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Short communication

Effects of L-tryptophan on gastric emptying evaluated by breath test in relation to gastric accommodation evaluated by Barostat in rats

Masayuki Uchida*, Orie Kobayashi, Chizuru Iwamoto

Food Science Institute, Division of Research and Development, Meiji Co., Ltd., 540 Naruda, Odawara, Kanagawa, 250-0862, Japan

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ABSTRACT

Gastric emptying has been known to correlate the pyloric sphincter contractile function and distention-induced gastric relaxation (gastric accommodation). In the present study, the effects of L-tryptophan on the gastric emptying and accommodation were evaluated by breath test using [1-^{13}C]acetic acid and Barostat study, respectively, in rats. L-Tryptophan significantly decreased Cmax and AUC120min and delayed Tmax, indicating the inhibition of gastric emptying. L-Tryptophan significantly enhanced the gastric accommodation. These findings show that L-tryptophan may inhibit the gastric emptying through the enhanced gastric accommodation. Therefore, L-tryptophan may be useful for the therapy of postprandial dyspepsia, especially for early satiety.

L-Tryptophan is one of the essential amino acids and has been known to inhibit gastric emptying. Waller et al. (1) reported that L-tryptophan delayed gastric emptying in the dog at molar concentrations below those required to stimulate duodenal osmoreceptors. Edelbroek et al. (2) also found that intraduodenal L-tryptophan increased basal pyloric pressure and stimulated localized phasic pyloric pressure waves, suggesting the inhibition of gastric emptying in healthy volunteers.

On the other hand, functional dyspepsia includes nausea, vomiting, abdominal pain, postprandial fullness, bloating and early satiety. In patients with functional dyspepsia, proximal stomach dysfunction has been suggested to be an important mechanism for symptom generation (3,4). Sanaka et al. (5) reported that the delay of gastric emptying may be related to the gastric adaptive relaxation in the proton pump inhibitor therapy, suggesting that the enhancement of gastric adaptive relaxation induces the delay of gastric emptying. These findings show that the inhibitory effect of L-tryptophan on gastric emptying may relate to the gastric accommodation. However, there was no report of L-tryptophan on the gastric accommodation.

Then, in the present study we aimed to clarify the correlation of L-tryptophan between the gastric emptying and accommodation using breath test and Barostat study, respectively.

The following animal studies were performed in accordance with the Guiding Principles for the Care and Use of Laboratory Animals approved by Meiji Co. Ltd.

Male Sprague–Dawley rats (230–280 g) were purchased from SLC (Shizuoka, Japan) and kept for 1 week in a room where the temperature and humidity were kept at 21 ± 2 °C and 55 ± 15%, respectively. The light and dark cycle was 12 h, and the light period was from 7:00 to 19:00. The animals were fasted in mesh cages for 18 h before each experiment in order to prevent coprophagy, but were allowed free access to drinking water during this period.

Breath test for evaluating the gastric emptying was performed according to our previous report (6). Briefly, a desiccator with a volume of 2000 ml was employed so that the rats could move freely within the chamber. The rats were placed in the chamber immediately after the oral administration of the test meal. The air in the chamber was continuously aspirated during experimental period with a pump and the expired air was able to be collected effectively in the breath-sampling bag. The $^{13}CO_2$ levels in the expired air were measured by placing the breath-sampling bags into the sample joint of the UBit-IR300 infrared analyzer (Ohtsuka Electrics Ltd., Osaka). Mixed gas composed of 5% CO_2 and 95% O_2 gas was used as the control. The measured values were represented as $\Delta^{13}CO_2$. Racol (enteral nutritional formulation) was used as a test meal by adding [^{13}C]acetic acid at a dose of 16 mg/kg (2.5 ml/kg).

Gastric accommodation was evaluated by the method previously reported by us (7). Rats were anesthetized by urethane (1.2 g/kg, i.p.). In this study, slightly improved balloon was used. Polyvinyl tube with as adherent polyethylene bag (maximum volume 7 ml; 3 cm maximum diameter) was introduced from the mouth to the

* Corresponding author. Tel.: +81 465 37 3664; fax: +81 465 37 3713.
E-mail address: masayuki.uchida@meiji.com (M. Uchida).
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stomach. Five milliliter of air was injected to the balloon from one of the balloon tube under the other side balloon tube closed for fitting the balloon to the stomach, and the balloon tubes were immediately opened to the air. After 5 min recovery time, the tubes of the balloon were connected to the Barostat (Barostat Distender IIR, G&J Electronics, Toronto, Canada). The pressure of the balloon was changed stepwise, 1, 2, 4 and 8 mmHg, at 1 min intervals. The volume of the balloon increased in a tick by the change of the pressure. The balloon volume just after the change of the pressure increased gradually and reached plateau about 1 min after the change of the pressure. Then, the increased volume was defined as distension-induced gastric adaptive relaxation (gastric accommodation).

L-Tryptophan suspended in the distilled water was administered orally at a dose of 0.5 and 1 g/kg 30 min before breath test and Barostat study. In the control rats Distilled water for injection was administered instead of L-tryptophan.

L-Tryptophan and [1-^{13}C]acetic acid were purchased from Wako Pure Chemical (Tokyo, Japan) and Cambridge Isotope Laboratories Inc. (MA, USA), respectively. Racol (nutrition formulae) and distilled water for injection were obtained from Ohtsuka Pharmaceutical Co. Ltd. (Tokyo, Japan) and Ohtsuka Seiyaku Industry (Tokushima, Japan), respectively.

All results are presented as the mean ± S.E.M. Statistical analyses were performed by using Stat View, Version 5.0.0.0 (SAS Institute Inc., USA), and P values <0.05 (Two-way repeated measures analysis of variance (ANOVA), Bonferroni post test and One-way ANOVA) were considered as statistically significant.

In the control rats, expired $^{13}CO_2$ air increased in course of time after the oral administration of the test meal (Fig. 1). Cmax, Tmax and AUC120min values were 386.6 ± 47.3‰, 28.8 ± 4.8 min and 2512 ± 1823‰ min, respectively (Table 1). By the treatment with L-tryptophan, gastric emptying was significantly inhibited and delayed (Fig. 1). Cmax and AUC120min values were significantly decreased, and Tmax value significantly delayed (Table 1).

In the control group, gastric accommodation increased pressure-dependent manner as shown in Fig. 2. By the treatment with L-tryptophan, gastric accommodation was enhanced pressure-dependent manner, and significant difference was observed at a dose of 1 g/kg as compared with control at 8 mmHg, but not at a dose of 0.5 g/kg (Fig. 2).

Peripheral serotonin (5-hydroxytriptamin: 5-HT) is involved in the regulation of gastrointestinal motility and sensation, whereas centrally it plays a role in mood regulation. It has been known that tryptophan is the substrate of 5-HT. Van Nieuwenhoven et al. (8) reported that lowering 5-HT level in the body using the acute tryptophan deficient method leads to a significantly delayed gastric emptying of a solid meal. Stephens et al. (9) reported that the tryptophan receptor is different from the osmoreceptor on the inhibition of gastric emptying. In addition, Cooke and Ward (10) found that tryptophan slowed gastric emptying by activating a receptor in the gut and not by a direct effect on the stomach or brain or via its major metabolites. These findings show that acute tryptophan deficiency and tryptophan administration showed the same delay of the gastric emptying. These results seem contradictory.

Table 1
Effect of L-tryptophan on the pharmacokinetic parameters of gastric emptying evaluated by breath test using Racol containing [1-^{13}C]acetic acid in rats.

	Cmax (‰)	Tmax (min)	AUC120min (‰ min)
Control	387.8 ± 4.4	30.0 ± 0.0	25,719 ± 340
L-Tryptophan	142.8 ± 16.9**	85.0 ± 5.0**	13,330 ± 1202**

Values represent the mean ± S.E. of used rats (n = 4).
**: Significant difference from the control group (P < 0.01).

In the present study, oral administration of L-tryptophan was found to delay the gastric emptying from the results of Tmax values. In addition, a significant decrease of Cmax and AUC120min values were observed by the oral administration of L-tryptophan, indicating the inhibition of gastric emptying. These results accord with the finding reported by Cooke and Ward (10).

On the gastric accommodation, Fan et al. (11) reported that both proximal gastric area and proximal gastric volume could be useful for assessment of the proximal gastric accommodation for the evaluation of proximal gastric accommodation disorder in patients with functional dyspepsia. In clinical study, Barostat study was considered to be gold standard for evaluating gastric accommodation. We recently improved the method to evaluate gastric accommodation in rats without surgical operation (7). Then in the present study we used our method for evaluating the effect of L-tryptophan on the gastric accommodation, and found that L-tryptophan significantly enhanced the gastric accommodation. Geeraerts et al. (12) reported that acute tryptophan depletion significantly decreased plasma tryptophan levels as compared with control, and that acute tryptophan depletion did not affect gastric sensitivity and compliance but decreased the sensation of nausea during balloon distension, suggesting the enhancement of accommodation in healthy volunteers. These findings show that acute tryptophan depletion significantly enhanced the gastric accommodation. However, in the present study, oral administration of L-tryptophan significantly enhanced the gastric accommodation. Above findings seem contradictory, although the reason of the

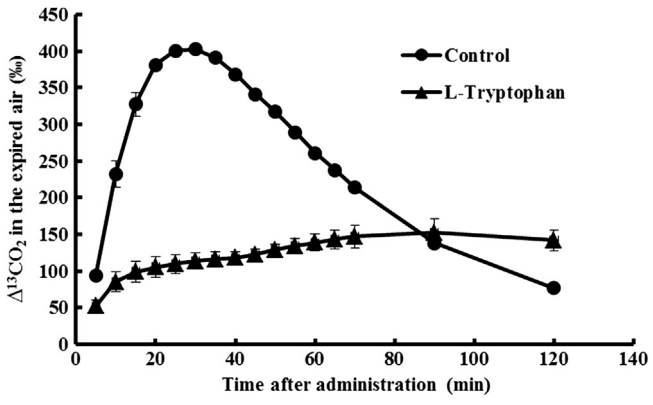

Fig. 1. Effect of L-tryptophan on the time course of expired $^{13}CO_2$ in rats. Symbols represent the mean ± S.E. of used rats (n = 4). Significant difference from the control from 5 to 70 min and 120 min (P < 0.01).

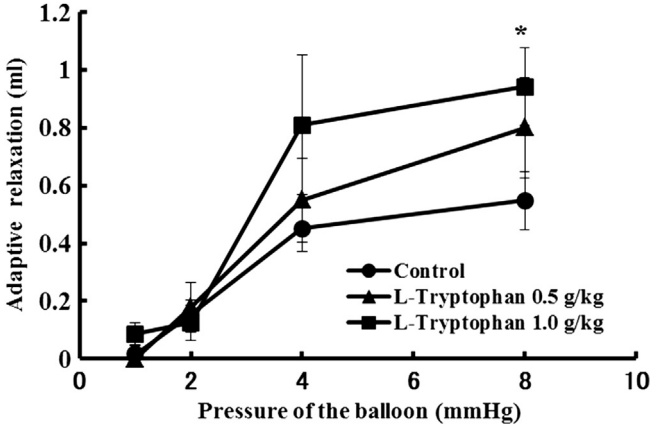

Fig. 2. Effect of L-tryptophan on the distension-induced gastric accommodation. Symbols represent the mean ± S.E. of used rats (n = 5 or 4). *: Significant difference from the control (P < 0.05).

difference is not clear. The acute tryptophan deficiency may act centrally, whereas the oral tryptophan may act peripherally.

5-HT is an important gastrointestinal signaling molecule. Serotonin is synthesized through the actions of L-tryptophan hydroxylase. 5-HT receptor subtypes enable modulate gastrointestinal motility, secretion, and sensation. 5-HT_1 agonists have been reported to enhance gastric accommodation for the treatment of functional dyspepsia (13). To clarify the involvement of 5-HT, it needs to evaluate the effects of 5-HT and 5-HT antagonist against the L-tryptophan-induced gastric accommodation, because L-tryptophan is the substrate of 5-HT.

Sanaka et al. (5) reported that the enhancement of gastric adaptive relaxation induced the delay of gastric emptying in the proton pump inhibitor therapy. In the present study, oral administration of L-tryptophan significantly inhibited gastric emptying and enhanced the gastric accommodation, supporting the report by Sanaka et al. (5). These findings may indicate that the inhibition of gastric emptying by L-tryptophan may be caused by the enhancement of gastric accommodation. The other amino acid which delays gastric emptying was found to enhance the gastric accommodation (data not shown).

In conclusion, the present findings show that L-tryptophan delayed the by gastric emptying through the enhanced the gastric accommodation. Therefore, L-tryptophan may become a useful material for the therapy of postprandial dyspepsia, especially for early satiety, because early satiety is caused by the dysfunction of gastric accommodation.

Conflict of interest

There is no conflict of interest in this study.

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