Intestinal Absorption and Urinary Excretion of Melamine in Male Wistar Rats

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Intestinal distribution and absorption of melamine and the disposition of this compound in blood and urine were examined in Wistar male rats. When rats were fed a diet containing 1% melamine for one week, the concentration of melamine in the stomach contents was 3,040 ±772 ppm. The concentration of melamine was highest in the upper part of the small intestine, 487±101 ppm, and it decreased gradually along the intestines, being 158±20.0 ppm in the lowest part. But there was more melamine in the cecum and the large intestine: 193± 38.8 ppm and 283±20.3 ppm, respectively. Further, 22.6±6.93 ppm and 5,050±2,010 ppm of melamine appeared in the blood and urine, respectively. Melamine was not absorbed in the stomach but was absorbed monoexponentially in the small intestine. Its half life in the ligated upper part of the small intestine was 37.9 min. The doubling time of melamine was 18.6 min in the blood and 2.9 min in urine. Melamine injected into a femoral vein was excreted monoexponentially into urine from blood. The excretion was suppressed between 15 and 20 min after the injection, then resumed monoexponentially after 20 min.

Key words: melamine; rat; digestive tract; absorption; urinary excretion

Melamine, 2,4,6-triamino-1,3,5-triazine, is the starting material for melamine-formaldehyde resin used in the production of a wide variety of tableware, such as cups, bowls and dishes. It is released from these products into some food-simulating solvents[1)~3)] and some beverages[4)], and the migration concentration increases at high temperature[3)] and under acidic conditions[1)]. Environmental water, including tap water, contains from 0.05 ppb to 0.23 ppm of melamine[5)]. In the U.S.A., tolerances were established for combined residues of cyromazine and melamine in eggs, poultry fat, meat and meat products[6)], celery, and lettuce[7)]. It is clearly likely that melamine is ingested by humans. The disposition and excretion of orally administered melamine were investigated by Mast et al.[8)] using male Fisher 344 rats.

This paper reports the absorption in the digestive tract, disposition in blood and urinary excretion of melamine *in situ* in male Wistar rats.

Materials and Methods

1. *Animals and maintenance*

Six-week-old male Wistar rats (Japan SLC Inc., Shizuoka) weighing 163±8 g were used in the present studies. The rats were fed on Oriental Yeast MF diet (Oriental Yeast Co., Ltd., Tokyo) and tap water for one week in a room maintained at 25±1℃ and 60% humidity, and on a 12 -hr light/dark cycle before the experiments. The diet was withheld overnight before the experimental dosing. Six rats were used for each group of experiments.

2. *Absorption and excretion tests*

Rats were anesthetized by i.p. injection of 35 mg of sodium pentobarbital/kg body weight and the abdominal cavity was opened. The stomach was ligated at both ends without closure of the blood vessels. In the small intestine, a length of about 5 cm was ligated without closure of the blood vessels. The ligated section of the small intestine was located 10 cm from the

pylorus. After ligation of the urethra, 1,000 μg of melamine in 0.5 ml of water was injected into each ligated organ. At definite times after the injection, blood was obtained by cardiac puncture, and urine in the bladder was obtained by bladder puncture after excision of the ligated part of the digestive tract. The excised part was cut open, and the contents were washed out with a small portion of water.

3. Injection of melamine into a femoral vein

Rats received 1,000 μg of melamine by injection into a femoral vein, then blood and urine were collected at definite times after the injection.

4. Feeding test and excision of the gastro-intestinal tract

After receiving the control diet for a week, the rats were fed the MF diet containing 1% (w/w) melamine for the following seven days. Between 10 and 11 a.m. on the 7th day, the rats were anesthetized and the abdominal cavity was opened. Blood and urine were collected by puncture, then the gastro-intestinal tract was excised and the stomach, small intestine, cecum and large intestine were separated. The small intestine was cut into five equal lengths. The contents of each section were washed out with a small portion of water.

5. Determination of melamine in the biological specimens

5.1 Gastro-intestinal contents

To 0.5 g of homogenized stomach contents, 0.5 ml of 1 % trichloroacetic acid was added. The mixture was pumped into a Sep-Pak TM Plus QMA® cartridge. The eluate from the cartridge was used as a test solution for high performance liquid chromatography (HPLC).

5.2 Blood and urine

Into a Bond Elut SCX® cartridge, 0.1 g of blood or urine was loaded. The cartridge was washed with 4 ml of water and 4 ml of 0.1 *N* hydrochloric acid (HCl), then melamine was eluted with 4 ml of 6 *N* HCl. The eluate was dried on a water bath, and the residue was dissolved in 1 ml of water. For blood, after the solution was filtered through a membrane filter, and the filtrate was used as a test solution for HPLC. The solution obtained from urine was charged into a Sep-pak TM Plus QMA® cartridge. The eluate was used as a test solution for HPLC.

5.3 Determination of melamine

Melamine was determined by HPLC[9]. The conditions for HPLC were as follows. Equipment, Shimadzu LC-6A. Column, Yana-pak ODS-A; 4.6 mm diameter and 250 mm length. Temperature, 40℃. Mobile phase, a 0.05 *M* phosphate buffer solution; pH 3.0. Flow rate, 0.6 ml/min. Detector, a Shimadzu SPD-6AV equipped with a D_2 lamp; 235 nm.

Melamine in biological specimens was confirmed with three-dimensional chromatograms[3]. The conditions for HPLC for this determination of melamine were as follows. Equipment, JASCO 880-PU. Column, Yana-pak ODS-A; 4.6 mm diameter and 250 mm length. Temperature, 40℃. Mobile phase, a 0.05 *M* phosphate buffer solution; pH 3.0. Flow rate, 0.6 ml/min. Detector, a Shimadzu SPD-M6A photodiode array (scan range, 200～300 nm).

Recoveries of melamine added to stomach contents (at 400 ppm), intestinal contents (300 ppm), blood (1 ppm) and urine (100 ppm) were 115.0±0.2%, 115.7±4.8%, 85.4±6.6% and 82.6±8.3%, respectively (mean±S.D. of five trials).

Results and Discussion

1. Gastro-intestinal distribution of melamine

The distribution of melamine in the contents of the different areas of the gastro-intestinal tract and the levels in blood and urine were determined after the rats had been fed on the 1% melamine-containing diet for one week (Fig. 1). The melamine concentration in the stomach contents was 3,040±772 ppm and the amount of melamine in the stomach contents/rat was 7.20±2.26 mg. The melamine concentrations in the small intestine were higher in the upper part of the tract (487±101 ppm in the first segment) than in the lower intestine (158±20.0 ppm in the fifth segment). But, the melamine concentrations in the cecum (193±38.8 ppm) and the large intestine (283±20.3 ppm) were higher than in the fifth segment of the small intestine. The higher concentration of melamine in the cecum and the large intestinal contents compared with that in the lower intestinal contents may

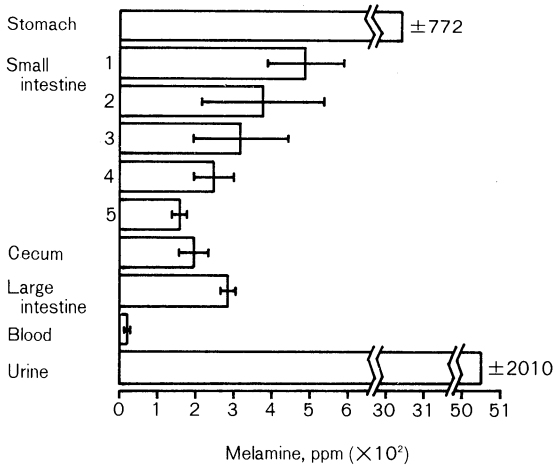

Fig. 1. Concentrations of melamine in the digestive tract and blood and urine of rats fed on the diet containing 1% melamine
Rats were given the diet containing melamine for one week, and killed between 10 and 11 a.m. after the feeding period

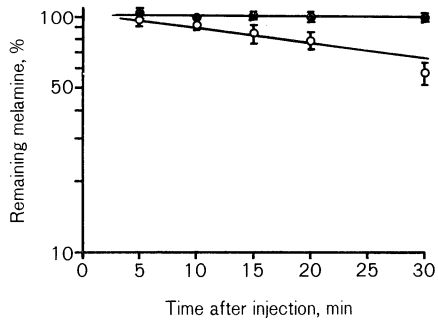

Fig. 2. Gastro-intestinal absorption of melamine in rats
Melamine (1000 μg) was injected into the ligated section of the stomach or the small intestine, and the amount of melamine remaining in the ligated part was determined at intervals.
●: stomach; ○: small intestine

be due to the adsorption of intestinal moisture. The concentrations of melamine in the blood and urine were 22.6±6.93 ppm and 5,050± 2,010 ppm, respectively. The average urinary volume at death was 0.41 ml (between 0.13 and 0.87 ml), and melamine amount in the urine was 1,852 μg (between 766 and 3,813 μg) per rat. Melamine concentrations in the digestive tract and body fluids may be the result of equilibrium between ingestion and excretion, when animals were fed melamine for a week. The melamine in these biological specimens were identified by means of three-dimensional chromatograms obtained by HPLC using a photodiode array detector.

The presence of melamine in the lower digestive tract suggests contact of this compound with intestinal microorganisms. The microbial flora in the lower intestine may be affected by the ingestion of large amounts of melamine since the growth of some microorganisms is inhibited by melamine[10]. Although melamine is not known to be metabolized in rats[8] or men[11,12], a *Pseudomonas* sp. isolated from sewage is known to utilize the amino group of melamine *in vitro* and to produce ammeline[13,14], a compound inducing blindness in chickens[15,16].

Kadokami and Shinohara[5] reported that 0.05 ppb of melamine was found in tap water. It was also reported that release of melamine from melamine tableware into beverages was less than 3 ppm when the tableware was heated with various beverages at 95℃ for 30 min[4]. The melamine residue in some agricultural products is regulated to less than 10 ppm in the U.S.[6,7]. However, there are several reports of the illegal addition of melamine to food[17] and feed[18]. Bisaz and Kummer[17] found up to 5.2% melamine had been added as an adulterant in potato protein used for food. The melamine concentration in the diet used in the present study is a possible level in the human environment.

2. Absorption of melamine from the ligated digestive tract

Mast et al.[8] reported that orally administered ^{14}C-melamine was rapidly absorbed and maximal plasma concentrations were reached within 60 min, but the absorption site in the digestive tract was not determined.

The rates of absorption of melamine from the ligated stomach and small intestine, after the injection of 1,000 μg of melamine into the ligated section, are shown in Fig. 2. Melamine was not absorbed in the stomach, but it was absorbed in the small intestine. This result on the absorption site may be supported by the pKa, 5.00[19], and by the fact that the *n*-octanol/water partition coefficient (P) was −2.03. The mela-

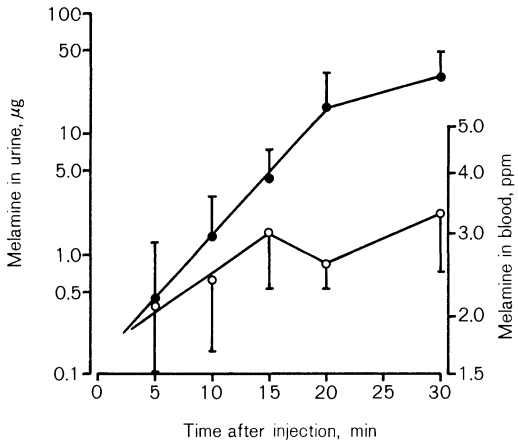

Fig. 3. Appearance of melamine in blood and urine of rats
Melamine (1000 μg) was injected into the ligatured part of the small intestine, and its appearance in blood and urine was monitored.
○: blood; ●: urine

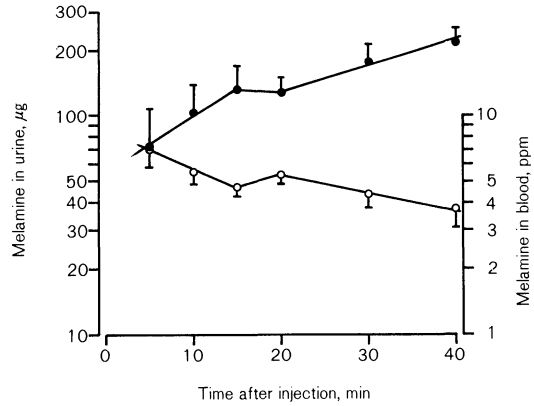

Fig. 4. Excretion of blood melamine into urine
Melamine (1000 μg) was injected into a femoral vein.
○: blood; ●: urine

mine concentration in the ligated small intestine decreased monoexponentially, and the biological half time of melamine in the ligated part of the upper small intestine was 37.9 min. So the melamine concentration found in the stomach in the feeding test may be regarded as reflecting the fact that melamine added to the diet is diluted with drinking water and gastric juice, but the decrease of melamine in the segments of the small intestine may be caused by absorption of melamine *in situ*.

The secretion of melamine into blood and excretion into urine when 1,000 μg of melamine was injected into the ligated small intestine are shown in Fig. 3. The melamine content in blood was 2.10±0.66 ppm at 5 min after the injection, and 2.99±0.68 ppm at 15 min. The melamine concentration in blood during the initial 15 min increased monoexponentially. The melamine in the blood decreased to 2.59±0.29 ppm at 20 min, but again increased to 3.31±0.80 ppm at 30 min. Urinary melamine increased throughout the 30-min experimental period. The melamine excretion in urine in the initial 20 min also increased monoexponentially. The excretion reached 29.8±19.8 μg/rat at 30 min. The melamine in the blood and urine after 30 min was determined by three-dimensional high performance liquid chromatograms. The doubling time of melamine in blood during the initial 15 min was 18.6 min and that in urine during the initial 20 min was 2.9 min.

3. Urinary excretion of melamine injected into a femoral vein

The decrease of melamine concentration in blood and the increase of urinary excretion when 1,000 μg of melamine was injected into a femoral vein are shown in Fig. 4. The blood melamine decreased monoexponentially during the initial 15 min and the urinary melamine increased monoexponentially in the same period. The half life of melamine in blood in the initial 15 min was 18.3 min and the doubling time in urine was 9.9 min. Melamine in the blood increased from 4.74±0.37 ppm at 15 min to 5.24±0.43 ppm at 20 min after it was injected. The urinary excretion of melamine was suppressed at the same time. After 20 min, the blood melamine decreased and the urinary melamine again increased monoexponentially.

The change in the melamine concentration in blood when the compound was injected into a femoral vein corresponded to the results obtained from the experiment in which the compound was injected into the ligated portion of the small intestine. While urinary excretion of melamine stopped between 15 and 20 min in the case of i.v. injection, it increased linearly for 20 min in the latter experiment. A probable reason

for the shoulder of the melamine concentration in blood observed in both experiments, the increase or decrease of melamine concentration at 20 min, is biliary excretion, since the melamine concentration decreased or increased again at 30 min. The suppression of the urinary excretion of melamine at 20 min may support this consideration. A similar phenomenon related to an entero-hepatic cycle has been observed in the case of dimethylamine when the compound was injected into a femoral vein of Wistar rats[20]. Concerning the decrease of melamine in blood, it seems unlikely that the administered melamine was metabolized since melamine is known to be little metabolized in rats[8]. Melamine present in the urine is shown as the amount, μg, in the present study to avoid the effect of dilution with the urine in the bladder which had been excreted before the ligation of the urethra. The standard deviation of the excreted melamine (in μg) in urine was small. The rate of absorption of melamine in the small intestine was very slow among the assimilation sites in the present experiments, so the excretion of melamine into urine would have been restricted by the absorption velocity.

The fate of melamine was studied in rats. The compound was not absorbed in the stomach but was absorbed in the small intestine, and was excreted in urine. Melamine ingested with feed was detected even in the cecum and the large intestine. This fact indicates that the growth of intestinal microorganisms may be affected by melamine[10,21] and that there is a possibility of microbiological degradation[13,14] of the compound in the intestine.

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