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Resiniferatoxin antagonizes cisplatin-induced emesis in dogs and ferrets

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Abstract

We evaluated the antiemetic activity of resiniferatoxin, an ultrapotent capsaicin analogue, on cisplatin- and apomorphine-induced emesis in dogs, and on cisplatin-induced acute and delayed emesis in ferrets. In the dog, resiniferatoxin (10 μg/kg, s.c.) 30 min before the injection of cisplatin markedly prevented acute emesis induced by cisplatin. When animals were given resiniferatoxin (10 μg/kg, s.c.) 24 h prior to cisplatin, the emesis was still inhibited, but not significantly. Resiniferatoxin (10 μg/kg, s.c.) 30 min before the administration of apomorphine also significantly reduced the emetic responses induced by apomorphine in dogs. In the ferret, resiniferatoxin (10 μg/kg, s.c.) 30 min prior to cisplatin completely inhibited acute emesis caused by cisplatin (10 mg/kg, i.p.). When ferrets were given resiniferatoxin (10 μg/kg, s.c.) 16 h prior to cisplatin, the emesis was still significantly inhibited. Cisplatin (5 mg/kg, i.p.) induced both acute (0–24 h) and delayed (24–72 h) phase emesis, and a single injection of resiniferatoxin (10 μg/kg, s.c.) at 36 h after cisplatin significantly reduced subsequent emetic responses during the 36–72 h period. These results suggest that resiniferatoxin-related vanilloids may be useful drugs against both acute and delayed emesis induced by cancer chemotherapy.

Keywords: Emesis, acute; Emesis, delayed; Apomorphine; Vanilloid; (Dog); (Ferret)

1. Introduction

The vagal afferent innervation of the gastrointestinal tract plays important functional roles in reflex control of motility, secretion, endocrine and exocrine function. It also provides the afferent signal for a variety of behavioral and integrative responses, for example, the emetic reflex. It has been hypothesized that several stimuli such as cytotoxic chemotherapeutic drugs and ingested chemical irritants either directly or indirectly activate vagal afferent nerves that trigger the central emetic pathway (Andrews et al., 1988). Therefore, vagotomy is known to reduce the acute emetic responses by the abovementioned peripherally acting agents in animals (Andrews et al., 1990; Fukui et al., 1992, 1993). Most of vagal afferent nerves are unmyelinated C-fibers, in other words, capsaicin-sensitive nerves (Andrews, 1986). Resiniferatoxin is a naturally occurring ultrapotent capsaicin analogue, and has a much more favorable ratio of desensitization to excitation than capsaicin (Szallasi and Blumberg, 1999). Surprisingly, previous studies demonstrated that subcutaneous injection of resiniferatoxin blocked emesis induced by both peripherally (e.g. copper sulfate and radiation) and centrally acting stimuli (e.g. provocative motion and loperamide) in ferrets and the house musk shrew (*Suncus murinus*), suggesting that the action of resiniferatoxin cannot be explained only by desensitization of the afferent fibers (Andrews and Bhandari, 1993; Andrews et al., 2000). In the paralyzed decerebrate dog, topical application of capsaicin and resiniferatoxin into the 4th ventricular abolished fictive retching induced by stimulation of abdominal vagal afferents (Shiroshita et al., 1997).

Nausea and vomiting following anticancer drugs such as cisplatin are a major cause of morbidity in oncology patient. Acute emesis, occurring within the first 24 h of chemotherapy, can be dramatically prevented by 5-HT3 receptor antagonists such as ondansetron, granisetron and tropisetron (Hesketh, 2000). In contrast to acute emesis, delayed emesis, which occurs 24 h or later after the start of chemotherapy, is poorly controlled with antiemetic regimens, including the 5-HT3 receptor antagonists (Gregory and Ettinger, 1998). As the precise mechanism involved in the

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Table 1
Effect of resiniferatoxin on cisplatin-induced acute emesis in dogs

Treatment[a]	Animals (vomits/total)	Latency[b] (min)	Vomits	Inhibition (%)
Control	8/8	72.4 ± 2.8	21.6 ± 3.9	–
Resiniferatoxin (10 μg/kg) 30 min	2/3	217.9 ± 47.4[c]	1.3 ± 0.9[c]	94.0
Resiniferatoxin (10 μg/kg) 24 h	4/4	147.9 ± 51.4	7.3 ± 2.9	66.2
Control	3/3	98.9 ± 2.1	16.7 ± 2.3	–
Resiniferatoxin (1 μg/kg) 30 min	3/3	133.0 ± 31.4	6.0 ± 3.2	64.0

[a] Resiniferatoxin was subcutaneously administered 30 min or 24 h before the injection of cisplatin.
[b] If a dog did not vomit, the latency period was taken to be equal to the observation time (300 min).
[c] Compared with the control, $P < 0.05$.

initiation of delayed emesis is unknown, there is currently no satisfactory treatment that can eliminate severe chemotherapy-induced delayed emesis in humans.

Although it was demonstrated that resiniferatoxin was effective on several emetic stimuli in ferrets and *S. murinus* (Andrews and Bhandari, 1993; Andrews et al., 2000), no reports are available concerning the systemic administration of resiniferatoxin in conscious dogs. This species is sensitive to various emetic stimuli, in a similar way to humans (Andrews et al., 1990). We evaluated the antiemetic activity of resiniferatoxin systemically administered on cisplatin- and apomorphine-induced emesis in dogs. To date, there have been no reports on the effect of resiniferatoxin on chemotherapy-induced acute and delayed emesis in an animal model. Therefore, the present study was also designed to define the effect of resiniferatoxin on cisplatin-induced acute and delayed emesis in ferrets.

2. Materials and methods

2.1. Animals

Beagle dogs of either sex weighing 8.0–14.5 kg and adult male ferrets weighing 1.0–2.3 kg were used in this study. Both dogs and ferrets were individually housed in each animal room at 23 ± 1 °C with lights on between 07:00 and 19:00, routinely fed a dry pellet, TC-1 (Maruha Pet Food, Japan) and Ferret Diet (PMI Feeds, USA), respectively; water was available ad libitum. In all experiments, animals were removed from their home cages and transferred to observation cages in a quiet room. All studies were performed in accordance with the guideline of the Fujisawa Pharmaceutical Animal Experimental Committee.

2.2. Dosage of resiniferatoxin

We selected a dose of 10 μg/kg as a non-lethal dose for ferrets in a preliminary experiment. Systemic administration of resiniferatoxin has not previously been studied in the conscious dog and therefore the dose to be used was selected on the basis of preliminary studies in ferrets. Although in most cases, in both ferrets and dogs, the respiratory rate was gradually stimulated by resiniferatoxin (10 μg/kg, s.c.), it returned to almost normal within 1 h. Resiniferatoxin (10 μg/kg, s.c.) failed to induce emetic and algesic responses, as indicated by continuous vocalization, piloerection, urination, defecation or scratching at the site of injection, in both species.

2.3. Experiments in dogs

2.3.1. Cisplatin-induced acute emesis in dogs

After the administration of cisplatin (3.2 mg/kg), animals were observed continuously for 5 h, and the number of incidences of emesis were counted. The presence of vomiting separated from the next bout by at least 1 min was considered as a single emetic episode. Resiniferatoxin (10 μg/kg) or vehicle was subcutaneously administered 30 min or 24 h before the injection of cisplatin. A separate group of animals was treated with resiniferatoxin (1 μg/kg, s.c.) 30 min prior to cisplatin.

2.3.2. Apomorphine-induced emetic responses in dogs

Apomorphine at 0.1 mg/kg was subcutaneously administered in dogs. The animals were observed continuously for 60 min for the emetic responses, the time of onset to vomiting and the number of both retches and vomits. Animals were retested 2 weeks later with apomorphine,

Table 2
Effect of resiniferatoxin on cisplatin-induced acute emesis in ferrets

Treatment[a]	Animals (vomits/total)	Latency[b] (min)	Retches + vomits	Inhibition (%)
Control	4/4	62.0 ± 5.6	264.5 ± 41.6	–
Resiniferatoxin (1 μg/kg) 30 min	3/3	59.7 ± 2.7	197.7 ± 40.7	25.3
Resiniferatoxin (10 μg/kg) 30 min	0/3	240.0 ± 0.0[c]	0.0 ± 0.0[c]	100
Resiniferatoxin (10 μg/kg) 16 h	3/3	73.0 ± 10.0	80.0 ± 14.1[d]	69.8
Resiniferatoxin (10 μg/kg) 24 h	3/3	49.0 ± 14.1	198.0 ± 24.6	24.9

[a] Resiniferatoxin was subcutaneously administered 30 min, 16 h or 24 h before the injection of cisplatin.
[b] If a ferret did not vomit, the latency period was taken to be equal to the observation time (240 min).
[c] Compared with the control, $P < 0.01$.
[d] Compared with the control, $P < 0.05$.

30 min after treatment with resiniferatoxin (1 and 10 μg/kg, s.c.). In preliminary experiments, identical dogs ($n = 13$) exhibited reproducible emetic responses to apomorphine (0.1 mg/kg, s.c.) between the first and second trial after 14 days. The latency and the number of retches + vomits in the first and second trial were 5.1 ± 0.4 min and 98.3 ± 9.2 and 4.7 ± 0.2 min and 100.8 ± 9.0, respectively.

2.4. Experiments in ferrets

2.4.1. Cisplatin-induced acute emesis in ferrets

Following administration of cisplatin (10 mg/kg, i.p.), animals were observed continuously for 4 h and the number of incidences of emetic responses consisting of retches plus vomits were counted. Resiniferatoxin (1 and 10 μg/kg) or vehicle was subcutaneously administered 30 min before the injection of cisplatin. A separate group of animals was treated with resiniferatoxin (10 μg/kg, s.c.) 16 or 24 h prior to cisplatin.

2.4.2. Cisplatin-induced delayed emesis in ferrets

Ferrets were intraperitoneally injected with cisplatin (5 mg/kg) at 08:30. The animal behavior was recorded using a video camera with an automatic night photography system, for up to 72 h, and analyzed at the end of the experiment. Resiniferatoxin (10 μg/kg) or vehicle was subcutaneously administered 36 h after cisplatin-treatment. A 12-h artificial light cycle (lights on between 07:00 and 19:00) was used throughout the study. Ferrets were given food (Ferret Diet, 70 g/day) and water ad libitum for 3 days.

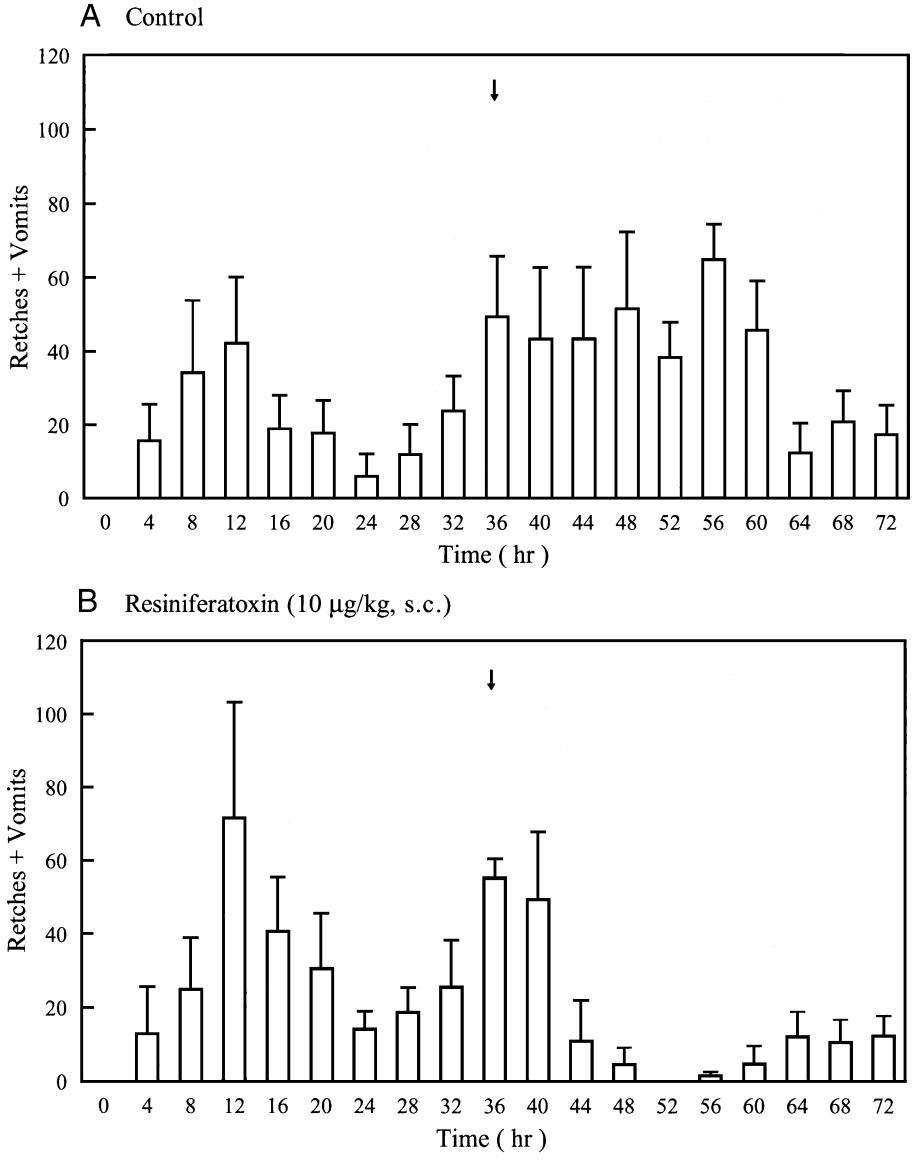

Fig. 1. (A) The profile of cisplatin-induced emetic response in vehicle-treated ferrets during a 72-h observation period ($n = 6$), and (B) effect of resiniferatoxin (10 μg/kg, s.c. at 36 h after cisplatin injection) on the cisplatin-induced emetic response ($n = 5$). Administration of drug or vehicle is indicated by (↓). Results are shown as the mean number of retches + vomits (vertical bar shows S.E.) occurring at 4-h time intervals after cisplatin injection.

2.5. Drugs

Cisplatin, apomorphine and resiniferatoxin were purchased from Sigma (St. Louis, USA). Cisplatin was prepared in saline at 70 °C followed by gradual cooling to 40 °C and administered immediately in a volume of 1 ml/kg for dogs or 5 ml/kg for ferrets. Apomorphine was dissolved in saline and administered in a volume of 0.5 ml/kg. Resiniferatoxin was made up in a mixture of Tween 80, ethanol and 154 mM NaCl at a ratio of 1:1:8. Resiniferatoxin or vehicle was injected in a volume of 1 ml/kg for both species.

2.6. Statistical analysis

Group results are expressed as mean ± S.E. Dunnett's test or a paired Student's *t*-test was used as a measure of significance. Values of $P < 0.05$ were regarded as statistically significant.

3. Results

3.1. Experiments in dogs

3.1.1. Cisplatin-induced acute emesis in dogs

As shown in Table 1, resiniferatoxin (10 μg/kg, s.c.) administered 30 min before cisplatin markedly reduced acute emesis caused by cisplatin, increased the latency to vomit and completely prevented emesis in 1 of 3 dogs. When given resiniferatoxin at 10 μg/kg, 24 h prior to cisplatin, emesis was antagonized, but not significantly. Decreasing the dose to 1 μg/kg of resiniferatoxin also antagonized the number of vomits but failed to reach statistical significance (Table 1).

3.1.2. Apomorphine-induced emetic responses in dogs

In the first trial, as control, apomorphine induced emesis within 3.4 ± 0.6 min and there were 80.5 ± 9.3 retches + vomits in four animals. After a rest period of 14 days, the vomiting began 24.1 ± 12.3 min after apomorphine, and the number of retches + vomits was 20.3 ± 11.3 ($P < 0.05$) in animals pretreated with resiniferatoxin (10 μg/kg, s.c.). In a separate experiment, resiniferatoxin at 1 μg/kg was ineffective on both latency (3.6 ± 0.2 min vs. control 4.0 ± 0.5 min) and number of retches + vomits (96.7 ± 1.2 vs. control 115.7 ± 14.7) in three animals.

3.2. Experiments in ferrets

3.2.1. Cisplatin-induced acute emesis in ferrets

As shown in Table 2, pretreatment with resiniferatoxin (10 μg/kg, s.c.) 30 min before the injection of cisplatin completely antagonized cisplatin-induced acute emesis, while resiniferatoxin at 1 μg/kg was ineffective. Resiniferatoxin at 10 μg/kg given 16 h, but not 24 h, prior to cisplatin significantly prevented emetic episodes.

Table 3
Effect of resiniferatoxin on cisplatin-induced delayed emesis in ferrets

Treatment	*N*	Retches + vomits		
		Time after cisplatin (h)		
		0–24	24–36	36–72
Control	6	135 ± 57	85 ± 29	339 ± 63
Resiniferatoxin (10 μg/kg)	5	195 ± 67	100 ± 19	107 ± 33[a]

Resiniferatoxin was subcutaneously administered at 36 h after cisplatin injection.
[a] Compared with the control, $P < 0.05$.

3.2.2. Cisplatin-induced delayed emesis in ferrets

The pattern of emesis induced by cisplatin at 5 mg/kg in ferrets is shown in Fig. 1. Until 36 h after the injection of cisplatin, there was no difference in the mean number of retches and vomits during 0–24 h (acute phase) and 24–36 h (delayed phase) in the resiniferatoxin group compared with the control (Table 3). A single subcutaneous injection of resiniferatoxin (10 μg/kg) at 36 h after cisplatin apparently reduced delayed emesis during the subsequent 36–72 h by 68% (Fig. 1, Table 3).

4. Discussion

In this study, we first demonstrated that systemically administered resiniferatoxin significantly reduced emesis induced by cisplatin and apomorphine in conscious dogs, without severe side effects.

It has been hypothesized that cisplatin-induced acute emesis is mediated by increased 5-HT levels in the small intestine, which in turn stimulate vagal, but not splanchnic, afferent fibers through 5-HT3 receptors. Indeed, it was demonstrated that the occurrence of acute emesis induced by cisplatin required intact abdominal vagal afferent innervation in animals (Andrews et al., 1990; Fukui et al., 1992, 1993), and that splanchnicectomy alone was without effect on retching and vomiting evoked by cisplatin in ferrets (Andrews et al., 1990). Vagal afferent nerves are capsaicin-sensitive (Andrews, 1986; Shiroshita et al., 1997), and capsaicin analogue, including resiniferatoxin, mediate their actions through a specific membrane receptor that was recently determined to be vanilloid VR1 receptor (Caterina et al., 1997). A previous study showed that vanilloid VR1 receptor immunoreactivity was observed in the terminals of afferent fibers projecting to the superficial layers of the spinal cord dorsal horn and the trigeminal nucleus caudalis, which were closely associated with nociception in central nervous system (CNS) (Tominaga et al., 1998). In addition, it was also observed in peripheral nervous system (PNS) such as vagus and sciatic nerves (Tominaga et al., 1998). These areas were also identified by [^{3}H] resiniferatoxin binding (Szallasi and Blumberg, 1999). Interestingly, the present study showed that resiniferatoxin at 1 μg/kg was ineffective on

apomorphine-induced emesis, but, although not significantly, did reduce the emesis caused by cisplatin in dogs. In addition, the antiemetic activity of resiniferatoxin at 10 μg/kg on emetic response to cisplatin was more effective than apomorphine. Fukui et al. (1992) reported that apomorphine-induced emesis in dogs was unaffected by vagotomy and splanchnicectomy. The different antiemetic potency of resiniferatoxin on cisplatin- and apomorphine-induced emesis in this study may be associated with the activation or non-activation of vagal afferent nerves, respectively. In the ferret, resiniferatoxin was also more efficacious against peripherally acting emetic stimuli than centrally acting stimuli (Andrews and Bhandari, 1993). Thus, it seems likely that the desensitization of vagal afferent neurons by resiniferatoxin through vanilloid VR1 receptor may be initially involved in suppression of the incidence of acute emesis induced by cisplatin, while it is suggested that resiniferatoxin may mostly act by depletion of a neurotransmitter (substance P and/or calcitonin gene related peptide) in the brainstem (Andrews and Bhandari, 1993; Andrews et al., 2000).

There are several clinical indications for use of topical application of capsaicin, while topical resiniferatoxin has mainly been targeted at patients with urge incontinence due to neurological diseases (Szallasi and Blumberg, 1999). Intravesical resiniferatoxin in patients with overactive bladder produced functional changes, and this improvement persisted up to 4 weeks (Szallasi and Blumberg, 1999). It has also been reported that desensitization of bladder afferents following intravesical resiniferatoxin in rats lasted up to 4 weeks (Craft et al., 1995). In the present study, resiniferatoxin given 24 h prior to cisplatin inhibited, but not significantly, acute emesis in dogs, when continuously observed for 5 h after cisplatin administration. Furthermore, it was also demonstrated that ferrets pretreated with resiniferatoxin 16 h prior to emetic challenge showed a significant inhibition of cisplatin-induced acute emesis. These observations suggest that the duration of antiemetic action of resiniferatoxin given subcutaneously may be about 24 h in both dogs and ferrets.

A novel finding of the present study is that resiniferatoxin significantly reduced the delayed phase of cisplatin-induced emesis in ferrets. The pathogenesis of delayed emesis, in contrast to acute emesis, is not well understood. Although delayed emesis is generally less severe than acute emesis (Kris et al., 1985), unsatisfactory control of delayed emesis can potentially result in a patient's refusal to continue with successful chemotherapeutic treatments. The profile of emetic response in the ferret following cisplatin (5 mg/kg, i.p.) was similar to that described previously (Rudd et al., 1994; Rudd and Naylor, 1994). The treatment of the animals with a single injection of resiniferatoxin, 36 h after cisplatin, significantly inhibited subsequent emetic responses during 36–72 h. As shown in Fig. 1, an apparent inhibition of emetic episodes by resiniferatoxin was observed until 60 h, although the antiemetic action of resiniferatoxin was gradually attenuated with the passage of time after that. This is reasonable, given that duration of antiemetic action of resiniferatoxin is about 24 h. Future studies plan to investigate the antiemetic activity of repeated injection of resiniferatoxin on delayed emesis.

Interestingly, treatment with resiniferatoxin did not immediately inhibit delayed emetic response. This was different to the case of acute emesis experiments where resiniferatoxin acted sufficiently within 6 h. It was reported that section of abdominal visceral afferent nerves had no effect on methotrexate-induced delayed emesis in dogs (Fukui and Yamamoto, 1999), a model suggested to be useful for studies of the mechanisms of delayed emesis induced by chemotherapy in humans (Fukui and Yamamoto, 1999; Yamakuni et al., 2000). It seems unlikely that abdominal afferent innervation is an essential participant in the production of delayed phase emesis in the present study. Previous studies by Shiroshita et al. (1997) suggested that the medial region of nucleus tractus solitarius was the site of action of systemic resiniferatoxin. This region has also been demonstrated to be a site where vanilloid VR1 receptor mRNA expressed (Mezey et al., 2000). Therefore, our results suggest that the antiemetic activity of resiniferatoxin against the delayed emesis induced by cisplatin may act through vanilloid VR1 receptor in CNS, but not PNS.

In *S. murinus*, the mechanism of antiemetic action of resiniferatoxin against nicotine-induced emesis is still debated. Pretreatment with subcutaneous resiniferatoxin 1 h prior to nicotine was highly effective on nicotine-induced emesis (Andrews et al., 2000), while resiniferatoxin intracerebroventricularly administered 3 h before nicotine failed to reduce emesis by nicotine (Rudd and Wai, 2001). In the present study, resiniferatoxin had a slow effect on delayed emesis by cisplatin, compared to apomorphine, a centrally acting stimulus. These results suggest the possibility that resiniferatoxin may have at least two antiemetic mechanisms in CNS that are dependent on the passage of time after administration of resiniferatoxin; consisting of quick and slow onsets. Further studies though are required to resolve the precise mechanisms of antiemetic action of resiniferatoxin.

Unfortunately, a previous (Andrews and Bhandari, 1993) and the present study demonstrated that resiniferatoxin at antiemetic doses causes side effects, such as increasing respiratory rate and decreasing body temperature in animals. Thus, it is unlikely that resiniferatoxin itself may be developed as an antiemetic drug. As the dog is a widely used non-rodent species in pharmacology, pharmacokinetic and toxicity studies, results from the present study are likely to be useful in developing resiniferatoxin analogues for clinical utility. Although the pathogenesis of delayed emesis remains poorly understood in comparison to recent improved understanding of the pathophysiology of acute emesis, our results suggest that resiniferatoxin-related vanilloids may be useful drugs against both acute and delayed emesis induced by cancer chemotherapy.

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