Effect of Mosapride on Prednisolone-Induced Gastric Mucosal Injury and Gastric-Emptying Disorder in Dog

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ABSTRACT. Previous report demonstrated that prokinetic agent mosapride has anti-ulcerogenic action in rat-indomethacin gastric mucosal injury model. Here, we assessed the prophylactic effect of mosapride on gastric mucosal injury and emptying disorder induced by prednisolone in dogs. Crossover study design was employed. Six healthy beagles were administered prednisolone alone (2 mg/kg, twice a day [BID] subcutaneously) and prednisolone with mosapride (1 mg/kg, BID, orally), followed by an interval of at least 6 weeks. In each treatment, gastric mucosal injury was scored endoscopically according to the modified Lanza scale, and gastric emptying was assessed with 13C-octanoic acid breath test. The incidence of gastrointestinal adverse events was also investigated. Coadministration of mosapride with prednisolone significantly (P<0.05) reduced the gastric mucosal injury score (mean ± SD, 17.67 ± 6.96), compared with that of prednisolone treatment alone (25.50 ± 13.03). Prednisolone treatment delayed the half-emptying time (184 ± 45 min) compared with that of controls (137 ± 19 min), and coadministration of mosapride improved this gastric-emptying delay (143 ± 29 min). Furthermore, the incidence of the gastrointestinal adverse event vomiting became less frequent upon coadministration with mosapride. In addition to its prokinetic action, our study suggests that mosapride has an anti-ulcerogenic action in dogs. The use of mosapride in combination with prednisolone is effective for attenuating prednisolone-induced gastrointestinal adverse events.

KEY WORDS: 5-HT4R agonist, corticosteroid, gastric ulcer, gastrointestinal adverse event, prokinetic agent.

The corticosteroid prednisolone has been widely used for treating immune-mediated diseases such as immune-mediated hemolytic anemia, idiopathic polyarthritis, and atopic dermatitis [2, 18, 19]. However, this drug may cause gastric mucosal injury when administered at high doses [22]. Moreover, a previous study indicated that prednisolone can alter gastrointestinal motility [21, 22]. Mosapride, a selective 5-hydroxytryptamine-4 receptor (5-HT4R) agonist, promotes the release of acetylcholine in enteric nerves by activating 5-HT4R and thereby enhancing gastrointestinal motility [5, 17]. Since cisapride was withdrawn from the market for its cardiac arrhythmia side effect, mosapride has been used as a prokinetic agent for the treatment of dyspeptic symptoms of gastrointestinal disorders such as chronic gastritis, functional dyspepsia, irritable bowel syndrome, and gastric esophageal reflux disease [5, 17]. Mosapride also enhances upper gastrointestinal motility in dogs [29, 33] and has been approved as a prokinetic agent for canines in Japan. We have previously demonstrated that mosapride is effective for treating vincristine-induced gastrectic motility disorder in dogs [28]. In addition to its prokinetic action, recent findings suggest that mosapride mediates novel actions through 5-HT4R. Fujisawa et al. [7] demonstrated that mosapride attenuated gastric mucosal damage in a rat indomethacin gastric mucosal injury model. They demonstrated that this anti-ulcerogenic action of mosapride was mediated through cholinergic anti-inflammatory pathway; mosapride activates acetylcholine release from the enteric nervous system by 5-HT4R activation, which finally acts on alpha7 nicotinic acetylcholine receptor (α7nAChR) expressed on immune cells [4, 7]. Their report suggests that mosapride can be used as an anti-ulcerogenic agent for the prevention of ulcers caused by drugs such as nonsteroidal anti-inflammatory drugs (NSAIDs) or corticosteroids. However, this action of mosapride has not yet been investigated in larger species, and its clinical significance has not been clarified. The effect of mosapride on gastrointestinal clinical symptoms, as well as on gastric mucosal injury, has also not been investigated yet.

In this study, we evaluated the prophylactic action of mosapride on prednisolone-induced gastric mucosal injury and motility disorder in dog.

MATERIALS AND METHODS

Animals: Six healthy beagles were used in this study (1 male, 5 females). The age of the dogs ranged from 4 to 5
years (median, 4.8 years), and their body weights ranged from 8.7 to 13.7 kg (median, 12.0 kg). The dogs were acclimatized for at least 1 month before the experiment. We confirmed the absence of gastrointestinal clinical signs, and physical examinations showed no abnormalities. Absence of any abnormality in the blood test results (complete blood count, neutrophil count, blood urea nitrogen, creatinine, alkaline phosphatase, and alanine aminotransferase) was also confirmed. Experiments and animal care procedures were approved by the Animal Use and Care Committee of the University of Tokyo.

**Study procedure:** This study was carried out with open-label crossover study design. The 6 dogs were randomized into one of 2 treatment groups (group A or B). Group A firstly received prednisolone alone (2 mg/kg, BID, SC, PREDNISOLONE INJECTION SOLUTION KS®; Kyoritsu Seiaku Corporation, Tokyo, Japan) for 3 days. After at least 6 weeks-washout period, the dogs received the same dose of prednisolone with mosapride (1 mg/kg, BID, PO) for 3 days. Group B received the prednisolone and mosapride 1st, and after the washout period, prednisolone alone was administered. Mosapride powder was diluted in 15 ml of distilled water, and the solution was administered PO. After each 3-day medication, gastric emptying, gastric mucosal injury, and gastrointestinal adverse events were assessed in both treatments. Gastric mucosal injury was assessed with gastroscopy, and gastric emptying was evaluated with the 13C-octanoic acid breath test. Before the medication study, the baseline gastric-emptying time was determined. Gastroscopy was performed at pretreatment and 2 weeks before the 2nd medication to confirm the absence of gastric lesions.

**Evaluation of gastric mucosal injury:** Gastric mucosal injury was evaluated by gastroscopy according to the modified Lanza scale—a systematic scoring system for the severity of gastric lesions [11, 14, 30]. After an overnight fast, an intravenous catheter was placed, and anesthesia was induced with propofol and maintained with isoflurane. Dogs were intubated with a plastic mask, and the expired air from dogs was collected through the mask. Breath samples were collected before giving the test meal (baseline), every 15 min after feeding for 4 hr and every 30 min for an additional 2 hr. The 13CO2 rate was analyzed using a 13CO2-infrared spectrophotometry analyzer (POC One, Otsuka Pharmaceutical Co., Ltd.). The amount of 13C in the samples was expressed as the change (Δ13CO2, %) in the 13CO2/12CO2 ratio before and after feeding of the test meal. Resting 13CO2 production was assumed stable at 0.194 l (m2·min) [31]. The body surface area (m2) was computed using the following formula: 10.1 × body weight (g)2/3/10,000 [31]. The 13C excretion rate (13C%/dose/hr) was calculated according to a previous report [8] by fitting the following formula: y=a+be−(ct+dt2) (y, %dose/hr; t, time; a, b, and c, constants; e, exponential). The cumulative 13C recovery in the breath (C%D%) was determined using the following formula: %D=m(1−e−kβ/t) (t, time; k and β, constants). The lag phase (t_lag) and half-emptying time (t_1/2) were used as gastric-emptying parameters in the present study and were calculated using the following formulae: t_lag=b/c and t_1/2=(−1/k) × ln (1−2e−βt). The parameter t_lag reflects the time to maximum emptying speed of the substrate, and t_1/2 indicates the estimated time during which half of the total 13CO2 is excreted. These gastric-emptying parameters were calculated using the Excel software program.

**Assessment of gastrointestinal clinical symptoms:** The incidence of gastrointestinal adverse events was assessed in the 6 dogs during each 3-day medication period. The gastrointestinal clinical symptoms investigated in the present study included anorexia, vomiting, diarrhea, and hematochezia. A veterinarian carefully observed the dogs during the medication period.

**Expression of α7nAChR mRNA:** The gastric mucosal samples were endoscopically obtained from the gastric ulcer lesions in 3 dogs treated with prednisolone alone. Total RNA was extracted with a commercially available kit (RNeasy Mini RNA Isolation Kit; GE Healthcare UK Ltd., Buckinghamshire, UK) according to the manufacturer’s manual. Reverse transcription was performed using a Prime Script RT Reagent Kit (Takara Bio Inc., Shiga, Japan). The PCR amplification was performed using a 13C-octanoic acid breath test, as described in previous reports [31]. The dogs were fasted at least 12 hr before performing the breath test. A breath bag (Otsuka Pharmaceutical Co., Ltd., Tokyo, Japan) was connected to a plastic mask, and the expired air from dogs was collected through the mask. Breath samples were collected before giving the test meal (baseline), every 15 min after feeding for 4 hr and every 30 min for an additional 2 hr. The 13CO2 rate was analyzed using a 13CO2-infrared spectrophotometry analyzer (POC One, Otsuka Pharmaceutical Co., Ltd.). The amount of 13C in the samples was expressed as the change (Δ13CO2, %) in the 13CO2/12CO2 ratio before and after feeding of the test meal. Resting 13CO2 production was assumed stable at 0.194 l (m2·min) [31]. The body surface area (m2) was computed using the following formula: 10.1 × body weight (g)2/3/10,000 [31]. The 13C excretion rate (13C%/dose/hr) was calculated according to a previous report [8] by fitting the following formula: y=a+be−(ct+dt2) (y, %dose/hr; t, time; a, b, and c, constants; e, exponential). The cumulative 13C recovery in the breath (C%D%) was determined using the following formula: %D=m(1−e−kβ/t) (t, time; k and β, constants). The lag phase (t_lag) and half-emptying time (t_1/2) were used as gastric-emptying parameters in the present study and were calculated using the following formulae: t_lag=b/c and t_1/2=(−1/k) × ln (1−2e−βt). The parameter t_lag reflects the time to maximum emptying speed of the substrate, and t_1/2 indicates the estimated time during which half of the total 13CO2 is excreted. These gastric-emptying parameters were calculated using the Excel software program.

<table>
<thead>
<tr>
<th>Table 1. Criteria of the gastric mucosal injury scoring based on the modified Lanza scale [29]</th>
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<td>Score</td>
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<td>1</td>
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Table 2. Gastric mucosal injury scores in each treatment

<table>
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<tr>
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<th>Prednisolone</th>
<th>Prednisolone + Mosapride</th>
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<tbody>
<tr>
<td>Total score*</td>
<td>25.50 ± 13.03</td>
<td>17.67 ± 6.96</td>
</tr>
<tr>
<td>Cardia</td>
<td>5.50 ± 3.95</td>
<td>3.66 ± 2.27</td>
</tr>
<tr>
<td>Body*</td>
<td>7.17 ± 4.38</td>
<td>3.83 ± 2.14</td>
</tr>
<tr>
<td>Angularis incisura*</td>
<td>6.33 ± 2.82</td>
<td>3.16 ± 1.60</td>
</tr>
<tr>
<td>Pyloric antrum</td>
<td>6.50 ± 2.76</td>
<td>7.00 ± 2.65</td>
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Data are expressed as mean ± SD. *P<0.05.

Table 3. Incidence of each gastrointestinal clinical symptom in the 6 dogs in each treatment

<table>
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<tr>
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<th>Prednisolone</th>
<th>Prednisolone + Mosapride</th>
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<tbody>
<tr>
<td>Anorexia</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Vomiting*</td>
<td>5</td>
<td>1</td>
</tr>
<tr>
<td>Diarrhea</td>
<td>0</td>
<td>0</td>
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<tr>
<td>Hematochezia</td>
<td>0</td>
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*P<0.05.

RESULTS

During the study period, none of the dogs displayed fatal events, and any gastric mucosal injury induced by the 1st treatment was healed before the 2nd treatment. The typical endoscopic findings of the gastric mucosa after prednisolone treatment in the dogs are shown in Fig. 1A. When prednisolone alone was administered, multiple erosions were observed in 5 of the 6 dogs, and 3 had more than 1 ulcer lesion. The remaining dog had no erosion or ulcer lesions, but showed multiple hemorrhages in the gastric body, angularis incisura, and pyloric antrum. Histopathological findings of biopsy samples from the erosion and ulcer sites revealed defects in mucosal epithelia with mucosal hemorrhage and infiltration of neutrophils and mononuclear cells (Fig. 1B). In the ulcer tissues, mRNA expression of α7nAChR was detected (Fig. 2).

The gastric mucosal injury scores in each treatment and in each region are shown in Table 2. The mean ± SD value of the total gastric lesion score in 6 dogs after prednisolone treatment was 25.50 ± 13.03 (range, 13–36). No significant difference was found in gastric mucosal lesion scores among the cardia (5.50 ± 3.95), gastric body (7.17 ± 4.38), angularis incisura (6.33 ± 2.82), and pyloric antrum (6.50 ± 2.76), and pyloric antrum (6.50 ± 2.76) regions after prednisolone treatment. When mosapride was coadministered with prednisolone, the mean ± SD value of total gastric lesion score was 17.67 ± 6.96 (range, 14–20). Treatment with mosapride significantly decreased the total gastric lesion score compared with that of prednisolone treatment alone. The attenuation of gastric lesions by mosapride administration was especially marked in the gastric body (Fig. 3) and angularis incisura.

The results of gastric-emptying analysis in each treatment are shown in Fig. 4. The mean ± SD values of tlag (min) and t1/2 (min) in the 6 dogs were 98 ± 20 and 137 ± 19 in the control, 132 ± 28 and 184 ± 45 in prednisolone treatment, and 100 ± 18 and 143 ± 29 in prednisolone with mosapride treatment, respectively. When prednisolone alone was administered, there was a significant increase in tlag and t1/2, compared with that of the control. Mosapride treatment significantly decreased tlag and t1/2 compared with prednisolone treatment, and no significant difference was observed with that of the control.

The incidence of each gastrointestinal clinical symptom in each treatment is shown in Table 3. When prednisolone alone was administered, vomiting was observed in 5 of the 6 dogs at least once during the medication period. In contrast, only 1 dog had vomiting when mosapride was coadministered with prednisolone. Anorexia, diarrhea, and hematochezia were not observed in either treatment. The difference in the occurrence of vomiting between the treatments was statistically significant.

DISCUSSION

We have shown that prednisolone induces prominent gastric mucosal injury, which can be attenuated by coadministration with mosapride. A deficiency of endogenous prostaglandins has been reported to be associated with corticosteroid-induced gastric mucosal injuries [16]. Prostaglandin inhibition causes mucosal ischemia, gastric acid and mucus imbalance, and HCO3− diffusion, which lead to local gastric mucosal necrosis [3, 13, 15, 16, 25]. In necrotic tissues, endothelial and infiltrating mononuclear cells produce pro-inflammatory cytokines and activate local fibroblasts or neutrophils. This inflammatory process has a role in ulcer formation and healing [1, 9, 26, 27]. A previous study demonstrated that the expression of inflammatory cytokines is elevated in aspirin-induced gastritis in humans [10].

According to previous reports, the attenuation of gastric...
Fig. 1. Endoscopic and histopathological findings of the gastric mucosa after prednisolone treatment in a dog. (A) Endoscopic view of the stomach (cardia, angularis incisura, and pyloric antrum) after prednisolone treatment in a dog. In the cardia and pyloric antrum regions, multiple erosions (arrow) with bleeding were observed. An ulcer (arrowhead) was found in the angularis incisura. (B) Microscopic image of a gastric mucosal erosion site in a dog. A defect of the mucosal epithelia with infiltration of neutrophils and mononuclear cells (arrowheads) was observed.

Fig. 2. Expression of α7nAChR and GAPDH mRNA. (1) Brain. (2-4) Gastric ulcer lesions in 3 dogs.

Fig. 3. Endoscopic findings in the gastric body after prednisolone administration (left) and mosapride citrate coadministration with prednisolone (right) in the same dog. In this dog, multiple ulcers (arrowheads) were observed in the gastric body after prednisolone administration. When mosapride citrate was coadministered, petechial hemorrhages and 1 erosion site (arrow) was observed. However, there was no ulcer lesion in the whole stomach. The gastric mucosal injury scores of the gastric body in each treatment were 10 and 5, respectively.
Mucosal damage by mosapride may be associated with cholinergic receptor stimulation following 5-HT₄R activation. The cholinergic anti-inflammatory pathway has been recently recognized; this pathway modulates inflammation through the cholinergic neuron stimulation by acting on the α7 nicotinic acetylcholine receptor (α7nAChR) expressed in immune cells such as macrophages or neutrophils [4, 20, 23]. A previous study on rodents demonstrated that mosapride attenuated gastric mucosal damage through the acceleration of acetylcholine release, followed by activation of 5-HT₄R, which acts on α7nAChR located in macrophages [7]. Another rodent study reported that activation of α7nAChR ameliorates indomethacin-induced intestinal ulceration by modulating neutrophil activity in mice [12]. We have determined the mRNA expression of α7nAChR in gastric mucosal lesions in dogs treated with prednisolone. However, to definitively confirm the association of α7nAChR with the anti-ulcerogenic action of mosapride in dogs, a further large-group experiment with an α7nAChR agonist and antagonist is needed. Other conventional prokinetic agents have also been reported to have an anti-ulcerogenic action in rat gastric mucosal injury models. In a previous study, the dopamine D₂ receptor antagonists metoclopramide and domperidone ameliorated small intestinal ulcers through cholinergic receptor stimulation followed by dopamine D₂ receptor inhibition in an indomethacin ulcer model [32]. Further studies may be required to assess the anti-ulcerogenic action of conventional prokinetic agents such as metoclopramide in dogs.

In this study, gastric emptying was delayed by prednisolone administration. The pathogenesis of the prednisolone-induced gastric-emptying disorder may be associated with prostaglandin deficiency and gastric mucosal injury. Prostaglandin E₂ inhibition promotes contractile force generation in circular muscles, while the force in longitudinal muscles is suppressed in the stomach [24]. This action may cause gastric motility abnormalities. Gastric mucosal injury may also alter gastric motility. A previous report described gastric hypomotility in human patients with gastric ulcers [6]. Although the pathogenesis of gastric-emptying disorders was not definitively determined, our study indicates that mosapride is effective for the improvement of prednisolone-induced gastric-emptying abnormalities.

The present study demonstrated that the incidence of prednisolone-induced vomiting was attenuated by mosapride coadministration. The pathogenesis of this vomiting may be associated with gastric mucosal injury and the gastric-emptying disorder. The decreased incidence of vomiting in dogs treated with mosapride could be a result of mosapride’s anti-ulcerogenic and prokinetic actions. From the present study, it is indicated that mosapride is effective for the attenuation of prednisolone’s adverse reaction, which will be contribute to the management of prednisolone’s adverse reaction in dog. As anatomical and physiological properties of canine stomach are close to humans, this study may also be served as a preclinical study towards the novel clinical application of mosapride as an anti-ulcerogenic agent for the attenuation of drug adverse reaction of corticosetroids.

In summary, mosapride ameliorates prednisolone-induced gastric mucosal injury and gastric-emptying disorder in dogs. The preventive administration of mosapride reduces the incidence of prednisolone-induced gastrointestinal adverse reaction by prokinetic and anti-ulcerogenic action.

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