Tetrahydroberberine, an Isoquinoline Alkaloid Isolated from Corydalis Tuber, Enhances Gastrointestinal Motor Function

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Received March 22, 2011; accepted June 8, 2011

ABSTRACT

Because delayed gastric emptying and impaired gastric accommodation are regarded as pathophysiological mechanisms underlying functional dyspepsia (FD), prokinetics and fundic relaxants have been suggested as a new treatment for FD. We isolated tetrahydroberberine (THB), an isoquinoline alkaloid (5,8,13,13a-tetrahydro-9,10-dimethoxy-6H-benzo[g]1,3-benzodioxolo[5,6-a]quinoline) from Corydalis tuber, and found that it has micromolar affinity for dopamine D2 (pKᵢ = 6.08) and 5-HT₁A (pKᵢ = 5.38) receptors but moderate to no affinity for other relevant serotonin receptors (i.e., 5-HT₁B, 5-HT₁D, 5-HT₃, and 5-HT₄; pKᵢ < 5.00). Oral administration of THB not only resulted in significantly accelerated gastric emptying of normal rats in a bell-shaped relation-ship, with a maximal efficacy at a dose of 30 μg/kg, but also restored the delayed gastric emptying caused by apomorphine, which might be mediated by an antidopaminergic effect. Data from electromyography indicated enhanced motor function of the upper gastrointestinal tract by THB, which occurred through strengthening contractility and shortening the contraction interval. Furthermore, in rats stressed by repeated restraint, a significantly higher shift in the pressure-volume curve by THB (10 μg/kg, p < 0.05), which was inhibited by [O-methyl-3H]N-[2-(4-(2-methoxyphenyl)-1-piperazinyl)(ethyl)]-N-(2-pyridinyl)cyclohexanecarboxamide trihydrochloride (WAY-100635), a 5-HT₁A antagonist, and N-nitro-L-arginine methyl ester, a nitric-oxide synthase inhibitor but not a vasoactive intestinal peptide antagonist, was observed. Oral administration of THB resulted in a drastic increase of gastric accommodation in Beagle dogs. Area under the volume versus time curve was increased significantly by THB (30 μg/kg, p < 0.01) and comparable with that of sumatriptan (3 mg/kg), a potent fundic relaxant. Taken together, our data suggested that THB, with D₂ receptor antagonist and 5-HT₁A receptor agonist properties, has significant potential as a therapeutic for treatment of FD.

This study was supported by grants from the Plant Diverse Research Center of 21C Frontier R&D Programs, Ministry of Science and Technology [Grants PF06205-01, PF06205-02, PF06205-03]; National Research Foundation of Korea funded by the Government of Korea [Grants 2011-0003580, 2011-0004984]; and Technology Development Program for Agriculture and Forestry, Ministry of Food, Agriculture, Forestry and Fisheries, Republic of Korea.

Article, publication date, and citation information can be found at http://jpet.aspetjournals.org.

doi:10.1124/jpet.111.182048.

Introduction

Functional dyspepsia (FD) is one of the main syndromes associated with gastrointestinal motor dysfunction (Tack, 2007). Delayed gastric emptying and impaired gastric accommodation are known to contribute to clinical manifestations, such as postprandial fullness, early satiation, epigastric pain, and burning sensation (Talley et al., 2006). Currently, prokinetics and fundic relaxants appear to be the drugs of choice for the treatment of FD (Tack, 2008; Brun and Kuo, 2010). Prokinetics stimulate smooth muscle contractions, leading to enhanced gastric emptying and acceleration of both small and large intestinal transit (Karamanolis and Tack, 2006; Tack, 2008). Fundic relaxants target impaired gastric accommodation of the upper gastrointestinal tract (Kindt and Tack, 2006; Tack, 2008). One of the major categories of prokinetic drugs is antidopaminergic agents (Tonini et al., 2004). The gastrointestinal tract actually has the ability to produce a substantial amount of dopamine. Enteric dopamine has been known to mediate inhibition of gut motility and decrease antrudoenal coordination in gut muscle, thereby inhibiting acetylcholine release from the cholinergic nerve by the activation of the neuronal dopamine D₂ receptor...
(Iwanaga et al., 1990). Domperidone, a D₂ receptor antagonist, has been reported to induce an increase in gastric muscle contraction in guinea pigs (Reddymasu et al., 2007). Ito-pride is a benzamide derivative antagonizing the D₂ dopamine receptor, which has demonstrated acceleration effects on gastrointestinal motility and gastric emptying in both animals and humans (Tsubouchi et al., 2003; Holtmann et al., 2006). Another significant category of prokinetic drugs is serotonergic agents (Tonini and Pace, 2006). The main effects relevant to gastrointestinal motility are mediated by 5-HT₄ receptors and humans (Tsubouchi et al., 2003; Holtmann et al., 2006).

Introduction and duodenal motility and gastric emptying in both animals and humans (Tsubouchi et al., 2003; Holtmann et al., 2006). The main effects relevant to gastrointestinal motility are mediated by 5-HT₄ receptors (Tsubouchi et al., 2003; Holtmann et al., 2006). Tegaserod, an aminodindole compound, is a partial 5-HT₄ receptor agonist that has been shown to induce acceleration of gastric emptying in FD patients as well as healthy volunteers (Degen et al., 2001; Vakil et al., 2008). Cisapride is a benzamide derivative antagonizing the D₂ dopamine receptor, which has demonstrated acceleration effects on gastrointestinal motor functions using several compounds isolated from Pharbitis semen and Corydalis tuber (Lee et al., 2008). We previously reported on the strong gastroprokinetic effects of DA-9701, an herb-based novel prokinetic agent formulated with DA-9701 not only resulted in accelerated gastric accommodation in Beagle dogs (Lee et al., 2000).

We previously reported on the strong gastroprokinetic effects of DA-9701, an herb-based novel prokinetic agent formulated with Pharbitis semen and Corydalis tuber (Lee et al., 2008). Administration of DA-9701 not only resulted in accelerated gastric emptying and gastrointestinal transit in normal rats as well as abnormally induced conditions but also induced an increase in gastric accommodation in Beagle dogs (Lee et al., 2008). In an effort to determine the active component(s), we performed binding assays for various receptors controlling gastrointestinal motor functions using several compounds isolated from DA-9701. Among them, tetrahydroberberine (THB), an isoquinoline alkaloid, 5,8,13,13a-tetrahydro-9,10-dimethoxy-6H-benzo[6,1,3]benzodioxolo[5,6-a]quinoline (Fig. 1), isolated from Corydalis tuber, was found to be bound to both dopamine D₂ and 5-HT₁A receptors but not other relevant serotonin receptors. In this study, we show that THB works not only as a prokinetic but also a fundic relaxant in animal models and that it has potential as a therapeutic for gastrointestinal motor dysfunctions such as FD.

![Fig. 1. Structure of THB.](image-url)
TABLE 1
Experimental conditions for the determination of affinities at native and recombinant receptors in vitro

<table>
<thead>
<tr>
<th>Receptor</th>
<th>Source</th>
<th>Radioligand</th>
<th>Kᵢ</th>
<th>Nonspecific</th>
<th>Included Buffer*</th>
<th>Included Time and Temperature</th>
</tr>
</thead>
<tbody>
<tr>
<td>5-HT₁A</td>
<td>CHO K1</td>
<td>[³H]8-OH-DPAT (0.25)</td>
<td>0.29</td>
<td>Metergoline (10)</td>
<td>A</td>
<td>60 min, 27°C</td>
</tr>
<tr>
<td>5-HT₂A</td>
<td>Sprague-Dawley cerebral cortex</td>
<td>[¹¹C]Cyanopindolol (0.01)</td>
<td>0.19</td>
<td>Serotonin (10)</td>
<td>B</td>
<td>90 min, 37°C</td>
</tr>
<tr>
<td>5-HT₃A</td>
<td>Human embryonic kidney 293</td>
<td>[³H]GR65630 (0.55)</td>
<td>0.57</td>
<td>MDL-72222 (10)</td>
<td>C</td>
<td>60 min, 25°C</td>
</tr>
<tr>
<td>5-HT₄</td>
<td>Hartley guinea pig striatum</td>
<td>[³H]GR113808 (0.70)</td>
<td>0.14</td>
<td>Serotonin (30)</td>
<td>D</td>
<td>30 min, 25°C</td>
</tr>
<tr>
<td>5-HT₅A</td>
<td>CHO K1</td>
<td>[⁹]Hiodosipiperone (0.27)</td>
<td>0.12</td>
<td>Haloperidol (5)</td>
<td>E</td>
<td>120 min, 25°C</td>
</tr>
</tbody>
</table>

* Buffer A, 50 mM Tris-HCl (pH 7.4), 10 mM MgSO₄, 0.5 mM EDTA, and 0.1% ascorbic acid; buffer B, 50 mM Tris-HCl (pH 7.4), 154 mM NaCl, 10 μM garypirine, and 30 μM isopenanline; buffer C, 50 mM Tris-HCl (pH 7.5), 5 mM MgCl₂, and 1 mM EDTA; buffer D, 50 mM Tris-HCl (pH 7.4); buffer E, 50 mM Tris-HCl (pH 7.4), 120 mM NaCl, 5 mM KCl, 5 mM MgCl₂, and 1 mM EDTA.

**International Ltd., Kent, UK** previously soaked in 0.5% polyethylene- mine and a filter washed with ice-cold 50 mM Tris-HCl buffer (pH 7.4) at 25°C. Radioactivity was measured on a beta counter, the data were analyzed graphically with inhibition curves, and IC₅₀ values were derived. Kᵢ values were calculated according to the equation Kᵢ = IC₅₀/[1 + (C/Kᵢ)] with C as the concentration of each radioligand and Kᵢ the equilibrium dissociation constant of each radioligand.

Binding assays for other serotonin (5-HT₁B and 5-HT₄) receptors were conducted at Ricerca Biosciences (Taipei, Taiwan) and Caliper Life Sciences (Hopkinton, MA).

**Animals.** Male Sprague-Dawley rats (200–220 g) were purchased from Orient Bio, Inc. (Gapyeung, South Korea) and had free access to water and a standard pellet diet. Before the experiments, rats were kept in a environment-controlled room in groups of four. For restraint stress-induced impaired gastric compliance, rats were restrained in a plastic cage for 4 h per day for 4 days. Rats were fasted for 24 h before the start of the experiment (water was available ad libitum). At the beginning of the experiment, rats were sedated with a single dose of ketamine (50 mg/kg i.m.; Yulan Corporation). Thereafter, a dose of 25 mg/kg was administered intramuscularly every hour to maintain sedation. During the experiment, the animals always were positioned lying down on their right sides. Ketamine sedation allowed the rats to tolerate swallowing of the finely powdered polycyline balloon that was adhered to the polycyline tube and intubation of the tube during the experiment while spontaneous breathing was preserved. The bag was positioned in the stomach via a guide wire. A heating pad was used throughout the experiments to maintain the body temperature of the animal at 37°C. Gastric pressure–volume relationships were studied using a gastric barostat (Distenter Series II; G&J Electronics, Toronto, ON, Canada). The system consists of an ultrathin polycyline balloon (10-mL maximal capacity; Mui Scientific, Mississauga, ON, Canada), which was finely folded and can be inserted through the mouth into the proximal stomach, and has infinite compliance at the volumes used for distention of the rat stomach. A polycyline balloon was adhered to a single lumen polycyline tube (1.2-mm diameter; Natsume, Tokyo, Japan). The tube was connected to a pressure recording port and an air inflation port on a computer-driven barostat for recording of volume changes while the pressure was kept constant. Before the start of the experiment, the balloon was connected to the barostat, and the intrabag pressure was raised to 10 mm Hg. Monitoring of the constant intrabag volume ensured that there was no leak (Janssen et al., 2004). Drugs were administered after the intragastric volume maintained a stable baseline value during a period of at least 10 min at the constant pressure used. A single dose of THB was administered per experiment. Different doses (1, 3, 10, 30, or 100 μg/kg i.v.) of THB were divided randomly over different experiments. The doses of the drugs (WAY-100635, L-NAME, L-arginine, and VIP antagonist) used for mechan- ism studies were chosen based on previous publications (Takahashi and Owyang, 1997; Zhou et al., 2008).

**Canine Gastric Accommodation.** Experiments were performed on four adult female Beagle dogs (7–9 kg b.wt.). Dogs were trained to stand quietly in a sling without sedation. Experiments were performed on conscious dogs after allowing at least 15 days for recovery after surgery. Before each experimental session, the dogs were fasted for at least 18 h; water was available ad libitum. Between consecutive experimental sessions with the same animal, a washout period...
of at least 72 h was allowed. Dogs were observed throughout the experiment, and any sign of discomfort or anomalous behavior was noted. The gastric cannula was opened, and after verification that the stomach did not contain any food residues, the bag of the barostat was introduced into the proximal stomach (position of the bag checked radiographically). Before and at the end of the in vivo tests, the bag was checked for air leaks by increasing the pressure to 20 mm Hg (Chen et al., 2009). To determine the influence of 5-HT1 receptor antagonists in the mediation of canine gastric accommodation induced by THB, WAY-100635 (0.1 mg/kg) was tested versus 30 μg/kg THB and administered intravenously 10 min before THB.

Data Analysis. Results were expressed as mean ± S.E.M. Differences in the data were evaluated using paired t test for the comparison of two groups or one-way analysis of variance (ANOVA) followed by Dunnett’s test for multiple comparisons. A difference was considered significant if p < 0.05.

Results

Identification and Characterization of THB. In an effort to determine active component(s) from DA-9701, we have fractionated the ethanol extract of Corydalis tuber according to polarity and purified several compounds by column chromatography, as described under Materials and Methods. We have screened and chased the affinities of the compounds for relevant receptors involved in the control of gastrointestinal motor function, which included various serotonin receptors and dopamine D2 receptor, by radioligand competition binding assays, according to the conditions presented in Table 1. We found that a compound known as SF-2 has micromolar affinities for dopamine D2 receptor (pK_i = 6.08) and 5-HT1A receptor (pK_i = 5.38) but moderate to no affinity for other relevant serotonin receptors (i.e., 5-HT1B, 5-HT1D, 5-HT3, and 5-HT6; pK_i < 5.00), as presented in Table 2. SF-2 showed a similar affinity for D2 receptor to that of itopride (in this study) and a 1434-fold lower affinity than that of domperidone compared with values reported previously (Takuma et al., 1998). In terms of 5-HT1A receptor affinity, SF-2 showed 132 times lower affinity than that of buspirone (pK_i = 7.50). The structure of SF-2 was identified by NMR and mass spectroscopy. Spectroscopic data, including 1H NMR and 13C NMR, are as below. Yellow powder; m.p. 167°C; fast atom bombardment mass spectroscopy, m/z 340 [M + H]+; 1H NMR (500 MHz, CDCl3), δ 6.87 (1H, d, J = 8.5 Hz, H-12), 6.80 (1H, d, J = 8.5 Hz, H-11), 6.74 (1H, s, H-1), 6.60 (1H, s, H-4), 6.08 (2H, s, -OCH2O-), 4.27 (1H, d, J = 15.5 Hz, H-8), 3.86 (6H, s, OCH3-9, OCH3-10), 3.58 (2H, d, J = 15.5 Hz, H-8, H-14), 3.25 (1H, m, H-6), 3.22 (1H, m, H-13), 3.14 (1H, m, H-5), 2.86 (1H, dd, J = 15.0, 12.0 Hz, H-13), 2.68 (1H, m, H-6), 2.65 (1H, m, H-5); 13C-NMR (125 MHz, CDCl3), δ 150.2 (C-10), 146.1 (C-2), 145.8 (C-3), 145.0 (C-9), 130.6 (C-14a), 128.5 (C-8a), 127.7 (C-4a), 127.6 (C-12a), 123.8 (C-12), 110.9 (C-11), 108.3 (C-4), 105.4 (C-1), 101.0 (C-8), 101.0 (-OCH2O-), 95.9 (OCH3-9), 59.5 (C-14), 55.8 (OCH3-10), 53.8 (C-8), 51.3 (C-6), 36.3 (C-13), 29.4 (C-5). Spectral data for THB were determined to be identical to that of the source material obtained from Trademax (Shanghai, China). SF-2 is identified as THB, an isoquinoline alkaloid. The chemical structure is shown in Fig. 1. To the best of our knowledge, there have been no reports on serotonin receptors, although it has been known as an antidopaminergic agent (Niwa et al., 1991). On the basis of the above data, we performed testing to determine whether THB has potential as a prokinetic as well as a fundic relaxing agent.

Effects of THB on Gastric Emptying. To estimate the prokinetic effects of THB, we performed gastric emptying experiments using semisolid meals. In normal rats, compared with the control group, THB induced significant acceleration of gastric emptying. As shown in Fig. 2A, data from the control group indicated that only 28.1 ± 1.1% was emptied; however, the decrease in residual meal by THB was significant. The effect was a bell-shaped relationship, with a maximal efficacy at the dose of 30 μg/kg. The residual percentage of the meal was 50.3 ± 1.2% (p < 0.05), and the maximal effect of THB was comparable with that achieved with cisapride at a dose of 10 mg/kg p.o. (54.8 ± 1.5, n = 8, p < 0.05). We next used delayed models of gastric emptying to determine whether THB, known to function as a D2 receptor antagonist (Wu and Jia, 1996, 1997), is capable of correcting abnormally depressed gastric emptying. Apomorphine has inhibitory effects on gastric emptying through its action as a dopamine agonist (Blancquaert et al., 1982). Administration of apomorphine (0.05 mg/kg s.c.) resulted in a marked delay of gastric emptying of a semisolid meal by approximately 50% compared with that of naive rats (37.8 ± 2.4 versus 68.9 ± 7.4). Delayed gastric emptying was restored by THB. At doses of 10 and 100 μg/kg, the gastric emptying rates were 48.8 ± 5.2 (p < 0.05) and 55.9 ± 5.7 (p < 0.01), respectively. The effect of 100 μg/kg THB was comparable with that of itopride at a dose of 30 mg/kg (Fig. 2B). These results suggested that THB could induce acceleration of gastric emptying under normal conditions as well as apomorphine-induced abnormally delayed gastric emptying.

<table>
<thead>
<tr>
<th>Receptor</th>
<th>THB</th>
<th>Cisapride</th>
<th>Mosapride</th>
<th>Sumatriptan</th>
<th>Buspirone</th>
<th>WAY-100635</th>
<th>GRI27935**</th>
</tr>
</thead>
<tbody>
<tr>
<td>5-HT1A</td>
<td>5.38</td>
<td>&lt;6.00</td>
<td>&lt;5.00</td>
<td>6.43</td>
<td>7.50</td>
<td>9.05</td>
<td>7.58</td>
</tr>
<tr>
<td>5-HT1B</td>
<td>&lt;5.00</td>
<td>&lt;6.00</td>
<td>&lt;5.00</td>
<td>7.60</td>
<td>&lt;5.00</td>
<td>5.88</td>
<td>9.18</td>
</tr>
<tr>
<td>5-HT1D</td>
<td>N.D.</td>
<td>&lt;5.00</td>
<td>N.D.</td>
<td>7.92</td>
<td>6.48</td>
<td>8.41</td>
<td></td>
</tr>
<tr>
<td>5-HT3</td>
<td>&lt;5.00</td>
<td>3.69</td>
<td>&lt;6.00</td>
<td>N.D.</td>
<td>N.D.</td>
<td>N.D.</td>
<td></td>
</tr>
<tr>
<td>5-HT4</td>
<td>&lt;5.00</td>
<td>7.40</td>
<td>7.01</td>
<td>N.D.</td>
<td>N.D.</td>
<td>N.D.</td>
<td></td>
</tr>
<tr>
<td>D2</td>
<td>6.08</td>
<td>3.43</td>
<td>&lt;4.00</td>
<td>N.D.</td>
<td>N.D.</td>
<td>N.D.</td>
<td></td>
</tr>
</tbody>
</table>

N.D., not determined.

* In this study.

1 Yoshikawa et al., 1998.

2 Kakigami et al., 1998.

3 Meyer et al., 1996.

4 Koek et al., 1998.

5 Gommeren et al., 1998.

Effects of THB on Motor Activity in the Upper Gastrointestinal Tract. To further evaluate the question of whether THB enhances gastric motor function, we measured contractility using EMG. Rats received surgical implantation with 41 pairs of electrodes in the antrum, duodenum, and jejunum. The effects of THB on gastrointestinal contractile activity upon intravenous injection under fasting conditions are presented in Fig. 3. Control rats injected with saline showed a typical pattern of motor activity; however, injection of THB (10 μg/kg i.v.) resulted in significant enhancement of contractile force in the gastrointestinal tract, and an overall increase in the motor activity index was observed. As shown in Fig. 3A, peak count was increased significantly by THB, not only in the gastric antrum but also the duodenum and jejunum, compared with that of the control. Amplitude and integral areas showed a significant increase, as shown in Fig. 3, B and C, respectively, indicating that THB induced stronger contractility compared with that of the control. The effects were prominent in the jejunum, resulting in approximately 2-fold increases, compared with that of the control. Furthermore, the interval of MMC was measured from the end of one activity to the end of the next one. The MMC occurred at regular intervals of approximately 10.1 ± 1.3 min in the control group. Injections of THB resulted in significant shortening of the interval between the MMCs in the duodenum (7.3 ± 1.1 min, p < 0.05) and jejunum (7.4 ± 0.4 min, p < 0.01), although significant effects were not observed in the antrum (Fig. 3D). These results suggested that THB could enhance gastric motor function in the upper gastrointestinal tract through strengthening contractility and shortening the contraction interval.

Fundic Relaxing Effects of THB. Because DA-9701 not only enhanced gastric accommodation in Beagle dogs, we also determined the binding affinity of THB to the 5HT1A receptor (Table 2) and attempted to determine whether THB has the ability to perform fundic relaxant activities, using two animal models. We first used rats with impaired gastric compliance by repeated restraint stress. Rats were restrained in small plastic bags for 4 h per day for 4 consecutive days; balloons connected with a barostat then were inserted into the stomach for the measurement of the volume-pressure relationship. A significant shift (p < 0.05) of the pressure-volume curve was observed in stressed rats toward the lower volume compared with that of control rats, indicating im-

Fig. 2. Effects of THB on gastric emptying. A, in normal rats, after 24 h of fasting, animals (n = 8 for each group) were orally administered THB and cisapride (Cisa) at the indicated doses or 3% hydroxypropyl methylcellulose as a vehicle (Con). B, effects of THB on delayed gastric emptying. In an amorphine-induced delay model, animals were orally administered THB and itopride (Ito) at the indicated doses or 3% hydroxypropyl methylcellulose as a vehicle (Con) and were injected simultaneously with amorphine (0.05 mg/kg s.c.). Naive animals were not injected with amorphine and were orally administered the vehicle. Percentage gastric emptying was calculated as described under Materials and Methods. *, p < 0.05; **, p < 0.01 versus control. ‡, p < 0.01 versus normal (one-way ANOVA with post hoc Dunnett’s test).

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Fig. 3. Effects of THB on gastrointestinal motor activity in conscious rats. THB (10 μg/kg i.v.) versus control (intravenous saline) on the different EMG parameters (A, peak count; B, amplitude; C, integral area; D, interval of MMC) from the rat gastric antrum at 5 mm proximal to the pylorus, the duodenum, and the jejunum, respectively, at 5 and 15 cm distal to the pylorus. *, p < 0.05; **, p < 0.01 versus control (one-way ANOVA with post hoc Dunnett’s test).
paired gastric compliance, resulting in a significantly lower maximal gastric volume (data not shown). Intravenous administration of THB after maintenance of the intragastric volume at a stable baseline value resulted in a significantly higher shift in the pressure–volume curve in rats treated with the doses of 10 μg/kg (Fig. 4C) and 30 μg/kg (Fig. 4D), whereas doses below 10 and 100 μg/kg showed no significant effects. At $\frac{1}{2}P_{\text{max}}$, the gastric volume was $9.6 \pm 0.8$ ml (rats treated with a dose of 10 μg/kg, $p < 0.05$) versus $7.6 \pm 0.8$ ml (control), and in rats treated with a dose of 30 μg/kg, the value was $9.5 \pm 1.0$ ml ($p < 0.05$) versus $6.8 \pm 0.9$ ml (control). The maximum delta volume (approximately 2 ml) at $\frac{1}{2}P_{\text{max}}$ was obtained in rats treated with 10 μg/kg THB (Fig. 4F). In addition, in an effort to understand the mode of action for gastric relaxation, we conducted an antagonist study. WAY-100635, a 5HT1A receptor antagonist, was chosen based on data from the receptor binding assay of THB to the 5HT1A receptor, and, additionally, other antagonists of signaling molecules, including NO and VIP, also were used (Desai et al., 1991). WAY-100635 was administered (0.1 mg/kg i.v.) 10 min before the administration of THB (10 or 30 μg/kg i.v.) at doses presenting significant efficacy. THB induced an increase of gastric volume that was inhibited significantly by WAY-100635. The delta volume at $\frac{1}{2}P_{\text{max}}$ was lowered to 0.02 ml (Fig. 5). Administration of L-NAME (10 mg/kg), a NO synthase inhibitor, also resulted in significant inhibition of delta volume at $\frac{1}{2}P_{\text{max}}$ ($0.2 \pm 0.1$ vs $1.5 \pm 0.3$ ml, $p < 0.05$). In addition, to exclude nonspecific effects of L-NAME, we used L-arginine, a NO precursor. When L-arginine (100 μM/kg) was administered, L-NAME restored the gastric volume to the levels induced by THB, indicating involvement of NO. However, administration of the VIP antagonist (30 nmol/kg) did not result in any changes in gastric volume induced by THB. As shown in Fig. 5, the delta volume at $\frac{1}{2}P_{\text{max}}$ was lowered to 0.3 ml (rats treated with THB (Fig. 5B). Taken together, these data suggested the potential of THB as a fundic relaxant for an increase of gastric accommodation.

**Discussion**

This study began from DA-9701, an herb-based gastroprokinetic agent formulated with Pharbitis semen and Corydalis tuber (Lee et al., 2008). A phase III clinical trial of DA-9701 for FD has been completed recently, and we are anticipating DA-9701 as a novel natural medicine for treatment of FD in South Korea. In this study, we provided several pieces of evidence to show that THB, an isoquinoline alkaloid isolated from Corydalis tuber, is one of the active component(s) responsible for gastroprokinetic and fundic relaxing activity. THB has been known as an antidopaminergic agent; however, for the first time, we recognized THB as an agonist for the 5-HT1A receptor and explored the possibility of its possible use for the treatment of FD. We demonstrated that THB stimulates gastric emptying and gastrointestinal contractility as well as enhances gastric accommodation in conscious animals, which might be mediated by dopamine D2 and/or serotonin 5-HT1A receptors. These findings suggested that THB may have potential as a novel agent in the treatment of FD associated with gastric motor dysfunction.
Tetrahydroberberine as a Prokinetic and a Fundic Relaxant

The extent of dopaminergic innervation and its roles in the gut are not completely understood; however, dopamine is known to cause potent inhibition of motility, reduced lower esophageal sphincter tone, reduced gastric tone and intragastric pressure, and decreased antral and pyloric motor function. Although the question of whether and/or how anticholinergic drugs act on the 5-HT1A receptor is not yet clear, anticholinergic drugs are known to cause potent inhibition of motility, reduced lower esophageal sphincter tone, reduced gastric tone and intragastric pressure, and decreased antral and pyloric motor function.

Several antidopaminergic agents, including domperidone, metoclopramide, and itopride, are used primarily for the treatment of motility disorders of the upper digestive tract, such as FD, and some agents seem to be beneficial for the treatment of visceral pains. However, the toxicity issue is still under consideration. Itopride at a dose of 100 mg/kg and metoclopramide at a dose of 10 mg/kg were reported to produce reduced activity, tremor, and abnormal behavior in dogs (Koizumi et al., 1992). The development of safer and more effective antidopaminergic agents for the treatment of gastric functional disorders is clearly needed.

THB was found to enhance gastric emptying in a bell-shaped, dose-dependent manner, and the maximal effective dose was 30 μg/kg, whereas cisapride, a conventional prokinetic used as a control in this experiment, showed an effect comparable with that at a much higher concentration (10 mg/kg), indicating that THB has an effect superior to that of conventional drugs.

Data from a delayed gastric emptying model using apomor-
Authorship Contributions
Participated in research design: T.H. Lee, Son, and Jin.
Conducted experiments: Kim and S.O. Lee.
Performed data analysis: K.R. Lee and Jin.
Wrote or contributed to the writing of the manuscript: Jin.

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