Beneficial Effect of Dietary Fiber on the Upper Gastrointestinal Transit Time in Rats Suffering from a Toxic Dose of Amaranth

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Gobo dietary fiber (GDF) obtained from the roots of edible burdock (Arctium lappa L.) was examined for its protective role against amaranth (Food Red No. 2, Am) toxicity in the upper gastrointestinal tract (from the mouth to the ileal end) of rats. Ileorectostomized rats were examined for their growth response to feeding with a purified basal diet containing 4% Am with or without 7.5% GDF. The transit half-time (TTs0) through the upper gastrointestinal tract was also examined with ileostomized rats by recovering the small intestinal contents from the ileal end, using Cr-EDTA as a nonabsorbable water-soluble marker. Although feeding Am to the ileorectostomized rats resulted in a 50% mortality, concurrent feeding of Am and GDF not only protected the rats from death but also significantly promoted their growth rate when compared to the effect on the survivors fed only with the Am-containing diet. The results of TTs0 measurements on ileostomized rats showed that the TTs0 was decreased by half in the presence of Am, but was restored to the value for the control without Am when the Am-containing diet was supplemented with GDF. These and previous results imply that Am toxicity develops mainly in the upper gastrointestinal tract as a result of decreased availability of nutrients that is produced by the rapid transit and inhibitory effect of Am on the digestion-absorption process. The beneficial effect of GDF appears to be in normalizing the rapid transit through the upper gastrointestinal tract of chyme containing Am and not in the cecum or colon.

Ershoff and Thurston11 first reported that dietary fiber counteracted the toxic effect of Am when rats were fed with a purified diet containing 5% Am. They suggested in a later report23 that the protective effect of the dietary fiber was mainly due to its anion exchange capacity. This assumption was based on the observation that such an anion exchange resin as cholestyramine also had anti-Am activity.21 We9 have previously measured the capacity of various dietary fibers and activated charcoal to bind Am at different concentrations of the dye (20, 50, and 200 ppm) in vitro and found that the active dietary fibers did not bind sufficient amount of the Am to fully account for their anti-Am activity. Although activated charcoal showed, as expected, the highest capacity to adsorb Am, it had no anti-Am effect on rats.

Our previous observation indicated that the restoration of growth found in rats concurrently given Am and GDF was achieved in part by an improvement in the protein digestibility that was decreased by Am.4 However, dietary fiber had no protective effect on such digestion-absorption processes in vitro as protein digestion, membrane digestion of dipeptides and transmural L-valine transport when it was added to an assay medium containing Am.5 In other words, it seemed that dietary fiber exerted its beneficial effect only when it passed through the intestinal lumen together with chyme containing Am in the whole animal. We speculated from these observations that the regulation of small intestinal transit by dietary fiber would be important in counteracting Am toxicity, because the absorption of sucrose and glycyglycine from the jejunum perfused in situ was increased by slowing the perfusion rate, even when Am was present in the perfusate.5

The objectives of the present study are to examine to what extent GDF affects the upper gastrointestinal transit in rats fed with Am, and to examine the proposition that GDF exerts its beneficial effect during the time when it passes through the small intestine rather than the large intestine. The former was carried out by measuring the transit time from the mouth to the terminal ileum, using ileostomized rats. For the latter, we used ileorectostomized rats and examined the growth response to the concurrent feeding with Am and GDF.

Materials and Methods

Materials. As the source of dietary fiber, we used the roots of edible burdock (gobo), which is one of the vegetables commonly available all the year round in Japan and which has been reported to contain 3.8% dietary fiber on a raw weight basis4 as determined by the method of Prosky et al.7

The procedure for preparing GDF was the same as that described previously.5 Briefly, the edible portion of fresh roots was finely mashed with water by passing through a waste disposer (ISE IN-SINK-ERATOR, Model 333SS, Emerson Electric Co., Racine, WI). After being rinsed in running water, the residue was stirred in boiling water to remove starches and other water-soluble materials, separated by filtration, dehydrated with 99% ethanol and dried in air.

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Fig. 2. Feeding Schedule for Transit Time Measurements in ileostomized Rats.

Hatched areas indicate the experimental feeding period and excreta collection period. A nonabsorbable water-soluble marker (Cr-EDTA) was incorporated at a 0.5% level into each experimental diet, which was fed only for the first hour. After the nutrient proportions. The control and test diets refer to the basal diet to which Am was added and to the basal diet to which Am plus GDF were concurrently added, respectively. The dietary levels of Am and GDF are described for each experiment.

Surgical procedures. Ileostomy was carried out on 9 male rats of the Wistar strain, weighing about 260 g, by a modification of the technique of Lambert. After 12 hours of fasting, the rats were anesthetized by an intraperitoneal injection of Nembutal (sodium pentobarbital, Abbott Laboratories, North Chicago, IL), their abdomens were shaved, and a laparotomy performed via a midline incision. The ileum about 10 cm proximal to the ileocecal valve. The proximal cut end of the intestine was brought out through an opening (4 to 5 mm in diameter) made in the abdominal wall, and stitched to the peritoneum and skin with interrupted sutures. An elastic silicon tube (10 mm x 4 mm diameter) was attached to the opening to quantitatively collect excreta. The cecum was cut out after ligating the mesenteric blood vessels, and the colon was left in the abdominal cavity after closing the cut end by suturing.

To avoid free access to the excreta, the post-operative rats were then transferred to restrainable cages devised in our laboratory. In preliminary experiments, we had confirmed the suitability of the cages for a prolonged feeding period of at least 4 weeks. The rats were fed with the basal diet during the recovery period of 15 days. For the first 3 days of the recovery period, the rats were intramuscularly injected with 10 μl/100 g of body weight of Mycamin Sol (a mixture of procaine penicillin G and dihydrostreptomycin sulfate; Meiji Seika, Tokyo).

Ileorectostomy was also carried out on rats weighing about 200 g according to the method of Lambert. After opening the abdomen in the same manner as that described already, the intestine was transected both at 1 cm proximal to the ileocecal valve and at about 5 cm proximal to the anus. The proximal cut end of the ileum was anastomosed to the distal cut end of the rectum in end-to-end fashion, using an interrupted suturing technique with No. 8-0 silk thread. The cecum and colon were then cut out after ligating the mesenteric blood vessels. For control rats of surgery, a sham operation was performed, in which the ileum was transected at about 1 cm from the ileocecal valve and then immediately anastomosed.

The animals were fasted for 12 hours after surgery, and then fed with the basal diet for the recovery period of 17 days. Three of the 20 ileorectostomized rats were excluded from the study because they failed to recover their body weight. The other animals grew at the rate of 74% that obtained with intact animals during the recovery period.

Analytical method. Excreta collected within the same time period from individual ileostomized rats was combined for each diet, homogenized with an appropriate volume of distilled water, and then centrifuged at 15,000 × g for 10 min. The resultant supernatant was analyzed for Cr content with an atomic absorption spectrophotometer (Shimadzu AA-640-12, Kyoto) as described by Kim et al. The Cr content in the diets was also determined for the supernatant after extracting with distilled water.

The recovery of Cr from the excreta and diet was in the range of 96 to 107%. The time required for excreting 50% of the ingested marker (transit halftime, Tt50) was calculated from the equation of a logarithmic curve obtained by plotting the cumulative excretion of Cr against time.

Experiment 1. Effect of 10% GDF on Tt50 for the ileostomized rats with 4% Am loading. After the ileostomized rats had recovered from the surgery, they were evaluated for the upper gastrointestinal transit by measuring a nonabsorbable marker recovered from the ileal end. The Am level in the control and test diets was 4% and that of GDF in the test diet was 10%. According to the feeding schedule (Fig. 2), the ileostomized rats were fed with one of the 3 diets ad libitum for 24 hours after 24

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**Table 1. Composition of the Basal Diet**

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>Casein</td>
<td>25.0 g</td>
</tr>
<tr>
<td>Corn oil</td>
<td>5.0 g</td>
</tr>
<tr>
<td>Minerals mixture</td>
<td>4.0 g</td>
</tr>
<tr>
<td>Vitamins mixture</td>
<td>4.0 g</td>
</tr>
<tr>
<td>Choline chloride</td>
<td>0.2 g</td>
</tr>
<tr>
<td>Vitamin E granule</td>
<td>0.05 g</td>
</tr>
<tr>
<td>Sucrose</td>
<td>100 g</td>
</tr>
</tbody>
</table>

1 Lactic casein (30mesh), purchased from New Zealand Dairy Board, Wellington.
2 Just prior to preparing the diet, retinyl palmitate and ergocalciferol were mixed with corn oil to provide 600 IU and 600 IU, respectively, per kg of diet.
3 The mineral mixture contained (in g/kg) CaCO3, 11.72; NaCl, 9.65; KH2PO4, 13.72; MgSO4, 7H2O, 3.99; MnSO4·H2O, 0.37; Fe(C6H5O7)2, 0.5; CaHPO4, 2H2O, 0.17; (in mg/kg of diet) CuSO4·H2O, 62.4; ZnCl2, 56.8; Na2MoO4·2H2O, 2.82; Co(CH3COO)2·4H2O, 0.84; Na2SeO3, 0.59; KIO3, 0.56. This mix was essentially the same as mineral mixture-2 formulated by Eibiha, Inamura and Kiriya, with the exception of omitting the following trace elements: Na2SiO3·9H2O, Na2B4O7, 10H2O, NiCl2·6H2O, NaF, Na2HAsO4·7H2O, CrCl3·6H2O, SnSO4, and NH4VO3.
4 This was identical with harper’s mixture with sucrose as a diluent.
5 Added as a 50% solution in 50% ethanol.
6 Trade name is “Juvera E granule,” purchased from Eisai Co., Tokyo. It contained 200 mg of all-rac-α-tocopheryl acetate per gram.

The resultant dry residue with a recovery of about 4% from the fresh roots was then powderized in a Wiley mill with a 1 mm diameter pore sieve, and used as such in the feeding experiments (this powder is subsequently referred to as GDF). The composition of GDF is shown in Fig. 1. As a nonabsorbable, water-soluble marker, Cr-EDTA was prepared by the method of Binnerts et al. and used in the form of a dry powder. Am as the trisodium salt of 1-(4-sulfo-1-naphthylazo)-2-naphthol-3,6-disulfonic acid was obtained from a commercial source (San-ei Chemical Industries, Osaka).

**Diet.** A purified feed (Table 1) which satisfies all the nutrient for rats requirements was used as the basal diet. Supplementation of Am and GDF was done by replacing equal amounts of the whole diet so as not to

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hours of fasting.

Cr-EDTA was incorporated into each experimental diet at a 0.5% level and fed only for the first 1 hour. The excreta were collected hourly for 24 hours from the beginning of feeding. This test was repeated with a different experimental diet at 3-day intervals, during which time the rats were maintained on the basal diet to remove any residual marker from the intestine. The excreta resulted from a different experimental diet were then collected in the same manner.

Experiment 2. Growth response of the ileorectostomized rats to feeding on 4% Am with or without 7.5% GDF. This experiment was to ascertain the main site where Am toxicity develops and where the beneficial effect of GDF appears; either in the small intestinal lumen or in the large intestinal lumen. If GDF counteracts the Am toxicity in the large intestinal lumen, the venefical effect of GDF would disappear in the rats whose cecum and colon had been removed.

Twenty-two postoperative ileorectostomized rats, including 5 sham-operation cases, were individually housed in suspended cages with screen-bottom of stainless steel, which were placed in a room maintained at 23 ± 2°C and with a 12-hour light (0800 to 2000) and 12-hour dark (2000 to 0800) cycle. The rats were divided into 4 groups (with the sham-operation rats in one group) and fed on the respective diets. The body weight and food intake of each were recorded every morning for 14 days.

Statistical methods. Data were subjected to an analysis of variance19 and to Duncan's multiple range test.20 Any difference with p<0.05 was evaluated as significant.

Results

Experiment 1

The food intake (g/24 hours) of the basal, control and test diets was 35.1, 20.7, and 20.9, respectively. Therefore, the ileostomized rats consumed the same amount of Am when the control and test diets were fed to them. The cumulative excretion of Cr is shown in Fig. 3. The marker was observed in the excreta within the first 1 hour after ingesting the control or test diet, whereas it took more than 1 hour until its appearance in the excreta when the rats were fed with the basal diet. However, the subsequent Cr excretion was quite different in amount between the control and test diets.

When fed with the control diet, the rats required about 8 hours to excrete 50% of the ingested marker, whereas

Table II. Food Intake, Body Weight Gain, Food Efficiency, and Mortality when 4% Amarath Was Fed with or without 7.5% GDF to Ileorectostomized Rats1

<table>
<thead>
<tr>
<th>Diet</th>
<th>Rat</th>
<th>No. of rats</th>
<th>Food intake</th>
<th>Net food efficiency</th>
<th>Mortality (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Basal diet</td>
<td>Sham</td>
<td>5</td>
<td>366 ± 8.5</td>
<td>0.35 ± 0.01</td>
<td>0</td>
</tr>
<tr>
<td>Basal diet</td>
<td>IRS</td>
<td>5</td>
<td>360 ± 15*</td>
<td>0.34 ± 0.05*</td>
<td>0</td>
</tr>
<tr>
<td>Basal diet</td>
<td>+4% amaranth</td>
<td>IRS</td>
<td>190 ± 23b</td>
<td>-0.03 ± 0.02b</td>
<td>50</td>
</tr>
<tr>
<td>Basal diet</td>
<td>+7.5% GDF</td>
<td>IRS</td>
<td>301 ± 8.5a</td>
<td>0.27 ± 0.02c</td>
<td>0</td>
</tr>
</tbody>
</table>

1. Average initial body weight was 212 g (range from 163 to 258 g).
2. Calculated on the basis of the nutrient intake, excluding such non-nutritive fractions as amaranth and GDF in diets consumed.
3. Mean ± SEM; values not sharing a common superscript letter within a column are significantly different (p<0.05) as determined by Duncan's multiple range test.
4. IRS refers to rats subjected to ileorectostomy.
5. Dietary fiber prepared from "gobo," the roots of edible burdock (Arctium lappa L.).

![Fig. 3. Cumulative Excretion of Cr in Ileostomized Rats.](image)

The time necessary to excrete 50% of the marker (T1/2) was calculated from each equation of the curve obtained by plotting the cumulative excretion of Cr versus time. The curves at the top, center and bottom were obtained from feeding the basal diet, control diet and test diet, respectively. Excreta collected from individual rats within the same collection period was combined for each diet and analyzed for Cr.

![Fig. 4. Change in Body Weight Gain of Ileorectostomized Rats Fed on Three Different Diets.](image)
TT50 obtained with the basal diet was more than 17 hours, indicating that the upper gastrointestinal transit time was reduced by half in the presence of Am. On the other hand, TT50 of 18.2 hours with the test diet was almost equivalent to the value obtained with the basal diet.

**Experiment 2**

The growth rate of the ileorectostomized rats after the recovery period was almost equal to that of the sham-operated rats when the animals were fed with the basal diet for 14 days (Table II), indicating that these animals were suitable after surgery for evaluating the effect of diets on their growth response. When the ileostomized rats were fed with the control diet, they suffered from severe diarrhea, and a marked decrease in food intake, body weight gain and food efficiency, 3 of 6 the rats dying in the course of the study (Fig. 4, Table II).

However, such toxic effects of Am were alleviated by supplementing with 7.5% GDF, as judged by the improvement in body weight gain and non-mortality (Table II). The rats fed with the test diet grew uniformly by 77% as compared with the basal diet-fed rats after a 3-day lag (Fig. 4). This improved growth rate was attributed to the increased food efficiency (g of gain/g of food intake) to a considerable extent (Table II). Diarrhea also disappeared when the rats were fed with the test diet.

**Discussion**

Fifty % of the marker was recovered from the excreta during about 18 hours after ingesting the basal diet (Fig. 3). This TT50 value is somewhat greater than the values from other investigators whose experimental conditions were different from ours. Marcus and Lengemann and Verga have reported that the transit half-time from the mouth to the terminal ileum was 4.3 and 7.3 hours, respectively, for normal rats fed with a radioactive tracer (85Sr-labelled microspheres or 91Y). The greater TT50 value obtained for the ileostomized rats suggests that intestinal motility would be adaptively slowed down to compensate for the functional role of the cecum and colon. In practice, it was observed that ileostomy excreta changed in form from fluid to a soft mass during the course of the recovery period.

In fact that TT50 was markedly deceased by feeding the control diet (Fig. 3) suggests that dietary Am accelerated gastric emptying and/or small intestinal transit. Although stimulation of the intestinal motility by Am may be a protective response to intraluminal toxicants, rapid emptying and transit through the small intestine would aggravate the malabsorption of nutrients as well as inhibitory effect of Am on the digestion-absorption systems.

On the other hand, the decreased TT50 value was considerably improved by concurrent feeding with 10% GDF. The whole gut transit time is often regarded as a reflection of the large intestinal event, since the time required for the chyme to traverse the gastrointestinal tract is mainly spent in the large intestine, and dietary fiber, in general, is well known to shorten this time in human subjects and rats.

Our findings, however, raise the possibility that dietary fiber can affect small intestinal transit as well. In this regard, other investigators have also observed that the passage through the stomach and small intestine was delayed in dogs fed on meal with guar-gum (5 and 10 g/liter). A similar effect was observed in rats fed on a diet incorporating guar-gum, cellulose or bran at 6% level.

It seems likely that such bulk-forming capacity of GDF as determined by the settling volume in water is responsible for normalizing the rapid transit through the upper gastrointestinal tract by resisting the propulsion of digesta. This retarding action of GDF would be beneficial for absorbing nutrients from the small intestine, especially when the digestion-absorption activities are depressed in the presence of Am, because the reduced absorption of nutrients can be restored during prolonged gastric emptying and/or intestinal transit. Indeed, we have observed in an experiment on the jejunal perfusion system of anesthetized rats that the absorption of sucrose and glycyglycine from the perfusate increased even in the presence of Am (25 mg/ml) when the perfusion rate was slowed down.

As compared with the previous observations obtained with normal rats, the ileorectostomized rats also developed Am toxicity when fed with 4% Am (Fig. 4). Even in the rats without a cecum and colon, supplementation with GDF enabled them to survive and grow to a statistically significant extent (Fig. 4). Therefore, it is reasonable to speculate that the large bowel microorganisms in rats are neither involved in the development of Am toxicity nor in the beneficial effect of dietary fiber. To support this view, we have also observed that such a microorganism-resistant material as powdered polystyrene foam also had a protective effect when it was added to the control diet at a 5% level.

Takaji et al. have already observed a similar growth response to that of the ileorectostomized rats when the same kinds of diet as those used here were fed to rats after total gastrectomy. Therefore, the stomach can also be excluded as the site at which Am and GDF interact. From these and previous results, we conclude that GDF exerts its beneficial effects by normalizing small intestinal transit of the chyme, whose passage through the intestinal lumen was appreciably accelerated by a large amount of Am.

**References**