Effect of Polycarbophil on the Absorption of Nutrients

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The effects of polycarbophil on the absorption of various nutrients were evaluated by several in situ methods. Polycarbophil reduced the absorption of 3-O-methyl-D-glucose (3-OMG) and L-phenylalanine in the in situ loop and the in situ perfusion methods, but it did not affect the absorption of these nutrients in an open system, the in situ modified loop method, which is closer to physiological conditions. It also did not affect the absorption of vitamin A or phosphatidylycholine-1,2-dipalmitoyl in the latter system. These results indicate that the absorption of nutrients is probably not altered by polycarbophil under physiological conditions.

Key words calcium polycarbophil; polycarbophil; nutrient absorption; in situ modified loop method

Materials and Methods

Calcium polycarbophil is a macromolecular water-absorbing polymer under evaluation as a candidate drug for the treatment of diarrhea and/or constipation in irritable bowel syndrome (IBS).1) Calcium polycarbophil is decalcified under acidic conditions in the stomach, and the resultant polycarbophil produces the pharmacological effects.1,2) Polycarbophil also has a bioadhesive character.3,4) However, the polymer adsorbs some compounds in an irreversible manner, and it has been suggested that it might reduce the absorption of nutrients or drugs.5,6)

The objective of the present study was to establish whether the absorption of various nutrients is inhibited by polycarbophil. We evaluated the effects of polycarbophil on the absorption of a sugar (3-O-methyl-D-glucose, 3-OMG), an amino acid (L-phenylalanine), a vitamin (vitamin A) and a lipid (phosphatidylycholine-1,2-dipalmitoyl) using several in situ methods.

Materials and Methods

Chemicals Polycarbophil was prepared in our laboratory from calcium polycarbophil (Lee Laboratories, U.S.A.) according to the following procedure. Calcium polycarbophil was decalcified with 0.1 N hydrochloric acid and washed with purified water, and the polycarbophil thus obtained was freeze-dried. [3H]-3-OMG (sp act 3241.2 GBq/mmol), [3H]-L-phenylalanine (sp act 1838.9 GBq/mmol), [3H]-vitamin A (sp act 721.5 GBq/mmol) and [3H]-phosphatidylycholine-1,2-dipalmitoyl (sp act 1850 GBq/mmol) were purchased from Amersham Co., Ltd. [14C]-Polyethylene glycol 4000 (PEG 4000, sp act 2.22 MBq/mmol), used as a non-absorbable marker, was purchased from New England Nuclear Corp. All other regents were of analytical grade.

Animals Male Wistar rats, weighing 200–300 g, were used. Animals were acclimatized to the breeding environment for at least 1 week and were starved for at least 20 h prior to use.

Buffer Solutions The isotonic buffer solution used for jejunal loop and perfusion was a modified 2-(N-morpholino)ethanesulfonic acid (MES) buffer adjusted to 290 mOsm/kg with 85 mM mannitol and adjusted to pH 6.5 with Tris. Modified MES buffer contained 5 mM KCl, 100 mM NaCl, 10 mM MES and 0.01% PEG 4000 traced with [14C]-PEG 4000 (0.185 kBq/ml). The isotonic buffer solution used to investigate the influence of sodium ion concentration on the effect of polycarbophil was modified to contain a sodium ion concentration of 100 or 60 mM and a mannitol concentration of 85 or 160 mM. [3H]-Nutrients were dissolved in these buffers and polycarbophil was emulsified with these buffers at a concentration of 1%.

Absorption of Nutrients The studies of the effects of polycarbophil on the absorption of nutrients were conducted by means of the in situ loop, the in situ perfusion and the in situ modified loop methods. Animals were anesthetized by intramuscular injection of carbamic acid ethylester (1.5 g/kg body wt.). About 30 min after the injection, a central longitudinal incision was made into the abdominal wall and the small intestine was exposed.

In the in situ loop experiment, the loop was made between 2–4 cm distal to Treitz's ligament and 22 cm distal to the proximal end. The intestine was flushed with isotonic saline, then the saline left in the lumen was expelled with air and the cannulas were removed. A syringe was inserted into the distal end and a point 1 cm proximal to the distal end was ligated with a silk suture. A syringe containing 1 ml of the drug solution ([3H]-3-OMG, 55.5 kBq/ml) was inserted into the proximal end and a point 1 cm distal to that end was ligated, then the solution was injected into the loop. The contents of the loop were thoroughly mixed for 5 min using the syringes and 0.1–0.5 ml of the contents was drawn out as the time 0 sample. After removal of the syringes and ligation of both ends of the loop with silk sutures, the loop was replaced inside the abdominal cavity. The loop was isolated 30 min after the injection and the contents were collected.

The in situ modified loop method used in the present study was as follows. After ileocecal ligation with a silk suture, 1 ml of the drug solution (3-OMG, 55.5 kBq/ml; L-phenylalanine, 55.5 kBq/ml; vitamin A, 37 kBq/ml; phosphatidylycholine, 92.5 kBq/ml) was injected into the duodenum using a syringe and the modified loop (from duodenum to ileum) was replaced inside the abdominal cavity. The loop was isolated 1 h after the injection and the contents were collected.

The in situ perfusion experiment was conducted according to the method of Lu et al.7) Polyethylene tubing

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was inserted 2—4 cm distal to Treitz’s ligament and 8 cm distal to the first cannula. The intestine was flushed with isotonic saline, then the saline left in the lumen was expelled with air and the cannulas were removed. Polyethylene tubing for the perfusion was inserted to a position 1 cm from each incision and ligated; therefore, the length of the intestine available for perfusion was 6 cm. Each perfusion solution containing $^{3}$H]-3-OMG or $^{3}$H]-L-phenylalanine in 1.85 or 1.11 mcg/ml was pumped through the inlet tubing at a flow rate of 200 ml/min (perfusion pump Model SJ-1210, Miyumi Scientific Inc., Japan). Samples from the outlet tubing were collected every 10 min for 3 h.

To maintain the body temperature at 36 to 37°C, the animals were warmed with lamps until the designated time in these in situ methods.

**Measurement of Sodium Ion** Intestinal fluids were collected by centrifugation at 3000 rpm for 10 min from loops in the same manner as in the in situ loop and the in situ modified loop experiments. The concentrations of sodium ion in the intestinal fluid were measured by an automated electrolyte analyzer, model 710 (Hitachi, Japan).

**Measurement of Radioactivity** A part of the intestinal fluids after administration of $^{3}$H]-nutrient and $^{14}$C]-PEG 4000 was taken into a vial and Creasol I cocktail (Nakarai Tesque Inc., Japan) added. Scintillation counting was carried out with an LSC-1000 liquid scintillation counter (Aloka Co., Ltd., Japan).

**Analytical Methods** Recoveries (%) of $^{3}$H]-labelled nutrients by the in situ loop and in situ modified loop technique were determined according to Eq. 1.

$$\text{Recovery} (\%) = \frac{R_{\text{actual}}}{D_{\text{nutrient}}} \times \frac{D_{\text{PEG 4000}}/R_{\text{PEG 4000}} \times 100}{1}$$

where $R$ and $D$ represent the recovered amount at time $t$ and the dose, respectively.

Recovery (%) of nutrients was obtained from the difference between the inlet and the outlet concentrations of radioactivities in accordance with Eq. 2.

$$\text{Recovery} (\%) = \left(1 - \frac{CH_{\text{outlet}}/CH_{\text{inlet}}} {CC_{\text{total}}/CC_{\text{outlet}}} \right) \times 100$$

where $CH$ and $CC$ represent the radioactivity concentrations of $^{3}$H]-nutrients and $^{14}$C]-PEG 4000 in the perfusate, respectively.

An unpaired $t$ test was used to compare the mean values between treatments for groups of rats and controls. The criterion for a statistically significant difference was $p < 0.01$.

**RESULTS AND DISCUSSION**

**Effect of Polycarbophil on the Absorption of Nutrients**

**Determined by the in Situ Perfusion Method and by the in Situ Loop Method**

Generally water-absorbing polymers such as polyacrylic acid are known to swell after absorbing water owing to the difference of osmotic pressure produced by uptake of electrolytes such as sodium ion as a driving force. Therefore, the effects of polycarbophil on the absorption of 3-OMG and L-phenylalanine, known to be cotransported with sodium ion, were evaluated by the in situ loop and the in situ single-pass perfusion methods. In the in situ perfusion method, the recovery (%) of L-phenylalanine and 3-OMG is shown in Table 1. The recoveries of 3-OMG and L-phenylalanine in 1% polycarbophil-treated rats increased in comparison with those in the control rats, suggesting that the absorption of these nutrients was significantly reduced by polycarbophil under this experimental condition.

Sodium concentrations in the supernatant and the gel were determined to be 59.7 and 122.7 mm, respectively, when 1% polycarbophil was placed in the MES buffer containing 99.8 mm sodium ion used for perfusion. Therefore, the influence of sodium ion concentration on the effect of polycarbophil in the in situ perfusion method was examined at sodium ion concentrations of 60 and 100 mm. As shown in Table 1, the recovery at 60 mm Na$^+$ in the absence of polycarbophil was identical with that obtained in the presence of 100 mm Na$^+$ with 1% polycarbophil, suggesting that the increase of recovery in 1% polycarbophil-treated rats is due to the decrease of sodium ion.

Next, the effect of polycarbophil on 3-OMG absorption was tested by the in situ loop method. As shown in Table 1, the recovery in 1% polycarbophil-treated rats was increased in comparison with that in the control rats, indicating that the absorption of 3-OMG was significantly suppressed by polycarbophil under this experimental condition. The concentrations of sodium ion determined at 30 min were 104.0 ± 1.7 (the mean ± S.E.) and 75.5 ± 1.6 mm, respectively, when the control MES buffer solution and 1% polycarbophil were applied to the closed loops. These results indicate that the increase of recovery in 1% polycarbophil-treated rats is due to the decrease of sodium ion.

**Table 1. Recoveries of $^{3}$H]-Nutrients by the in Situ Perfusion Method and by the in Situ Loop Method**

<table>
<thead>
<tr>
<th>Methodology</th>
<th>Nutrient</th>
<th>Recovery (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>1% Polycarbophil</td>
</tr>
<tr>
<td></td>
<td>(100 mm Na)</td>
<td>(60 mm Na)</td>
</tr>
<tr>
<td>In situ perfusion$^a$</td>
<td>3-OMG</td>
<td>90.4 ± 1.4</td>
</tr>
<tr>
<td></td>
<td>1-Phenylalanine</td>
<td>85.5 ± 0.9</td>
</tr>
<tr>
<td>In situ loop$^b$</td>
<td>3-OMG</td>
<td>86.1 ± 0.9</td>
</tr>
</tbody>
</table>

$a$) Determined every 10 min for 3 h after perfusion. $b$) Determined 30 min after administration. Each value represents the mean ± S.E. of three to five rats. *Significantly different from the control value ($t$ test, $p < 0.01$).

**Effect of Polycarbophil on the Absorption of Nutrients**

**Determined by in Situ Modified Loop Method**

Though polycarbophil reduced the absorption of L-phenylalanine and 3-OMG in both the in situ loop and the in situ perfusion methods, ample sodium ion would be supplied from gastric juice, bile, pancreatic juice and intestinal juice under physiological conditions. So, the effect of polycarbophil on the absorption of nutrients was further examined by the in situ modified loop method. Under this experimental condition, the recovery of 3-OMG in 1% polycarbophil-treated rats was identical with that in the control, indicating that the absorption of 3-OMG was not affected by polycarbophil in this case (Table 2). For L-phenylalanine also, the recovery was not different from
Table 2. Recoveries of [3H]Nutrients by the in Situ Modified Loop Method

<table>
<thead>
<tr>
<th>Nutrient</th>
<th>Recovery (%)&lt;sup&gt;a&lt;/sup&gt;</th>
<th>1% Polycarbophil</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>41.9 ± 1.5</td>
</tr>
<tr>
<td>3-OMG</td>
<td>26.2 ± 1.2</td>
<td>27.2 ± 1.3</td>
</tr>
<tr>
<td>l-Phenylalanine</td>
<td>58.0 ± 1.2</td>
<td>57.1 ± 2.2</td>
</tr>
<tr>
<td>Vitamin A</td>
<td>49.8 ± 5.5</td>
<td>54.0 ± 5.7</td>
</tr>
</tbody>
</table>

<sup>a</sup> Determined 1 h after administration. Each value represents the mean ± S.E. of three to five rats.

the control (Table 2), suggesting that the absorption of l-phenylalanine was also not affected by polycarbophil in this case. Next, the effects of polycarbophil on the absorption of vitamin A and phosphatidylycholine-l-α-dipalmitoyl, known to be absorbed by sodium ion-independent transport mechanisms,<sup>11,12</sup> were evaluated by the in situ modified loop method. As shown in Table 2, the recovery of these two substances in controls were not significantly different from those in 1% polycarbophil-treated rats. These results suggest that polycarbophil does not influence the absorption of either vitamin A or phosphatidylycholine-l-α-dipalmitoyl from the intestine. In the in situ modified loop method, the sodium ion concentrations 1 h after applying 1% polycarbophil solution with 100 mM Na<sup>+</sup> and the control MES buffer to the loops were 138.2 ± 7.6 and 131.5 ± 4.1 mM, respectively. Therefore, it was confirmed that sufficient sodium ion was supplied in the intestine from body fluids under physiological conditions.

In conclusion, polycarbophil did reduce the absorption of 3-OMG and l-phenylalanine in the in situ loop and the in situ perfusion methods, but did not affect the absorption of these nutrients, or vitamin A or phosphatidylycholine-l-α-dipalmitoyl in an open system (the in situ modified loop method), which is closer to physiological conditions. These results indicate that the absorptions of nutrients after oral administration are unlikely to be significantly altered by polycarbophil.

REFERENCES