kg i.v. DAU 6215 but not ondansetron afforded complete protection from emesis in all animals tested (Fig. 2). The latency period between cisplatin administration and the onset of vomiting (controls, 123.9 ± 4.2 min) was gradually lengthened by increasing the doses of the test compounds (data not shown).

The antiemetic effect of s.c. DAU 6215 was comparable with that obtained in i.v. experiments, whereas an

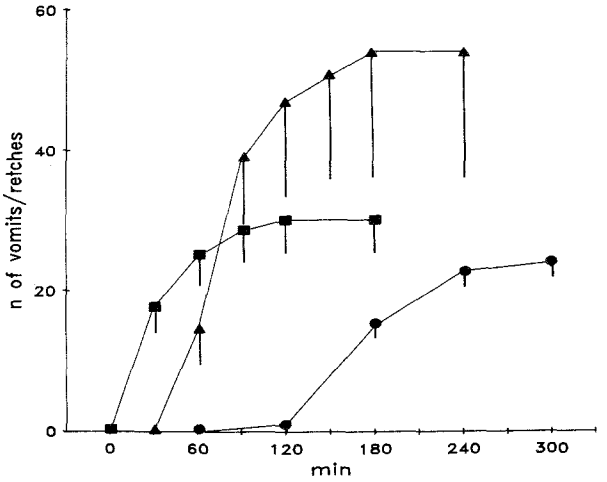

Fig. 1. Cumulative time-courses of the emetic response to 3 mg/kg i.v. cisplatin in dogs (●; n = 14), 10 mg/kg i.v. doxorubicin in ferrets (▲; n = 6) and 800 cGy whole-body radiation in ferrets (■; n = 4)

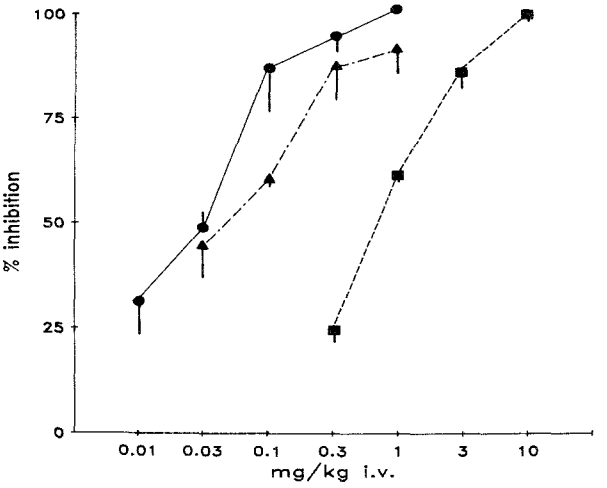

Fig. 2. Dose-related inhibition of cisplatin-induced emetic episodes by i.v. DAU 6215 (●), ondansetron (▲) and metoclopramide (■) in dogs. *Abscissa*, doses of antiemetics; *ordinate*, percentage of inhibition of the mean number of emetic episodes in the control group. Each data point represents the mean ± SEM of 3–4 experiments

Table 1. ID_{50} doses of DAU 6215, ondansetron and metoclopramide against cisplatin-induced emesis in the dog

Agent	Route of administration		
	i.v.	p.o.	s.c.
DAU 6215	25 (16–40)[a]	6 (3–11)	13 (7–24)
Ondansetron	46 (25–87)[a]	70 (14–342)	
Metoclopramide	740 (635–861)		

[a] From Turconi et al. [22]

Data represent mean values expressed in μg/kg; 95% confidence limits are shown in parentheses

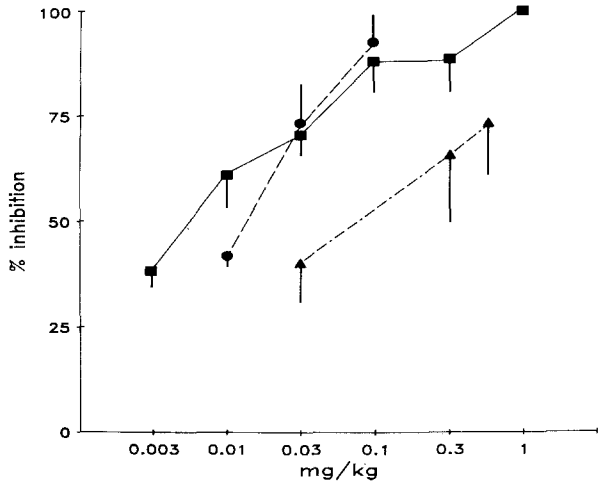

Fig. 3. Dose-related inhibition of cisplatin-induced emetic episodes by increasing doses (*abscissa*) of p.o. DAU 6215 (■), s.c. DAU 6215 (●) and p.o. ondansetron (▲) in dogs. On the *ordinate*, the percentages of inhibition of the mean number of episodes in the control group are shown. Each data point represents the mean ± SEM of 3–4 experiments

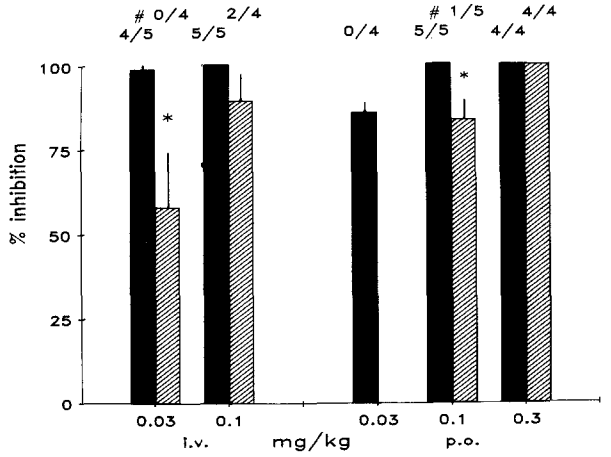

Fig. 4. Antiemetic effect of DAU 6215 (*filled columns*) and ondansetron (*hatched columns*) against doxorubicin-evoked emesis in ferrets. *Abscissa*, doses and administration routes; *ordinate*, percentage of inhibition of the mean number of vomits/retches in the control group. *Figures at the top* indicate the number of ferrets completely protected vs those treated (controls, 0/6). * Significantly different ($P < 0.05$) from DAU 6215 at the same dose (Student's *t*-test for unpaired data, number of vomits/retches); # Significantly different ($P < 0.05$) from DAU 6215 at the same dose (Fischer's exact test, frequency of completely protected animals)

increase in potency was observed when the compound was given p.o. (Fig. 3, Table 1); in both sets of experiments, the onset of emesis was delayed in a fashion similar to that noted for i.v. injection (data not shown). As in the i.v. protocol, complete protection from vomiting was provided by 1 mg/kg DAU 6215 given p.o. Oral ondansetron exerted a dose-related antiemetic effect, although it exhibited lower potency (Fig. 3, Table 1).

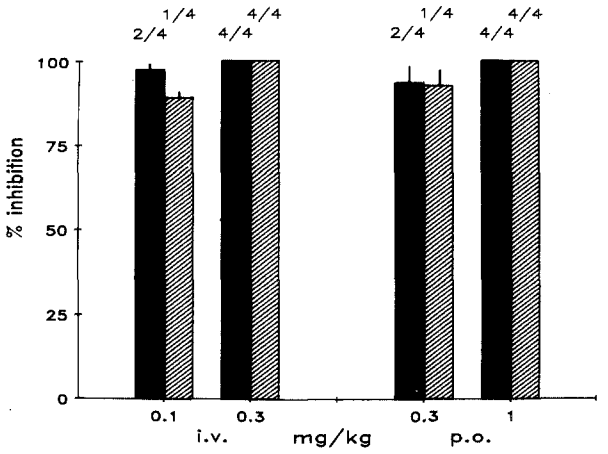

Fig. 5. Antiemetic effect of DAU 6215 (*filled columns*) and ondansetron (*hatched columns*) against X-ray-induced emesis in the ferret. *Abscissa*, doses and administration routes; *ordinate*, percentage of inhibition of the mean number of vomits/retches in the control group. *Figures on the top* represent completely protected vs treated animals (controls, 0/4)

Doxorubicin-induced emesis in the ferret

Doxorubicin produced a marked emetic response in all of the control ferrets after a latency period of 50.8 ± 4.2 min ($n = 6$). Vomiting /retching was most severe in the first 2 h post-dosing; the total number of vomits/retches over the 4-h observation period was 53.5 ± 17.7 (Fig. 1). When given i.v. at 0.1 mg/kg, DAU 6215 provided complete antiemetic protection, whereas after an i.v. dose of 0.03 mg/kg, one animal produced few retches during the 4-h observation period; ondansetron was less effective, giving incomplete protection at 0.1 mg/kg i.v. (Fig. 4).

Oral DAU 6215 given at 0.3 and 0.1 mg/kg completely abolished the response to doxorubicin, and at 0.03 mg/kg it substantially decreased the number of emetic episodes in all animals (Fig. 4). Ondansetron afforded full protection at 0.3 mg/kg p.o., whereas 0.1 mg/kg p.o. only reduced the emetic response in most of the animals (Fig. 4).

Radiation-induced emesis in the ferret

Neither retching nor vomiting was observed in two ferrets that had been exposed to 100 cGy X-ray irradiation, whereas all animals that had received 200 ($n = 2$), 400 ($n = 2$) or 800 cGy ($n = 4$) showed an emetic response. A linear relationship was found between the doses of irradiation and both the number of vomits/retches and the time of their onset (data not shown). After 800 cGy exposure, the mean number of retching/vomiting episodes was 29.7 ± 4.9, with the mean latency period being 11 ± 1.1 min ($n = 4$); the emetic response was prominent during the 1st h post-irradiation but disappeared after the 2nd h (Fig. 1).

DAU 6215 and ondansetron displayed comparable antiemetic activity, with both drugs affording full protection at 0.3 mg/kg i.v. and 1 mg/kg p.o. (Fig. 5) and significantly delaying the onset of emetic symptoms at lower

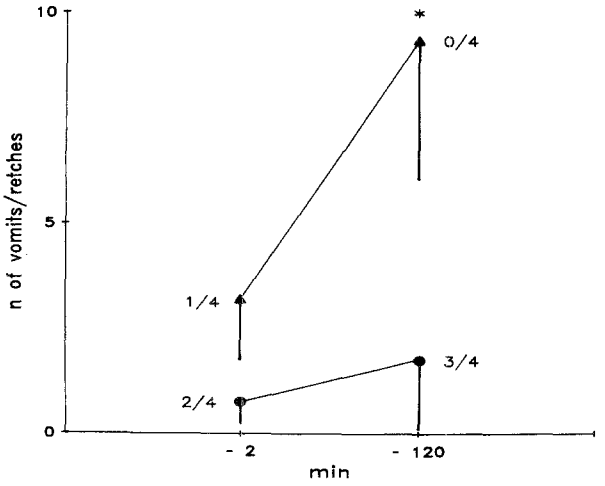

Fig. 6. Antiemetic effect of 0.1 mg/kg DAU 6215 (●) and ondansetron (▲) given i. v. at 2 min or 2 h prior to whole-body radiation. *Figures next to points* represent completely protected vs treated animals (controls, 0/4). * Significantly different ($P < 0.05$) from DAU 6215 (Neuman-Keuls test, number of vomits/retches)

doses (data not shown). When 0.1 mg/kg of either compound was given i. v. at 2 h prior to irradiation, there was a significant decrease in the antiemetic effect of ondansetron as compared with DAU 6215 (Fig. 6).

Discussion

DAU 6215, a novel 5-HT_3 receptor antagonist derived from benzimidazolone, is endowed with high potency and selectivity of action at this receptor subtype [21, 22]. In keeping with the evidence for a pivotal role of 5-HT_3 receptors in the occurrence of cytotoxic treatment-induced emesis, the present investigation demonstrated that DAU 6215 is a potent and highly effective antiemetic agent against chemotherapy- and irradiation-induced emesis in two animal species.

DAU 6215 was markedly effective against all of the emetogenic stimuli applied, completely preventing vomiting at doses of between 0.1 (doxorubicin) and 1 mg/kg (cisplatin). The compound proved to be more effective than ondansetron in inhibiting doxorubicin- but not radiation-induced emesis in ferrets and exhibited slightly more potent activity against cisplatin-induced emesis in the dog. In this species, metoclopramide was significantly less potent, in agreement with its low affinity for 5-HT_3 receptors [12]. In each experimental protocol, the antiemetic activity of DAU 6215 was rather uninfluenced by a change in the administration route, and a similar finding was obtained for ondansetron. However, in this respect, DAU 6215 displayed peculiar behaviour in cisplatin experiments in which the lower ID_{50} value was obtained following oral administration of the compound. Although a precise explanation for this phenomenon cannot be proposed at present, these results altogether are in agreement with the high bioavailability of the compound, which was previously demonstrated in pharmacokinetic studies (unpublished results).

An additional interesting feature of DAU 6215 was indicated by the results obtained using the 2-h pretreatment protocol in radiation experiments. In fact, lengthening of the pretreatment time did not significantly alter the effectiveness of DAU 6215, whereas it decreased that of ondansetron. The result obtained for DAU 6215 denotes a long-lasting antiemetic effect, which is a valuable property in view of the proposed administration of this compound to patients undergoing chemotherapy. On the basis of this result, one might also speculate that the longer duration of action of DAU 6215 as compared with ondansetron contributed to its higher potency versus the reference compound in doxorubicin and cisplatin experiments, in which the interval between the antiemetic treatment and the onset of emesis was considerably longer that that recorded for the standard irradiation protocol. However, this hypothesis needs to be confirmed by further experiments.

The disparity observed between the doses affording full protection in the different protocols, notably for DAU 6215, is likely to be ascribed to the different emetogenic properties of the treatments applied as well as to species-related pathogenetic mechanisms implied in this type of emesis. In the ferret, the main mechanism by which cytotoxic drugs and irradiation elicit emesis appears to involve the stimulation of abdominal (mainly vagal) afferent fibres, which project either directly or through the area postrema to the so-called vomiting centre [4]. Serotonin is a major candidate for consideration as an endogenous mediator of such stimulation: ileal mucosal levels of the amine increase as a consequence of chemotherapeutic treatment in ferrets [20], and cisplatin-induced release of 5-HT from enterochromaffin cells has been described in the isolated small intestine of guinea pigs [18]. The amine might in turn activate serotonin receptors located on the peripheral endings of vagal afferents, which have been shown to belong to the 5-HT_3 subclass [1, 4]. Other findings, however, such as the inefficacy of vagotomy in providing complete protection from chemotherapy- or irradiation-induced emesis [2, 4], indicate an additional role for 5-HT_3 receptors located in areas of the CNS, such as the nucleus tractus solitarius and the area postrema, that display a high density of this receptor subtype [4, 15].

As to the mechanism operating in the emetic response of the dog to cisplatin, rather conflicting data have been reported thus far. Pharmacological evidence suggests that 5-HT_3 receptors located in CNS beyond the blood-brain barrier play a role in the occurrence of cisplatin-induced emesis in this species [16]; on the other hand, major involvement of the area postrema [3] and of peripheral endings of afferent fibres [10] have been proposed following experiments that implied ablation or lesion of the aforementioned structures.

In conclusion, the present results demonstrate that DAU 6215 is a highly effective, long-lasting antiemetic agent in animal models of chemotherapy- and irradiation-induced emesis; these properties, together with the high bioavailability of the drug and its lack of antidopaminergic activity [22], should be of great clinical value when they have been confirmed in humans.

Acknowledgements. The authors thank Prof. L. Leonardi for the helpful collaboration in carrying out the irradiation experiments and Drs. P. Cigarini and S. Dell'Orso for the statistical analysis of the results.

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