Alleviation of Oxidative Damage in Multiple Tissues in Rats with Streptozotocin-Induced Diabetes by Rice Bran Oil Supplementation

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ABSTRACT: The possibility of 8-hydroxy-2′-deoxyguanosine (8-OHdG) serving as a sensitive biomarker of oxidative DNA damage and oxidative stress was investigated. Reactive oxygen species (ROS) have been reported to be a cause of diabetes induced by chemicals such as streptozotocin (STZ) in experimental animals. In this study, we examined oxidative DNA damage in multiple tissues in rats with STZ-induced diabetes by measuring the levels of 8-OHdG in the liver, kidney, pancreas, brain, and heart. Levels of 8-OHdG in mitochondrial DNA (mtDNA) and nuclear DNA (nDNA) were also determined in multiple tissues of rats treated with rice bran oil. Levels were 0.19 ± 0.07, 0.88 ± 0.30, 1.97 ± 0.05, and 9.79 ± 3.09 ($1/10^5$ dG) in the liver of nDNA of normal rats, nDNA of STZ-induced diabetic rats, mtDNA of normal rats, and mtDNA of STZ-induced diabetic rats, respectively. Levels of mtDNA of 8-OHdG were 10 times higher than those of nDNA in multiple tissues. Significant reductions in mtDNA 8-OHdG levels were seen in the liver, kidney, and pancreas of diabetic rats treated with rice bran oil compared with diabetic rats without intervention. Our study demonstrated that oxidative mtDNA damage may occur in multiple tissues of STZ-induced diabetics rats. Intervention with rice bran oil treatment may reverse the increase in the frequency of 8-OHdG.

KEYWORDS: 8-OHdG; diabetes; oxidative stress; streptozotocin

INTRODUCTION

DNA is susceptible to damage by reactive oxygen species (ROS). ROS, such as superoxide, hydrogen peroxide, the hydroxyl radical, and singlet oxygen, are produced during normal and pathophysiological processes, and production of such radicals is further enhanced by ionizing radiation, environmental mutagens, and carcinogens.[1] In terms of oxidative DNA damage, nucleic acids exposed to oxygen radicals generate various modified bases, and more than 20 oxidatively altered pu-

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rines and pyrimidines have been detected.[2,3] 8-Hydroxyguanine (7,8-dihydro-8-oxoguanine) is believed to be an important compound because of its role as the marker of mutagenicity and carcinogenicity in bacterial and mammalian cells.[4,5]

The diabetogenic agent streptozotocin (STZ) is a D-glucopyranose derivative of *N*-methyl-*N*-nitrosourea (MNU).[6,7] STZ has broad-spectrum antibiotic activity and is often used to induce diabetes mellitus in experimental animals through its toxic effects on pancreatic β cells. Diabetic complications in target organs arise from the chronic elevation of glucose levels. The pathogenic effect is mediated to a significant extent via increased production of ROS and/or reactive nitrogen species (RNS) and subsequent oxidative stress.[8,9] This study was undertaken to examine oxidative DNA damage in various tissues of STZ-induced diabetic rats by measuring the levels of 8-OHdG.

MATERIALS AND METHODS

STZ-Induced Diabetic Rats

At 8 weeks of age, Sprague-Dawley rats were injected intraperitoneally with STZ at 45 mg/kg body weight twice in a 3-day period. Nicotinamide (200 mg/kg) was injected before the STZ injection as a protection reagent. The development of diabetes was verified by the presence of hyperglycemia. On day 3 post–STZ treatment, rats were randomly assigned to two groups ($n = 6$ per group): diabetes with soybean oil (control) and diabetes with rice bran oil (intervention group). Rice bran oil was extracted by the supercritical CO_2 extraction method.[10] There are 33.90 mg of γ-oryzanol, 0.5 mg of γ-tocotrienol, and 3.02 mg of α-tocopherol per gram of rice bran oil, all of which were analyzed by high-performance liquid chromatography.[11] The saturated fatty acid (SFA), monounsaturated fatty acid (MUFA), and polyunsaturated fatty acid (PUFA) levels of rice bran oil were 13.61%, 50.67%, and 35.5%, respectively, as analyzed by gas chromatography.[12] On day 28, rats were anesthetized with ether, and various tissues were quickly removed and stored at –70°C until DNA was extracted.

Enzyme Digestion of DNA and ELISA for 8-OHdG

The presence of 8-OHdG was detected as reported previously.[13] Aliquots of DNA equivalent to 50 μg were freeze-dried and reconstituted in 20 mM sodium acetate, pH 4.8, containing 45 mM zinc chloride. Samples were heated in a boiling-water bath for 3 min and cooled quickly on ice prior to the addition of nuclease P1 to yield 0.1 U/μg of DNA, and then samples were incubated at 37°C for 1 h. Samples were made alkaline by the addition of 1.5 M Tris–HCl, pH 8.0, and alkaline phosphatase was added to give ~0.05–0.075 U/μg of DNA; then samples were incubated at 37°C for 30 min.

Enzymatic digests of DNA were analyzed by competitive enzyme-linked immunosorbent assay (ELISA) using a monoclonal antibody to 8-OHdG (8-OHdG Check; Genox, Baltimore, MD). Fifty microliters of DNA digest or standard 8-OHdG solution was added to the wells of a 96-well plate precoated with 8-OHdG, and then 50 μL of primary antibody (mouse anti–8-OHdG) was added; the plate was then incubated at 37°C for 1 h. After incubation, wells were washed with 200 μL/well of

TABLE 1. Contents of 8-OHdG in mtDNA and nuclear DNA from multiple tissues of normal rats and STZ-induced diabetic rats

	Liver	Kidney	Pancreas	Brain	Heart
N-nDNA	0.19 ± 0.07*	0.17 ± 0.08*	0.16 ± 0.09	0.05 ± 0.03	0.07 ± 0.03
D-nDNA	0.88 ± 0.30*	1.06 ± 0.39*	0.75 ± 0.36	0.34 ± 0.15	0.34 ± 0.19
N-mtDNA	1.97 ± 0.65†	1.77 ± 0.68†	1.73 ± 0.71†	0.64 ± 0.39†	0.63 ± 0.33†
D-mtDNA	9.79 ± 3.09†	9.20 ± 3.04†	8.03 ± 4.37†	3.28 ± 1.38†	3.76 ± 1.51†

Results are presented as the mean ± SD. *,†Statistically significant difference ($P < 0.05$).
N, normal rats; D, diabetic rats; nDNA, nuclear DNA; mtDNA, mitochondrial DNA.

0.05% (vol/vol) Tween 20 in 0.01 M phosphate-buffered saline (PBS), pH 7.4, and then 100 μL/well secondary antibody (peroxidase conjugated anti–mouse immunoglobulin G) was added and the plate was incubated for 1 h at 37°C. Following incubation, peroxidase substrate (*o*-phenylenediamine/hydrogen peroxide/PBS) was added, followed 30 min later by 2 N sulfuric acid; the absorbance was then read at 450 nm.

RESULTS

Five tissues, including the liver, kidney, pancreas, brain, and heart, were collected from normal rats and from STZ-induced diabetic rats. DNA extracted from various tissues was classified into mitochondrial DNA (mtDNA) and nuclear DNA (nDNA) groups. The 8-OHdG contents of mtDNA and nDNA were determined and are listed in TABLE 1. Hepatic levels of 0.19 ± 0.07, 0.88 ± 0.30, 1.97 ± 0.05, and 9.79 ± 3.09 ($1/10^5$ dG) of nDNA of normal rats, nDNA of STZ-induced diabetic rats, mtDNA of normal rats, and mtDNA of STZ-induced diabetic rats were found, respectively. mtDNA had levels of 8-OHdG that were 10 times higher than those of nDNA in multiple tissues. There were significantly increased 8-OHdG levels of nDNA in liver and kidney of STZ-induced diabetic rats compared with the control group. Significant

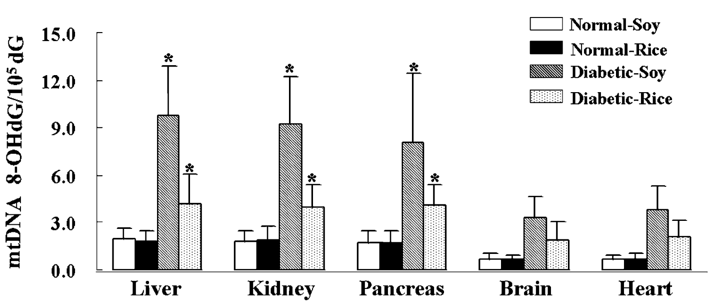

FIGURE 1. Levels of 8-OHdG in mtDNA of multiple tissues compared between normal rats and diabetic rats supplemented with rice bran oil.

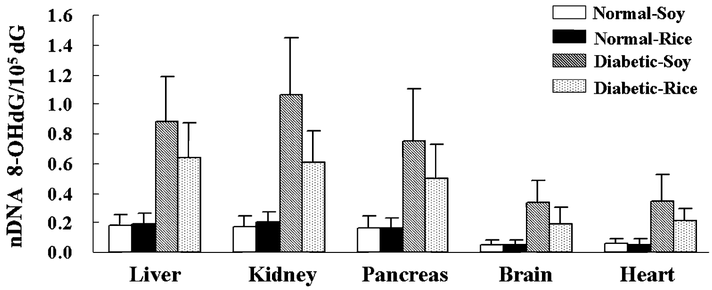

FIGURE 2. Contents of 8-OHdG in nDNA determined from diabetic rats supplemented with rice bran oil.

differences between mtDNA of diabetic rats and normal rats were detected in all examined tissues.

Protective effects of oxidative damage to DNA by supplementation with rice bran oil were determined. Levels of 8-OHdG in mtDNA of various tissues were also compared between normal and diabetic rats supplemented with rice bran oil (FIG. 1). There were significant decreases in mtDNA 8-OHdG contents in the liver, kidney, and pancreas from diabetic rats treated with rice bran oil. Intervention using rice bran oil treatment may reverse the increase in the frequency of 8-OHdG from mtDNA. Contents of 8-OHdG in nDNA from diabetic rats supplemented with rice bran oil were also determined (FIG. 2). Lower 8-OHdG proportions in the liver, kidney, and pancreas were found. However, there were no significant differences in 8-OHdG levels in nDNA between control subjects and rice bran oil–treated rats.

DISCUSSION

STZ is a well-known genotoxic agent, and it has been shown to induce DNA damage in mouse liver and kidney, which are the tumor target organs.[14,15] This study found higher levels of 8-OHdG in mtDNA from the liver, kidney, and pancreas of STZ-induced diabetic rats than in controls. The results indicate that diabetes-induced oxidative stress may cause major damage in the above-mentioned tissues. Diabetic nephropathy is the major cause of morbidity and mortality in diabetic patients. Several mechanisms have been proposed for the pathogenesis of diabetic nephropathy, such as hyperfiltration,[16] increased production of advanced glycation end products (AGEs),[17] activation of protein kinase C,[18,19] and enhanced oxidative stress.[20,21] Our findings are consistent with the notion that oxidative stress is an etiological factor in the occurrence of nephropathy.

Increased oxidative stress has been hypothesized to contribute to the pathological processes of diabetic complications. However, the detailed biomolecular mechanism has not been ascertained. In general, oxidative stress can modify nucleic acids such as 8-OHdG, affect its exact linkage, and play a crucial role in mutagenesis.[22] Increased levels of 8-OHdG have been reported in urine,[23,24] mononuclear cells,[23,25] skeletal muscles,[26] pancreas,[27] liver,[28,29] and kidney[28,29] of diabetic patients.

It is widely accepted that mtDNA is ~10–20 times more vulnerable to oxidative damage and subsequent mutations than is nDNA.[30] Higher levels of oxidative DNA damage of mtDNA than nDNA occur for the following three reasons. First, the lack of protective histones renders mtDNA more vulnerable to oxidative attack. Second, it has been argued that the proximity of mtDNA to the mitochondrial electron transport chain, a site of superoxide anion generation, predisposes it to oxidative damage. Third, it has been pointed out that oxidative DNA damage may be less efficiently repaired in mtDNA. This argument has been reiterated in many research reports.[31,32] However, there are only a few studies reporting on oxidative DNA damage in mtDNA in various tissues of diabetic rats.

In this study, we further examined the 8-OHdG levels in mtDNA and nDNA of various tissues. We found increased levels of 8-OHdG in mtDNA of the liver, kidney, and pancreas of diabetic rats at 4 weeks after the onset of diabetes. In contrast, levels of 8-OHdG in nuclear DNA were not significantly increased in diabetic rats compared with controls. These results are consistent with previous findings that mtDNA is more sensitive to oxidative stress than nDNA. Interestingly, previous studies indicated that modified mtDNA may contribute to increased levels of 8-OHdG. The possible consequence of increased formation of 8-OHdG in mtDNA is the increase in mtDNA mutation, including mispairing and deletions. Kakimoto *et al.*[33] also showed that increases in mtDNA deletion were in concert with higher levels of 8-OHdG in the kidney of diabetic rats. Deleted mtDNA may accumulate with the duration of diabetes in multiple tissues of diabetic rats.

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