Original Article

Differential colon DNA damage induced by azo food additives between rats and mice

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ABSTRACT — Azo dyes, amaranth, allura red and new coccine, which are currently used as food color additives in Japan, have been reported to cause colon specific DNA damage in mice. To examine species difference in the DNA damage between rats and mice, each of dyes was administered to male mice (1 and 10 mg/kg) and male rats (10, 100 and 1,000 mg/kg) by gavage. Brain, lung, liver, kidney, glandular stomach, colon, urinary bladder and bone marrow were sampled 3 hr (for mice) and 3, 6, 12 and 24 hr (for rats) after the treatment. The alkaline comet assay showed DNA damage in the mouse colon 3 hr after the administration of all of the dyes at 10 mg/kg. In rats, however, none of the dyes damaged DNA. Azo dyes should undergo metabolic reduction in the colon to be adducted to DNA. To determine transit time of the dyes to the colon after their administration, gastric emptying and intestinal transport in mice and rats were examined using brilliant blue FCF (BB) as an indicator. The half times of gastric emptying were 70 and 80 min for mice and rats, respectively; and about 60% of the BB was removed from the stomach 1 hr after the gastric intubation in both mice and rats. BB reached the mouse and rat colon 1 and 3 hr after the administration, respectively. Considering the wide dose range and sampling times well covering the transit time to the colon, rats may be insensitive to these azo dye-induced DNA damage.

Key words: Rat, Mouse, DNA damage, Colon, Food additive

INTRODUCTION

The synthetic azo dyes, amaranth, allura red and new coccine are used as food additives in Japan. These dyes have been negative in Ames test with or without S9 mix (Fujita and Sasaki, 1993; Fujita *et al.*, 1995; IARC, 1975; Prival *et al.*, 1988; JECFA 487. Allura red AC; JECFA 565. Ponceau 4R; JECFA 575. Amaranth). Tsuda *et al.* (2001) however, reported that these dyes induced colon specific DNA damage in mice when examined with the *in vivo* comet assay.

Water-soluble azo dyes such as amaranth, allura red and new coccine have charged sulfo groups that preclude significant absorption of the ingested dye. When the intact dye reaches the colon, it can undergo extensive metabol-

ic reduction by intestinal microflora (Levine, 1991), and the reduced aromatic amines are rapidly absorbed (Chung *et al.*, 1992). Aromatic amines are oxidized and acetylated by P450 and acetyltransferase, respectively, and the activated aromatic amines show genotoxicity (Kato and Kamataki, 1995). The complex metabolic pathway of the azo dyes including azo reduction may be one of the reasons for the negative results in Ames tests even with S9 mix. The *in vivo* comet assay that has the advantage of reflecting absorption, distribution, metabolism and excretion would be a more effective tool for detecting the organ specific genotoxicity of these food additives undergoing the complex metabolism.

There have been no reports, however, that examined DNA damage of these azo dyes in the *in vivo* comet assay

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in rats. The information about the gastrointestinal transit of the compound is important when the oral toxicity is assessed, especially to justify the sampling time. Intestinal transport data were reported for mice (Tsuda *et al.*, 1998), but no available data on the bowel transit time have been reported for rats.

In this study, therefore, we examined DNA damage of these azo dyes using the *in vivo* comet assay in rats, in relation to the gastric emptying and the intestinal transport in rats.

MATERIALS AND METHODS

Chemicals

All samples of amaranth (Food red No. 2, CAS 915-67-3), allura red (Food Red No. 40, CAS 25956-17-6) and new coccine (Food Red No. 102, CAS 2611-82-7) were from certified lots approved for food use in Japan and were purchased from Tokyo Chemical Industry Co., Ltd (Tokyo, Japan). Brilliant blue FCF (BB), which is approved in Japan for use in food, drugs and cosmetics, was purchased from Tokyo Chemical Industry (Tokyo, Japan). Regular (GP-42) and low melting point (LGT) agarose were obtained from Nacalai Tesque (Kyoto, Japan) and diluted respectively to 1% and 2% in physiological saline. BB was used as a marker dye, because it confers a distinctive blue color and is not practically absorbed from the intestine. BB was dissolved in distilled water to make 10 mg/ml solution.

Animals

Male ICR (CD-1) mice or male Fischer (F344) rats were obtained from Charles River Japan, Inc. (Kanagawa, Japan) at 7 weeks of age and used for the experiments after 1 week acclimatization. They were fed with commercial pellets MF (Oriental Yeast Co., Ltd., Tokyo, Japan) and tap water *ad libitum* throughout the acclimatization period and the experiment. The animal room was kept at $22 \pm 2^\circ\text{C}$ and 50-70% humidity with a 12-hr light-dark cycle. All procedures were approved by the Animal Research Committee, Faculty of Agriculture, Iwate University, and were conducted under both Guidelines for Animal Experiment in Iwate University and the "Guiding Principles in the Use of Animals in Toxicology," which were adopted by the Society of Toxicology in 1989.

Comet assay

Each dye was dissolved in distilled water and administered by gavage in a volume of 10 ml/kg. Each animal was administered with the dye at 10 to 11 a.m., and was fed with the pellets and tap water *ad libitum* during

whole experimental period. For the mouse study, four to six mice were assigned to each treatment group. For the rat study, four to five rats were assigned to each treatment group. In previous *in vivo* comet assays (Sasaki *et al.*, 1997a, 1997b and 1998; Tsuda *et al.*, 1998, 2001; Sekihashi *et al.*, 2002), there were no significant differences in mean migration between vehicle control groups and corresponding untreated groups at any time for any organ in rats and mice. The results enabled us to use untreated (zero time) control animals rather than a larger number of concurrent sampling time control animals.

From shortly after treatment until just before they were sacrificed, the animals were carefully observed for pharmacotoxic signs. Mice were sacrificed by cervical dislocation 3 hr after the treatment of azo dyes (1 and 10 mg/kg), and rats were sacrificed by anesthesia with ether 3 hr after the treatment of azo dyes (10 mg/kg), and 6, 12 and 24 hr after the treatment of azo dyes (100 and 1,000 mg/kg), and necropsied; brain, lung, liver, kidney, stomach, colon, urinary bladder and bone marrow were removed.

The assay was conducted as previously described (Tsuda *et al.*, 1998, 2001; Hashimoto *et al.*, 2007). The protocol used was in accordance with the published comet assay genotoxicity guidelines (Tice *et al.*, 2000). The brain, lung, liver and kidney were minced, suspended in chilled homogenizing buffer (pH 7.5) containing 0.075 M NaCl and 0.024 M Na_2EDTA , and then homogenized gently using a Potter type homogenizer. The mucosa of glandular stomach, colon and urinary bladder was scraped with a paper knife into chilled homogenizing buffer and homogenized in the same manner. To obtain nuclei, the homogenates were centrifuged at $700 \times g$ for 10 min at 0°C , and the precipitates were resuspended in chilled homogenizing buffer. Slides prepared from nuclei isolated by homogenization were placed in a chilled lysing solution (2.5 M NaCl, 100 mM Na_4EDTA , 10 mM Trizma, 1% Sarkosyl, 10% DMSO and 1% Triton X-100, pH 10), and were kept at 0°C in the dark for more than 60 min, then in chilled alkaline solution (300 mM NaOH and 1 mM Na_2EDTA , pH 13) for 10 min in the dark at 0°C . Electrophoresis was conducted at 0°C in the dark for 15 min at 1 V/cm and approximately 250 mA. The slides were neutralized and stained with 50 μl of 20 $\mu\text{g/ml}$ ethidium bromide (Wako Pure Chemicals, Osaka, Japan).

We examined and photographed 50 nuclei per slide at $200 \times$ magnification with the aid of a fluorescence microscope. The length of the whole comet ("length") and diameter of the head ("diameter") were measured for 50 nuclei per organ per animal. We calculated migration as the differences between length and diameter for each of 50 nuclei. Mean migration of 50 nuclei from each organ

was calculated for each individual animal. The differences between the averages of treated animals and the untreated control animals were compared with the Dunnett test after one-way ANOVA. A *p*-value less than 0.05 was considered statistically significant.

Histopathology

After removal of mucosa, the rest of the glandular stomach and colon tissues were processed for histopathology to examine if the entire mucosa was removed. The rest of these tissues were fixed in Bouin fixative, decolorized into saturated 70% ethanol with lithium carbonate, dehydrated, embedded in paraffin, sectioned at 5 μ m, and stained with hematoxylin and eosin.

Gastric emptying

Three mice and two to four rats were used. Mice and rats were orally administered with 0.35 ml/head and 1.8 ml/head of BB, respectively, at 10 to 11 a.m. They were fed with the pellets and tap water *ad libitum* during whole experimental period. Mice were sacrificed by the cervical dislocation 1, 5, 10, 30 and 60 min after the administration. Rats were sacrificed by cervical dislocation 1, 5 and 10 min after the administration or with ether anesthesia 1 and 3 hr after the administration. The gastric content was sampled, weighted (average weights of the contents were ca. 0.7 g and ca. 3.1 g for a mouse and a rat, respectively), and was mixed well to ensure the uniformity of the distribution of BB in the gastric content. Distilled water was added to the 0.05 g portion of the gastric content, amounting to 5 ml suspension, then centrifuged at 15,000 rpm for 10 min at the room temperature. The supernatant was collected. The extraction procedure from the precipitate was repeated until the supernatant color became practically zero (less than the color of 0.5 μ g/ml of BB solution). All the supernatant samples were pooled together, and the BB density was measured spectrophotometrically (wave length: 630 nm).

The amount of BB in the stomach and the gastric emptying (%) was decided with the following formulas.

$$\begin{aligned} &\text{The amount of BB in the stomach (mg)} \\ &= \{\text{the supernatant density (mg/ml)}\} \times \{\text{total pooled} \\ &\quad \text{volume of the supernatant (ml)}\} \times \{\text{the weight of} \\ &\quad \text{gastric content (g)} / 0.05 \text{ (g)}\} / \{\text{the recovery rate} \\ &\quad \text{(\%)}\} \end{aligned}$$

$$\begin{aligned} &\text{The gastric emptying (\%)} \\ &= 100 \times \{\text{the amount of BB in the stomach at the sam-} \\ &\quad \text{pling time (mg)}\} / \{\text{dose of BB}\} \end{aligned}$$

Recovery test was conducted as follows: 0.35 ml and

1.8 ml of BB was mixed with the stomach content of the mouse and rat, respectively. The extraction procedure was the same as that for the gastric emptying measurement. The amount of recovered BB and the recovery rate were decided with the following formulas.

$$\begin{aligned} &\text{The amount of recovered BB (mg)} \\ &= \{\text{the supernatant density (mg/ml)}\} \times \{\text{total pooled} \\ &\quad \text{volume of the supernatant (ml)}\} \times \{\text{the weight of the} \\ &\quad \text{mixture (g)}\} / 0.05 \text{ (g)} \end{aligned}$$

$$\begin{aligned} &\text{The recovery rate (\%)} \\ &= 100 \times \{\text{the amount of recovered BB (mg)}\} / \{3.5 \\ &\quad \text{(mg) or 18 (mg) of BB in the mouse or rat, respec-} \\ &\quad \text{tively}\} \end{aligned}$$

Intestinal transport

Four to eight mice and three to four rats were assigned to each treatment group. Mice and rats were administered orally 0.35 ml/head and 1.8 ml/head of BB, respectively, at 10 to 11 a.m. They were fed with the pellets and tap water *ad libitum* during whole experimental period. Mice were sacrificed by the cervical dislocation 1, 5, 10, 15, 30, 60 and 180 min after the administration. Rats were sacrificed by the cervical dislocation 1, 5 and 10 min or with ether anesthesia 1, 3 and 6 hr after the administration.

When BB was observed only in the small intestine, the mobility of the dye (%) there was determined by comparing the distance the dye was transported in the small intestine with the total length of small intestine. When the dye was observed in the large intestine, the mobility of the dye (%) there was determined by comparing the distance the dye was transported in the large intestine with the total length of large intestine. The small intestine was $84.5 \pm 0.3\%$ ($n = 36$) and $86.4 \pm 0.3\%$ ($n = 25$) of the length of the total intestine in mice and rats, respectively. Thus, the mobility of the dye in the entire intestine was calculated using a fixed value of 84.5% and 86.4% for the proportion of small intestine (the cecum is between the small and large intestine) for each mouse and rat, respectively (Tsuda *et al.*, 1998).

RESULTS

Comet assay

No death, morbidity, or distinctive clinical signs were observed after any treatment. Necropsy revealed no treatment effect on any organ examined. Thus, any DNA damage observed was not likely to be due to general cytotoxicity (Tsuda *et al.*, 2001). The histopathology of the glandular stomach and colon tissues left after the scraping

showed that the entire mucosa was removed.

An increase in DNA damage was indicated by an increase in migration length of the stained DNA. In mice, as shown in Table 1, each of amaranth, allura red and new coccine induced colon specific DNA damage at a dose of 10 mg/kg at 3 hr after the administration. In rats, shown in Tables 2 and 3, neither the colon specific DNA damage nor the damages in the other organs tested were observed for any tested dyes with a wide dose range of 10 to 1,000 mg/kg at any sampling times from 3 to 24 hr except for the DNA damage in the stomach shown 3 hr after the administration of amaranth with the dose of 10 mg/kg. The DNA damage in the stomach was considered sporadic because of lack of dose-related response.

Gastric emptying

The recovery rates were 98.5% and 92.9% for mice and rats, respectively. The gastric content 1 min after the administration varied greatly, thus these data were not used for the regression analysis. The regression lines of the gastric emptying after 5 min were $y = 77e^{-0.0099t}$ and $y = 70e^{-0.0087t}$ for mice and rats, respectively. Y: gastric content (%) in the stomach, t: time after the gastric intubation.

$T_{1/2}$ of the gastric emptying was about 70 min for mice and about 80 min for rats. The average contents of 57% and 58% were removed from the stomach 60 min after the oral administration to mice and rats, respectively. The gastric contents at time zero were about 80% and 70% for mice and rats, respectively, while the recovery rates were almost 100%. The observation made 1 min after the administration of BB showed stained gastric mucosa but no outflow of the gastric content was observed. The BB stained in the mucosa could not be easily extracted. The reason for the low gastric content at time zero is not clear.

Intestinal transport

As shown in Fig. 1, BB reached the colon 1 hr and reached almost the end of the intestine 3 hr after its administration to mice. In rats, BB arrived at the colon 3 hr after the administration. After the administration, BB traveled about 90% and 100% of the rat intestine for 6 and 12 hr, respectively.

DISCUSSION

Our present results showing that amaranth, allura red and new coccine induced colon specific DNA damage at 10 mg/kg in mice confirmed the previously reported results (Tsuda *et al.*, 2001).

Present results of *in vivo* rat comet assay showed that none of the dyes tested induced colon specific DNA damage with a wide dose range of 10 to 1,000 mg/kg at any sampling times from 3 to 24 hr. Colon DNA damage in mice induced by the dyes is detected 3 hr after the administration, and the damage remains for up to 24 hr (Tsuda *et al.*, 2001). If we assume that BB moves similarly to the azo dyes, about 60% of the azo dyes may have been removed from the mouse stomach 1 hr after the administration, and reach the colon 1 hr and almost the end of the intestine 3 hr after the administration. From this kinetics, colon DNA damage in mice caused by these dyes may appear 2 hr after the arrival at the colon, and the damage may persist for about 24 hr. Gastric emptying for rats was similar to that of the mice. After the administration the azo dyes may arrive at the rat colon in 3 hr and at the end of intestine within 6 hr. Thus, the chance that the sampling times of rat colon epithelium from 3 to 24 hr would have missed the dye-induced DNA damage may be low. Based on the wide range of dose selection and the sampling times well covering bowel transit time, the absence of azo-induced DNA damage in rats may be intrinsic to the rat.

The *in vivo* comet assay with a similar protocol to the present study where the sampling time covers from 3 to 24 hr showed that all of the 23 rodent genotoxic carcinogens tested that were positive in at least one organ of mice also showed the positive effect in at least one organ of rats, although the two species differed in target organs (Sekihashi *et al.*, 2002). The study also revealed that 15 rodent carcinogens which caused DNA damage in mouse colon also caused it in rat colon. Because many chemicals induce colon DNA damage in a similar way in mice and rats, this unique azo-induced differential DNA damage might reflect the different metabolism of these dyes between mice and rats. There are marked differences in enzyme activities associated with the hindgut microflora such as caecal azoreductase between rats and mice (Rowland *et al.*, 1986). A species difference in the extent of N-oxidation of azo compounds is also evident between rats and mice (Koh and Gorrod, 1989). Thus, the different metabolism of these azo dyes between rats and mice may be due to the differences in microflora and/or other enzymes such as microsomal N-oxidation.

A compendium of bioassay results organized by target organ for 533 chemicals that are carcinogenic at least one species shows that none of the 299 chemicals evaluated as carcinogenic in mice have the large intestine as target organ, although it shows that 4% of 354 chemicals evaluated as carcinogenic in rats have the large intestine as target organ (Gold *et al.*, 1991). The mice with

Table 1. Migration of nuclear DNA from organs of male mice treated with red food dyes

	Dose (mg/kg)	Sampling time (hr)	Migration							
			Colon	Stomach	Urinary bladder	Lung	Kidney	Liver	Brain	Bone marrow
Control n = 6	0	0	4.22 ± 0.52	3.57 ± 0.33	2.73 ± 0.65	2.16 ± 0.33	1.53 ± 0.38	0.84 ± 0.38	0.30 ± 0.14	1.16 ± 0.56
Amaranth n = 4	1	3	3.39 ± 0.32	3.01 ± 0.79	1.89 ± 0.24	1.40 ± 0.41	1.33 ± 0.63	0.29 ± 0.18	0.36 ± 0.29	0.77 ± 0.45
	10	3	12.56 ± 1.34**	3.76 ± 0.47	2.41 ± 0.69	3.10 ± 0.93	1.37 ± 0.38	1.81 ± 0.84	0.37 ± 0.14	0.37 ± 0.37
Allura red n = 4	1	3	5.69 ± 1.44	4.73 ± 0.77	2.01 ± 0.24	0.87 ± 0.23	1.41 ± 0.58	0.47 ± 0.37	0.23 ± 0.09	0.49 ± 0.40
	10	3	13.80 ± 1.15**	5.64 ± 1.06	3.90 ± 0.42	3.19 ± 1.16	1.93 ± 1.05	1.60 ± 0.58	0.80 ± 0.30	0.71 ± 0.20
New cocine n = 4	1	3	3.34 ± 0.77	4.97 ± 1.03	3.33 ± 1.03	1.89 ± 0.30	1.54 ± 0.70	0.54 ± 0.34	0.99 ± 0.65	1.31 ± 0.55
	10	3	12.34 ± 0.87**	4.76 ± 1.34	3.66 ± 0.51	3.14 ± 0.46	1.33 ± 0.76	0.59 ± 0.48	1.00 ± 0.69	1.90 ± 0.86

Red food dyes dissolved in distilled water were given to mice (4~6 mice per group) by gavage with a volume of 10 ml/kg. Differences between treatments and the control were compared with the Dunnett test after one-way ANOVA. Values (µm) are given as mean ± S.E. ** Significant differences between control and treatment group, 0.001 < *p* < 0.01.

Table 2. Migration of nuclear DNA from organs of male Rats treated with red food dyes

	Dose (mg/kg)	Sampling time (hr)	Migration							
			Colon	Stomach	Urinary bladder	Lung	Kidney	Liver	Brain	Bone marrow
Control n = 5	0	0	3.67 ± 1.19	5.55 ± 0.92	6.83 ± 1.91	3.10 ± 0.91	7.49 ± 3.62	0.47 ± 0.23	0.49 ± 0.44	0.45 ± 0.15
Amaranth n = 4	10	3	4.93 ± 1.15	9.70 ± 1.48*	7.64 ± 1.27	2.73 ± 1.05	11.21 ± 3.08	0.24 ± 0.16	0.51 ± 0.28	0.53 ± 0.36
Allura red n = 4	10	3	3.97 ± 2.19	7.00 ± 0.55	5.77 ± 1.01	3.14 ± 0.97	6.54 ± 2.76	1.00 ± 0.39	0.30 ± 0.23	0.23 ± 0.16
New cocine n = 4	10	3	3.54 ± 0.69	3.61 ± 0.48	8.87 ± 1.33	2.64 ± 0.99	5.97 ± 1.52	0.87 ± 0.32	0.10 ± 0.08	0.41 ± 0.41

Red food dyes dissolved in distilled water were given to rats (4~5 rats per group) by gavage with a volume of 10 ml/kg. Differences between treatments and the control were compared with the Dunnett test after one-way ANOVA. Values (µm) are given as mean ± S.E. * Significant differences between control and treatment group, 0.01 < *p* < 0.05

Table 3. Migration of nuclear DNA from organs of male rats treated with red food dyes

			Migration							
	Dose (mg/kg)	Sampling time (hr)	Colon	Stomach	Urinary bladder	Lung	Kidney	Liver	Brain	Bone marrow
Amaranth n = 4	0	0	8.06 ± 2.94	9.93 ± 1.89	6.33 ± 2.05	2.81 ± 0.62	5.89 ± 1.79	1.99 ± 0.29	1.49 ± 0.31	1.73 ± 0.54
		6	5.99 ± 1.80	5.79 ± 2.48	8.24 ± 1.16	2.19 ± 0.75	3.96 ± 1.58	1.59 ± 0.31	0.91 ± 0.36	0.86 ± 0.52
	100	12	11.61 ± 1.20	6.31 ± 1.03	13.36 ± 1.24	2.63 ± 0.62	7.10 ± 2.71	2.61 ± 0.99	1.11 ± 0.39	0.61 ± 0.38
		24	8.36 ± 2.90	6.24 ± 0.26	10.84 ± 2.55	2.94 ± 0.80	4.67 ± 2.48	1.81 ± 0.28	0.99 ± 0.63	0.50 ± 0.29
	1000	6	6.77 ± 1.38	6.87 ± 1.12	8.14 ± 1.59	2.76 ± 0.69	3.10 ± 0.55	2.53 ± 0.62	1.57 ± 0.54	1.29 ± 0.37
		12	12.84 ± 2.48	10.43 ± 0.95	11.14 ± 1.54	4.03 ± 0.91	6.94 ± 1.42	1.89 ± 0.61	2.41 ± 0.79	1.53 ± 0.74
		24	9.57 ± 1.68	9.67 ± 1.41	13.86 ± 1.79	3.17 ± 1.35	8.86 ± 1.26	3.06 ± 0.35	2.86 ± 1.29	1.06 ± 0.32
Allura red n = 4	0	0	6.77 ± 2.16	6.71 ± 0.62	6.34 ± 2.03	3.54 ± 0.78	7.36 ± 1.95	1.94 ± 0.66	1.86 ± 1.16	1.07 ± 0.56
		6	6.29 ± 1.97	6.36 ± 2.44	9.44 ± 3.86	2.47 ± 0.70	4.27 ± 1.49	1.81 ± 0.52	1.37 ± 0.50	0.67 ± 0.37
	100	12	6.84 ± 2.19	8.16 ± 0.33	9.51 ± 2.54	1.47 ± 0.36	3.76 ± 1.04	1.00 ± 0.37	1.41 ± 0.53	0.31 ± 0.22
		24	7.00 ± 1.48	7.06 ± 1.24	8.37 ± 1.96	3.53 ± 1.39	6.34 ± 1.77	1.49 ± 0.40	0.37 ± 0.14	1.41 ± 0.87
	1000	6	7.61 ± 1.71	8.64 ± 1.29	11.00 ± 1.31	2.59 ± 0.98	5.93 ± 2.06	1.43 ± 0.12	0.53 ± 0.22	0.24 ± 0.17
		12	12.76 ± 3.40	9.43 ± 1.82	19.73 ± 6.51	3.79 ± 1.07	10.26 ± 3.11	2.57 ± 0.88	1.86 ± 0.64	1.03 ± 0.50
		24	9.06 ± 2.21	7.87 ± 1.71	12.21 ± 1.24	2.44 ± 0.76	6.64 ± 0.55	2.07 ± 0.39	2.21 ± 0.39	1.01 ± 0.07
New coccine n = 4	0	0	7.49 ± 2.17	7.19 ± 1.46	4.47 ± 1.62	2.44 ± 0.30	7.69 ± 2.12	2.33 ± 0.85	2.21 ± 0.82	1.46 ± 0.92
		6	5.23 ± 1.34	5.04 ± 0.73	7.04 ± 1.34	0.96 ± 0.25	1.89 ± 0.49	0.77 ± 0.10	0.56 ± 0.25	0.09 ± 0.09
	100	12	9.64 ± 3.31	5.29 ± 1.51	8.49 ± 2.26	2.07 ± 0.64	5.89 ± 1.34	0.86 ± 0.31	1.47 ± 0.39	0.97 ± 0.56
		24	9.76 ± 1.48	8.80 ± 1.88	11.87 ± 1.98	1.79 ± 0.35	2.51 ± 0.84	1.17 ± 0.19	1.67 ± 0.42	0.59 ± 0.21
	1000	6	9.11 ± 1.66	8.70 ± 1.39	6.80 ± 2.70	1.77 ± 0.34	8.61 ± 1.10	2.14 ± 0.27	1.19 ± 0.27	0.71 ± 0.28
		12	9.64 ± 2.02	8.56 ± 1.40	14.06 ± 3.37	1.89 ± 0.83	12.07 ± 2.33	2.74 ± 0.32	1.97 ± 0.35	1.21 ± 0.88
		24	7.89 ± 2.38	5.34 ± 2.03	11.60 ± 1.49	3.16 ± 0.91	5.84 ± 1.84	2.49 ± 0.51	1.10 ± 0.41	1.43 ± 0.55

Red food dyes dissolved in distilled water were given to rats (4 rats per group) by gavage with a volume of 10 ml/kg. Differences between treatments and the control were compared with the Dunnett test after one-way ANOVA. Values (µm) are given as mean ± S.E. There was no significant difference between any corresponding control and treatment group.

Differential colon DNA damage between rats and mice

Fig. 1. Intestinal transport of mice and rats. The time courses of intestinal transport (%) after the BB administration to mice ($n = 4$ to 8) and rats ($n = 3$ to 4). All data are mean $\pm$ S.E. The small intestine (the cecum is between the small and large intestine) was 84.5% ($n = 36$) and 86.4% ($n = 25$) the length of the total intestine in mice and rats, respectively.

the *Apc* gene mutation, which is an important suppresser gene of the human colon cancer, induced the cancer mostly in the small intestine, but not in the colon (Fodde *et al.*, 1994; Oshima *et al.*, 1995). Poul *et al.* (2009) reported that amaranth did not induce micronucleus in mice guts and discussed that the colon DNA damage in mice comet assay was unable to be fixed in stable genotoxic lesions. Heterocyclic amines including PhIP induce colon cancer in rats but not in mice (Nagao and Sugimura, 1993), although PhIP induces colon DNA damage in both mice and rats (Sekihashi *et al.*, 2002). Therefore, the detection power of mice for colorectal cancer may be much lower than rats.

The carcinogenicity of amaranth has been tested in mice, rats and dogs, and those results were considered to be inconclusive by the Working Group of IARC (1975, 1987). A long-term toxicity study of new coccine in rats exposed *in utero* showed no carcinogenicity (Brantom *et al.*, 1987). Allura red is not carcinogenic in rats (Borzelleca *et al.*, 1989) or mice (Borzelleca *et al.*, 1991). The present results showing comet positive and negative in mouse and rat colons respectively are in good agreement with the negative results of rat and mouse carcinogenicity studies of the dyes. Because the sensitivity of carcinogenicity of mouse colon seemed to be very low compared to rat colon.

For human cancer risk assessment of these dyes, however, further studies are required to clarify the underlying mechanism of differential effect of these dyes on the colon DNA damage between rats and mice.

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