JNEPHROL 2012; 25(01):56-62

DOI:10.5301/JN.2011.7741

Impact of short-duration administration of N-acetylcysteine, probucol and ascorbic acid on contrast-induced cytotoxicity

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Abstract

Background: **The best pharmaceutical prevention of contrast-medium-induced nephropathy for emergency procedures remains unknown. The aim of this study was to examine the impact of short-duration antioxidant pretreatment on contrast-medium-induced cytotoxicity.**

Methods: **Human embryonic kidney cells were treated with three different contrast media: ionic ioxitalamate, non-ionic low-osmolar iopromide, and iso-osmolar iodixanol. The doses and durations of pretreatment with antioxidants were 2 mM/L N-acetylcysteine for 15 minutes, 40 μM/L probucol for 30 minutes, and 30 μM/L ascorbic acid for 30 minutes. A supplementary dose of 2 mM/L N-acetylcysteine was administered 12 hours after contrast medium treatment. Cell viability was determined by tetrazolium MTT assay.**

Results: **All three contrast media caused significant reduction of cell viability at 24 hours ($p<0.001$). In the groups receiving iopromide or iodixanol, N-acetylcysteine pretreatment significantly improved cell viability compared with no N-acetylcysteine pretreatment ($p<0.001$). In the group receiving ioxitalamate, N-acetylcysteine pretreatment followed by a supplementary dose of N-acetylcysteine at 12 hours rather than N-acetylcysteine pretreatment alone significantly improved cell viability compared with no N-acetylcysteine pretreatment ($p=0.038$). Probucol or ascorbic acid pretreatment was unable to reduce cell death caused by the three contrast media.**

Conclusions: **Short-duration pretreatment with N-acetylcysteine significantly reduced contrast-medium-induced cytotoxicity. These findings provide new insight into the prevention of contrast-medium-induced nephropathy in clinical emergency scenarios.**

Key words: *Antioxidant, Ascorbid acid, Contrast-induced cytotoxicity, N-acetylcysteine, Probucol*

Introduction

Ever since the widespread use of contrast media for diagnostic and interventional procedures, contrast-medium-induced nephropathy (CIN) has been a major cause of iatrogenic renal failure (1-4). The development of CIN increases

the short- and long-term mortality of patients undergoing percutaneous coronary interventions (5, 6). Prevention of CIN is therefore important to reduce substantial morbidity, and even mortality.

Contrast-medium-induced cytotoxicity and reactive oxygen species are the major known mechanisms of CIN (7-12). Volume expansion by saline infusion before the administration of contrast media is the most widely used strategy for its prevention (13, 14). However in some diseases, such as severe congestive heart failure, oliguric renal failure, or end-stage renal failure, patients are unable to tolerate large amounts of saline infusion. For these patients, pharmaceutical treatment plays a pivotal role in the prevention of CIN.

N-acetylcysteine (NAC), which acts as a potent antioxidant either directly by scavenging reactive oxygen species or indirectly through production of glutathione, can prevent oxidative tissue damage (15). Probucol is also a potent antioxidant and has been used to protect the kidney from progressive diabetic nephropathy and gentamicin-induced nephrotoxicity (16). Our previous animal study showed the renal protective effects of pretreatment with NAC and probucol against contrast-induced oxidative stress (17). Ascorbic acid is an antioxidant vitamin capable of scavenging oxygen-derived free radicals (18, 19).

A large number of studies and several meta-analyses have shown the value of prophylactic use of NAC (20). CIN was found to be significantly reduced when 600 mg NAC was given orally 24 hours before the administration of iopromide compared with hydration only (21). The optimal dose, the allowable time and frequency of administration of NAC remain unclear, especially in emergency scenarios, which is a practical issue.

This in vitro study tested the hypothesis that a single dose of antioxidant 15 minutes or 30 minutes before contrast medium administration may have a protective effect against contrast-medium-induced cytotoxicity. We examined the effects of pretreatment with NAC 15 minutes, probucol 30 minutes, and ascorbic acid 30 minutes before the administration of different contrast media on kidney cell viability. These experimental findings should provide new insights into the prevention of CIN in clinical emergency scenarios.

Subjects and methods

Culturing 293T cells and antioxidant pretreatment

Human embryonic kidney 293T cells were maintained at 37°C in Dulbecco's Modified Eagle Medium supplemented with 10% fetal bovine serum and 1% glutamine in a water-saturated atmosphere of 95% O_2 and 5% CO_2. The cells were administered 1 of the 3 antioxidants: 2 mM/L NAC (Flumucil; Zambon, Bresso, Milan, Italy) for 15 minutes, 40 μM/L probucol (Sigma, Saint Louis, MO, USA) for 30 minutes, and 30 μM/L ascorbic acid (Sigma) for 30 minutes. The doses were selected according to the available data in the clinical setting (22-24). In the supplementary NAC experiment, 2 mM/L NAC was administered 12 hours after contrast medium administration.

Contrast administration

Three different contrast media were used: ionic ioxitalamate (Telebrix-35; Mallinckrodt Inc., Hazelwood, MO, USA); non-ionic low-osmolar iopromide (Ultravist-370; GE Health Care, Cork, Ireland); and non-ionic iso-osmolar iodixanol (Visipaque-320; GE Health Care) (Tab. I). Before administration of the contrast media, the culture media with anti-

TABLE I
CONTRAST MEDIA USED IN THIS STUDY

Contrast agent	Trade name	Type	Iodine concentration (mg I/mL)¶	Osmolality (mOsm/kg)
Ioxitalamate	Telebrix-35	Ionic	350	2130
Iopromide	Ultravist-370	Non-ionic	370	774
Iodixanol	Visipaque	Non-ionic	320	290

¶mg I/mL = milligrams of iodine per milliliter.

oxidants were aspirated as completely as possible. Then the contrast medium was mixed with new culture medium without serum to reach a concentration of 5% for the experiments. The treatment duration for contrast media was 24 hours.

MTT assay of cell viability

Cells prepared in 96-well plates were treated with 5% contrast medium for 24 hours with or without antioxidant pretreatment. Then 20μL thiazolyl blue tetrazolium bromide (MTT) solution (Sigma) was added to each well. After incubation for 3 hours, formazan in 200μL DMSO was mixed thoroughly. The reaction product was measured using a microplate reader (Sunrise; Tecan, Inc., Austria) and reading the absorbance at 570 nm.

Evaluation of apoptotic cells

To investigate contrast-medium-induced apoptosis, cells were treated with 5% contrast medium in the presence or absence of NAC for 24 hours. Cultures were then stained with 10 μM Hoechst 33258 (Sigma) for 10 minutes at room temperature. Three fields randomly selected by fluorescence microscopy (Nikon ECLIPSE TS100; Nikon Corporation, Tokyo, Japan) at 200× magnification were counted in a blind manner. Cell nuclei were homogeneously blue under a wavelength of 350 nm. Cells with densely packed and fragmented chromatin were considered positive for apoptosis. The results were expressed as percentage of Hoechst-positive cells/bright field cells. The total cell count for each experiment was more than 5,000 cells.

Western blot analysis

Cells were lysed in a solution containing 150 mM NaCl, 10 mM Tris-HCl, 5 mM EDTA, 1% Triton X-100, and protease inhibitors (10 μg/mL aprotinin, 1 μg/mL leupeptin, 1 μg/mL pepstatin, and 0.1 mM phenylmethylsulfonyl fluoride) at pH 7.4. Proteins were analyzed by SDS-PAGE as follows: after electrophoresis, the proteins were transferred from the gel onto PVDF membranes. The membranes were blocked with a skim milk solution. Specific primary antibodies used for immunoblotting (anti-caspase-3, anti-Bax, and anti-Bcl2) were purchased from BD Pharmingen (San Diego, CA, USA). Detection was performed using an enhanced chemiluminescence detection method (Millipore Corp., Billerica, MA, USA). Densitometric analysis was done using LabWorks 4.5 Image Acquisition and Analysis Software (Ultra-Violet Products Ltd., Cambridge, UK).

Statistical analysis

Data are expressed as mean ± SD. Continuous variables among the 4 groups were analyzed by 1-way ANOVA followed by the Least Significant Difference test with Bonferroni correction. Statistical analyses were performed using SPSS for Windows, version 17 (SPSS Inc., Chicago, IL, USA). The level of statistical significance was set at $p<0.05$.

Results

The cell viability, determined by tetrazolium MTT assay, of the control and contrast-medium-treated groups with or without antioxidant pretreatment is shown in the Figures. The results of probucol pretreatment and ascorbic acid pretreatment are shown in Figures 1 and 2, respectively. In both experiments, the 3 contrast media caused significant reduction of cell viability at 24 hours (all $p<0.001$). Ioxitalamate significantly caused more cell death than iopromide and iodixanol with or without antioxidant pretreatment. Probucol pretreatment could not reduce the cell death caused by the 3 contrast media. Additionally, probucol pretreatment significantly induced more cell death compared with the control group ($p=0.002$) (Fig. 1). Similarly, ascorbic acid pretreatment did not reduce the cell death caused by the 3

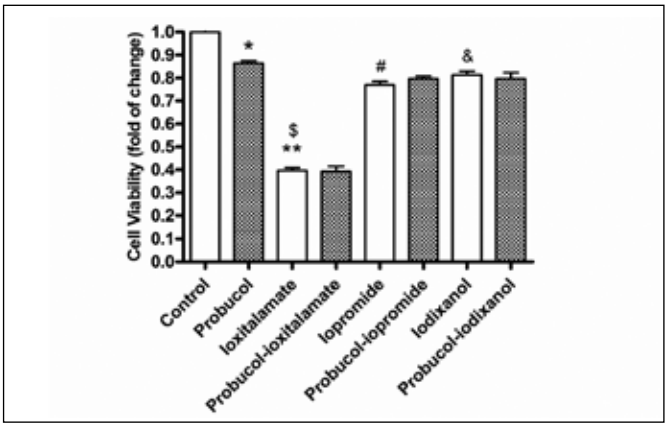

Fig. 1 - Cell viability, determined by tetrazolium MTT assay, after contrast medium treatment with and without pretreatment of probucol. Values of cell viability of control and contrast-medium-treated groups with or without antioxidant pretreatment are demonstrated by folds of control. All 3 contrast media caused significant reduction of cell viability at 24 hours (all $p<0.001$, **ioxitalamate vs. control; #iopromide vs. control; &iodixanol vs. control). Ioxitalamate significantly caused more cell death than iopromide and iodixanol with or without antioxidant pretreatment ($p<0.01, ioxitalamate vs. iopromide and iodixanol). Cell viability after contrast medium treatment was not different between groups with and without probucol pretreatment. Probucol even significantly reduced the cell viability compared with control groups (*p=0.002).

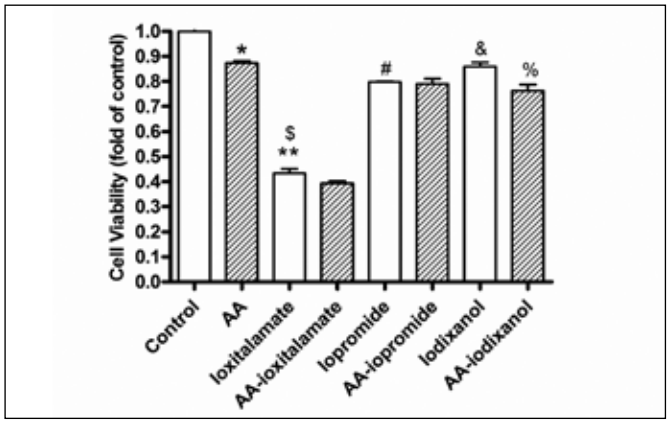

Fig. 2 - Cell viability, determined by tetrazolium MTT assay, after contrast medium treatment with and without pretreatment of ascorbic acid. Values of cell viability of control and contrast-medium-treated groups with or without ascorbic acid (AA) pretreatment are demonstrated by folds of control. All three contrast media caused significant reduction of cell viability at 24 hours (all p<0.001, **ioxitalamate vs. control; #iopromide vs. control; &iodixanol vs. control). Ioxitalamate significantly caused more cell death than iopromide and iodixanol with or without antioxidant pretreatment ($p<0.01). Cell viability after contrast medium treatment was not different between groups with and without pretreatment of ascorbic acid. Ascorbic acid even significantly reduced cell viability compared with control (*p=0.002, AA vs. control) and iodixanol groups (%p=0.022, AA-iodixanol vs. iodixanol).

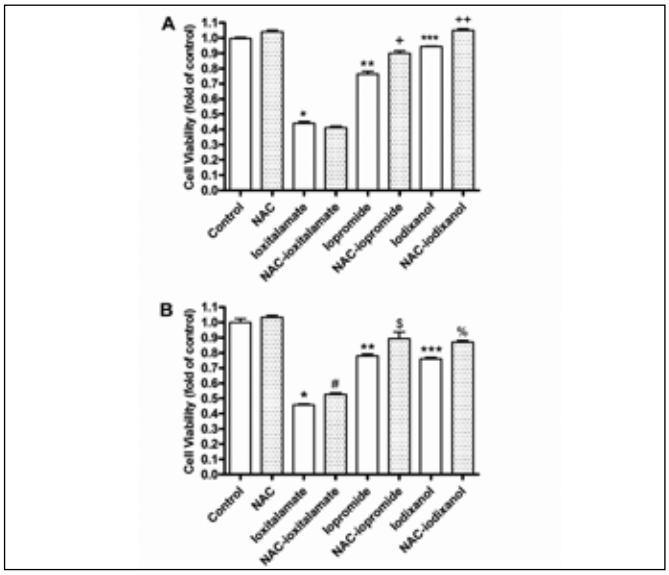

Fig. 3 - Cell viability after contrast medium treatment with or without pretreatment or supplementary dose of N-acetylcysteine (NAC). Values of cell viability of control and contrast-medium-treated groups with or without NAC pretreatment are demonstrated by folds of control. All 3 contrast media caused significant reduction of cell viability at 24 hours (all p<0.001, *ioxitalamate vs. control; **iopromide vs. control; ***iodixanol vs. control). N-acetylcysteine pretreatment significantly improved cell viability after low-osmolar iopromide and iso-osmolar iodixanol treatment (both p<0.001, +NAC-iopromide vs. iopromide; ++NAC-iodixanol vs. iodixanol). However, cell viability after ioxitalamate treatment was not different between groups with or without single-dose NAC pretreatment (p=1) (3A). Interestingly, a supplementary dose of NAC at 12 hours significantly improved cell viability in all 3 contrast medium groups (#p=0.038, NAC-ioxitalamate vs. ioxitalamate; $p<0.001, NAC-iopromide vs. iopromide; %p<0.001, NAC-iodixanol vs. iodixanol) (3B).

contrast media. Moreover, ascorbic acid pretreatment significantly induced more cell death in the control (p=0.002) and iodixanol-treated groups (p=0.022) (Fig. 2).

The impact of NAC pretreatment on contrast-medium-induced cytotoxicity is shown in Figures 3A and 3B. Unlike probucol and ascorbic acid pretreatment, NAC pretreatment did not induce more cell death compared with the control group (p=0.123). In the group treated with low-osmolar iopromide, NAC pretreatment significantly improved cell viability compared with no NAC pretreatment (p<0.001). In the group treated with iso-osmolar iodixanol, NAC pretreatment significantly improved cell viability compared with no NAC pretreatment (p<0.001). However, in the group treated with ionic ioxitalamate, NAC pretreatment did not improve cell viability compared with no NAC pretreatment (p=1) (Fig. 3A).

We then investigated whether 15 minutes' pretreatment followed by a supplementary dose of NAC at the same concentration 12 hours after contrast medium treatment could reduce the cytotoxicity caused by ioxitalamate. Interestingly, in the group treated with ionic ioxitalamate, a supplementary dose of NAC significantly improved cell viability compared with no supplementary dose of NAC (p=0.038) (Fig. 3B).

Hoechst-positive apoptotic cells were identified in the ioxitalamate-treated group and the NAC-pretreated ioxitalamate group but not in the control and NAC-treated groups (Fig. 4A). However, the percentage of these positive cells was less than 1% in the ioxitalamate group and NAC-pretreated ioxitalamate group. Activated caspase-3, Bax, and Bcl2 were used to verify the contrast-induced cell damage. None of the contrast media used in this study at a concentration of 5% over 24 hours induced an increase in the expression of activated caspase-3, Bax, and Bcl2 (Fig. 4B).

Discussion

This study examined the impact of short-duration treatment with different antioxidants including probucol, ascorbic acid, and NAC on contrast-medium-induced cytotoxicity. Several important conclusions can be drawn. Firstly, at the low concentration used in this study (5%) contrast media, even the

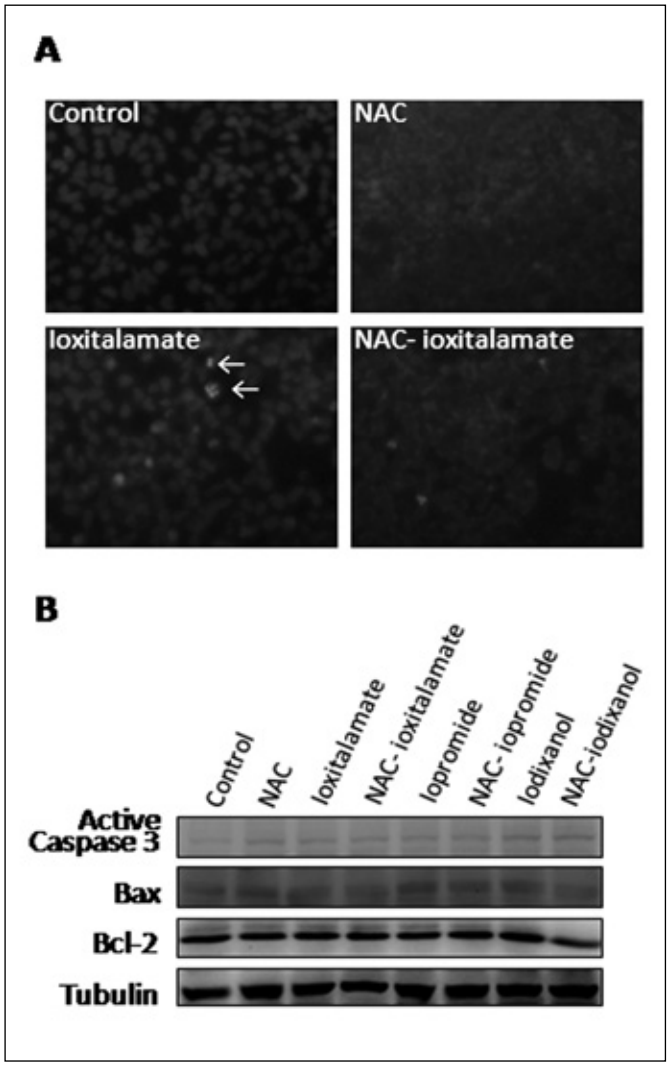

Fig. 4 – Apoptotic cells were identified as densely packed and fragmented chromatin in Hoechst stain. (A) Hoechst-positive apoptotic cells (arrows) were demonstrated in the ioxitalamate-treated and ioxitalamate with N-acetylcysteine (NAC)-pretreated groups. (B) Expression of active caspase-3, Bax, and Bcl2 in 293T cells 24 hours after contrast administration with and without NAC pretreatment. There was no difference in the expression of active caspase-3, Bax, and Bcl between groups treated with the 3 different contrast media with or without NAC pretreatment.

iso-osmolar iodixanol, can induce a significant reduction in cell viability at 24 hours, as determined by tetrazolium MTT assay. Secondly, probucol and ascorbic acid did not reduce the contrast-medium-induced cytotoxicity. Thirdly, single-dose NAC pretreatment for 15 minutes significantly reduced the cytotoxicity caused by non-ionic low- and iso-osmolar contrast media. Finally, a supplementary dose of NAC at 12 hours, in addition to 15 minutes' pretreatment, significantly reduced the cytotoxicity caused by ionic contrast media.

In this study Hoechst staining and apoptotic protein expression were used to examine the presence or degree of apoptosis. Both experiments failed to show any significant increase in apoptosis in the contrast-medium-treated groups (Fig. 4). Dose- and time-dependent cytotoxicity of contrast media has been reported by others (9, 25). Compared with previous studies showing contrast-induced apoptosis (9, 26-28), the concentration of contrast medium used in this study was relatively low. Thus, it stands to reason that no significant increase in positive Hoechst staining or expression of apoptotic proteins was observed in this study.

The prophylactic role of probucol in CIN was studied in a randomized clinical trial (29). The group receiving pretreatment with probucol, 500 mg orally twice daily for 3 days before and after coronary procedures, had a significantly lower serum creatinine level than the hydration alone group.

The preventative effect of ascorbic acid for CIN caused by iso-osmolar and low-osmolar contrast media has been reported in clinical trials. Ascorbic acid, administered at a dose of 3 g before the index procedure and 2 g after the procedure, significantly reduced the incidence of contrast-induced acute kidney injury compared with placebo (30). However, high-dose NAC was shown to be more effective in reducing the incidence of CIN than ascorbic acid, especially in high-risk patients (31). In our study of short-duration pretreatment with antioxidants, only NAC showed beneficial effects in terms of kidney cell viability after contrast medium administration.

The preventive role of NAC for CIN has been documented by multiple clinical trials. A meta-analysis showed beneficial effects of NAC in the prevention of CIN caused by low-osmolar or iso-osmolar contrast media such as iopromide, iohexol, and iodixanol (20, 21, 32). A randomized clinical trial showed that oral NAC along with hydration prevented the reduction in renal function induced by iopromide (33). Another meta-analysis showed that prophylactic NAC intervention less than 2 hours before contrast administration significantly reduced the incidence of CIN (34). However, the NAC protocols in these studies were initiated at the most 1 hour before contrast administration (34). In most percutaneous coronary interventions for acute coronary syndrome or emergency contrast-enhanced computed tomography, patients were unable to receive a prophylactic NAC regimen 1 hour or 1 day earlier as in previous clinical trials. This study provided in vitro evidence that 15 minutes' pretreatment with NAC reduced non-ionic low- and iso-osmolar contrast-medium-induced cytotoxicity and a supplementary dose of NAC after 12 hours in addition

to 15 minutes' pretreatment significantly reduced the cytotoxicity caused by ionic contrast media. These findings provided new insight into the prevention of CIN for clinical emergency procedures. Further clinical studies of short-duration prophylactic NAC regimens in the prevention of CIN are warranted.

The study has some limitations. Firstly, this was an in vitro experiment. Secondly, no control conditions, such as the use of hyperosmolar solutions, were used in assessing the role of hypertonicity in CIN. Finally, no adult proximal tubule cell line was used.

Conclusion

This work provides new evidence demonstrating that short-duration NAC pretreatment can significantly reduce the cytotoxicity of non-ionic, low-osmolar and iso-osmolar contrast media. With a supplementary dose 12 hours after contrast medium administration, NAC can significantly reduce ioxitalamate-induced cytotoxicity. Short-duration probucol or ascorbic acid pretreatment does not have a protective effect against contrast-induced cytotoxicity. This study provides new insight into the prevention of CIN, especially in emergency clinical settings.

Financial support: None.

Conflict of interest statement: None.

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References

1. Esplugas E, Cequier A, Gomez-Hospital JA, Blanco BGD, Jara F. Comparative tolerability of contrast media used for coronary interventions. Drug Saf. 2002;25:1079-1098.
2. Mehran R, Nikolsky E. Contrast-induced nephropathy: definition, epidemiology, and patients at risk. Kidney Int Suppl. 2006;100:S11-S15.
3. Katzberg RW, Haller C. Contrast-induced nephrotoxicity: clinical landscape. Kidney Int Suppl. 2006;100:S3-S7.
4. Aspelin P, Aubry P, Fransson SG, Strasser R, Willenbrock R, Berg KJ. Nephrotoxic effects in high-risk patients undergoing angiography. N Engl J Med. 2003;348:491-499.
5. McCullough PA, Adam A, Becker CR, et al. Epidemiology and prognostic implications of contrast-induced nephropathy. Am J Cardiol. 2006;98:5K-13K.
6. Finn WF. The clinical and renal consequences of contrast-induced nephropathy. Nephrol Dial Transplant. 2006;21:i2-i10.
7. Persson PB, Hansell P, Liss P. Pathophysiology of contrast medium-induced nephropathy. Kidney Int. 2005;68:14-22.
8. Zhao J, Huang Y, Song Y, et al. Low osmolar contrast medium induces cellular injury and disruption of calcium homeostasis in rat glomerular endothelial cells in vitro. Toxicol Lett. 2009;185:124-131.
9. Lee HC, Sheu SH, Yen HW, Lai WT, Chang JG. JNK/ATF2 pathway is involved in iodinated contrast media-induced apoptosis. Am J Nephrol. 2010;31:125-133.
10. Lee HC, Yen HW, Sheu SH. Effects of different contrast media on glutathione peroxidase and superoxide dismutase activities in the heart and kidneys of normal and streptozotocin-induced diabetic rats. J Formos Med Assoc. 2006;105:530-535.
11. Heinrich M, Scheer M, Heckmann M, Bautz W, Uder M. Reversibility and time-dependency of contrast medium induced inhibition of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) conversion in renal proximal tubular cells in vitro: comparison of a monomeric and a dimeric nonionic iodinated contrast medium. Invest Radiol. 2007;42:732-738.
12. Haller C, Hizoh I. The cytotoxicity of iodinated radiocontrast agents on renal cells in vitro. Invest Radiol. 2004;39: 149-154.
13. Solomon R, Deray G. How to prevent contrast-induced nephropathy and manage risk patients: practical recommendations. Kidney Int Suppl. 2006;100:S51-S53.
14. Mueller C, Buerkle G, Buettner HJ, et al. Prevention of contrast media-associated nephropathy: randomized comparison of 2 hydration regimens in 1620 patients undergoing coronary angioplasty. Arch Intern Med. 2002;162:329-336.

15. DiMari J, Megyesi J, Udvarhelyi N, Price P, Davis R, Safirstein R. N-acetylcysteine ameliorates ischemic renal failure. Am J Physiol. 1997;272:F292-F298.
16. Nishimura M, Sasaki T, Ohishi A, et al. Angiotensin-converting enzyme inhibitors and probucol suppress the time-dependent increase in urinary type IV collagen excretion of type II diabetes mellitus patients with early diabetic nephropathy. Clin Nephrol. 2001;56:96-103.
17. Yen HW, Lee HC, Lai WT, Sheu SH. Effects of acetylcysteine and probucol on contrast medium-induced depression of intrinsic renal glutathione peroxidase activity in diabetic rats. Arch Med Res. 2007;38:291-296.
18. Frei B, England L, Ames BN. Ascorbate is an outstanding antioxidant in human blood plasma. Proc Natl Acad Sci USA. 1989;86:6377-6381.
19. Bendich A, Machlin IJ, Scandurra O, Burton GW, Wayner DDM. The antioxidant role of vitamin C. Adv Free Radic Biol Med. 1986;2:419-444.
20. Kelly AM, Dwamena B, Cronin P, Bernstein SJ, Carlos RC. Meta-analysis: Effectiveness of drugs for preventing contrast-induced nephropathy. Ann Intern Med. 2008;148: 284-294.
21. Fishbane S, Durham JH, Marzo K, Rudnick M. N-acetylcysteine in the prevention of radiocontrast-induced nephropathy. J Am Soc Nephrol. 2004;15:251-260.
22. Zager RA, Johnson AC, Hanson SY. Radiographic contrast media–induced tubular injury: Evaluation of oxidant stress and plasma membrane integrity. Kidney Int. 2003;64:128-139.
23. Tang XY, Yang XD, Peng YF, Lin JH. Protective effects of lycopene against H2O2-induced oxidative injury and apoptosis in human endothelial cells. Cardiovasc Drugs Ther. 2009;23:439-448.
24. Yeom CH, Lee G, Park JH, et al. High dose concentration administration of ascorbic acid inhibits tumor growth in BALB/C mice implanted with sarcoma 180 cancer cells via the restriction of angiogenesis. J Transl Med. 2009;7:70-78.
25. Heinrich MC, Scheer M, Heckmann M, Kuefner MA, Uder M. Iodixanol induces apoptotic and antiproliferative effects but no necrotic cell death in renal proximal tubular cells in vitro. Rofo. 2009;181:349-354.
26. Yano T, Itoh Y, Kubota T, et al. A prostacyclin analog prevents radiocontrast nephropathy via phosphorylation of cyclic AMP response element binding protein. Am J Pathol. 2005;166:1333-1342.
27. Hizóh I,, Sträter J, Schick CS, Kübler W, Haller C. Radiocontrast-induced DNA fragmentation of renal tubular cells in vitro: role of hypertonicity. Nephrol Dial Transplant. 1998;13:911-918.
28. Hizoh I, Haller C. Radiocontrast-induced renal tubular cell apoptosis: hypertonic versus oxidative stress. Invest Radiol. 2002;37:428-434.
29. Li G, Yin L, Liu T, et al. Role of probucol in preventing contrast-induced acute kidney injury after coronary interventional procedure. Am J Cardiol. 2009;103:512-514.
30. Alexopoulos E, Spargias K, Kyrzopoulos S, et al. Contrast-induced acute kidney injury in patients with renal dysfunction undergoing a coronary procedure and receiving non-ionic low-osmolar versus iso-osmolar contrast media. Am J Med Sci. 2010;339:25-30.
31. Jo SH, Koo BK, Park JS, et al. N-acetylcysteine versus ascorbic acid for preventing contrast-Induced nephropathy in patients with renal insufficiency undergoing coronary angiography: NASPI study—a prospective randomized controlled trial. Am Heart J. 2009;157:576-583.
32. Birck R, Krzossok S, Markowetz F, Schnülle P, van der Woude FJ, Braun C. Acetylcysteine for prevention of contrast nephropathy: meta-analysis. Lancet. 2003;362:598-603.
33. Tepel M, van der Giet M, Schwarzfeld C, Laufer U, Liermann D, Zidek W. Prevention of radiographic-contrast-agent-induced reductions in renal function by acetylcysteine. N Engl J Med. 2000;343:180-184.
34. Sinert R, Doty CI. Prevention of contrast-induced nephropathy in the emergency department. Ann Emerg Med. 2007;50:335-345.

Accepted: December 29, 2010