Protective Effect of the Mold *Monascus Anka* Against Acetaminophen-Induced Liver Toxicity in Rats

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ABSTRACT—Antioxidant and hepatoprotective actions of the mold *Monascus anka* (also called *Beni-Koji* in Japan) against acetaminophen (AAP)-induced liver toxicity were investigated. Serum aspartate aminotransferase and glutathione *S*-transferase (GST) activities increased by AAP (180 mg/kg, i.p.) treatment were depressed when the *Beni-Koji* preparation (4 ml/kg, i.p.) was given 15 and 1 hr before AAP administration. The decrease in liver cytosolic GST activity by AAP, reflecting the release of the enzyme into serum, was also blocked by the mold. Cytochrome P450 activity was inhibited by the *Beni-Koji* preparation. These results suggest that *M. anka* prevents AAP-induced liver toxicity by both antioxidant action and the inhibition of AAP metabolism.

Keywords: Acetaminophen, Mold, Antioxidant

Acetaminophen (AAP) is an analgesic/antipyretic drug that causes hepatic necrosis when large doses are ingested. The hepatotoxicity of AAP is due to its biotransformation by the cytochrome P450 system to the reactive metabolite, *N*-acetyl-*p*-benzoquinone imine (NAPQI) (1, 2). This metabolite is normally detoxified by conjugation with glutathione (GSH). However, after large doses of AAP ingestion, NAPQI is formed in amounts sufficient to deplete GSH subsequently; sulfhydryl groups of hepatic proteins react with the reactive metabolite, resulting in hepatic necrosis. Recently, the involvement of reactive oxygen species (ROS) in AAP induced hepatotoxicity has been reported (3–5). In our laboratory, it was found that the mold preparation from *Monascus anka* (also called *Beni-Koji* in Japan) has antioxidant action and can prevent galactosamine plus endotoxin-induced hepatotoxicity in which ROS is also generated (unpublished data). Thus we examined whether the *Beni-Koji* preparation can prevent AAP-induced hepatotoxicity.

The mold *M. anka* cultured on rice, was used for the experiments. One gram of the mold was incubated at 37°C for 60 min with 4 ml of water and then centrifuged at 3000 rpm for 15 min. The supernatant was chromatographed on a Sephadex G75 column, which was equilibrated with water. The fraction with 1,1-diphenyl-

2-picrylhydrazyl (DPPH) radical scavenging action eluted from the column was concentrated by an evaporator, followed by lyophilization. The powder thus obtained was dissolved in water and used as the *Beni-Koji* preparation. Antioxidant action of the *Beni-Koji* preparation was assayed by DPPH radical scavenging action as follows: the reaction mixture contained 1 ml of 0.1 mM DPPH in ethanol, 0.95 ml of 0.05 M Tris-HCl (pH 7.4), 1 ml of ethanol and 50 μ l of *Beni-Koji* preparation or deionized water (control). The absorbance of the mixture was measured at 517 nm exactly 30 sec after adding the *Beni-Koji* preparation. Male Sprague-Dawley rats (150–170 g) from Nihon SLC Co. (Shizuoka) were randomly divided into the groups: control, AAP-treated and AAP plus *Koji* (*Beni-Koji* preparation)-treated. AAP (180 mg/kg) dissolved with 30% dimethyl sulfoxide was given intraperitoneally 24 hr after the administration of a cytochrome P450 inducer, 3-methylchoranthrene (25 mg/kg, i.p.). Control rats were given the 30% dimethyl sulfoxide solution. *Beni-Koji* preparation with 50% DPPH scavenging activity (4 ml/kg) was given intraperitoneally 15 and 1 hr before AAP treatment. Rats were fasted overnight and then injected with AAP. Three hours after the injection, the rats were killed by decapitation, and blood was collected from the stump. The serum was stored at –20°C and used within 2 days. The liver was

removed after perfusion with 1.15% potassium chloride solution in situ and homogenized in 2 volumes of the same solution with a teflon-glass homogenizer. The liver cytosol and microsomes were prepared as reported previously (6). GSH *S*-transferase activity for 1-chloro-2,4-dinitrobenzene and GSH peroxidase for cumene hydroperoxide were measured by the methods of Habig et al. (7) and Reddy et al. (8), respectively. Aniline hydroxylase activity in the microsomes was evaluated by measuring *p*-aminophenol (9). GSH content in the liver homogenate and aspartate aminotransferase (AST) activity in serum were measured by the method of Reed et al. (10) with high performance liquid chromatography (Toyo Soda, Tokyo) and by Assay Kit (Kainos, Tokyo), respectively. Lipid peroxide in the microsomes was determined by the thiobarbituric acid (TBA) assay and expressed as malondialdehyde equivalents (11). Protein concentration in each fraction of the liver was determined by the method of Lowry et al. (12). Data were expressed as the mean $\pm$ S.D. The significance of difference was calculated by Student's *t*-test and differences with $P < 0.05$ were considered significant. All experiments conformed to the guidelines set by the Animal Care and Use Committee of University of the Ryukyus.

Lipid peroxidation in liver microsomes was inhibited dose-dependently by the *Beni-Koji* preparation, and the DPPH scavenging action also followed a similar pattern (Fig. 1). When AAP was given to rats, serum GSH *S*-transferase and AST activities were increased to 2106%

Fig. 1. Effect of the *Beni-Koji* preparation on lipid peroxidation. Microsomes were incubated at 25°C for 30 min with various concentrations of the *Beni-Koji* preparation which was diluted with H₂O and then incubated with 1 mM H₂O₂ for a further 10 min. Lipid peroxide in the microsomes was determined as described in the Text. Each point shows a mean of duplicate determinations. ●, lipid peroxidation; ▲, DPPH scavenging action.

and 598% of the control, respectively, and it was decreased to 1061% and to 249% of the control, respectively, by pretreatment with *Beni-Koji* preparation (Fig. 2). Cytosolic GSH *S*-transferase and GSH peroxidase activities were decreased by AAP treatment to 53% and to 46% of the control, respectively, and the cytosol GST and

Fig. 2. Effect of the *Beni-Koji* preparation on AAP-induced hepatotoxicity. Rats were given AAP (180 mg/kg, i.p.) 24 hr after 3-MC (25 mg/kg, i.p.) administration and killed 3 hr later. Each column shows the mean $\pm$ S.D. from 3 to 6 rats. SGST, serum GSH *S*-transferase (GST); AST, aspartate aminotransferase; cyt, cytosol; mic, microsomes; GSHpx, GSH peroxidase; LPO, lipid peroxidation; AN, aniline hydroxylase. ▨, AAP-treated; ▤, AAP plus *Beni-Koji* preparation-treated. * $P < 0.05$, ** $P < 0.01$ vs control. # $P < 0.05$ vs AAP-treated.

GSH peroxidase activities were raised to 65% and to 55% of the control, respectively, by pretreatment with *Beni-Koji* preparation. On the other hand, the increase in microsomal GSH *S*-transferase (176% of control) and GSH peroxidase (126% of control) activities observed after AAP treatment were not affected by *Beni-Koji* preparation pretreatment. Aniline hydroxylase activity in microsomes was not changed by AAP treatment. Liver GSH content was almost depleted by AAP or AAP plus *Beni-Koji* treatment. When rats were given the *Beni-Koji* preparation alone 15 and 1 hr before killing, liver and serum enzyme activities were not changed, while aniline hydroxylase activity was decreased significantly (85% of the control). Since a depression of aniline hydroxylase activity in the liver microsomes was observed in the *Beni-Koji* preparation-treated rats, the effect of the *Beni-Koji*

preparation on cytochrome P450 activity was examined in vitro. As shown in Fig. 3, aniline hydroxylase activity was inhibited dose-dependently by the *Beni-Koji* preparation.

In the present study, it was observed that GSH *S*-transferase and AST activities in serum increased by AAP treatment were depressed by pretreatment of the rats with the *Beni-Koji* preparation. Although serum AST is a well-known marker for liver injury, it has been reported that liver cytosolic GSH *S*-transferase is also released into the serum during liver damage (13, 14). In this study, we observed a significant decrease in the cytosolic GSH *S*-transferase activity in rats treated with AAP and a slight rise in the transferase activity after the *Beni-Koji* preparation pretreatment. It is likely that cytosolic GSH *S*-transferase was released into the serum by AAP-induced liver injury, and the pretreatment of rats with the *Beni-Koji* preparation depressed the release. Thus it was demonstrated that the *Beni-Koji* preparation prevents AAP-induced liver toxicity. AAP-induced liver toxicity is due to covalent binding of the reactive metabolite NAPQI formed via cytochrome P450 system with liver proteins and also due to reactive oxygen species that are generated via reaction of NAPQI with a ferrous-oxyform of cytochrome P450 (5), or the activation of macrophages and neutrophils (3, 4). Since the *Beni-Koji* preparation scavenged the lipid soluble radical DPPH and depressed lipid peroxidation in microsomes, it was assumed that the AAP-derived metabolite NAPQI or reactive oxygen species might be scavenged by the *Beni-Koji* preparation. Previously we demonstrated that NAPQI could bind the microsomal GSH *S*-transferase, resulting in an increase in the activity of the transferase (14). In the present study, microsomal GSH *S*-transferase increased by AAP treatment was not decreased by the *Beni-Koji* preparation treatment, and lipid peroxidation was slightly decreased by the treatment. It has been observed by an electron spin resonance spectrometer that the component isolated from the *Beni-Koji* preparation could scavenge superoxide anion and DPPH radical (Y. Aniya, unpublished data). Taken together, these observations indicate that the *Beni-Koji* preparation may scavenge oxygen radicals rather than NAPQI. When the *Beni-Koji* preparation alone was given to rats, there were no significant effects on serum and liver parameters except for a slight decrease in aniline hydroxylase activity. Furthermore, aniline hydroxylase activity was inhibited in vitro by treatment of microsomes with the *Beni-Koji* preparation, showing that the *Beni-Koji* preparation can inhibit cytochrome P450 activity both in vitro and in vivo. Considering that the reactive metabolite NAPQI is produced via the cytochrome P450 system, the inhibitory effect of the *Beni-Koji* preparation on cytochrome P450 activity may be beneficial for the prevention of AAP-induced liver in-

Fig. 3. Effect of the *Beni-Koji* preparation on aniline hydroxylase activity in liver microsomes. Microsomes (7.2 mg/ml) prepared from nontreated rats were incubated in 0.05 M Tris-HCl (pH 7.4) with the *Beni-Koji* preparation at 37°C for 15 min and then aniline hydroxylase activity was measured. Δ , control; $\circ$, *Beni-Koji* preparation with 50% DPPH scavenging action; $\blacksquare$, *Beni-Koji* preparation with 30% DPPH scavenging action. A: Velocity versus substrate concentration curves, B: Eadie-Hofstee plots.

jury.

In conclusion, it is suggested that the *Beni-Koji* preparation from the mold *M. anka* protects against AAP-induced liver injury, firstly by the radical scavenging action/antioxidative action where reactive oxygen species-mediated liver injury is depressed and secondly by inhibition of the cytochrome P450, leading to reduced generation of toxic metabolite from AAP, resulting in protection against the metabolite-mediated liver injury.

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