JNEPHROL 2011; 24(03): 376-380

DOI:10.5301/JN.2010.5784

Ionic contrast media induced more apoptosis in diabetic kidney than nonionic contrast media

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Abstract

Background: **Contrast-induced nephropathy is a major cause of hospital-acquired acute renal failure, and its risk is significantly increased in patients with diabetes mellitus. This study aimed to examine both the role of apoptosis in low-osmolar contrast media–induced kidney injury in normal and diabetic rats and the difference in the induced kidney injury between ionic and nonionic contrast media.**

Methods: **Normal and streptozotocin-induced diabetic Wistar rats were administered with ionic low-osmolar ioxaglate, nonionic low-osmolar iopromide or normal saline injection. Apoptosis in kidney tubular cells was determined by the presence of positive terminal deoxynucleotidyl transferase–mediated dUTP in situ nick end-labeling (TUNEL) stain.**

Results: **At 24 hours after administration, both ioxaglate and iopromide injections induced more apoptosis in diabetic (49.7% vs. 25.3% for ioxaglate; 37.7% vs. 25.3% for iopromide; both p<0.001) and normal (36.2% vs. 27.4%, p=0.002, for ioxaglate; 33.6% vs. 27.4%, p=0.029, for iopromide) kidney tubular cells than normal saline injections. Additionally, ioxaglate induced more apoptotic tubular cells in diabetic kidneys than in normal kidneys (p<0.001). Moreover, ioxaglate significantly induced more apoptotic cells than iopromide in diabetic kidneys, but not in normal kidneys (p<0.001, for diabetic rats; p=0.345, for normal rats).**

Conclusion: **Ionic low-osmolar contrast media induced more apoptosis in tubular cells in diabetic kidneys than in normal kidneys. Notably, ionic contrast media induced more apoptosis than nonionic contrast media in diabetic kidneys.**

Key words: *Apoptosis, ATF2, Contrast media, Diabetes mellitus, Nephropathy*

Introduction

Contrast media–induced nephropathy (CIN) is associated with increased mortality and morbidity in patients receiving coronary angiography and intervention (1-3). The incidence of CIN is the third leading cause of hospital-acquired acute renal failure, and for high-risk patients, the risk of CIN can be as high as 50% (4, 5). Diabetes mellitus is one of the most important risk factors for CIN (6-9). Additionally, diabetic patients with CIN had a significantly decreased survival rate compared with nondiabetic patients over a 2-year follow-up (7).

Although the mechanisms of CIN remain unclear (10), reactive oxygen species generation and apoptosis are recognized as the most likely mechanisms (11-14). Our previous study demonstrated that oxidative stress is induced in the rat kidney by contrast media administration (15). Additionally, we demonstrated that a histone acetyltransferase, activating transcriptional factor 2 (ATF2), plays a pivotal role in contrast media–induced cytotoxicity (apoptosis), and ionic high-osmolar contrast media and ionic low-osmolar contrast media induce more ATF2 expression in kidney cell lines than nonionic low-osmolar contrast media (16). Diabetic patients have a higher prevalence of CIN than nondiabetic patients (6-9). However, the difference in the induction of apoptosis in the kidney by ionic and nonionic low-osmolar contrast media between diabetic subjects and nondiabetic subjects remains unclear. Accordingly, this study tested the hypothesis that ionic contrast media could induce more apoptosis in a diabetic rat kidney than in a normal rat kidney. This study compared the differences in the induction of apoptosis between diabetic rats and normal rats after ionic and nonionic low-osmolar contrast media administration. Additionally, this study examined the expression of ATF2 in the diabetic rat kidney and the nor-

mal rat kidney after ionic and nonionic low-osmolar contrast media administration.

Materials and methods

Contrast media

Two contrast media were used: ionic low-osmolar ioxaglate (Hexabrix-320; Guerbet, Aulnay-sous-Bois, France; osmolality 600 mOsm/kg, iodine content 320 mg/mL) and nonionic low-osmolar iopromide (Ultravist-370; Schering AG, Berlin, Germany; osmolality 880 mOsm/kg, iodine content 370 mg/mL).

Animal studies

This animal study was approved by the Animal Care and Treatment Committee of our institution. All animals received humane care in compliance with the "Guide for the Care and Use of Laboratory Animals" prepared by the Institute of Laboratory Animal Research, Commission on Life Sciences, National Research Council, and published by National Academy Press (in 1996). Twenty-eight male Wistar rats (National Laboratory Animal Breeding and Research Center, National Science Council, Nankang, Taiwan), 6-8 weeks old and weighing 150-180 g, were used. Diabetes mellitus was induced by a single peritoneal injection of 55 mg/kg streptozotocin (Sigma Chemical, St. Louis, MO, USA). Diabetes mellitus was confirmed by checking blood glucose (Precision Plus; Abbott Laboratories, North Chicago, IL, USA). After successful induction of diabetes mellitus, the blood glucose level was greater than 200 mg/dL within 24-36 hours after streptozotocin injection. If the blood glucose of the rats was below 200 mg/dL, another dose of 55 mg/kg streptozotocin was injected. The blood glucose of diabetic rats was monitored daily, and insulin (Monotard HM; Novo Nordisk, Copenhagen, Denmark) was injected subcutaneously to maintain a blood glucose level of around 350 mg/dL. All rats were fed with a regular diet and water until 4 weeks after the day of streptozotocin injection. All rats fasted for 24 hours prior to this in vivo experiment. Normal and streptozotocin-induced diabetic rats were each separated into 3 groups and administered with single injections of ioxaglate (11.6 ml/kg) (normal group, n=5; diabetes group, n=5), iopromide (10 ml/kg) (normal group, n=4; diabetes group, n=5) or normal saline (10 ml/kg) (normal group, n=5; diabetes group, n=4) via tail veins. At 24 hours after injection, the rats were anesthetized with pentobarbital sodium (Abbott Laboratories, North Chicago, IL, USA) and sacrificed for dissection. The kidneys were harvested and dissected into pieces, which were then stored at -80°C or fixed in 4% buffered formalin until study.

Quantitative real-time PCR to analyze the expression of ATF2 gene

Total RNA was the isolated from homogenized kidney tissue using an RNeasy kit (Qiagen, Germantown, MD, USA) according to instructions of the manufacturer. The cDNA was synthesized from total RNA with transcriptor reverse transcriptase (Roche Applied Science, Indianapolis, IN, USA) using oligo (dT) primers. The mRNA expression levels in the kidney tissue were quantified by real-time polymerase chain reaction (PCR) using a LightCycler (Roche Applied Science, Indianapolis, IN, USA) with the universal probe system. Melting curves were acquired, and the mRNA levels were normalized to relative amounts of hydroxymethylbilane synthase (HMBS). The following primers were used: ATF2 forward, 5'-CTGGTGGCTGAAAGGAACAT-3'; ATF2 reverse, 5'-TCCCAAGTTGCCATCTAGTGT-3'; HMBS forward, 5'-TCCCTGAAGGATGTGCCTAC-3'; HMBS reverse, 5'-ACAAGGGTTTTCCCGTTTG-3'.

TUNEL assay

Kidney tissues fixed in 4% buffered formalin were used for the terminal deoxynucleotidyl transferase–mediated dUTP in situ nick end-labeling (TUNEL) assay. Samples were initially deparaffinized. Sections of 5 μm were then postfixed in a pre-cooled ethanol:acetic acid (2:1 v/v) solution for 5 minutes. Proteinase K (Roche, Applied Sciences, Mannheim, Germany) was applied for 2 minute using an In Situ Cell Death Detection Kit, POD (Roche Applied Sciences, Mannheim, Germany) according to the manufacturer's protocol. Chromogen diaminobenzidine (Sigma, St. Louis, MO, USA) was utilized to visualize TUNEL staining with hematoxylin used as a counterstain. Positive controls were obtained by incubating both fixed and permeabilized sections with DNAse I (Roche Applied Sciences, Mannheim, Germany). Negative controls included both the omission of terminal deoxynucleotidyl transferase labeling reaction mixture and terminal deoxynucleotidyl transferase to eliminate the potential inappropriate inclusion of nucleotides. Only stained kidney tubular cells with dark brown nuclei, in addition to nuclear condensation, were considered to be apoptotic. All cells were counted using a ×40 objective microscope in 4 different views per section. The TUNEL index was defined as the TUNEL-positive cells/total cells per high-power microscopy field × 100.

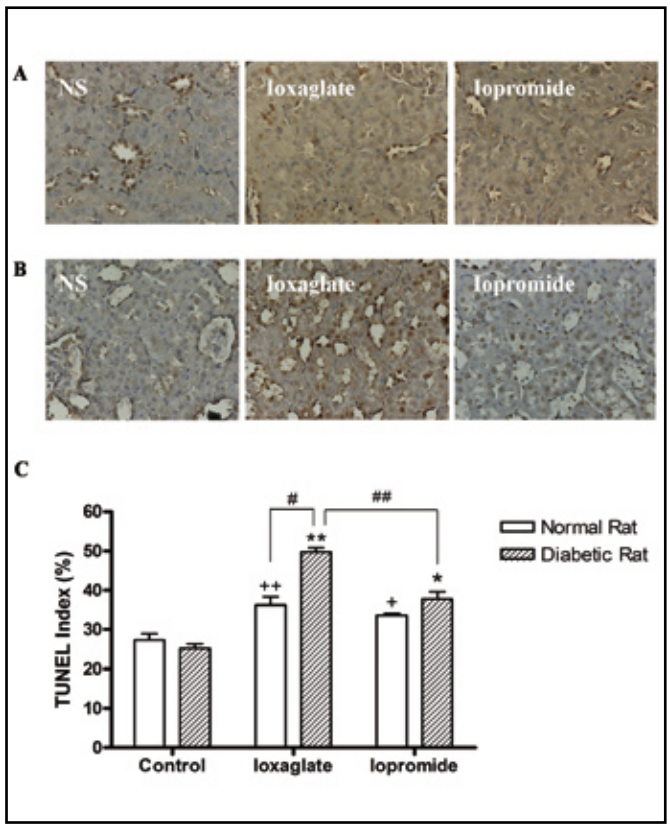

Fig. 1 - Histopathological examination (magnification ×400) of normal rat kidney (A) and diabetic rat kidney (B) after normal saline (NS), ioxaglate and iopromide treatment. Apoptosis is characterized by positive TUNEL staining. In normal saline-treatment group (control), there is no difference in the TUNEL index between normal and diabetic rat kidneys. Ioxaglate-treated group exhibits more TUNEL-positive cells in normal rat kidney than normal saline-treated group ($^{*}p=0.002$) (A, C). Iopromide-treated group also exhibits more TUNEL-positive cells in normal rat kidney than normal saline-treated group ($^{**}p=0.029$) (A, C). In diabetic rats, ioxaglate-treated and iopromide-treated groups exhibit more TUNEL-positive cells in rat kidney than normal saline-treated group ($^{+}p<0.001$ and $^{++}p<0.001$, respectively) (B, C). Ioxaglate treatment ($^{\#}p<0.001$), but not iopromide treatment (p=0.141), induced more TUNEL-positive cells in diabetic kidneys than in normal kidneys (C). Notably, ioxaglate significantly induced more TUNEL-positive cells than iopromide only in diabetic rats ($^{\#\#}p<0.001$) (C).

Statistical analysis

Data are expressed as means ± SEM. Continuous variables among groups were analyzed by 1-way ANOVA followed by the least-significant difference procedure. Statistical analyses were performed using SPSS for Windows, version 17 (SPSS Inc., Chicago, IL, USA). The level of statistical significance was set as a p value <0.05.

Results

Ioxaglate and iopromide treatment induced apoptosis in normal and diabetic rat kidneys

Normal Wistar rats and diabetic rats were treated with intravenous injections of ioxaglate, iopromide and normal saline. Apoptosis was characterized by positive TUNEL staining. In the normal saline-treatment group, there was no difference in the expression of apoptosis between normal and diabetic rats (Fig. 1). In normal rats, ioxaglate-treated kidney tubular cells exhibited more TUNEL-positive cells at 24 hours after intravenous injection than did normal saline-treated kidney tubular cells (36.2% ± 2.6% vs. 27.4% ± 1.6%, p=0.002) (Fig. 1). Similarly, iopromide-treated kidney tubular cells exhibited more TUNEL-positive cells at 24 hours after intravenous injection than did normal saline-treated kidney tubular cells in normal rats (33.6% ± 0.6% vs. 27.4% ± 1.6%, p=0.03) (Fig. 1). In diabetic rats, ioxaglate-treated and iopromide-treated kidney tubular cells exhibited more TUNEL-positive cells than did normal saline-treated kidney tubular cells (49.7% ± 1.2% and 37.7% ± 2.4 vs. 25.3% ± 1.1%, respectively; both p<0.001). Ioxaglate induced more TUNEL-positive cells in diabetic kidneys than in normal kidneys (p<0.001). However, iopromide did not induce more TUNEL-positive cells in diabetic kidneys than in normal kidneys (p=0.14). Moreover, ioxaglate significantly induced more TUNEL-positive tubular cells than iopromide in diabetic kidneys, but not in normal kidneys (p<0.001, for diabetic rats; p=0.35, for normal rats) (Fig. 1).

Ioxaglate and iopromide treatment induced ATF2 expression in kidneys

The ATF2 mRNA expression in homogenized kidney tissue at 24 hours after intravenous injection was measured. The values of relative amount of transcripts were derived by normalization to the mRNA level of normal rats with normal saline injection. Diabetic rats had higher ATF2 expression than normal rats in the normal saline-treated group (1.23 ± 0.2 in diabetic kidney, vs. 1.0 in normal kidney), although the difference did not reach statistical significance. Ioxaglate induced increased ATF2 expression in the kidney compared with normal saline, especially in the diabetic kidney, although the difference did not reach statistical significance (1.52 ± 0.42 vs. 1.0 in normal kidney, and 1.89 ± 0.69 vs. 1.23 ± 0.2 in diabetic kidney), although the difference did not reach statistical significance.

Discussion

This study examined the impact of low-osmolar iodinated contrast media on apoptosis and ATF2 expression in normal and diabetic kidneys. Several important conclusions were obtained from the study. Firstly, intravenous administered low-osmolar contrast media can significantly induce apoptosis in normal and diabetic kidneys. Secondly, ionic low-osmolar contrast media induced more apoptosis in diabetic kidneys than in normal kidneys. Thirdly, ionic contrast media induce more apoptosis in diabetic kidneys than do nonionic contrast media. Finally, there is no difference in the expression of apoptosis in normal rat kidney between ionic and nonionic contrast media treatment.

Organizations representing the disciplines of cardiology, nephrology and radiology have addressed formal recommendations in their practice guidelines on reducing contrast-induced nephropathy (17). The American Heart Association and American College of Cardiology, in their joint guidelines for the management of patients with unstable angina or non-ST elevation myocardial infarction and percutaneous coronary intervention, have specified that iso-osmolar contrast agents are indicated and preferred in chronic kidney disease patients receiving angiography (18, 19). The American College of Radiology and the European Society of Urogenital Radiology guidelines also recommend the use of low-osmolar or iso-osmolar contrast media rather than high-osmolar contrast media (20, 21). All of these guidelines have highlighted the importance of choosing low-osmolar and iso-osmolar contrast media for the prevention of CIN in high-risk patients. However, the issue of choosing ionic or nonionic low-osmolar contrast media had never been mentioned. This study showed that ionic contrast media induced more apoptosis in diabetic kidneys than nonionic contrast media. Accordingly, we recommend that nonionic low-osmolar contrast media should be preferred for patients with diabetes mellitus.

Our previous in vitro study showed that contrast media can activate the JNK/ATF2 pathway. ATF2 mRNA expression significantly increased in kidney cell lines after ionic high-osmolar and ionic low-osmolar contrast media administration, and peaked at 4 hours after contrast media administration. By knockdown ATF2 expression, cell death was significantly increased. The finding indicated an inducible and protective role of ATF2 in contrast media-induced apoptosis (16). In this in vivo study, a single intravenous injection of low-osmolar contrast media could induce increased expression of ATF2 in the kidney. Although the patterns of induced ATF2 expression in diabetic and normal rats were similar to those of the induced apoptosis, the magnitude of induced ATF2 expression in diabetic and normal rats did not reach statistical significance, while in contrast, the magnitude of induced apoptosis in diabetic and normal rats did reach statistical significance. Thus, the induced ATF2 level was not enough to overcome the cytotoxic effects (apoptosis) of contrast media.

In most CIN cases, serum creatinine begins to rise within 24 hours but typically peaks at 2-3 days after contrast exposure. Thus, serum creatinine may not be a sensitive marker for early diagnosis of CIN (22-24). This in vivo study provided evidence that contrast media could significantly induce apoptosis in kidney tubular cells as early as within 24 hours. Therefore, the induced apoptosis by contrast media might precede the significant rise of serum creatinine.

Several study limitations should be addressed. Firstly, this was an animal experiment. Secondly, dose-dependent effects of contrast media on apoptosis in kidney tubular cells were not assessed in this study. Finally, renal function was not measured in this study. However, this should not have any significant impact on our main findings.

Conclusion

This work provides scientific evidence that ionic low-osmolar contrast media induce more apoptosis in diabetic kidneys than in normal kidneys. Notably, ionic low-osmolar contrast media induce more apoptosis in diabetic kidneys than nonionic contrast media. Accordingly, nonionic low-osmolar contrast media rather than ionic low-osmolar contrast media should be indicated and preferred for patients with diabetes mellitus, to reduce the incidence of CIN.

Acknowledgements

The authors would like to thank Dr. Sheau-Fang Yang, MD and Wan-Tzu Chen for completing the TUNEL staining.

Financial support: This study was supported by a grant from the Kaohsiung Medical University Hospital (KMUH96-6R17).

Conflict of interest statement: None declared.

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Accepted: June 11, 2010