Comparison of the Effects of Pantethine and Fursultiamine on Plasma Gastrointestinal Peptide Levels in Healthy Volunteers

Yosuke Suzuki, Hiroki Itoh, Tomohide Abe, Fumihiro Nishimura, Yuhki Sato, and Masaharu Takeyama

Department of Clinical Pharmacy, Oita University Hospital; Hasama-machi, Oita 879–5593, Japan.
Received April 22, 2011; accepted July 20, 2011

Pantethine and fursultiamine have been evaluated for their clinical usefulness in the treatment and prevention of uncomplicated postoperative adhesive intestinal obstruction. In recent years, the actions of drugs used to treat gastrointestinal diseases have been elucidated pharmacologically from the viewpoints of gastrointestinal peptide levels. We examined the effects of pantethine and fursultiamine on plasma levels of calcitonin gene-related peptide (CGRP)-, vasoactive intestinal polypeptide (VIP)-, motilin- and substance P (SP)-like immunoreactive substances (IS) in healthy subjects. An open-labeled study was conducted on five healthy volunteers. Each subject was administered a single oral dose of pantethine, fursultiamine and placebo at intervals of one month. Venous blood samples were collected before and at 20, 40, 60, 90, 120, 180 and 240 min after each administration. Plasma peptide levels were measured using a highly sensitive enzyme immunoassay. A single oral dose of pantethine resulted in significant increases of plasma CGRP- and VIP-IS levels compared to placebo. Furthermore, areas under the plasma concentration–time curves (AUC0–240) of CGRP- and VIP-IS were significantly higher after pantethine administration compared with placebo. On the other hand, fursultiamine had no effect on plasma levels and AUC0–240 of CGRP-, VIP-, motilin- and SP-IS. This study demonstrated the different effects of pantethine and fursultiamine from the viewpoint of plasma gastrointestinal peptide changes. The pharmacological effects of pantethine may be closely related to the changes in plasma CGRP- and VIP-IS levels.

Key words pantethine; calcitonin gene-related peptide; vasoactive intestinal polypeptide; fursultiamine; motilin; substance P

Materials and Methods

Materials Pantethine (Pantosin tablet) was purchased from Daiichi Sankyo Co., Ltd. (Tokyo, Japan). Fursultiamine (Alinamin-F sugar-coated tablet) was purchased from Takeda Pharmaceutical Co., Ltd. (Osaka, Japan). Maize starch (Wako Pure Chemicals Co., Ltd., Osaka, Japan) was used as placebo. Synthetic human CGRP and its fragment (8–37), VIP, motilin and SP were purchased from Peptide Institute, Inc. (Osaka, Japan). Anti-serum to CGRP (14160) was purchased from Peptide Institute, Inc. (Osaka, Japan), VIP (T-4116) from Peninsula Laboratories (California, U.S.A.), and motilin (Y121) and SP (Y150) from Yanaihara Institute (Shizuoka, Japan). All other reagents were analytical reagent grade from commercial sources.

Subjects Five healthy male nonsmoking volunteers aged 25—31 (median 27) years and weighing 58—70 (median 64) kg participated in this study. All subjects had no history of gastrointestinal disease and their baseline fasting plasma levels of CGRP-, VIP-, motilin- and SP-IS were within the normal ranges for healthy subjects reported previously. The study was approved by the Ethics Committee of Oita Medical University. Each subject received information about the scientific purpose of the study, and gave informed consent. No subject received any medication during one month before the study. The subjects refrained from taking food rich in pantothenic acid and vitamin B1 from the day before the.
study. They then fasted for at least 2 h before the study was commenced and during the experiments.

**Study Schedule** We performed an open-labeled study. A single dose of pantethine 600 mg, fursultiamine 100 mg, or placebo was administered orally with 100 ml water. Each subject was administered the three drugs in the order of pantethine, fursultiamine, and placebo at intervals of one month. The doses of pantethine and fursultiamine in this study were the maximum daily doses used in clinical therapy. Venous blood samples (10 ml) were collected before and at 20, 40, 60, 90, 120, 180 and 240 min after administration of pantethine, fursultiamine or placebo. All subjects finished lunch (standardized lunch of less than 800 kcal) before 12:00. Each study was conducted from 14:00 to 18:00, during which the subjects maintained a resting and relaxed state.

**Preparation of Plasma Extracts** Blood samples were collected into chilled tubes containing 500 kallikrein inhibitor units/ml of aprotinin and 1.2 mg/ml of ethylenediaminetetraacetic acid (EDTA). After centrifugation, plasma samples were diluted with 4% acetic acid buffer (pH 4.0), and loaded onto C18 reverse-phase cartridges (Sep-Pak C18; Millipore Corp., Milford, MA, U.S.A.). After washing with 4% acetic acid buffer, plasma peptides were eluted with 70% acetonitrile in 0.5% acetic acid buffer (pH 4.0). Elutes were concentrated by spin-vacuum evaporation, lyophilized and stored at -80 °C until use. The recovery of plasma CGRP-, VIP-, motilin- and SP-IS was greater than 90% using this extraction procedure.15—18)

**Enzyme Immunoassays for CGRP-, VIP-, Motilin- and SP-IS** Plasma peptide levels were measured using a highly sensitive enzyme immunoassay for CGRP,15) VIP,16) motilin17) and SP18) as described previously. The assay was performed by a delayed addition method. An anti-rabbit IgG- (55641, ICN Pharmaceuticals, Inc., Ohio, U.S.A.) coated immunoplate (Nunc-Immuno Module Maxisorp F8, InterMed, Denmark) was used to separate bound and free antigens. Human CGRP fragment (8—37), VIP fragment (11—28), motilin and SP were conjugated with β-galactosidase by N-(ε-maleimido-caproyloxy)-succimide according to the methods of Kitagawa et al.19) The enzyme immunoassays for CGRP-, VIP-, motilin- and SP-IS were specific and highly sensitive, with detection limits of 4.0, 8.0, 0.25 and 0.25 pg/ml, respectively.

**Data Analysis and Statistics** Total release of peptides was calculated as the areas under the plasma concentration–time curves (AUC\(_{0—240}\)) using the trapezoidal method. All values are expressed as means ± standard derivation (S.D.). Differences in plasma peptide-IS levels and AUC\(_{0—240}\) between the pantethine or fursultiamine and placebo groups were analyzed by Dunnett test. A p value less than 0.05 was considered as statistically significant. Statistical analyses were performed using the SPSS software package (version 17.0; SPSS Inc., IL, U.S.A.).

**RESULTS** The plasma CGRP-IS level–time profile and the release of CGRP-IS (AUC\(_{0—240}\)) after a single oral dose of pantethine, fursultiamine or placebo are shown in Fig. 1a and Table 1. Oral administration of pantethine resulted in significant increases in plasma CGRP-IS level at 40, 60 and 120 min (23.5±2.7, 27.8±5.0, 20.9±4.0 pg/ml, respectively) com-

![Fig. 1](image-url)

*Fig. 1. Effects of a Single Oral Dose of Pantethine (●), Fursultiamine (○) or Placebo (□) on Plasma Levels of Calcitonin Gene-Related Peptide (CGRP)- (a), Vasoactive Intestinal Polypeptide (VIP)- (b), Motilin- (c) and Substance P (SP)-Immunoreactive Substance (d)

Values represent means ± S.D., n=5. *p<0.05, **p<0.01, vs. placebo.*
pared with the levels after placebo administration (13.1±2.9, 14.3±1.3, 12.8±1.6 pg/ml, respectively). Furthermore, \(AUC_{0-240}\) was significantly higher after pantethine administration (9368.1±2287.9 pg min/ml) compared with placebo (6410.4±809.0 pg min/ml). Although plasma CGRP-IS level decreased significantly at 120 min after fursultiamine administration (7.9±1.2 pg/ml) compared with placebo (12.8±1.6 pg/ml), the levels generally remained unchanged after fursultiamine administration similar to the profile of placebo, and \(AUC_{0-240}\) was not significantly different from that of placebo.

Figure 1b and Table 1 show the plasma VIP-IS level–time profile and the release of VIP-IS \(AUC_{0-240}\) after a single oral dose of pantethine, fursultiamine or placebo. Pantethine administration resulted in significant increases in plasma VIP-IS level at 40 and 60 min (14.1±5.8, 14.5±8.8 pg/ml, respectively) compared with the levels after placebo administration (1.9±0.4, 2.0±0.8 pg/ml, respectively). The \(AUC_{0-240}\) was also significantly higher after pantethine administration (3702.7±2533.0 pg min/ml) compared with placebo (885.9±87.1 pg min/ml). On the other hand, no significant changes in plasma VIP-IS level and \(AUC_{0-240}\) were observed after the administration of fursultiamine.

The plasma motilin- and SP-IS level–time profiles and the releases of motilin- and SP-IS \(AUC_{0-240}\) after a single oral dose of pantethine, fursultiamine or placebo are shown in Figs. 1c, d and Table 1, respectively. Pantethine and fursultiamine administration did not alter the plasma levels or \(AUC_{0-240}\) of motilin- and SP-IS compared with placebo.

**DISCUSSION**

Recently, the actions of drugs used to treat gastrointestinal diseases have been elucidated pharmacologically from the viewpoints of gastrointestinal peptide levels.6—8) The traditional herbal medicines Dai-kenchu-to and Ninjin-to, which are used to treat postoperative ileus, have been reported to act systemically after absorption and distribution in circulation. Although plasma CGRP-IS level increased drastically after administration of pantethine, none of the subjects reported headache or lightheadedness due to blood pressure reduction by CGRP.24 On the other hand, fursultiamine administration did not induce major changes in plasma CGRP-IS level, except that plasma CGRP-IS level decreased significantly at 120 min after fursultiamine administration compared with placebo. It is possible that the absorbed fursultiamine may affect the CGRP sensory neurons through an as yet unknown mechanism, but there is no published data that could explain this change. However, it would appear that this effect of fursultiamine was minor because \(AUC_{0-240}\) of CGRP after fursultiamine administration was not significantly different from that of placebo. These results suggest that CGRP has little involvement in the action of fursultiamine in improving gastrointestinal motility which has been reported in animal experiments.25 It is likely that fursultiamine is not metabolized to thiamine triphosphate, an active metabolite of fursultiamine, at the time of absorption from the small intestine; as a result the direct action on CGRP sensory neurons in the gastrointestinal mucosa and is released with acetylcholine.26}

<table>
<thead>
<tr>
<th>Drugs</th>
<th>CGRP (AUC_{0-240}) (pg min/ml)</th>
<th>VIP (AUC_{0-240}) (pg min/ml)</th>
<th>Motilin (AUC_{0-240}) (pg min/ml)</th>
<th>SP (AUC_{0-240}) (pg min/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pantethine</td>
<td>9368.1±2287.9*</td>
<td>3702.7±2533.0*</td>
<td>16907.3±6378.0</td>
<td>1169.0±689.8</td>
</tr>
<tr>
<td>Fursultiamine</td>
<td>4693.6±1184.9</td>
<td>1286.1±811.5</td>
<td>18861.7±8065.0</td>
<td>966.9±433.0</td>
</tr>
<tr>
<td>Placebo</td>
<td>6410.4±809.0</td>
<td>885.9±87.1</td>
<td>17883.3±6881.4</td>
<td>1081.1±535.6</td>
</tr>
</tbody>
</table>

Values represent means±S.D., *p<0.05 vs. placebo.

VIP is both a vasodilator and a gastrointestinal motility regulator.19 VIP is considered to be a neurotransmitter released from nonadrenergic, noncholinergic inhibitory neurons in the gastrointestinal tract.26—28) In our study, pantethine administration significantly increased plasma VIP-IS level compared with placebo. This finding suggests that the vasodilating effect of VIP may contribute to the improvement of gastrointestinal motility and ileal function by pantethine. Meanwhile, fursultiamine had no significant effect on plasma VIP-IS level, indicating that VIP may not be involved in the enhancement of gastrointestinal motility by fursultiamine.

Motilin strongly stimulates fundic pouch activity and plays an important physiologic role in ileal contractility.29 Motilin is one of the most important factors controlling the regular occurrence of phase 3 interdigestive migrating contractions.30,31) In our study, pantethine and fursultiamine had no significant effect on plasma motilin-IS levels compared with placebo, suggesting that motilin may not be involved in the increase of gastrointestinal motility and improvement of ileal function by pantethine and fursultiamine.

SP coexists with CGRP in the sensory afferent neurons of the gastrointestinal mucosa and is released with acetyl-
choline in response to depolarizing stimulation in the enteric nervous system. They previously showed that SP neurons project into the myenteric plexus and circular muscle layer and may be involved in the regulation of the ascending contractile component of the peristaltic reflex. Furthermore, administration of tachykinins/SP to healthy volunteers increased intestinal mucosal blood flow. In our study, pantethine and fursultiamine had no significant effect on plasma SP-IS level. These suggest that SP may not be involved in the actions of pantethine and fursultiamine in increasing gastrointestinal motility and improving ileal function. In this study, pantethine administration resulted in significant increase of plasma CGRP-IS but not SP-IS level. While acetylcholine is known to be involved in the release of CGRP from the afferent nerve endings, the control mechanism of SP release by acetylcholine is unclear. Based on this finding, we hypothesize that pantethine increases acetylcholine secretion resulting in the release of CGRP but not SP in blood. On the other hand, fursultiamine may enhance gastrointestinal motility by cholinergic action via non-CGRP-mediated mechanisms.

Comparison of the effects of pantethine and fursultiamine on gastrointestinal motility has not been hitherto documented. This study revealed the differences of pantethine and fursultiamine from the viewpoint of gastrointestinal peptide changes, although it is uncertain whether these effects in healthy volunteers are the same as those in patients. Therefore, further studies are required to investigate the effects in patients with conditions such as ileus.

In conclusion, this study demonstrated the differences of pantethine and fursultiamine from the viewpoint of gastrointestinal peptide changes. A single oral dose of pantethine significantly increased plasma CGRP- and VIP-IS levels compared to placebo, although fursultiamine had no effect on plasma CGRP-, VIP-, motilin- and SP-IS levels. Our results suggest that the pharmacological effects of pantethine may be closely related to the changes in plasma CGRP- and VIP-IS levels, which are related to regulation of gastrointestinal function. Although further studies are needed, our findings may have implication for the treatment and prevention of postoperative ileus.

REFERENCES