Induction of emesis in *Suncus murinus* by pyrogallol, a generator of free radicals

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1 We investigated whether or not pyrogallol, a generator of free radicals, is emetogenic in *Suncus murinus*, the house musk shrew. Pyrogallol (i.p.) caused dose-dependent emesis in suncus with an ED₅₀ value of 77.3 mg kg⁻¹. At a dose of 128 mg kg⁻¹, all suncus vomited with mean latency of 18.8 ± 5.2 min and the number of vomiting episodes was 8.6 ± 2.9.

2 The prophylactic effects of N-(2-mercaptopropionyl)-glycine (MPG), an antioxidant, and tropisetron, a 5-hydroxytryptamine₃ (5-HT₃) receptor antagonist, were studied. Pyrogallol (128 mg kg⁻¹, i.p.)-induced emesis was prevented by treatment with MPG (i.p.) or tropisetron (s.c.) with ID₅₀ values of 149 mg kg⁻¹ and 117 µg kg⁻¹, respectively.

3 Pyrogallol-induced emesis was completely prevented by surgical abdominal vagotomy.

4 The present results indicate that pyrogallol-induced emesis is characteristically very similar to that caused by cisplatin and support the idea that generation of free radicals causes the release of peripheral 5-HT, which stimulates vagal afferent sensory nerves to cause emesis.

Keywords: Emesis; radical; pyrogallol; cisplatin; 5-hydroxytryptamine; suncus

Introduction

Recently, many cancer chemotherapeutic agents have been developed, but the concomitant side effects, including bone marrow suppression, nephrotoxicity, nausea, vomiting and hair loss remain major problems in cancer chemotherapy. In particular, nausea and vomiting cause serious discomfort to patients. Considering the quality of life of the cancer patients, it is very important to overcome such side effects. Among cancer chemotherapeutic agents, cisplatin is known to have potent anticancer effects and also known to cause severe vomiting.

Recently, it has been shown that 5-hydroxytryptamine₃ (5-HT₃) receptor antagonists have prophylactic effects on the emesis, and many 5-HT₃ receptor antagonists have been developed (Andrews *et al.*, 1988; Matsuki *et al.*, 1992). It is recognized that cisplatin is converted to an active metabolite(s) (Mutoh *et al.*, 1992), which it is proposed releases 5-HT from the enterochromaffin cells, and that the released 5-HT stimulates the abdominal visceral afferent nerves which transmit impulses to the 'vomiting centre' to cause emesis. 5-HT₃ receptor antagonists are less effective against delayed emesis (Aapro, 1992); therefore, drugs that can prevent the release of 5-HT may be more useful in cisplatin-induced emesis than 5-HT₃ receptor antagonists.

However, little is known about the mechanism of cisplatin-induced release of 5-HT. Cisplatin is known to cause lipid peroxidation of various tissues (Hannemann & Baumann, 1988; Torii *et al.*, 1993a). We have already reported that cisplatin-induced emesis was completely prevented by preadministration of N-(2-mercaptopropionyl)-glycine (MPG), an antioxidant (Torii *et al.*, 1993a). Moreover, cisplatin-induced emesis was exaggerated by ferric chloride, which is known to catalyze the production of cytotoxic oxygen radicals, and ameliorated by deferoxamine, an iron chelator (Matsuki *et al.*, 1994). These results suggest that oxygen radicals are involved in cisplatin-induced emesis. In the present study, we found that pyrogallol, a generator of free radicals, caused emesis in *Suncus murinus*.

Methods

Animals

Experiments were performed on healthy adult female *Suncus murinus* (the house musk shrew) weighing 35–50 g. All animals were housed under a 12 h light/dark cycle with free access to food and water.

Experimental procedures

Drug treatment Pyrogallol (1,2,3-trihydroxybenzen) was used as a generator of free radicals. The agent was administered intraperitoneally in suncus and behavioural changes including vomiting were observed for 90 min. The number of vomiting episodes and the latency to the first vomit were recorded. Initially vomiting is always accompanied by an expulsion of gastric contents. The dose of pyrogallol was increased from 32 mg kg⁻¹, in two fold steps. The ED₅₀ value of the emetic effect was calculated by Brownlee's up-and-down method (Brownlee *et al.*, 1953).

The antiemetic effects of tropisetron (s.c.), a 5-HT₃ receptor antagonist, and MPG (i.p.) on pyrogallol (128 mg kg⁻¹, i.p.)-induced emesis were investigated. These agents were administered 30 min prior to the injection of pyrogallol. The ID₅₀ values for pyrogallol-induced emesis were also calculated by the up-and-down method.

Vagotomy In five animals, an abdominal vagotomy was performed. Details of the method of surgical vagotomy have been described elsewhere (Torii *et al.*, 1991b). In brief, the animals were anaesthetized with pentobarbitone, 35 mg kg⁻¹ i.p., after which the bilateral vagus attached to the oesophagus just below the diaphragm was isolated and sectioned. These animals were allowed to recover for at least 1 week before being tested.

Drugs

The drugs used in the experiments were as follows: pyrogallol (Tokyo Kasei Kogyo Co., Ltd., Tokyo, Japan) and MPG (Sigma Chemical Co.). Tropisetron was provided by Sandoz.

These drugs were dissolved in saline. The volume of drug solutions injected was adjusted to 0.1 ml 50 g⁻¹ body weight.

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Table 1 The emetic effect of pyrogallol in *Suncus murinus*

Dose (mg kg ⁻¹)	No. of suncus vomiting/tested	No. of vomiting episodes	Latency (min)	ED ₅₀ value (mg kg ⁻¹)
128	5/5	8.6 ± 2.9	18.8 ± 5.2	77.3
64	1/5	3	23	
32	0/1	—	—	

Values for the number of vomiting episodes and the latency per vomiting suncus are mean ± s.e.mean, but actual values are indicated when the number of vomiting suncus was less than three.

— No suncus vomited at the dose.

Table 2 Prophylactic effects of N-(2-mercaptopropionyl)-glycine (MPG) and tropisetron on pyrogallol-induced emesis in *Suncus murinus*

Treatment	Dose	No. of suncus vomiting/tested	No. of vomiting episodes	Latency (min)	ID ₅₀ value
MPG (i.p.)	320 (mg kg ⁻¹)	0/2	—	—	149 (mg kg ⁻¹)
	160	2/5	3,3	14,16	
	80	3/3	7.0 ± 2.1	16.3 ± 3.4	
	Control	1/1	7	12	
Tropisetron (s.c.)	1000 (µg kg ⁻¹)	0/1	—	—	117 (µg kg ⁻¹)
	500	0/1	—	—	
	250	0/3	—	—	
	126	2/5	2,1	4,12	
	63	3/3	3.7 ± 1.8	5.0	
	Control	1/1	12	13	

Values for the number of vomiting episodes and the latency per vomiting animal are means ± s.e.mean.

MPG and tropisetron were injected 30 min before the administration of pyrogallol (128 mg kg⁻¹, i.p.).

Table 3 The effects of the surgical vagotomy on pyrogallol-induced emesis in *Suncus murinus*

Treatment	No. of suncus vomiting/tested	No. of vomiting episodes	Latency (min)
Sham operation	4/4	8.3 ± 0.9	20.8 ± 1.1
Vagotomy	0/5	—	—

The operated animals were used for experiments after 1 week.

Pyrogallol (128 mg kg⁻¹) was intraperitoneally injected.

Values for the number of vomiting episodes and latency are mean ± s.e.mean.

Results

Intraperitoneal injection of pyrogallol induced dose-dependent emesis in suncus (Table 1). The calculated ED₅₀ value was 77.3 mg kg⁻¹. At the dose of 128 mg kg⁻¹, all five suncus vomited with a latency of 18.8 ± 5.2 min and the number of vomiting episodes was 8.6 ± 2.9 times (*n* = 5). The duration of pyrogallol (128 mg kg⁻¹)-induced emesis, time between the first and the last episode, was 15.0 ± 6.5 min. The duration was significantly shorter than emesis caused by cisplatin (20 mg kg⁻¹, i.p.), reported by Mutoh *et al.* (1992) (57.6 ± 12.7 min, *P* < 0.05 by Student's *t* test). Injection of pyrogallol caused salivation, grooming and transient immobilization before the start of emesis. Such behavioural effects were observed after administration of cancer chemotherapeutic agents, nicotine, veratrine, copper sulphate and X-irradiation. Pyrogallol was not fatal at any of the doses studied, all the animals being alive 1 week after the injection of pyrogallol. Pyrogallol also did not affect the body weights significantly.

Table 2 shows the prophylactic effects of MPG and tropisetron on pyrogallol (128 mg kg⁻¹, i.p.)-induced emesis. Both MPG and tropisetron prevented pyrogallol-induced emesis with the respective ID₅₀ values of 149 mg kg⁻¹ and 117 µg

kg⁻¹. As reported previously, neither MPG nor tropisetron itself caused apparent behavioural change of animals (Torii *et al.*, 1991a; 1993a).

The effect of surgical vagotomy on pyrogallol-induced emesis was investigated (Table 3). In a sham operated group, pyrogallol-induced emesis was not influenced (*n* = 4). However, the pyrogallol-induced emesis was completely prevented by surgical vagotomy (*n* = 5). In a separate experiment we confirmed that vagotomized suncus did not vomit after receiving copper sulphate (40 mg kg⁻¹, p.o.). This indicates that the nervous connections involved in the vomiting reflex were retained in the vagotomized suncus.

Discussion

Cisplatin is a very effective antineoplastic agent against various types of solid tumours (Rozenzweig *et al.*, 1977). However, clinical use of cisplatin is limited because of bone marrow suppression, nephrotoxicity, nausea and vomiting. For bone marrow toxicity, haematopoietic cytokines, such as granulocyte-colony stimulating factor (G-CSF) and granulocyte-macrophage-colony stimulating factor (GM-CSF), have been introduced to treat granulocytopenia developed by cancer chemotherapy (Hollingshead & Goa, 1991). As to nephrotoxicity, it was reported that cisplatin elevated blood urea nitrogen and creatinine concentration and that antioxidants and radical scavengers attenuated cisplatin-induced nephrotoxicity (McGinness *et al.*, 1978; Dobyan *et al.*, 1986; Sugihara & Gemba, 1986). Sangeetha *et al.* (1990) reported that generation of free radicals following chemotherapy was increased in cancer patients. We propose that cisplatin-induced emesis is also mediated by oxygen free-radicals for the following reasons (Torii *et al.*, 1993a): (1) MPG blocks cisplatin-induced emesis; (2) cisplatin causes lipid peroxidation in the various tissues of suncus and (3) X-irradiation, which is known to release OH· from the water molecule, also causes emesis (Torii *et al.*, 1993b).

In the present study, we showed that pyrogallol, a potent

Table 4 Comparison of cisplatin-, pyrogallol- and 5-hydroxytryptamine (5-HT)-induced emesis in *Suncus murinus*

	Emetic stimuli			
	Cisplatin (20 mg kg ⁻¹ , i.p.)	X-irradiation (800 cGy)	Pyrogallol (128 mg kg ⁻¹ , i.p.)	5-HT (10 mg kg ⁻¹ , i.p.)
Tropisetron (s.c.) (ID ₅₀ value)	Effective ^a (25 µg kg ⁻¹)	Effective ^b (29 µg kg ⁻¹)	Effective (117 µg kg ⁻¹)	Effective ^c (7.8 µg kg ⁻¹)
Surgical vagotomy	Effective ^c	Effective ^b	Effective	Effective ^c
MPG (i.p.) (ID ₅₀ value)	Effective ^d (130 mg kg ⁻¹)	NT	Effective (149 mg kg ⁻¹)	Not effective ^d

NT: not tested.

^aTorii *et al.*, 1991a; ^bTorii *et al.*, 1993b; ^cTorii *et al.*, 1991b; ^dTorii *et al.*, 1993a; ^eMutoh *et al.*, 1992.

generator of superoxide anion (O₂⁻) (Marklund & Marklund, 1974), induced emesis in *suncus*. Pyrogallol is known to be both competitive and non-competitive inhibitor of catechol *O*-methyl-transferase (COMT) (Crout, 1961; Baldessarini & Greiner, 1973). The latter noncompetitive inhibitory activity increases with pre-incubation of pyrogallol (Crout, 1961). The pyrogallol is rapidly autoxidized and generates O₂, hydrogen peroxide (H₂O₂), and hydroxyl radical (OH·) by the Haber-Weiss reaction [O₂ + H₂O₂ → OH· + OH⁻ + O₂]. These reactive species are thought to contribute significantly to the noncompetitive effects of pyrogallol on COMT, which contains an essential sulphhydryl group (Axelrod & Tomchick, 1958). 5-HT is present in high concentrations in the gastrointestinal tract, predominantly in the enterochromaffin cells (ECs) (Erspamer, 1966). Because 5-HT is emetogenic in *Suncus* (Torii *et al.*, 1991b), inhibition of enzymes which break down 5-HT may promote emetic response. However, 5-HT is not metabolized by COMT because 5-HT has no catechol structure. The great majority of 5-HT is metabolized to 5-hydroxyindoleacetaldehyde by monoamine oxidase (MAO), and then to 5-hydroxyindoleacetic acid (5-HIAA) by aldehyde dehydrogenase. The release of 5-HT from the intestinal mucosa is regulated by a complex pattern of neuronal and humoral inputs to the ECs (Racké & Schwörer, 1991). Noradrenaline (NA) is shown to be present in abdominal vagal fibres (Lundberg *et al.*, 1976). There is a possibility that inhibition of COMT increases the concentration of NA that may affect the release of 5-HT. However, stimulation of α₂-adrenoceptor on ECs is thought to inhibit the release of 5-HT (Racké & Schwörer, 1991). Therefore, it is likely that the emetogenic activity of pyrogallol is not due to the inhibition of COMT, but to the production of oxygen radicals.

Pyrogallol-induced emesis was blocked by injection of MPG. In rabbit aortic rings, pyrogallol elicits endothelium-dependent contractions and the contractions are attenuated by MPG (Goldschmidt & Tallarida, 1991). They reported that endothelium-dependent vasodilatation depends on the presence of a sulphhydryl group that scavenges O₂⁻, produced from pyrogallol. Therefore, we think that MPG blocks pyrogallol-induced emesis by scavenging O₂⁻, generated by pyrogallol.

Pyrogallol-induced emesis was prevented by tropisetron. The surgical abdominal vagotomy at the lower part of the oesophagus also prevented pyrogallol-induced emesis completely. These results suggest that pyrogallol shows its emetogenic effect peripherally, probably indirectly via the release of 5-HT and the stimulation of 5-HT₃ receptors on the vagal afferents.

Table 4 summarizes the overall data of our recent studies including the present data. There are marked similarities

among cisplatin-, X-irradiation- and pyrogallol-induced emesis. X-irradiation to the abdomen but not to the head causes emesis in *suncus* (Torii *et al.*, 1993b). All three types of emesis were blocked by surgical vagotomy and tropisetron. However, MPG has no inhibitory effect on 5-HT-induced emesis. These results suggest that the generation of free radicals occurs before the release of 5-HT from ECs. The ID₅₀ value for tropisetron vs pyrogallol was a little higher than against other emetogens. We selected the dose of each stimulus so that 100% animals vomit in control conditions. But, it is still possible that 128 mg kg⁻¹ pyrogallol was stronger than other stimuli. However, this is not likely since MPG blocked both cisplatin- and pyrogallol-induced emesis with similar efficacies. NA is known to cause emesis by stimulating α₂-adrenoceptors in the area postrema (Beleslin & Strbac, 1987). It is possible that the lower effectiveness of tropisetron is due to increase of NA by pyrogallol in the central nervous system. Both the central effect is probably not essential, because pyrogallol-induced emesis was completely blocked by vagotomy. Therefore, we do not have an explanation for the different efficacies of tropisetron.

The latency of pyrogallol-induced emesis (18.8 min) was also similar to that of X-irradiation (20.0 min: Torii *et al.*, 1993b) and *cis*-diaquodiammineplatinum (II) (a metabolite of cisplatin) (17.8 min: Mutoh *et al.*, 1992). Moreover, the latency of 5-HT (i.p.) was very short (less than 1 min) (Torii *et al.*, 1991b). Taken together, these results suggest that generation of free radicals at the peripheral site is an important step for the release of 5-HT, and that the release of 5-HT occurs or the concentration of 5-HT reaches the critical level about 20 min after the generation of free radicals.

The duration of pyrogallol-induced emesis was significantly shorter than that caused by cisplatin. Cisplatin is known to be accumulated in the cells and remains there in the long term (Oku *et al.*, 1988). On the other hand, pyrogallol is converted into several pyrogallol sulphate esters *in vivo* (Eccleston & Ritchie, 1973), and may be excreted by the kidney. Therefore, the short duration of pyrogallol-induced emesis is probably due to its fast turnover.

In summary, pyrogallol induced emesis in *suncus*, and the emesis was prevented by MPG, tropisetron and surgical vagotomy. MPG had no significant effect on antitumour activity of cisplatin *in vitro* (Torii *et al.*, 1993a). Therefore, antioxidants and radical scavengers may become new types of prophylactic drugs against cancer chemotherapeutic agent-induced emesis.

The authors are grateful to Dr P.L.R. Andrews for critical comments on the manuscript.

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(Received May 25, 1993)

Revised August 15, 1993

Accepted October 6, 1993)