Wheat Bran Protects Fischer-344 Rats from Diquat-Induced Oxidative Stress by Activating Antioxidant System: Selenium as an Antioxidant

Masashi Higuchi, Junichi Oshida, Koichi Orino, and Kiyotaka Watanabe

Laboratory of Veterinary Biochemistry, School of Veterinary Medicine, Kitasato University, Towada, Aomori 034-8628, Japan

Received October 6, 2010; Accepted November 24, 2010; Online Publication, March 7, 2011

Wheat bran had a protective effect against diquat toxicity in rats fed a purified diet (PD). We studied the effects of wheat bran on the antioxidant system in the liver of rats treated with saline and diquat. Although feeding wheat bran did not affect the concentration of hepatic non-protein sulfhydryl or the activity of glucose 6-phosphate dehydrogenase; W-PD, purified diet containing wheat bran; Se-PD, purified diet containing selenium; NPSH, non-protein sulfhydryl; W-PD, purified diet containing wheat bran; Se-PD, purified diet containing selenium; NPSH, non-protein sulfhydryl

Key words: diquat; oxidative stress; rat; selenium; wheat bran

The bipyridyl herbicide, diquat (DQ), stimulates the generation of reactive oxygen species by redox cycling metabolism, and has been extensively studied as a model of oxidant-mediated cellular injury. The superoxide anion radicals produced by DQ radicals form spontaneously or enzymatically (via superoxide dismutase) hydrogen peroxide and molecular oxygen. Ferrous ions that are reductively released from ferritin by both the superoxide and DQ radicals catalyze the Fenton reaction to produce the hydroxyl radical, which is an extremely potent oxidizing species, from hydrogen peroxide. Hydroxyl radicals can cause DNA strand breakage, inactivate enzymes, and initiate lipid peroxidation, resulting in cell death. It thus appears that DQ toxicity is induced by iron-mediated oxidative stress. Hydrogen peroxide is detoxified by the antioxidant system, including GSH, glutathione peroxidase (GPx), and glutathione reductase (GR). Enhanced DQ toxicity has been found in rats treated with chloroethyl-N-nitrosourea that inhibits GR and in GPx knockout mice, indicating that both GR and GPx were involved in protecting against DQ-induced oxidative stress.

Fischer-344 rats are much more sensitive to DQ toxicity than the other strains, and have been extensively used to clarify the fundamental mechanism for critical cell damage by reactive oxygen species. We have recently found that Fischer-344 rats fed a purified diet (PD) were much more susceptible to DQ-induced toxicity than rats fed a regular diet, suggesting that PD lacked the components present in the regular diet that had a protective effect against DQ toxicity. We also found that wheat bran has such an effect, although the protective mechanism of wheat bran remained to be disclosed. We therefore investigated the effects of wheat bran in the present study on the hepatic antioxidant system, including GSH, glucose 6-phosphate dehydrogenase (G6PDH), GPx, and GR, in rats injected with saline or DQ, and identified the antioxidant factor in wheat bran.

Materials and Methods

Animals, diets, and treatments. The test animals used in this study were 5-week-old male Fischer-344 rats (Clea Japan, Tokyo, Japan). Low-iron PD (no. A12501) consisting of 22% milk casein, 61% cornstarch, 5% crystalline cellulose, 4% purified soybean oil, 1% vitamin mix, and 7% mineral mix without iron was obtained from Clea Japan. This diet contained 5 ppm iron. Wheat bran containing 127 ppm iron and 0.6 ppm selenium was obtained from a local shop. The selenium content was measured by using an inductively coupled plasma atomic emission spectrometer by ESC Mitsukawa (Osaka, Japan). Ferric citrate and sodium selenate were purchased from Kanto Chemical (Tokyo, Japan). The rats were fed with PD, PD containing 20% (w/w) wheat bran (W-PD), or PD containing 0.15 ppm selenium (Se-PD) as recommended by the American Institute of Nutrition for 5 weeks unless otherwise indicated. Ferric citrate was added to each of these three diets to provide a total iron content of 320 ppm. Deionized distilled water in plastic bottles was freely available to the rats.

DQ dibromide was obtained from Labor Dr. Ehrenstorfer-Schäfers (Augsburg, Germany), and the rats were subcutaneously injected with DQ (0.065 or 0.1 mmol/kg body weight) in 0.9% NaCl. Control rats were injected with an equivalent volume of 0.9% NaCl. The rats were anesthetized with pentobarbital 3 h after DQ administration, and blood samples were collected by cardiac puncture. The animals were then sacrificed, and the liver of each was removed. Serum and tissue samples were stored at −25°C until needed. The survival time of the DQ-injected rats was measured in a survival experiment. All animal
experiments were carried out in accordance with the Guidelines for Animal Care and Use of Kitasato University School of Veterinary Medicine.

**Biochemical analyses.** The serum ALT activity was measured with an OLYMPUS AU400 Autoanalyzer (Olympus, Tokyo, Japan).

Liver samples were homogenized according to the methods described elsewhere. The liver non-protein sulfhydryl (NPSH) concentration was determined according to the method of Ellman, after the liver homogenate had been deproteinized with an equal volume of 4% sulfosalicylic acid by the method described by Smith et al. The concentration of NPSH in rat liver has been reported to be similar to that of GSH.

The liver G6PDH, GPx, and GR activities were respectively assayed according to the methods of Glaser and Brown, Lawrence and Burk, and Gupta et al.

**Statistical analyses.** Data were analyzed by one-way or two-way ANOVA, and followed by Tukey’s test for multiple comparisons.

**Results**

**Protective effect of wheat bran against DQ hepatotoxicity**

DQ-induced hepatotoxicity was assessed by measuring the serum ALT activity. The DQ treatment significantly elevated the serum ALT activity in the PD group, but not in the W-PD group (Fig. 1).

**Effect of wheat bran on the antioxidant system in rat liver**

No significant differences in liver weight were found in the PD and W-PD groups injected with saline and DQ (saline-injected PD group: 4.31 ± 0.30; saline-injected W-PD group: 4.28 ± 0.19; DQ-injected PD group: 4.30 ± 0.23; DQ-injected W-PD group: 4.26 ± 0.18 g of liver/100 g of body weight).

There were no significant differences in the hepatic NPSH concentration and G6PDH activity between the saline-injected PD and W-PD groups (Fig. 2). DQ administration significantly decreased the level of hepatic NPSH in the PD group, but had no effect on NPSH level in the W-PD group (Fig. 2A). The NPSH level was significantly higher in the DQ-injected W-PD group than in the DQ-injected PD group. Although DQ administration did not significantly affect the hepatic G6PDH activity in either the PD or W-PD group, the G6PDH activity in the DQ-injected W-PD group was significantly higher than that in the DQ-injected PD group (Fig. 2B).

**Effect of selenium on the DQ toxicity and hepatic GPx activity**

The effects of adding selenium to the diet on the DQ toxicity and hepatic GPx activity were assessed. DQ administration did not significantly elevate the serum ALT activity in either the Se-PD or W-PD group as compared with the PD group (Fig. 4A).

The hepatic GPx activity in both the saline- and DQ-treated rats was significantly higher in the Se-PD and W-PD groups than in the PD group (Fig. 4B). No significant difference in hepatic GPx activity was apparent in the PD, W-PD, and Se-PD groups after the DQ treatment from the respective saline-injected groups. The mean survival time was very short (4.0 ± 0.9 h) following the administration of 0.1 mmol DQ/kg to the PD group. The addition of selenium as well as wheat bran to PD significantly prolonged the survival time after administering DQ (26.9 ± 5.7 and 28.6 ± 3.3 h, respectively, p < 0.01 for each; Fig. 5) as compared with the PD.
Discussion

The present findings that Fischer-344 rats fed PD were more susceptible to DQ-induced hepatotoxicity, and that wheat bran attenuated the toxic effects of DQ are in accordance with our previous results.\(^7\) GPx is an important component of the cellular antioxidant system. GPx detoxifies such reactive oxygen species as \(H_2O_2\) and organic hydroperoxides with GSH, thereby conferring protection against oxidative damage. GSSG produced by the GPx reaction is then reduced by GR to GSH with NADPH generated through the pentose phosphate cycle. In the present study, the concentration of NPSH in the liver of DQ-treated rats fed PD was decreased to about 80% of that in the control rats at 3 h, although the NPSH concentration in the liver of the DQ-treated rats fed W-PD was the same as that in the control rats. The activity of hepatic G6PDH in the DQ-treated W-PD group was higher than that in the DQ-treated PD group, indicating an increase in the production of hepatic NADPH. The present study also showed that wheat bran markedly increased the activity of hepatic GPx in the control rats, and that administering DQ had no effect on the hepatic GPx activity in these animals. From these findings, we conclude that wheat bran activated the antioxidant system in the liver of Fischer-344 rats, resulting in the attenuation of DQ toxicity. An important question that remains to be determined, however, is whether the induction of antioxidant enzymes by wheat bran occurred transcriptionally or translationally.

Smith et al.\(^10\) have reported that a hepatotoxic dose of DQ did not affect the activity of GPx in rat liver in accordance with the present results, while Gupta et al.\(^6\) found elevated GPx activity in the liver of DQ-treated rats. Although both Smith et al.\(^10\) and Gupta et al.\(^6\) have reported elevated hepatic GR activity following DQ administration, we found in the present study that GR activity was decreased as a result of DQ administration. The reasons why coincident changes were not apparent in the activities of these hepatic antioxidant enzymes after the DQ treatment are unclear.

Some studies\(^4,5\) have reported that dietary selenium deficiency, which decreased the activity of hepatic GPx, rendered rats and mice susceptible to DQ-induced hepatotoxicity or lethality. Since GPx is a selenium-dependent enzyme, and we found that wheat bran increased the hepatic GPx activity, we examined the effect of adding selenium to PD on hepatic GPx activity and DQ toxicity. Selenium as well as wheat bran
markedly elevated the GPx activity in the liver of the saline-injected control rats, and attenuated the DQ-induced toxicity (Figs. 4 and 5). These results suggest that selenium as well as wheat bran had protective effects against DQ-induced oxidative stress. The selenium content of the wheat bran used in this study was determined to be 0.6 ppm, which is the same with the reported value (0.58 ppm), and is five times higher than that of okara and rice bran, both of which we have previously found did not protect against DQ toxicity. In conclusion, our findings indicate that wheat bran conferred a protective effect against DQ-induced oxidative stress by activating the hepatic antioxidant system, and that selenium was a key antioxidant contained in wheat bran. Adding wheat bran to a diet may therefore be an efficient way to remedy the selenium deficiency which frequently occurs in animals living in areas with low selenium content in the soil.

References