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

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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

Abbreviations: CAT, catalase; CIN, contrast-induced nephropathy; CM, contrast media; DNPH, 2,4-Dinitro-Phenyl-Hydrazine; DPPH, 2,2-Diphenyl-1-Picrylhydrazyl; DTNB, 5,50 -Dithiobis- (2-Nitrobenzoic Acid); EDTA, Ethylenediamine Tetraacetic Acid; GLC, glutamate cysteine ligase; GST, glutathione S-transferase; H&E, Hematoxylin & Eosin; HO-1, heme oxygenase-1; IV, intravenous; LLC-PK1, proximal porcine renal tubules; MDA, Malondialdeyde; MDCK, Mardin-Darby canine kidney distal tubular renal cells; NAD(P)H, nicotinamide adenine dinucleotide (phosphate); NQO1, quinone oxidoreductase 1; Nrf2, Nuclear factor (erythroid-derived 2)-like 2; PAS, Periodic- Acid Schiff; PC, Protein Carbonyls; ROS, Reactive Oxygen Species; SOD, superoxide dismutase; TAC, Total Antioxidant Capacity; TBARS, Thiobarbituric Acid Reactive Substances; 24hCIN group, animals euthanized at 24 h; 48hCIN group, animals euthanized at 48 h.

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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). Rabbits 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 iopromide 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. The supernatant was discarded and 1 mL of ethanol-ethyl acetate (1:1 v/v) 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 red 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 H_2O_2. Hemoglobin concentrations were determined by the hemoglobin cyanide (HiCN) 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.