Mechanism for Lowering Blood Ammonia Levels by Lactitol

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ABSTRACT—Lactitol has been reported to decrease the blood ammonia concentration in various experimental hyperammonemia models such as portacaval shunted rats. The mechanism responsible for this lowering of blood ammonia levels was investigated in rats. When lactitol was given orally twice a day for 7 days at doses of 3 and 10 g/kg/day and at half that daily dose on day 8, it significantly decreased the ammonia concentration of the portal blood by 27.3–43.2%, cecal ammonia contents by 49.2–57.6%, and the pH of the cecal contents from 6.52 to 5.92–5.54, 4 hr after the final administration. Lactitol also inhibited increases in the portal ammonia concentration induced by the intracecal administration of ammonium acetate (300 mg/kg) 4 hr after the final administration. When lactitol was given orally at bolus doses of 1 and 3 g/kg simultaneously with a charcoal meal, lactitol significantly facilitated small intestinal transit by 12–13%. At a bolus dose of 3 g/kg, given 1 hr before the administration of a charcoal meal into the proximal colon, lactitol significantly facilitated colonic transit by 29.5%. These effects of lactitol were similar to those of lactulose. These findings suggest that lactitol decreases blood ammonia concentration by inhibiting both the production and the absorption of ammonia through reducing intestinal pH and shortening the residence time of intestinal contents in the intestinal tract.

Keywords: Lactitol, Mechanism, Blood ammonia level, Intestinal transit

Hepatic encephalopathy is a metabolic disorder in which symptoms of psychosis/neuropathy and consciousness disorder caused by acute or chronic hepatic failure are manifested. The mechanism underlying the development of hepatic encephalopathy is not known. Since hyperammonemia is frequently observed in the development of this disease, however, ammonia has been noted as an important factor (1–3).

Lactitol has been shown to decrease blood ammonia levels in portacaval shunted (PCS) rats treated with carbon tetrachloride or dimethylnitrosamine (4). In addition, lactitol inhibits the increase of ammonia content in the brain and coma after administration of ammonium acetate in PCS rats treated with carbon tetrachloride or dimethylnitrosamine (4).

In the present study, we investigated the mechanism whereby lactitol lowers blood ammonia levels in rats.

MATERIALS AND METHODS

Animals

Male Sprague-Dawley rats, 5–7 weeks of age, were purchased from Japan SLC and used at 6–8 weeks of age.

The rats were fed F-2 chow (Funabashi Farm, Funabashi) and received tap water ad libitum. They were kept in a room that was ventilated more than 15 times/hr, at a temperature of 21–25°C and humidity of 45%–65%.

Drugs

Lactitol monohydrate [(+)-4-0-α-D-galactopyranosyl-D-glucitol monohydrate; NS-4, Zyma, Nyon, Switzerland; regarded as lactitol only] (Fig. 1) is a white odorless crystalline or crystallic powder that has a sweet taste. Lactitol was given as a solution in distilled water. As a reference drug, lactulose (Fig. 1, lactulose syrup containing 60% lactulose; Nikken, Tokyo) was given after dilution with distilled water.

Portal venous ammonia concentration, cecal ammonia content and pH of cecal contents

Each group consisted of eight 8-week-old rats. Drugs, at doses of 1, 3 and 10 g/kg/day, b.i.d., were given orally for 7 consecutive days; half of the daily dose was given on day 8. Control rats received the same volume of distilled water. Before and 4 hr after the final drug administration, the animals were anesthetized with ether and the abdo-
mens were opened; 0.3 ml of the portal blood was withdrawn. The cecum and its contents were removed and weighed after the ileo-cecal and colo-cecal junctions were ligated. The cecum was opened and its contents were placed in a test tube. The lumen of the cecum was washed with distilled water. The washed cecum was weighed, and this weight was subtracted from the total weight including the contents to obtain the weight of the contents. Aliquots (0.5 g) of the cecum contents were homogenized in a glass homogenizer for determination of ammonia content and aliquots of about 1 g were placed in a dish for pH measurement.

Determination of portal ammonia concentration
A sample (0.3 ml) of portal blood was added to 1.2 ml of protein elimination solution (from the ammonia test kit); this was mixed and then centrifuged at 3,000 rpm for 10 min. The ammonia concentration of the supernatant was determined by the method of Fujii and Okuda, using the Ammonia Test Wako (Wako Pure Chemicals, Osaka).

Determination of cecal ammonia content
Two milliliters of 3 M perchloric acid (Nacalai Tesque, Kyoto) was added to 0.5 g of cecal contents and homogenized. Five milliliters of 5 mM EDTA disodium salt was added to the homogenate, and this was mixed. Precipitated protein was discarded after centrifugation at 15,000 rpm for 15 min. Two milliliters of the supernatant was then mixed with 0.8 ml of 2 M potassium bicarbonate (Nacalai Tesque) until neutralized. The potassium perchlorate yielded during this neutralization was discarded by centrifugation at 10,000 rpm for 10 min. The supernatant was used for the determination of ammonia concentration, using an Amicheck Meter (AA-4120; Kyoto Daiichi Kagaku, Kyoto).

Measurement of pH of cecum contents
The cecum contents in a dish were suspended in 2 ml of distilled water and centrifuged at 3,000 rpm for 10 min; the pH of the supernatant was then measured with a pH meter (HM-26S; Toa, Tokyo).

Absorption of ammonia
Each group consisted of ten 8-week-old rats. Drugs, at doses of 1, 3 and 10 g/kg/day, b.i.d., were given orally for 7 consecutive days, and half of the daily dose was given on day 8. Control rats received the same volume of distilled water. Four hours after the final administration, the animals were anesthetized with ether, and ammonium acetate (Kanto Chemicals, Tokyo) at a dose of 300 mg/kg was injected into the cecum. Portal blood samples were withdrawn at given intervals, and the ammonia concentration in the blood was determined as described above.

Intestinal transit
Each group consisted of nine 8-week-old rats. The animals were fasted overnight before drug administration. Drugs, at doses of 0.3, 1 and 3 g/kg, and 0.2 ml of 15% active carbon suspension in 5% gum arabic/saline solution (charcoal meal) were simultaneously administered intragastrically; and 10 min later, the rats were sacrificed by cervical dislocation. The abdominal cavity was immediately opened, the distal area up to which the charcoal
meal reached was ligated, and then the entire intestine was removed. The total length (T) of the entire intestine and the charcoal-transit distance (D) were measured. Intestinal transit was calculated by the following equation:

\[ \text{Transit} \, (\%) = \frac{D \, (\text{cm})}{T \, (\text{cm})} \times 100 \]

**Colonic transit**

Each group consisted of ten 6-week-old rats. The animals were anesthetized with sodium pentobarbital (40 mg/kg, i.p.) and cannulated (silicone tube Fr. 5; Terumo, Tokyo) into the proximal colon through the cecum for charcoal meal administration. The cannula was exposed

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**Fig. 2.** Effects of lactitol and lactulose on portal ammonia concentration (A), cecal ammonia content (B) and pH of cecal contents (C) in rats. Drugs were administered orally twice a day for 7 consecutive days; half of the daily dose was administered on day 8. Before and 4 hr after the final drug administration, portal blood samples were withdrawn to determine the ammonia concentration, and the cecal contents were collected to determine the ammonia concentration and pH. Values represent means ± S.E. (N = 8). **P < 0.01, vs control (Dunnett's method).**
at the back through the subcutaneous tissues. Three days after cannulation, the rats were fasted overnight before drug administration. Drugs were given at doses of 0.3, 1 and 3 g/kg orally, and 1 hr later, 0.2 ml of 5% charcoal suspension was administered through the cannula. Thirty minutes later, the rats were sacrificed by cervical dislocation. The abdominal cavity was immediately opened, the distal area to which the charcoal had progressed was ligated, and the entire colon was then removed. The total length (T) of the colon and the charcoal-transit distance (D) were measured. Colonic transit was calculated by the equation given above.

Statistical analyses
Significance of differences between means was verified by the Dunnett’s method at P < 0.05 after analysis of variance.

RESULTS

Ammonia concentration in portal blood, ammonia content in cecum and pH of cecum contents

Ammonia concentration in portal blood: Figure 2A shows the effects of lactitol and lactulose on ammonia concentration in the portal blood. Lactitol, at doses of 1, 3 and 10 g/kg/day, decreased the ammonia concentration in portal blood by 12.60%, 27.3% and 43.2%, respectively, 4 hr after the final administration, but did not decrease the concentration before the final administration. Lactulose decreased the ammonia concentration in portal blood similarly to lactitol.

Ammonia content in cecum: Figure 2B shows the effects of lactitol and lactulose on ammonia content in the cecum. Lactitol, at doses of 1, 3 and 10 g/kg/day, decreased the ammonia content in the cecum by 8.4%, 49.2% and 57.6%, respectively, 4 hr after the final administration, but did not decrease the ammonia content before the final administration. Lactulose decreased the ammonia content in the cecum similarly to lactitol.

pH of cecal contents: Figure 2C shows the effects of lactitol and lactulose on the pH of the cecal contents. Neither drug affected the pH of the cecal contents before the final administration. However, both drugs, at doses of 3 and 10 g/kg/day, significantly reduced the pH of the cecal contents 4 hr after the final administration.

Absorption of ammonia
Figure 3 shows the effects of lactitol (A) and lactulose (B) on the absorption of ammonia. In control rats, the ammonia concentration in portal blood was increased immediately after the administration of ammonium acetate, reached a peak (about 9-fold elevation from the concentration before the administration of ammonium acetate) within 10 min, and gradually decreased thereafter. Both lactitol and lactulose inhibited the increases in the ammonia concentration in the portal blood after the administration of ammonium acetate.

Small intestinal transit
Lactitol, at doses of 1 and 3 g/kg, and lactulose, at a dose of 3 g/kg, significantly facilitated the small intestinal transit of the charcoal meal (Fig. 4A).

Colonic transit
Both lactitol and lactulose, at a dose of 3 g/kg, significantly facilitated the colonic transit of the charcoal meal.
Fig. 4. Effects of lactitol and lactulose on intestinal transit (A) and colonic transit (B) in rats. A: Drugs and charcoal meal were simultaneously administered orally, and intestinal transit was measured 10 min later. B: A charcoal meal was administered into the proximal colon 1 hr after the oral administration of drugs. Colonic transit was measured 30 min later. Values represent means±S.E. (N=9 in A and N=10 in B). *P<0.05, **P<0.01, vs control (Dunnett’s method).

DISCUSSION

Lactitol has been shown to decrease blood ammonia levels in experimental hyperammonemia (4) and in patients with chronic hepatic encephalopathy (5). In the preliminary experiments, lactitol given orally for 7.5 consecutive days decreased the ammonia concentration in the portal venous blood most substantially 4 hr after the final administration in rats (data not shown). Lactitol at doses of more than 3 g/kg/day also inhibited the onset of coma induced by ammonium acetate 4 hr after the final administration for 2 hr in PCS rats with hepatitis (4). The time showing the maximum effect and duration of the effect of lactitol were similar to those of lactulose. In the present study, lactitol, at doses of 3 and 10 g/kg/day for 7.5 consecutive days, reduced the ammonia concentration in the portal blood, ammonia content in the cecum, and the pH of the cecal contents 4 hr after the final administration. In addition, lactitol, at doses of 3 and 10 g/kg/day, inhibited the increase in the ammonia concentration in portal blood induced by the intracecal administration of ammonium acetate. These results indicate that lactitol may inhibit the absorption of ammonia from the colonic mucosa. This inhibition may be due to the ionization of ammonia through acidification of the lumen of the cecum and colon and to the facilitation of colonic transit, reducing the time during which ammonia is in contact with the colonic mucosa. The luminal acidification of the cecum and colon by lactitol may be due to the formation of volatile organic acids, including acetic, propionic and butyric acids, and nonvolatile lactic acid, which are yielded by intestinal flora through the metabolic pathway of lactitol (6). Acidification of the intestinal lumen has been reported to reduce ammonia generation from urea and amino acids (7, 8) and to prevent the absorption of ammonia by causing ionization of the ammonia (9, 10). Therefore, the decrease in ammonia content in the cecum induced by lactitol may also be important in decreasing ammonia concentration in the portal blood. In the lumen of the intestinal tract, the number of flora is relatively greater than the substrate, and therefore catabolism (production of ammonia through the deamination of amino acids) predominates over anabolism (utilization of ammonia as a nitrogen source to synthetize amino acids). Lactitol, which is not metabolized in small intestinal mucosa lacking β-galactosidase (11), may be utilized as an energy source by the intestinal flora, and may stimulate the anabolism in which ammonia is used (12), similarly to the action of lactulose (11). This fate of lactitol may explain the mechanism by which the ammonia concentration is reduced in the portal blood. All these effects of lactitol appear to be similar to those of lactulose.

Taken together, these results lead us to the conclusion that lactitol may reduce blood ammonia levels through its metabolism by intestinal flora and its facilitation of intestinal transit. Lactitol may be a more promising agent than lactulose for use in clinical practice, since lactitol has a more predictable cathartic effect and less sweet taste than lactulose (13).

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