Histopathological evaluation and redox assessment in blood and kidney tissues in a rabbit contrast-induced nephrotoxicity model

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Article history:
Received 5 July 2017
Received in revised form 27 July 2017
Accepted 28 July 2017
Available online 31 July 2017

Keywords:
Contrast media
Nephrotoxicity
Oxidative stress
Rabbit

ABSTRACT

Contrast-induced nephropathy (CIN) is a leading cause of hospital-acquired acute kidney injury as a result of iodinated contrast-media use for diagnostic purposes. Pathophysiology remains unclear. In the present study iopromide was administered to New Zealand white rabbits without any prior intervention. Oxidative stress was assessed in blood and tissue level at three anatomical kidney areas (medullary, cortical, juxtamedullary). Histopathological evaluation was also performed. Serum creatinine and urea increased in the CIN groups over 25% at two hours after administration and returned to baseline at 48 h. In kidney tissues, a significant reduction (40%) of catalase in renal cortexes of the CIN groups was observed. Necrosis and tubular vacuolization was also noted that correlated with urea and creatinine levels. Lipid peroxidation decreased at 10 h after administration (>45%) and remained low even at 48 h. Plasma protein carbonyls were significantly increased (67%) in 2 h and dropped later. Serum levels of creatinine and urea at 24 and 48 h significantly correlated with the Total Antioxidant Activity and lipid peroxidation, respectively. Oxidative stress is shown to be involved in CIN development in the rabbit, with more pronounced effects to be confined to the cortex and outer stripe of the outer medulla.

1. Introduction

Diagnostic and interventional medical procedures nowadays are associated with increased use of iodinated contrast-media (CM) rendering contrast-induced nephropathy (CIN) the third leading cause of hospital acquired acute kidney injury (Rancic, 2016). CIN pathophysiology remains unclear and is probably related to a combination of hemodynamic alterations, direct renal tubular cell toxicity and reactive oxygen species (ROS) production (Scoditti et al., 2013). A number of animal models were created for thorough study of CIN. Existing animal models are not perfect in extrapolating observations to humans, as the majority of animal protocols involve exposure to multiple renal insults for CIN induction in animals with previous normal renal function. CIN animal studies usually utilize rats, although the said model present difficulties in CIN induction (Aspelin et al., 2003). Researches usually induce prerenal azotemia via 16–24 h water deprivation (Buyuklu et al., 2015; de Almeida et al., 2010).
et al., 2016; Ozkan et al., 2012; Zhao et al., 2011), or via resection of large sections of animal's kidneys accompanied by 48-h water deprivation (Liu et al., 2014a). Other protocols describe rats' treatment with nephrotoxic drugs previous to CM administration (Kiss and Hamar, 2016). New Zealand White rabbit is increasingly used as an experimental model (Lauver et al., 2014; Pettersson et al., 2002). Although morphologically the rabbit resembles to rodents, protein sequence data suggest that rabbits are more closely related to primates than rodents (Graur et al., 1996), while their renal function is known to be sensitive to CM (Golman and Almen, 1984). Rabbis are shown to be more sensitive to CM than rats (Bhargava et al., 1990), with New Zealand white rabbits being more susceptible to CIN than other strains (Lauver et al., 2014). CIN can be induced in healthy rabbits with a single injection of iodinated CM rendering the said rabbit model a reliable model of nephrotoxicity (Kiss and Hamar, 2016) and this was the methodology applied in the present study in order to elucidate the involvement of oxidative stress, cell necrosis and injury in CIN development.

2. Materials and methods

2.1. Guidelines for animal experimentation

This study was carried out in strict accordance with the recommendations in the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health. The protocol was approved by the 103rd General Assembly of Specific Interest (09/03/2016) of the Department of Biochemistry and Biotechnology, University of Thessaly. All surgery was performed under sodium pentobarbital anesthesia, and all efforts were made to minimize suffering.

2.2. Experimental protocol

Nine male New Zealand white rabbits (3–4 months old and weighing approximately 3.5 kg) were housed at standard conditions with access to standard rabbit food and tap water ad libitum.

At the beginning of the study, animals were randomized and divided into three groups: i) 24-h CIN group (n = 3), ii) 48-h CIN group (n = 3), iii) Control group (n = 3).

The used rabbit CIN model was adapted based on the previously described rabbit renal toxicity test (Pettersson et al., 2002) and involved intravascular administration of the non-ionic, low-osmolar iodinated contrast agent, iopromide solution (Ultravist®, Bayer Healthcare, Berlin, Germany), at a dose of 8 g/kg iodine through the rabbit's marginal ear vein over a period of 30 min. NaCl 0.9% was administered in the Control group. Previous iodoprote administration, animals were weighed and anesthetized by intramuscular administration of Xylazine (Xylapan®, 4 mg/kg) and Ketamine (Narketan®, 40 mg/kg). An intravenous catheter was placed in a marginal ear vein for the administration of contrast agent. Blood collection was performed at 2 h, 10 h, 24 h and 48 h after contrast-media administration. The animals were euthanized with IV infusion of Pentobarbital Sodium (Dolethal®, 5 mL per animal) at 24 h (24 h CIN group) and 48 h (48 h CIN group and Control group) post contrast infusion. Immediately post sacrificing, animals were weighted and kidneys were harvested for histopathological evaluation and protein extraction.

The present study conformed to the National and European Union directions for the care and treatment of laboratory animals. All efforts were made to minimize animals' suffering.

2.3. Renal function parameters

Creatinine and Urea were analyzed by a standard absorbance photometry in the blood serum by the COBAS INTEGRA® 400 plus analyzer, Roche.

2.4. Oxidative stress biomarkers

Oxidative stress biomarkers were evaluated as previously described (Gerasopoulos et al., 2015). More specifically, for Thiobarbituric Acid Reactive Substances (TBARS) determination, a modified assay of Keles et al. (2001) was used. According to this assay, 100 µL of plasma or 50 mL of renal tissue homogenate (diluted 1:2) was mixed with 500 µL of 35% TCA and 500 µL of Tris-HCl (200 mmol/L, pH 7.4), and incubated for 10 min at room temperature. One mL of 2 M Na2SO4 and 55 mM of thiobarbituric acid solution were added, and samples were incubated at 95 °C for 45 min. Samples were then cooled on ice for 5 min and 1 mL of 70% TCA was added. Samples were vortexed and centrifuged at 15,000 g for 3 min and supernatant absorbance was measured at 530 nm. A baseline shift in absorbance was taken into account by running a blank along with all samples during measurement. Calculation of TBARS concentration was based on the molar extinction coefficient of malondialdehyde (MDA).

Protein carbonyls were evaluated based on the method of Patsoukis et al. (2004). In this method, 50 µL of 20% trichloroacetic acid (TCA) was added to 50 µL of plasma or renal tissue homogenate (diluted 1:2), and this mixture was incubated in ice bath for 15 min and centrifuged at 15,000 g for 5 min at 4 °C. The supernatant was discarded and 500 µL of 10 mM 2,4-dinitrophenyl hydrazine (DNPH) was added in 2.5 N HCl for the sample or 500 µL of 2.5 N HCl for the blank. Samples were incubated in dark at room temperature for 1 h with intermittent vortexing every 15 min and centrifuged at 15,000 g for 5 min at 4 °C. The supernatant was discarded and 1 mL of 10% TCA was added, vortexed and centrifuged at 15,000 g for 5 min at 4 °C. This step was repeated twice. The supernatant was discarded and 1 mL of 5 M urea (pH 2.3) was added, vortexed, and incubated at 37°C for 15 min. Samples were centrifuged at 15,000 g for 3 min at 4 °C and absorbance was read at 375 nm. Calculation of protein carbonyls concentration was based on the molar extinction coefficient of DNPH. Total plasma protein was determined using a Bradford reagent (Sigma-Aldrich Ltd.).

Determination of Total Antioxidant Activity (TAC) was based on the method of Janaszewska and Bartosz (2002), Briefly, 20 µL of plasma or 40 µL renal tissue homogenate (diluted 1:10 with PBS) were added, respectively, to 480 µL or 460 µL of 10 mM sodium potassium phosphate (pH 7.4) and 500 µL of 0.1 mM 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical and samples were incubated in the dark for 30 min at room temperature. Samples were centrifuged for 3 min at 20,000 g and absorbance was read at 520 nm. TAC is presented as mmol of DPPH reduced to 2,2-diphenyl-1-picrylhydrazine (DPPH:H) by antioxidants of plasma and renal tissue.

Catalase activity was determined using the method of Aebi (1984). Briefly, 4 µL of erythrocyte lysate (diluted 1:10) or 40 µL renal tissue homogenate (diluted 1:2) were added, respectively, to 2991 µL or 2955 µL of 67 mM sodium potassium phosphate (pH 7.4), and samples were incubated at 37°C for 10 min. A total of 5 µL of 30% hydrogen peroxide was added to samples and the change in absorbance was immediately read at 240 nm for 1.5 min. Calculation of catalase activity was based on the molar extinction coefficient of H2O2. Hemoglobin concentrations were determined by the hemoglobin cyanide (HbCN) method.

Each assay was performed in triplicate within 1 month of blood and tissue collection. Blood and tissue samples were stored in multiple aliquots at –80°C and thawed only once before analysis.
All reagents were purchased from Sigma-Aldrich (St. Louis, Mo.).

### 2.5. Histopathological examination of renal tissue

For histopathological examination, slices from both explanted kidneys were fixed in 10% phosphate-buffered formalin solution overnight. Left kidneys were sectioned longitudinally and right kidneys transversely. Following automated dehydration through a graded ethanol series, transverse kidney slices were embedded in paraffin and 5 μm sections were cut on a rotary microtome. Kidney sections were stained with Hematoxylin Eosin (H&E), Periodic Acid-Schiff (PAS), Masson’s Trichrome and Jone’s Silver Methenamine stains. Histopathological changes were analyzed for tubular necrosis, tubular vacuolization and proteinaceous casts.

Cortical alterations were graded as follows: 0: no damage, 1: mild (damage less than 25%), 2: moderate (damage between 25 and 50%), 3: severe (damage between 50 and 75%), 4: very severe (more than 75% damage). Light microscope was used to evaluate the sections from two experienced pathologists blinded to study data. Photographs were taken at 200× magnification.

### 2.6. Statistical analysis

The Statistical package SPSS 20.0 (Statistical Package for Social Sciences Inc., Chicago, IL, USA) was used for statistical analysis. Normally distributed continuous variables are expressed as the means ± standard deviations (sd). The independent sample t-test was applied to compare means from two different groups (n ≥ 2) (de Winter, 2013). One way Anova was also used in order to evaluate significant differences among treatment groups. Pearson and Spearman correlations between various parameters were also investigated. Differences among categorical variables were assessed by the Chi-square test. Statistical significant differences are considered for p < 0.05. Values are presented as mean ± SEM.

### 3. Results

#### 3.1. Clinical evaluation of the animals

In general, animals did not show any signs of discomfort after the CM administration and remained healthy until the last day of the experiment. Daily inspection, at least twice a day, was performed and no signs of anorexia or dehydration were noticed. Body and organ weight changes are summarized in Table 1. A slight increase is observed in kidney to body weight ratio in the CIN-treated groups.

#### 3.2. Renal function parameters

Creatinine levels increase even at 2 h over 25% after the administration of CM, while after 48 h levels seem to return to baseline values. Urea levels follow a similar pattern as expected (Fig. 1).

#### 3.3. Assessment of oxidative stress biomarkers in blood

Plasma TBARS in CM-treated animals decreased more than 45% at 10 h after administration (p < 0.05) and remained at low levels after 48 h (56% decrease, p = 0.038). Plasma’s protein carbonyls increased significantly 2 h after administration (67%, p < 0.001). The transient significant increase continued for 10 h (43%, p = 0.004), indicating protein oxidation, followed by a significant reduction of around 30% in 24 and 48 h (Figs. 2 and 3). No statistically significant changes were observed in TAC and CAT over the period of time. Nevertheless, a slight decrease (<10%) in TAC levels was observed at 2–10 h that was reversed after 24 h.

Nearly significant correlations between TAC at 24 h and creatinine and urea levels in CM-treated animals were observed (r = 0.912, p = 0.088; r = 0.916, p = 0.084, respectively). On the other hand, TBARS decrease significantly correlated with creatinine and urea levels at 48 h group (r = 0.981, p = 0.003; r = 0.985, p = 0.002, respectively). In addition, TAC and catalase levels at baseline were correlated with the respective levels at 24 h (r = 0.955, p = 0.045; r = 0.987, p = 0.013).

#### 3.4. Assessment of oxidative stress biomarkers in kidney tissue

Catalase measurement in kidneys’ cortical area showed a reduction of around 10% at 24 h that became significant and reached 40% at 48 h compared to controls. No statistically significant differences were found in other kidney areas (juxtamedullary, medullary) but the decrease in catalase levels was consistent. TAC increased in 24 h after CM administration in all anatomical areas and returned to baseline values or even lower at 48 h. Significant correlations in TAC levels between juxtamedullary and cortical areas (p = 0.048) and juxtamedullary and medullary areas (p = 0.015) were observed. A constant decrease in TBARS was observed, although not statistically significant. Nearly significant correlations were noted between cortical TBARS and serum urea (p = 0.065) and creatinine (p = 0.056), as well as between plasma levels and organ weight changes.

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**Table 1**

<table>
<thead>
<tr>
<th>Animal Groups</th>
<th>Initial Body Weight (Kg)</th>
<th>Final Body Weight (Kg)</th>
<th>Initial/Final BW</th>
<th>kidney/BW ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control Group</td>
<td>3.31 ± 0.208</td>
<td>3.21 ± 0.095</td>
<td>1.27 ± 0.452</td>
<td>0.006 ± 0.001</td>
</tr>
<tr>
<td>24hCIN Group</td>
<td>3.38 ± 0.316</td>
<td>3.39 ± 0.252</td>
<td>0.996 ± 0.023</td>
<td>0.006</td>
</tr>
<tr>
<td>48hCIN Group</td>
<td>3.55 ± 0.110</td>
<td>3.41 ± 0.095*</td>
<td>1.04 ± 0.027</td>
<td>0.006 ± 0.001</td>
</tr>
</tbody>
</table>

* A significant increase of 6% (p = 0.05) is observed in final body weight between 48CIN and control group.
juxtamedullary TBARS and serum urea \( (p = 0.055) \) at 48 h. Significant correlation was also observed in PC levels between juxtamedullary and cortical areas \( (p = 0.031) \). The medullary area seemed to be affected in a more generalized way (Table 2).

3.5. Histopathological evaluation of kidney tissue

No pathological findings were noted in kidney samples from control group with all stains used. In CM-treated animals, extensive tubular vacuolization and necrosis was observed in proximal convoluted tubules (mainly observed with H&E) that extended to the outer stripe of the outer medulla, whereas the inner stripe was almost normal. However, glomeruli were intact in both groups, assessed mainly with Jone's Methenamine Silver stain that is specialized in this specific part of the nephron. Proteinaceous casts in medullary tubular ducts, mostly in the inner zone of the medullary area were observed. In PAS stain the brush border of proximal convoluted tubules is well depicted and hyaline droplets are evident in CIN treated groups. Masson's trichrome stain did not show any stain-specific lesions (fibrosis), as the tubular damage was acute and collagenous connective tissue fibers were absent. There were no significant differences between 24-h and 48-h harvested kidney tissues. Vacuolization in proximal convoluted tubules was noted in both 24 h CIN and 48 h CIN groups although it was more extensive in the former. 48 h CIN group presented a higher percentage of necrotic tubules. Interstitial inflammation was also present in the cortical area (Figs. 4 and 5). Histopathological scores are presented in Table 3.

Fig. 2. Blood plasma TBARS levels. # Statistical significant decrease compared to 24hCIN group at baseline; Statistical significant decrease compared to 48hCIN group at baseline.

Fig. 3. Blood plasma protein Carbonyls. # Statistical significant increase compared to 48hCIN group at 2 h; Statistical significant increase compared to 48hCIN group at baseline.

4. Discussion

The rabbit CIN model of the present study fulfilled all three
conditions for an ideal preclinical CIN model, namely a) intravenous CM administration should lead to a significant increase in serum urea and creatinine levels, b) standard histopathological changes should be easily diagnosed with routine stains and c) the model should not be lethal, comparing comfortably with the usual clinical course of CIN (Linkermann et al., 2013). The rabbit nephrotoxicity model of the present study involved healthy animals with normal renal function unlike previous reports (Tumlin et al., 2006; Wang et al., 2001), which closely resembles CIN clinical conditions.

Serum biochemical parameters showed an increase in serum creatinine levels >25% at 2 h post contrast-media administration, which indicates impairment in renal function according to the European Society of Urogenital Radiology (Golshahi et al., 2014). The decline in creatinine and urea levels after 24 h leads to the assumption that a counterbalance mechanism from the rabbit’s defense system was triggered. Lauver et al. showed that creatinine begun to rise in 1–2 h and peaked at 48 h post contrast media administration (Lauver et al., 2014).

To our knowledge, this is the first study in New Zealand White rabbits examining the redox status separately in three different areas of kidney tissues (cortical, juxtamedullary, medullary regions). Different Oxygen partial pressure (PO2) and reduction in blood flow (Linkermann et al., 2013) in the said kidney areas may lead to different levels of ROS production. Histopathological lesions observed were associated well with the biochemical markers of CIN in our study.

Histopathological evaluation showed extended vacuolization in proximal convoluted tubules of kidney cortical region as well as in the outer zone of the medullary area in CIN group animals. Tubular vacuolization is a degenerative change associated with a variety of toxic substances. CM are taken up by tubular cells by pinocytosis and pinocytic vessels fuse with lysosomes forming larger vacuoles (Dickenmann et al., 2008). Vacuolization induced by xenobiotics is defined by clear to translucent spaces inside the cytoplasm. Usually cells are “swollen” and nuclei are dislocated (Sahota et al., 2013). The said changes are an early sign of CIN and without the presence of co-existing predisposing factors it might not progress to necrosis (Dickenmann et al., 2008). Because they are highly reversible. This condition was also described as “osmotic nephrosis” (Sahota et al., 2013). However, this term is misleading (Randhawa et al., 1999) because the cause of tubular epithelial cells’ swelling is not osmotic pressure but vacuoles formation (Dickenmann et al., 2008). As far as proteinaceous casts are concerned, they represent a fluid accumulation or cell breakdown products that fill the tubular lamina (Sahota et al., 2013). Literature refers to vacuolization (Hsu et al., 2010; Ozkan et al., 2012) and proteinaceous casts (Ari et al., 2012; Liu et al., 2014a; Ozkan et al., 2012) mainly in rat models.

The most important histopathological lesion related to CM acute nephrotoxicity is necrosis, especially in proximal convoluted tubules. Prevalence of necrotic phenomena in rats with CIN has
previously been reported (Ari et al., 2012; Buyuklu et al., 2015; Ozkan et al., 2012). The generalized necrosis present in animals with in this study is in agreement with previously published results on rabbits (Lauver et al., 2014). Proximal convoluted tubules are more prone to necrosis following xenobiotic administration than medullary collecting ducts or distal convoluted tubules. Proximal tubular epithelium is the kidney region where most of membrane-bound active transport activity occurs. It has increased permeability to ions and chemical influx than distal tubular epithelium, due to higher electrical resistance and tighter intracellular junctions (Sahota et al., 2013).

In chronic kidney injury a disruption of the basement membrane could be observed and result in tubular atrophy and interstitial fibrosis (Sahota et al., 2013). Masson’s Trichrome staining did not depict any signs of fibrous connective tissue in histopathological lesions probably because our study animals were euthanized in 24–48 h post CM in the acute phase of nephrotoxicity.

Catalase is a very important antioxidant enzyme that contributes to the repair system following CM administration, but does not seem to be activated systemically in the rabbit CIN model, despite the findings at the renal tissue level. Catalase down-regulation in the cortical area in CIN groups that climaxed at 48 h probably shows that the enzyme was “used” to counteract oxidative damage in the cortical area. Colbay and colleagues also showed that catalase is consumed in CIN rat kidneys and they attributed it to oxidative damage (Colbay et al., 2010). This reduction could be, also, attributed to cell necrosis and, as a consequence, the destruction of the enzyme. This observation is in accordance with the pattern of kidneys’ histopathological lesions, where tubular necrosis and vacuolization are prominent in the cortical region of the kidney tissues in the CIN groups.

Although the redox status of cortical area was more seriously affected, the medullary area seemed to suffer more generalized but with less severe effects. As mentioned above, physiologically, there is a difference in pO2 in the three renal areas; cortical region shows higher levels of oxygenation that gradually decrease to the papilla. Hence, cortical tubular cells are more vulnerable to ischemia, as medullary cells are prone to anaerobic metabolism making them resistant to oxygen fluctuations (Kalogeris et al., 2012). The renal medulla has a unique circulatory anatomy, which causes medullary thick ascending limbs of the Henle’s loop (located in the outer medulla) to perform energetically demanding ion transport in a situation of relative hypoxia compared with the renal cortex. It is thought that a mismatch between the metabolic demands of thick ascending limbs of the Henle’s loop and the medullary blood supply could cause a surplus of superoxide (O2•−), leading to oxidative tubular damage superimposed on ischemic damage (Liu et al., 2014b). Tubular transport is associated with ROS formation, mostly in the renal medullary thick ascending limb. The regional extremely dense mitochondrial population there represents a major source for generation of superoxide anions (O2•−) and hydroxyl radicals (OH•) by NAD(P)H-oxidase [nicotinamide adenine dinucleotide (phosphate) oxidase] (Pisani et al., 2013).

Vasoconstriction, reactive oxygen species (ROS) production and direct tubular cellular toxicity are thought as three interconnected mechanisms. In vivo animal CIN model studies demonstrated a

<p>| Table 3 |
| Histopathological Scoring after Hematoxylin &amp; Eosin staining (mean values ± standard deviation). |</p>
<table>
<thead>
<tr>
<th>Control group</th>
<th>Cortical</th>
<th>Juxtamedullary</th>
<th>Medullary</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tubular necrosis</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Vacuolization</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Proteinaceous casts</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>24h-CIN group</td>
<td>Cortical</td>
<td>Juxtamedullary</td>
<td>Medullary</td>
</tr>
<tr>
<td>Tubular necrosis</td>
<td>3.25</td>
<td>3.25</td>
<td>1</td>
</tr>
<tr>
<td>Vacuolization</td>
<td>3.25</td>
<td>3.25</td>
<td>2.25</td>
</tr>
<tr>
<td>Proteinaceous casts</td>
<td>0</td>
<td>0</td>
<td>2.6</td>
</tr>
<tr>
<td>48h-CIN group</td>
<td>Cortical</td>
<td>Juxtamedullary</td>
<td>Medullary</td>
</tr>
<tr>
<td>Tubular necrosis</td>
<td>3.67</td>
<td>3.67</td>
<td>1</td>
</tr>
<tr>
<td>Vacuolization</td>
<td>3</td>
<td>3</td>
<td>2.33</td>
</tr>
<tr>
<td>Proteinaceous casts</td>
<td>0</td>
<td>0</td>
<td>2.25</td>
</tr>
</tbody>
</table>

Fig. 5. The effects of iopromide in outer stripe of the outer medulla. Panels A,B are representative H&E stained sections (x 200 magnification) from control animals (A) and 24 h post-treatment animals (B), respectively. Panels C, D represent PAS stained sections (x 200 magnification) from animals 48 h post-treatment. Extended tubular vacuolization (1), proteinaceous tubular casts (2) and abundant hyaline droplets (3) are depicted.
prolonged vasoconstriction of the renal vasculature, especially in the cortical and outer medullary regions of the kidney (Heyman et al., 1991; Nygren, 1992) in accordance with histopathological findings of the present study. On the other hand, Garofalo and colleagues performed an in vitro experimental study using proximal porcine renal tubules (LLC-PK1) and Mardin-Darby canine kidney distal tubular renal cells (MDCK) and did not demonstrate a correlation between contrast media administration and ROS production, as MDA levels, hydrogen peroxide and superoxide anion were not increased. At the same time antioxidant agents administration did not offer any cell protection despite the presence of cell necrosis and apoptosis (Garofalo et al., 2007). In an in vitro study in proximal tubular renal cells derived from mice and humans contrast-media administration did not augment MDA levels and antioxidant agents administration as SOD, catalase, glutathione and oxyypirinol failed to protect cells against renal tubular injury (Zager et al., 2006). Oxidative stress link to CIN is a topic of intense medical research with conflicting results both at animal studies level and with antioxidant administration in CIN patients at clinical level. Characterization of the rabbit CIN model in respect to oxidative stress tissue levels for the different anatomical kidney areas holds promise for a deeper knowledge of CIN. Systemic disturbances of the redox status in animals exposed to CM was also observed in our study and correlated with the respective changes in the CIN markers. Increased protein oxidation was reversible after 24 h, along with the slight decrease in TAC observed, as the organism tries to counterbalance the injury after the renal damage. The exact opposite pattern of TAC changes was observed at the renal tissue level. Along with elevated protein carbonyls in serum there is a drop in TBARS starting at 10 h, showing hindered lipid peroxidation as a counteracting defense mechanism. Studies in rats had shown the instant increase in protein carbonyls and TBARS significantly after a single dose of CM administration, even in animals without any prior intervention (Deng et al., 2015; Toprak et al., 2008).

Future studies should focus more on a novel and interesting pathway of nephroprotection that was targeted in the latest studies on antioxidant defense in CIN involves the transcription nuclear factor erythroid 2-related factor 2 (Nrf2). Nrf2, when it is translocated to the nucleus, stimulates transcription of genes that encode detoxifying and antioxidant circulating enzymes, such as NADPH, quinone oxidoreductase 1 (NQO1), GSH (glutathione) S-transferase (GST), heme oxygenase-1 (HO-1), glutamate cysteine ligase (GCL) and peroxiredoxin 1. All these enzymes contribute to cellular protection by removing ROS including superoxide anions, hydrogen peroxide and hydroxyl radicals (Saito, 2013). Expression analysis of these genes by qPCR will possibly show the total picture of redox status in different areas in the kidney of the rabbit.

5. Conclusion

The present study describes the involvement of oxidative stress in the mechanisms of CIN induction in the rabbit animal model, both at a systemic and the renal tissue level. Histopathological findings show clear signs of nephrotoxicity confined to the cortex and outer stripe of the outer medulla with characteristic tubular necrosis and vacuolization. Although the redox status of cortical area was more seriously affected, the medullary area seemed to suffer more generalized but less severe effects. As CIN prevention strategies in humans usually involve the use of anti-oxidants, elucidation of the redox mechanistic pathway in CIN merits further investigation.

Conflicts of interest

The authors have declared that no competing interests exist.

Acknowledgments

This research was conducted in the framework of the “Toxicology” MSc Program in the Department of Biochemistry - Biotechnology, School of Health Sciences at the University of Thessaly.

Transparency document

Transparency document related to this article can be found online at http://dx.doi.org/10.1016/j.fct.2017.07.058.

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