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Metabolic Degradation of [6]-Gingerol in Rat Jejunal Mucosa

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To investigate the *in vitro* metabolism of [6]-gingerol, one of the major pharmacological constituents of ginger, in the intestine, [6]-gingerol was added to the jejunal mucosal and muscular tissue homogenates isolated from rats in high (50 $\mu\text{g}/\text{ml}$) and low (10 $\mu\text{g}/\text{ml}$) concentrations, and the residual [6]-gingerol concentration was periodically determined. In addition, the stability of [6]-gingerol at 20 $\mu\text{g}/\text{ml}$ in rat whole blood was also examined *in vitro* to compare with the metabolic degradation in rat jejunal tissues. In the incubation mixture of rat mucosal layer with the high concentration, the residual [6]-gingerol was $96.5 \pm 6.5\%$ following 60 min of incubation. For the low that initial [6]-gingerol concentration, [6]-gingerol concentration was significantly decreased to $81.0 \pm 6.1\%$ after 60 min ($p < 0.001$). On the other hand, cumulative amounts of [6]-gingerol decreased in jejunal mucosal homogenates with the high and low initial concentrations for 60 min being 0.236 ± 0.449 and 0.231 ± 0.084 $\mu\text{g}/\text{mg}$ protein, respectively. The residual [6]-gingerol in the incubation mixture of muscular homogenate was about 95–98% at 60 min after the incubation in both the initial concentrations. Furthermore, no change was observed in the [6]-gingerol concentration in the incubation mixture of whole blood. These results suggest that [6]-gingerol may be subject to some metabolic degradation in the mucosal layer of rat jejunum. This first-pass metabolism in the jejunal region would be one of the reasons for the considerable amount which was not recovered as [6]-gingerol in the previous *in situ* absorption study.

Keywords—[6]-gingerol, ginger, gastrointestinal metabolism, *in vitro*, incubation, jejunal mucosa, rat

Introduction

Ginger (*Zingiber officinale* Roscoe) is one of the most useful crude drugs in traditional Chinese herbal medicine¹⁾. It has been known to have various pharmacological and physiological effects, for example, analgesic, antipyretic, antihepatotoxic, cardiogenic and anticonvulsive activities, which have been reported to be predominantly based on [6]-gingerol (Fig. 1), one of the important pungent components of ginger²⁻⁴⁾. Recently, it has been reported that ginger is an effective and promising

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prophylactic antiemetic, which may be especially useful for day case surgery⁵). In our previous studies^{6,7}, we demonstrated that [6]-gingerol was cleared very rapidly from plasma in spite of relatively extensive binding of this compound to serum protein, and that [6]-gingerol was eliminated partly by the liver, but that there was no contribution of renal clearance to the total body clearance of [6]-gingerol. In addition, we investigated the *in situ* jejunal absorption of [6]-gingerol to determine the extent of its intestinal absorption after drug administration into the closed jejunal loop in rats. As the results, [6]-gingerol was substantially absorbed from the jejunal lumen but considerable amount of the drug administered was not recovered as [6]-gingerol from the closed loop system, i.e., the jejunal tissues, jejunal contents and mesenteric venous blood⁸). These findings indicated the possibility that [6]-gingerol might be subject to a first-pass metabolism at the gastrointestinal tract of rats. Gastrointestinal metabolism has been shown *in situ* or *in vitro* for various compounds⁹⁻¹⁵). In the present study, we determined [6]-gingerol remaining during the incubation in the mucosal and muscular tissue homogenates derived from rat jejunum to investigate the metabolism and/or decomposition of this compound in the jejunal region *in vitro*. In addition, stability of [6]-gingerol in rat whole blood was examined to compare with the results obtained from the jejunal tissues.

Fig. 1. Chemical Structure of [6]-Gingerol

Materials and Methods

Materials [6]-Gingerol was obtained from the rhizome of ginger. The details for the methods of isolation and purification of [6]-gingerol have been given in a previous report¹). Bovine serum albumin was purchased from Sigma Chemical Co. (St. Louis, U.S.A.). All other reagents and solvents used were commercially available and of either analytical or liquid chromatographic grade.

Animals Male Wistar rats (Japan SLC Inc., Hamamatsu, Japan), weighing 312 to 398 g (11 to 13 weeks old), were used. All rats were housed in a well-controlled, specific pathogen-free room. In case of collecting jejunum, rats were fasted with free access to water overnight before the operation. Any other rats employed to collect blood samples were cannulated in the right external jugular vein with silicone tubing (i.d. 1.0 mm, o.d. 1.5 mm; Dow Corning, Tokyo, Japan) under anesthesia with pentobarbital (40 mg/kg, i.p.).

[6]-Gingerol Solution [6]-Gingerol was quickly weighed and dissolved in acetonitrile. An aliquot of this solution was taken in a glass-tube and the solvent was completely evaporated under a gentle stream of nitrogen at 40°C. The residue was reconstituted as uniformly dispersed in pH7.4 Krebs-Ringer bicarbonate buffer containing 5% Tween 80. The final concentrations of [6]-gingerol solutions

prepared were 20, 100 $\mu\text{g}/\text{ml}$ and 2 mg/ml .

***In Vitro* Incubation of Jejunal Mucosa, Muscle and Blood with [6]-Gingerol** Immediately after abdominal midline incision (about 5 cm) under pentobarbital anesthesia (40 mg/kg , i.p.), the second and third jejunal segments were isolated by modification of the methods reported previously^{11,14, 15)}. The jejunal mucosal layer was gently scraped off with a slide glass and the residual muscular layer was minced after everting the segment lumen. The wet weights of the jejunal mucosal and minced muscular tissues were carefully measured. One gram (as wet weight) of the tissue was suspended in 10 ml of pH7.4 Krebs-Ringer bicarbonate buffer solution, and then each of these suspension was homogenized for 5 min by using an ultrasonic homogenizer SONIFIER® (Branson Sonic Power Co., Danbury, U.S.A.). Tissue homogenate (10 ml) was added with an equal volume of [6]-gingerol solution of 20 or 100 $\mu\text{g}/\text{ml}$. Under the aeration with 95% O_2 -5% CO_2 , incubation was carried out at 37°C by means of mechanical agitation. For the blank experiments, pH7.4 Krebs-Ringer bicarbonate buffer solution (10 ml) without jejunal tissue homogenate was used to incubate with [6]-gingerol. During the incubation, aliquots (100 μl) of the incubation mixture were collected at 0, 10, 20, 30, 40, 50 and 60 min to determine the [6]-gingerol concentration.

For the incubation study with blood, fresh whole blood was withdrawn through the cannula and collected into the heparinized tubes. Incubation mixture was prepared from whole blood (4.95 ml) with 2 mg/ml of [6]-gingerol solution (0.05 ml). Incubation was carried out in the same condition as described above. Aliquots (100 μl) of the incubation mixture were collected periodically and hemolysed by mixing with the distilled water (100 μl) to determine the whole blood concentration of [6]-gingerol.

Residual percentage of [6]-gingerol was calculated by dividing the residual concentration at each time point by the concentration at time zero. From the blank experiment, the residual [6]-gingerol in the incubation mixture was found to be always about 100% in both drug concentrations. For tissue homogenates, the protein concentrations in the homogenates were determined by the method of Lowry *et al.*¹⁷⁾ to normalize the decreased amount of [6]-gingerol during the incubation by protein contents.

Analytical Procedures [6]-Gingerol concentrations in the incubation mixtures of jejunal mucosa, muscle and whole blood were determined by the HPLC method reported previously⁶⁾. Briefly, 500 μl of acetonitrile was added to 100 μl of the sample. The mixture was centrifuged and 500 μl of supernatant was evaporated at 40°C under a gentle stream of nitrogen. The residue was reconstituted in 75 μl of the mixture of ethanol-distilled water (4:6) and an aliquot (20 μl) was applied onto the liquid chromatograph equipped with ultraviolet spectrophotometric detector SPD-10A (Shimadzu, Kyoto, Japan) set at 280 nm.

Results

Figure 2 shows time-courses for residual percentage of [6]-gingerol at the initial concentration of 50 and 10 $\mu\text{g}/\text{ml}$ in the incubation mixture of jejunal mucosal homogenate. In the high concentration (50 $\mu\text{g}/\text{ml}$), the residual [6]-gingerol was $96.5 \pm 6.5\%$ at 60 min after the incubation, whereas it was significantly decreased to $81.0 \pm 6.1\%$ in the low concentration (10 $\mu\text{g}/\text{ml}$) after 60 min ($p < 0.001$). After 40 min, there were significant differences in the residual percentage between these

Fig. 2. Residual Rate of [6]-Gingerol in the Incubation Mixture of Rat Jejunal Mucosa. Each point represents the mean $\pm$ S.D. of 4-6 rats. a) Significantly different from the initial value ($p < 0.01$). b) Significantly different from the initial value ($p < 0.001$). c) Significantly different from the "High" ($p < 0.01$). d) Significantly different from the "High" ($p < 0.05$).

Fig. 3. Residual Rate of [6]-Gingerol in the Incubation Mixture of Rat Jejunal Muscle. Each point represents the mean $\pm$ S.D. of 5-6 rats.

two initial concentrations.

Figure 3 shows time-courses for residual percentage of [6]-gingerol at the initial concentration of 50 and 10 $\mu\text{g/ml}$ in the mixture of jejunal muscular homogenate. Residual [6]-gingerol percentages in the incubation mixtures at both the initial concentrations were almost the same over the whole time-course and were about 95-98% at 60 min after the incubation. There was no significant decrease of the [6]-gingerol concentration in both the initial concentrations.

Table 1. Cumulative Amount of [6]-Gingerol Decreased for 60 min

Tissue	Initial concentration	Decreased amount ($\mu\text{g}/\text{mg protein}$) ^{a)}
<i>Mucosa</i>	High	0.236 ± 0.449
	Low	0.231 ± 0.084
<i>Muscle</i>	High	0.122 ± 0.355
	Low	0.055 ± 0.146 ^{b)}

a) Each value represents the mean $\pm$ S.D. of 4–6 rats.

b) Significantly different from the result of low concentration in the mucosal homogenate ($p < 0.001$).

Fig. 4. Residual Rate of [6]-Gingerol in the Incubation Mixture of Rat Blood. Each point represents the mean $\pm$ S.D. of 6 rats.

Cumulative amounts of [6]-gingerol decreased for 60 min in the incubation mixtures of jejunal mucosa and muscle are shown in Table 1. Decreased amounts of [6]-gingerol in the jejunal mucosal homogenates with the high and low initial concentrations were 0.236 ± 0.449 and 0.231 ± 0.084 $\mu\text{g}/\text{mg protein}$, respectively, and there was no significant difference between these normalized data. In the muscular homogenates, however, the low concentration induced less decrease in [6]-gingerol amount than the high concentration did. In addition, decreased amount in the muscular homogenate at the low concentration was significantly lower than that in the mucosal homogenate ($p < 0.001$).

Figure 4 shows time-course for [6]-gingerol concentration at the initial concentration of 20 $\mu\text{g}/\text{ml}$ in the incubation mixture of rat blood. Residual [6]-gingerol percentage in the incubation mixture was $105.4 \pm 10.9\%$ at 60 min after incubation.

Discussion

Ginger has been prescribed in about 50% of Chinese medical preparations¹⁾, and it is principal-

ly administered orally. Therefore, it is important to investigate the gastrointestinal absorption of [6]-gingerol, one of the major pharmacological and pungent components of ginger. In our previous study using *in situ* rat jejunal loop⁸⁾, it was observed that about 10% of [6]-gingerol administered appeared in the mesenteric venous blood but the total [6]-gingerol amount detected in jejunal contents, jejunal tissues and mesenteric venous blood was only 60% of the administered amount. These findings suggested that [6]-gingerol would be translocated from the jejunal lumen to the blood through the mucosal and muscular layers and that [6]-gingerol would be decomposed in these jejunal tissues and/or blood. The present work was, therefore, designed to investigate the *in vitro* metabolism or decomposition of [6]-gingerol in rat jejunal mucosa, muscle and blood.

In the first place, metabolic degradation of [6]-gingerol in the incubation mixture of rat jejunal mucosa was investigated. Significant extent of [6]-gingerol was decreased with time in the mucosal incubation mixture with relatively low [6]-gingerol concentration (10 $\mu\text{g/ml}$). It was reported that [6]-gingerol was decomposed in alkaline solution¹⁾. Since no change was observed in the blank experiment in which pH7.4 Krebs-Ringer bicarbonate buffer solution was used in place of the tissue homogenate, it is considered that the decrease of [6]-gingerol concentration when incubated with the mucosal homogenate should be due to the metabolic degradation by the tissue constituents. In contrast to the low concentration, the residual percentage of [6]-gingerol was not significantly changed through 60 min in a high concentration (50 $\mu\text{g/ml}$). However, normalization of the cumulative amount of [6]-gingerol decreased during 60-min incubation by the tissue protein content yielded almost the same value of about 0.23 $\mu\text{g/mg}$ protein for both the high and low concentrations of [6]-gingerol. From these results, it is suggested that [6]-gingerol is metabolically decomposed in the mucosal tissue and this kinetic process may be non-linear. In addition, this process would be saturated at the high concentration employed in the present study. On the other hand, the residual [6]-gingerol amount was not significantly decreased and was not changed at all through 60 min after the incubation by rat jejunal muscular tissue and whole blood, respectively. These results indicate that jejunal muscular tissue has little capacity for metabolizing [6]-gingerol in comparison with jejunal mucosal tissue and that blood has no capacity for it. Approximate extent of 20% of the initial amount as the decrease over 60 min in the mucosal incubation mixture with the low concentration could not sufficiently explain the previous results that 40% of initial amount of [6]-gingerol was not recovered in the *in situ* loop experiment⁸⁾. In general, *in vitro* procedures may give useful information on the relative capacity of mucosal enzymes derived from various regions of the intestine to metabolize drugs on the one hand, cell viability and/or enzyme activity would be affected by various experimental conditions on the other hand. It is considered that the present *in vitro* preparations of jejunal mucosal layer isolated from rats might possibly lose physiological functionality to some extent in comparison with *in situ* preparations.

Drug metabolism in the intestine has been reported for some compounds. For example, glucuronide conjugation of salicylamide occurs in rabbit and rat intestine^{9,10,13,14)}, and the glucuronidation of morphine and narorphine has been observed in the rat intestine as well as liver¹¹⁾. Intestinal decarboxylation was found to be a predominant factor in the overall first-pass metabolism of levodopa in rats¹⁵⁾. These intestinal metabolic processes are considered to occur preferably in the jejunal mucosal layer which has a large part of intestinal drug metabolic enzymes. Considering the facts that

[6]-gingerol is highly lipophilic and that liver contributes largely to the elimination of this compound from plasma, it is likely that [6]-gingerol would be metabolically decomposed in the intestinal mucosal tissue as well as in the liver. Thus, [6]-gingerol is probably subject to metabolic degradation when transported across the mucosal membrane in the intestine.

It has been reported that [6]-shogaol is one of the components of ginger as well as [6]-gingerol^{1,17}. Surh and Lee¹⁸ reported that [6]-shogaol was transformed into 1-(4-hydroxy-3-methoxyphenyl)-decan-3-one (paradol) and 1-(4-hydroxy-3-methoxyphenyl)-decan-3-ole (reduced paradol) by a reductive metabolic system when it was incubated with the rat liver cytosolic fraction in the presence of NADPH or NADPH-generating system. Reductive metabolic system fortified with NADPH is also identified in small intestine¹⁹. Since [6]-gingerol is a saturated ketone which has a similar structure to [6]-shogaol, it may be metabolized to the corresponding saturated alcohol, 1-(4-hydroxy-3-methoxyphenyl)-decan-3,4-ole(gingediol)¹⁸, by the reductive metabolism in a similar fashion to that of [6]-shogaol.

In conclusion, it is suggested that [6]-gingerol may be subject to decomposition, probably metabolic degradation, in the mucosal layer of rat jejunum. This possible first-pass metabolism in the jejunum would be one of the reasons for the considerable amount which was not recovered as [6]-gingerol in the previous *in situ* absorption experiments.

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