Can Paricalcitol Increase the Effectiveness of N-Acetylcysteine in Contrast Induced Acute Kidney Prophylaxis in Rats? A Biochemical and Histopathological Study

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SUMMARY: N-Acetylcysteine (NAC) is used for contrast induced acute kidney injury (CI-AKI) prophylaxis because of its antioxidant effects. Paricalcitol, which has renoprotective effects, is likely to provide a more effective prophylaxis when added to NAC treatment. The study was designed based on this hypothesis. The study was organized to include 4 groups each consisting of 7 rats. Group 1 was the control group, and Group 2 included rats with CI-AKI. Rats in Group 3 were administered NAC at a dose of 100 mg/kg via oral gavage once a day for 5 days. Rats in group 4 were administered paricalcitol at a dose of 0.4 mcg/kg once a day for 5 days in addition to NAC. CI-AKI was induced after the treatments in both groups. The study was terminated on the sixth day. Samples were collected from the rats’ sera and kidney tissues to study oxidant and antioxidant parameters; kidney function tests were also studied. There were significant differences between the contrast nephropathy group (Group 2) and NAC and NAC+paricalcitol groups with respect to serum urea and creatinine levels. When the same groups were compared regarding oxidant (TOS-MDA) and antioxidant (TAC-Paraoxonase) parameters, we observed that the oxidant parameters increased in serum and kidney tissue samples with NAC use, and that effect was strengthened by the addition of paricalcitol to NAC treatment. However, despite increased antioxidant effectiveness, we observed no decrease in urea and creatinine levels when paricalcitol was added for CI-AKI in rats. There was no significant difference between Group 3 and Group 4. Paricalcitol provides a more potent antioxidant effect in both serum and kidney tissue samples when added to NAC treatment in rats with CI-AKI. Despite increased antioxidant parameters, however, paricalcitol does not provide a significant decrease in urea and creatinine levels.

KEY WORDS: CI-AKI; Paricalcitol; NAC; Oxidative stress; Antioxidants.

INTRODUCTION

Contrast-induced acute kidney injury is a clinical disorder with a usually reversible course, which occurs after radiocontrast agent use. Intravascular administration of iodinated radiocontrast agents has been reported to cause acute renal dysfunction. Even minor changes in renal function have been closely associated with increased morbidity and mortality. For this reason, it is an important reference point for the prevention of radiocontrast nephropathy (Weisbord & Palevsky, 2005). It is more common in hospitalized patients. Contrast-induced nephropathy, a form of acute kidney injury, has been the focus of attention in recent years, with new information on its pathogenesis, proliferation of innovative approaches to its prevention and in recent years. The recognition that contrast-induced nephropathy is associated
with long-term adverse events has been reported to be an important point that should not be overlooked by clinicians (Solomon et al., 2009). In a randomized prospective study, the incidence of contrast nephrotoxicity among non-ionic contrast media, iohexol and ionic contrast media, diatrizoate, was investigated in a large population of both 'low' and 'high risk' patients undergoing cardiac angiography. It can even occur with a lower incidence in patients having no risk factor (Rudnick et al., 1995).

It has been stated that the prevalence of increased oxidative stress and acute phase inflammation in patients with chronic kidney disease has not been fully investigated. Few prevalence studies have been reported to examine biomarkers of oxidative stress and inflammation in the larger patient population with chronic kidney disease. Given the high prevalence of cardiovascular disease in the chronic kidney disease population, it was hypothesized that this population would show an increase in inflammation and oxidative stress biomarkers, and these biomarkers would be inversely proportional to glomerular filtration rate. Therefore, commonly used biomarkers of acute phase inflammation and oxidative stress status in a cohort of patients with stage 3-5 chronic kidney disease not receiving renal replacement therapy were examined (Oberg et al., 2004). It has also been reported that there is an increase in oxidative stress parameters due to uremia in chronic renal failure (Himmelfarb & McMonagle, 2001). It was concluded that AdipoRon, an agonist of adiponectin receptors, benefits many organs, including the kidney. AdipoRon (50 mg/kg) was found to significantly reverse serum creatinine, blood urea nitrogen, creatinine clearance, and urinary kidney injury molecule-1 levels induced by iopromide in Sprague-Dawley rats. They found that preservation of AdipoRon was accompanied by enhanced activated protein kinase (AMPK) phosphorylation. Both in vivo and in vitro studies concluded that compound c, an AMPK inhibitor, reversed AdipoRon-mediated development in the contrast-induced nephropathy model (Gu et al., 2020).

Izquierdo et al. (2012) observed that the initial levels of oxidation markers MDA, nitric oxide and protein carbonyl groups decreased significantly after three months of paricalcitol treatment, while the levels of GSH, thioredoxin, catalase and SOD activity increased significantly. It has been reported that after paricalcitol treatment, the levels of inflammatory markers CRP, TNF-a, IL-6 and IL-18 were significantly decreased in serum and the level of the anti-inflammatory cytokine IL-10 increased. It has been suggested that paricalcitol-induced reduction in albuminuria and inflammation may be mediated independently of its effects on hemodynamics or parathyroid hormone suppression. They stated that long-term randomized, controlled studies are needed to confirm these benefits of vitamin D analogues. Despite the use of angiotensin converting enzyme inhibitors and angiotensin receptor blockers, it has been reported that patients with chronic kidney disease show no improvement in endothelial function within 1 month of treatment with paricalcitol, but a reduction in inflammation and albuminuria (Alborzi et al., 2008).

The aim of this experimental study was investigated based on the hypothesis whether a further level of kidney function protection could be achieved by adding paricalcitol therapy to NAC to treat contrast induced acute kidney injury in rats.

**MATERIAL AND METHOD**

This study was approved by Dicle University Prof. Dr. Sabahattin PAYZIN Health Sciences Research and Practice Center Experimental Animals Ethics Committee and conducted in Dicle University Prof. Dr. Sabahattin PAYZIN Health Sciences Research and Practice Center. Biochemical analyses were performed in University of Dicle, Faculty of Medicine Biochemistry Department Central Laboratory, and histopathological studies in University of Dicle Veterinary Faculty Department of Histology and Embryology Research Laboratory. Our experimental study was funded by Scientific Research Project Unit of Dicle University (Project No: 13-TF 90).

**Experimental protocol and groups.** The study was conducted with a total of 28 adult Wistar Albino rats that were allocated equally into 4 groups. The rats had a mean weight of 200-250 grams. The animals were fed on ad libitum and water under standard conditions for 5 days in the DUSAM. The study was designed with 28 rats allocated equally into four groups.

1. **Group (Control group) :** No agent administered.

2. **Group (Contrast nephropathy group):** Diatrizoate (urografin 76 %, Schering AG, Germany) was administered at a dose of 6 ml/kg via the tail vein under ether anesthesia on day 5 to create experimental contrast agent nephropathy (n=7) (Fig. 1).

3. **Group (Contrast nephropathy + NAC group):** The rats were administered 100 mg/kg NAC (Asist ampoule, Bilim Ilac, Turkey) via oral gavage once a day for 5 days. Contrast nephropathy was induced by administering 6 ml/kg diatrizoate (Urografin 76 %, Schering AG, Germany) via the tail vein under ether anesthesia on day 5 (n=7) (Fig. 1).
4. Group (Contrast nephropathy + Paricalcitol + NAC group): The rats were administered 0.4 mcg/kg paricalcitol (Zemplar ampoule, Abbott USA) once a day and 100 mg/kg NAC (Asist ampoule, Bilim Ilac Turkey) via oral gavage for 5 days. Contrast nephropathy was induced by administering 6 ml/kg diatrizoate (Urografin 76 %, Schering AG, Germany) via the tail vein under ether anesthesia on day 5 (n=7) (Fig. 1).

The rats were administered xylazine (10 mg/kg) and ketamine HCl (70 mg/kg) via intramuscular (I.M) route prior to the surgical procedure on the final study day. The rats were fixed in the supine position under general anesthesia; a midline section was made on the anterior abdominal wall for histopathological and biochemical studies. The right kidney of each animal was removed for histopathological study. A piece of kidney tissue samples put into 10 % neutral formaline for further histopathological investigation. Another piece of kidney tissue samples taken for biochemical study were placed into aluminum foils. Then, a 5-ml blood sample was collected from the heart after sternotomy for biochemical study. This step was also used to sacrifice the animals. Blood samples collected on the first and sixth days were used for biochemical analysis.

Evaluation of renal function. AEROSET c8000 analyzer was used for measurement of serum urea and creatinine.

Biochemical analysis: Tissue samples were weighed and homogenized. Both serum and kidney tissue specimen were examined for oxidant malondialdehyde (MDA), total oxidant status (TOS) and antioxidant parameters paraoxonase and total antioxidant capacity (TAC) were measured. MDA were determined with the thiobarbituric acid method. TOS and TAC were determined through use of a new measurement method developed by Erel (2004, 2005). Paraoxonase was measured by employing kits available.

Results

The comparison of the control group and the CI-AKI group showed a significant difference between the serum urea and creatinine levels of both groups. The same difference was also found when the groups that developed CI-AKI after NAC and NAC+paricalcitol were administered (groups 3-4). When compared with groups 3 and 4, CI-AKI group (group 2) showed significant reduction in serum urea and creatinine levels. We interpreted this finding as the clinical effectiveness of NAC and NAC+Paricalcitol.
However, there was no significant difference between group 3 and group 4 with respect to serum urea and creatinine levels (Table I).

The comparison of the oxidant (TOS-MDA) and antioxidant (TAC-paraoxonase) parameters in the serum and kidney samples of the control group (group 1) and the CI-AKI group (group 2) showed a significant increase in the oxidant parameters and a drop in antioxidant parameters. When the groups that developed CI-AKI after being treated with NAC and NAC+ Paricalcitol (group 3 and group 4) were compared with the contrast nephropathy group, a significant increase in antioxidant parameters and a significant decrease on oxidant parameters were observed. The comparison of group 3 and group 4 with each other with respect to antioxidant and oxidant parameters revealed that adding paricalcitol to NAC treatment showed statistical significance unlike serum creatinine and urea levels. Addition of paricalcitol increased antioxidant effect and significantly reduced oxidant parameters (Table II).

In the evaluation of the control group sections, normal histologic view has been observed (Fig. 2a). Strong tubular necrosis and atrophy were seen in CI-AKI group sections (Fig. 2b). Tubular necrosis and atrophy levels were decreased in CI-AKI+NAC (Fig. 2c) and CI-AKI+NAC+Paricalcitol (Fig. 2d) as compared to CI-AKI group sections. The histopathological comparison of the groups has been shown in Table III.

The examination of kidney tissue samples demonstrated that the histopathological changes were more severe in the CI-AKI group than the CI-AKI+NAC and CI-AKI+NAC+Paricalcitol groups.

Table I. Renal function tests in rats of the groups.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Control (n=7)</th>
<th>CIN (n=7)</th>
<th>CIN+NAC (n=7)</th>
<th>CIN+NAC+Paricalcitol (n=7)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum Urea (mg/dl)</td>
<td>36.29±5.85</td>
<td>98.29±5.56</td>
<td>74.57±3.41</td>
<td>71±6.56</td>
</tr>
<tr>
<td>Creatinine (mg/dl)</td>
<td>0.67±0.04</td>
<td>1.09±0.8b</td>
<td>0.76±0.14ab</td>
<td>0.78±0.04ab</td>
</tr>
</tbody>
</table>

a p<0.001 in comparison with the control group. b p<0.01 in comparison with the CIN group. c p<0.01 in comparison with CIN+NAC. d p<0.05 in comparison with the control group.

Table II. Oxidant and antioxidant parameters of serum and kidney tissue in the study groups.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Control (n=7)</th>
<th>CIN (n=7)</th>
<th>CIN+NAC (n=7)</th>
<th>CIN+NAC+Paricalcitol (n=7)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum MDA (mmol/ml)</td>
<td>4.44±0.47</td>
<td>32.59±3.28d</td>
<td>20.4±2.13de</td>
<td>16.81±1.56de</td>
</tr>
<tr>
<td>TOS (µmol/L)</td>
<td>42.4±2.16</td>
<td>281.21±15.72d</td>
<td>132.63±5.46de</td>
<td>119.6±6.41de</td>
</tr>
<tr>
<td>TAC (mmol/L)</td>
<td>0.85±0.12</td>
<td>0.96±0.13</td>
<td>1.96±0.28e</td>
<td>2.02±0.16de</td>
</tr>
<tr>
<td>Paraoxonase (U/L)</td>
<td>93.6±7.62</td>
<td>158.43±39.28</td>
<td>195.03±24.72</td>
<td>212.45±16.02</td>
</tr>
</tbody>
</table>

Kidney Tissue

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Control (n=7)</th>
<th>CIN (n=7)</th>
<th>CIN+NAC (n=7)</th>
<th>CIN+NAC+Paricalcitol (n=7)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MDA (mmol/ml)</td>
<td>16.73±3.58</td>
<td>38.90±5.17d</td>
<td>34.35±3.22a</td>
<td>29.28±2.68de</td>
</tr>
<tr>
<td>TOS (µmol/L)</td>
<td>81.79±4.63</td>
<td>205.8±19.97d</td>
<td>129.71±9.37de</td>
<td>123.15±9.23de</td>
</tr>
<tr>
<td>TAC (mmol/L)</td>
<td>4.92±0.87</td>
<td>3.21±0.34d</td>
<td>4.03±0.24b</td>
<td>4.26±0.38b</td>
</tr>
<tr>
<td>Paraoxonase (U/L)</td>
<td>51.97±2.19</td>
<td>44.34±3.13e</td>
<td>106.66±5.82de</td>
<td>124.07±4.53deef</td>
</tr>
</tbody>
</table>

MDA: Malondialdehit, TOS: Total oxidant status, TAC: Total antioxidant capacity, NO: Nitric oxide. a: p<0.05 in comparison with the control group. b: p<0.05 in comparison with the CIN group. c: p<0.001 in comparison with the CIN group. d: p<0.001 in comparison with CIN+NAC. e: p<0.001 in comparison with CIN+NAC+NAC. f: p<0.001 in comparison with CIN+NAC.

Table III. Comparison of histopathological parameters of all experimental groups.

<table>
<thead>
<tr>
<th>Histopathological Findings</th>
<th>Control (n=7)</th>
<th>CI-AKI (n=7)</th>
<th>CI-AKI+NAC (n=7)</th>
<th>CI-AKI+NAC+Paricalcitol (n=7)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tubular necrosis</td>
<td>0</td>
<td>2.86±0.38c</td>
<td>1.71±0.49bc</td>
<td>1.57±0.78cde</td>
</tr>
<tr>
<td>Tubular atrophy</td>
<td>0</td>
<td>2.71±0.49c</td>
<td>1.72±0.76bc</td>
<td>1.55±0.60cde</td>
</tr>
<tr>
<td>Regenerative atypia</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Hydropic degeneration</td>
<td>0</td>
<td>2.85±0.38c</td>
<td>1.71±0.49bc</td>
<td>1.57±0.79cde</td>
</tr>
<tr>
<td>Interstitial fibrosis</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Loss of brush margin</td>
<td>0</td>
<td>2.85±0.37c</td>
<td>1.85±0.66bc</td>
<td>1.71±0.95cde</td>
</tr>
</tbody>
</table>

a: p<0.05 in comparison with control group. b: p<0.05 in comparison with CI-AKI group. c: p<0.001 in comparison with control group. d: p<0.001 in comparison with CI-AKI group.
Diagnosis of many clinical conditions is only possible with radiological studies. Thus, contrast agent use is required in an increasingly higher number of diagnostic and therapeutic procedures. This causes a higher incidence of acute kidney injury due to contrast agent use. Therefore, hospital admissions due to acute kidney injury after contrast agent use are the third most common cause of acute kidney failure (Nash et al., 2002). CI-AKI also causes prolonged hospital stay and increased hospital costs (Rihal et al., 2002). Because of all these reasons, CI-AKI prophylaxis is greatly important.

Medullary ischemia caused by vasoconstriction in the renovascular bed and the toxic effects of increased reactive oxygen species secondary to contrast agent use are the most likely causes of CI-AKI (Murphy et al., 2000). However, direct renal tubular toxicity may be another important cause (Tumlin et al., 2006). It is known that there occurs an increase in oxidant parameters and oxidative stress with the progression of acute kidney injury (Himmelfarb & McMonagle, 2001). MDA and TBARS are the end products of the peroxidation of membrane polyunsaturated fatty acids by free oxygen radicals; they are markers of oxidative injury. It is known that these metabolites increase in experimental CI-AKI models (Cetin et al., 2008; Devrim et al., 2009). In accordance with the literature, our study demonstrated an increase in oxidant MDA and TAC levels in both tissue and serum samples (Table II). All these studies indicate that the variability of oxidant and antioxidant parameters in CI-AKI may be important for determining clinical injury and the benefits of preventive measures. Our study investigated the results of paricalcitol treatment in addition to NAC treatment in rats with CIN. N-acetylcysteine is the precursor of glutathione synthesis; it clears free oxygen radicals (Shalansky et al., 2005a). The use of NAC is associated with increased levels of antioxidants (Quintavalle et al., 2011). A handicap of the drug is its rapid metabolism in its hepatic first pass, which reduces its bioavailability (Olsson et al., 1988). Therefore, it is widely accepted that its high-dose use via intravenous route may be more effective for preventing CI-AKI (Shalansky et al., 2005b). In our study, the comparison of the contrast nephropathy group (group 2) and the group with induced contrast nephropathy after being treated with NAC (group 3) showed that the oxidant parameters MDA and TOS were reduced in serum and kidney tissue samples. There occurred a significant increase in

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**DISCUSSION**

Fig. 2 (A-D). Kidney histopathological findings of the study groups (Haematoxylin and Eosin staining, original magnification, $\times$ 200). (A) Group 1 (control); (B) Group 2 (CI-AKI); (C) Group 3 (CI-AKI+NAC); (D) Group 4 (CI-AKI+NAC+Paricalcitol).
antioxidant parameters TAC and paraoxonase in group 3 compared with group 2. In our study it is evident that NAC administration reduces oxidative stress. The comparison of serum urea and creatinine levels of group 2 and group 3 revealed that NAC administration was effective. There are many studies on the use of NAC in CI-AKI prophylaxis. In some of them, the beneficial effects of prophylactic use of NAC have been shown.

Tepel et al. (2000) showed that the risk of CI-AKI was reduced by 90.5 % and Shyu et al. (2002) by 86.6 % after NAC use. Our results are in parallel with the literature data. However, there are also several studies that have shown that NAC is ineffective on CI-AKI. Although Boccalandro et al. (2003), NAC dose is debated, the study by Vallero et al. (2002), represents this group of studies. Another important point is that whether NAC is effective in patients with increased serum creatinine levels. Durham et al. (2002) showed that NAC was ineffective in this group of patients (serum creatinine >1.7 mg/dl). The main rationale of our study was to test whether adding another drug with renoprotective properties and known antioxidant effects would provide a more effective CI-AKI prophylaxis.

Paricalcitol is an active vitamin D analog that has non-hypercalcemic effects. Paricalcitol use in hemodialysis patients is associated with as much increase in antioxidant parameters as with active vitamin D (Yeter et al., 2021).

Antioxidant parameters were higher in both serum and kidney tissue in group 4, which was treated with paricalcitol, than the contrast nephropathy group (group 2) and CI-AKI+NAC group (group 2). These results agree with the literature data. There are other studies indicating paricalcitol’s pleiotropic effects. It is known to prevent cyclosporine-induced nephrotoxicity in rats (Park et al., 2010) and to reduce proteinuria in diabetic patients (de Zeeuw et al., 2010). Paricalcitol also has antifibrotic effects (Piao et al., 2012). Bae et al. (2020) showed that paricalcitol with known renoprotective effects mitigated mitochondrial injury in tissue samples with contrast nephropathy.

CONCLUSIONS

In accordance with the literature data, we observed no statistically significant reduction in serum urea and creatinine levels despite an increase in antioxidant parameters when we added paricalcitol to NAC treatment. Paricalcitol shows a more potent antioxidant effectiveness when added to NAC in CI-AKI prophylaxis. However, paricalcitol provides no additional benefit in kidney function over NAC treatment.

REFERENCES


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