Strain Gauge Force Transducer and Its Application in a Pig Model to Evaluate the Effect of Probiotic on Colonic Motility

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Summary The aim of this study was to investigate the effect of probiotic, i.e., fermented milk prepared with Lactobacillus casei strain Shirota, on colonic motility by the strain gauge force transducer (SGFT) in a pig model. The contractions of the circular muscle layer of the cecum, upper colon, lower colon, and terminal colon in pigs were directly measured in conscious status by this method. This method was useful for quantitatively evaluating the effects of stimuli on colonic motility. Feeding significantly stimulated the motilities of the upper and lower colon. Defecation significantly stimulated the motilities of the upper and terminal colon. Two weeks' feeding of the fermented milk significantly activated the response to feeding in four portions of the large intestine. It increased motility of the terminal colon that did not promote defecation. The frequency of defecation from 9:00 to 10:00 (the period just after the morning meal) increased significantly, but from 0:00 to 1:00 (the midnight period) it decreased as a result of the ingestion of fermented milk. Such effects of the fermented milk on motility of the terminal colon are discussed in relation to the movement of digesta. The effects may relate to the stimulation of colonic fermentation as shown by a decrease in fecal pH.

Key Words strain gauge force transducer (SGFT), colon, motility, probiotic

Probiotics and prebiotics, which are defined respectively as the supplement of live bacteria, e.g., Bifidobacteria and Lactobacilli, and as the supplement of a specific substrate for health-promoting bacteria, i.e., Bifidobacteria, have beneficial effects on health (1-6). They are applied to prevent and treat diarrhea and constipation. The major targets of these products are therefore control of the transit of digesta and defecation frequency. However, their effects on colonic motility are still obscure largely because of the technical limitations in the methodology. Indeed, difficulties in measuring colonic movement under conscious conditions are apparent (7-9). Furthermore, inaccessibility of the colon by the usual methods has prevented scientists from measuring colonic motor events (7-9).

A strain gauge force transducer (SGFT) is a potent method of measurement (7-14). By the use of SGFT, the contraction of intestinal muscle, especially the circular muscle layer, can be measured directly. Moreover, SGFT is advantageous because it can be used to measure intestinal motility during physiological activity with minimal interference (8). However, the studies have been limited to dogs and rats, and these animals seem inappropriate for a human colonic model because of significant differences in the anatomy and function of the large intestine (7, 8, 15). One of the best models for the physiological study of the human colon is the pig because of its similarities in diet, anatomy at a microscopic level, contractile activity, and habits of defecation (7, 16, 17). The size of a pig’s large intestine is another advantage for the application of SGFT. The length and width easily accommodate the proper placement of transducer probes.

We tried during this experiment to establish a methodology for the application of SGFTs in the pig model. We investigated the effect of probiotic, i.e., fermented milk prepared with Lactobacillus casei strain Shirota, on colonic motility by this method.

MATERIALS AND METHODS

Chemicals. Chemicals used through this experiment were purchased from Wako Pure Chemical Industries, Osaka, Japan, or Nacalai Tesque Inc., Kyoto, Japan, unless otherwise noted.

Surgical preparation of experimental animals. Five 10-week-old female pigs (Landrace×Large white×Durock crossbred) weighing about 20 kg each were used. They were anesthetized with halothane (fluothane: Takeda Chemical Industries, Osaka, Japan) and incised with a midline. SGFTs (F-12I5; Star Medical Inc., Tokyo, Japan) were sutured onto the surface of the cecum, upper colon, lower colon, and terminal colon to measure the contractions of the circular muscle layer (Fig. 1). One

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351
transducer was placed in the middle of the cecum. The transducers on the upper colon and the lower colon were fixed about 1 m and 2 m distal to the junction of the cecum and colon, respectively. The transducer on the terminal colon, which was 3 to 5 cm proximal to the pelvic cavity, was placed at about 20 cm proximal to the anus.

The wires of the transducers were guided from the body between the 14th and 15th ribs. They were then connected to a WPS-16PA connector (Star Medical Inc.) that was placed in a jacket on the pig. The jacket was necessary to prevent the materials from being scratched. The wires were connected to a personal computer.

The pigs were housed individually in cages in a light-controlled room (09:00–21:00 light; 21:00–09:00 dark) and fed 400 g meals twice a day at 09:00 and 21:00. The meals consisted of cracked maize (65.3%), soybean meal (18.7%), alfalfa meal (9.3%), wheat bran (3.7%), meat bone meal (1.9%), CaCO3 (0.5%), NaCl (0.3%), and a vitamin mineral premixture (0.3%, Tonmisshu 1). These feed ingredients were purchased from Kumiai Shiryo (Kobe, Japan). Water was accessible to the pigs at all times. All animals were handled with due regard for their welfare, as approved by the Experimental Animal Care and Use Committee, Kyoto Prefectural University, Kyoto.

Experimental schedule. The pigs recovered two weeks after surgery. After recovery, the motility of the large intestine was continuously recorded on a computer for three days. Each defecation was observed and recorded. All feces from the first day of the recording period were collected for weight and pH measurement. Measurements were taken immediately after the feces were collected. The pigs were thereafter given 130 mL of commercially available fermented milk containing Lactobacillus casei strain Shirota (Yakult, Yakult Honsha Co., Ltd., Tokyo, Japan) with the morning meal for 17 d. This amount of fermented milk (130 mL) contained more than 10^{10} L. casei strain Shirota. The motility of the large intestine and defecation time were recorded during the last three days, and feces were collected and treated as described above.

Recording and analysis of motility of the large intestine. The contraction signals of the circular muscle layer of the cecum and colon were recorded on a computer through an amplifier (MS-08M, Star Medical Inc.). The recorded data were analyzed by computer software (Eight Star, Star Medical Inc.) to calculate the Motor Index (MI) that was the sum of areas between the lines of the base and contraction waves. The MI of each 1 h period was then calculated, and the MI for a 1 h period before defecation was compared to that after defecation to evaluate the response of the large intestine to feeding. To evaluate the response of the large intestine to defecation, the MI for a 0.5 h period, from 15 min before to 15 min after defecation, was calculated. These were compared to the mean MI of a 0.5 h period, excluding the defecation period, from the record of the 24 h period.

The total daily MI was calculated from the records of a 24 h period, and a mean daily MI was further calculated from three whole-day MI values. In this study, the MI for each 1 h period was expressed as a percentage of the total MI, which refers to the MI ratio. These parameters were compared during two experimental periods to evaluate the effects of the fermented milk on the motility of the large intestine.

Statistical analyses. The results were expressed as means for five animals with standard deviations. Statistical analyses were done by Tukey’s LSD after one-way ANOVA.

RESULTS
Response to feeding (Fig. 2)
The MI for the 1 h period after feeding was significantly higher than that before feeding in the upper and lower colon for both day and night (p<0.01 in the day, p<0.05 in the night). The same tendency was observed at the cecum and terminal colon with insignificant manner (p>0.05). The MI for the 1 h period after feeding was significantly increased by the administration of the fermented milk in four portions of the large intestine. The differences were statistically significant for the cecum and the upper and lower colons during the day (p<0.05), whereas they were significant for the cecum (p<0.01) and terminal colon (p<0.05) in the night.

Response to defecation (Fig. 3)
The MI for the 0.5 h period was higher during the defecation period in four portions of the large intestine than the mean MI for the 0.5 h period calculated from data during the nondefecating phase before administration of the fermented milk. The difference was significant for the upper and terminal colon (p<0.05), but insignificant for the remaining two portions. However, the MI for the 0.5 h at defecation in three portions of the colon was not different from the mean MI during supplementation of the fermented milk.
Effect of Probiotic on Colonic Motility

Fig. 2. Large-intestine response to feeding in pigs before and during administration of the fermented milk. (A) 09:00 meal and (B) 21:00 meal. Motor activities were expressed as a relative motor index (MI). MIs for 1 h periods before feeding (open bar) and during feeding (solid bar) were presented (MI before feeding = 1). Values are means and SD for five animals; *, p<0.05; **, p<0.01. For details, see text.

Fig. 3. Large-intestine response to defecation in pigs before and during administration of the fermented milk. Motor indexes for 0.5 h at defecation (solid bar) and in nondefecating state (open bar) are presented (MI in nondefecating state = 1). Values are means and SD for five animals; *, p<0.05. For details, see text.

Hourly MI ratio (Fig. 4 and Table 1)

Figure 4 shows the 24 h transition of the MI ratio of the cecum and the upper, lower, and terminal colon before and during administration of the fermented milk. The MI ratio during the day (from 09:00 to 21:00) was significantly higher than during the night (from 21:00 to 09:00) in the upper colon (p<0.05) and terminal colon (p<0.01) before administration of the fermented milk (Table 1).

The transitions of the hourly MI ratio in the cecum and upper and lower colon during the day were not influenced so much by the supplementation of the fermented milk. The administration of the fermented milk decreased the MI ratio during the day in the terminal colon.

Fecal analyses

The fermented milk did not significantly influence the daily defecation frequency (times/d), daily fecal excretion (g/d), and mass of individual feces (g/feces) (Table 2). However, the feeding of the fermented milk after the morning meal (from 09:00 to 10:00) caused a significant increase in the frequency of defecation (0.13 vs.
Fig. 4. Effects of *L. casei* strain Shirota-fermented milk on the hourly MI ratio (%) in the cecum, upper colon, lower colon, and terminal colon of pigs. Total motor index per 24 h = 100. For calculation, see text. C, cecum; UC, upper colon; LC, lower colon; TC, terminal colon; broken line, before administration; solid line, during administration.

Table 1. Effects of *L. casei* strain Shirota-fermented milk on MI ratio (%) in the day and in the night in the large intestine of pigs.

<table>
<thead>
<tr>
<th></th>
<th>Before administration</th>
<th>During administration¹</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Day²</td>
<td>Night³</td>
</tr>
<tr>
<td>Cecum</td>
<td>49.8 ± 5.6</td>
<td>50.2 ± 5.6</td>
</tr>
<tr>
<td>Upper colon</td>
<td>52.6 ± 4.9</td>
<td>47.7 ± 4.9*</td>
</tr>
<tr>
<td>Lower colon</td>
<td>51.0 ± 7.4</td>
<td>49.0 ± 7.4</td>
</tr>
<tr>
<td>Terminal colon</td>
<td>58.3 ± 8.2</td>
<td>41.7 ± 8.2*</td>
</tr>
<tr>
<td></td>
<td>49.3 ± 4.3</td>
<td>50.7 ± 4.3</td>
</tr>
<tr>
<td></td>
<td>50.8 ± 7.8</td>
<td>49.2 ± 7.8*</td>
</tr>
<tr>
<td></td>
<td>49.6 ± 5.3</td>
<td>50.4 ± 5.3</td>
</tr>
<tr>
<td></td>
<td>45.3 ± 10.0</td>
<td>54.7 ± 10.0*</td>
</tr>
</tbody>
</table>

¹ *L. casei* strain Shirota-fermented milk was orally administrated to pigs for two weeks.

² Day, from 09:00 to 21:00.

³ Night, from 21:00 to 09:00.

Total motor index per 24 h = 100. For details, see text. Values are the means and SD for five pigs; *, p<0.05 (day vs. night); #, p<0.05 (before administration vs. during administration).

Table 2. Effects of *L. casei* strain Shirota-fermented milk on the defecation frequency, fecal mass, and fecal pH.

<table>
<thead>
<tr>
<th></th>
<th>Before administration</th>
<th>During administration¹</th>
</tr>
</thead>
<tbody>
<tr>
<td>Frequency (times/d)</td>
<td>3.9 ± 2.1</td>
<td>3.3 ± 1.4</td>
</tr>
<tr>
<td>Daily excretion (g/d)</td>
<td>358.0 ± 85.0</td>
<td>327.2 ± 86.9</td>
</tr>
<tr>
<td>Individual excretion (g/time)</td>
<td>100.3 ± 8.7</td>
<td>119.4 ± 21.9</td>
</tr>
<tr>
<td>pH</td>
<td>6.24 ± 0.39</td>
<td>5.87 ± 0.18*</td>
</tr>
</tbody>
</table>

¹ *L. casei* strain Shirota-fermented milk was orally administrated to pigs for two weeks.

Weight and pH of feces were measured on day 1 in the sampling period. For details, see text. Values are the means and SD for five pigs; *, p<0.05 (before administration vs. during administration).
0.73 time/h). Inversely, the defecation frequency decreased (0.33 vs. 0.00) in the midnight hour (from 0:00 to 1:00). The pH of feces decreased significantly as a result of the feeding of fermented milk (p<0.05) (Table 2).

**DISCUSSION**

It is well known that colonic motor activity increases with eating (8, 9, 15, 16). Our present results on the upper and lower colons agree with other published reports. However, motor activities of the cecum and the terminal colon tended to increase with feeding. Chemical and physical characteristics of digesta, i.e., water content and the concentration of organic acid, are dependent on a portion of the colon (18–20). These factors are assumed to affect the control of colonic motility (15). For example, it is probable that higher amplitude contractions were required to propel hard feces with a low-water content. In our study, motor activities in the terminal colon were stimulated by defecation (Fig. 3). This increased motor activity agreed with the observation of high-amplitude contractions during a dog's defecation (9, 11). Increased motor activity in our study, therefore, corresponds to giant migrating contractions. However, motor activities in the cecum and upper and lower colon were not stimulated by defecation. The motility of these portions may not be strongly affected by defecation because the major function of these portions, which is fermentation, absorption of water and electrolytes, retention of digesta, and fecal mass formation, must not be disturbed by defecation. In the case of terminal colon, contractions that were not associated with defecation were observed. The function of these contractions may be to retain digesta and to compact fecal mass.

The MI ratio for 12 h was lower during the night than during the day for the upper, lower, and terminal colon. This agrees with the previous observations in man and pig in which colonic motor activity decreased in the night (7, 16, 21, 22). Pigs continued to sleep nearly the entire night in this experiment. Therefore sleeping may lower the motility of the colon, which leads to a low frequency of defecation. However, the MI ratio for 12 h in the cecum did not decrease as it did in the colon during the night. This may be explained by the inflow of digesta into the cecum that originated from the 21:00 meal. According to Clemens et al. (23), a liquid digesta marker in pigs of 180 kg body weight reached the cecum 2 to 4 h after the feeding, whereas a solid digesta marker took 4 h after administration with diet. The arrival of digesta within those times should elicit motor activity that does not promote defecation. The activation of cecal fermentation by fermented milk had been anticipated because of a decline in the pH value of feces. The fermented milk also affected the response of the terminal colon to defecation. Little defecation was observed during the night as fermented milk was administered, though MI ratio during the night increased. The fermented milk appeared to elicit motor activity that does not promote defecation. An increase in the frequency of defecation observed from 09:00 to 10:00 explains the specific digesta movement and motor activity in the terminal colon when the fermented milk was fed; digesta (or feces) seemed to be retained longer in the terminal colon during the night as a result of the fermented milk, and the motor activity in the terminal colon was stimulated by the retained digesta. In other words, these motor activities may play a role in the retention of digesta and the compaction of feces. This may cause a delay in the transit of digesta.

The direct measurement of the colonic contractions by the SGFT method in pigs was successful in this study. This method was useful for quantitatively evaluating the effects of probiotic on colonic motility.

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