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Effect of platycodin D on gastric motility, gastric emptying and gastrointestinal transit*)

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Summary

Diarrhea is a condition which causes malabsorption and dehydration. Recently, the anti-motility effect of several herbal compounds for the treatment of hypermotility-induced diarrhea has been studied. The root of Platycodon grandiflorum has been widely used in oriental medicine for the treatment of various chronic inflammatory diseases. The aim of the present study was to assess the effects of Platycodin D (PD), the major triterpene saponin in the root of P. grandiflorum, on gastrointestinal (GI) motility by assessing both gastric emptying (GE) and intestinal transit (IT) in mice with different treatment protocols. Mice were randomly allocated to 5 groups (n = 15/group) according to their treatment protocols (control, administered with antikinetics: atropine, dopamine, or with pro-kinetics: itoride, bethanechol) for each GE and IT test. Each group was subsequently divided into 3 subgroups (n = 5) pre-treated with different PD doses (0, 2.5, and 5 mg/kg). Pre-treatment with PD in the control treatment group of mice showed reduced GE and IT in a dose-dependent manner. At the maximum PD effect, GE and IT were reduced by 63% and 50%, respectively, compared with those in the normal control group. In the groups given atropine or dopamine, pre-treatment with PD further reduced GE and IT by 35% to 58%, respectively. The PD pre-treatment dramatically reduced the GI motility enhanced by itopride and bethanechol. On the whole, these results suggest that PD treatment might be beneficial in motility-induced diarrhea.

Keywords: Platycodin D, gastrointestinal motility, gastric emptying, intestinal transit

Diarrhea is a symptom of several diseases and is characterized by rapid and frequent passage of liquefied fecal material through the bowel. Motility-related diarrhea is one of several different types of diarrhea and is caused by excessive movements of the bowel, which result in both enhanced motility and decreased absorption of fluid in the gastrointestinal (GI) tract (1). In animal husbandry, diarrhea can result in economic losses because of reduced feed absorption with increased body fluid loss (23). During motility-induced diarrhea, anti-motility compounds, such as diphenoxylate, loperamide, opium alkaloids, and anticholinergics, have been tested. However these drugs can readily evoke side effects if used for a prolonged time (5). Thus, there is a need for new compounds that have anti-motility activity. Several herbal plants have been well described for their anti-motility properties, such as *Lantana camara*, *Plumbago indica*, and *Achillea millefolium* among others (3, 4, 21).

The root of *Platycodon grandiflorum* has long been used in traditional medicine in East Asia (25). More than 20 triterpenoid saponins have been found in the roots of *P. grandiflorum* including platycosides (A, B, C, D, E and F), platycodins (A, D, D2 and D3), polygalacin (D and D2), and platyconic acid A (14). Triterpenoid saponins from *P. grandiflorum* have been shown to exhibit a variety of pharmacological activities, such as anti-inflammatory, anti-cancer, and hepatoprotective effects (13, 14, 17, 27). Among these terpenoid saponins, platycodin D (PD) is the most potent in various pharmacological activities, including anti-obesity effect (10, 14, 28). Most herbal plants containing terpenoid saponins have demonstrated anti-motility activity against drug-induced hyper-motility (18, 22). However to the best of the Authors' knowledge, there is no report on the effect of PD on GI motility.

*) Publikacja dofinansowana przez Ministerstwo Nauki i Szkonictwa Wyższego w ramach programu Index Plus, nr umowy 77/JxP/dem 2012.

Hence, the aim of the present study was to evaluate the effect of PD, as a pure ingredient, on GI motility, using gastric emptying (GE) and intestinal transit (IT) tests. Moreover, the effect of PD pre-treatment in situations of altered GI motility was also evaluated with the use of different drugs, including atropine, dopamine, itopride, and bethanechol.

Material and methods

Chemicals. PD (≥ 98% purity) from *P. grandiflorum* roots was purchased from Innoten (Seoul, Korea). Atropine (≥ 99% purity), dopamine hydrochloride (≥ 98% purity), bethanechol chloriode (≥ 99% purity), and itopride hydrochloride (≥ 98% purity) were from Sigma Chemicals (St. Louis, MO, USA). All other reagents were from Sigma Chemicals (St. Louis, MO, USA).

Animals. Male Crl:CD-1 (ICR) mice (6-7 weeks of age) were purchased from Orient Bio (Seongnam, Korea) and acclimated for 1 week before the experiments. The mice were housed under a 12 h light/dark cycle (22-23°C) and provided with standard mice food pellets and water *ad libitum*. The animals were fasted for 16 h before the experiment. The experimental protocols were approved by the Institutional Animal Care and Use Committee of the Chungnam National University (Daejeon, Korea).

Gastric emptying. For the pre-treatment, after 16 h of food deprivation, a pure compound (2.5 or 5 mg/kg) of PD or distilled water (DW) was administered by oral gavage. The rationale for these doses is that this range was successfully used in earlier studies (13-14). Fifteen minutes later, all mice in a given group were administered atropine (1 mg/kg, subcutaneous injection, sc) or dopamine hydrochloride (1 mg/kg, intraperitoneal injection, ip) or bethanechol chloride (10 mg/kg, sc) or itopride hydrochloride (50 mg/kg, peroral, po) or DW (0.1 mL/10 g BW, po). A liquid meal containing blue dextran (0.2 g), corn starch (1.5 g), and dextran sulfate (1 g) suspended in DW (10 mL) was administered orally (0.1 mL/10 g BW) 30 min after the last treatment. Each mouse was sacrificed by cervical dislocation 4 min after receiving the liquid meal. The stomach was immediately removed to collect blue dextran in gastric contents. Then, the gastric contents from each mouse were individually homogenized in DW (7 mL). The homogenates were then centrifuged at 2000 × *g* for 15 min, and the 1 mL of supernatant was mixed with the same amount of 0.025 N NaOH. Absorbance was measured with a spectrophotometer at 620 nm. Gastric emptying was calculated as follows: Gastric emptying (%) = 100 − (X × 100/Y) where X is the absorbance of blue dextran recovered from the stomachs of animals sacrificed 4 min after the administration of the marker, and Y is the mean (n = 5) absorbance of blue dextran recovered from the stomachs of DW pre-treated control animals which were killed at 0 min following the administration of the marker (24).

Intestinal transit. Fasted mice were pre-treated by the same method as in the gastric emptying evaluation test. Fifteen minutes later, all mice from a given group were treated with atropine (1 mg/kg, subcutaneous injection, sc) or dopamine hydrochloride (1 mg/kg, intraperitoneal injection, ip) or bethanechol chloride (10 mg/kg, sc) or itopride hydrochloride (50 mg/kg, peroral, po) or DW (0.1 mL/10 g BW, po). Thirty minutes after these treatments, a meal containing charcoal (0.5 g), soluble starch (0.5 g), corn starch (1.5 g), and corn oil (1 g) suspended in water (10 mL) was administered orally (0.1 mL/10 g BW). Each mouse was sacrificed by cervical dislocation 20 min after receiving the charcoal-containing meal, and the small intestine was carefully dissected from the pylorus to the ileocaecal junction. Intestinal transit (IT) was measured as the distance traveled by the marker in the small intestine. The total length of the small intestine and the distance traveled by the charcoal-containing meal were then measured. IT was expressed in percentage and calculated as follows: IT = X/Y × 100. Where X = distance traveled by the charcoal-containing meal and Y = total length of the small intestine (24).

Statistical analysis. The results were expressed as mean ± standard error (SEM). Significant differences among the experimental groups were determined by the one-way analysis of variance (ANOVA) test or the non-parametric Kruskal-Wallis test. Where significant effects were found, post hoc analysis was performed by Tukey's multiple comparison test or the Mann-Whitney U-test, and $P < 0.05$ was considered statistically significant.

Results and discussion

Many plant extracts containing tannins and tannic acid, flavonoids, alkaloids, sesquiterpenes, diterpenes, terpenes, and terpenoids have been found to possess anti-diarrheal activity. They act by reducing gastrointestinal motility and/or secretions (22). In the present study, the effect of PD, a pure terpenoid saponin from *P. grandifrolum*, on GI motility was tested. The results from normal control group mice demonstrated that treatment with PD decreases GE and IT in normal mice in a dose-dependent manner (table 1; $p < 0.05$). This result is in line with earlier pharmacokinetic reports in rats (20). It showed a relatively low oral bioavailability for a limited GI absorption. Indeed, saponins

Tab. 1. The effect of platycodin D (PD) on gastric emptying (GE) and intestinal transit (IT) in mice

(n = 5/each treatment)	Control (DW)		PD 2.5 mg/kg		PD 5 mg/kg	
	GE (n = 15)	IT (n = 15)	GE (n = 15)	IT (n = 15)	GE (n = 15)	IT (n = 15)
DW	74.7 ± 1.1	71.7 ± 1.3	65.4 ± 5.8	58.5 ± 1.8[a]	27.4 ± 6.4[a, b]	35.7 ± 5.8[a, b]
Dopamine	72.0 ± 2.9	62.0 ± 1.8	57.9 ± 2.1	47.5 ± 2.0[a]	29.9 ± 2.0[a, b]	27.7 ± 1.8[a, b]
Atropine	61.5 ± 6.0	51.7 ± 2.6	61.5 ± 1.7	40.1 ± 2.2	35.9 ± 1.1[a, b]	33.4 ± 1.5[a]

Explanations: [a] $p < 0.05$, a significant difference as compared with the control group; [b] $p < 0.05$, a significant difference as compared with the PD 2.5 mg/kg group

have been reported to have limited intestinal absorption because sugar moieties increase the hydrogen bond count, polar surface area, and the molecular flexibility of the molecules to unfavourable levels (8). This might suggest a local rather than systemic action of PD.

Ealier reports showed that GI motility is regulated not only by nitric oxide and gastrointestinal peptides, but also by various neurotransmitters, including dopamine, catecholamines, and acetylcholine (5-7, 16, 19). In the present study, treatment with atropine or dopamine slightly decreased both GE and IT compared with the normal control group. The inhibition of GI motility has been suggested as due to the anti-muscarinic action of atropine and the sympathetic nerve stimulation of dopamine (12). In the present study, it has been shown that PD, used in combination with these drugs, produced a further 55% and 35% decrease in motility compared to a single treatment of dopamine or atropine, respectively (Tab. 1).

To characterise its pharmacological action, PD was examined under different conditions of hypermotility, and the effects of PD on GE and IT against the itopride (dopaminergic antagonist) and the bethanechol (muscarinic agonist) were evaluated. Many anti-dopamine agents have been used to stimulate gastrointestinal motility. They inhibit dopamine by antagonistically binding to dopamine receptors (15). Itopride has an affinity for the dopamine D2-receptor. It stimulates post prandial rhythmic contractions in the gastric antrum and enhances gastric motility. These effects combine to accelerate the emptying of gastric contents and to reduce reflux from the duodenum and from the stomach into the esophagus (11). In addition, it is well known that the release of acetylcholine also stimulates gastric emptying and gastrointestinal motility (9). Acetylcholine increases tonus, contraction amplitude, and peristaltic activity in the stomach and intestine (4). Bethanechol acts as a prokinetic agent by activating acetylcholine release. In the present study, treatment with itopride and bethanechol caused a slight increase in GE (15% and 14%, respectively) and IT (19% and 11%, respectively) compared to the control group. Pre-treatment with PD decreased motility due to itopride or bethanechol in a dose-independent manner. Although there were slight differences between itopride- and bethanechol-treated groups, they did not show strict correlation after PD pre-treatment (Fig. 1 and 2).

In the veterinary field, the anti-motility effect of PD could be used not only for the treatment of motility-induced diarrhea, but also to increase feed conversion efficiency, a critical factor in the economics of animal husbandry. It can be increased through the administration of a compound that decreases the transit time of nutrient matter in the digestive tract, increasing absorption. Andersen and Fogh (2) report that anti-motility herbal material can be useful in obesity patients through its reduction of upper gut motility, which influences appetite and satiety, and prevents over-consumption. Previously, PD has been shown to effectively reduce cholesterol levels in hypercholesterolemic mice and to ameliorate obesity induced by high fat diet (26, 28). The anti-motility effect of PD might indicate that this terpenoid saponin could be a candidate therapy for overweight patients, particularly given its additional anti-cholesterolemic activity.

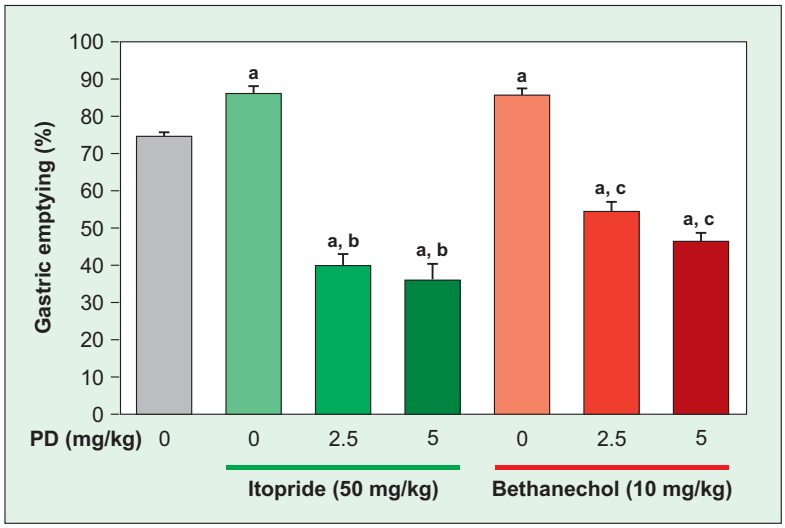

Fig. 1. Effects of PD on gastric emptying in pro-kinetics treated mice (n = 5/each column). Values are expressed as means ± SEM. [a] $p < 0.05$, a significant difference as compared with the control group; [b] $p < 0.05$, a significant difference as compared with the itopride alone treated control group; [c] $p < 0.05$, a significant difference as compared with the bethanechol alone treated control group

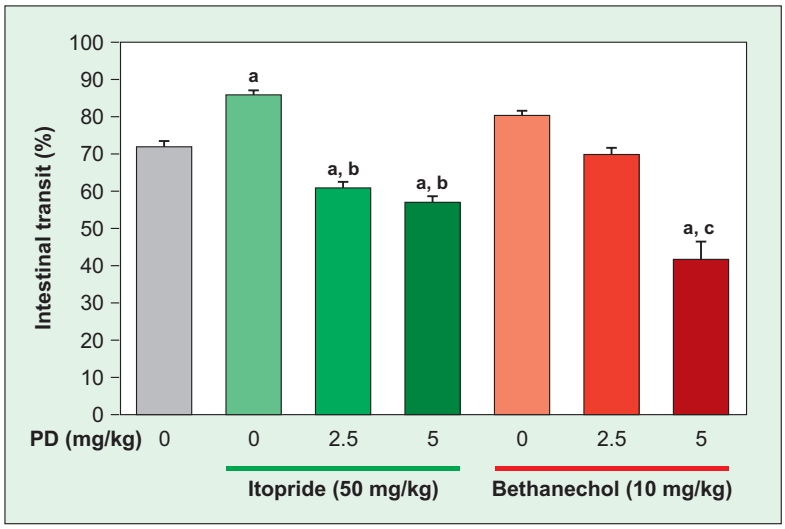

Fig. 2. Effects of PD on gastric emptying in pro-kinetics treated mice (n = 5/each column). Values are expressed as means ± SEM. [a] $p < 0.05$, a significant difference as compared with the control group; [b] $p < 0.05$, a significant difference as compared with the itopride alone treated control group. [c] $p < 0.05$, a significant difference as compared with the bethanechol alone treated control group

In conclusion, PD, a pure terpenoid saponin from *P. grandifrolum* has an anti-motility effect in the gastrointestinal tract. However, its mechanism of action still needs to be understood. Further studies should follow to determine the therapeutic range in its clinical application.

Acknowledgement

This work was supported by research funds from Chungnam National University.

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