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Protective Effect of Colored Rice over White Rice on Fenton Reaction-based Renal Lipid Peroxidation in Rats

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Rice has been one of the most important grains. While polished white rice is favored, colored strains of rice, red, or black, have been maintained for religious purposes in Japan. We studied whether feeding of unpolished colored rice instead of white rice ameliorates oxidative renal tubular damage in rats induced by ferric nitrilotriacetate. Whereas renal lipid peroxidation was exacerbated in white rice-fed group in comparison with standard chow group, this exacerbation was not observed in red or black rice-fed groups. These changes were dependent on the proportion of colored rice to standard chow in the diet. Cyanidin 3-*O*-β-D-glucoside was detectable neither in the serum nor kidney after one week of colored rice diet, but serum protocatechuic acid was significantly increased after black rice diet. There was a generalized decrease in the renal glutathione peroxidase activity in rice diet groups. Renal enzymatic activities of superoxide dismutase, glutathione *S*-transferase and NAD(P)H quinone reductase were not associated with the levels of lipid peroxidation. However, renal catalase activity was significantly increased in black rice-fed groups. These may partly explain the antioxidative effect. Furthermore, colored strains of rice are rich in proteins. Thus, our data warrants further investigation of the antioxidative effect of colored rice.

Keywords: Colored rice; Anthocyanin; Iron; Kidney; Lipid peroxidation; Antioxidant enzymes

INTRODUCTION

Rice (*Oryza sativa L.*) has been one of the most important grains for the world's inhabitants especially in Asian countries.[1] While the pericarp or testa of wild-genotype rice is either red or black in color (Fig. 1), white rice, a recessive trait, has been favored and selected during the past centuries presumably because of its appearance and good flavor as well as the high production efficiency. In Japan, colored rice, especially red rice, has been maintained in limited areas such as in Souja (Okayama Prefecture), Tsushima Island (Nagasaki Prefecture), and Tanegashima Island (Kagoshima Prefecture) for religious purposes as an offering to natural God in Shinto shrines. Black rice has been cultivated in some Asian countries including China and Indonesia for religious purposes or as traditional foods.[2]

Since approximately 10 years ago, wild-genotype rice has become popular as "ancient rice" among natural food lovers in Japan because they are stronger than white rice against pathologic insects and weeds, thus requiring less insecticides, herbicides, and chemical fertilizers. Furthermore, natural pigments in plants attracted much attention because

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FIGURE 1 Spikes and grains of rice. (A) Spikes; from above to below, white rice (c.v. Koshihikari); red rice (c.v. Beni-roman); black rice (c.v. Okuno-murasaki; Ouu-368). (B) Grains; from left to right, white rice, red rice and black rice.

of its possible antioxidant activity in human body based on a hypothesis that endogenous antioxidants play an important role in plant defense systems against oxidative stress. For example, we have isolated cyanidin 3-*O*-β-D-glucoside (C3G) from black rice[3,4] and revealed that this potent antioxidant anthocyanin is absorbed and metabolized in rats.[5] Furthermore, its administration was shown to protect against ischemia-reperfusion injury of liver in rats.[5] However, as far as we know, there has been no data available on whether diet of colored rice has any beneficial effects *in vivo*.

In the present study, we have used ferric nitrilotriacetate (Fe-NTA)-induced oxidative renal injury model that causes Fenton-like reaction in the lumina of renal proximal tubules in rats.[6–10] Whereas the reaction induced by Fe-NTA *in vivo* is rather drastic at the first injection,[11] repeated injections induced a state of sustained oxidative stress in association with iron overload. This is one of the most finely-studied models of free radical-induced carcinogenesis. After repeated intraperitoneal administration for 12 weeks, a high incidence of renal cell carcinoma (60–90%) is observed in rodents after one year.[12–14] Recently, we have identified *p16*^{INK4A} and *p15*^{INK4B} tumor suppressor genes as one of the major target genes in this model with a genetic analysis.[15] Furthermore, we have identified 20 independent transcripts that revealed marked difference in expression during carcinogenesis.[16] Since it has been established that levels of lipid peroxidation at an early stage have been associated with the incidence of renal cell carcinoma,[17,18] this is an ideal model to ask whether certain components of diet could reduce oxidative injury *in vivo*. In the present paper, we show for the first time that diet containing colored rice has advantageous effects over white rice *in vivo*. Possible mechanisms and its implication will be discussed.

MATERIALS AND METHODS

Rice

White rice (c.v. Koshihikari), red rice (c.v. Beni-roman), and black rice (c.v. Okuno-murasaki; old line name, Ouu-368) used in the present study were

cultivated under controlled conditions in the experimental fields of Department of Bioresources, Mie University, or School of Bioresources, Hiroshima Prefectural University. Koshihikari is one of the most popular strains in Japan. Beni-roman and Okuno-murasaki are newly bred from the descendants of crosses between a Japanese local strain of red rice, or an Indonesian local strain of black rice and modern white varieties under the Japanese Ministry of Agriculture, Forestry, and Fisheries. Grains of each strain of rice were threshed, but not polished, stored at 4°C and used within one year. Figure 1 shows the spikes and unpolished grains of each strain of rice used in the present study. Rice was given to rats either as uncooked rice with or without standard chow (F-2; Funabashi, Chiba, Japan), or steam-cooked rice with an electric rice cooker (SR-03F, National; Yashiro-cho, Hyogo, Japan). This is a common household method of cooking rice in Japan.

Analysis of Nutrients and Trace Metals in Rice

Diet composition was analyzed according to the standard procedure.[19] The metal contents were measured with a Hitachi Z-7000 polarized Zeeman atomic absorption spectrophotometer (Tokyo, Japan) as previously reported[20] with slight modification. Acid extraction was done with 10N nitric acid for 24 h at 95°C.

Animals

Five-week old specific-pathogen-free male Wistar rats (Shizuoka Laboratory Animal Center, Shizuoka, Japan) weighing 90–100 g were purchased. They were kept in plastic cages in an air-conditioned room (22–24°C) with a light/dark cycle of 12 h each. A total of 95 animals were used: (1) Fe-NTA-treatment group with simple diet: untreated normal rats given standard chow, and Fe-NTA-treated control rats given standard chow ($N = 8$, respectively); Fe-NTA-treated rats given only cooked rice (white, red or black; $N = 4$, respectively); Fe-NTA-treated rats given only uncooked rice (white, red or black; $N = 4$, respectively); (2) Fe-NTA-treatment group with mixed diet; the proportion of uncooked rice to standard chow was modified (9:1, 3:1, and 1:1 by weight for white, red, or black rice to standard chow; $N = 3$, respectively); (3) no Fe-NTA treatment group for the measurement of renal enzymatic activities: untreated normal rats given standard chow ($N = 4$); untreated rats given only cooked rice (white, red, or black; $N = 4$, respectively); untreated rats given only uncooked rice (white, red and black; $N = 4$, respectively).

Rats were given deionized water (Millipore Japan, Osaka) and ample diet *ad libitum* during the experiments (50 g cooked rice per rat, or 30 g uncooked rice per rat). They were maintained for six days with each diet and thereafter used for the study. Weight of each animal was recorded every other day. Fe-NTA of 10 mg iron/kg body weight was injected intraperitoneally approximately at 9 a.m. All the plasma and kidney samples were obtained approximately at 10 a.m.

Chemicals

Ferric nitrate enneahydrate, sodium hydrogen carbonate, 1-chloro-2,4-dinitrobenzene (CDNB), glutathione (GSH), nitroblue tetrazolium, 3,3′-methylene-bis(4-hydroxycoumarin), tween 20 and NAD(P)H:quinone reductase were from Wako (Osaka, Japan); nitrilotriacetic acid disodium salt and 2,6-di-*tert*-butyl-*p*-cresol (BHT) were from Nacalai Tesque (Kyoto, Japan). EDTA was from Dojin (Kumamoto, Japan). *tert*-Butyl hydroperoxide (*t*-BHP) was from Aldrich (Tokyo, Japan). Sodium cholate, xanthine, and xanthine oxidase were from Sigma (St Louis, MO). 2-Thiobarbituric acid was from Merck (Darmstadt, Germany). Glutathione reductase, NADPH, and FAD were from Oriental Yeast (Tokyo, Japan). All the chemicals used were of analytical quality; deionized water was used throughout. Fe-NTA solution was prepared as previously described immediately before use.[21]

Determination of TBA-reactive Substances

The rats were killed, 1 h after Fe-NTA administration by decapitation, and kidneys were immediately obtained. This protocol was based on our previous results that lipid peroxidation reaches its peak 1 h after Fe-NTA administration.[21] Preliminary experiments further confirmed that lipid peroxidation reaches its peak at 1 h in the groups fed with rice. One kidney was used for the determination of TBA-reactive substances (TBARS) and the other was used for histological evaluation. TBA-reactive substances was determined according to the method of Buege and Aust[22] with slight modification. To prevent additional chromophore formation during the assay, 0.1% BHT was added to the reaction mixture.

Histology

Kidneys were transversely cut at 5 mm thickness and fixed with 10% neutral formalin solution. Then, they were subjected to paraffin embedding, cut at 3.5 μm and stained with hematoxylin and eosin.

TABLE I Dietary composition (unpolished rice)

	White rice	Red rice	Black rice	Standard chow
Chemical composition				
Water (g/kg)	169	185	169	80
Crude protein (g/kg)	63	87	73	208
Crude fat (g/kg)	31	26	29	48
Crude ash (g/kg)	12	12	13	50
Crude fiber (g/kg)	23	33	33	32
Vitamins				
Ascorbic acid	n.d.	n.d.	n.d.	n.d.
α-Tocopherol (mg/kg)	14	15	13	85
Minerals and metals				
Iron (mg/kg)	13.5	12.4	11.9	300
Manganese (mg/kg)	22.4	23.5	38.5	100
Nickel (mg/kg)	0.28	0.14	0.19	–
Cobalt	3.74*	3.47*	2.26*	2†
Copper (mg/kg)	3.11	1.34	5.97	11
Pigments				
C3G, cooked (μmol/kg)	n.d.	n.d.	193.0	–
C3G, uncooked (μmol/kg)	n.d.	n.d.	45.5	–
PC, cooked (μmol/kg)	n.d.	10.9	257.9	–
PC, uncooked (μmol/kg)	0.834	6.07	24.9	–

n.d.: not detected; –: not determined; C3G: cyanidin-3-*O*-β-D-glucoside; PC: protocatechuic acid. Measurement of pigments were done after methanol extraction with or without cooking rice as described in the "Materials and methods section". Detection limit; ascorbic acid, 10 mg/kg; C3G and PC, 0.04 μmol/kg. *Unit: μg. dagger; Unit: mg/kg.

Immunohistochemistry of 8-hydroxy-2′-deoxyguanosine (8-OHdG)

The avidin–biotin complex method with alkaline phosphatase was used as described.[8] After antigen retrieval with autoclaving in 10 mM citrate buffer, pH 6.0 for 10 min, normal rabbit serum (Dako; diluted to 1:75) for the inhibition of non-specific binding of secondary antibody, monoclonal antibody against 8-hydroxy-2′-deoxyguanosine (N45.1, 10 μg/ml; Japan Institute for the Control of Aging, Fukuroi, Shizuoka), biotin-labeled rabbit anti-mouse IgG serum (Dako; diluted to 1:300), and avidin–biotin complex (Vector Laboratories, Burlingame, CA; diluted to 1:100) were sequentially applied. Black substrate for alkaline phosphatase (Vector) was used

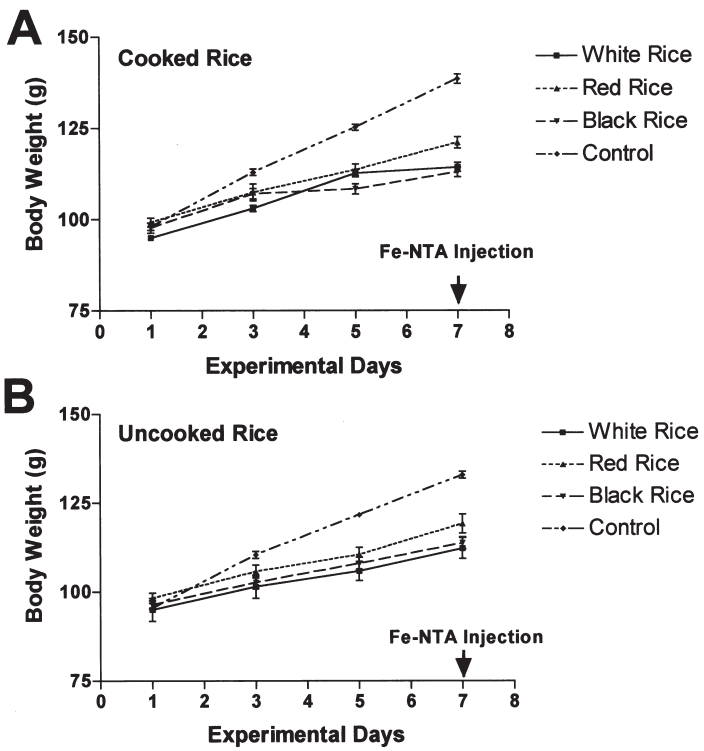

FIGURE 2 Body weight of each experimental group. (A) Cooked rice groups. (B) Uncooked rice groups. Only groups with simple diet are shown. Refer to "Materials and methods section" for details (means ± SEM, $N = 4$).

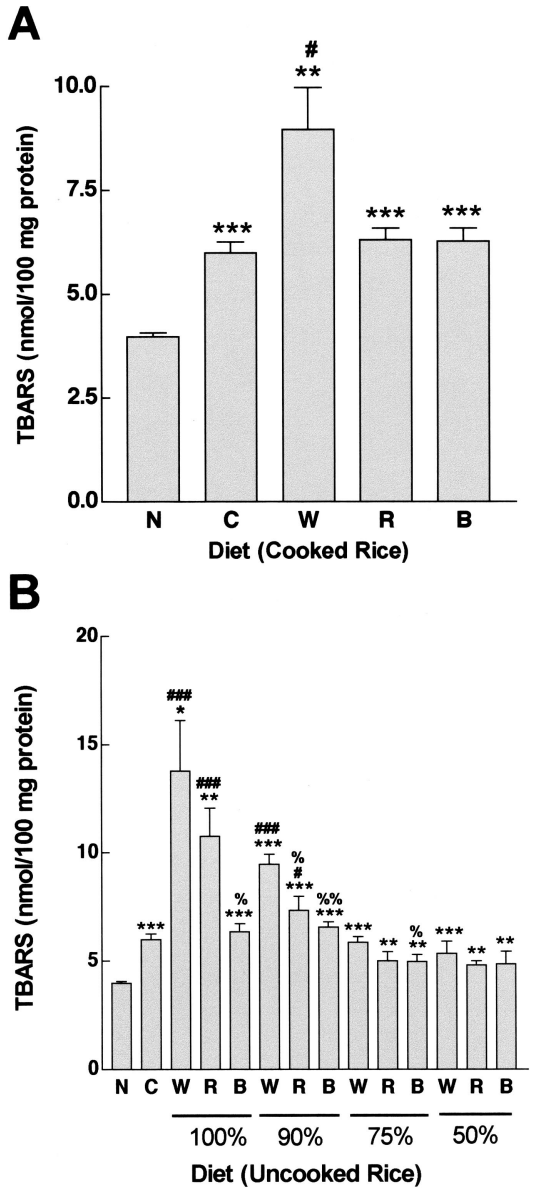

FIGURE 3 Determination of TBA-reactive substances. (A) Cooked rice groups. (B) Uncooked rice groups. Each animal received an intraperitoneal injection of 10 mg iron/kg of Fe-NTA. Kidney was taken out 1 h after Fe-NTA administration, and the homogenates were subjected to TBARS determination. N: normal untreated control (standard chow); C: Fe-NTA-treated control (standard chow); W: white rice-fed group; R: red rice-fed group; B: black rice-fed group. Percentage indicates the fraction of rice in the mixed diet. Refer to "Materials and methods section" for detail. *, $p < 0.05$, **, $p < 0.01$, ***, $p < 0.001$ vs. normal untreated group; #, $p < 0.05$, ###, $p < 0.001$ vs. Fe-NTA-treated control (standard chow); %, $p < 0.05$, %%, $p < 0.01$ vs. white rice-fed group of the same mixture fraction (means ± SEM, $N = 3–4$).

for color presentation. Procedures using phosphate-buffered saline or IgG fraction of normal mouse serum instead of anti-8-OHdG antibody showed no or negligible immunostaining.

Enzyme Assay

Glutathione peroxidase (GP*x*) activity was measured by NADPH oxidation in a coupled reaction system containing *tert*-butyl hydroperoxide, glutathione, and glutathione reductase.[23] Glutathione *S*-transferase (GST) activity toward CDNB as a substrate was measured according to the method of Habig *et al.*[24] Catalase[25,26] and total superoxide dismutase (SOD)[27,28] activities were determined as described. Assays of NAD(P)H:quinone reductase (NAD(P)H:QR) activity was determined by a procedure reported by Benson *et al.*[29]

Determination of C3G and Protocatechuic Acid Level in Rice, Plasma, and Kidney

Determination of C3G and protocatechuic acid (PC) in plasma and kidney samples was done with a high-performance liquid chromatography as previously described.[5] Determination of C3G and PC in uncooked as well as cooked rice was done using the protocol for plasma samples after methanol extraction. The retention time for standard C3G and PC was 7.30 and 5.45 min in the protocol for plasma samples, and 6.70 and 4.65 min in the protocol for tissue samples.

Statistical Analyses

All the data are shown as means ± SEM. Statistical analyses were performed by an unpaired *t*-test, which was modified for unequal variances when necessary. An analysis of variance was also used in the mixed diet study.

RESULTS

Nutrients, Trace Metals, C3G, and PC in Rice

Nutrients and levels of trace metals, C3G, and PC in each strain of rice used and standard chow are summarized in Table I.

Cooked Rice Diet

The weights of rats fed only with cooked rice for a week were significantly lower than those of the rats fed with standard chow (white rice, 114.3 ± 1.3 g; red rice, 121.0 ± 1.6 g; black rice, 112.9 ± 1.3 g; $p < 0.001$ vs. standard chow, 138.5 ± 1.3 g; means ± SEM, $N = 4$) (Fig. 2A). In white rice group, TBARS was significantly higher after Fe-NTA administration as compared with those fed with standard chow. However, this was reversed to the levels of standard chow-fed rats when cooked red or black rice was given (Fig. 3A).

Uncooked Rice Diet

This experiment was performed to rule out the possibility that some constituents of rice is either

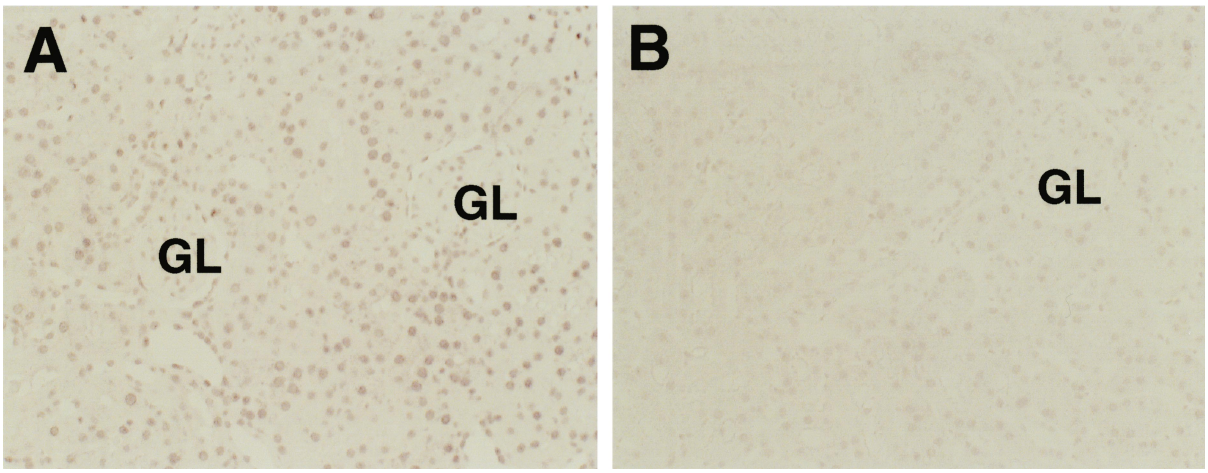

FIGURE 4 Representative immunohistochemical analysis of 8-hydroxy-2′-deoxyguanosine. (A) Uncooked white rice-fed group. (B) Uncooked black rice-fed group. Uncooked rice was mixed with standard chow at a ratio of 9:1. Each animal received an intraperitoneal injection of 10 mg iron/kg of Fe-NTA. Kidney was taken out 1 h after Fe-NTA administration, fixed with neutral formalin, and subjected to routine paraffin embedding process and immunohistochemical analysis. White-rice fed group revealed more intense nuclear immunostaining in the renal proximal tubular cells. Refer to "Materials and methods section," and Fig. 3B. GL, glomerulus (without nuclear counterstaining, ×115).

activated or inactivated during the cooking process. The weights of rats fed with uncooked rice of any color for a week were significantly lower than those of the rats fed with standard chow (white rice, 112.2 ± 2.9 g; red rice, 119.2 ± 2.7 g; black rice, 113.8 ± 1.7 g; $p < 0.001$ vs. standard chow, 132.9 ± 1.0 g; $N = 4$) (Fig. 2B). Only in uncooked white rice group, lipid peroxidation as seen by TBARS was significantly increased before Fe-NTA administration (5.483 ± 0.157 nmol/100 mg protein; $p < 0.05$ vs. standard chow, 4.542 ± 0.275 nmol/100 mg protein; $N = 4$). Furthermore, in white rice group, TBARS was significantly higher after Fe-NTA injection as compared with the standard chow group. However, this was completely reversed to the levels of standard chow-fed rats when black rice was given, but partially reversed when red rice was given (Fig. 3B).

Then, we undertook to modify the diet by changing the proportion of rice between 100 and 50% (w/w) in combination with standard chow. In accordance with the higher mixture ratio of standard chow, TBARS in white or red rice-fed groups decreased (Fig. 3B). Furthermore, red or black rice group revealed more weight gain (75% mixture; white rice, 148.0 ± 5.6 g; red rice, ∗152.2 ± 3.1 g; black rice, ∗151.6 ± 2.4 g; ∗$p < 0.01$ vs. normal chow, 140.2 ± 1.2 g; means ± SEM, $N = 4$). When standard chow was mixed to 50% level, there was no more difference in the TBARS level among each group (Fig. 3B). An analysis of variance revealed that there is a significant difference in TBARS among the four groups (comparison of standard chow, white rice, red rice, and black rice groups) after Fe-NTA administration at the rice mixture levels of 100 and 90% ($p < 0.001$), and also among the same rice groups (comparison of 100, 90, 75, 50, and 0% groups; white and red rice groups, $p < 0.0001$; black rice group, $p < 0.05$).

Histology and Immunohistochemistry of 8-OHdG

Histology of the kidney in all the animals used in the present study was examined. There was no significant change in hematoxylin- and eosin-stained specimens after rice feeding. Histology after Fe-NTA treatment was closely associated with the TBARS levels as we had reported previously;[21] namely, according with the increase in the renal TBARS level, more severe degeneration was

TABLE II Levels of protocatechuic acid

	White rice	Red rice	Black rice	Standard chow
Kidney (nmol/g tissue)				
Cooked	*13.3 ± 2.0	3.2 ± 1.4	*13.3 ± 1.6	6.7 ± 0.1
Uncooked	10.8 ± 2.5	10.1 ± 2.8	7.8 ± 3.0	6.7 ± 0.1
Serum (nmol/ml)				
Cooked	n.d.	0.048 ± 0.021	*1.17 ± 0.42	0.053 ± 0.014
Uncooked	n.d.	n.d.	*1.59 ± 0.65	0.053 ± 0.014

n.d.: not detected. Either cooked or uncooked rice diet was given as described in the "Materials and methods section". Detection limit, 0.04 nmol/g tissue or 0.04 nmol/ml serum ($N = 3$–4; means ± SEM; ∗$p < 0.05$ vs. standard chow).

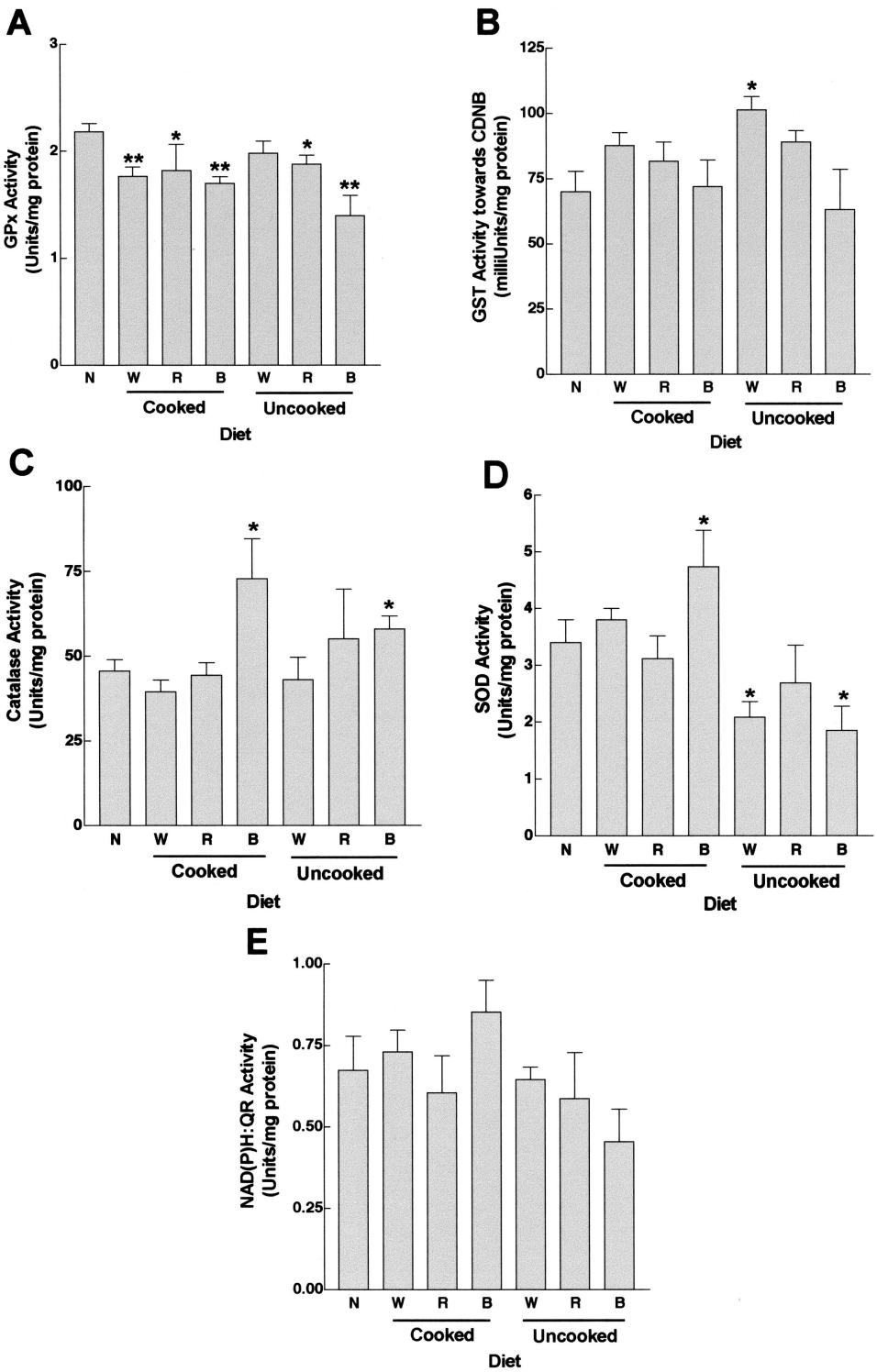

FIGURE 5 Enzymatic activities in the kidney after six days of rice feeding. (A) GP*x* activity; (B) GSH-transferase activity; (C) catalase activity; (D) total SOD activity; (E) NAD(P)H:quinone reductase activity. N: standard chow-fed group; W: white rice-fed group; R: red rice-fed group; B: black rice-fed group. Note that Fe-NTA is not administered. Refer to "Materials and methods section" for details. *, $p < 0.05$, **, $p < 0.01$ vs. standard chow-fed group (*N*).

observed in the renal proximal tubules of rats treated with Fe-NTA (data not shown). Nuclear immunostaining of 8-OHdG was observed in the proximal tubular cells with degenerative changes as shown in Fig. 4.

Enzyme Activity

Glutathione peroxidase activity was significantly decreased in the rice-fed groups except the group fed with uncooked white rice (Fig. 5A). GST activity was

significantly increased only in the group fed with uncooked white rice (Fig. 5B). Catalase activity was increased in the groups fed with cooked or uncooked black rice (Fig. 5C). Total SOD activity was significantly increased in rats fed with cooked black rice while it was significantly decreased in rats fed with uncooked white or black rice (Fig. 5D). There was no significant change in NAD(P)H:QR activity in each group (Fig. 5E).

In summary, among the antioxidant enzymatic activity evaluated after cooked or uncooked rice feeding for six days, only catalase activity of the black rice-fed group revealed a logical inverse association with the TBARS levels.

Levels of C3G and PC in the Kidney and Serum

C3G was detected neither in the kidney nor serum of any group in the present study (detection limit: < 0.04 nmol/g or ml). The levels of PC in the kidney of each group shown in Table II were not associated with the antioxidative effect of colored rice. However, serum levels of PC were significantly higher in the groups of cooked and uncooked black rice (Table II).

DISCUSSION

Recently, possible antioxidant nature of plant constituents in the diet has attracted much attention of researchers and medical doctors from the standpoint of preventing a variety of adult-onset diseases. Indeed, a number of common human pathologic conditions are reported to be associated with oxidative stress. By the use of immunochemical and immunohistochemical techniques with specific antibodies against 8-hydroxy-2′-deoxyguanosine and 4-hydroxy-2-nonenal-modified proteins, we have systematically demonstrated the presence of oxidative damage in the following pathologic conditions.[8,30] These include renal cell carcinoma,[31] colon cancer,[32] atherosclerosis of aorta,[33] pancreatic β-cells in diabetes mellitus,[34] substantia nigra of Parkinson's disease,[35] alcoholic liver disease, [36] type C viral hepatitis,[37] arsenic-induced skin cancer,[38] ultraviolet-induced epidermal damage[39] and ischemia-reperfusion injury of liver.[40]

Rice has been a staple diet in Asian countries, and is one of the most important grains in the world.[1] While white polished rice is generally favored, strains of colored rice have been preserved for religious purposes in Japan. Now the consumption of colored rice is increasing because of the additional value based on anticipated antioxidant activity and organic cultivation using a low dose of herbicides and insecticides, and of curiosity for "ancient" rice.

Recently, several reports have been published regarding rice and its associated products. Hudson *et al.* showed that white unpolished rice (brown rice) and bran contain compounds with putative cancer preventive properties by looking at cell viability and colony-forming ability of human cancer-derived cultured cells and that certain phenols (e.g. tricin) present in brown rice bran may be associated with this activity.[41] Koide *et al.* showed that the mice fed with red rice survived longer than the groups fed with either white rice or standard chow after intraperitoneal inoculation of syngeneic lymphoma cells, and suggested that anthocyanidins are responsible for this activity based on cultured cell experiments.[42] We previously isolated C3G from black rice,[3] and its administration was protective against ischemia-reperfusion injury of liver in rats.[5] C3G was also identified as an antioxidative pigment in other plant materials including black and red beans, purple cabbage, red grape, plum and purple corn.[4] Furthermore, it was recently shown that Kuro-su (black vinegar), a traditional vinegar produced from unpolished black rice, suppressed lipid peroxidation *in vitro* and in mouse skin,[43] and that aqueous extracts of red rice fermented with *Monascus ruber* induced nitric oxide-mediated endothelium-dependent relaxation of rat thoracic aorta.[44]

In the present paper, we asked whether feeding of colored rice show any beneficial effects on free radical-induced tissue injury in comparison with white rice. Our study for the first time demonstrated that colored rice diets have protective effect on oxidative injury as compared with white rice by determination of lipid peroxidation, histology, and immunohistochemistry. For excluding the possibility that certain components are lost or inactivated during the cooking process of rice, we carried out experiments both with uncooked as well as cooked rice. Both experiments showed that colored rice, red or black, are more protective against Fe-NTA-induced renal proximal tubular injury. This effect was dose-dependent on the proportion of rice mixed with commercial standard chow, and when mixed with equivalent weight of standard chow, no difference was observed among the three kinds of rice. Therefore, this effect of colored rice might be obscured by well-balanced diet.

While grains of black rice contained C3G, no C3G was detectable in the serum or kidney in the present study. However, PC, a possible metabolite of C3G,[5] was significantly increased in the serum of black rice-fed groups (Table II). Protocatechuic acid was also detected in the kidney of all the groups studied, thus suggesting that there are several sources for PC and that PC content in the kidney is not associated with the antioxidative effect.

There are four possible explanations for the antioxidative effect of colored rice; namely, direct and indirect antioxidative effects, effect on iron absorption, and nutritional factor. Since C3G was present in an undetectable level *in vivo* in the present experiments, we need further investigation for drawing any conclusion on its direct antioxidant activity. Especially, detailed analyses of its absorption and metabolism would be necessary. The available C3G amount may be different since the extracted C3G was different between cooked and uncooked black rice (Table I). Increased PC levels in the serum (Table II) may indicate that C3G was absorbed and metabolized.

There was a general tendency of decreased GP*x* activity after feeding rice for a week. However, catalase activity was significantly increased in black rice-fed group, and probably supplemented the GP*x* activity for metabolizing H_2O_2. This may at least partly explain the antioxidative effect of colored rice since Fe-NTA-induced renal tubular damage is Fenton reaction-based. GST, SOD and NAD(P)H: quinone reductase activities were not associated with the degree of lipid peroxidation and histology. Iron overload has been associated with carcinogenesis.[6] It is possible that colored rice may decrease the absorption of iron from peritoneum via portal vein with a mechanism of chelation. Furthermore, colored rice is superior to white rice in nutrition, especially protein amount (Table I) since rats fed with mixed diet of colored rice and standard chow gained more weight when the mixture ratio was 3:1. Other chemical composition, vitamins, minerals, and metals were not significantly different between the three kinds of rice.

Our results were obtained in a near physiological condition since we had used cooked and uncooked rice as the diet. Diets containing colored rice have a possibility to work for prevention or supportive therapy of oxidative stress-associated diseases as discussed above especially in a situation when nutrition of food is not well considered. However, we have to be careful about the general use of colored rice diet since the present study was focused on the free radical-induced renal tubular injury in rats. Further studies are warranted to identify which component is important for the antioxidative effect and to evaluate for which type of pathologic conditions this kind of diets are useful.

Since the whole genome of rice is going to be sequenced within a short period of time,[45] transgenic rice might be a promising tool for producing more efficient strains of rice for protection against free radical-induced injury if the precise molecular mechanism is clarified in our future experiments. At last, an interesting observation recently reported was that genetic diversity increases disease control in rice.[46] Mixed cultivation of white and colored rice may work for decreasing insecticides in the fields. This is another possibility of using colored rice for environmental safety. In conclusion, we have for the first time shown that diet of colored rice can be more protective than white rice against Fenton reaction-based tissue injury in a specific situation.

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