L-Carnitine on contrast-induced nephropathy in diabetic rats

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Abstract

**Background:** The use of contrast media in coronary interventions favors diagnostic success. Contrast-induced nephropathy (CIN) is considered a frequent adverse event in the presence of the risk factor diabetes mellitus (DM). L-Carnitine is a lipid metabolism compound with a possible antioxidant effect.

**Objectives:** to evaluate the effect of L-Carnitine on contrast-induced nephropathy in rats with diabetes mellitus.

**Method:** twenty-eight male Wistar rats (250-300g) were randomized into four groups: Citrate (control); Diabetes (65mg/kg of intravenous streptozotocin); Diabetes+Iodinated contrast (meglumine sodium ioxathalamate 6ml/kg intraperitoneal-i.p, single dose); Diabetes+Iodinated contrast+L-Carnitine (50 mg/kg i.p. administered for 5 days). Physiological parameters were evaluated (weight, feed and water intake, glycemia and ratio of kidney weight to animal weight); renal function (inulin clearance, serum creatinine, urinary NGAL and urinary albumin); renal hemodynamics and oxidative profile (urine peroxides, thiols in renal tissue, thiobarbituric acid reactive substances in urine and urinary nitric oxide).

**Results:** Contrast-induced nephropathy was confirmed by reduced renal blood flow, increased renal vascular resistance, reduced inulin clearance, increased serum creatinine, and urinary NGAL with increased oxidative peroxide and consumption of antioxidant enzymes. L-Carnitine favored the improvement of renal function and hemodynamics with recovery of redox imbalance.

**Conclusion:** the use of L-Carnitine demonstrated a renoprotective and antioxidant effect.

**Keywords:** Diabetes mellitus; Acute Kidney Injury; Contrast Media; Oxidative Stress; Antioxidants.
Introduction

The indiscriminate use of Iodinated Contrast (IC) means in coronary interventions contributes to the third cause of in-hospital acute kidney injury (AKI); contrast-induced nephropathy (CIN), which has an incidence of 2% in the general population and 40% in the population with risk factors (1-4). CIN is defined by the KDIGO criteria as an increase in serum creatinine of 0.5 mg/dl (44mmol/l) or an increase of 25% in relation to the baseline value, evaluated within 48 hours after contrast administration (5).

IC has a direct nephrotoxic effect on the renal tubular epithelium resulting in decreased renal function, apoptosis and necrosis through the generation of reactive oxygen species (ROS) (6, 7). The pathophysiological mechanism is still not fully understood, but it is known that there is redistribution of the Na+/K+-ATPase pump from the basolateral to the luminal surface with an increase in sodium in the distal tubules, which favors vasoconstriction, which is also mediated by endothelin and adenosine. Additionally, blood viscosity changes in the presence of IC, favoring the plasticity of erythrocytes oriented towards the presence of microvascular thrombosis (8).

There are risk factors that increase contrast nephrotoxicity, such as previous renal dysfunction resulting from chronic kidney disease or comorbidities such as diabetes mellitus (DM), with the diabetic kidney being particularly susceptible to intense hypoxia and oxidative stress (7,9). The mechanism by which DM predisposes to contrast-induced nephropathy is still not fully elucidated, however, it may be related to the hemodynamic changes caused by the hyperglycemic action and by the decrease in the renal antioxidant capacity (7, 10).

In this context, this study aims to elucidate the action of L-Carnitine (β-hydroxy-γ-N-trimethyl ammonium-butyrate), which is a compound of lipid metabolism mainly produced by the liver and kidney. However, it can also be obtained through diet in foods such as red meat and dairy products or as a food supplement, currently being prescribed for athletes due to its benefits for energy metabolism (11 - 14).

L-Carnitine induces antioxidant proteins such as endothelial nitric oxide synthase (eNOS), heme oxygenase-1 (HO-1) and superoxide dismutase (SOD), and has a protective action against lipid peroxidation in membranes and against oxidative stress in membranes. endothelial cells (11, 15).

Considering the scarcity of current scientific evidence that investigates evaluating the renoprotective role of this compound, this investigation aims to identify the antioxidant action of L-Carnitine in contrast-induced nephropathy in the preclinical model of a risk factor, diabetes mellitus.
Methods

Animals and experimental design

Twenty-eight male Wistar rats, weighing between 250-290g, were maintained with free access to water, food (Nuvilab CR-1, Nuvital, Brazil) and remained under thermal conditions (25 °C/77 °F) with a light-light cycle. 12 hours dark. All procedures performed in this study are in accordance with the Ethical Principles of the National Council for the Control of Animal Experimentation (CONCEA) – and were approved by the Ethics Committee on the Use of Animals of the Faculty of Medicine of the University of São Paulo (CEUA - FMUSP), according to protocol nº 126/16. The animals were randomized into the following experimental groups: Citrate (Control): animals that received citrate buffer (streptozotocin-STZ vehicle) at pH 4.2, intravenously-i.v.; Diabetes (DM): animals that received 65 mg/kg of STZ (16), i.v., diluted in 0.1M citrate buffer at pH 4.2 on the 1st day of the protocol and continued in follow-up until the 28th day of the experimental protocol; Diabetes + Iodized Contrast (DM+IC): DM animals that received 6 ml/kg of iodinated contrast (meglumine sodium ioxathalamate intraperitoneally - i.p), a single dose on the 26th day of the experimental protocol; Diabetes + Iodinated Contrast + L-Carnitine (DM+IC+LCar): DM+IC animals that received 50mg/kg i.p of L-Carnitine on the 23rd day, for five days. The animals submitted to the DM model had their glycemia evaluated by hemoglucotest, 48 hours after induction, using Advantage reagent strips (Advantage – Roche®, Brazil). Animals that presented glycemia above 250mg/dl during this period and after one week signs of polyuria, polydipsia and polyphagia were considered to have diabetes mellitus. All DM animals had their body weight and blood glucose monitored for 4 weeks (28 days), once a week.

At the end of the protocol, the animals were placed in individual metabolic cages (27th day) for the collection of 24-hour urine for evaluation of renal function and oxidative metabolites. After removal from the metabolic cages (28th day), the animals were anesthetized with ketamine/xylazine (75mg/kg/10mg/kg; Anasedan®, Vetbrands) intraperitoneally and submitted to the Inulin clearance procedure, for renal function studies. Afterwards, a blood sample was collected by puncture of the abdominal aorta. During the procedure, the animals were kept on a heated surface at 38°C to avoid hypothermia. The left kidney was removed and refrigerated at -80°C for quantification of the antioxidant enzyme. The animal was euthanized using the physical method of exsanguination, in accordance with ethical standards for animal handling in a research laboratory (17).

Kidney function

Renal function was assessed using urinary inulin clearance, serum creatinine, lipocalin
associated with neutrophilic gelatinase (NGAL) and urinary albumin. After anesthetic induction, the animal received a dose of 100 mg/kg of diluted inulin and 10 mg/kg throughout the experiment at a rate of 0.04 ml/min through jugular vein puncture. After a 30-minute stabilization period, urine collection was started by catheterization of the bladder and a blood sample was collected every 60 minutes for two hours, for analysis of urinary and plasma inulin concentration using the Antrona method(18); the Jaffé colorimetric method was used to determine serum creatinine values and the results were expressed in mg/dl (19); the ELISA kit (Rat-NGAL, BioVendor, research and diagnostic products) was used to analyze the urinary NGAL, which was expressed in pg/mL (19); albumin concentrations in 24-hour urine samples were evaluated using commercially available enzyme-linked immunosorbent assays (ELISA) (Betil Laboratories, USA). The optical density of each sample was determined using an Ultra Microplate Reader (EL808; Bio-Tek Instruments, Winooski, VT, USA) and expressed as ng/24 h for urine concentrations (20).

Renal Hemodynamics

Concomitant with Inulin clearance, the carotid artery was punctured with a polyethylene catheter to measure mean arterial pressure (MAP) and heart rate (HR). At the end of the Inulin clearance, a laparotomy was performed to visualize the left renal pedicle and an ultrasonic micro-probe was placed to measure the renal blood flow (RBF). Renal vascular resistance (RVR) was calculated using the following formula: RVR = MAP / FSR(21).

Oxidative profile

The evaluation of urinary peroxides was performed using the FOX-2 method, in which the use of iron-xylenol orange oxidizes the Fe2+ ion and produces a blue-purple complex (α= 4.3 x 104 M-1 cm-1), values were expressed in nmol/g of urinary creatinine (22). The evaluation of urinary peroxides and urinary thiobarbituric acid reactive substances (TBARS) allows the identification of final products of the lipid peroxidation cascade that react in the presence of thiobarbituric acid in body fluids (α= 1.56 x 105 M-1 cm-1), values were expressed in nmol/g of urinary creatinine (23). The quantification of thiols was performed from samples of renal tissue ground and homogenized with a 10 nM solution of sodium acetate, 0.5% tween-20 and DTPA (pH 6.5). The results infer the amount of one of the main thiol compounds is glutathione (GSH) which acts as a redox buffer (23), the values were expressed in nmol/mg of total proteins. The synthesis of Nitric Oxide (NO) was evaluated by means of the quantification of nitrite (NO2-), a stable metabolite of NO, by the Griess method, the values were expressed in µmol/g of urinary creatinine (24).

Statistical analysis
Continuity variables were expressed as mean and standard deviation (±). The assumptions of normality and homogeneity were performed using the Shapiro-Wilk and Levene test and the variance between groups was analyzed using the ANOVA One Way test, followed by the post-hoc Student-Newman-Keuls (SNK) multiple comparisons of the program Statistical Graph-Pad Prism version-8 for Windows®. Values of \( p < 0.05 \) were considered significant.

**Results**

**Physiological Parameters**

The groups that were induced to DM showed signs of polyphagia, polydipsia, polyuria, renal hypertrophy and chronic hyperglycemia (\( p < 0.05 \)). L-Carnitine resulted in a decrease in kidney weight and kidney weight/animal weight ratio compared to the DM+CI group (\( p < 0.05 \)). Data summarized in table 1.

Table 1. Physiological parameters of Control groups; MD; DM+CI and DM+CI+LCar. São Paulo, 2023.

<table>
<thead>
<tr>
<th>Groups</th>
<th>n</th>
<th>Glicemia (mg/dl)</th>
<th>Kidney wight (g)</th>
<th>Kidney wight / Body weight (%)</th>
<th>Food Intake (g)</th>
<th>Water Intake (ml)</th>
<th>Urinary Output (ml/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>7</td>
<td>102±35</td>
<td>1.31 ± 0.07</td>
<td>0.35 ± 0.05</td>
<td>22.7 ± 1.8</td>
<td>30.0 ± 6.4</td>
<td>0.013 ± 0.003</td>
</tr>
<tr>
<td>DM</td>
<td>7</td>
<td>489±23*</td>
<td>1.54 ± 0.16</td>
<td>0.59 ± 0.06*</td>
<td>34.0 ± 4.0*</td>
<td>122.8 ± 31.4*</td>
<td>0.059 ± 0.009*</td>
</tr>
<tr>
<td>DM + CI</td>
<td>7</td>
<td>501±18*</td>
<td>1.72 ± 0.14*†</td>
<td>0.64 ± 0.13*</td>
<td>39.1 ± 4.9*</td>
<td>149.2 ± 25.2*</td>
<td>0.071 ± 0.009†</td>
</tr>
<tr>
<td>DM+CI+LCar</td>
<td>7</td>
<td>472±30*</td>
<td>1.58 ± 0.11‡</td>
<td>0.52 ± 0.04‡</td>
<td>31.7 ± 3.7*</td>
<td>120.7 ± 14.8*</td>
<td>0.064 ± 0.010*</td>
</tr>
</tbody>
</table>

*\( p < 0.05 \) versus Control; †\( p < 0.05 \) versus DM; ‡\( p < 0.05 \) versus DM+CI.

**Effect of L-Carnitine on Kidney Function**

The DM+IC group showed a significant increase in serum creatinine and urinary NGAL biomarkers with a respective decrease in glomerular filtration rate (GFR) (Inulin clearance) \( p < 0.05 \). L-Carnitine demonstrated recovery of renal function through significant elevation of GFR and reduction in serum creatinine and urinary NGAL values \( p < 0.05 \). Urinary albumin showed increased concentrations in the groups that were induced in the model of diabetes mellitus \( p < 0.05 \). Data were expressed in Table 2.
Table 2. Renal function of Control groups; MD; DM+CI and DM+CI+LCar. São Paulo, 2023.

<table>
<thead>
<tr>
<th>Grupos</th>
<th>n</th>
<th>Creatinina sérica (mg/dl)</th>
<th>Clearance de Inulina 100g (ml/min)</th>
<th>NGAL Urinário (pg/mL)</th>
<th>Albumina Urinária (ng/24h)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Controle</td>
<td>7</td>
<td>0,33 ± 0,09</td>
<td>0,89 ± 0,23</td>
<td>45,6 ± 0,9</td>
<td>0,48 ± 0,18</td>
</tr>
<tr>
<td>DM</td>
<td>7</td>
<td>1,04 ± 0,18*</td>
<td>0,52 ± 0,12*</td>
<td>53,7 ± 21,0</td>
<td>2,97 ± 0,98*</td>
</tr>
<tr>
<td>DM+CI</td>
<td>7</td>
<td>1,29 ± 0,88†</td>
<td>0,16 ± 0,04†</td>
<td>138,1 ± 74,8*†</td>
<td>3,78 ± 0,81*</td>
</tr>
<tr>
<td>DM+CI+LCar</td>
<td>7</td>
<td>0,44 ± 0,09†</td>
<td>0,51 ± 0,21†</td>
<td>65,4 ± 12,2*†</td>
<td>3,19 ± 0,33*</td>
</tr>
</tbody>
</table>

*p<0.05 versus Controle; †p<0.05 versus DM; ‡p<0.05 versus DM+CI.

**Effect of L-Carnitine on the oxidative profile**

In figure 1, urinary peroxides and TBARS showed a significant increase in the DM and DM+CI groups compared to the control group (Peroxides: DM=13.5 ± 5.7 and DM+CI=21.3 ± 9.8 vs Control =2.0 ± 0.9; TBARS: DM=12.9 ± 3.0 and DM+CI=22.5 ± 5.2 vs Control=0.8 ± 0.1). The additional increase in the DM+CI group stands out (Peroxides: DM+CI=21.3 ± 9.8 vs DM=13.5 ± 5.7; TBARS: DM+CI=22.5 ± 5.2 vs DM=12.9 ± 3.0). The DM+CI+LCar group showed a significant reduction in oxidative metabolites when compared to the DM and DM+CI groups (Peroxides: DM+CI+LCar=4.5 ± 2.4 vs DM=13.5 ± 5.7 and DM +CI=21.3 ± 9.8; TBARS: DM+CI+LCar=0.6 ± 0.1 vs DM=12.9 ± 3.0 and DM+CI=22.5 ± 5.2). As for urinary NO, the DM+IC and DM+IC+LCar groups showed a significant increase in relation to the Control groups (DM+IC=142.8 ± 33.2 and DM+IC+LCar=130.2±38, 4 vs Control=37.6 ± 10.1 and DM=70.8 ± 14.1). Regarding thiols, the DM and DM+CI groups showed a significant decrease in antioxidant reserve when compared to the Control group (DM=17.2 ± 2.9 and DM+CI=10.6 ± 2.1 vs Control=33 .8 ± 6.1). The group treated with L-Carnitine revealed a reestablishment of the antioxidant reserve when compared to the DM and DM+CI group (DM+CI+LCar=36.0 ± 13.9 vs DM=17.2 ± 2.9 and DM+ CI=10.6 ± 2.1).
Figure 1. Oxidative profile of Control groups; MD; DM+CI and DM+CI+LCar. São Paulo, 2023. (A) Urinary Peroxides (nmol/gr cr), (B) Urinary TBARS (nmol/gr cr), (C) Thiols (nmol/mg total pr), (D) Urinary NO (nM/mg cr). Cr: creatinine. Pr: protein. NO: nitric oxide. U: urinary. Data were expressed as mean±standard deviation for each group (n=7). *p<0.05 versus Control; †p<0.05 versus DM; ‡p<0.05 versus DM+CI.

Discussion

Chronic noncommunicable diseases (NCDs), such as DM, systemic arterial hypertension (SAH) and cancer, have progressively become a challenge for global public health in both spheres of health action: prevention and treatment. In this sense, comorbidities and risk factors for cardiovascular events that require hemodynamic interventions with IC means become important, characterizing CIN as an in-hospital iatrogenic event (4,7,9). In the search for inexpensive therapeutic alternatives, the present study revealed that L-Carnitine, which has been widely used as a nutritional supplement by athletes (13,25), demonstrated a renoprotective effect through recovery of renal function and hemodynamics, reduction of oxidative metabolites and restoration of endogenous antioxidant reserve in the preclinical model of DM with IC. Chronic hyperglycemia is considered an important precursor of renal pathophysiological mechanisms, as it increases NADPH oxidase levels and mitochondrial protein glycation resulting in oxidative stress, inflammation and cell apoptosis (26,27). The present study revealed clinical signs of DM such as polyphagia, polydipsia, polyuria, albuminuria and renal hypertrophy due to streptozotocin induction. These data corroborate the validation and potential translation of the experimental model for clinical DM comorbidity.

In this sense, in the presence of a risk factor for DM, the administration of a single dose of contrast medium sought to mimic the clinical event of percutaneous coronary intervention (PCI)
with IC. Despite generally being well tolerated in healthy kidneys, ICS infusion can lead to the development of CIN (4, 28, 29). Mechanisms responsible for the development of CIN include the cytotoxic effect on renal tubules; hemodynamic changes; rheological alterations with the production of ROS and renal ischemia (6, 8, 30).

The animals that received IC showed a significant increase in serum creatinine, which is the standardized biomarker for evaluating renal function in the clinic. Therefore, its alteration is considered a trigger for the clinical management of interventions in the search for protection of the renal parenchyma (3, 4). Moitinho et al demonstrated the presence of CIN by means of a 25% increase in serum creatinine in relation to the baseline value after 48 h after PCI (29). On the other hand, studies with new biomarkers have been used for earlier identification of kidney damage, since, generally, changes in serum creatinine levels can occur over days to weeks. The NGAL biomarker stands out, which is a glycoprotein belonging to the lipocalin superfamily, which can be found in blood and urine (31). Adults and children undergoing PCI showed the predictive role of NGAL in the early diagnosis of CIN (32). This investigation revealed a significant increase in urinary NGAL in animals that received IC, corroborating other preclinical studies (16,19). Additionally, in the experimental context, inulin clearance constitutes the gold standard for GFR assessment. It is a complex technique, but it absolutely reveals glomerular and tubular activity. Couto et al and Fernandes et al demonstrated an increase in inulin clearance in animals that were induced to CIN (19, 21). Additionally, an increase in urinary albumin was observed in the aforementioned groups, with albuminuria closely associated with changes in basal glomerular thickening in DM (27). Recent studies have demonstrated a significant reduction in renal function in diabetic rats treated with IC (10, 19, 21). In this unfavorable scenario of renal dysfunction, L-Carnitine reversed the parameters described above, inferring a renoprotective action that can be via anti-inflammatory and vasodilator.

In renal hemodynamics, a progression in the reduction of renal blood flow was observed between the DM and DM+IC groups, with inversely proportional values in the evaluation of renal vascular resistance. Nephrotoxic drugs act directly on tubular cells and vascular endothelium. There is a chemical interaction in favor of cell injury, which generates the release of inflammatory cytokines IL-6, TNF-α and reactive oxygen species such as hydrogen peroxide and superoxide radical (6, 8, 33, 34). Hypoxia sets in and generates an imbalance between vasodilator substances (nitric oxide and prostaglandins) and vasoconstrictors (endothelin, adenosine, vasopressin, angiotensin II and dopamine – 1), which are also activated via the endothelium by contrast osmolarity, contrast viscosity and erythrocyte aggregation that results
in decreased oxygen supply (6,35). Therefore, CIN's mechanism of action is associated with concomitant cellular and hemodynamic events that converge to the worsening of renal function. Elevated glucose, in addition, is related to increased action of the renin-angiotensin system, intensifying vasoconstriction. Studies that evaluated hemodynamic parameters in models with cytotoxic drugs revealed results similar to those found in this investigation (10,21,33). L-Carnitine improved renal perfusion with decreased renal vascular resistance. Experimental investigations have demonstrated that L-Carnitine and its derivative acetylcarnitine influence the activities of mitochondrial metabolism by inhibiting oxidation, adipogenesis and cell death (36,37). Barhwal et al demonstrate the renoprotection of L-Carnitine against the toxicity of drugs that damage mitochondria (38) and Furuno et al revealed that beta-oxidation stimulated by L-Carnitine reduced the toxic effects of fatty acids (39). Therefore, investigations with L-Carnitine demonstrate that it is possible to have an increase in the synthesis of mitochondrial ATP and formation of antioxidant enzymes, containing the advance of the injury in the renal tissue (12, 36).

Based on its action on energy metabolism, L-Carnitine has been used as a nutritional supplement considering its benefits in restoring antioxidant defenses, increasing protein and albumin levels and improving nutritional status and muscle (including smooth and cardiac muscle) (37). In clinical practice, its supplementation is often used to manage endogenous carnitine deficiency in hemodialysis patients with chronic heart failure and genetic disorders (40). Complementarily, in clinical studies the administration of L-Carnitine has been associated with improvements in cardiovascular injury in patients on hemodialysis, as well as in patients with myocardial infarction, angina, enlargement or dilation of the left ventricle and arrhythmias (37,40,41). Its antioxidant effects have also been demonstrated in preclinical models, as well as in the present study (36,37).

The perpetuation of hypoxia favors ATP depletion and generation of ROS (OH-, O2- and H2O2), which are harmful to the renal parenchyma in favor of lipid peroxidation, resulting in death, apoptosis and DNA damage(42). In this investigation, the elevation of urinary peroxides and TBARS confirm the participation of free radicals in diabetic animals, being intensified in those that received IC. Additionally, the analysis of non-protein soluble thiols in the kidney tissue demonstrated the consumption of the endogenous antioxidant reserve, through the reduction of this variable in the DM and DM+IC groups. On the other hand, treatment with L-Carnitine reversed these parameters.

Regarding the NO variable, increases were observed in the diabetic groups, with a slight numerical reduction in the group treated with L-Carnitine. The participation of nitric oxide in
renal injuries is still little known. It is known that NO can interact with the O2- radical and form nitrite peroxide (ONOO-), which reacts with proteins and induces oxidation, reducing the bioavailability of this molecule (NO), which has vasodilator properties (43). Some studies have shown that L-Carnitine has anti-inflammatory and antioxidant action by increasing the production of nitric oxide and inducing the enzymes superoxide dismutase catalase and glutathione peroxidase (11, 12,15).

Conclusion

The results of the present study reinforce that DM is an important risk factor for nephrotoxicity caused by drugs such as iodinated contrast. It was possible to observe that the presence of DM contributed to the dysfunctions of renal function, hemodynamics and oxidative stress in the development of CIN, evidenced by the potentialization of renal damage when IC was administered in the diabetic group. Pre-conditioning with L-Carnitine in diabetic animals with CIN showed its renoprotective and antioxidant effect, confirming the hypothesis of this study and highlighting it as a possible therapeutic alternative to revert or prevent CIN in the DM risk factor, as well as to corroborate its nutritional supplementation in clinical practice. However, new preclinical studies, with encouragement from these authors for clinical studies, are necessary to strengthen the results obtained in this investigation.

Authors' contribution


Potential conflict of interest

The authors declare that there is no conflict of interest.

References


