

Oxidant stress due to non ionic low osmolar contrast medium in rat kidney

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Background & objectives: Contrast media may cause contrast-induced nephropathy (CIN) in risk group. This study was taken up to establish possible effects of non ionic low osmolar contrast medium administration on oxidant/antioxidant status and nitric oxide (NO) levels in rat kidney tissues.

Methods: Fourteen female, 14 wk old Wistar-albino rats were divided into 2 groups of 7 rats each (control and contrast groups). Non ionic low osmolar contrast medium was administered iv to the animals in the contrast group. The day after, animals were sacrificed and malondialdehyde (MDA) and NO levels and activities of antioxidant [superoxide dismutase (SOD), glutathione peroxidase (GSH-Px) and catalase (CAT)] and oxidant [xanthine oxidase (XO)] enzymes were measured in kidney tissues. Serum creatinine levels were measured to evaluate kidney functions.

Results: Contrast medium administration caused an increase in MDA levels and a decrease in NO levels in kidney tissues.

Interpretation & conclusions: The results suggest that non ionic low osmolar contrast medium administration leads to accelerated oxidant reactions and decreased NO level in rat kidney tissues. Further studies need to be done to assess the role of these changes in CIN.

Key words Nephrotoxicity - non ionic low osmolar contrast medium - oxidant/antioxidant status

Contrast induced nephropathy (CIN) is defined as an acute deterioration of renal function following administration of contrast medium in the absence of any other known reason. It is characterized by an increase in serum creatinine of more than 25 per cent above baseline level within 48 h. The risk factors for CIN are pre-existing renal failure, presence of diabetes mellitus (DM), volume of contrast medium used, dehydration, congestive heart failure, advanced age and simultaneous

usage of nephrotoxic drugs¹. Renal medullary ischaemia following contrast induced intrarenal vasoconstriction, direct cytotoxicity, oxidative tissue damage and apoptosis are possible pathophysiological mechanisms implied for CIN². Various properties of contrast media such as osmolality, ionic or non ionic nature and viscosity have been suggested to contribute to CIN³.

It has been proposed that there is a balance between oxidants and antioxidant defense mechanisms under

normal conditions, and disturbance in this balance lead to oxidative stress. Reactive oxygen species (ROS) such as superoxide anion radicals ($O_2^{\cdot-}$) are known as potent oxidants and ischaemia-reperfusion injury is an important cause of oxidative stress⁴. Nitric oxide (NO) has physiological functions such as vasodilator in regulation of blood pressure, neurotransmitter in the brain, and inhibitor of platelet aggregation⁵.

In this study, it was aimed to investigate the effects of non ionic low osmolar contrast medium administration on oxidant/antioxidant status and NO levels in rat kidney tissues.

Material & Methods

Contrast medium: Low osmolar and non ionic iomeprol (Iomeron 300 produced by Santa Farma, Italy) having 300 mg iodine per milliliter was used as contrast medium in this study. Osmolality and viscosity of the contrast medium were 521 ± 24 mOsm/kg-water at 37 °C and 4.5 ± 0.4 mPas at 37 °C, respectively.

Animals: Female Wistar-albino rats (14 wk old, 200 ± 10 g body weight) were purchased from Laboratory Animals Unite of Ankara Teaching and Research Hospital, Ankara. They were divided into 2 groups of 7 rats each (control and contrast groups). The study was approved by the Ethics Committee of Ankara Teaching and Research Hospital. The contrast medium was given as a single dose of 10 ml/kg (iodine load in 3 g/kg) by iv route to the animals in the contrast group⁶. This dose is higher than the clinical doses⁷ (approx. 600 mg/kg of human body) used in basic clinical care. Animals in the control group were given physiological saline (0.9 % NaCl solution in distilled water) as control vehicle at the dose of 10 ml/kg⁶. Twenty four hours after the administration of contrast medium or control vehicle, the animals were treated with ketamine - HCl (100 mg/kg) and were sacrificed. Their kidney tissues were removed for biochemical analyses. Blood samples were obtained from inferior vena cava of the animals just before sacrifice for the determination of serum creatinine levels.

Biochemical analysis: Levels of malondialdehyde (MDA) and NO, and activities of antioxidant [superoxide dismutase (SOD), glutathione peroxidase (GSH-Px) and catalase (CAT)] and oxidant (xanthine oxidase, XO) enzymes were measured in kidney tissues. The tissues were homogenized in physiological saline (1 g in 5 ml) using a homogenizer (B. Braun Melsungen AG 853202, Germany) and then, centrifuged at 4000 x g for 20 min (Heraus Labofur 200, Germany). Clear

supernatants were removed to be used in the analyses. Protein levels were measured by using the Lowry's method⁸, MDA levels by the thiobarbituric acid reactive substances method⁹, and XO activity was determined by measuring uric acid formation from xanthine substrate at 293 nm¹⁰. GSH-Px activity was measured by following changes in NADPH absorbance at 340 nm¹¹, and CAT activity by measuring decrease of H_2O_2 absorbance at 240 nm¹². In the activity calculations (IU - international unit), extinction coefficients of uric acid, H_2O_2 and NADPH were used for XO, CAT and GSH-Px, respectively. SOD activity was measured by the method based on nitroblue tetrazolium (NBT) reduction rate. One unit for SOD activity was expressed as the enzyme protein amount causing 50 per cent inhibition in NBT reduction rate¹³. Level of NO was measured by the method based on the Griess reaction¹⁴. Since nitrate anion does not give reaction, the samples were treated with cadmium to reduce nitrate anions into nitrite anions before NO assay¹⁵. Serum creatinine levels were measured by the method based on the colour reaction between alkaline picrate and creatinine¹⁶. All spectrophotometric measurements were made by using an UV-visible spectrophotometer (Unicam Helios alpha, England).

Statistical analysis: Student's t test was used to determine differences between the groups. $P < 0.05$ were considered as significant.

Results & Discussion

It was found that MDA levels increased (0.804 ± 0.176 vs. 0.553 ± 0.068 nmol/mg; $P < 0.01$) and NO levels decreased (2.160 ± 0.247 vs. 2.768 ± 0.412 μ mol/mg; $P < 0.01$) significantly in contrast group as compared with control group (Table).

Table. Measured parameters in serum and kidney tissues from rats

Parameters	Control group	Contrast group
<i>Kidney</i>		
MDA (nmol/mg)	0.553 ± 0.068	$0.804 \pm 0.176^*$
XO (mIU/mg)	0.153 ± 0.011	0.158 ± 0.011
SOD (U/mg)	47.87 ± 3.61	45.57 ± 3.37
GSH-Px (mIU/mg)	92.03 ± 12.06	88.16 ± 12.12
CAT (IU/mg)	85.30 ± 8.22	78.98 ± 14.88
NO (μ mol/mg)	2.768 ± 0.412	$2.160 \pm 0.247^*$
<i>Serum</i>		
Creatinine (mg/dl)	0.44 ± 0.09	0.40 ± 0.13

CAT, catalase; GSH-Px, glutathione peroxidase; MDA, malondialdehyde; NO, nitric oxide; SOD, superoxide dismutase; XO, xanthine oxidase
 Values are mean \pm SD (n=7)
 * $P < 0.01$ compared to control group

CIN is known to be one of the most important complications of the use of contrast media. It causes hospital-acquired acute renal failure (ARF)¹⁷. Three mechanisms *viz.*, direct or indirect haemodynamic effects, direct contrast medium molecule tubular toxicity, and endogenous biochemical disturbances have been proposed for the pathophysiology of contrast-induced ARF¹⁸. Haemodynamic effects include pre-renal dehydration and hypotension, medullary ischaemia, increased endothelin and adenosine, and decreased NO. Endogenous biochemical disturbances are increases in ROS production and/or decreases in antioxidant defense capacity resulting in oxidative stress. Any of these mechanisms may cause CIN separately or together¹⁸. It is suggested that serious vasoconstriction can contribute to additional renal injury by the release of ROS¹⁹. Sandhu *et al*²⁰ showed that urinary MDA to creatinine ratio increased following contrast medium infusion and suggested a relation between contrast medium infusion and free radical generation. In another study, Ribeiro *et al*²¹ investigated NO production in rat renal artery smooth muscle cells primary culture (rVSMC) exposed to contrast medium and found that non ionic iobitridol, low-osmolar ioxaglate and high-osmolar ioxitalamate caused decreases in NO levels as compared to control. They suggested that decreased NO may explain vasoconstriction and ARF by contrast media use²¹.

We found that non ionic low osmolar iomeprol administration to rats caused an increase in MDA and a decrease in NO levels in rat kidney tissues. No difference was however observed in creatinine levels between the groups indicating that contrast medium did not cause ARF. Oxidant and antioxidant enzyme activities did not change after contrast administration as compared with those of the control group. Increase in MDA level indicated that contrast medium use caused oxidative stress in rat kidney tissues. NO levels decreased following contrast medium administration. This might cause vasoconstriction in rat kidneys, which may be the reason of contrast-induced ARF. Haemodynamic effects like decreased NO levels and endogenous biochemical disturbances resulting in oxidative stress may be the mechanisms that cause CIN¹⁸. In this study, non ionic low osmolar contrast medium caused oxidative stress and some haemodynamic changes like decrease in NO level in the rat kidney tissues. However, the alterations in MDA and NO levels may not be associated with clinically apparent changes as no significant changes were observed in the analysis parameters relevant to kidney function like serum creatinine levels between the two groups.

In conclusion, our results showed that non ionic low osmolar contrast medium administration led to an increase in MDA levels indicating accelerated oxidant reactions, and a decrease in NO levels in rat kidney tissues. Further studies are needed to evaluate possible roles of vasoconstriction caused by the decrease in kidney tissue NO level together with oxidative stress in the pathophysiology of CIN.

References

1. Soma VR, Cavusoglu E, Vidhun R, Frishman WH, Sharma SK. Contrast-associated nephropathy. *Heart Dis* 2002; 4 : 372-9.
2. Oudemans-van Straaten HM. Contrast nephropathy, pathophysiology and prevention. *Int J Artif Organs* 2004; 27 : 1054-65.
3. Davidson C, Stacul F, McCullough PA, Tumlin J, Adam A, Lameire N, *et al.* CIN Consensus Working Panel. Contrast medium use. *Am J Cardiol* 2006; 98 : 42K-58K.
4. Shah AM, Channon KM. Free radicals and redox signalling in cardiovascular disease. *Heart* 2004; 90 : 486-7.
5. Murad F. Discovery of some of the biological effects of nitric oxide and its role in cell signaling. *Biosci Rep* 2004; 24 : 452-74.
6. 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 : 530-5.
7. Suzuki H, Oshima H, Shiraki N, Ikeya C, Shibamoto Y. Comparison of two contrast materials with different iodine concentrations in enhancing the density of the aorta, portal vein and liver at multi-detector row CT: a randomized study. *Eur Radiol* 2004; 14 : 2099-104.
8. Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. Protein measurement with the folin phenol reagent. *J Biol Chem* 1951; 193 : 265-75.
9. Dahle LK, Hill EG, Holman RT. The thiobarbituric acid reaction and the autoxidations of polyunsaturated fatty acid methyl esters. *Arch Biochem Biophys* 1962; 98 : 253-61.
10. Hashimoto S. A new spectrophotometric assay method of xanthine oxidase in crude tissue homogenate. *Anal Biochem* 1974; 62 : 426-35.
11. Paglia DE, Valentine WN. Studies on the quantitative and qualitative characterization of erythrocyte glutathione peroxidase. *J Lab Clin Med* 1967; 70 : 158-69.
12. Aebi H. Catalase. In: Bergmayer HU, editor. *Methods of enzymatic analysis*. New York: Academic Press Inc.; 1974. p. 673-7.
13. Durak İ, Canbolat O, Kavutcu M, Oztürk HS, Yurtarlanı Z. Activities of total, cytoplasmic, and mitochondrial superoxide dismutase enzymes in sera and pleural fluids from patients with lung cancer. *J Clin Lab Anal* 1996; 10 : 17-20.
14. Durak İ, Kavutcu M, Kaçmaz M, Avcı A, Horasanlı E, Dikmen B, *et al.* Effects of isoflurane on nitric oxide metabolism and oxidant status of guinea pig myocardium. *Acta Anaesthesiol Scand* 2001; 45 : 119-22.

15. Ridnour LA, Sim JE, Hayward MA, Wink DA, Martin SM, Buettner GR, *et al.* A spectrophotometric method for the direct detection and quantitation of nitric oxide, nitrite, and nitrate in cell culture media. *Anal Biochem* 2000; 281 : 223-9.
16. Vasiliades J. Reaction of alkaline sodium picrate with creatinine: I. Kinetics and mechanism of formation of the mono-creatinine picric acid complex. *Clin Chem* 1976; 22 : 1664-71.
17. Aspelin P. Nephrotoxicity and the role of contrast media. *Radiat Med* 2004; 22 : 377-8.
18. Katzberg RW. Contrast medium-induced nephrotoxicity: which pathway? *Radiology* 2005; 235 : 752-5.
19. Tumlin J, Stacul F, Adam A, Becker CR, Davidson C, Lameire N, *et al.* CIN Consensus Working Panel. Pathophysiology of contrast-induced nephropathy. *Am J Cardiol* 2006; 98 : 14K-20K.
20. Sandhu C, Newman DJ, Morgan R, Belli AM, Oliveira D. The role of oxygen free radicals in contrast induced nephrotoxicity. *Acad Radiol* 2002; 9 (Suppl 2) : S436-7.
21. Ribeiro L, de Assunção e Silva F, Kurihara RS, Schor N, Mieko E, Higa S. Evaluation of the nitric oxide production in rat renal artery smooth muscle cells culture exposed to radiocontrast agents. *Kidney Int* 2004; 65 : 589-96.

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