ORIGINAL ARTICLE

Effects of Different Contrast Media on Glutathione Peroxidase and Superoxide Dismutase Activities in the Heart and Kidneys of Normal and Streptozotocin-induced Diabetic Rats

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Background/Purpose: Hemodynamic changes and contrast nephropathy are well known complications of contrast media injection. However, the mechanisms of toxicity leading to these complications remain unclear. We hypothesized that contrast media toxicity would manifest as a change in antioxidant enzyme activity, thus leading to tissue damage.

Methods: This study investigated the effects of injection of ionic high-osmolar diatrizoate, ionic low-osmolar ioxaglate, and nonionic low-osmolar iopromide on the activities of two antioxidant enzymes, glutathione peroxidase (GPX) and superoxide dismutase (SOD), in the heart and kidney tissue of normal male Wistar rats ($n=51$) and streptozotocin (STZ)-induced diabetic rats ($n=54$). Activities of GPX and SOD were assayed spectrophotometrically.

Results: Renal GPX activities were significantly decreased in both normal (458.3 ± 64.6 to 385.5 ± 63.4 mU/mg, $p=0.005$) and diabetic rats (669.0 ± 98.1 *vs.* 564.0 ± 153.3 mU/mg, $p=0.035$) at 1 hour after diatrizoate injection. Renal SOD activities were not affected after contrast injection. Ioxaglate and iopromide injection did not cause any change in renal antioxidant enzyme activity. In contrast to kidney tissue, there was no significant change in GPX and SOD activities in heart tissue at 1 hour after injection of different contrast media.

Conclusion: Intravenous injection of ionic high-osmolar diatrizoate reduced renal GPX activity during the first hour in both normal and STZ-induced diabetic rats. Heart tissue was not prone to antioxidant enzyme activity changes after intravenous contrast media injection. GPX activity reduction can be an important mechanism of nephrotoxicity after contrast media injection. [*J Formos Med Assoc* 2006;105(7):530–535]

Key Words: antioxidant enzyme, contrast media, contrast nephropathy, reactive oxygen species, streptozotocin

Contrast media has been used in angiography for more than 80 years.[1] Low-osmolar and nonionic contrast media are safer in cardiac angiography with fewer hemodynamic changes and lower incidence of malignant arrhythmias.[2–5] Nephrotoxicity is still the major problem, and an increase in serum creatinine of more than 1.5 mg/dL has been observed in 6% of patients

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using nonionic contrast media.[6] Diabetes mellitus (DM) and preexisting renal insufficiency are the major risk factors for contrast nephropathy. The pathophysiology of contrast media nephropathy is not well understood.[7] Animal experiments have shown that contrast nephropathy is accompanied by increased production of reactive oxygen species.[8] Other studies have shown that administration of radiocontrast agents increase lipid peroxidation in rat kidneys.[9] This study assessed the effects of intravenous injection of ionic high-osmolar, ionic low-osmolar, and nonionic low-osmolar contrast media on antioxidant enzyme activity in the heart and kidneys of normal and streptozotocin (STZ)-induced diabetic rats.

Methods

Contrast media

The effects of the following three types of contrast media were studied: (1) ionic high-osmolar diatrizoate meglumine/diatrizoate sodium (DTZ) (Hypaque-75, Squibb, USA; osmolarity 2016 mOsm/kg, iodine content 370 mg/mL); (2) ionic low-osmolar ioxaglate (IXG) (Hexabrix-320, France; osmolarity 600 mOsm/kg, iodine content 320 mg/mL); and (3) nonionic low-osmolar iopromide (IPM) (Ultravest-370, Schering, Germany; osmolarity 880 mOsm/kg, iodine content 370 mg/mL).

Study animals

This study was approved by the Animal Care and Treatment Committee of our institution. Male Wistar rats (purchased from the National Laboratory Animal Breeding and Research Center, Taiwan), 6–8 weeks old and weighing 150–180 g were used. DM was induced by a single peritoneal injection of 55 mg/kg STZ (Sigma Chemical, St Louis, MO, USA). Diabetes was confirmed by checking blood glucose (Precision Plus, Abbott Laboratories, IL, USA). With successful DM induction, blood glucose level was greater than 200 mg/dL within 24–36 hours after STZ injection. If the rat's blood glucose was below 200 mg/dL, another dose of 55 mg/kg STZ was injected. The blood glucose of diabetic rats was monitored daily, and insulin (Monotard HM, Novo Nordisk, Denmark) was injected subcutaneously to maintain a blood glucose level of around 350 mg/dL. All were fed a regular diet and water until 4 weeks after the day of STZ injection. All rats fasted for 24 hours prior to the start of experiments.

Normal and STZ-induced diabetic rats were separated into three different contrast media or normal saline injection groups for injection with a volume of 10 mL/kg for DTZ, 11.6 mL/kg for IXG, 10 mL/kg for IPM and 10 mL/kg for normal saline. Injections were administered via tail vein puncture. One hour after injection, the rats were anesthetized with sodium pentobarbital (Abbott Laboratories) and sacrificed for dissection. The heart and kidneys were obtained for analysis.

Measurements of antioxidant enzyme activities

The dissected kidney and heart tissue was immediately rinsed in ice-cold phosphate-buffered saline. Cleaned specimens were chipped and homogenized in appropriate buffer solution, which contained 50 mM Tris-HCl at pH 7.5, 5 mM EDTA and 1 mM 2-mercaptoethanol. Antioxidant enzyme activities were determined by spectrophotometric assays. Glutathione peroxidase (GPX) activity was assayed in a 1-cm path cuvette containing 350 µL assay buffer (GPx-340, OxisResearch, Portland, USA), 350 µL NADPH reagent and 70 µL test sample that was prepared and diluted into assay buffer just prior to analysis. The mixture in the cuvette was added to 350 µL diluted tert-butyl hydroperoxide and then mixed. Absorbance at 340 nm was recorded at 23°C for 3 minutes. Superoxide dismutase (SOD) activity was measured with an assay kit (SOD-525, OxisResearch) by the suggested procedure summarized as follows: 30 µL sample was incubated in a 1-cm optical path cuvette with 500 µL 10 mM hydrogen peroxide for 1 minute and finally chromogen reagent. The absorbance of samples was recorded at 525 nm at 37°C. All activities of GPX and SOD were normalized by correction of sample protein

content. All measurements were repeated three times with intra-variation less than 8%.

Statistical analysis

All values were expressed as mean ± standard deviation. Two-way ANOVA was performed to test whether the DM/normal rats or contrast media/normal saline affected the results of enzyme activity. Statistical significance was analyzed using the unpaired Student's *t* test. A p value < 0.05 was considered to be statistically significant.

Results

A total of 105 male Wistar rats were used. Fifty-four STZ-induced DM rats had a mean body weight of 243.6 ± 49.6 g, and the mean body weight of 51 normal rats was 314.0 ± 49.9 g. The mean blood glucose of diabetic rats was 379.6 ± 83.3 mg/dL.

In the saline injection group, DM rats had higher renal GPX activities than normal rats (669.0 ± 98.1 *vs.* 458.3 ± 64.6 mU/mg, $p < 0.0001$). In contrast to renal GPX, renal SOD activity tended to be lower in DM than normal rats, but this difference was not significant ($p = 0.264$; Table 1). In heart tissue, both GPX (456.5 ± 62.5 *vs.* 424.9 ± 63.5 U/mg) and SOD activities (306.8 ± 61.0 *vs.* 279.1 ± 66.0 U/mg) were higher in DM rats than in normal rats, although this difference was not significant ($p = 0.182$ and 0.373) (Table 2).

DTZ, IXG and IPM injections were given to 14, 15 and 11 normal rats and 15, 15, 12 DM rats, respectively. Renal GPX activity was significantly lower in the DTZ group than in the saline groups in both normal (458.3 ± 64.6 *vs.* 385.5 ± 63.4 mU/mg, $p = 0.005$) and STZ-induced DM rats (669.0 ± 98.1 *vs.* 564.0 ± 153.3 mU/mg, $p = 0.035$) (Figure). Renal GPX activities were not significantly different among the IXG (449.2 ±

Table 1. Antioxidant enzymes in kidney tissue 1 hour after injection of various contrast media in normal and streptozotocin-induced DM rats*

	Normal rats			DM rats		
	n	GPX (mU/mg)	SOD (U/mg)	*n*	GPX (mU/mg)	SOD (U/mg)
NS	11	458.3 ± 64.6	628.4 ± 106.4	12	669.0 ± 98.1	583.3 ± 109.7
DTZ	14	385.5 ± 63.4†	675.8 ± 148.3	15	564.0 ± 153.3‡	635.1 ± 113.1
IXG	15	449.2 ± 94.7	642.6 ± 113.9	15	642.9 ± 99.0	602.7 ± 102.5
IPM	11	444.8 ± 68.0	571.5 ± 78.7	12	656.0 ± 125.0	575.6 ± 83.9

**All values of enzyme activity are presented as mean ± standard deviation; †GPX activity in DTZ group of normal rats was significantly lower than in NS group and p = 0.005; ‡GPX activity in DTZ group of DM rats was significantly lower than in NS group and p = 0.035. DM = diabetes mellitus; GPX = glutathione peroxidase; SOD = superoxide dismutase; NS = normal saline; DTZ = diatrizoate; IXG = ioxaglate; IPM = iopromide.*

Table 2. Antioxidant enzymes in heart tissue 1 hour after injection of various contrast media in normal and streptozotocin-induced DM rats*

	Normal rats			DM rats		
	n	GPX (mU/mg)	SOD (U/mg)	*n*	GPX (mU/mg)	SOD (U/mg)
NS	11	424.9 ± 63.5	279.1 ± 66.0	12	456.5 ± 62.5	306.8 ± 61.0
DTZ	14	428.4 ± 69.5	271.0 ± 57.4	15	460.1 ± 69.4	310.6 ± 73.0
IXG	15	433.3 ± 67.3	267.1 ± 78.7	15	458.0 ± 84.1	280.0 ± 62.9
IPM	11	460.1 ± 104.1	304.1 ± 102.3	12	464.1 ± 74.3	299.0 ± 86.2

**All values of enzyme activity are presented as mean ± standard deviation. DM = diabetes mellitus; GPX = glutathione peroxidase; SOD = superoxide dismutase; NS = normal saline; DTZ = diatrizoate; IXG = ioxaglate; IPM = iopromide.*

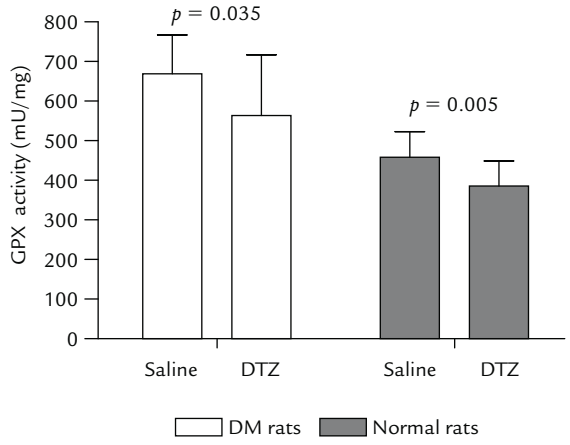

Figure. Glutathione peroxidase (GPX) activity 1 hour after diatrizoate (DTZ) injection was significantly lower than after saline injection in both normal and streptozotocin-induced DM rats.

94.7 mU/mg), IPM (444.8 ± 68 mU/mg) and saline control groups (Table 1). Renal SOD activities are also listed in Table 1. There was no significant difference in SOD activities among the three contrast media groups, both in normal and DM rats. GPX and SOD activities in heart tissue were not significantly different among contrast media groups, both in normal and DM rats.

Discussion

This study demonstrated that injection of ionic, high-osmolar contrast media diatrizoate reduced renal GPX activity in both normal Wistar and STZ-induced DM rats. Ionic low-osmolar ioxaglate and nonionic low-osmolar iopromide, however, did not have a significant effect on renal GPX activity. Ionic high-osmolar contrast media has been shown to cause more advanced and prolonged renal function depression than nonionic low-osmolar contrast media in patients with normal renal function and in patients with preexisting renal dysfunction.[10,11] While experimental and laboratory studies have suggested that ionic high-osmolar contrast media have the greatest nephrotoxic potential, this study demonstrated that GPX activity was reduced by high-osmolar contrast media injection.

Antioxidant enzymes serve as a defense mechanism against reactive oxygen species. In the kidney, proximal tubular cells and, to a lesser extent, glomeruli show activities of copper, zinc and manganese SOD, catalase and GPX.[12] SOD forms H_2O_2 out of $O_2^{-}\bullet$, which can then be converted to H_2O and O_2 by the catalase system. Additional H_2O_2 can be detoxified by peroxidases, mainly by GPX.[13] Among the antioxidant enzymes, GPX has been reported to be the most clearly influenced by lifestyle and environmental factors, such as intake of dietary supplements and smoking behavior.[14] A clinical study showed that a decrease in plasma GPX activity was associated with progression of renal disorders.[15] An animal study showed that GPX-deficient mice had increased oxidative stress, impaired endothelial function and structural vascular abnormalities.[16] In this study, renal SOD activity did not change significantly after contrast media injection in both normal and DM rats. This suggests that reduction of GPX rather than SOD was involved in the mechanism of contrast nephropathy. Reduction of renal GPX after the injection of high-osmolar contrast media may cause an imbalance between the availability of the defense mechanism and the presence of reactive oxygen species, thus resulting in oxidative stress-induced glomerular injury. In addition, more severe GPX depletion may serve as a contributory factor to contrast-induced renal vasoconstriction.

Unlike the kidneys, GPX and SOD activity in the heart tissue of normal and DM rats were not significantly different between groups of rats treated with contrast media or normal saline. The activity of antioxidant enzymes is variable within different tissues and cell types.[12] Therefore, different organs and tissues have different susceptibilities to reactive oxygen species. Another factor associated with the extent of tissue injury is the exposure concentration and time. With intravenous injection in humans, arterial enhancement decays almost within 30 seconds as each heart beat dilutes the contrast medium. In first pass dynamics of

pharmacokinetics, if more blood is ejected per unit of time, the contrast medium injected per unit of time will be diluted.[17,18] The majority of contrast media is excreted unmetabolized via the kidney. Contrast media are small molecules and are filtrated freely through the basal membrane of renal glomeruli. They are concentrated 100 times in the urine. Consequently, the osmotic load of contrast media that is presented to the kidney tubules during the first hour after injection is very high.[19] Due to the different exposure conditions to contrast media *in vivo*, the response of antioxidant enzymes in the heart cannot be the same as in the kidneys after intravenous contrast media injection.

Glomerular expression of antioxidant enzymes can be modulated. In this study, renal GPX activity in DM rats was 46% higher than in normal rats. Diabetes is characterized by high glucose concentrations that lead, via several mechanisms, to increased oxidative stress.[20,21] Animal studies have shown high renal activities of GPX and decreased renal catalase and SOD in STZ-induced DM compared to controls.[12,22] Our findings are consistent with these studies.

In a previous experiment, we found that serum creatinine levels were within normal limits in both normal and DM rats and were not significantly different. There was also no significant change in serum creatinine level 1 hour after contrast media injection. Clinically, contrast nephropathy generally presents as a rise in serum creatinine 24–48 hours after contrast exposure, peaking within 3–5 days.[23] Our results suggest that change in antioxidant enzyme activity, such as GPX activity reduction in renal tissue, which occurs in the first hour after administration, is a rapid response to contrast media exposure and precedes the detectable rise of serum creatinine.

Previous studies have demonstrated the presence of complex alterations in the activities of antioxidant enzymes in various tissues of STZ-induced DM rats.[22] In this study, antioxidant enzymes in heart tissue were not different between normal and DM rats. This implies that antioxidant enzyme alteration did not develop during 4 weeks of hyperglycemic status. A longer period of hyperglycemia would be needed to cause significant oxidative stress in the heart tissue of STZ-induced DM rats.

The patterns of antioxidant enzyme alterations in chemically-induced DM are independent of the diabetogenic agent used. In addition, comparative studies involving red blood cells from DM rats and human diabetics revealed a number of common changes.[22] Our results showing renal GPX activity reduction in rats may reflect a similar change in human beings, and represent an important mechanism of renal function deterioration after contrast media injection, especially when ionic high-osmolar contrast media are used.

The relatively high analytical variation of SOD and GPX in this study is probably attributable to unavoidable differences in manual weighing and pipetting of reagents as well as to small variations between kits.

In conclusion, intravenous injection of ionic high-osmolar diatrizoate reduced renal GPX activity during the first hour in both normal and STZ-induced DM rats. Neither ionic low-osmolar ioxaglate nor nonionic low-osmolar iopromide injection changed renal GPX or SOD activities. The heart tissue of normal and DM rats were not prone to antioxidant enzyme activity changes after intravenous contrast media injection. These results suggest that GPX activity reduction is an important mechanism of contrast media-induced nephrotoxicity, especially that which occurs when using ionic high-osmolar contrast media.

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