Protective Effects of Wheat Bran against Diquat Toxicity in Male Fischer-344 Rats

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After injection with 0.1 mmol diquat/kg body weight, survival time was markedly shorter in Fischer-344 rats fed a purified diet than in rats fed a regular diet, and much more severe hepatotoxicity and nephrotoxicity were observed in the former than in the latter. The longer the feeding period on the purified diet, the shorter the survival time after diquat administration. These results indicate that the purified diet lacked components present in the regular diet that had protective effects against diquat toxicity. These two diets had nearly the same composition and content of vitamins and minerals. We tested the ingredients of the regular diet to determine which ones reduce diquat toxicity. We found that wheat bran had a protective effect, but that rice bran and bean-curd refuse (okara) did not.

Key words: wheat bran; oxidative stress; diquat; Fischer-344 rat

Diquat (DQ) is a bipyridyl herbicide that is hepatotoxic and nephrotoxic in vivo.1,2) The DQ radical, which is formed by NADPH-cytochrome P-450 reductase, transfers an electron to molecular oxygen to generate a superoxide anion radical.3,4) Both the DQ radical and superoxide cause ferritin to release ferrous iron, which is involved in the Fenton reaction.3–6) It thus appears that DQ toxicity is due to iron-mediated oxidative stress. This suggests that variations in the levels of stored iron in the body can affect the response to DQ. That would be consistent with our recent finding that higher levels of stored iron are associated with more severe DQ toxicity in Fischer-344 rats.7)

In a recent study, we observed 100% mortality 4 h after administration of 0.1 nmol DQ/kg to Fischer-344 rats fed a purified diet.7) In contrast, Smith et al.1) reported that animal mortality was very low even 24 h after they injected the same dose of DQ into Fischer-344 rats. This difference in survival appears to be due to differences between the purified diet used in our previous study and the regular diet used by Smith et al.1) Therefore, in the present study, we compared DQ toxicity between rats fed a purified diet and rats fed a regular diet, and tested the components of the regular diet to determine which ones had protective effects against DQ-induced toxicity.

Materials and Methods

Animals and treatments. The test animals were 5-week-old male Fischer-344 and Wistar rats (Clea Japan, Tokyo). Purified powdered feed A12501 (22% milk casein, 61% cornstarch, 5% crystalline cellulose, 4% purified soybean oil, 1% vitamin mix, and 7% mineral mix without iron) and regular powdered feed CE-2 (soybean meal, whitefish meal, soybean oil, alfalfa meal, wheat, corn, wheat bran, yeast, wheat germ, defatted rice bran, grain sorghum, vitamins, and minerals) were obtained from Clea Japan. These two diets had nearly the same composition and content of vitamins and minerals, except that A12501 contains 4 to 5 ppm iron and CE-2 contains 320 ppm iron. Wheat bran, rice bran, and okara (bean-curd refuse), containing 127, 70, and 48 ppm iron respectively, were obtained from a local shop. CE-2, wheat bran, rice bran, or okara was added (10 or 20%, w/w) to the purified diet. Ferric citrate (Kanto Chemical, Tokyo) was added to these diets and to the purified diet without other additives, for a total iron content of 320 ppm. Rats were fed the regular diet or the purified diet for 6 weeks, after which they were assigned to experimental groups

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Abbreviations: DQ, diquat; ALT, alanine aminotransferase; UN, urea nitrogen
diet with or without additives for 5 weeks, unless otherwise indicated. Deionized distilled water was freely available to the rats in plastic bottles.

DQ dibromide was obtained from Labor Dr. Ehrenstorfer-Schäfers (Augsburg, Germany). The rats were injected subcutaneously with DQ (0.1 mmol/kg body weight) in 0.9% NaCl. Control rats were injected with an equivalent volume of 0.9% NaCl. Three h after DQ administration, the rats were anesthetized with pentobarbital, and blood samples were collected by cardiac puncture. The rats were then sacrificed, and the livers were removed. Serum and tissue samples were stored at $-25^\circ$C until use. In the survival experiment, the survival time of DQ-injected rats was measured. The animal experiments were done in accordance with the Guidelines for Animal Care and Use of Kitasato University School of Veterinary Medicine and Animal Sciences.

Biochemical analyses. Total nonheme iron and ferritin in the liver were measured by a method described elsewhere. Serum alanine aminotransferase (ALT) and urea nitrogen (UN) were analyzed with an Olympus AU400 Autoanalyzer (Olympus, Tokyo).

Statistical analyses. Data from experiments with two groups were analyzed by Student’s $t$-test. Data from experiments with more than two groups were analyzed by one-way or two-way ANOVA, followed by Tukey’s test for multiple comparisons.

Results

Comparison of DQ toxicity between the regular diet and the purified diet

Following administration of 0.1 mmol DQ/kg to the Fischer-344 rats, the mean survival time of rats fed the purified diet was much shorter than that of rats fed the regular diet ($2.91 \pm 0.43$ vs. $26.8 \pm 4.6$ h respectively, $p < 0.001$; Fig. 1A). After injection of 0.1 mmol DQ/kg into Wistar rats, the mean survival time of rats fed the purified diet was shorter than that of rats fed the regular diet ($68.8 \pm 25.5$ vs. $142.8 \pm 67.1$ h respectively, $p < 0.01$; Fig. 1B). These results indicate that Fischer-344 rats are much more sensitive to DQ than are Wistar rats. Hence the subsequent experiments were performed using Fischer-344 rats.

After DQ administration, serum ALT activity and UN levels were significantly higher in the rats fed the purified diet than in those fed the regular diet (Table 1).

Effects of feeding duration of purified diet on survival of DQ-administered rats

Rats were fed a purified diet for various periods of time, and were then injected with DQ. As Fig. 2 shows, longer feeding duration of the purified diet correlated with shorter survival time.

Effects of additives to purified diet on survival time of DQ-administered rats

Among rats fed a purified diet for 5 weeks, there was no difference in growth between the diet with additives and that without additives (CE-2, okara, rice bran, or wheat bran) (Table 2). Although okara and rice bran had little effect on the survival of DQ-injected rats, wheat bran had the same protective effect as CE-2 (Table 3).

Protective effects of wheat bran against DQ hepatotoxicity and nephrotoxicity

DQ hepatotoxicity and nephrotoxicity were assessed in rats fed a purified diet with or without wheat bran (10% or 20%). In DQ-administered rats, wheat bran
Additives (Figs. 3 and 4, Table 1). After DQ injection, in rats fed a purified diet containing 20% wheat bran, serum ALT activity and UN levels were almost the same as in the rats fed the regular diet (Figs. 3 and 4, Table 1).

Table 1. Comparison of DQ Hepatotoxicity and Nephrotoxicity between the Regular Diet and the Purified Diet

<table>
<thead>
<tr>
<th>Diet</th>
<th>Serum ALT (U/l)</th>
<th>Serum UN (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Saline</td>
<td>DQ</td>
</tr>
<tr>
<td>Regular diet</td>
<td>46 ± 4</td>
<td>266 ± 121</td>
</tr>
<tr>
<td>Purified diet</td>
<td>34 ± 4</td>
<td>19.067 ± 5.296b</td>
</tr>
</tbody>
</table>

Fischer-344 rats were fed a regular diet or a purified diet for 5 weeks, and were then injected with 0.1 mmol DQ/kg. Each value is the mean ± SD for 6 rats.

aSignificantly different from the saline-treated (control) group fed the same diet (p < 0.01).
bSignificantly different from the DQ-treated group fed a regular diet (p < 0.01).

Table 2. Growth of Rats Fed a Purified Diet with or without Additives

<table>
<thead>
<tr>
<th>Additives</th>
<th>Body weight (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>5 weeks old</td>
</tr>
<tr>
<td>None</td>
<td>70.8 ± 5.6</td>
</tr>
<tr>
<td>CE-2</td>
<td>70.8 ± 5.8</td>
</tr>
<tr>
<td>Okara</td>
<td>70.7 ± 4.8</td>
</tr>
<tr>
<td>Rice bran</td>
<td>70.8 ± 5.0</td>
</tr>
<tr>
<td>Wheat bran</td>
<td>70.7 ± 4.6</td>
</tr>
</tbody>
</table>

Fischer-344 rats were fed a purified diet with or without 20% (w/w) additives for 5 weeks, and were then injected with 0.1 mmol DQ/kg. Each value is the mean ± SD for 6 rats.

Fig. 2. Effects of Feeding Period of Purified Diet on Survival of DQ-Administered Rats. Fischer-344 rats, fed a regular diet for 0, 2, 3, 6, or 5 weeks, were then fed a purified diet for 5, 3, 1.4, or 0 weeks. They were then injected with 0.1 mmol DQ/kg at the age of 10 weeks. Data are the means ± SD, with the number of animals in parentheses. aSignificantly different from the group that was not fed a purified diet; p < 0.05.

Table 3. Effects of Additives to Purified Diet on Survival Time of DQ-Administered Rats

<table>
<thead>
<tr>
<th>Additives</th>
<th>Survival time (h)</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>2.28 ± 0.29</td>
</tr>
<tr>
<td>CE-2</td>
<td>30.64 ± 8.96a</td>
</tr>
<tr>
<td>Okara</td>
<td>3.30 ± 0.83</td>
</tr>
<tr>
<td>Rice bran</td>
<td>2.74 ± 0.42</td>
</tr>
<tr>
<td>Wheat bran</td>
<td>35.91 ± 30.11a</td>
</tr>
</tbody>
</table>

Fischer-344 rats were fed a purified diet with or without 20% (w/w) additives for 5 weeks, and were then injected with 0.1 mmol DQ/kg. Each value is the mean ± SD for 6 rats. aSignificantly different from the value of no additives (p < 0.05).

Fig. 3. Protective Effects of Wheat Bran against DQ Hepatotoxicity. Fischer-344 rats were fed a purified diet with or without wheat bran (10% or 20%) for 5 weeks, and were then injected with saline (open circle) or 0.1 mmol DQ/kg (closed circle). Each datum is the mean ± SD for 6 rats. aSignificantly different from the saline-injected groups fed the same diet; p < 0.05. bSignificantly different from the DQ-treated group fed a purified diet without wheat bran; p < 0.05. cSignificantly different from the DQ-treated group fed a purified diet with 10% wheat bran; p < 0.05.

Effects of wheat bran on body iron stores

Among the rats fed a purified diet, there were no significant differences in liver nonheme iron and ferritin concentrations between diets containing 0, 10, and 20% wheat bran (Table 4).
Rats Fed a Purified Diet Containing Various Amounts of Wheat Bran

Discussion

Table 4. Nonheme Iron and Ferritin Concentrations in the Liver of Rats Fed a Purified Diet Containing Various Amounts of Wheat Bran

![Graph showing Serum UN (mg/dl) vs Wheat bran (%)]

Fischer-344 rats were fed a purified diet with or without wheat bran (10% or 20%) for 5 weeks, and were then injected with saline (open circle) or 0.1 mmol DQ/kg (closed circle). Each datum is the mean ± SD for 6 rats. aSignificantly different from the saline-treated groups fed the same diet; p < 0.05. bSignificantly different from the DQ-treated group fed a purified diet without wheat bran; p < 0.05. cSignificantly different from the DQ-treated group fed a purified diet with 10% wheat bran; p < 0.05.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Content of wheat bran (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nonheme iron (µg/g wet weight)</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>41.6 ± 5.3</td>
</tr>
<tr>
<td>Ferritin (µg/g wet weight)</td>
<td>107 ± 20</td>
</tr>
</tbody>
</table>

Fischer-344 rats were fed a purified diet containing 0%, 10%, and 20% (w/w) wheat bran for 5 weeks. Each value is the mean ± SD for 6 rats.

DQ-induced oxidative stress. He et al.8 also reported that in Wistar rats, a purified diet resulted in increased sensitivity to the drugs lipopolysaccharide and d-galactosamine (which induce liver injury), as compared with a crude pelleted diet.

As shown in Fig. 1, Fischer-344 rats were more sensitive to DQ toxicity than Wistar rats. Smith et al.3 found that very severe hepatic damage was induced by DQ in Fischer-344 rats, although the damage in Sprague-Dawley rats was minimal. Few differences in the activities of glutathione peroxidase and reductase, involved in primary intracellular defense against oxidative stress, were found between Fischer and Sprague-Dawley rats. Since their report, many studies of DQ toxicity have been carried out using Fischer-344 rats that are highly sensitive to DQ.2,7,9–11 The reason for this strain difference in DQ toxicity remains to be elucidated.

The present finding that a longer period of consumption of the purified diet is associated with shorter survival times suggests that the protective effect of the regular diet against DQ toxicity gradually fades with time. We examined the effects of wheat bran and rice bran (ingredients in the regular diet but not in the purified diet) on survival after DQ injection, and also the effects of okara (as a substitute for soybean meal in the regular diet). Wheat bran had the same protective effect as the regular diet, but rice bran and okara had no protective effect. After DQ administration, the survival time of rats fed the purified diet containing only 20% regular diet was almost the same as that of the animals fed 100% regular diet. Feeding the purified diet with 10% wheat bran to the rats resulted in less significant protection against DQ hepatotoxicity and nephrotoxicity as compared with the purified diet with 20% wheat bran, or the regular diet only. Based on these results, the protective effect of the regular diet against DQ toxicity is not considered to be entirely attributable to wheat bran alone, although no information about the amounts of ingredients except for vitamins and minerals in the regular diet has been made public. In the present study, we tested only three ingredients in the regular diet that were not contained in the purified diet. It remains to be determined whether other ingredients in the regular diet have protective effects against DQ toxicity.

Frølich and Lysø2 examined the bioavailability of iron from wheat bran in anemic pigs (by measuring hemoglobin and serum iron concentrations), and found that wheat bran did not inhibit iron absorption; however, they did not measure body iron stores. In contrast, Bjørn-Rasmussen3 found that the addition of 7% wheat bran to white bread decreased iron absorption in humans who were assumed to have satisfactory iron stores. In the present study, we found no effect of wheat bran on body iron stores in the rats. Although our recent study suggested that lower body iron stores are associated with less severe DQ toxicity in rats,7 the protective effect of wheat bran against DQ toxicity does not appear to be due to a decrease in iron stores.
In the present study, rats that were fed the purified diet for 3 weeks beginning at 7 weeks of age and were then fed the purified diet supplemented with 20% wheat bran for 1 week had almost the same survival time as the rats fed the regular diet (data not shown), indicating that consumption of wheat bran for even a short period of time can confer protection against DQ toxicity in rats. Petry et al. found that pre-treatment with the antioxidants U-74006F and U-78517G markedly reduced the hepatotoxicity and nephrotoxicity of DQ in Fischer-344 rats. The available evidence suggests that wheat bran contains an antioxidant factor. Phenolic acids, including ferulic, vanillic, and syringic acids, have been found to exist in wheat bran and to have strong antioxidant activities in vitro. Therefore, there is a possibility that phenolic acids are responsible for protection against DQ-induced oxidative stress in vivo.

In conclusion, the present findings indicate that the purified diet lacked a factor that protects rats against DQ toxicity, and that this factor is present in wheat bran. Further study is needed to identify the factor in wheat bran that has antioxidant activity in vivo, and to clarify how this factor protects against DQ-induced oxidative injury.

References