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Acute effect of ionic high osmolar contrast medium on renal antioxidant enzyme activity in streptozotocin-induced diabetic rats

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Abstract Acute contrast medium-induced nephrotoxicity was estimated in 3%–12% of patients receiving cardiac angiography, especially in advanced age, renal insufficiency and diabetic patients. As intrinsic renal antioxidant enzyme activities may play a crucial role in defence against renal oxidant injury, this study was designed to investigate the acute effect of ionic high osmolar diatrizoate meglumine/diatrizoate sodium on renal antioxidant activities in normal or streptozotocin (STZ)-induced diabetic rats at two time points (1 h and 24 h). A total of 40 Wistar rats were separated to normal and STZ-induced diabetic groups. Ten of each group were injected with diatrizoate (10 ml/kg) via tail vein and 10 with 10 ml/kg of 0.9% NaCl as control. This study shows that diabetic rats had higher renal glutathione peroxidase (GPx) activities than those of normal rats. GPx activities decreased significantly after diatrizoate injection at the first hour (717.4±104.0 to 578.6±92.1 mU/mg in the diabetic group, 466.4±30.6 to 371.4±75.5 mU/mg in the normal group, all P=0.032) but the difference faded 24 h later. The increase of superoxide dismutase (SOD) activities was enhanced (673.5±100.2 to 750.4±129.8 U/mg, P=0.04) in the normal group, but not in the diabetic group (624.1±156.6 to 671.1±136.7 U/mg, P=0.15) after diatrizoate injection at the first hour. At 24 h, renal SOD activities were still significantly higher in the diatrizoate injection group. In summary, intrinsic renal antioxidant activities are adapted in STZ-induced diabetes and ionic high osmolar diatrizoate could modify their activities. Furthermore, diabetics have abnormal response of renal antioxidant activities by contrast media and are at risk for contrast-mediated nephrotoxicity.

Key words Diabetes • Diatrizoate • Nephrotoxicity • Glutathione peroxidase • Superoxide dismutase

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Introduction

Acute renal failure could be induced by contrast media. It is estimated that 3%–12% of patients suffer from nephrotoxicity after cardiac angiography [1–3]. Patients at high risks, such as those with diabetes, previous renal insufficiency or dehydration are even more easily insulted by contrast medium. Several mechanisms are thought to explain this phenomenon, such as renal vasoconstriction, decreased blood flow, decreased glomerular filtration rate and direct renal cell toxicity [4–6]. Free oxygen radical production from reduction-oxidation reactions is one of the important events of pathogenesis of tissue injury. It is known that reactive oxygen species will be generated from the formation of advanced glycation end-products in diabetes. It is clinically correlated with an increased incidence of nephropathy [7]. These reactive oxygen species are normally scavenged by a defence system of intracellular antioxidant enzymes, such as catalase, superoxide dismutase (SOD) and glutathione

peroxidase (GPx) [8]. However, when the production of reactive oxygen species exceeds the scavenge capacity of antioxidant enzymes, tissue damage may occur. Reduced activity of antioxidant enzymes was illustrated in dehydrated animals after ionic high osmolar contrast medium injection [9]. However, the change of intrinsic renal antioxidant enzyme activity under the influence of ionic high osmolar contrast medium like diatrizoate has not yet been reported in diabetes. As intrinsic antioxidant enzyme activities may play a role in defence against renal injury, we hypothesise that there will be a difference in antioxidant enzyme activities in diabetics as compared with normal subjects.

Materials and methods

Type of contrast media

Diatrizoate meglumine/diatrizoate sodium (DTZ, Hypaque-75; Squibb, New Brunswick, NJ, USA) is an ionic, high osmolar contrast medium. The osmolality is 2016 mOsm/kg and its pH is 7.0–7.6. The iodine load (3700 mg/kg) of DTZ is calculated to be 10 ml/kg for injection. This dose was reported to induce renal injury in animal models. Normal saline (0.9% NaCl) was used as a control.

Study animal groups

Male Wistar rats (purchased from National Laboratory Animal Breeding and Research Center, Taiwan) around 6–8 weeks old, weighing 150–180 g, were used to create diabetic rat model. The experimental animals were induced by a single peritoneal injection of 55 mg/kg of streptozotocin (STZ) (Sigma Chemical, St. Louis, MO, USA). Diabetes was confirmed by checking blood glucose (Accutrend Glucose, Boehringer Mannheim, East Sussex, UK). Insulin was injected peritoneally according to blood glucose, which was maintained at around 350 mg/dl. These animals were maintained on a regular diet and water *ad libitum* for 4 weeks after the injection of STZ. A total of 40 male Wistar rats were equally separated into two groups: normal and STZ-induced diabetic groups. Ten of each group were injected with 10 ml/kg of DTZ via tail vein and 10 with 10 ml/kg of 0.9%NaCl as controls. Five of each group were sacrificed at one hour and the other five at 24 h after DTZ or normal saline injection. These rats were anaesthetised with sodium pentobarbital (Abbott Laboratories, Chicago, IL, USA). This study was approved by the Animal Care and Treatment Committee of our institution.

Antioxidant enzyme assay

The dissected kidney was soon rinsed in ice-cold phosphate-buffered saline (PBS). The clean specimens were chipped and homogenised in an appropriate buffer solution, which contained 50 mM Tris-HCl at pH 7.5, 5 mM EDTA and 1 mM 2-mercaptoethanol. GPx activity was assayed in a 1.5-ml cuvette containing 350 µl of assay buffer (GPx-340, OxisResearch, USA), 350 µl of NADPH reagent and 70 µl of test sample that was prepared and diluted into assay buffer just prior to analysis. Then, the whole mixture was added to 350 µl of diluted tert-butyl hydroperoxide and mixed. The absorbance was read at 340 nm and the change was recorded for three minutes. SOD activity was measured with an assay kit (SOD-525, OxisResearch, USA) by the suggested procedure, summarised as follows: 30 µl of sample was incubated with 500 µl 10 mM hydrogen peroxide for one minute and finally chromogen reagent. The absorbance of samples was recorded at 525 nm. All the activities of GPx or SOD were normalised after correction of sample protein content. All the measures were repeated three times with intra-variation less than 8%.

Statistical analysis

All values were expressed as the mean±SEM. Mann-Whitney *U*-test was performed to compare difference between groups and $P<0.05$, two-tailed was considered to be significant.

Results

A total of 40 male Wistar rats were used for study. Twenty diabetic rats had mean body weight of 254.5±43.8 g, which is 23% lower than that of the twenty normal rats. Mean body weight of 20 normal rats was 330.5±42.5g. Mean blood sugar of diabetic rats and normal rats before sacrifice was around 390±60.4 mg/dl and 103.2±17.4 mg/dl respectively.

Diabetic rats had higher renal GPx activities than those of normal rats (717.4±104.0 *vs*. 466.4±30.6 mU/mg at first hour, 622.9±114.4 *vs*. 494.6±60.8 mU/mg at 24th hour after normal saline injection, all $P<0.0001$) (Fig. 1). GPx

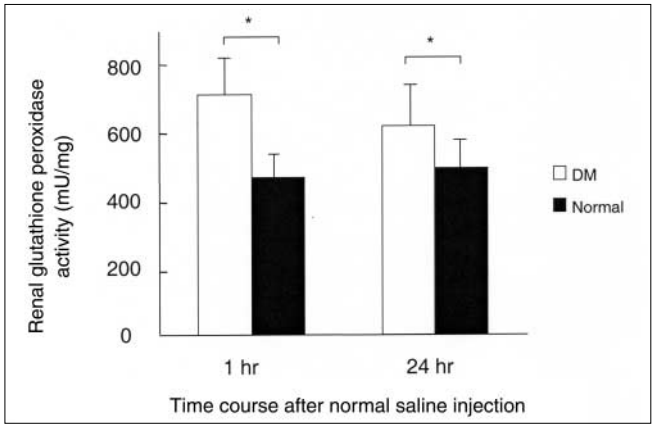

Fig. 1 Comparison of renal glutathione peroxidase activity between STZ-induced diabetic and normal rats at 1 and 24 h after normal saline injection. Increased renal GPx activities are noted in diabetic rats as compared to those of normal rats. *$P<0.0001$. (Open bar denotes diabetic group. Solid bar denotes normal group, $n=5$)

activity decreased significantly after DTZ injection in both groups at the first hour (717.4±104.0 to 578.6±92.1 mU/mg in diabetic group, 466.4±30.6 to 371.4±75.5 mU/mg in normal group, all P=0.032) (Fig. 2). We observed that GPx activities at 24 h after DTZ had no significant change as compared to those after normal saline injection in both groups (652.7±154.9 *vs*. 622.9±114.4 mU/mg in diabetic group, 434.5±64.3 *vs*. 494.6±60.8 mU/mg in normal group, all P=0.22).

Contrary to renal GPx increase, renal SOD activity had the tendency to be decreased in the diabetic group as compared to that of the normal group at the 1st hour after normal saline injection (624.1±156.6 *vs*. 673.5±100.2 U/mg), although the P value was 0.12. Renal SOD activity remained lower in diabetics than in the normal group (500.9±55.1 to 590.4±51.9 U/mg, P=0.01) at 24 h after saline injection (Fig. 3). In order to recognise the different

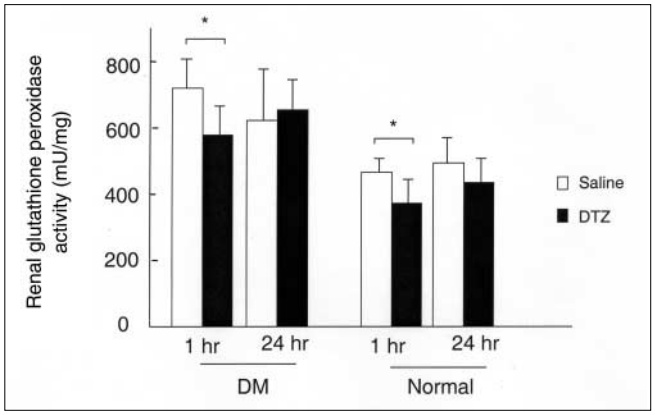

Fig. 2 Effect of diatrizoate on renal GPx activities in both diabetic and normal rats. Decreased renal GPx activities are noted in both diabetic and normal rats at 1st hour after diatrizoate injection. *P=0.032. There is no significant difference of renal GPx at 24th hour as compared to 1st hour. P=0.22. (Open bar denotes normal saline injection as control. Solid bar denotes diatrizoate injection. n=5)

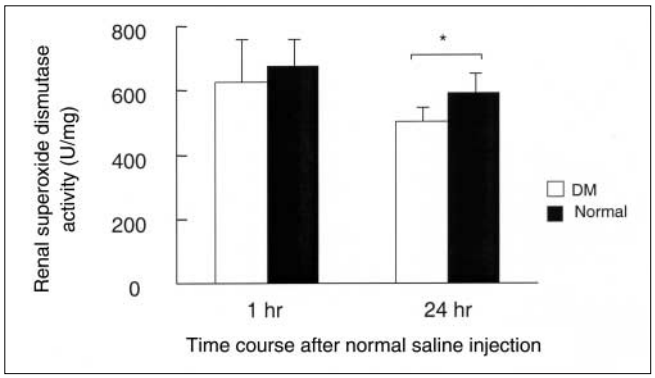

Fig. 3 Comparison of renal superoxide dismutase activity between STZ-induced diabetic and normal group at 1 and 24 h after normal saline injection. Diabetic group has decreased renal SOD activity as compared to normal group but it did not reach statistical significance at 1st hour. (Open bar denotes diabetic group. Solid bar denotes normal group)

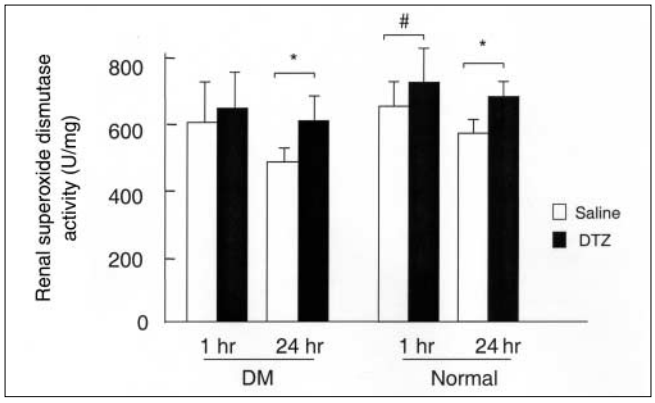

Fig. 4 Effect of diatrizoate on renal SOD activities in both diabetic and normal rats. Increased renal SOD activities are noted in both diabetic and normal rats at 1st hour after diatrizoate injection but P=0.043 in normal group, P=0.15 in diabetic group. Renal SOD at 24th hour after diatrizoate injection is significantly increased as compared to that after normal saline injection, all *P<0.05. (Open bar denotes normal saline injection as control. Solid bar denotes diatrizoate injection)

influence of diatrizoate on renal SOD activity, we measured and compared this activity with normal saline in both groups. At the first hour after diatrizoate injection, renal SOD activity was elevated insignificantly in the diabetic group as compared to saline injection (671.1±136.7 *vs*. 624.1±156.6 U/mg, P=0.15). However, in normal groups, the elevation was statistically significant (750.4±129.8 *vs*. 673.5±100.2 U/mg, P=0.043). Twenty four hours after diatrizoate, SOD activities were still high as compared to those after normal saline injection (706.3±86.5 *vs*. 590.4±51.9 U/mg, P=0.01, in normal group and 631.2±58.4 *vs*. 500.9±55.1 U/mg, P=0.032, in diabetic group) (Fig. 4).

Discussion

The present study shows that intrinsic renal cellular GPx activities in STZ-induced diabetic rats are significantly higher than those of normal rats. It is well known that cellular GPx is an important enzyme to catalyse the reduction of hydrogen peroxide and lipid peroxides to water and lipid alcohol, respectively. One of the obvious manifestations of diabetes is hyperglycaemia, which could promote the formation of glycation or non-enzymatic glycosylation of proteins and lipoproteins by the Maillard reaction. The formation of advanced glycation end-products will produce reactive oxygen species in diabetes [10, 11]. High levels of GPx suggest it may result from chronic upset of oxidant stress in diabetes. It is reasonable to expect that cellular GPx prevents cells from oxidant injury [12, 13]. From experiments of GPx gene deficiency, it further confirms that this enzyme reveals its cellular protection in many tissues [14–16]. Besides, antioxidant states are apparently altered in diabetic

tissues at different ages [17–19]. Thus, the increased activity of renal GPx in diabetes could be the compensatory or adaptation mechanism for renal cells to resist the increased oxidant stress in a hyperglycaemic situation.

As we know, ionic high osmolar contrast medium, like diatrizoate, has the highest toxicity to renal cells. There is a high incidence of acute renal failure caused by diatrizoate. Many mechanisms have been proposed to explain renal function deterioration, including cellular energy failure, a disruption of calcium homeostasis, a disturbance of tubular cell polarity, renal medullary hypoxia and programmed cell death [20, 21]. Reactive oxygen species generated by contrast media also play a role in acute nephropathy [21–24]. Meanwhile, much evidence shows that exogenous antioxidants N-acetylcysteine could reduce the incidence of contrast medium-induced nephrotoxicity [25, 26]. However, the modulation of endogenous antioxidant activity by diatrizoate is not entirely clear in diabetes. In our study, we disclose that renal GPx activities were significantly depressed by diatrizoate at the 1st hour both in diabetic and normal groups and the decreased GPx gradually recovered at 24 h also in both groups. We observe that the influence of diatrizoate on diabetic rats is not different from normal rats. The depressed GPx activity by diatrizoate may indicate the vulnerability of renal cellular toxicity. Yoshioka et al. showed the important protection effect of GPx on renal function in a post-ischaemic model. They demonstrated that renal GPx activity was depressed 3 days after ischaemia but rose up to around 2-fold at 6 days. The higher renal GPx activity would protect from developing proteinuria even when hydrogen peroxide was directly infused into renal artery at 6 days [27]. Renal GPx activity reduced by diatrizoate in our study could be potentially harmful to renal function.

In contrast to elevation of renal GPx, baseline renal SOD activity in the diabetic group was lower than that of the normal group, although it did not reach statistical significance. This change of activity may also reflex chronic adaptation of the antioxidative system in defence against reactive oxygen species in diabetes [19]. When we compared the SOD activities after diatrizoate to those after normal saline, we found that SOD activities were significantly elevated in the normal group at the 1st hour after diatrizoate but there was no significant increase in diabetic group. This implies some inertial response of diabetic renal SOD activity to acute insult. Diatrizoate does not suppress the intrinsic renal SOD activity. Interestingly, SOD activities at 24 h after diatrizoate remained higher than those after normal saline injection in both groups. These results further suggest that normal rats preserve this intrinsic antioxidant activity with faster and more persistent response to exogenous insult than diabetes do. In this study, we did not directly measure renal superoxide or reactive radical metabolites produced by diatrizoate. However, it is reasonable to suppose superoxide could be the immediate insult to impair renal function. Bakris et al. demonstrated that allopurinol and SOD infusion could protect glomerular filtration rate and decrease renal venous malondialdehyde within 30 min after diatrizoate injection [23]. Increased renal SOD after DTZ in our study may point out the important defence role of antioxidant enzyme in normal rats. In contrary to normal response in normal rats, the abnormal response in diabetes indicates the risk of renal toxicity.

Our data show that there is reduced renal SOD activity at 24 h after normal saline or contrast medium injection both in diabetic and normal rats. It is unclear whether psychological stress or anaesthesia agent caused the acute change of renal SOD. Zimmermann et al. studied the antioxidant status of blood in acute stroke patients and demonstrated that acute stroke would lead to GPx elevation in the first day but no significant differences of SOD [28]. However, Cherubini et al. demonstrated the immediately reduced antioxidants after an acute ischaemic stroke [29]. The patients with lower erythrocyte SOD activity had the worst early outcomes. Besides, chronic psychological stress was also associated with altered antioxidative status in diabetic rats that revealed low catalase and high thiobarbituric acid reactive substance in kidney [30]. In the present study, renal SOD activities in diabetic rats fall much lower than those in normal rats at 24 h. The same phenomenon could also be observed after diatrizoate. Accordingly, psychological or physical stress seems to involve the modulation of intrinsic antioxidant activity. As we did not perform the experiment for longer, we could not study the chronologic change of antioxidant activities of GPx and SOD. However, this study implicates that the intrinsic renal SOD activities in diabetes present an abnormal response caused by diatrizoate and diabetics are at risk for contrast-mediated nephrotoxicity.

In summary, intrinsic renal antioxidant enzyme activities to defend against reactive oxygen species are altered with obviously increased renal GPx activity in STZ-induced diabetic rats. Acute modulation of antioxidant activity by diatrizoate is quite different. Diatrizoate suppresses renal GPx and induces renal SOD activities. Furthermore, diabetics have a relatively abnormal response of renal antioxidant SOD activity to diatrizoate and are at risk of contrast-induced nephrotoxicity.

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