

## Effect of Ringer's solution submitted to magnetic field in renal ischemia-reperfusion model in rats

### Efeito da solução de Ringer submetida a campo magnético em modelo de isquemia-reperusão renal em ratos

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#### ABSTRACT

**Purpose:** To evaluate the effect of hydration with simple Ringer's solution submitted to magnetic field in rats under renal ischemia and reperfusion (I/R) model, using biochemical and histopathological evaluation. **Methods:** Wistar rats were allocated into 2 equal groups of 6 animals each: Renal ischemia/reperfusion group + hydration with magnetic Ringer (mag Ringer). The left kidney underwent ischemia for 30 minutes. The right kidney was not manipulated. Thereafter, i.v. hydration was performed with 3 ml mag Ringer; Group renal I/R + hidration with simple Ringer. The simple Ringer solution was submitted to a magnetic field of 20mT intensity for 2 hours immediately prior to the surgical procedure. After 24 hours, under anesthesia blood was collected for dosing serum urea and creatinine. Histopathological analysis was performed on the left kidney (HE staining). **Results:** There was no significant difference between the creatinine ( $p=0.764$ ) and urea ( $p = 0.926$ ) values between the control and test groups (non-parametric Mann-Whitney test). Comparing the histopathological scores, there was a significant difference between the groups, with higher levels of lesions in the mag Ringer

treated group ( $p=0.025$ ) - Mann-Whitney test. **Conclusion:** The use of Ringer's solution submitted to magnetic field for intravenous hydration in a renal ischemia/reperfusion model did not alter the renal function and had a negative influence on the histopathological findings on the kidney, when compared to control.

**Keywords:** Ischemia. Reperfusion. Kidney. Fluidtherapy. Magnetism.

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## RESUMO

**Objetivo:** Avaliar o efeito da hidratação com solução de Ringer simples submetida à ação de campo magnético, em ratos sob modelo de isquemia e reperfusão (I/R) renal, através de métodos bioquímicos e histopatológicos. **Métodos:** Ratos Wistar foram divididos por sorteio em 2 grupos iguais de 6 animais cada: Grupo Isquemia/reperfusão renal + hidratação com Ringer tratado com magnético (Ringer mag) – Provocada isquemia no rim esquerdo durante 30 minutos. O rim direito não foi manipulado. Em seguida, realizou-se a hidratação de infusão i.v. com 3 ml de Ringer mag; Grupo I/R renal + reposição volêmica com Ringer simples. A solução de ringer simples foi submetida a campo magnético de intensidade 20mT. A solução foi exposta ao CM durante 2 horas imediatamente antes da realização do procedimento cirúrgico. Após 24 horas, sob anestesia foi colhido sangue para dosagem de Ureia e Creatinina. Análise histopatológica foi realizada no rim esquerdo (coloração HE). **Resultados:** não houve diferença significativa entre os valores de creatinina ( $p=0,764$ ) e ureia ( $p=0,926$ ) entre os grupos controle e tratado, usando o teste não paramétrico de Mann-Whitney. Comparando os escores histopatológicos, houve diferença estatisticamente significativa entre os grupos, observando-se maiores níveis de lesões no grupo tratado  $p$ -valor= 0,025 (teste de Mann-Whitney). **Conclusão:** O uso de solução de Ringer submetida a campo magnético para hidratação intravenosa em modelo de isquemia/reperfusão renal não alterou as provas de função renal e influenciou negativamente nos achados histopatológicos do rim, quando comparado com o controle.

**Descritores:** Isquemia. Reperfusão. Rim. Fluido terapia. Magnetismo.

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## INTRODUCTION

Renal injury due to ischemia-reperfusion syndrome (IRS) occurs in most surgical interventions in which there is a deficit on kidney blood supply. Therefore, it can be observed during renal transplantation, aortic and kidney arteries vascular surgical interventions and partial nephrectomy. Regarding kidney transplantation, it is known

that the incidence of graft dysfunction, acute rejection and chronic nephropathy are directly related to the degree of ischemic injury. The rejection is also influenced by the time of normothermic ischemia, when organs from donors with heart arrest are used, owing to highest failure rate of the surgical procedure<sup>1</sup>.

The consequences of ischemia-reperfusion injury depend on the duration of ischemia, intensity of reoxygenation, temperature and the nature of the organ, as these factors determine the degree of injury<sup>2</sup>. Experimental studies performed in the 1970s, used to deploy kidney hypothermia in-situ to decrease metabolic activity and administered mannitol to reduce cell edema in cases of prolonged intraoperative kidney artery clamping. As for normothermic ischemia, the surgical time can reach 90 minutes<sup>3-4</sup>. However, in clinical practice, the maximum tolerable time limit for normothermic ischemia is 30 minutes. In performing open partial nephrectomy, this time interval is sufficient and it is easy to promote renal hypothermia. Nevertheless, in laparoscopic partial nephrectomies, the technical challenge is greater, both to perform renal hypothermia and the procedure. Therefore, the 30-minute interval is limiting for many surgeons. Consequently, the therapeutic option in many centers for patients with kidney tumors is an open partial nephrectomy or total laparoscopic nephrectomy.

As a result of these limitations, several strategies and resources have been investigated to prevent or reduce the cellular injury caused by IRS. A recent alternative considered is the use of liquid, under magnetic field, for hydration. The passage in a permanent magnetic field activates the water molecules, changing its structure, pH and electrical activity making it similar to body water<sup>6,7</sup>. Among the effects of magnetized water, an increase in the activity of enzymes such as glutamate decarboxylase<sup>8</sup>, as well as increased permeability through the membranes have been reported<sup>9,10</sup>, and the magnetic field can accelerate biochemical reactions in body tissues<sup>11,12</sup>. The activation of these electrolytes theoretically ease their binding to other organic compounds useful in cases of ischemia - as antioxidants - just as acting on the autonomic nervous system, improving blood circulation and also improving the effect of treatment on various diseases related to blood circulation, including IRS<sup>13</sup>.

Some studies on the physiological activity of water submitted to magnetic field in humans were reported during 1980 and 1990, mainly in China<sup>7,8</sup>. Nonetheless, the scientific basis for the physiological activity of magnetic field-treated water intake in the

prevention and treatment of diseases in humans are still quite limited. For this reason, we consider meaningful a study in order to understand the metabolic changes resulting from the renal ischemia and reperfusion process and its correlation with the rehydration with simple Ringer's solution submitted to magnetic field, in order to minimize intra and postoperative complications.

## **OBJECTIVE**

The objective of the present experimental study was to evaluate the effect of hydration with simple Ringer's solution submitted to magnetic field, in rats under ischemic model and kidney reperfusion, through biochemical and histopathological evaluation.

## **METHODS**

This study consists is an exploratory, prospective and experimental analysis. Twelve (12) Wistar rats (*Rattus norvegicus*), weighing  $415.3 \pm 58.27$ , from the Health Center Facility of the Federal University of Rio Grande do Norte (UFRN) - Brazil were used. After 7 days of acclimatization at the Nucleus of Experimental Surgery-UFRN, with *ad libitum* access to water and food for rats (Prevence<sup>®</sup>), the animals were anesthetized with ketamine 70 mg/kg body weight + Xilazine 10 mg/kg i.p, operated under aseptic technique and observed in individual cages (one rat per cage). A median laparotomy of 05 cm was performed from the xiphoid appendix.

The animals were randomly chosen and allocated into 2 equal groups of 6 animals each: Renal ischemia/reperfusion group + treatment with Ringer submitted to magnetic field (MF), which will be named Magnetized Ringer. Ischemia in the left kidney was done by total occlusion of the left kidney artery with a bulldog clip for 30 minutes, with posterior release of the clamp and reperfusion, guaranteed by the observation of the renal artery beats return. The right kidney, just as its vascular hilum, was not manipulated. During the entire period of ischemia, the abdomen was closed by suturing. Soon after, through the dissection of the right femoral vein, with the aid of catheter No. 24 and surgical microscope (DF Vasconcelos - 10X magnification), it was performed the slow infusion of 3 ml of magnetized Ringer. In kidney ischemia/reperfusion group the

volume replacement was performed with with 3 ml Ringer (without magnetization). In order to yield the solution of Magnetized Ringer, the simple Ringer's solution was previously submitted to magnetic field of 20mT intensity produced by 6 magnets in an adapted model (Figure 1). The solution was exposed to MF for 2 hours immediately before the surgical procedure.



**Figure 1** – Model constructed to produce the magnetic field (20 mT)

After 24 hours of monitoring in the postoperative observation room of the Nucleus of Experimental Surgery, the animals were anesthetized again and whole blood was collected by cardiac puncture. Serum separation was performed by centrifugation at 3,000 rpm, stored at -20°C for subsequent dosing of Urea and Creatinine. For histopathological analysis, the left kidney was removed after euthanasia of the animals with anesthetic overdose (Thiopental 100 mg/kg i.p.). The kidney was fixed in 10% buffered formalin in individual vials. The criteria and histopathological findings were: microscopic changes compatible with acute tubular necrosis - tubular lumen dilation, tubular cell vacuolization, intratubular cylinders and tubular cell necrosis (Table 1). The animals were weighed before the start of the experiment and 24 hours later.

**Table 1** - Degree of renal tubular lesion attributed to histological scores.

Score	Histopathological Pattern
0	Normal
0.5	Small injured focal areas
1	< 10% of cortical area damaged
2	10 a 25% of cortical area damaged
3	25 a 75% of cortical area damaged
4	> 75% of cortical area damaged

Regarding statistical analysis, it was initially evaluated whether the quantitative variables met normality, using the Shapiro Wilk and Kolmogorov-Smirnov tests. To test the hypothesis of the difference between the two groups, the Student's t-test for independent samples and the non-parametric Mann-Whitney test were used in the variables that did not present normality. The analysis were performed with the SPSS®20 software, considering the significant differences when  $p < 0.05$ .

## RESULTS

All animals survived the experiment. In the control group, one animal was excluded from the study because the results were discrepant within the group. Contrast analysis showed no significant difference in creatinine values between the control and treated groups ( $p = 0.764$  by the non-parametric Mann-Whitney test). The same non-significant difference was observed between groups when compared to the urea variable ( $p = 0.926$  by the non-parametric Mann-Whitney test). Results regarding body weight loss in the 24 hours post-procedure, as well as dosage of Urea and Creatinine are shown in Table 2.

**Table 2** – Descriptive statistics and inferential statistical tests

Variables	Group		p-value <sup>1</sup>
	Control (N = 5)	Treated (N = 6)	
Body weight loss (%)	4.26 ± 0.32	3.21 ± 2.95	0.424
Urea (mg/dL)	52.4 ± 20.53	45.5 ± 4.28	0.926 <sup>2</sup>
Creatinine (mg/dL)	0.84 ± 0.21	0.88 ± 0.25	0.764

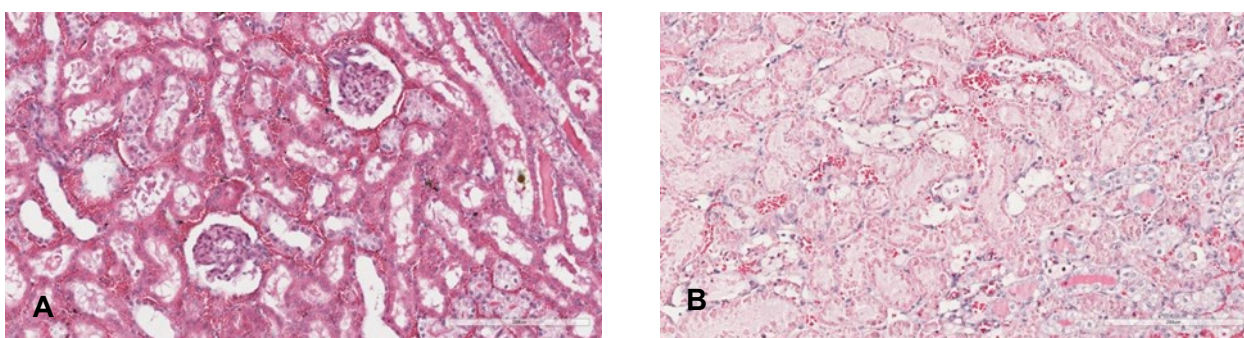
Mean ± standard deviation. 1 - P-value <0.05 Student's t-test. 2 - P-value <0.05 non-parametric Mann-Whitney test.

As far as histopathological analysis, two animals from each group (control and treated) were excluded from the analysis because of divergent results of the sample. The histopathological evaluation of the left kidney, exposed to ischemia and reperfusion, was performed through classification using the degree of injury. According to histology, it was obtained by semi-quantitative evaluation, which varied from 0 to 4, in which zero corresponded to the absence of injury and four to the intense lesion.

Therefore, for a greater analytical comprehension, it was proposed to analyze the data from a numerical point of view. Different degrees of injury were found in both groups. As shown in Table 3, lesion scores classified in 1, 2 and 4, namely above 10%, 10 to 25% and greater than 75%, respectively, were the most found in the slides analyzed. Only 1 sample from the control group did not present any degree of quantifiable lesion. Images related to the histopathological changes found in the slides analyzed can be visualized in Figure 2.

**Table 3** - Histopathological examination of the analyzed kidney fragments of each animal.

Case	Score	Case	Score
Treated 1	4	Control 1	0
Treated 2	3	Control 2	1
Treated 3	4	Control 3	1
Treated 4	2	Control 4	2
Treated 5	-	Control 5	-
Treated 6	-	Control 6	1



**Figure 2** - Histology of the ischemic left kidney (H/E staining, amplified original 20x). Ischemic glomerulus (A); acute tubular necrosis (B).

Therefore, statistically significant difference were obtained between the groups. P-value of 0.025 by the non-parametric Mann-Whitney test, which showed a higher level of lesions in the magnetized Ringer group (Table 4).

**Table 4** – Descriptive statistics and respective inferential statistical tests of histopathological evaluation

Variable	Group		p-value <sup>1</sup>
	Control (N = 4)	Treated (N = 4)	
Histopathological exam	1.00(0.75)	3.50(1.75)	0.025

Median (interquartile deviation - Q1-Q3). 1 - P-value <0.05 non-parametric Mann-Whitney test

## DISCUSSION

This study has demonstrated that the use of Ringer submitted to magnetic field did not modify, from a functional point of view, the outcome of kidney ischemia and reperfusion in an experimental model in rats. The use of the magnetic field treatment technique for cellular activation of a simple Ringer's solution did not result in a significantly positive effect when compared to the same non-magnetized solution when serum levels of biochemical markers of renal function (urea and creatinine) were compared. In addition, at the histopathological level, the use of this solution, treated with a magnetic field, resulted in a higher renal score of acute tubular necrosis, comparing with controls.

Acute renal injury (ARI) is defined as an abrupt decrease in the rate of glomerular filtration with retention of nitrogenous slags, creatinine and urea. These are used as the main criteria to define and classify ARI, being considered as specific tests, but delayed and with spare sensitivity<sup>14,17</sup>. Serum creatinine concentration is the most used kidney function test in specialized hospital settings, being widely used as a biomarker of chronic kidney disease and acute kidney injury. However, despite being an advantageous test, serum creatinine values do not increase significantly until 50% of its function is compromised<sup>15,17</sup>. Therefore, considering that the maximum tolerable time limit for normothermic ischemia is 30 minutes, which is the time used in the methodology of our work in order to simulate the practical scenario, linked to the 24 hours of postoperative observation, these steps may have been insufficient for the significant alteration of those biomarkers.

Concerning to urea, in spite of being the first biomarker to be used for renal function analysis<sup>16</sup>, it is important to emphasize its incongruity as a kidney function test.



This is due to several factors, such as oscillating production during the day, variable plasma concentration according to protein intake, gastrointestinal bleeding and the use of some medications; decrease in production under conditions such as hepatic insufficiency and undernutrition<sup>16</sup>. Although Urea shows a slightly earlier increase, it is considered a marker that is likely to be susceptible to physiological and environmental variations, decreasing its sensitivity in analyzes of ischemia and reperfusion in which there is an intense modification of biological systems. In our study, on account of intraoperative and postoperative losses, along with loss of body mass throughout the study, may have been factors of oscillation of this parameter.

As a result of a process of acute kidney injury because of I/R there was, as expected, the earlier increase in values obtained from Urea, which has a normal value between 20-40 mg/dL<sup>16</sup>. Despite of that, this increase was not statistically significant. In addition, due to its delayed characteristic, no difference in serum creatinine levels was observed comparing the groups; its reference values were defined between 0.6 to 1.3 mg/dL<sup>16</sup>. In the evaluation of body mass loss, the control group showed a slightly greater weight loss, but not significant.

In the perioperative setting and in critically ill patients, ischemic acute tubular necrosis (ATN) is the most common cause of acute kidney injury, most often by the pre-renal component, with underlying hypovolemia<sup>17</sup>. For the development of ATN, microvascular and tubular lesions occur, and inflammatory responses induced by ischemia and reperfusion (I/R) play a predominant role<sup>18,19</sup>. In the kidney, histological changes secondary to severe perioperative ischemia are markedly defined by acute tubular necrosis with hemorrhage. Therefore, kidney injury was similar in the groups in which the magnetized simple ringer was used, ranging between high percentage of ATN scores (2 to 4). Contrasting this result, the ATN values found in the slides of the control group, namely the group treated with simple non-magnetized ringer, scores were observed with significant lower levels of acute renal injury (between 0 and 2).

Although the scientific bases and experimental tests focused on the study of the physiological activity in use of magnetized fluids in humans are still highly limited, there are some researches that already elucidated what effects this technique can generate in the most diverse biological systems. It is currently suggested that long-term ingestion of magnetized water (over 8 weeks) might be beneficial in both the prevention

and treatment of complications in diabetic patients, as well as their important antioxidant action<sup>20</sup>. However, its effects under the ischemia and reperfusion process still require further analysis. In our study, through the non-statistically significant difference between the analyzed groups, it was shown that the performance of magnetic field over the perfusion solution did not result in better outcomes in the I/R scenario. In the I/R process there are different and intense changes in the biological systems, which in turn is susceptible to variation according to factors such as: duration of ischemia, intensity of reoxygenation, temperature and the nature of the organ.

In conclusion, the use of Ringer's solution submitted to magnetic field for intravenous hydration in kidney ischemia/reperfusion model did not modify the evidence of renal function and had a negative influence on the histopathological findings of the kidney, when compared to the control.

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