Effects of Corydaline from Corydalis Tuber on Gastric Motor Function in an Animal Model

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The aim of this study was to evaluate the prokinetic and gastric-relaxing effects of the isoquinoline alkaloid corydaline, which was extracted from Corydalis tubers (CT). Corydaline is a marker compound used for quality control of DA-9701, a prokinetic agent formulated from extracts of Pharmitidis semen and Corydalis tuber that is currently in clinical trials in Korea for the treatment of functional dyspepsia (FD). DA-9701 was previously reported to be a potential therapeutic agent for the treatment of abnormalities in gastrointestinal motor function in FD patients; however, the therapeutic effects of corydaline on FD have yet to be demonstrated in an in vivo study. In the current study, oral administration of corydaline not only significantly accelerated gastric emptying in normal rats but also improved delayed gastric emptying to near normal levels. Furthermore, corydaline induced significant gastric relaxation, shifting the pressure–volume curve towards higher volumes compared to controls. These results suggest that corydaline promotes gastric emptying and small intestinal transit and facilitates gastric accommodation.

Key words corydaline; Corydalis tuber; functional dyspepsia; gastric motor function

Functional dyspepsia (FD) is a highly prevalent chronic gastrointestinal (GI) disorder that causes a considerable burden to both the patient and society. Although the etiology and pathogenesis of FD are poorly understood, FD is associated with pathophysiologic abnormalities including delayed gastric emptying (GE), impaired gastric accommodation, and visceral hypersensitivity. Delayed GE has been reported in 30—40% of FD patients, and a number of studies have demonstrated impaired gastric accommodation after meals in approximately 40% of patients. Given these results, we hypothesized that GE of a semi-solid meal, GI transit, and gastric accommodation would be suitable markers for evaluating motor effects in animal models.

Corydalis tubers, the roots of Corydalis yahuosuo W.T. WANG (Papaveraceae), have long been used as herbal medicine for their analgesic and anti-ulcer effects. It has been reported that Corydalis tuber extracts exhibit several biological activities, acting as both an anti-spasmodic agent in the GI tract and as an analgesic. Previous phytochemical studies reported that Corydalis tubers are made up primarily of alkaloid compounds including corydaline, berberine, protopine, and palmatine. These alkaloid compounds are reported to provide the analgesic, anti-spasmodic, and anti-acetylcholinesterase activities associated with Corydalis tubers. Of these compounds, corydaline is considered the marker compound for quality control of Corydalis extract; however, the pharmacological effects of corydaline on GI motor function have not yet been studied.

We previously investigated the prokinetic activities of corydaline to determine its potential bioactivity in FD and elucidated the basis of the selection of corydaline as the principal marker compound for quality control. In the present study, we evaluated the effects of corydaline on GE and GI transit in rats and gastric accommodation in conscious dogs.

MATERIALS AND METHODS

Plant Material Plant material was commercially purchased at Kyungdong herbal market (Seoul, Korea) and identified by Dr. S. Choi at the Research Center of Dong-A Pharm. Co., Ltd. (Yongin-si, Korea). A voucher specimen has been deposited in our laboratory at Kyung Hee University.

Extraction and Isolation Dried Corydalis tuber (30 kg) was extracted with 50% aqueous ethanol three times at room temperature. Upon removal of the solvent under vacuum, the ethanolic extract yielded 2.85 kg of material (Corydalis tuber extract, CTE; 7.6% by dry weight). We found that the CTE contained corydaline, palmatine, berberine, and other isoquinoline alkaloids. Based on HPLC analysis, the prescribed range of index components including corydaline and palmatine was detected in the CTE. The CTE was suspended in H2O and partitioned successively with n-hexane, ethyl acetate (EtOAc), and n-butanol. The EtOAc-soluble fraction (15 g) of the extract was subjected to a silica gel column using mixtures of n-hexane–EtOAc of increasing polarity as eluents to give 12 sub-fractions (fr. 1—12). From these 12 fractions, fr. 4 (2.4 g) was chromatographed on a silica gel column eluting with a gradient of n-hexane–acetone to yield corydaline (37 mg). Spectral data and chromatographic behavior of the isolated compound was determined to be identical to that of the source material (WAKO, Japan).

Chemicals Apomorphine, cisapride, domperidone, and hydroxypropylmethyl cellulose (HPMC) were purchased from Sigma Chemical Co. (St. Louis, MO, U.S.A.). Fluorescein isothiocyanate (FITC)-dextran was purchased from Fluka (Tokyo, Japan). Other reagents used were of analytical grade or higher and were used without further purification.

Animals and Experimental Procedure Male Sprague-Dawley (SD) rats were purchased from OrientBio Inc. (Gapyeung, Korea) and allowed at least one week of acclima-
ization before use. The initial body weights of all rats were within the range of 220—250 g. The animals were housed in a regulated environment with a 12-h light/dark cycle. Food and water were given *ad libitum*, except for the duration of the experimental period. Female Beagle dogs were purchased from Central Lab. Animal Inc. (Seoul, Korea), individually housed in air-conditioned boxes, and given dog food in pellet form (Purina, Dog Chow®). All experiments were performed in accordance with the guidelines of the Institutional Animal Care and Use Committee of Research Center, Dong-A Pharm. Co., Ltd.

**Drug Administration** The following drugs were administered to experimental animals: cisapride, domperidone, corydaline, and CTE. All drugs were suspended in 3% HPMC (vehicle) and administered orally in a volume of 5 ml/kg for rats and 1 ml/kg for dogs.

**Gastric Emptying** GE was measured according to the method of Ozaki and Sukamoto with some modifications.15) Male Sprague-Dawley rats (220—250 g) were fasted for 18 h with *ad libitum* access to water. Normal rats were given 2 ml of semi-solid meals by gavages at 60 min following drug administration. After 30 min, animals were sacrificed, and the weights of their stomachs and contents were measured in order to determine GE. GE was calculated using the following equation, as previously described:

GE (％) = [1 − weight of test stomach/weight of zero time control stomach] × 100

To investigate the effects of CTE and corydaline on GE in normal animals, 80 rats were randomly divided into nine groups (*n*=8—9); four CTE treatment groups (0.3, 1, 3, 10 mg/kg), three corydaline treatment groups (0.1, 1, 10 µg/kg), one reference drug treatment group (cisapride, 10 mg/kg) and one control group (vehicle).

In the next set of experiments, GE was abnormally delayed by administration of apomorphine. Rats were given 2 ml of a semi-solid meal at 60 min following drug administration and simultaneously injected with apomorphine (s.c., 0.05 mg/kg). After 50 min, GE was determined by the method described above. Ninety rats were randomly divided into 11 groups (*n*=8—9); four CTE treatment groups (0.3, 1, 3, 10 mg/kg), three corydaline treatment groups (0.1, 1, 10 µg/kg), two reference drug treatment groups (cisapride, 10 mg/kg; domperidone, 10 mg/kg), one negative control group (vehicle), and one normal group without apomorphine treatment.

**Gastrointestinal Transit** Gastrointestinal transit was estimated using the geometric center (GC) of non-digestible fluorescein (FITC)-labeled dextran, as previously described.16—18) Male Sprague-Dawley rats (220—250 g) were fasted for 18 h with *ad libitum* access to water. Under ether anesthesia, the median abdomen was opened by a 3-cm midline laparotomy. The small intestine was exteriorized and gently manipulated, then the abdominal muscles and skin were closed with EZ clip applier (Stoelting Co., Wood Dale, IL, U.S.A.). The surgery was completed within 5 min and no analgesic agent was used post-operatively. The drugs were administered orally 4 h post-procedure, and the animals were given a test meal of FITC-dextran by gavages 60 min after drug administration. After 15 min the animals were sacrificed by cervical dislocation, the abdomen opened, and the stomach and small intestine carefully removed. As previously described,18) the small intestine was divided into ten equal segments (1—10) and the FITC-dextran concentration in each segment was measured using a microplate reader (POLARstar OPTIMA, BMG Labtech, Offenburg, Germany). The GC of the distribution of FITC-dextran was calculated using the following equation, as previously described19):

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\text{GC} = \sum \left(\frac{\text{fraction of FITC-labeled dextran per segment}}{\text{segment number}}\right)
\]

To determine the effects of CTE on intestinal transit in rats subjected to laparotomy, 80 rats were randomly divided into 10 groups (*n*=8): four laparotomy plus CTE treatment groups (0.3, 1, 3, 10 mg/kg CTE), three laparotomy plus corydaline treatment groups (0.1, 1, 10 µg/kg corydaline), one laparotomy plus reference drug treatment group (cisapride, 10 mg/kg), one sham-operated group (vehicle control), and one normal group.

**Gastric Accommodation** The effects of CTE on gastric accommodation were examined according to the method of De Ponti et al. with some modification.20) Five female Beagle dogs (7—9 kg) were operated on under general anesthesia with Tiletamine/Zolazepam (Zoletil®, Virbac Laboratories, Carros, France; i.v., 10 mg/kg). A chronic gastric fistula was inserted *via* a midline laparotomy in the larger curvature of the stomach between the glandular portion and forestomach and was exteriorized through the anterior aspect of the abdomen. A recovery period of at least 15 d was allowed before starting the gastric accommodation experiments. Each dog was used only once per week in the experiments. On the morning of the experiments, the dogs were weighed and placed in slings. The gastric fistula was opened and any remaining gastric content was removed with a syringe. We introduced a bag of barostat (Distender Series II TM, G&J Electronics Inc., Toronto, Canada) into the proximal stomach. Animals were administered test drugs and, following an equilibration period, the intrabag pressure was increased by 2-mmHg increments every 3 min, starting from the minimal distending pressure up to 16 mmHg without deflating the barostat bag at each pressure step. Changes in intragastric volume occurring after the administration of CTE or corydaline were automatically measured at the peak of the response. In each experiment, three pressure-volume curves were obtained, allowing a 50-min interval between the end of a distention cycle and the beginning of the subsequent cycle. Volume and pressure data were analyzed using a program in which different parameters were measured and/or calculated (Protocol Plus Deluxe 6.7R, G&J Electronics Inc.).

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

**RESULTS**

In normal rats, GE of semi-solid meals during the 30 min period was 30.9±1.8, 27.8±7.9, 44.6±2.6, and 32.9±3.7% for 0, 0.1, 1, and 10 µg/kg doses of corydaline, respectively (*n*=8—9 for each dose) (Fig. 1A). Treatment with CTE also significantly increased GE (*p*<0.05) compared with that of the controls, and the maximal GE effect of CTE was compa-
rable to that achieved with 10 mg/kg cisapride. When apomorphine was administered, the GE of a semi-solid meal was markedly delayed by approximately 50% compared with untreated rats (29.8±2.9% vs. 61.7±2.9%, p<0.05). The delayed GE was rescued by administration of corydaline at a dose of 0.1 μg/kg (44.6±8.3%), and the maximal GE effect was observed in rats treated with corydaline at dose of 1 μg/kg (45.5±5.4%, p<0.05) (Fig. 1B). Treatment with CTE at a dose of 1 mg/kg (57.7±4.4%) significantly restored the delayed GE to a near normal level. These results suggest that corydaline may accelerate GE under normal conditions, and furthermore, can correct the abnormal delay in GE that is induced by apomorphine.

Laparotomy with mild intestinal manipulation produced a significant delay in GI transit (GC=2.56±0.09, n=8, p<0.05) compared with the normal group (GC=3.83±0.19, n=8). CTE was associated with normalization of laparotomy-induced delayed GI transit at doses of 0.3 mg/kg (GC=3.48±0.24, n=8, p<0.05) and 1 mg/kg (GC=3.87±0.09, n=8, p<0.05), results comparable to those obtained with cisapride (GC=3.62±0.09, n=8, p<0.05) (Fig. 2).

The effects of corydaline and CTE on gastric accommodation were assessed in Beagle dogs using a barostat. The first distension cycle was used to unfold the intragastric bag, and the recorded values were discarded. The second distension cycle was used as a control. For the third distention cycle, corydaline was gavaged 40 min prior to initiating the distension cycle. Baseline gastric volumes were measured at 2 mmHg were 16±9 ml and 13±3 ml before and after corydaline treatment, respectively (Table 1). Corydaline (1 μg/kg) was associated with relaxation of the proximal stomach. With the use of the exponential model, a significant shift in the pressure–volume curve was observed with corydaline compared to control, resulting in a large increase in slope at 1/2P_max by 19.06 ml/mmHg (vs. 13.81, p<0.01), and the volume at 1/2P_max by 69±12 ml (vs. 59±18). There were signifi-

| Volume–Pressure Relationship in Controls and Following Oral Administration of Corydaline According to the Exponential (V=V_0 e^{k_P P}) Equation^a |
|------------------|-----------------|-----------------|------------------|------------------|------------------|
| Exponential model |                    | Slope at 1/2P_max, ml/mmHg | Vol. at 1/2P_max, ml/mmHg |
|                  | V_0, ml          | k_P, mmHg^{-1}    |                  |                  |
| Control          | 20±5             | 0.18±0.01         | 12.68            | 65±22            |
| Corydaline (0.3 μg/kg) | 21±8             | 0.21±0.01         | 14.49            | 72±24            |
| Control          | 16±9             | 0.27±0.04         | 13.81            | 59±28            |
| Corydaline (1 μg/kg) | 13±3             | 0.28±0.01         | 19.06**          | 69±12            |
| Control          | 4±2              | 0.43±0.12         | 17.91            | 43±6             |
| CTE (3 mg/kg)    | 5±3              | 0.40±0.08         | 18.10            | 47±6             |

^a^ V is volume (in ml), V_0 is theoretical volume when P=0 mmHg, P is pressure (in mmHg), and k_P is the rate constant. The overall fit for each curve was summarized as an r^2 value. Values are the mean±S.E.M. (n=4). **p<0.01 vs. control (paired t-test); slope at 1/2P_max was calculated as follows: y=V_0 e^{k_P P}, with P=6 mmHg.
M1/M2 muscarinic receptor, significantly improves the symptoms of FD and is more effective in reducing symptoms in patients with the postprandial distress syndrome of FD than in patients with epigastric pain syndrome.23)

We previously demonstrated that DA-9701, a multi-herbal extract formulated from Pharthitis semen and Corydalis tuber that is currently in clinical trials for the treatment of FD, is a potential safe and effective therapeutic agent for patients with abnormalities in GI motor function.13) Choi et al. showed that the pacemaker currents in interstitial cells of Cajal (ICC) are cellular and molecular targets underlying the effect of DA-9701 on GI motor function by means of intracellular mobilization of Ca^{2+} through phospholipase C.24)

As a representative compound extract of Corydalis tubers, corydaline can significantly enhance GI motor functions that accelerate GE and GI transit as well as gastric accommodation. GE and GI transits are well-established models used in numerous studies to evaluate prokinetics. Corydalis enhanced GE in normal rats with an effective dose of 1 μg/kg, much lower than that of conventional prokinetics. Following treatment with corydaline, GE increased from 30.9±1.8 to 44.6±2.6%, comparable to treatment with cisapride (50.9±5.5%). When apomorphine was administered, GE was reduced to 29.8±2.9% compared to the normal group (61.7±2.9%). Treatment with corydaline at a dose of 1 μg/kg significantly abrogated the delayed GE induced by apomorphine (45.5±5.4%, p<0.05). Apomorphine is known to inhibit GE by acting as a dopamine agonist, and domperidone (D_{2} receptor antagonist) and cisapride (5-HT_{3} antagonist and 5-HT_{4} agonist) also accelerated delayed GE in this model (62.4±2.6, 65.6±3.0%, respectively).

CTE possesses moderate affinity for D_{2}, 5-HT_{1B}, and 5-HT_{4} receptors (K_{i}=0.2, 6.3, 7.6 μg/ml, respectively). In our previous studies, CTE produced 5-HT_{4} receptor-mediated relaxation of the rat esophagus (EC_{50}=12.9 μg/ml) and contraction of the guinea pig colon (EC_{50}=1.4 μg/ml). It was suggested that CTE and its main component, corydaline, contributed in part to the ameliorative effect on the delayed GE induced by apomorphine, and that the action mechanism of CTE and corydaline may be related to serotonergic or dopaminergic activity.

The present study showed a mild improvement in delayed GI transit by treatment with corydaline. Delayed GI transit induced by laparotomy with mild intestinal manipulation was partially recovered with administration of corydaline: the GC was increased from 2.56 to 3.12 by corydaline compared to the normal group (p<0.05) and p<0.01, respectively) (Fig. 3). These results suggest that corydaline may induce gastric relaxation and increase gastric compliance.

**DISCUSSION**

There is no single therapy available that is capable of providing relief to the majority of FD patients due to the pathophysiological heterogeneity of the disease. There are currently several relevant drugs marketed or under development, including prokinetics and fundic relaxant drugs. Prokinetics, such as cisapride and mosapride, are a group of drugs that stimulate smooth muscle contraction to enhance GE and intestinal transit.20,21) Recent studies have demonstrated that impaired gastric accommodation in FD may contribute to symptom generation.22) Matsueda et al. showed that acotiamide, a fundus-relaxing drug that acts via blockade of the...
possible explanations. First, the dose range used may be at the upper end of the dose–response curve. Second, the drugs may not act via a specific receptor-mediated mechanism enhancing GI motor activity. Third, the drug may bind to the activation site or the inhibition site on the receptor with different affinity.28 Finally, the drug may act at receptors other than D2 receptors at higher doses, thereby inhibiting its potential to enhance GI motility or obscuring its prokinetic effects. It has been reported that several receptors are associated with GI disorders.29,30 Dopamine decreases the tone and motility of the GI tract, and dopamine antagonists, such as sulpiride, domperidone, and metoclopramide, enhance the peristaltic contractions of the esophageal body, increase the muscle tone of the lower esophageal sphincter, and stimulate gastrointestinal motor activity.30,31 It appears that stimulation of the 5-HT1B receptor has a relaxation effect on gastric tone in dogs since the antimiigraine 5-HT1B/D receptor agonist sumatriptan induces an immediate gastric relaxation and gastric accommodation mediated by 5-HT1B receptors.19

The gastric barostat technique is the most common method for measuring gastric compliance and accommodation in both humans and animals. Corydaline induced gastric accommodation at a dose of 1 μg/kg but had no measurable effect on gastric tone. The slope at 1/2Pmax of the pressure–volume curve, which provides an estimation of gastric compliance, was significantly increased following corydaline treatment (p<0.01). Corydaline had a moderate effect on the pressure–volume curve that fitted the exponential model better than the linear model. It has been suggested that an exponential equation fits the concept of adaptive accommodation better than the linear model.30,31 Furthermore, corydaline has weak affinity for the 5-HT1A, 5-HT1B/D and non-selective 5-HT1 receptor subtypes (pK<sub>I</sub><sub>A</sub>, 5; data not shown), thus excluding the 5-HT1 receptors from the gastric-relaxing effect. Further study is necessary to clarify the mechanisms of action of corydaline.

In summary, the current study provides the first in vivo evidence that corydaline is an active compound of Corydalis tuber potentially responsible for improvement of GI motor function.

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