Standardization of the experimental model of acute renal failure for bidirectional study with an apical periodontitis

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ABSTRACT

Introduction: Acute Renal Failure (ARF) occurs in about 5% of hospitalizations and up to 30% of ICU admissions, with mortality rates between 15% and 60%. The objective of this study was to analyze different protocols for the induction of IRA in an animal model, seeking the standardization of the model for the study of bidirectional relationship with apical periodontitis (AP). **Methods:** Twenty-four rats were divided into three groups (n = 8): G1 - Administration of Gentamicin at a dose of 80mg/kg/day for 5 days; G2 - Administration of Gentamicin at the dose of 100mg/kg/day for 8 days; G3 - Administration of Gentamicin at the dosage of 100mg/kg/day for 10 days. The AP was induced in all groups by means of pulp exposure of the first and second upper and lower molars from the right side. After 30 days, the animals were euthanized, the kidneys collected for histological analysis, and the jaws removed for radiographic analysis. **Results:** The presence of AP was confirmed in all specimens by radiographic examination. The G3 group had a mortality rate of 75% while G1 and G2 had no mortality. Histologically, the renal tissue of the G2 protocol presented tissue changes such as dilation and more severe tubular necrosis when compared to the G1 protocol (p <0.05). **Conclusion:** In view of the high mortality rate observed in G3 and the histological findings observed in G1 and G2, the indicated protocol is 100mg/kg/day for 8 days to study the bidirectional relationship with apical periodontitis.

Keywords: Acute Kidney Injury. Periapical Periodontitis. Kidney Diseases.

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Introduction

The bidirectional relationship between apical periodontitis (AP) and systemic health has been reported in the literature both in animal models and in humans. In a standardized animal model, apical periodontitis, when associated with diabetes and periodontal disease, may increase triglyceride levels,[1] creatinine,[2] glycated hemoglobin,[3] proinflammatory mediators such as IL-17,[4] besides altering some parameters of the blood count, especially the white series[5] to alter the insulin signal of blood and muscle tissue.[6,7] In addition, four infectious foci were able to increase triglyceride levels in normal rats.[8] In humans with type 2 diabetes, increased glycated hemoglobin was observed, as well as a higher number of infectious foci in these patients.[9] The prevalence of apical periodontitis in these patients compared to patients without the disease was observed in patients with chronic kidney disease.[10] These findings, both in animal and human models, demonstrate the importance of knowledge about the relationship between infection of endodontic origin and systemic health.

The kidneys are complex, regulating organs that perform functions essential for the maintenance of organic homeostasis. Most of these functions involve a combination of excretion and reabsorption of substances, producing an input and output balance. As renal functions, it is possible to list fluid and electrolyte balance; control of blood pressure, red blood cell production and vitamin D production; excretion of metabolites; secretion of bioactive substances such as hormones; and promotion of the maintenance of acid-base balance.[11,12]

Acute renal failure (ARF) is defined as rapid loss of renal function with azotemia, ie elevation of creatinine and urea levels.[13] This condition is usually considered an inpatient illness. The incidence may range from 2 to 5%.[14-16] In a prospective study with a 5% incidence,[15] when 2216 hospitalizations were evaluated, 79% of the episodes were correlated with hypovolemia, post-surgery, contrast administration for XR and aminoglycosides.

Aminoglycosides are antimicrobials used primarily in the treatment of patients with severe infections caused by gram-negative aerobic bacteria, but with high nephrotoxic power.[17] The major representatives of this group are gentamicin, amikacin, streptomycin and tobramycin. Nephrotoxicity, induced mainly by gentamicin, is related to dose and duration of treatment and is characterized by tubular necrosis,[18] marked decreases in glomerular filtration rate (GFR), ultrafiltration coefficient (KF) and glomerular plasma flow[19]. It should be noted that the use of gentamicin (80 mg/kg) for more than seven days in the therapeutic routine has been a common cause of nephrotoxicity in approximately 30% of patients.[20] Thus, due to the high nephrotoxicity presented by this drug, it has been the method of choice for the induction of ARF in animal models.[21-26]

In the literature there are several protocols for the induction of ARF in rats using gentamicin, the most common route being intraperitoneal. Among the most used dosages we can highlight that of one dose of 100mg / kg daily for 8 days;[23,27-29] one dose of 100mg / kg daily for 10 days[23,27,30] and the dose of 80mg / kg daily divided in two daily applications for 5 days.[31]

In view of the above, the objective of this study was to analyze different protocols for the induction of ARF in an animal model, seeking the standardization of the model for the bidirectional relationship with apical periodontitis (AP).

Material and methods

Experimental design

Twenty-four Wistar rats (Rattus norvegicus albinus), with an initial mean weight of 280g, were used in this study. The rats were housed in mini-isolators for rats (Alesco, São Paulo, SP, Brazil), kept in temperature-controlled rooms (22-24°C), and given ad libitum access to water. The experimental procedures were approved by and conducted in accordance with the guidelines of the institutional ethics committee and in compliance with the U.K. Animals (Scientific Procedures) Act 1986 (00373-2017).

Induction of acute kidney injury

Acute kidney injury was induced in rats by intraperitoneal (i.p.) injections of Gentamicin (Gentatec 100mg/kg), according to each protocol described: G1 -Administration of Gentamicin (Gentatec®), intraperitoneally (i.p.) at the dose of 80mg/kg/day, divided into two applications, for 5 days; G2 - Administration of Gentamicin (Gentatec®) intraperitoneally (i.p.) at the dosage of 100mg/kg/day for 8 days;

G3 - Administration of Gentamicin (Gentatec®), intraperitoneally (i.p.), at the dosage of 100mg/kg/day for 10 days.

Induction of oral infection

On the day of the last i.p. injection of gentamicin of each protocol the animals were anesthetized using intramuscular administration of ketamine (87 mg/kg) and xylazine (13 mg/kg) (Rompum - Bayer SA, São Paulo, SP, Brazil).

For inducing AP, the dental pulps of the first and second upper and lower right molars of each rat were exposed using surgical round burs LN (Burs Long Neck - Maillefer, Dentsply Ind. e Com. Ltda, Petrópolis, RJ, Brazil)[1-4]. The dental pulps were exposed in the oral cavity for 30 days, until the end of the experiment.

Rats euthanasia and confirmation of AP and ARF induction

The rats were euthanized 30 days after AP induction and the last dose of the antibiotic by using an anesthetic overdose of thiopental sodium (Thiopentax; Cristália, Itapira, SP, Brazil). In previous studies, it was observed that this period is sufficient for the development of apical periodontitis.[1-4] To confirm the induction of AKI, the kidneys were collected for histological processing in hematoxilin and eosin (H.E.). After kidney collection, the upper and lower jaws were dissected, processed, and subjected to radiographic analysis.

Results

After the experimental period, the G3 group presented a mortality rate of 75% (25% survival) when combined with apical periodontitis. Both the G1 and G2 protocols did not present mortality (survival of 100%), as shown in Figure 1.

The presence of apical periodontitis was confirmed in all animals by radiographic examination after 30 days of pulp exposure. It was possible to observe a radiolucent area in periapical area of the teeth that were had the pulps exposed to the oral medium, suggestive of periapical lesions of endodontic origin (Fig 2).

Renal tissue analysis was performed in all rats after the 30-day experimental period. The effect of gentamicin, a drug used for the induction of renal disease, was investigated, and then compared among the different protocols. Macroscopic changes in the kidneys of normal and diseased rats can be observed in Figure 3. Microscopic changes in renal tissue can be observed in Figure 4. Both macroscopically and microscopically, it is possible to observe the differences between healthy tissue and diseased tissue.

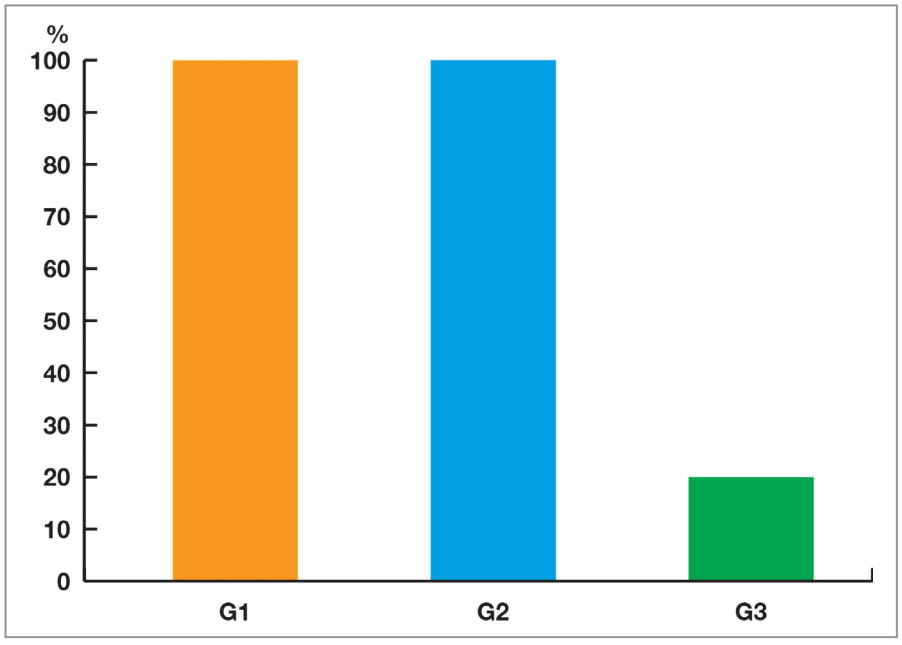

Figure 1. Survival rate of the different experimental groups (%).

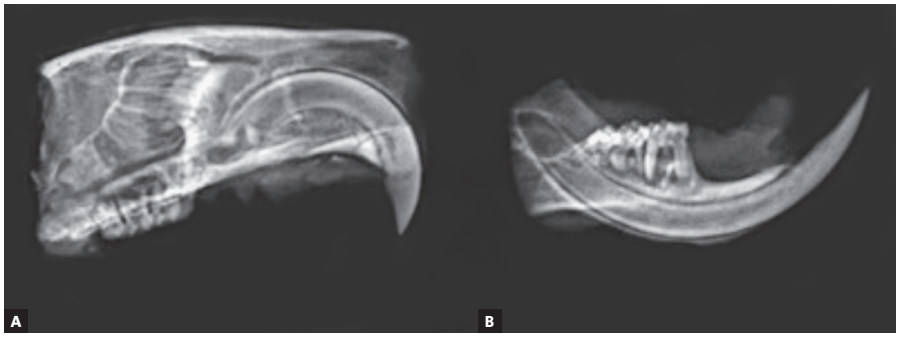

Figure 2. Radiographic images of the maxilla (**A**) and mandible (**B**), respectively, proving the presence of endodontic infection. It is evidenced a radiolucent area next to the peripapices of the molars that had their pulps exposed.

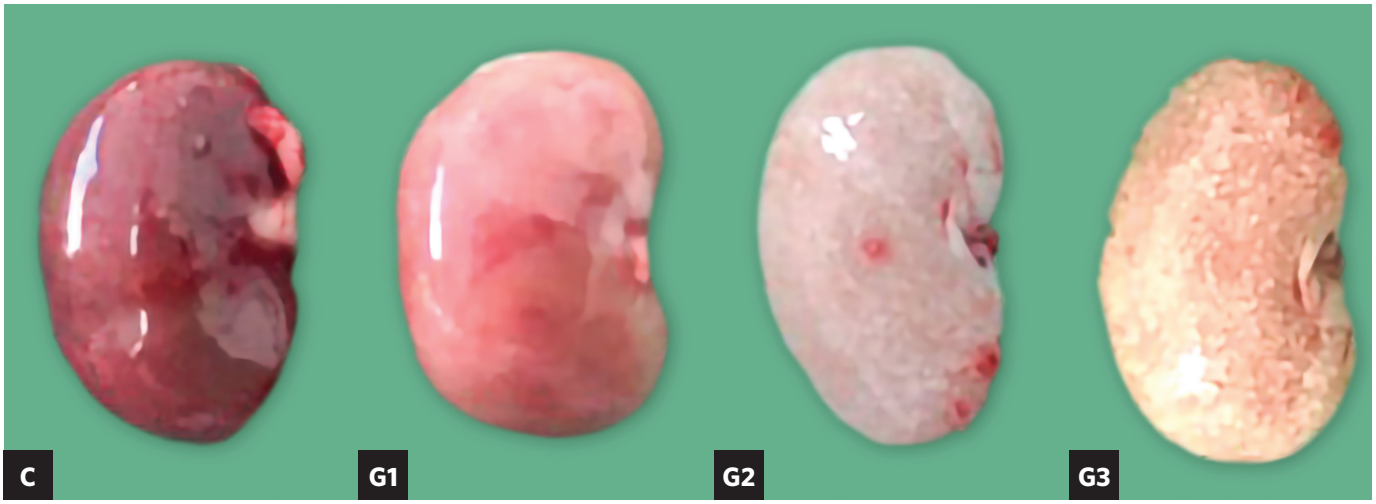

Figure 3. Macroscopic image of the kidney (**C**): left, normal animal kidney; followed by the animal kidney of the experimental protocol **G1**; to the center, protocol animal's kidney **G2**; and, on the right, animal kidney of the **G3** protocol.

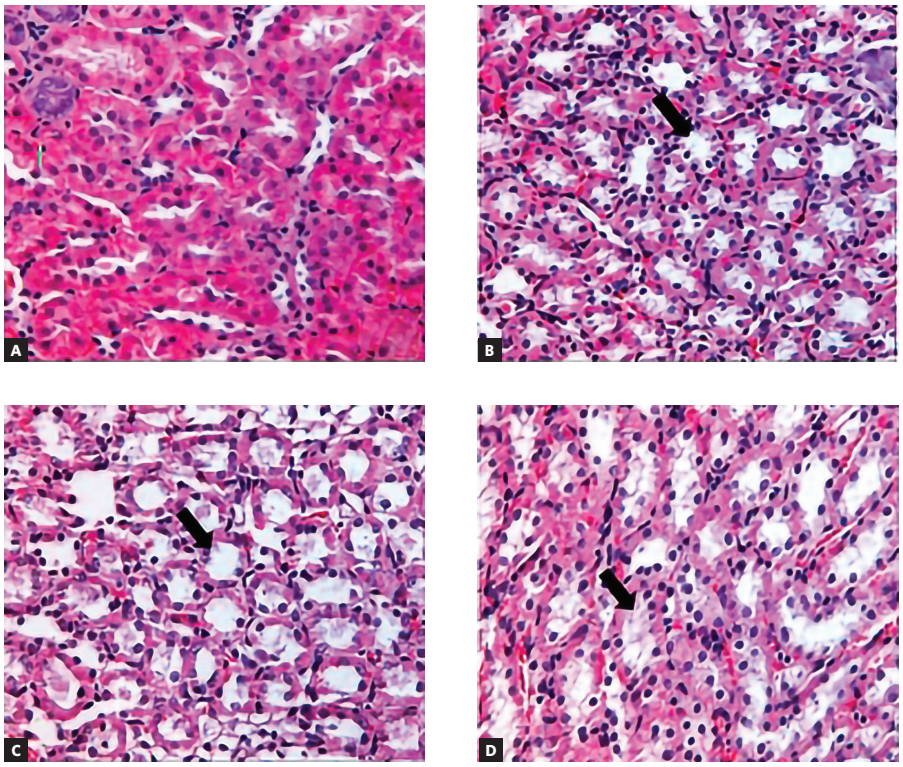

Figure 4. HE staining of the kidneys at 400x magnification. **A**, corresponds to the normal animal's kidney; **B**, G3 protocol; **C**, G2 protocol; and **D**, G3 protocol. In images **B**, **C** and **D**, one can observe the presence of tubular necrosis with dilated tubules (arrows) and cells with pycnotic nuclei.

Discussion

Apical periodontitis was induced by pulp exposure of rat molars, allowing bacterial infection from the oral environment.[1,4] This exposure lasted for 30 days, as it is a sufficient period of time to observe the development of apical periodontitis and complete pulp necrosis[4] and was confirmed radiographically in the present study.

Gentamicin is an aminoglycoside antibiotic that is commonly used for the treatment of severe infections caused by gram-negative bacteria. However, frequent use of gentamicin is inadequate because of its high nephrotoxic power.[27] The present study demonstrates the nephrotoxic power of gentamicin in three experimental protocols.

Administration of high doses of gentamicin (40 mg/kg or more) is a prerequisite for the induction of renal dysfunction and acute nephrotoxicity in animals.[32,33] For the study of the different protocols, the first protocol chosen was the dosage of 80 mg/kg/i.p. of Gentamicin divided into two daily administrations for 5 days.[31] In this protocol macroscopically, the kidneys presented necrosis characteristics, which were confirmed in the histological analysis due to the presence of renal alterations compatible with acute tubular necrosis, being such alterations, cytoplasmic vacuolization, the presence of cells with pynotic nuclei and inflammatory infiltrate.

The second dose chosen (G2) is the most commonly used, with 100 mg/kg /day i.p for 8 days in a single dose. This dose has been reported by others and has been shown to be capable of developing renal dysfunction and nephrotoxicity in rats[34,35] which is in agreement with our findings, In the histological sections obtained in the present study, we can also observe renal changes compatible with acute tubular necrosis, which suggests the presence of loss of renal function. These histological characteristics were also observed in the G1 protocol, however, with greater intensity and prevalence in G2. Macroscopically, in the G2 protocol there was a notable chromatic alteration of the organ, where reddish-brown, typical of a healthy kidney, gave rise to a more whitish color suggestive of necrosis. In addition to a more pronounced chromatic alteration, the kidneys of this protocol were smaller when compared to the kidneys of the G1 protocol. These findings, micro and macroscopic, suggest a greater nephrotoxic power of the second protocol when compared to the first.

The last protocol used (G3) at the dose of 100 mg/kg/day i.p. for 10 days in a single dose, also demonstrates a high power of nephrotoxicity,[30] with a greater presence of pathognomonic signs of renal disease, both microscopically and macroscopically, when compared to G1 and G2. However, for the study of bidirectional relation with apical periodontitis, the animals should survive during the 30 day period for the development of the lesions, which did not occur, since this group had a high mortality rate (75 %), by associating the two conditions, being thus excluded as an option of an experimental model for the study of bidirectional relation with apical periodontitis.

According to the Brazilian Society of Nephrology, approximately 13 million Brazilians have some degree of renal problem, of which 5,000 are in a severe stage depending on hemodialysis or in the transplant queue. In addition, the number of cases is increasing 10% per year. The main risk groups are patients with a history of renal disease in the family, elderly, obese, diabetic and hypertensive. Thus, the standardization of this study model allows the understanding of the functioning of the metabolism of patients in these conditions, as well as the best materials accepted by it and its response to endodontic treatment.[36]

Conclusion

In view of the high mortality rate observed in G3 and the histological findings observed in G1 and G2, the protocol most suitable for the bidirectional relationship between acute renal failure and apical periodontitis is the G2 protocol, of 100 mg/kg/day for 8 days.

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