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EVALUATION OF THE APPLICABILITY OF DOPPLER VELOCIMETRY FOR MONITORING ACUTE KIDNEY INJURY IN RATS

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ABSTRACT

Animal models of renal disease have been used in the study of pathogenesis and therapeutic protocols. In this study, doppler ultrasound was used to evaluate dysfunction in the renal vasculature in acute kidney injury in an animal model. Eight male Wistar rats received gentamicin (80 mg/kg) for 10 days. The blood urea and creatinine levels were measured to assess renal function. All doppler ultrasound measurements were performed on both kidneys and a colour map of the renal circulation was generated. Renal function and Doppler ultrasound measurements were performed 10 days before GM treatment and on the 5th and 10th days of the assay. Gentamicin treatment led to increased serum creatinine and blood urea levels at 5 days and 10 days post initial inoculation. A significant reduction in renal artery blood flow was observed after 5 days. However, these levels remained unchanged until the 10th day, demonstrating a lack of correlation with serum creatinine and blood urea levels. Therefore, the assessment of flow blood velocity of renal arteries by doppler ultrasound is not useful for monitoring acute kidney injury in rats.

Keywords: Renal circulation, Ultrasonography Doppler, Disease models, Animal.

INTRODUCTION

Acute renal injury (AKI) is defined as an abrupt reduction in kidney function and is associated with a high mortality rate.1 Drugs are a common cause of AKI. Gentamicin (GM) is an antibiotic widely used to treat infections caused by Gram-negative bacteria.2,3,4 However, it causes nephrotoxicity as a major adverse reaction in up to 30% of treated patients. The pathogenesis of GM involves multiple pathways, including oxidative stress, inflammation, increased nitric oxide (NO) and reduced renal blood flow.5

Biochemical measurements of blood or urine along with clinical observations have been used to diagnose AKI in human and animal models. Rats are one of the experimental models used most frequently to study pathogenesis and therapeutic protocols for AKI. To establish an AKI model, based on biochemical parameters, successive blood samplings must be performed to assess the renal injury.

The imaging techniques, such as magnetic resonance, computerised tomography and nuclear medicine imaging, are being increasingly used both clinically and experimentally, contributing to advances in understanding the pathophysiology of many diseases. However, the use of these techniques for the assessment of renal tissue perfusion in small animals is complicated by many issues, such as the degrees of spatial and temporal resolution that are obtainable or by the use of X-rays, radioisotopes or contrast agents that are potentially nephrotoxic.6,7 Advances in high frequency ultrasound imaging methods have

opened up new opportunities for the analysis of blood flow in vivo.

The laser-Doppler flowmeter (LDF) reads the frequency of the oscillation produced by the Doppler frequency shift of the red blood cells and is a non-invasive and accurate method to measure microcirculatory blood flow in tissue.

The aim of this study was to evaluate the applicability of doppler ultrasound for monitoring AKI in rats.

MATERIALS AND METHODS

Animals

Adult males Wistar Rats (Rattus norvegicus-salbinus) weighing between 200-250 gwere obtained fromthe animal house of the Laboratory of Animal Experimentation of the National Institute of Pharmacology and Molecular Biology (LEA INFAR), Federal University of São Paulo (UNIFESP). All animals were cared for in accordance with the standards of the institute under a protocol approved by the Animal Experimentation Ethics Committee (Number of Process: 5509151014).

Acute kidney injury model

Rats (n = 8) were treated with GM (Mantecorp Indústria Química e Farmacêutica S.A., Rio de Janeiro, Brazil) (80 mg / kg, i.p.; n = 8) for 10 consecutive days. At the end of the assay, the rats were euthanized using 3 times the dose of ketamine and xylazine required for anaesthesia by intraperitoneal administration. The experimental design is shown in Figure 1.

Blood collection

Blood samples for biochemical testing were obtained by puncturing the tail vein under inhalation anaesthesia with isoflurane (Cristália, Lindóia, São Paulo, Brazil).Collections occurred10 days before the start of induction and on days 5 and 10 after inoculation with GM.

Briefly, blood was collected under ether anaesthesia and after immersion of the tail in warm water (40ºC). It was then transferred to an Eppendorf tube (1.5 ml) and centrifuged (2500 rpm, 10 min, room temperature), and the serum was separated and stored at - 20ºC until dosage.

Biochemical analyses

The serum creatinine and blood urea nitrogen (BUN) were evaluated in the serum samples and in the 24-hour urine using the Cobas Mira Plus (Roche) with a Labtest kit (Labtest, Belo Horizonte, Brazil).

Ultrasound examination

To obtain ultrasound images, the animals were anaesthetised with isoflurane and posi-

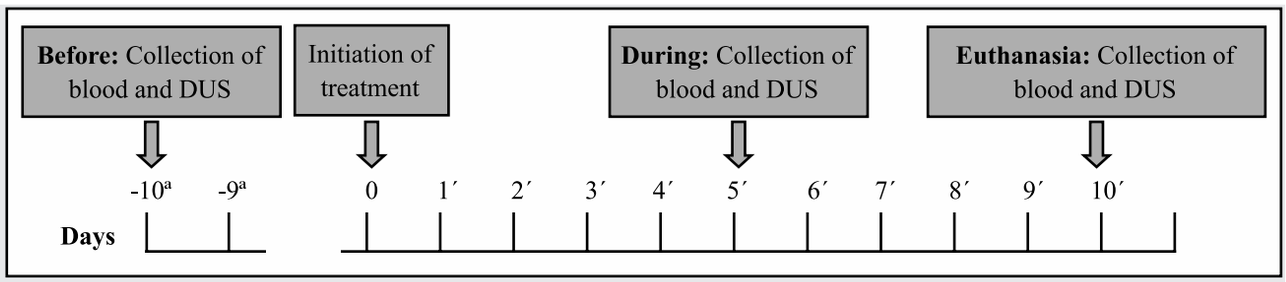

Figure 1: Experimental design: Eight rats were used in the experiment. Blood collection and ultrasound examination were performed 10 days before and on the 5th and 10th days after treatment with GM.

tioned supine on a MousePad (THM100) with integrated temperature sensor, heater and ECG electrodes (Indus Instruments, Houston, TX). The paws of the animals were placed in ECG sensors allowing constant monitoring of the heart rate and body temperature. The removal of hair in the region of interest was performed with a depilatory cream, and a gel (gel 100 ™ ECO ECO-Med Pharmaceutical INC. Mississango, Ontario, Canada) was used as a fluid coupling between the animal's skin and the translator in real time. The tests were carried out in a Vevo 770 (VisualSonics, Toronto, ON, Canada) system with a 40 MHz linear transducer, with a 6 mm focal length and lateral and axial resolutions of 68.2 and 38.5 mM, respectively. B-mode images in the axial and longitudinal planes of both kidneys, with measurement of the longitudinal, transverse and antero-posterior kidney diameters, were obtained.

Colour Doppler ultrasonography

Doppler and spectral modes (Colour and PW--mode) were used for US of the aorta and renal arteries (RA), and the peak systolic velocity and end diastolic velocity were measured. The intra--renal arteries were evaluated by measuring the resistive index, acceleration time and acceleration index.

Statistical analyses

The results are presented as the means ± SE. Single comparisons of mean values were performed using Student's t test. Multiple comparisons of the mean values were performed using one-way ANOVA followed by the Bonferroni's test using GraphPad Prism version 4.0 for Windows (GraphPad Software, San Diego, Calif., USA; http://www.graphpad.com). $P < 0.05$ was considered statistically significant.

RESULTS

Serum creatinine and blood urea levels

In this work, we studied an animal model of nonlethal kidney injury. GM treatment led to increased serum creatinine and blood urea levels at 5 days and 10 days post initial inoculation (Figure 2).

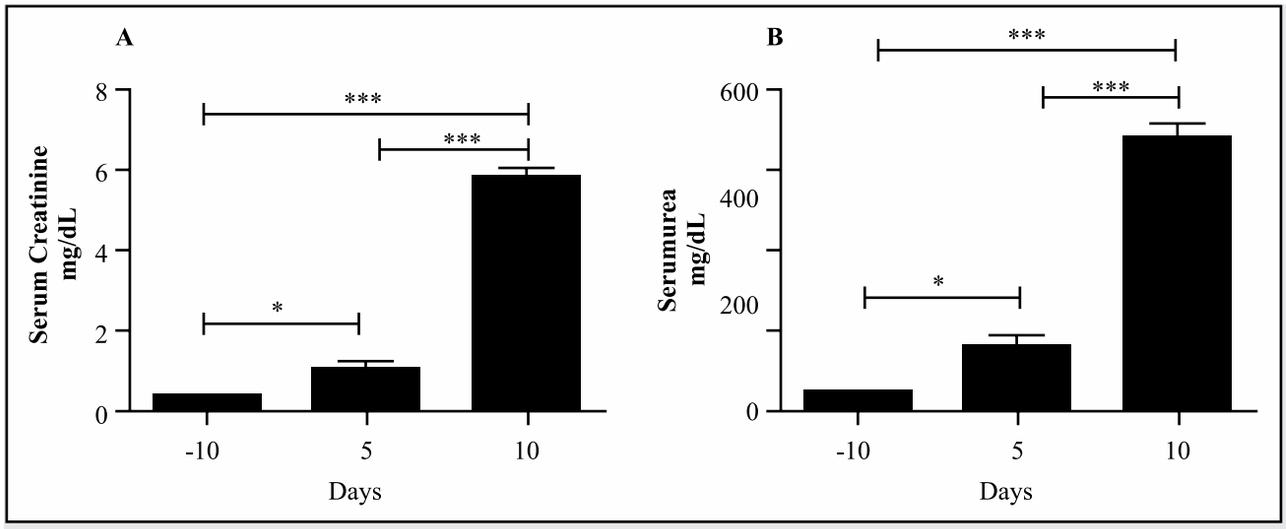

Figure 2: Biochemical parameters of renal function after GM injury (A) Serum creatinine (pCr) and (B) blood urea nitrogen (BUN) levels. One-way ANOVA (**p<0.05, *** p<0.001).

Colour Doppler analysis

Doppler velocimetry analysis of RA was performed (Figure 3). Blood velocity values were assessed before and after GM inoculation (day -10). On the 5th day of GM treatment, RA velocimetric indices decreased significantly compared to the pre-assay value (day -10). After 10 days of treatment, the blood velocity of the RA levels remained unchanged (Figure 4).

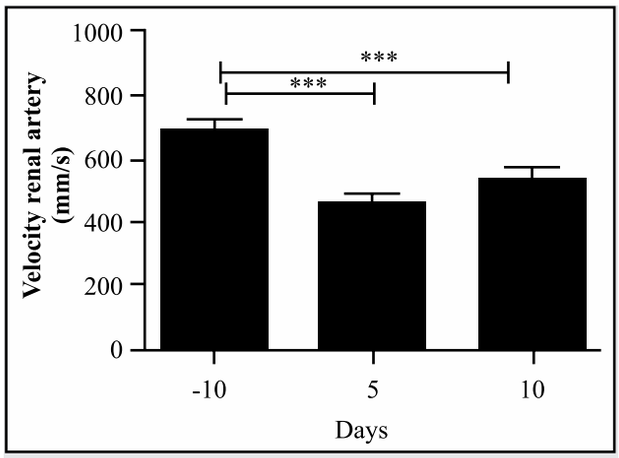

Figure 4: Doppler velocimetry findings of the RA before and after GM treatment. Significant decrease of RA blood velocity was observed after 5 and 10 days of treatment (***p<0.01 and *p<0.05, respectively) (ANOVA One-Way, with Tukey test).

DISCUSSION

Animal testing in nephrology was and continues to be responsible for advancing the understanding of the pathophysiology of acute and chronic kidney injury. To validate the animal models, repeated blood samples are required to assess renal function by measuring serum creatinine and blood urea nitrogen. Blood collection is an additional stress for the animals and also has an impact on the outcome of the research data.8 Doppler US is a non-invasive diagnostic method that is used widely in human and veterinary medicine.9 The present study, involving a nephrotoxic animal model of AKI, was used to evaluate the potential utility of Doppler velocimetry in rats.

Changes in the serum parameters indicated that treatment with GM caused a decrease in renal function after 5days, which was even more pronounced at the end of the treatment at 10 days.

Doppler velocimetry analysis was used as a tool to assess renal circulation. Significant reduction in renal artery blood flow was verified after 5 days. This decrease was inversely pro-

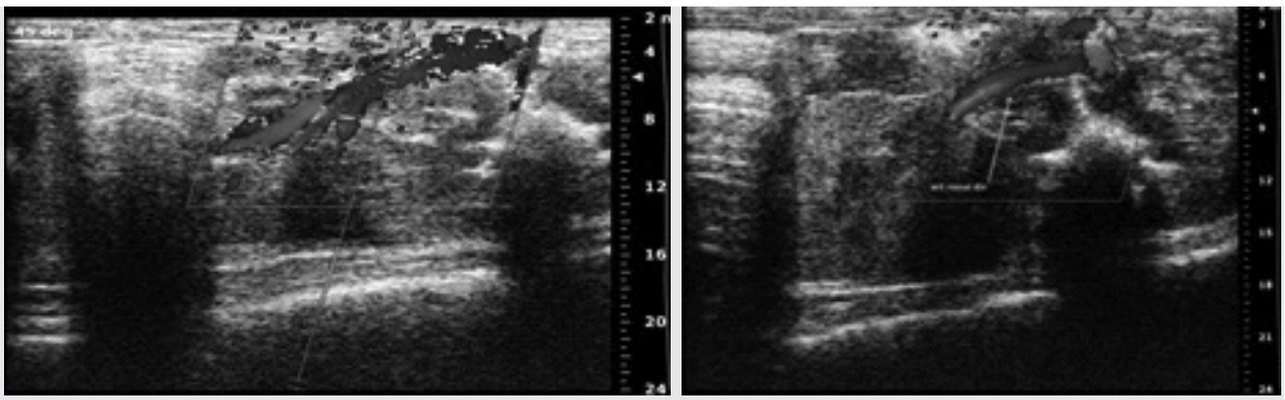

Figure 3: Colour Doppler map of the right renal artery showing the blood drawn with a velocity profile of the parabolic flow.

portional to the increase of blood urea and serum creatinine, which are parameters typically used to diagnose AKI.10 The decrease of RA blood flow can be explained by renal vasoconstriction, which is a known consequence of aminoglycoside nephrotoxicity.10 After 10days of GM treatment, the blood urea and serum creatinine levels were elevated compared with those presented at day 5, whereas renal artery blood flow remained unchanged. These data indicate that decreased renal blood flow may be the first signal of dysfunction, as reported by Carvalho and Chammas.9 Additionally; these data indicated that intrarenal vascular hemodynamic alteration didn´t contribute to the elevation of urea and creatinine blood levels.

CONCLUSION

The results presented in this paper indicate that Doppler velocimetry of renal arteries could be a useful and non-invasive method for monitoring acute kidney injury in animal models. However, Doppler velocimetry is not useful for monitoring acute kidney injury in in rats.

ACKNOWLEDGMENT

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AVALIAÇÃO DA APLICABILIDADE DA DOPPLERVELOCIMETRIA PARA O MONITORAMENTO DE LESÃO RENAL AGUDA EM RATOS

RESUMO

Modelos animais têm sido utilizados tanto no estudo da patogênese quanto no desenvolvimento de protocolos terapêuticos de doenças renais. No presente trabalho, a ultrassonografia com doppler foi usada para avaliar disfunções na vascularização renal em um modelo animal de doença renal aguda. Oito ratos Wistar machos receberam gentamicina (80 mg/kg) durante 10 dias. Os níveis de ureia e creatinina do sangue foram medidos para avaliar a função renal. Todas as medições foram realizadas em ambos os rins, gerando um mapa da circulação renal. As medições da função renal e da ultrassonografia com doppler foram feitas 10 dias antes do tratamento com gentamicina e no 5° e 10° dia do ensaio. O tratamento levou ao aumento de creatinina sérica e ureia no sangue no 5° e 10° dia após o início da inoculação da gentamicina. Uma redução significativa no fluxo sanguíneo da artéria renal foi observada após 5 dias. No entanto, estes níveis permaneceram inalterados até o 10° dia, o que demonstra uma falta de correlação com os níveis de creatinina sérica e ureia. Portanto, a avaliação da velocidade do fluxo sanguíneo das artérias renais, por ultrassonografia com doppler não é útil para monitorar lesão renal aguda em ratos.

Palavras-chave: Circulação renal, Ultrassonografia Doppler, Modelos animais.

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