Effect of Dietary Fiber on Morphine-induced Constipation in Rats

Takashi Niwa,1 Makoto NakaO,1 Seiko Hoshi,2 Kiyofumi Yamada,1 Kazuhiro Inagaki,3 Mikio Nishida,3 and Toshitaka Nabeshima1,7

1Department of Neuropsychopharmacology and Hospital Pharmacy, Nagoya University Graduate School of Medicine, 65 Tsuruma-cho, Showa-ku, Nagoya 466-8560, Japan
2Department of Nutritional Research, Nutrition Science Institute, Meiji Milk Products Co. Ltd, 540 Naruda, Odawara 250-0862, Japan
3Department of Clinical Pharmacy, Faculty of Pharmacy, Meijo University, 150 Yagotoyama, Tempaku-ku, Nagoya 468-8503, Japan

Received October 19, 2001; Accepted February 2, 2002

Morphine is used to alleviate chronic cancer pain. However, constipation is a major adverse effect that often detracts from the patient’s quality of life. In this study, we investigated the effectiveness of dietary fiber on morphine-induced constipation. Rats were fed on a normal diet or one containing either 10% or 20% apple fiber for two weeks before morphine was administered. In the control diet group, the fecal number and dry weight were decreased by treating with morphine in a dose-dependent manner. Moreover, the motility of the small and large intestines was reduced. The fecal number and weight were increased and the colon motility was promoted by dietary fiber, regardless of whether morphine was being administered. The dietary fiber increased the concentration of short-chain fatty acids (SCFAs) in the cecum. These results suggest that dietary fiber has a preventative effect on morphine-induced constipation by increasing SCFAs in the cecum, and thereby promoting colon motility in rats.

Key words: constipation; dietary fiber; morphine; SCFAs

Treatment for the relief of cancer pain is based on World Health Organization (WHO) guidelines. These guidelines consist of a 3-step analgesic ladder and, depending on the strength of the pain, recommend non-opioid analgesics, then weak opioids and finally strong opioids. Oral morphine is the first choice for treating the third level of pain, that is, moderate to severe cancer pain, and the WHO guidelines have introduced morphine as the main medicine for the treatment of cancer pain.1

Morphine has several pharmacological actions in addition to its analgesic action and, therefore, many adverse effects such as constipation, nausea, vomiting and drowsiness.2,3 These adverse effects can cause patients to stop taking morphine. Constipation is a serious side effect that frequently occurs soon after morphine administration. Since tolerance is developed very slowly to the effects of morphine on the intestines, constipation may last the entire period that morphine is being administered.4 Untreated constipation may progress to obstruction, which can lead to life-threatening complications associated with bowel obstruction.5 Since evacuation control can become more difficult than the control of the cancer pain, the use of a laxative is recommended when administering morphine.

The first choice of a laxative to treat morphine-induced constipation is usually senna. However, since the constipation is so severe, high doses and different types of laxatives are often needed. The result is that some patients show diarrhea, while others have persistent constipation.

The effectiveness of dietary fiber in relieving constipation has recently been reported.6 Since dietary fiber has no adverse effects, it can be used safely. However, it has not been reported whether dietary fiber is effective in preventing morphine-induced constipation. In this study, we investigated the effectiveness of dietary fiber in preventing morphine-induced constipation in rats.

Materials and Methods

Animals. Male Sprague-Dawley rats aged four weeks (initial body weight, 70–90 g; Japan SLC Inc., Hamamatsu, Japan) were used in this study. Five animals were allotted to each of nine groups. The rats

To whom correspondence should be addressed. Phone: +81-52-744-2674; Fax: +81-52-744-2682; E-mail: tnabeshi@med.nagoya-u.ac.jp
Abbreviations: SCFAs, short-chain fatty acids; Bis Tris, bis (2-hydroxyethyl)-iminotris (hydroxymethyl) methane; CTZ, chemoreceptor trigger zone
were individually housed in wire drop-bottom cages to minimize coprophagy. Following 1 week of preliminary rearing, two groups of rats were fed on either a 10% or 20% apple fiber-containing diet (CLEA Japan, Inc., Tokyo, Japan), while a control group was fed on control food (CE-2; CLEA Japan, Inc., Tokyo, Japan) that contained 4.13% dietary fiber (Table 1). The rats had free access to food and water.

This study was approved by the Institute for Laboratory Animal Research at Nagoya University School of Medicine under the animal experiment guidelines of Nagoya University School of Medicine.

The apple fiber-containing diet was given for two weeks, and the then seven-week old rats were used for the experiments. Table 2 indicates the composition of the apple fiber.

**Drugs.** Morphine hydrochloride (Shionogi Pharmaceutical Co. Ltd., Osaka, Japan), apple fiber (Miyarisan Co. Ltd., Nagano, Japan), carmine (Sigma Chemical Co., St. Louis, MO, U.S.A.), and sodium carboxymethylcellulose (Katayama Chemical Industries, Osaka, Japan) were used. Crotonic acid, sodium acetate, sodium propionate, sodium isobutyrate, sodium n-butyrate, i-valeric acid, n-valeric acid, p-toluene sulfonic acid, EDTA, and bis (2-hydroxyethyl)-iminotris (hydroxymethyl) methane (Bis Tris) were purchased from Wako Pure Chemical Industries (Osaka, Japan).

Morphine hydrochloride (1 or 3 mg/kg) was dissolved in saline. The morphine was subcutaneously administered twice a day for 3 days (days 22-24) (Fig. 1). The control group rats were subcutaneously administered with saline.

**Body weight gain.** The body weight was measured every 3 days at 9:00 a.m., and the body weight gain was calculated as the difference between day 1 and day 25.

**Food intake.** The weight of remaining food was measured every day during the morphine administration period (days 22-24) at 9:00 a.m., and the food intake was calculated as the difference between this remainder and the food weight on the previous day.

**Total number, dry weight, and water content of fecal pellets.** The fecal number, weight, and water content were examined during the morphine administration period (days 22-24). Every day, at 9:00 a.m., feces were collected from each rat. The number of fecal pellets was counted, and the feces after each defecation were weighed. Each fecal specimen was then lyophilized and weighed to determine the water content. The total number and dry weight of the fecal pellets were calculated as the average per 24 hours for each rat.

**Gastrointestinal transit ratio.** Gastrointestinal motility was measured according to the methods of Nagakura et al.7 and Suzuki et al.9 with a minor modification. On day 22, carmine was orally administered to each rat in a volume of 1 ml (3 g of carmine suspended in 50 ml of 0.5% carboxymethylcellulose). One hour after administering the marker, the animal was killed by exsanguination from the abdominal aorta under ether anesthesia, and the small intestine was removed. The distance over which the carmine had traveled and the total length of the small
intestine were measured. The gastrointestinal transit ratio is expressed as a percentage of the distance the carmine had traveled relative to the total length of the small intestine. Each rat was subcutaneously treated with either saline or a single dose of morphine before administering the marker.

**Bead evacuation time.** The colonic propulsive activity was measured according to the method of Takasaki et al.\(^{(9)}\) with a minor modification. On day 22, each rat was subcutaneously treated with either saline or a single dose of morphine, and a Teflon bead (3.2 mm in diameter) was then inserted into the distal colon 3 cm above the anus 30 min after the injection. The time required to evacuate the bead was measured as an index of the colonic propulsive activity.

**Cecal pH value and short-chain fatty acid concentration.** On the day after the final administration of morphine (day 25), the rats were killed by exsanguination from the abdominal aorta under pentobarbital anesthesia. The ileocecal and ceco-colonic junctions were ligated, and the cecum was removed. The cecum was immediately frozen and stored at \(-20^\circ\)C until further analyses. The cecal contents were drained from the ceco-colonic junction into a 50-ml vial, and then used for analyses. The cecal pH value was measured with a needle-type pH meter (6251-10C, Horiba, Kyoto, Japan).

Short-chain fatty acids (SCFAs) in the cecum were measured by HPLC (Shimadzu, Kyoto, Japan) with an internal standard. Approximately 300 mg of the cecal content was homogenized by ultrasonication in 1 ml of a 10 mm sodium hydroxide aqueous solution containing 5 mm crotonic acid as an internal standard, and the mixture centrifuged at 10,000 \(\times\) g for 15 min. The fat-soluble substances in the supernatant were removed by extracting with chloroform. This neutralization of the cecal contents with sodium hydroxide prevented the extraction of short-chain fatty acids and crotonic acid by the chloroform. The aqueous phase was then passed through a membrane filter (cellulose acetate, 0.20 \(\mu\)m pore size; DISMIC-13cp; Advantec Toyo, Tokyo, Japan). The sample was applied to an HPLC column (Shimadzu) for an analysis of SCFAs.

SCFAs were measured according to the method of Hoshi et al.\(^{(10)}\) with a minor modification. SCFAs were separated in an ion-exclusion column and detected by a post-column pH-buffered electroconductivity detection method, using a double-connected H-type cation exchanger column (Shim-pack SCR-102H, 8 mm i.d. \(\times\) 30 cm long; Shimadzu), a column temperature of 45°C, a mobile phase of a 5 mm p-toluene sulfonic acid aqueous solution (0.8 ml/min flow rate, 45°C), an electroconductivity detector of positive polarity at 45°C (type CDD-6A; Shimadzu) and a detection reagent of a 20 mm bis Tris aqueous solution containing 5 mm p-toluene sulfonic acid and 100 \(\mu\)M EDTA (0.8 ml/min flow rate, 45°C).

The cecal concentration of SCFAs is indicated as \(\mu\)mol/g cecal content. The total SCFA concentration was calculated as the sum of acetate, propionate, n-butyrate, i-butyrate, and i-valerate.

**Statistical analyses.** Each value is indicated as the mean \(\pm\) standard error of the mean (S.E.M.). The significance of a difference was tested by a one-way analysis of variance (ANOVA) followed by the Dunnett multiple-comparison test. Differences with p values of \(<0.05\) are considered to be statistically significant.

### Results

**Body weight gain and food intake**

There was no significant difference in body weight gain among the experimental groups (Table 3). When the apple fiber content of the diet was increased, the tendency to take food increased, but this change was not significant. The food intake by the control and apple fiber diet groups was decreased dose-dependently by the morphine treatment, although this difference was not significant.

**Total number and dry weight of fecal pellets**

In the control groups, morphine administration significantly decreased the number of fecal pellets (Fig. 2A) and total fecal dry weight (Fig. 2B) in a dose-dependent manner, suggesting the induction of morphine-induced constipation (\(p<0.01\)). In the saline-treated groups, when the apple fiber content of the diet was increased, the total number and dry weight of fecal pellets increased dose-dependently, the increase being significant with the 20% apple fiber diet.
In the control diet groups, morphine administration markedly prolonged the bead evacuation time in

![Fig. 2](image)

**Fig. 2.** Effect of Dietary Fiber on the Decrease in Total Number (A) and Weight (B) of Fecal Pellets Induced by Morphine and Effects of Morphine and Dietary Fiber on the Fecal Water Content (C).

Each value is the mean ± S.E.M. (n = 5). **p < 0.01 vs. saline-treated/control diet group. *p < 0.05, **p < 0.01 vs. the corresponding control diet group.

diet compared with the control diet group (p < 0.05). In the morphine-treated groups, the total number and dry weight of fecal pellets increased according to the apple fiber content when compared with the corresponding control. In the morphine-treated/20% apple fiber diet group, the total number and dry weight of fecal pellets were significantly higher (Figs. 2A and 2B).

![Fig. 3](image)

**Fig. 3.** Effect of Dietary Fiber on the Decrease in Gastrointestinal Transit Ratio (A) and Delay in Bead Evacuation Time (B) Induced by Morphine.

Each value is the mean ± S.E.M. (n = 5). **p < 0.01 vs. saline-treated/control diet group. *p < 0.05, **p < 0.01 vs. the corresponding control diet group.

**Fecal water content**

In the three control diet groups, there was no significant difference in the fecal water content even after the morphine treatment (Fig. 2C). In the saline-treated groups, the fecal water content was significantly increased by the apple fiber diet in comparison with the control diet (p < 0.05). In the morphine-treated groups, no significant difference in the fecal water content was apparent between the apple fiber diet and control diet groups (Fig. 2C).

**Gastrointestinal transit ratio and bead evacuation time**

In the control diet groups, morphine administration decreased the gastrointestinal transit ratio in a dose-dependent manner. A significant lower the ratio was observed in the 3 mg/kg-treated group than in the saline-treated group (p < 0.01) (Fig. 3A). The apple fiber diets (10% and 20%) had no effect on the gastrointestinal transit ratio at any dose of morphine or saline.

In the control diet groups, morphine administration markedly prolonged the bead evacuation time in
a dose-dependent manner \((p<0.01)\) (Fig. 3B). In the saline-treated groups, the bead evacuation time was significantly shortened by the apple fiber diet \((p<0.01)\). In the morphine-treated groups, when the apple fiber content of the diet was increased, the bead evacuation time was significantly shortened when compared with the corresponding control diet group \((p<0.01)\).

**Cecal pH**

In the control diet groups, morphine had no effect on the cecal pH value (Fig. 4). In the apple fiber diet groups, the cecal pH value was significantly lower relative to the corresponding control \((p<0.01)\).

**Cecal short-chain fatty acids**

In the control diet groups, morphine had no effect on the cecal concentration of acetate, propionate, butyrate and total SCFAs, apart from an increase in the acetate level in the morphine 1 mg/kg group. In the apple fiber diet groups, the cecal acetate concentration was significantly higher, dependent on the apple fiber content of the diet, but independent of the morphine treatment. On the other hand, apple fiber had no effect on the cecal propionate concentration. In the saline-treated and morphine 1 mg/kg-treated groups, cecal n-butyrate was significantly increased by the apple fiber diet \((p<0.05, p<0.01)\). The total SCFA concentration was increased by both the 10% and 20% apple fiber-containing diets (Fig. 5).
Discussion

To make an animal model of the constipation induced by morphine, we subcutaneously administered 1 or 3 mg/kg of morphine to rats twice a day for 3 days. As an index of constipation, we used the total number and dry weight of fecal pellets. Koyuncuoglu et al.\textsuperscript{10} have reported that the fecal weight was significantly decreased by morphine administration to rats. In the present study, both the total number and dry weight of fecal pellets were significantly decreased by the morphine treatment. Therefore, it became clear that the doses of morphine we used reduced the amount of feces discharged. On the other hand, it is known that morphine can cause nausea and vomiting by stimulating the chemoreceptor trigger zone (CTZ).\textsuperscript{25} A reduction in food intake caused by nausea may lead to a reduction in the amount of feces. Although the food intake tended to decrease dependent on the morphine dose in our study, the change was not significant. We consider that the reduction in the amount of feces following morphine administration, which was confirmed in this study, was not due to the secondary effect of reduced food intake. Accordingly, we consider it possible to make a constipation model with rats by using our morphine administration schedule.

We investigated the clinical application of dietary fiber in this study. Apple fiber is one of the dietary fiber products already on the market, and we consider that apple fiber might be easily accepted by patients because of its pleasant smell. Furthermore, apple fiber is a water-insoluble dietary material which has been reported to increase the fecal weight and promote colon motility.\textsuperscript{10} We selected apple fiber as the dietary material for these reasons.

We found that the total number and dry weight of fecal pellets were both increased by apple fiber, the increase being unaffected by the morphine administration. These results suggest that the effect of apple fiber on feces was independent of the effect of morphine.

Morphine diminishes or abolishes propulsive peristaltic waves in the intestine.\textsuperscript{13,14} The opiate also promotes water absorption in the intestine.\textsuperscript{15} It is possible, therefore, that the fecal water content could be decreased by morphine administration. However, no significant decline in the fecal water content was apparent from morphine administration in this study. On the other hand, it has been reported that the fecal water content was increased by dietary fiber which has a water-holding effect.\textsuperscript{19} In this experiment, in the saline-treated groups, the fecal water content was significantly increased by the apple fiber, but in the morphine 1 and 3 mg/kg-treated groups, no such effect of apple fiber was observed. It has been reported that the normal fecal water content in humans is 72.0 ± 1.2%.\textsuperscript{17} In the present study, the normal fecal water content in the rats was 52.6 ± 2.0%, much lower than that in humans. We suggest that, because of this, the effect of morphine on the fecal water content in rats would be extremely small, and that when the fecal water content was increased by dietary fiber, the effect of morphine to promote water absorption in the intestine became apparent.

It has been reported that dietary fiber swelled feces by its water-holding effect and improved bowel movement.\textsuperscript{18} However, in the morphine-treated/apple fiber diet group, no significant increase in the fecal water content was apparent. In other words, the swelling of feces caused by the water-holding effect of the dietary fiber was suppressed by the morphine administration. It is unlikely, therefore, that the preventative effect of dietary fiber on morphine-induced constipation was due to the water-holding effect.

To elucidate the mechanism for the effect of apple fiber, we studied its influence on the suppression of intestinal motility by morphine. In the control diet groups, a decrease in the motility of the small and large intestines was induced by the morphine treatment, consistent with the results of Williams et al.\textsuperscript{19} It is suggested that the suppressive effect on colon motility greatly contributes to morphine-induced constipation. In this respect, dietary fiber apparently promoted large intestinal motility at the time of morphine administration. It is suggested from these results that apple fiber improved morphine-induced constipation by promoting large intestinal motility. In the morphine-untreated groups, the promoting effect of dietary fiber on colon motility was little, although it was significant. However, colon motility was strongly promoted at the time of morphine administration, indicating the possibility that the effect of dietary fiber was stronger under the suppression of colon motility.

It has been reported that short-chain fatty acids, which are the products of colonic bacterial fermentation of dietary fiber, had a laxative effect by affecting the enteral mucosa.\textsuperscript{20} We therefore examined the effects of dietary fiber on the cecal SCFA content to clarify its promoting effect on large intestinal motility.

SCFAs are the major anions of the colonic contents and the main products from the colonic bacterial fermentation of unabsorbed starch and dietary fiber. The term SCFA refers to a group of C2-C6 monocarboxylic acids, although only acetate, propionate, and butyrate are produced in significant amounts in the human colon.\textsuperscript{21} Yajima\textsuperscript{22} has reported the contracting effect of SCFAs on an isolated rat colon. Moreover, it has been reported that the potency of SCFAs was inversely related to the chain length, acetate being more potent than butyrate.\textsuperscript{23} In the present study, acetate was markedly increased by the apple fiber. Yajima\textsuperscript{22} has reported that acetate
did not induce contraction in an isolated rat colon. However, in that report, sodium acetate was used. Cherbut et al. have reported that acetic acid induced concentration-dependent contractions in the rat terminal ileum in vitro, whereas sodium acetate, the dissociated form, had no effect. It is suggested from this fact that, in the apple fiber diet group, the increase in acetic acid promoted colon motility, and thereby promoted defecation. SCFAs are readily absorbed and metabolized by the colonic epithelial cells. Moreover, it has been reported that colonic epithelial cells used SCFAs in the order of butyrate > propionate > acetate. It is possible that propionate and butyrate are promptly metabolized in the colon because they are more easily used than acetate. It should be noted that it has been reported that large amounts of SCFAs suppressed the movement of the large intestine. Therefore, if dietary fiber is administered in excess, there is a possibility that constipation would be worsened. When using dietary fiber to improve defecation, therefore, the dosage is important.

In summary, our study clearly demonstrates that dietary fiber was effective in preventing morphine-induced constipation in rats. Furthermore, it is suggested that SCFAs, which are products of the colonic bacterial fermentation of dietary fiber, especially acetate, were involved in the defecation-improving effect of dietary fiber by promoting colon motility. Our study supports the use of apple fiber in preventing morphine-induced constipation in patients.

Acknowledgments

This study was supported, in part, by a Grant-in-Aid for Health Science Research on Pharmacutical and Medical Safety from the Ministry of Health, Labour and Welfare of Japan, and by Special Coordination Funds for Promoting Science and Technology, Target-Oriented Brain Science Research program from the Ministry of Education, Culture, Sports, Science and Technology of Japan.

References

20) Spiller, R. C., Pharmacology of dietary fibre. Phar-


