Note

Effect of *Streptococcus thermophilus* on the Trp-P-1 Level in the Blood

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We examined the 3-amino-1,4-dimethyl-5H-pyrido[4,3-b]indole (Trp-P-1) concentration in the blood after administering Trp-P-1 (0.25 mg/ml) with or without *Streptococcus thermophilus* 1131 cells (10 mg/ml) to rats. The Trp-P-1 concentration in the blood from the portal vein was significantly lower in the rats that had been administered with Trp-P-1 with the strain 1131 cells than without them. However, there was no difference in the Trp-P-1 concentration in the blood taken from the abdominal aorta of these rats.

**Key words:** portal vein; abdominal aorta; blue rayon

Many kinds of heterocyclic amines are present in broiled, fried and smoked foods, and have mutagenic and/or carcinogenic activities.1) Humans are exposed to heterocyclic amines such as 3-amino-1,4-dimethyl-5H-pyrido[4,3-b]indole (Trp-P-1), 2-amino-3-methylimidazo[4,5-f]quinoline (IQ), and 2-amino-3,8-dimethylimidazo[4,5-f]quinoxaline (MeIQx). In contrast, the consumption of vegetables and fruit may decrease the incidence and help in preventing the development of cancer.2) The consumption of fermented milk products is also associated with a reduced risk of cancer of the breast, colon, and pancreas.3) In a previous *in vivo* study, *Bifidobacterium* supplementation to the diet inhibited IQ-induced colon and liver tumors in F344 rats.4) However, the mechanism for the anticarcinogenic effects of lactic acid bacteria is unclear. Orrhage *et al.* have reported one possible mechanism in an *in vitro* study.5) They showed that many lactic acid bacteria had antimutagenic activity by binding to heterocyclic amines. Nevertheless, the physiological effect of bacterial binding to heterocyclic amines is not clear. To investigate the mechanism for the anticarcinogenic effect of lactic acid bacteria, we have previously examined the effects of *Streptococcus thermophilus* 1131 cells on Trp-P-1 absorption by the small intestine by using an *in situ* loop technique.6) Strain 1131 cells bound to Trp-P-1 at the same pH as that in the small intestine and decreased its absorption. In this present study, the effects of strain 1131 cells on Trp-P-1 absorption via the gastrointestinal tract were examined *in vivo*; we measured the Trp-P-1 concentration in the blood after its administration with or without *S. thermophilus* 1131 cells to rats.

Male F344 rats, 10 weeks old, were purchased from Japan SLC (Hamamatsu, Japan) and fed on a certified MF diet (Oriental Yeast, Tokyo, Japan) *ad libitum* until they were tested. The care of the rats in this study was according to the Standards Relating to the Rearing and Keeping of Industrial Animals (the Prime Minister’s Office, Japan). The rats (210–250 g for each experimental group, n = 5) were starved for 16 h prior to the experiments, but were provided with water *ad libitum*. The rats were then administered with a single oral dose of 1 ml of a skimmed milk solution (10%) containing Trp-P-1 (0.25 mg/ml) with or without strain 1131 cells (10 mg/ml) after starving. The *S. thermophilus* cells were prepared as described previously,7) and Trp-P-1 was purchased from Wako Pure Chemical Industries, Osaka, Japan. The rats were anesthetized with diethyl ether, and then blood was taken from the portal vein and abdominal aorta 15, 30 and 60 min after the administration. First, 2 ml of blood was taken from the portal vein with a syringe. Next, 2 ml of blood was taken from the abdominal aorta with another syringe, before removing the first syringe. The blood samples were taken from 5 rats at each sampling time (15, 30, and 60 min after the Trp-P-1 administration). One ml of the blood was added to 29 ml of saline, and the mixture was treated three times with blue rayon purchased from Funakoshi (Tokyo, Japan); blue rayon (80 mg) was used with 30 min of shaking for each treatment. The combined blue rayon (240 mg) was washed four times with water (100 ml each time), and then Trp-P-1 was extracted three times with methanol-ammonia (40:1; 10 ml of methanol-ammonia was used with 15 min of shaking each time). The extracts were evaporated to 0.5 ml and mixed with 0.5 ml of acetonitrile, before being filtered (0.45 μm pore size, GL 13A Chromatodisc; GL Science Co.,

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*Abbreviations:* Trp-P-1, 3-amino-1,4-dimethyl-5H-pyrido[4,3-b]indole; IQ, 2-amino-3-methylimidazo[4,5-f]quinoline; MeIQx, 2-amino-3,8-dimethylimidazo[4,5-f]quinoxaline
Trp-P-1 in the filtrate was measured by HPLC as previously described. The recovery of Trp-P-1 from the blood was 89.5 ± 1.1% (mean ± SD, n = 4). Statistical analyses were carried out by Student's t-test.

As shown in Fig. 1, the Trp-P-1 concentration in the blood from the portal vein of rats administered with Trp-P-1 and strain 1131 cells (0.136 ± 0.026 μg/ml) was lower than that of rats administered with Trp-P-1 and no bacterial cells (0.180 ± 0.039 μg/ml) 30 min after the administration. Moreover, the Trp-P-1 concentration in the portal venous blood of rats administered with Trp-P-1 and strain 1131 cells (0.077 ± 0.015 μg/ml) was also lower than that of animals treated without bacterial cells (0.103 ± 0.021 μg/ml) 60 min after dosing. These results support those of our previous report that strain 1131 cells might inhibit Trp-P-1 absorption from the small intestine using an in situ loop technique. However, there was no difference in the Trp-P-1 concentration in the blood from the abdominal aorta between the rats given strain 1131 cells and those given no bacterial cells (Fig. 2). This might have been because a large fraction of Trp-P-1 is metabolized in the liver or is excreted in the bile and/or urine. The Trp-P-1 concentration in the abdominal aorta was markedly lower than that in the portal vein, probably because of its metabolism and excretion in the bile. If the blood accounts for 7% of the whole body weight, the total amount of Trp-P-1 in the blood is less than 1% of the dose at 30 or 60 min, after dosing. After a single dosing of IQ or MelQx solution in mice, these concentrations in the blood were also at very low levels.

However, Zhang and Ohta have reported that the Trp-P-1 level in the abdominal aorta of rats given bacterial cells, i.e., Bacteroides fragilis, Escherichia coli, Lactobacillus acidophilus and Streptococcus cremoris, was significantly lower than that of the animals given no such bacterial cells. Moreover, they showed high levels of Trp-P-1 (about 30% of the amount administered to the rat) in the abdominal aorta. These results are very different from our observations. Zhang and Ohta used male Wistar rats (110–130 g) that had been starved for 4 days in their study. After the rats had been starved for 3 days, the dry weight of the ileum had decreased by half. Therefore, it can be presumed that the 4-day fast influenced the Trp-P-1 absorption and Trp-P-1 level in the abdominal aorta because the physical conditions of the rats’ intestines had been changed by the 4-day fast.

In conclusion, this study has shown that S. thermophilus 1131 cells inhibited Trp-P-1 absorption by the gastrointestinal tract of rats. However, it is not clear whether this inhibition of Trp-P-1 absorption could help to prevent carcinogenesis.

Fig. 1. Effect of Streptococcus thermophilus 1131 Cells on the Trp-P-1 Concentration in Blood Taken from the Portal Vein. A Trp-P-1 solution (0.25 mg/1 ml) was administered with or without strain 1131 cells (10 mg). Blood was taken from the portal vein at 15, 30 and 60 min after dosing. Trp-P-1 in the blood was extracted with blue rayon and measured by HPLC. Each value is the mean ± S.D. of n = 5 experiments. *p < 0.05 (Student’s t-test).

Fig. 2. Effect of Streptococcus thermophilus 1131 Cells on the Trp-P-1 Concentration in Blood Taken from the Abdominal Aorta. A Trp-P-1 solution (0.25 mg/1 ml) was administered with or without strain 1131 cells (10 mg). Blood was taken from the abdominal aorta at 15, 30 and 60 min after dosing. Trp-P-1 in the blood was extracted with blue rayon and measured by HPLC. Each value is the mean ± S.D. of n = 5 experiments.

References


