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**Pharmacological Characterization of T-2328, 2-Fluoro-4'-methoxy-3'-[[[(2S,3S)-2-phenyl-3-piperidinyl]amino]methyl]-[1,1'-biphenyl]-4-carbonitrile Dihydrochloride, as a Brain-Penetrating Antagonist of Tachykinin NK<sub>1</sub> Receptor**

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**Abstract.** The pharmacological properties of T-2328 were evaluated as an antagonist of the tachykinin neurokinin 1 (NK<sub>1</sub>) receptor. T-2328 inhibited the specific binding of [<sup>3</sup>H][Sar<sup>9</sup>,Met(O<sub>2</sub>)<sup>11</sup>]substance P to tachykinin NK<sub>1</sub> receptors in human lymphoblastic IM9 cells with K<sub>i</sub> of 0.08 nM. In the same assay, K<sub>i</sub> for aprepitant, a brain-penetrating NK<sub>1</sub> antagonist, was 1.3 nM. The antagonism of T-2328 is highly selective for the human NK<sub>1</sub> receptors since the affinities for human NK<sub>2</sub>, NK<sub>3</sub> receptors, and 13 other kinds of receptors and ion channels were >1000-fold lower than for NK<sub>1</sub> receptors. Reduction in B<sub>max</sub> with no change in affinity suggests the non-competitive nature of T-2328 interaction with the NK<sub>1</sub> receptor. T-2328 (0.03 – 0.1 mg/kg, i.v.) and aprepitant (1 – 3 mg/kg, i.v.) significantly prevented the GR73632 (i.c.v.)-induced foot tapping response in gerbils. The potencies of T-2328 in both in vitro and in vivo studies were more than 10 times greater than those of aprepitant. I.v. administration of T-2328 (0.1 – 0.3 mg/kg) potently blocked both acute and delayed emetic responses induced by cisplatin (5 mg/kg, i.p.) in ferrets. It is concluded that T-2328 is a potent, centrally active NK<sub>1</sub> antagonist. T-2328 may have potential as a novel therapeutic agent for the treatment of chemotherapy-induced emesis.

**Keywords:** NK<sub>1</sub> antagonist, brain-penetrative, anti-emetic, cisplatin, delayed emesis

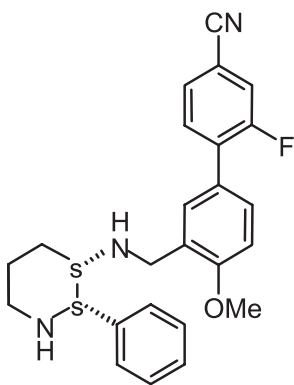
**Introduction**

The mammalian tachykinins (substance P, neurokinin A, and neurokinin B) are widely distributed throughout the central and peripheral nervous systems, where they act as neurotransmitters or neuromodulators (1, 2). Three types of G-protein-coupled receptors, denoted NK<sub>1</sub>, NK<sub>2</sub>, and NK<sub>3</sub>, mediate a wide range of biological activities of tachykinins. Neuroanatomical studies have demonstrated that the NK<sub>1</sub> receptor is predominant in the human brain, whereas the expression of NK<sub>2</sub> or NK<sub>3</sub>

receptors is either weak or absent (3). NK<sub>1</sub> receptors are widely expressed throughout the central nervous system (3) and are involved in the regulation of various behavioral, endocrine, and autonomic functions. Based on the pharmacological data and recent clinical trials, it has emerged that blockade of brain tachykinin NK<sub>1</sub> receptors may provide a novel treatment of emesis (4, 5), major depression (6), and anxiety problems (7). Therefore, there have been subsequent developments of selective and potent NK<sub>1</sub>-receptor antagonists (8).

In order to seek a novel NK<sub>1</sub>-receptor antagonist, T-2328 (2-fluoro-4'-methoxy-3'-[[[(2S,3S)-2-phenyl-3-piperidinyl]amino]methyl]-[1,1'-biphenyl]-4-carbonitrile dihydrochloride) was recently synthesized in the laboratories of Tanabe Seiyaku Co., Ltd. (Osaka) (Fig. 1). In

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**Fig. 1.** Chemical structure of T-2328 (2-fluoro-4'-methoxy-3'-[[[(2*S*,3*S*)-2-phenyl-3-piperidinyl]amino]methyl]-[1,1'-biphenyl]-4-carbonitrile dihydrochloride).

the present paper, the pharmacological properties of T-2328 were examined and compared with those of aprepitant (9–11), a brain-acting NK<sub>1</sub> antagonist. Furthermore, we investigated the antiemetic potential of T-2328 against cisplatin-induced emesis in ferrets.

## Materials and Methods

### Animals

Male gerbils (48–62 g; Nippon SLC, Shizuoka) were housed as a group in a breeding room (lighted on from 7:00 to 19:00) with the room temperature of  $23 \pm 2^\circ\text{C}$  and free access to pellet chow (CRF-1; Oriental Yeast, Tokyo) and drinking water. Male adult ferrets (1.1–1.6 kg; Marshall Farms, North Rose, NY, USA) were individually kept in a breeding room (lights on from 7:00 to 19:00) with the room temperature of  $25 \pm 1^\circ\text{C}$  and free access to dry pellet diet (cat food: CS, Oriental Yeast) and drinking water. All animal experiments procedures were performed under the Guiding Principles for the Care and Use of Laboratory Animals approved by The Japanese Pharmacological Society and also under the guidelines of the Animal Ethics Committee of Tanabe Seiyaku Co., Ltd.

### Tachykinin NK<sub>1</sub> receptor binding

Human lymphoblastoma IM-9 cells (Dainippon Pharmaceutical Co., Ltd., Osaka) were grown in RPMI1640 culture medium supplemented with 10% fetal bovine serum under an atmosphere of 5% CO<sub>2</sub>–95% air. For the binding experiment, the medium was changed to the assay buffer A of the following composition: 50 mM Tris-HCl (pH 7.4), 150 mM NaCl, 3 mM MnCl<sub>2</sub>, 0.02% BSA, 40 µg/ml bacitracin, 4 µg/ml chymostatin, 4 µg/ml phosphoramidon, and 4 µg/ml leupeptin. The binding assay for NK<sub>1</sub> receptors was

carried out by incubating  $4 \times 10^6$  cells with 0.3 nM [<sup>3</sup>H][Sar<sup>9</sup>,Met(O<sub>2</sub>)<sup>11</sup>] substance P and increasing concentrations (1:10 dilution) of T-2328 for 60 min at room temperature. Nonspecific binding was determined in the presence of 2 µM L-703,606, a specific antagonist for the NK<sub>1</sub> receptor. Specific [<sup>3</sup>H][Sar<sup>9</sup>,Met(O<sub>2</sub>)<sup>11</sup>] substance P binding in the competition binding study, defined as the difference between the total and nonspecific binding, was about 85%–95% of the total binding. The interaction was terminated by vacuum filtration onto a GF/C glass filter presoaked in 0.3% polyethylenimine. The radioactivity on the filter was measured by a liquid scintillation counter. The inhibition constant (K<sub>i</sub>) value was calculated with the GraphPad Prism software (GraphPad, San Diego, CA, USA).

### Tachykinin NK<sub>2</sub> and NK<sub>3</sub> receptor binding

The interaction of T-2328 with tachykinin NK<sub>2</sub> receptors was determined in membranes from CHO cells stably expressing the human NK<sub>2</sub> receptor (CRM033; NEN Life Science Products, Inc., Boston, MA, USA). Cell membranes of 10 µg protein were incubated with 0.4 nM [<sup>3</sup>H]SR-48968 in the presence of T-2328 for 60 min at room temperature in the assay buffer B of the following composition: 20 mM Tris-HCl (pH 7.4), 1 mM MnCl<sub>2</sub>, and 0.1% BSA. Nonspecific binding was determined in the presence of 10 µM neurokinin A, an NK<sub>2</sub> agonist. In this assay, K<sub>i</sub> for NKA was 8.7 nM.

The interaction of T-2328 with tachykinin NK<sub>3</sub> receptors was investigated in membranes from CHO cells stably expressing the human NK<sub>3</sub> receptor (CRM065, NEN Life Science Products). Cell membranes of 1 µg protein were incubated with 0.3 nM [<sup>3</sup>H]SR-142801 in the presence of T-2328 for 60 min at room temperature in assay buffer B. Nonspecific binding was determined in the presence of 3 µM SB-223412, a specific antagonist for the NK<sub>3</sub> receptor. In this assay, K<sub>i</sub> for SB-223412 was 10.3 nM.

The incubation was terminated by vacuum filtration onto a GF/C glass filter presoaked in 0.3% polyethylenimine. The radioactivity on the filter was measured by a liquid scintillation counter. K<sub>i</sub> values were calculated with the GraphPad Prism software.

### Binding to receptors/ion channels unrelated to tachykinin receptors

The selectivity of T-2328 was evaluated in various receptor- and ion-channel-binding assays. These include receptors for dopamine D<sub>1</sub>, dopamine D<sub>2S</sub>, GABA<sub>A</sub> agonist site, GABA<sub>A</sub> Cl<sup>−</sup> channel, GABA<sub>B</sub>, glutamate nonselective, strychnine-sensitive glycine, histamine H<sub>1</sub>, opiate κ, opiate μ, serotonin 5-HT<sub>1</sub>, serotonin 5-HT<sub>3</sub>, and N-type Ca<sup>2+</sup>-channel. These were carried out at MDS

Panlabs Pharmacology Services (now MDS Pharma Services, Bothell, WA, USA).

#### *GR73632-induced foot tapping in gerbils*

Male gerbils were briefly anesthetized by inhalation with halothane. An incision was made in the middle of scalp to expose the skull. GR73632 (0.05–50 pmol in 5  $\mu$ l), an NK<sub>1</sub> agonist, was administered directly into the lateral ventricle by vertical insertion of a cuffed 25-gauge needle to 1-mm lateral and 4.5-mm below bregma. Immediately following the recovery of the righting reflex, the duration of repetitive hind foot tapping was recorded for 5 min in a clear observation box (15.5 cm  $\times$  22 cm  $\times$  12 cm). Foot tapping was defined as rhythmic, repetitive tapping of the hind foot. To examine brain penetration, T-2328 (0.01–0.1 mg/kg) and aprepitant (0.3–3 mg/kg) were peripherally administered via a vein of the penis 0–2 min before the injection of GR73632 under halothane anesthesia. After the measurement, gerbils were sacrificed by an overdose of diethyl ether.

#### *Cisplatin-induced emesis in ferrets*

Two hours before cisplatin treatment, ferrets were transferred to observation cages. Cisplatin was intraperitoneally injected at a dose of 5 mg/kg under halothane anesthesia. T-2328 (0.1 and 0.3 mg/kg) was intravenously administered via the tail vein at 0, 24, and 48 h after cisplatin treatment. Animal behavior was recorded remotely by a video camera (TK-N1100; Victor, Yokohama) equipped to a recording system (HM-DR10000, Victor) for 72 h. Emesis was characterized by rhythmic abdominal contractions that were either associated with oral expulsion of solid or liquid material from the gastrointestinal tract, that is, vomiting, or not associated with the passage of material, that is, retching movements (12). Each emetic episode was considered separate when the animal changed its location in the observation cage or when the interval between retches and/or vomits exceeded 5 s. Total number of emetic episodes was calculated in each 1-h period during

the experiment. At the end of the experiment, the ferrets were sacrificed by an overdose of diethyl ether.

#### *Chemicals*

T-2328, aprepitant, and SB-223412 were synthesized at Tanabe Seiyaku Co., Ltd. [<sup>3</sup>H][Sar<sup>9</sup>,Met(O<sub>2</sub>)<sup>11</sup>] substance P (47 Ci/mmol) and [<sup>3</sup>H]SR-48968 were purchased from NEN Life Science Products, Inc. L-703,606 oxalate salt (Research Biochemicals International RBI, Natick, MA, USA), neurokinin A (Calbiochem-NoVabiochem Co., Ltd., San Diego, CA, USA), [<sup>3</sup>H]SR-142801 (Amersham Pharmacia Biotech UK, Ltd., Buckinghamshire, England), GR73632 (Research Biochemicals International), cisplatin (cisplatinum(II) diammine dichloride; Sigma-Aldrich, Inc., St. Louis, MO, USA), and other reagents were purchased from commercial sources.

T-2328 was dissolved in DMSO and diluted in assay buffers for the binding experiments. For the in vivo experiments, T-2328 was dissolved in 1% DMSO-saline and aprepitant was dissolved in EtOH/PEG400/H<sub>2</sub>O (1:2:1). They were administered to gerbils and ferrets in the volume of 5 and 2 ml/kg, respectively. Cisplatin was dissolved in saline at 70°C–75°C followed by gradual cooling to 40°C–50°C and intraperitoneally administered in a volume of 5 ml/kg.

#### *Statistical analysis*

The results were expressed as the mean  $\pm$  S.E.M. Statistical analysis was performed by means of ANOVA followed by Dunnett's multiple comparison test. *P* values less than 0.05 were considered as statistically significant.

## **Results**

#### *Binding studies*

Table 1 shows the K<sub>i</sub> of T-2328 and aprepitant to tachykinin receptors. Both T-2328 and aprepitant potently inhibited the binding of [<sup>3</sup>H][Sar<sup>9</sup>,Met(O<sub>2</sub>)<sup>11</sup>] substance P to human tachykinin NK<sub>1</sub> receptors. The

**Table 1.** Inhibition constants (K<sub>i</sub>) of T-2328 and aprepitant in radioligand binding assays for human tachykinin receptors

Drugs	Receptors	K <sub>i</sub> or IC <sub>50</sub> * (nM)	95% Confidence interval (nM)
T-2328	hNK <sub>1</sub>	0.08	0.06–0.09
	hNK <sub>2</sub>	3537	2921–4284
	hNK <sub>3</sub>	309	202–473
Aprepitant	hNK <sub>1</sub>	1.30	1.07–1.57
	hNK <sub>2</sub>	>10000*	
	hNK <sub>3</sub>	1205	756–1922

affinity for T-2328 was about 16 times higher than that for aprepitant. The affinities of T-2328 for human NK<sub>2</sub> and NK<sub>3</sub> receptors were about 44,000 and 3,800 times lower than that for the human NK<sub>1</sub> receptor, respectively. Table 2 shows affinities of T-2328 with 13 kinds of receptors and ion channels. The K<sub>i</sub> values of T-2328 for binding of opiate  $\kappa$  and  $\mu$  receptors were of micromolar ranges and IC<sub>50</sub> values for other receptors and ion channels were over 10  $\mu$ M.

The nature of T-2328 antagonism was studied by the Scatchard analysis of specific binding of [<sup>3</sup>H][Sar<sup>9</sup>,Met(O<sub>2</sub>)<sup>11</sup>]substance P to the human tachykinin NK<sub>1</sub> receptor. As shown in Fig. 2, [<sup>3</sup>H][Sar<sup>9</sup>,Met(O<sub>2</sub>)<sup>11</sup>]substance P-binding was saturable with the K<sub>d</sub> value of 0.35 nM and B<sub>max</sub> of 6.2 fmol/10<sup>6</sup> cells. In the presence of 0.3 nM T-2328, the total number of receptors labeled by [<sup>3</sup>H][Sar<sup>9</sup>,Met(O<sub>2</sub>)<sup>11</sup>]substance P

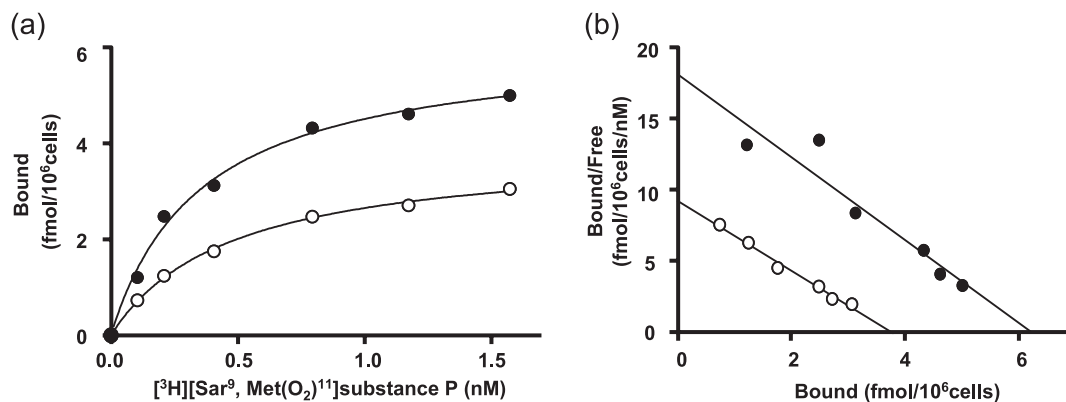
was reduced to 3.8 fmol/10<sup>6</sup> cells without change in the K<sub>d</sub> of 0.41 nM.

#### GR73632-induced foot tapping in gerbils

Intracerebroventricular (i.c.v.) injection of GR73632 (0.05 – 50 pmol) dose-dependently induced foot tapping responses in gerbils with a minimum effective dose of 0.5 pmol (Fig. 3a). The dose of 50 pmol produced constant foot tapping responses during the 5-min observation period. The foot tapping response was not observed following administration of the vehicle. The dose of 5 pmol GR73632 was chosen for antagonism studies as it represented a submaximal dose. T-2328 (0.01 – 0.1 mg/kg) and aprepitant (0.3 – 3 mg/kg) dose-dependently inhibited the foot tapping response (Fig. 3b). Almost complete inhibition was achieved by T-2328 and aprepitant at 0.1 and 3 mg/kg, respectively.

**Table 2.** Affinities for various receptors and ion channels

Receptor	Source	Radioligand	IC <sub>50</sub> or K <sub>i</sub> * ( $\mu$ M)
Dopamine D <sub>1</sub>	human recombinant	[ <sup>3</sup> H]SCH-23390	>10
Dopamine D <sub>2S</sub>	human recombinant	[ <sup>3</sup> H]spiperone	>10
GABA <sub>A</sub> , agonist site	rat brain	[ <sup>3</sup> H]muscimol	>10
GABA <sub>A</sub> , Cl <sup>-</sup> channel	rat cerebral cortex	[ <sup>3</sup> H]TBOB	>10
GABA <sub>B</sub>	rat cerebellum	[ <sup>3</sup> H]GABA	>10
Glu, non-selective	rat brain	[ <sup>3</sup> H]L-Glu	>10
Gly, strychnine-sensitive	rat spinal cord	[ <sup>3</sup> H]strychnine	>10
Histamine H <sub>1</sub>	guinea pig brain	[ <sup>3</sup> H]pyrilamine	>10
Opiate $\kappa$	human recombinant	[ <sup>3</sup> H]diprenorphine	1.89*
Opiate $\mu$	human recombinant	[ <sup>3</sup> H]diprenorphine	3.65*
5-HT <sub>1</sub> , non-selective	rat cerebral cortex	[ <sup>3</sup> H]5-HT	>10
5-HT <sub>3</sub>	human recombinant	[ <sup>3</sup> H]GR-65630	>10
Ca <sup>2+</sup> channel, N-type	rat brain	[ <sup>125</sup> I] $\omega$ -conotoxin GVIA	>10



**Fig. 2.** The nature of T-2328 antagonism. a: Saturation of [<sup>3</sup>H][Sar<sup>9</sup>,Met(O<sub>2</sub>)<sup>11</sup>]substance P binding to NK<sub>1</sub> receptors in human IM9 cells. b: Scatchard analysis of specific [<sup>3</sup>H][Sar<sup>9</sup>,Met(O<sub>2</sub>)<sup>11</sup>]substance P binding in human IM9 cells in the absence or presence of 0.3 nM T-2328. Cells were incubated with increasing concentrations of [<sup>3</sup>H][Sar<sup>9</sup>,Met(O<sub>2</sub>)<sup>11</sup>]substance P in the absence (closed circle) or presence of 0.3 nM T-2328 (open circle).

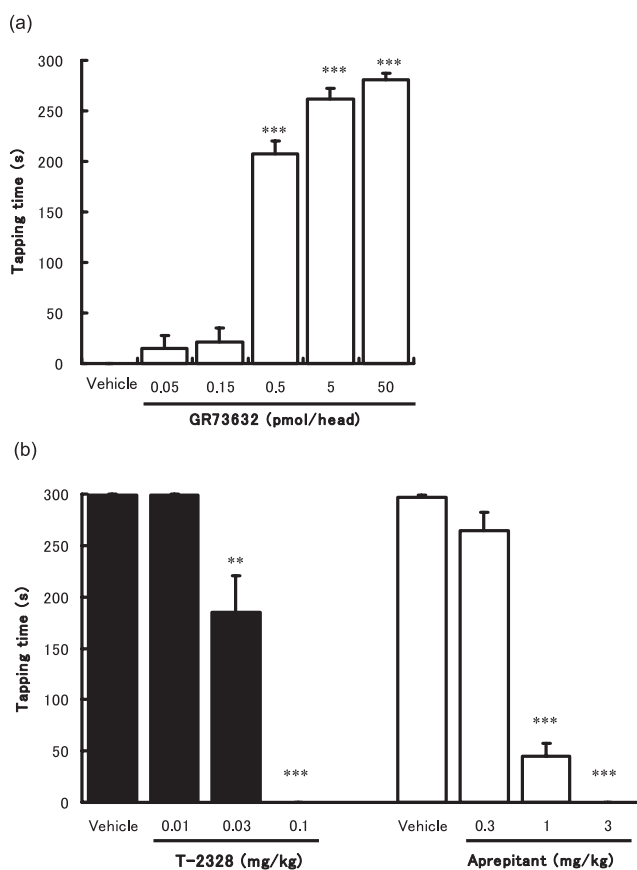
### Cisplatin-induced emesis in ferrets

Cisplatin (5 mg/kg, i.p.) induced emetic responses 24 h after administration, that is, delayed emesis (Fig. 4a). The number of emetic episodes induced by cisplatin was  $4.0 \pm 2.1$  and  $70.3 \pm 9.5$  in the first 24 h and in the next 24–72 h period, respectively. Daily administration of T-2328 at 0.1 and 0.3 mg/kg, i.v. potently inhibited both acute (0–24 h) and delayed (24–72 h) phases of emesis in a dose-dependent manner (Fig. 4: b and c). In the delayed phase of emesis, the total number of emetic episodes with 0.1 and 0.3 mg/kg T-2328 were  $10.5 \pm 3.1$  and  $3.0 \pm 1.2$ , respectively. The reduction of delayed emesis in the T-2328-treated groups was statistically significant ( $P < 0.001$ ).

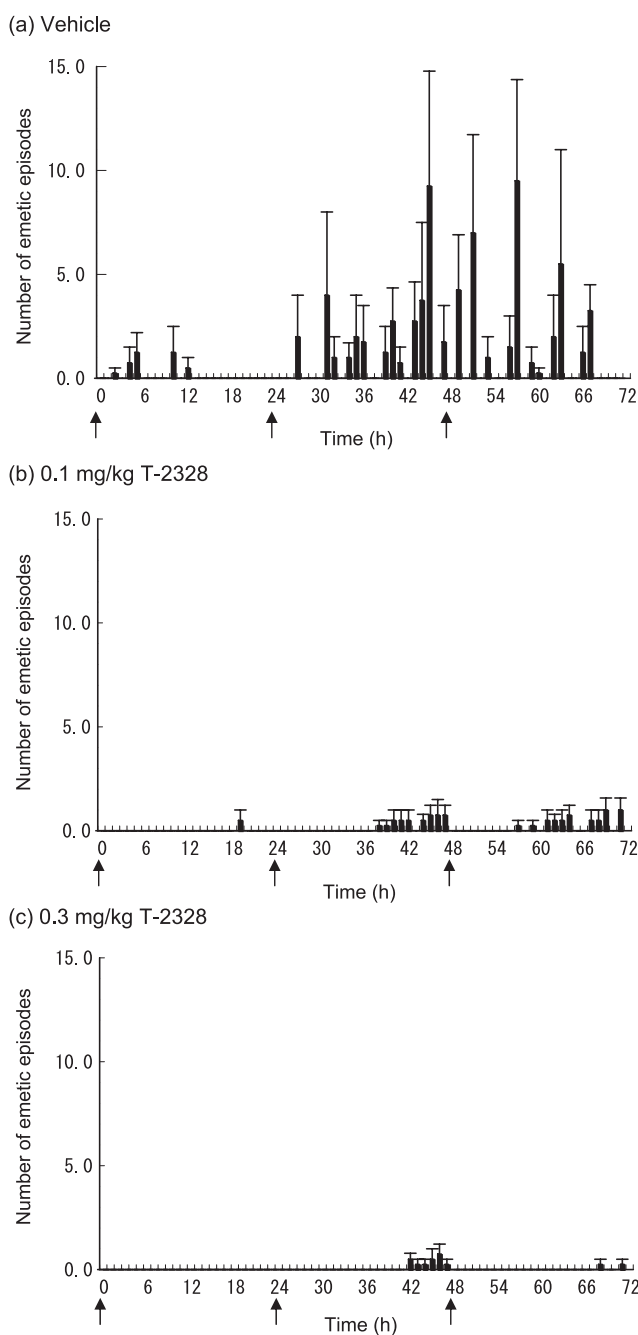
### Discussion

T-2328 potently inhibited the binding of the radioactive ligand to human NK<sub>1</sub> receptors. The  $K_i$  was of a

subnanomolar range and 16 times lower than that of aprepitant. The inhibition of the NK<sub>1</sub> receptor by T-2328 was highly selective because  $K_i$  values for NK<sub>2</sub> and NK<sub>3</sub>



**Fig. 3.** Effects of T-2328 and aprepitant on GR73632-induced foot tapping in gerbils. a: Dose-response to GR73632 following i.c.v. administration. b: Inhibition by T-2328 and aprepitant of GR73632-induced foot tapping in gerbils. Results are shown as the mean  $\pm$  S.E.M. from 5 animals per group. \*\* $P < 0.01$ , \*\*\* $P < 0.001$ , compared with vehicle.



**Fig. 4.** Effect of T-2328 on the cisplatin-induced emesis in ferrets. Vehicle (a) or T-2328 (b and c) was intravenously administered at 0, 24, and 48 h after cisplatin administration. The arrows indicate the administration time of vehicle or T-2328. Results are shown as the mean numbers of emetic episodes ( $\pm$  S.E.M.) in 1 h during the 72-h observation period from 4 animals per group. In the delayed phase of emesis (24–72 h), total number of emetic episodes in each group was  $70.3 \pm 9.5$ ,  $10.5 \pm 3.1$ , and  $3.0 \pm 1.2$ , respectively. The reduction of delayed emesis in both T-2328-treated groups (b and c) was statistically significant ( $P < 0.001$ ).

receptors were >10000-fold and >1000-fold higher, respectively, than that for NK<sub>1</sub> receptors. In contrast, there was no affinity of T-2328 to dopamine (D<sub>1</sub>, D<sub>2S</sub>), histamine (H<sub>1</sub>), 5-HT (5-HT<sub>1</sub> and 5-HT<sub>3</sub>), GABA (GABA<sub>A</sub>, B), glutamate (non-selective), glycine (strychnine-sensitive), and N-type Ca<sup>2+</sup> channel at the concentration of 10  $\mu$ M. Although an interaction was detected for opiate  $\kappa$  and  $\mu$  receptors, these K<sub>i</sub> values were above 1  $\mu$ M. The present study shows that T-2328 is a novel, selective and potent nonpeptide antagonist of the tachykinin NK<sub>1</sub> receptor.

Most peptide antagonists of NK<sub>1</sub> receptors display competitive inhibition of the binding of a specific ligand. However, it is difficult to predict the manner of antagonism from the chemical structure of non-peptide antagonists. For example, some non-peptide antagonists including CP-122,721 (13) and SDZ NKT 343 (14) are non-competitive antagonists, whereas CP-99,994, an analogue of CP-122,721, is a competitive inhibitor (15). In the present study, the B<sub>max</sub> for saturation binding studies of the ligand to IM9 cells was suppressed by 0.3 nM T-2328 without any change in K<sub>d</sub>. This result indicates that T-2328 interacts with the NK<sub>1</sub> receptor in a manner that is apparently noncompetitive.

Because the brain NK<sub>1</sub> receptor is likely to be involved in symptoms of various CNS diseases, we examined the potential of T-2328 as a central acting NK<sub>1</sub> antagonist. I.c.v. administration of an NK<sub>1</sub>-receptor agonist induces a characteristic foot tapping response in gerbils (16, 17). Since this readily quantifiable response is inhibited by brain-penetrating NK<sub>1</sub> antagonists (17) but not by peptide antagonists with systemic administration, this model measures activation of brain NK<sub>1</sub>-receptor activity. Inhibition of central NK<sub>1</sub> receptors by T-2328 was thus assessed in NK<sub>1</sub>-agonist-induced foot tapping of gerbils. The foot tapping induced by GR73632 (i.c.v.) was potently antagonized by systemic administration of T-2328 with 30 times lower dosage than aprepitant. It is suggested that T-2328 penetrates into the brain by peripheral administration and antagonizes central NK<sub>1</sub> receptors.

Long-lasting emesis is a major burden during chemotherapy in cancer treatment (18). Chemotherapy-induced emesis seems to consist of acute (up to 24 h) and delayed (24 h and after) phases in both humans and animals. While the acute phase emesis responds to 5-HT<sub>3</sub> antagonists, the delayed phase remains difficult to control (19). Involvement of substance P and NK<sub>1</sub> has been suggested in the generation of delayed emesis following the treatment with chemotherapeutic agents (20). Recent studies further demonstrated the suppression of delayed emesis by aprepitant in both humans and animals (4, 5, 10), although brain-nonpenetrating NK<sub>1</sub> antagonists are

ineffective (21). Cisplatin induced long-lasting emesis in ferrets for 72 h. T-2328 inhibited the cisplatin-induced delayed emesis by i.v. treatment at doses equivalent to inhibition of foot tapping in gerbils. While the acute phase emesis may be initiated by activation of 5HT<sub>3</sub> receptors in sensory nerves (19), T-2328 was inactive against the 5-HT<sub>3</sub> receptor. It is thus suggested that T-2328 exerts the antiemetic effect through acting on the brain NK<sub>1</sub> receptor.

We examined the anti-emetic effect of T-2328 in cisplatin-induced emesis by intravenous administration. Many patients undergoing chemotherapy have severe oral mucositis that prevents the administration of oral medication because they have difficulty in swallowing and are unable to drink (22). Since aprepitant is available only with an oral formulation, injectable drug is a useful tool for treatment of chemotherapy-induced emesis (23).

In summary, the present study demonstrates that human NK<sub>1</sub> receptors are inhibited by T-2328 with high affinity and selectivity in a non-competitive manner. Brain NK<sub>1</sub> receptors were antagonized and the cisplatin-induced emesis was controlled by T-2328 with systemic administration. Therefore, T-2328 is a novel potential drug for treatment of diseases in which blockade of central NK<sub>1</sub> receptors is of therapeutic relevance including chemotherapy-induced emesis.

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