The antiemetic properties of a novel neurokinin-1 (NK₁) receptor antagonist, FK886 ([3,5-bis(trifluoromethyl)phenyl][2R]-2-(3-hydroxy-4-methylbenzyl)-4-[2-[25]-2-(methoxymethyl)morpholin-4-yl]ethyl)piperazin-1-yl)methanone dihydrochloride), were studied in dog models of cisplatin- and apomorphine-induced emesis. Intravenously administered FK886 (0.32–1 mg/kg) significantly inhibited cisplatin-induced acute emesis during the 5-h observation period. Nearly complete inhibition was observed at 1 mg/kg. At an equivalent dose range, orally administered FK886 also significantly inhibited emesis, indicating good oral absorption. Similarly, FK886 inhibited apomorphine-induced emetic responses effectively following both intravenous and oral administration. The effects were long lasting, with 1.6 mg/kg of FK886 completely blocking apomorphine-induced retching and vomiting after a 12-h pretreatment period. Furthermore, FK886 showed fast onset of antiemetic activity after oral administration. At doses of 0.32 mg/kg or more, a pre-treatment period of 0.5 h was sufficient for complete inhibition of apomorphine-induced emetic responses. This fast onset after oral administration was supported by pharmacokinetic data, which demonstrated plasma levels of FK886 after oral administration reached levels similar to those 30 min after intravenous administration. These results suggest that FK886 has excellent antiemetic properties in dogs, and that its rapid-onset and long-lasting properties might make it a promising antiemetic agent.

Key words

FK886; neurokinin-1 antagonist; emesis; cisplatin; apomorphine; dog

MATERIALS AND METHODS

Animals

Beagle dogs of either sex, weighing 7.3–9.5 kg (Kitayama Labes, Nagano, Japan) were used. The dogs were individually housed in temperature- and humidity-controlled rooms with a 12:12 h light/dark cycle (lights on 07:00–19:00 h), routinely fed with dry pellet food (TC-1; Maruha Pet Food, Tokyo, Japan), and had water available ad libitum. They were allowed to acclimatize for at least 1 week before the experiments. Before an experiment, the dogs were deprived of food but not water for 24 h. On the day of the experiment, they were transferred to observation cages in a quiet room. Emetic was characterized by rhythmic abdominal contractions that were associated with (vomiting) or without (retching) oral expulsion of materials from the gastrointestinal tract. If episodes of vomiting or retching were separated by 1 min, they were considered separate episodes. In dogs completely protected from emetic responses, the latency period was taken as equal to the observation period. All animal experimental procedures were approved by the Institutional Animal Care and Use Committee of Astellas Development Center (Ibaraki, Japan).
Animal Care and Use Committee of Astellas Pharma Inc.

**Cisplatin-Induced Acute Emesis** Dogs were randomly assigned to different treatment groups and treated with intravenous (i.v.) injection of cisplatin (3.2 mg/kg). They were observed for the onset of vomiting (latency period) and the number of vomits for 5 h from cisplatin injection. Each animal received i.v. or oral (per os (p.o.)) FK886 (0.1–1 mg/kg), or a corresponding volume of vehicle just prior to the cisplatin injection. In another group, the effect of a 5-HT3 antagonist, granisetron, was studied. Because of its relatively short duration of action, granisetron (0.001–0.1 mg/kg, i.v.) was administered at 30 min before and 90 min after the cisplatin injection.

**Apomorphine-Induced Emetic Responses** In this study, we assessed the relationship between the dose and the duration of action of FK886. We employed a pre-post study design, because the emetic responses induced by apomorphine vary greatly between dogs, hampering efforts to determine efficacy when using a parallel study design with only a small number of animals in each group. Reproducibility of the apomorphine-induced emetic responses after a two-week recovery period in animals in each group was omitted to minimize the number of animals used in the experiments.

Apomorphine at 0.1 mg/kg was administered subcutaneously (s.c.) to induce emetic responses. The number of retches and vomits over a 60-min observation period were counted (first round). Two weeks later, the emesis study (second round) was performed similarly to the first round. In this round, dogs received i.v. or p.o. FK886 (0.032–1.6 mg/kg, n=2) at various pretreatment intervals prior to the apomorphine injection to assess the duration of action. In another group (n=2), the effect of granisetron was studied. Granisetron has been reported ineffective against apomorphine-induced emesis. Thus, a dose of 1 mg/kg, which is 10 times higher than that which completely inhibits cisplatin-induced acute emesis, was injected i.v. 5 min before the apomorphine injection. Administration conditions that protected at least one of the two dogs from emetic responses were regarded as effective.

**Pharmacokinetics of FK886** Male beagle dogs were deprived of food but not water overnight and received FK886 i.v. or p.o. at a dose of 1 mg/kg (n=3). Water and food were re-supplied 4 and 8 h after FK886 administration, respectively. Plasma samples were assayed for parent drug after liquid–liquid extraction. A reversed-phase high performance liquid chromatography with tandem mass spectrometry (HPLC/MS/MS) was used to determine FK886. The lower limit of quantification was 0.5 ng/mL. Pharmacokinetics parameters, were calculated by non-compartmental analysis using the software MOMENT(Excel).23)

**Chemicals** FK886 and granisetron hydrochloride were synthesized at the Chemistry Research Laboratories of Astellas Pharma Inc. Cisplatin and apomorphine hydrochloride were obtained from Sigma-Aldrich (St. Louis, MO, U.S.A.). For i.v. administration, FK886, granisetron or apomorphine were dissolved in saline and administered at a volume of 0.5 mL/kg. For p.o. administration, FK886 was dissolved in distilled water and administered at a volume of 1 mL/kg. Cisplatin was prepared in prewarmed (70°C) saline followed by gradual cooling to 40°C and immediately administered i.v. at a volume of 1 mL/kg.

**Data Analysis** Data were expressed as the mean±S.E.M. unless otherwise noted. Statistically significant differences in the latency and number of vomits between control and compound-treated animals were determined with one-way analysis of variance followed by Dunnett’s multiple comparison test. p Values less than 0.05 were regarded as significant.

**RESULTS**

**Cisplatin-Induced Acute Emesis** Cisplatin administered at a dose of 3.2 mg/kg i.v. induced a reproducible and characteristic emetic response in dogs. The responses occurred within 88.8±4.6 min (range 65–102 min) and included 14.0±1.1 (range 11–19) vomits during the 5-h observation period in vehicle-control dogs (n=8).

When FK886 (0.1–1 mg/kg) was injected i.v. just prior to

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**Table 1. Effects of Intravenously Administered FK886 on Cisplatin-Induced Emesis in Dogs**

<table>
<thead>
<tr>
<th>Treatment (mg/kg)</th>
<th>Protected/Tested</th>
<th>Vomits</th>
<th>% Inhibition</th>
<th>Latency (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vehicle</td>
<td>0/4</td>
<td>13.8±1.0</td>
<td>0</td>
<td>85.3±6.9</td>
</tr>
<tr>
<td>0.1</td>
<td>0/4</td>
<td>9.8±3.0</td>
<td>29</td>
<td>107.3±6.2</td>
</tr>
<tr>
<td>0.32</td>
<td>1/4</td>
<td>3.0±1.7**</td>
<td>78</td>
<td>175.8±41.8</td>
</tr>
<tr>
<td>1</td>
<td>2/4</td>
<td>0.5±0.3**</td>
<td>96</td>
<td>236.3±36.9**</td>
</tr>
</tbody>
</table>

Values are mean±S.E.M. **p<0.01 vs. control group as calculated by Dunnett’s multiple comparison test. FK886 was given intravenously just prior to cisplatin (3.2 mg/kg, i.v.) administration. The number of vomits and the latency to the first vomit were determined for a 5-h observation period following cisplatin administration.

**Table 2. Effects of Orally Administered FK886 on Cisplatin-Induced Emesis in Dogs**

<table>
<thead>
<tr>
<th>Treatment (mg/kg)</th>
<th>Protected/Tested</th>
<th>Vomits</th>
<th>% Inhibition</th>
<th>Latency (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vehicle</td>
<td>0/4</td>
<td>14.3±1.7</td>
<td>0</td>
<td>92.3±6.6</td>
</tr>
<tr>
<td>0.1</td>
<td>0/4</td>
<td>12.3±1.4</td>
<td>14</td>
<td>84.8±3.2</td>
</tr>
<tr>
<td>0.32</td>
<td>0/4</td>
<td>5.5±1.2**</td>
<td>62</td>
<td>97.3±9.5</td>
</tr>
<tr>
<td>1</td>
<td>2/4</td>
<td>0.8±0.5**</td>
<td>94</td>
<td>223.3±44.8**</td>
</tr>
</tbody>
</table>

Values are mean±S.E.M. **p<0.01 vs. control group as calculated by Dunnett’s multiple comparison test. FK886 was given orally just prior to cisplatin (3.2 mg/kg, i.v.) administration. The number of vomits and the latency to the first vomit were determined for a 5-h observation period following cisplatin administration.
cisplatin administration, there was a dose-dependent decrease in the number of vomits, with this decrease being significant at doses of 0.32 mg/kg and higher (Table 1). One of the four dogs treated with 0.32 mg/kg and two dogs treated with 1 mg/kg were completely protected from emesis. There was a significant increase in latency until the first vomit at 1 mg/kg. Oral FK886 (0.32–1 mg/kg) given immediately before cisplatin also produced a dose-dependent and significant decrease in the number of vomits (Table 2). Two of the four dogs in the 1 mg/kg treatment group were completely protected from emesis. A significant increase in the latency period was observed at 1 mg/kg.

The administration of a 5-HT3 receptor antagonist, granisetron (2×0.01–0.1 mg/kg, i.v.), dose-dependently and significantly decreased the number of vomits and prolonged the latency in cisplatin-induced acute emesis (Table 3). All dogs in the 2×0.1 mg/kg treatment group were completely protected from emesis.

The latency periods in dogs that were not completely protected from emesis by FK886 or granisetron were further plotted against the number of vomits. The relationship was evaluated by the Pearson product-moment correlation coefficient, which showed that the latency period negatively and significantly correlated with the number of vomits in both in FK886- (r = −0.648, p < 0.01; Fig. 1A) and granisetron-treated (r = −0.942, p < 0.01; Fig. 1B) animals.

**Apomorphine-Induced Emetic Responses**

The effects of i.v. or p.o. administered FK886 were also evaluated for apomorphine-induced emetic responses. In the present study, we assessed the relationship between the dose and duration of action of FK886. Apomorphine (0.1 mg/kg, s.c.) evoked emesis in all dogs (n=32) in the first (control) round that was characterized by an onset within 4.5±0.3 min of delivery (range 2.5–8.3 min) and 75.7±4.8 (range 43–143) retches and vomits.

![Fig. 1](image-url)  
**Fig. 1.** Relationship between the Number of Vomits and the Latency in Cisplatin-Induced Emesis in Dogs That Were Not Completely Protected from Emesis by FK886 (A) or Granisetron (B)

### Table 3. Effects of Intravenously Administered Granisetron on Cisplatin-Induced Emesis in Dogs

<table>
<thead>
<tr>
<th>Treatment (mg/kg)</th>
<th>Protected/Tested</th>
<th>Vomits (mean±S.E.M.)</th>
<th>% Inhibition</th>
<th>Latency (min) ± S.E.M.</th>
</tr>
</thead>
<tbody>
<tr>
<td>2×Vehicle</td>
<td>0/5</td>
<td>18.0±2.5</td>
<td>0</td>
<td>103.8±5.2</td>
</tr>
<tr>
<td>2×0.001</td>
<td>0/3</td>
<td>10.7±0.9</td>
<td>44</td>
<td>121.0±5.5</td>
</tr>
<tr>
<td>2×0.01</td>
<td>0/3</td>
<td>7.0±0.6*</td>
<td>61</td>
<td>161.0±8.5***</td>
</tr>
<tr>
<td>2×0.1</td>
<td>2/2</td>
<td>0±0**</td>
<td>100</td>
<td>300±0***</td>
</tr>
</tbody>
</table>

*Values are mean±S.E.M. *p<0.05, **p<0.01, ***p<0.001 vs. control group as calculated by Dunnett’s multiple comparison test. Granisetron was given intravenously 30 min before and 90 min after cisplatin (3.2 mg/kg, i.v.) administration. The number of vomits and the latency to the first vomit were determined for a 5-h observation period following cisplatin administration.

### Table 4. Effects of Intravenously Administered FK886 and Granisetron on Apomorphine-Induced Emetic Responses in Dogs

<table>
<thead>
<tr>
<th>Treatment (mg/kg)</th>
<th>Pretreatment interval (h)</th>
<th>Control Protected/Tested</th>
<th>Mean episodes</th>
<th>Treated Protected/Tested</th>
<th>Mean episodes</th>
</tr>
</thead>
<tbody>
<tr>
<td>FK886</td>
<td></td>
<td>0.083</td>
<td>0/2</td>
<td>85.5</td>
<td>0/2</td>
</tr>
<tr>
<td>0.1</td>
<td>2</td>
<td>0/2</td>
<td>58.0</td>
<td>1/2</td>
<td>4.5</td>
</tr>
<tr>
<td>0.1</td>
<td>4</td>
<td>0/2</td>
<td>63.0</td>
<td>0/2</td>
<td>31.5</td>
</tr>
<tr>
<td>0.32</td>
<td>4</td>
<td>0/2</td>
<td>90.5</td>
<td>1/2</td>
<td>15.0</td>
</tr>
<tr>
<td>0.32</td>
<td>8</td>
<td>0/2</td>
<td>85.5</td>
<td>0/2</td>
<td>20.0</td>
</tr>
<tr>
<td>0.32</td>
<td>12</td>
<td>0/2</td>
<td>83.5</td>
<td>0/2</td>
<td>45.5</td>
</tr>
<tr>
<td>Granisetron</td>
<td>1</td>
<td>0.083</td>
<td>0/2</td>
<td>71.0</td>
<td>0/2</td>
</tr>
</tbody>
</table>

*Values are mean±S.E.M. Test compounds were given intravenously at various pretreatment intervals before apomorphine administration (0.1 mg/kg, s.c.). The number of emetic episodes (retching and vomiting) was determined for a 60-min observation period following apomorphine administration.*
over the 60-min observation period.

Single i.v. injection of FK886 potently prevented the apomorphine-induced emetic responses, with 0.1 mg/kg FK886 administered at 2 h before apomorphine administration inhibiting the number of retches and vomits almost completely (Table 4). The duration of action of FK886 was long, and substantial potency was still retained 4 h after i.v. treatment at 0.32 mg/kg. Like the i.v. injection, p.o. administered FK886 also exerted long-lasting inhibitory activity (Table 5), with a single dose of 1.6 mg/kg completely abolishing emetic responses after a 12-h pretreatment period. In addition to the above long-lasting properties, FK886 also displayed rapid onset after oral administration, with nearly complete protection from emesis provided by administration of 0.32 mg/kg as late as 0.5 h before apomorphine.

Granisetron had no effect on apomorphine-induced emetic responses at a dose of 1 mg/kg, i.v. (Table 4).

Pharmacokinetics of FK886 Plasma concentrations of FK886 after i.v. or p.o. administration at a dose of 1 mg/kg are shown in Fig. 2. After i.v. administration, the plasma concentration declined in a bi-phasic fashion. The pharmacokinetic parameters of FK886 were evaluated by non-compartmental analysis according to the trapezoidal rule. The terminal t_{1/2} was 2.8 h, and total body clearance was 0.378 l/h/kg. When administered p.o., FK886 was absorbed rapidly and reached nearly equal levels as those after i.v. administration at 30 min after the administration. The time required to reach maximum concentration (T_{max}) after p.o. administration was 0.7 h, and the maximum concentration (C_{max}) was 488 ng/ml. The terminal t_{1/2} was 4.3 h, and bioavailability was 81%.

**DISCUSSION**

Even though multiple neurotransmitters are involved in triggering emetic responses, we considered whether NK₁ receptor activation plays a key role, on the basis that NK₁ antagonists possess broad-spectrum anti-emetic activity in experimental animal models. Indeed, we verified that NK₁ receptor antagonists block both cisplatin- and apomorphine-induced emesis, while 5-HT₃ receptor antagonists block cisplatin-induced emesis only.²²,²⁴ The dorsal vagal complex, which includes the area postrema (AP), nucleus tractus solitarii (NTS) and dorsal motor nucleus of the vagus nerve (DMN), and the central pattern generator area in the brainstem have been proposed as key mediators of the emetic reflex, and are known to be heavily innervated by NK₁ receptor-expressing fibers.⁵,¹⁸,¹⁹ Various emetic stimuli, including cisplatin and apomorphine, increase the neuronal activity of this area in various species.²⁵–²⁷ Furthermore, Darmani et al. recently reported that cisplatin causes an over-expression of NK₁ receptor proteins in the brainstem, and that this over-expression is closely associated with the peak immediate- and delayed-phase vomiting frequencies in least shrew (Cryptotis parva).²⁸ Thus, brain penetration is an important determinant of the influence of NK₁ receptor antagonists on the onset and duration of anti-emetic responses.

Intravenously administered FK886 potently and dose-dependently attenuated the number of cisplatin-induced vomits,
reaching near complete inhibition at 1 mg/kg. At an equivalent dose range, orally administered FK886 also potently inhibited the number of cisplatin-induced vomits, indicating good oral absorption. FK886 also inhibits apomorphine-induced emesis effectively. The effect was long lasting, particularly when dosed orally, with a single p.o. administration of FK886 (1.6 mg/kg) producing complete blockade of retching and vomiting up to 13 h after administration. Importantly, FK886 not only has a long-lasting duration of action, it also shows fast onset after p.o. administration. Even after a 0.5-h pretreatment period, FK886 exerts a maximum effect, i.e., nearly complete inhibition of emetic responses, at doses of 0.32 mg/kg or higher.

These excellent anti-emetic properties are supported by pharmacokinetic data that demonstrate that plasma levels of FK886 in dogs, we have previously demonstrated good and quick brain penetration by FK886 in rats; a brain/plasma ratio of 0.87 and dogs, we have previously demonstrated good and quick brain penetration by FK886 in rats; a brain/plasma ratio of 0.87 and dog brain levels of 1412 ng eq/g tissue was observed 5 min after a single i.v. administration of 3.2 mg/kg radiolabeled FK886. Taken together, these findings indicate that FK886 may be efficiently distributed to the brain after p.o. administration and exert anti-emetic activity in dogs. On the other hand, aprepitant, another antiemetic drug, has been reported to be absorbed and distributed to the brain rather slowly after p.o. administration in ferrets; after 1 mg/kg p.o. administration, both plasma and brain T_max were about 10 h and C_max was 353 ng/mL in plasma and 160 ng/g tissue in brain. Aprepitant is prescribed only for the prevention of chemotherapy-induced and postoperative nausea and vomiting. FK886, however, is expected to be used not only as a prophylactic, but also for therapeutic antiemetic treatment due to its rapid-onset and long-lasting properties.

It has been reported that NK_1 receptor antagonists, such as CP-99,994, CP-122,721, aprepitant and GR205171 do not significantly increase the latency of cisplatin-induced acute emesis at doses that do not completely block the emetic responses. In contrast to this, FK886 displayed a dose-dependent and significant increase in latency. Even when the dogs completely protected from emesis were excluded from analysis, the dose-dependency and significance in the prolonged latency was obvious. Furthermore, the latency period in dogs that were not completely protected from emesis by FK886 significantly correlated with the number of vomits. Taken together, the increased latency seems to be a specific response. It is well known that 5-HT_3 antagonism increases latency in cisplatin-induced emesis models. However, FK886 has no appreciable affinity (IC_{50}>1 μM) for the [^{3}H]serotonin binding site in rat striatum or for another 53 sites including receptors, ion channels and transporters. Thus, the prolonged latency by FK886 does not appear to be explained by 5-HT_3 receptor antagonism or blockade of non-NK_1 receptors. Further study is necessary to clarify this point.

In conclusion, the present results suggest that FK886 is an effective antiemetic in dogs. FK886 exerted excellent activity following i.v. and p.o. administration against cisplatin- and apomorphine-induced emetic responses. These results agree with previous studies on centrally active NK_1 receptor antagonists, and suggest that FK886 has the potential to block a broad spectrum of emesis. In addition, its rapid-onset and long-lasting properties are attractive features that make FK886 a potential antiemetic agent.

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**REFERENCES**


