Protective effect of sodium bicarbonate on radiological contrast medium-induced nephropathy in rats

ABSTRACT
Radiological iodinated contrasts (IC) agents cause acute kidney injury (AKI). To evaluate the renoprotective effect of sodium bicarbonate (Bic) on renal function (creatinine clearance [Clcr], Jaffé, and Clcr/mL/min⁻¹·10⁵ g⁻¹) and the oxidative profile (peroxide excretion, urinary peroxides, urinary malondialdehyde, FOX-2 expression, and thibobituric acid reactive substance [TBARS]) in rats treated with an IC agent. Adult male Wistar rats weighing 250–300 g were treated once daily for 5 days with one of the following treatments: saline (0.9%, 3 mL/kg/d, intraperitoneally [i.p.]), IC agent (sodium and meglumine ioxitalamate, 3 mL/kg, i.p.), Bic + Saline (3 mL/kg Bic, i.p., 1 h before and after saline treatment), and Bic + IC (3 mL/kg Bic, i.p., 1 h before and after the IC treatment). The IC agent induced AKI, and the antioxidant renoprotective effect of Bic was confirmed (Clcr/UBARS/Urinary peroxide: saline group, 0.59 ± 0.03 A/0.11 ± 0.02 A/1.29 ± 0.24 A vs Bic+Saline group, 0.58 ± 0.03 A/0.13 ± 0.02 A/1.32 ± 0.64 A; IC group, 0.22 ± 0.02 A/0.19 ± 0.02 A/4.77 ± 0.24 A vs Bic+CI group, 0.51 ± 0.04 A/0.13 ± 0.13 ± 0.80 ± 0.04 A; p<0.05). The protective effect of Bic in the IC-induced AKI was confirmed; hence, Bic administration may be considered as a therapeutic option for patients undergoing IC-enhanced radiography.

DESCRIPTORS
Acute kidney injury
Contrast media
Iodine
Sodium bicarbonate
Therapeutics

RESUMO
Contrastes radiológicos iodados - CI são causa de lesão renal aguda – LRA. Avaliar o efeito renoprotetor do bicarbonato de sódio (Bic) sobre a função renal (clearance de creatinina, Jaffé, Clcr/mL/min/100mg) e o perfil oxidativo (excreção de peróxidos, PU e de malonialdeído urinários, FOX-2 e TBARS, nmol/mgCr ) em ratos com CI. Ratos machos adultos Wistar, 250-300g, tratados 1x/dia, por 5 dias, foram divididos nos grupos: Salina (solução salina 0,9%, 3ml/kg/dia, intraperitoneal-i.p.); CI (ioxitalamato de meglumina e sódio, 3ml/kg,i.p.); Bic+Salina (Bic 3ml/kg,i.p. 1 hora antes e 1 hora depois da Salina); Bic+CI (Bic 3ml/kg,i.p. 1 hora antes e 1 hora depois do CI). CI induziu LRA e o Bic confirmou seu efeito renoprotetor antioxidante (Clcr/TBARS/PU Salina: 0.59±0.03/0.11±0.02/2.98±0.24 vs Bic+Salina 0.58±0.03/0.13±0.02/1.97±0.64 vs CI 0.22±0.02/0.19±0.02/4.77±0.24 vs Bic+CI 0.51±0.04/0.13±0.80 ±0.04 A; A/R<0.05). O Bic confirmou efeito protetor na LRA por CI, podendo ser considerado como possibilidade terapêutica para pacientes submetidos a CI.

DESCRIPTEOS
Lesão renal aguda
Meios de contraste
Iodo
Bicarbonato de sódio
Terapêutica

RESUMEN
Contrastes radiológicos iodados - CI son causa de lesión renal aguda–LRA. Evaluar el efecto renoprotector del bicarbonato de sodio (Bic) en la función renal (clearance de creatinina, Jaffé, Clcr/mL/min/100mg) y el perfil antioxidativo (excreción de peróxidos, PU y de malonialdehído urinarios, FOX-2 e TBARS, nmol/mgCr ) en ratones con CI. Ratones machos adultos Wistar, 250-300g, tratados 1x/día durante 5 días, fueron divididos en grupos: Salina (solución salina 0,9%, 3ml/kg/día, intraperitoneal-i.p.); CI (ioxitalamato de meglumina y sodio, 3ml/kg,i.p.); Bic+Salina (Bic 3ml/kg,i.p. 1 hora antes y 1 hora después de la Salina); Bic+CI (Bic 3ml/kg,i.p. 1 hora antes y 1 hora después del CI). CI indujo LRA y el Bic confirmó su efecto renoprotector antioxidante (Clcr/TBARS/PU Salina: 0.59±0.03/0.11±0.02/2.98±0.24 vs Bic+Salina 0.58±0.03/0.13±0.02/1.97±0.64 vs CI 0.22±0.02/0.19±0.02/4.77±0.24 vs Bic+CI 0.51±0.04/0.13±0.80 ±0.04 A; A/R<0.05). El Bic confirmó efecto protector en la LRA por CI, pudiendo considerársele posibilidad terapéutica para pacientes sometidos a CI.

DESCRIPTEOS
Lesión renal aguda
Medios de contraste
Iodo
Bicarbonato de sódio
Terapéutica

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INTRODUCTION

Acute kidney injury (AKI) is a complication with a high incidence rate among patients admitted to intensive care units (ICU), ranging from 17% to 35% of cases. It is characterized by high morbidity and mortality, ranging from 50% to 90%, which have not shown significant improvement for at least the past 2 decades[1]. These data are sufficiently significant to have justified ongoing investments in research to elucidate their physiopathological intimacy.

Some progress can already be seen in the clinical setting in the application of new diagnostic nomenclature in which new biomarkers and proposed systems of classification of the severity of the injury have been revaluated. These initiatives have confirmed that only prophylactic measures may bring some impetus for the epidemiology of this injury, such as the identification of agents that demonstrate protective roles in AKI.

The complexity of the pathological mechanisms of AKI and the scientific advances of the diagnostic and therapeutic methods for AKI, which increase the survival of the patient population, led to the use of drugs. Nephrotoxic drug agents, despite the disadvantage of AKI development as an adverse effect, are essential not only in the treatment of diseases such as bacterial infections, inflammations, and cancer, but also in their diagnoses, as in the case of imaging tests using iodine-based radiological contrast media. Such agents are currently considered the second most nephrotoxic agents used in clinical practice, demonstrating its relevance to the evolution of renal dysfunction.

Iodinated contrast (IC) agents are used widely in diagnosis. Its use is emphasized in coronary angiography, intravenous urography, computed tomography (CT), and myelography. These compounds are known to absorb radiation from diagnostic tools in proportion to the iodine concentration in the solution. This constitution complicates the interaction with the body. However, adverse effects such as systemic hypersensitivity reactions, vascular effects, and adverse reactions in the heart and kidney have been observed in clinical practice. With regard to the kidney, IC agent-induced nephropathy (CIN) has taken on alarming clinical dimensions, being considered the third leading cause of AKI in current hospital settings.

With regard to the kidney, IC agent-induced nephropathy (CIN) has taken on alarming clinical dimensions, being considered the third leading cause of AKI in current hospital settings.

IC agents present physicochemical characteristics that allow classifying them according to dissociation in water (ionic or nonionic), iodine concentration, molecular size (monomer or dimer), and osmolality compared with the solution (high, low, or iso-osmolar)[6]. Thus, IC agents are classified into 4 types as follows: ionic high-osmolar, ionic low-osmolar, nonionic low-osmolar, and nonionic iso-osmolar. Among the many physicochemical characteristics, the osmolality of the IC solution is, in principle, the most related to the risk of nephrotoxicity[7].

However, the superiority of low osmolality IC solution in terms of toxicity remains unclear. When administered to patients with normal renal function in the absence of other factors, both contrast agents (high and low osmolality) had the same risk of nephrotoxicity. The advantage of the low-osmolality IC solution was evidenced when used in patients with preexisting renal failure or other risk factors, who had a lower incidence of AKI after receiving the low-osmolality IC medium[8].

The incidence of CIN, approximately 10% of hospitalized patients[10], may increase to >50% when the patient presents risk factors[3], with high serum creatinine level being the highest impact factor[9]. Besides creatinine level, other factors contribute to the occurrence of AKI, including advanced age, dehydration, concomitant administration of nephrotoxic drugs, large volume of administered contrast medium, hyperosmolar contrast agent administered intravenously or arterially (cardiac and abdominal aortic angiography), reexposure to the contrast agent in a short interval of time, or even pathological conditions such as multiple myeloma, heart failure, and liver disease, glomerulopathies, or systemic arterial hypertension[10]. Given that patients in need of diagnostic intervention with the use of a contrast agent are rarely free of all the aforementioned factors, the number of nephrotoxicity cases by IC administration is a concern.

CIN is characterized by a decline in acute renal function, in the absence of other causes, with a relative increase of 25% or absolute 0.5 mg/dL in serum creatinine level compared to previous levels or serum creatinine level increase of 50% or 1 mg/mL[9] from 24 to 48 h or from 72 h to 7 days after administration of the agent, with a duration at these levels of 2–5 days, returning to baseline levels in 10–14
days for mild cases and in 14–21 days for severe cases(2,8,9). Complications may range from discrete transient alterations in creatinine level to the need for temporary or permanent renal replacement therapy(2,8,10), with an alarming mortality prognosis of 34%.

The pathophysiological mechanisms of CIN in humans are complex and not completely understood. Studies confirm its multifactorial pathogenesis. The combined action of selective hypoxemia, caused by glomerular vasoconstriction, and obstruction and direct tubular injury resulting from precipitation of proteins and oxidative mechanisms seems to describe in a more coherent and acceptable manner the pathophysiological mechanism of CIN(11-12). The complete dissociation of the effects of tubular injury and the secondary effects of renal ischemia are difficult to achieve in practice as well as in animal models(13).

Given these complications, the strengthening of prophylactic measures for the prevention of CIN is invigorated because most procedures using contrast media are programmed and are therefore capable of identifying the characteristics that cause CIN by historical review and physical examination. Such attention allows the adoption of preventive measures such as adjusting the volume of the contrast, choosing the nature of the compound, hydration precautions before and after examination, restriction in the concomitant use of anti-inflammatories, nonhormonal, and other nephrotoxic drugs(13), and repeated exposure to the radiological agent in short intervals. However, considering that the exclusion of risk factors is almost impractical in clinical emergency diagnostic scenarios, preventive therapeutic measures are the only alternative.

Some pharmacological maneuvers took shape in the clinic seeking to change the trifold urgency, need, and non-modifiable risk. Emphasized among these maneuvers are the use of diuretics (furosemide/mannitol/captopril), fentanyl, atrial natriuretic peptide, calcium channel blockers, nonselective antagonists of the endothelin receptor, and antioxidants (N-acetylcysteine and sodium bicarbonate), which has the most data available.

The interest of this study is the antioxidant effect of sodium bicarbonate because of its superiority to the commonly used solution, 0.9% sodium chloride(14,15). This superiority can be explained by the protective effect of sodium bicarbonate in the intratubular fluids, reducing the formation of free radicals(2,12). This antioxidant characteristic of sodium bicarbonate emphasizes preventative expression in AKI by IC administration and can result in significant clinical benefits in diagnostic interventions.

Considering the high prevalence of CIN and the difficulty of establishing consistent preventive measures, the continuing focus of investigations has been on drugs capable of alleviating this complication, with a more recent emphasis on sodium bicarbonate based on the above-mentioned evidence.

With regard to preventive aspects, the nurse assumes the principal role in this health-promoting action in different clinical settings. Professionals in the ICU, where patients with AKI are normally admitted, have the opportunity for care improvement and risk prevention as well as the preservation of organ function through the knowledge of the pathophysiological mechanisms involved in acute syndromes and improvement of technical and scientific skills. The details of these mechanisms and the possible drug interference in the intensive care of patients at risk of or with AKI will influence the epidemiological aspects of this disease. Moreover, research undertakings such as experiments on animal models, as proposed in this study, will allow the academic nursing setting to increase the scope of its science and expand the possibilities for dissemination of these results.

This study aimed to verify the efficacy of sodium bicarbonate treatment by measurement of the renal function and renal oxidative profile of rats treated with iodinated radiological contrast using a creatinine clearance test, measurement of peroxides and urinary malondialdehyde (MDA).

**METHOD**

We performed a quantitative study with an experimental design after obtaining approval from the Ethics Committee on Animal Experimentation of the Institute of Biological Sciences, University of São Paulo, under protocol no. 43/08/CEEA. The study was performed at the Laboratory of Experimental Research in Animal Models (LEMA) at the School of Nursing of the University of São Paulo.

We used 8.4% sodium bicarbonate (therapeutic and homeopathic compounding pharmacy, Herbes Naturelles), Telebrix Coronar iodinated contrast medium, 100-mL sodium and meglumine ioxitalamate (50 Cueber), and adult male Wistar rats weighing 250–300 g that were divided into 4 groups to receive the following treatments:

- Saline (controls): 3-mL·kg⁻¹·day⁻¹ 0.9% saline (SF) intra-peritoneally (i.p.) once daily for 5 days
- Bicarbonate + saline (Bic + Saline): 3-mL·kg⁻¹·day⁻¹ sodium bicarbonate solution, 1 h before and after the administration of 0.9% saline solution (SF), 3 mL·kg⁻¹·day⁻¹, once daily for 5 days
- IC: 3 mL·kg⁻¹·day⁻¹ IC sodium and meglumine ioxitalamate (SMI) i.p., once daily for 5 days
- Bicarbonate + IC (Bic + IC): 3 mL·kg⁻¹·day⁻¹ sodium bicarbonate solution, 1 h before and after the administration of SMI i.p., once daily for 5 days

All the groups received the drugs at the same time, had free access to standardized food and water, and remained in adequate thermal conditions. On treatment day 5, the animals were placed in a metabolic cage after drug(s) administration for collection of 24-h urine samples, for subsequent measurement of peroxides and urinary malondialdehyde (MDA).
verification of creatinine clearance (Clcr) by the Jaffé method, using absorbance spectrophotometry at 520 nm. Clcr along with plasma and urinary creatinine levels were as parameters for the evaluation of overall renal function.

After 24 h in the metabolic cage, the animals were anesthetized for puncture of the abdominal aorta by laparotomy. Approximately 10 mL of aortic blood was collected, which was the sample used for the measurement of plasma creatinine level. At the end of the procedure, the animals were euthanized according to the recommended regulations for the use of experimental animals.

The oxidative profile was interpreted based on the peroxide and urinary MDA levels. The measurement of urinary peroxide levels was performed by FOX-2, which involves the determination of hydroperoxide levels by the iron-xylenol orange method that exhibits high iron ion selectivity. This reaction produces a bluish purple color that is read by absorbance spectrophotometry at 560 nm. The values were calculated using the coefficient for urinary peroxides of 4.3 \times 104 Mcm⁻¹ and stabilized per gram of urinary creatinine.

The dosage of the thiobarbituric acid reactive substance (TBARS) was used to measure the urinary excretion of MDA. MDA is an aldehyde frequently analyzed in quantitative and qualitative analytical methods for determining the levels of lipid peroxidation. MDA is a major product of lipid peroxidation cascades and can be detected by various methods including the use of a high-pressure ultraviolet light in a high-performance liquid chromatography system and thiobarbituric acid, which reacts with various substances, including MDA (TBARS).

The analysis of variance (ANOVA) and Tukey multiple comparison test were used for the statistical analysis of the data from all the groups.

**RESULTS**

The results as presented in the tables are expressed as mean and standard deviation values. The significance level was set at p = 0.05. The data in Table 1 indicate no significant variations in weight and urine flow between the groups.

**Table 1** – Body weight, urinary flow, and overall renal function of the Saline, Bic + Saline, IC, and Bic + IC groups - São Paulo, 2011

<table>
<thead>
<tr>
<th>Groups</th>
<th>n</th>
<th>W (g)</th>
<th>U (ml/min)</th>
<th>Clcr/100 g (ml/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saline</td>
<td>9</td>
<td>253±14</td>
<td>0.0137±0.0004</td>
<td>0.59±0.03</td>
</tr>
<tr>
<td>Bic + Saline</td>
<td>9</td>
<td>251±9</td>
<td>0.0142±0.0004</td>
<td>0.58±0.03</td>
</tr>
<tr>
<td>IC</td>
<td>9</td>
<td>255±8</td>
<td>0.0088±0.0009</td>
<td>0.22±0.02</td>
</tr>
<tr>
<td>Bic + IC</td>
<td>5</td>
<td>252±14</td>
<td>0.0135±0.0004</td>
<td>0.51±0.04</td>
</tr>
</tbody>
</table>

Key: W, weight; U, urine flow; and Clcr/100 g, creatinine clearance/100 g of body weight
The data represent the mean ± standard error

The results show that the saline and Bic + Saline groups showed no difference in the mean Clcr/100 g (0.59 ± 0.03 vs. 0.58 ± 0.03), indicating that the administration of Bic did not impair renal function in these animals. Thus, the Bic + Saline group was used as a control for this study.

The group of rats that received the IC agent alone showed a significant reduction in glomerular filtration rate compared with the saline group, rendered by the mean Clcr/100 g, which was significantly lower (0.22 ± 0.02 vs. 0.59 ± 0.03). This fact confirmed the episodes of nephrotoxicity by IC administration while maintaining urinary flow. Comparing the group of animals treated with the IC medium and that pretreated for 5 days with Bic + IC, a significant improvement by 43% was observed in the mean Clcr/100 g (0.22 ± 0.02 vs. 0.51 ± 0.04), confirming the renoprotective effect of the antioxidant Bic when administered before and after the IC treatment.

The urinary peroxide (WU) levels measured by the FOX-2 method demonstrated that the mean WU in the IC group was 3.6 times greater than that in the saline group (4.77 ± 0.24 vs. 1.29 ± 0.24). The opposite can be observed in the group that received pretreatment with Bic (Bic + IC), which maintained a 2.6 times lower mean, indicating a significant reduction compared with the IC group (1.80 ± 0.04 vs. 4.77 ± 0.24).

**Table 2** – Urinary TBARS values in the saline, Bic + Saline, IC, and Bic + IC groups - São Paulo, 2011

<table>
<thead>
<tr>
<th>Groups</th>
<th>n</th>
<th>TBARS (nmol/mgCr)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saline</td>
<td>9</td>
<td>0.11±0.02</td>
</tr>
<tr>
<td>Bic + Saline</td>
<td>9</td>
<td>0.13±0.02</td>
</tr>
<tr>
<td>IC</td>
<td>9</td>
<td>0.19±0.02</td>
</tr>
<tr>
<td>Bic + IC</td>
<td>5</td>
<td>0.13±0.03</td>
</tr>
</tbody>
</table>

The data represents the mean ± standard deviation

The oxidative status assessed in this study by measuring the urinary MDA (TBARS) was significantly greater in
the group treated only with IC medium than in the saline group (0.19 ± 0.02 vs. 0.11 ± 0.02). This fact reiterated that the AKI by IC administration has an oxidizer component in its pathophysiological aspect. In contrast, as expected, the group pretreated with Bic + IC was similar to the control group with regard to this parameter (BicIC + Saline; 0.13 ± 0.02 vs. 0.13 ± 0.02).

**DISCUSSION**

IC agents are widely used in clinical practice as diagnostic aids, and their deleterious effects are well known[10]. In particular, CIN, currently the third leading cause of in-hospital AKI, presents as a complication of IC administration, with an alarming mortality rate of 34%(13).

Several studies show that the pathophysiological mechanism of CIN is multifactorial, a synergistic combination of both vascular factors resulting from the glomerular and tubular vasoconstrictions associated with cellular damage by direct toxicity, tubular obstruction, and osmotic alterations(3,12). Several causes have been described for the occurrence of these vascular and tubular factors, such as an increase in the vasoconstrictor agents (endothelin and angiotensin II), a decrease in the vasodilators (nitric oxide and prostaglandins), and the formation of reactive oxygen species (ROS).

ROS are produced naturally and continuously in small quantities by the body in oxidative metabolic processes(16). They are generated in the cytoplasm, mitochondria, or cell membrane by the catalytic action of enzymes during the electron transfer processes occurring in cellular metabolism and by exposure to exogenous factors(17). Under normal conditions, these free radicals are extremely useful and essential for cellular growth and adaptation, activation of the immune system, drug detoxification, and production of the relaxing factor—nitric oxide—derived from the endothelium(16).

However, when these substances are found in high concentrations in the bloodstream, they become the main mediators of injuries to tissues because of the damage caused by the proteins, and to the DNA and cells by the lipid peroxidation of the membrane. This results in oxidative stress production in the tissues and cells when there is an exacerbated production of the ROS or a decrease in antioxidant generation or a possible combination of these 2 causes(18).

The experimental model of AKI reproduced in this study, through the administration of IC medium in rats, was aimed at confirming the occurrence of CIN as determined through the significant Clcr reduction in the IC group and oxidative mechanism involvement by the significant elevation of the cellular injury markers, MDA and urinary peroxide, both of which are oxidative stress markers.

Complications generated by the oxidative stress range from simple discrete changes in renal function to more severe kidney failure. From 13% to 50% of patients who progress to permanent dialysis require renal replacement therapy(10). However, the choice of traditional treatment strategies such as the function replacement methods, has not provided satisfactory results on the impact on the epidemiological aspect of the injury, as there has been a persistence of AKI mortality rates for >2 decades, which reinforces the need for studies with a preventive approach.

In recent years, antioxidant agents have been highlighted in the field, and more recently, the use of sodium bicarbonate has been asserting itself as an interesting therapeutic option. As a result, evaluating the effect of antioxidants such as sodium bicarbonate may effectively prevent or reduce CIN(19). This is because several studies have demonstrated its performance in intratubular fluid, reducing the formation of free radicals generated by the contact of the contrast medium with the renal tubules(3,11).

Scientific evidence shows the superiority of the Bic solution as compared with sodium chloride at 0.9%. In a randomized prospective study, the use of such substances at a concentration of 154 mEq/L in 119 subjects with serum creatinine levels ≥1.1 mg/dL resulted in 13.6% of the patients developing CIN with the use of preventive sodium chloride and only 1.7% with the use of sodium bicarbonate(19).

In this study, the preventive function of Bic was observed when comparing the group of animals treated with the IC agent and those that received pretreatment with Bic + IC, in which there was an improvement of 43% in the mean Clcr/100 g, confirming the renoprotective effect of the antioxidant Bic when administered before and after IC treatment. These findings make the study significant and noteworthy in addition to the sample for the pretreated group being smaller than the number of those that developed CIN but sufficient for statistical analysis.

To reduce the currently high mortality and morbidity rates, renoprotection is essential for critically ill patients requiring admission to the ICU and several risk factors and hemodynamic instability that predispose them to the development of CIN. CIN patients, in turn, may ultimately require dialysis or even a kidney transplant.

Finally, the knowledge about the mechanisms and encouraging the preventive use of antioxidants such as sodium bicarbonate in patients at risk for AKI, as in the case of CIN, in nursing practice are favorable to the patient safety and may be considered a safe and practical method that is inexpensive and easy to use.

**CONCLUSION**

The present study confirmed the nephrotoxic effect of radio-iodinated contrast agents with the significant reduction in renal function, as depicted by Clcr.

The significant renoprotection of the Bic administered before and after the IC treatment, as evidenced by the
superior Clcr in the IC group, and its action as an antioxidant, as demonstrated by the reduction in the expression levels of the cellular injury markers, suggest that the oxidative mechanism is a component of IC-induced AKI.

REFERENCES


Acknowledgment
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