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 ($O_2^-$) are known as potent oxidants and ischaemia-reperfusion injury is an important cause of oxidative stress. Nitric oxide (NO) has physiological functions such as vasodilator in regulation of blood pressure, neurotransmitter in the brain, and inhibitor of platelet aggregation.

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. Osmolarity 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 ± 10g 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.

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**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 method, MDA levels by the thiobarbituric acid reactive substances method, and XO activity was determined by measuring uric acid formation from xanthine substrate at 293 nm. GSH-Px activity was measured by following changes in NADPH absorbance at 340 nm, and CAT activity by measuring decrease of $H_2O_2$ absorbance at 240 nm. 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 rate. Level of NO was measured by the method based on the Griess reaction. Since nitrate anion does not give reaction, the samples were treated with cadmium to reduce nitrate anions into nitrite anions before NO assay. Serum creatinine levels were measured by the method based on the colour reaction between alkaline picrate and creatinine. 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 μmol/mg; $P<0.01$) significantly in contrast group as compared with control group (Table).

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Control group</th>
<th>Contrast group</th>
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<tbody>
<tr>
<td><strong>Kidney</strong></td>
<td></td>
<td></td>
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<tr>
<td>MDA (nmol/mg)</td>
<td>0.553 ± 0.068</td>
<td>0.804 ± 0.176*</td>
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<tr>
<td>XO (mU/mg)</td>
<td>0.153 ± 0.011</td>
<td>0.158 ± 0.011</td>
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<tr>
<td>SOD (U/mg)</td>
<td>47.87 ± 3.61</td>
<td>45.57 ± 3.37</td>
</tr>
<tr>
<td>GSH-Px (mU/mg)</td>
<td>92.03 ± 12.06</td>
<td>88.16 ± 12.12</td>
</tr>
<tr>
<td>CAT (IU/mg)</td>
<td>85.30 ± 8.22</td>
<td>78.98 ± 14.88</td>
</tr>
<tr>
<td>NO (μmol/mg)</td>
<td>2.768 ± 0.412</td>
<td>2.160 ± 0.247*</td>
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<tr>
<td><strong>Serum</strong></td>
<td></td>
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<tr>
<td>Creatinine (mg/dl)</td>
<td>0.44 ± 0.09</td>
<td>0.40 ± 0.13</td>
</tr>
</tbody>
</table>

CAT, catalase; GSH-Px, glutathione peroxidase; MDA, malondialdehyde; NO, nitric oxide; SOD, superoxide dismutase; XO, xanthine oxidase

Values are mean ± 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)\(^7\). 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 ARF\(^8\). 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 together\(^8\). It is suggested that serious vasoconstriction can contribute to additional renal injury by the release of ROS\(^9\). Sandhu et al\(^10\) 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\(^11\) 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 use\(^11\).

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 CIN\(^8\). 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


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