Role of Intestinal Microflora in Metabolism of Glutathione Conjugates of 1-Nitropyrene 4, 5-Oxide and 1-Nitropyrene 9, 10-Oxide

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KINOUCI, T., KATAOKA, K., MIYANISHI, K., AKIMOTO, S. and OHNISHI, Y. *Role of Intestinal Microflora in Metabolism of Glutathione Conjugates of 1-Nitropyrene 4, 5-Oxide and 1-Nitropyrene 9, 10-Oxide.* Tohoku J. Exp. Med., 1992, **168** (2), 119-122 — DNA adduct formation in the liver of B6C3F1 mice after administration of 1-nitropyrene (1-NP) was shown by the ³²P-postlabeling technique. The major adduct was not *N*-(deoxyguanosin-8-yl)-1-aminopyrene, which was easily formed in in vitro nitroreduction of 1-NP in the presence of DNA, but the major spots migrated to the same position as the in vitro DNA adduct spots of K-region epoxides of 1-NP (1-NP 4, 5- and 9, 10-oxide). 1-NP oxides formed by the oxidative activation of 1-NP in the liver were excreted into the bile as detoxified glutathione conjugates which were changed to cysteine conjugates in the upper intestinal tract. The cysteine conjugates were degraded by cysteine conjugate β -lyase (β -lyase) of intestinal microflora in the lower intestinal tract. The mutagenicity of cysteine conjugates of 1-NP oxides for *Salmonella typhimurium* was enhanced by addition of β -lyase and was decreased by addition of aminooxyacetic acid, a β -lyase inhibitor. The in vitro binding of the cysteine conjugates to calf thymus DNA was increased by addition of β -lyase and xanthine oxidase. We administered glutathione conjugates of 1-NP oxides to two groups of mice that had been treated with antibiotics or saline by gavage and analyzed the DNA adducts in the lower intestinal mucosa. The specific DNA adducts were detected in the saline-treated group but not in the antibiotics-treated group. These results suggest that intestinal microflora play an important role in activation of glutathione conjugates of 1-NP oxides. ——— intestinal microflora; glutathione conjugates; 1-nitropyrene 4, 5-oxide, 1-nitropyrene 9, 10-oxide; cysteine conjugate β -lyase; DNA adduct formation

1-Nitropyrene (1-NP), one of the nitrated polycyclic aromatic hydrocarbons, is a major environmental pollutant and has been shown to be mutagenic for bacteria and cultured mammalian cells and also tumorigenic in the experimental animals (Tokiwa and Ohnishi 1986). 1-NP can be transformed into active derivatives through various pathways, which are either reduction of nitro group to *N*-hydroxy-1-NP, ring oxidation or combination of nitroreduction and ring oxidation (Tokiwa and Ohnishi 1986). A DNA adduct indicative of reductive

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metabolism, *N*-(deoxyguanosin-8-yl)-1-aminopyrene (dG-C8-AP), has been identified to be a minor adduct in vivo, and additional major DNA adducts have been observed (Roy et al. 1989). K-region epoxides of 1-NP (1-NP 4, 5- and 9, 10-oxide) have been detected as 1-NP microsomal metabolites (Fifer et al. 1986; Kataoka et al. 1991; Ohnishi et al. 1991). They are mutagenic for bacteria (Fifer et al. 1986; Ohnishi et al. 1986; Heflich et al. 1990) and responsible for the reaction with DNA (Smith et al. 1990). We did not detect the reductive metabolites in the germ-free animals administered 1-NP (Kinouchi et al. 1986). These results suggested the importance of ring oxidation pathway in the activation of 1-NP. We detected the glutathione conjugates of 1-NP oxides in the bile collected from 1-NP-administered rats and studied their further metabolism in the intestinal tract (Kinouchi et al. 1990).

In this paper we studied DNA adducts in the liver of 1-NP-administered mice and metabolic activation of detoxified glutathione conjugates of 1-NP oxides by bacterial β -lyase.

MATERIALS AND METHODS

1-NP (2 μ mol) was administered to 5-week-old B6C3F1 mice. The mice were sacrificed 24 hr after the administration and the livers were removed. Then DNA was extracted and DNA adducts were analyzed by using ^{32} P-postlabeling method (Gupta 1985). Reductive DNA adduct of 1-NP, dG-C8-AP, was prepared by incubation of DNA with 1-NP and xanthine oxidase (Djurić et al. 1986). Mutagenicity was measured by the preincubation method with *Salmonella typhimurium* strains TA98 and TA100 (Maron and Ames 1983). The extent of DNA binding of cysteine conjugates of [^{3} H] 1-NP oxides was measured by radioactive DNA extracted from the reaction mixture containing 20 μ M cysteine conjugates, 50 mM sodium citrate (pH 5.8), 500 μ g/ml hypoxanthine, 2 mg/ml calf thymus DNA, 75 μ mol/ml glucose, 2 U/ml glucose oxidase, 2 U/ml catalase, 0.1 U/ml xanthine oxidase and 97 μ g/ml β -lyase purified from *Peptostreptococcus magnus* (Kataoka et al. unpublished). Glutathione conjugates of 1-NP oxides were prepared (Kinouchi et al. 1990) and administered orally to two groups (antibiotics- and saline-treated) of ICR mice. After 48 hr, the mice were sacrificed and the DNA extracted from the lower intestinal mucosa was used for DNA adduct analysis.

RESULTS

The DNA adducts in the liver of 1-NP-treated mice and dG-C8-AP are shown in Fig. 1. The major adduct in the liver was not dG-C8-AP and dG-C8-AP was less than 4% of the total adducts in vivo. The major adducts migrated at almost the same positions as DNA adducts of 1-NP oxides in vitro and in vivo (data not shown). These results indicate that 1-NP is oxidatively metabolized in the liver.

1-NP oxides are detoxified as glutathione conjugates and excreted into bile. They are degraded and changed to cysteine conjugates by γ -glutamyltransferase and aminopeptidase in pancreatic juice or upper intestinal contents (Kinouchi et al. 1990). Lower intestinal contents degrade the cysteine conjugates, but the degradation is inhibited by addition of aminooxyacetic acid (AOAA), a β -lyase

Fig. 1. Autoradiograms of ^{32}P -postlabeled DNA adducts formed in vivo in the liver of mice treated with 1-NP (a) and formed in vitro in the reaction mixture containing calf thymus DNA, 1-NP, and xanthine oxidase (b). Arrow indicates the spot of dG-C8-AP.

inhibitor (Kinouchi et al. 1990). The mutagenicity of cysteine conjugates of 1-NP oxides was enhanced by addition of β -lyase and was decreased by addition of AOAA (Table 1). The binding of the cysteine conjugates to calf thymus DNA was increased by addition of β -lyase and xanthine oxidase (Table 2). The binding was not increased by addition of acetyl CoA. Glutathione conjugates of 1-NP oxides were administered to two groups of ICR mice that had been treated

TABLE 1. *Mutagenicity of cysteine conjugate of 1-NP 9, 10-oxide*

Cysteine conjugate (nmol/plate)	β -Lyase	Aminooxyacetic acid	Revertants/plate	
			TA98	TA100
0	—	—	39	123
5	—	—	112	153
5	+	—	1088	583
5	+	+	125	156

TABLE 2. *Binding of tritiated cysteine conjugate of 1-NP oxides to calf thymus DNA*

Xanthine oxidase (0.1 U/ml)	Allopurinol (1 mM)	β -Lyase	AOAA (1 mM)	Binding (pmol/mg DNA/hr)	
				1-NP 4, 5-oxide	1-NP 9, 10-oxide
—	—	—	—	6.0	7.2
+	—	—	—	18.6	44.6
+	+	—	—	5.7	28.9
—	—	+	—	9.0	14.4
+	—	+	—	76.9	153.3
+	+	+	—	33.0	47.3
+	—	+	+	34.0	51.6
+	+	+	+	14.0	27.0

with either antibiotics (bacitracin, neomycin and streptomycin) or saline (control group) orally. Antibiotics treatment decreased bacterial numbers and enzyme activities of intestinal contents. The DNA adducts in the lower intestinal mucosa were detected in control group but not in the antibiotics-treated group (data not shown). These results suggest that intestinal microflora play an important role in activation of glutathione conjugates of 1-NP oxides in vivo.

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