Urinary N-Acetyl-Beta-D-Glucosaminidase Activity in Rat Experimental Ischemic and Toxic Models of Acute Kidney Injury

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Bulletin UASVM Veterinary Medicine 74(1) / 2017,
Print ISSN 1843-5270; Electronic ISSN 1843-5378
DOI:10.15835/buasvmcn-vm: 12618

Abstract

The identification of a suitable prevention method which facilitates limiting the deleterious effects of acute kidney injuries is highly required. In order to identify a proper treatment for acute kidney injuries, a suitable experimental model that replicates the structural, metabolic and inflammatory lesions that occur in the natural acute injured kidney is highly necessary. Intense urinary NAG activity can be found in a variety of renal disease such as toxic nephropathies, ischemic renal injury following cardiac surgery or renal transplantation but also in glomerular disease especially in diabetic nephropathy. Rises in urinary NAG enzyme activity strongly suggests tubular cell damage and support NAG enzyme as a biomarker of renal tubular injury. The aim of this paper is to obtain a stable *in vivo* acute kidney injury experimental model, in Wistar, rats and to evaluate the urinary activity of N-acetyl- β -D-glucosaminidase (NAG) enzyme, blood levels of urea and creatinine and microstructural renal alterations induced by ischemia/reperfusion injury respectively gentamicin nephrotoxicity. For this purpose we have used a rat experimental model. Adult male Wistar rats weighing 250-300 g were randomly divided into 3 groups with 8 rats in each group. Group 1 served as a model for the renal ischemia/reperfusion injury experiment, group 2 served for toxic kidney injury experimental model and group 3 served as control group. All individuals in both groups 1 and 2 presented marked elevations in blood urea and creatinine at the moment of euthanasia (day 3 for group 1 and day 9 for group 2) compared to the control group where biochemical values remained within normal limits. Urine analysis of both group 1 and 2 showed marked urinary NAG index activity which suggests acute tubular injury, suggestion confirmed by histological evaluation of the renal parenchyma sampled from this subjects

Keywords: *N-Acetyl- β -D-Glucosaminidase, NAG, acute kidney injury, ischemia/reperfusion, gentamicin, nephrotoxicity.*

INTRODUCTION

Ischemia/reperfusion injury as a consequence of renal surgery, renal transplantation, shock, cardiac arrest renal together with various toxic injuries represents one of the major causes of acute kidney injury

(Gasanov *et al.*, 2011). Although extensive research performed in the last decades have substantially improved the knowledge of the mechanisms and pathological pathways involved in the development of renal ischemia/reperfusion injury and nephrotoxic

Fig. 1 Isolation of the right kidney

Fig. 2 Right renal pedicle clamping

injuries, an effective treatment for such lesions is still unavailable. Therefore, the identification of a suitable method to prevent and minimize the clinical effects of such injuries is highly required. In order to identify a proper treatment for acute kidney injuries, a stable experimental model that replicates the structural, metabolic and inflammatory lesions that occur in the natural acute injured kidney is mandatory.

N-Acetyl- β -D-Glucosaminidase (NAG) is a lysosomal enzyme located especially inside the epithelial cells of the renal proximal convoluted tubules. Rises in urinary NAG enzyme activity strongly suggests tubular cell damage and support NAG as a biomarker of renal tubular injury. During the course of renal disease, urinary NAG values remain permanently elevated. Intense urinary NAG activity can be found in a variety of renal disease such as toxic nephropathies, ischemic renal injury following cardiac surgery or renal transplantation but also in glomerular disease especially in diabetic nephropathy (Lerma *et al.*, 2014, Obermuller *et al.*, 2014).

Our aim was to evaluate the urinary activity of N-Acetyl- β -D-Glucosaminidase when ischemia/reperfusion injury or gentamicine toxicity is a contributing factor.

MATERIALS AND METHODS

Twenty-one healthy adult male Wistar rats weighing 250-300 g were selected for this study. All animals were cared for according to the experimental medicine protocols of University of Agricultural Sciences and Veterinary Medicine Cluj-Napoca. The animals were housed in an environmentally controlled animal house, in polypropylene rodent cages, at a temperature of $22 \pm 1^\circ \text{C}$ with a 12 h light/dark cycle and were allowed free access to drinking water and a standard granulated rodent laboratory rat diet.

The rats were randomly divided into 3 groups with 8 rats in each group. Group 1 served for the renal ischemia/reperfusion injury experiment, group 2 served for toxic kidney injury experiment and group 3 served as control group. Rats in group 1 were anesthetized with intramuscular injection of Xilazine (Xilazin Bio® 2%, Bioveta, Czech Republic) followed by 5mg/kg and Ketamine 50mg/kg (Ketamidol® 100mg/ml, Richter Pharma, Austria). After surgical preparation of the abdomen a midline celiotomy was performed and bilateral renal pedicles were isolated using minimal blunt dissection. Both renal pedicles were temporary occluded by surgical clamping for 1 hour using atraumatic microvascular clamps followed by reperfusion and abdominal wall closure (fig. 1 and 2). The animals were housed for three days in the same conditions. Enrofloxacin 5mg/kg/day (Enroxil® 10%, KRKA), intramuscular injection was given for postoperative infection prevention 3 days consecutively. Tramadol 20 mg/kg/day (Tramadol® 50mg/ml, KRKA) intramuscular injection was administered for postoperative analgesia 3 days consecutively. All animals were euthanized in the third day after surgery by means of Isoflurane euthanasia chamber. Whole blood was processed in order to measure serum urea and creatinine concentrations. Urinary NAG index activity was evaluated in urine samples obtained by cystotomy immediately after euthanasia. Kidney samples were obtained for histological examination.

Rats in group 2 were intraperitoneally injected with 100 mg/kg gentamicine sulphate (Gentamicin® 40mg/ml KRKA) for 8 consecutive days (Yaman and Balikci, 2009; Whiting and Brown, 1996). All individuals were euthanized in day 9 of the experiment by means of Isoflurane (Anesteran®, Rompharm, Romania) euthanasia chamber. Blood samples were collected and serum was obtained for urea and creatinine dosing.

Fig.3. Renal ischemia/reperfusion influence on urea

Fig.4. Renal ischemia influence on creatinine

Immediately after euthanasia urine was collected by cystotomy for NAG index activity evaluation. Complete necropsy was performed in all cases, and the kidneys were removed for histological examination.

Biochemical analysis:

Serum creatinine concentration was measured using a spectrophotometric kinetic method and Jaffe reaction while serum urea concentration was measured using a spectrophotometric kinetic method. In order to determine the Urinary NAG index, first urine samples were centrifuged at 1000 rpm for 5 min at 4°C then the enzymatic activity was measured by a spectrophotometric, colorimetric method. NAG index was measured using an end-point spectrophotometric reaction whereas the urinary concentration of creatinine was determined by a spectrophotometric, kinetic reaction using the Jaffe method. Urinary NAG index was calculated by the following equation: NAG index (U/g) = urinary NAG activity (U/l) / urinary creatinine concentration (g/l) (Sato *et al.*, 2002).

Statistical analysis: Results for each parameter were processed by average, standard deviation and verified by coefficient of variation. The experimental groups were compared with the control for each parameter.

Histological analysis:

A Complete necropsy was performed in all cases, and the kidneys were removed for histological analysis. The renal samples were fixed in 10% neutral buffered formalin and embedded in paraffin wax. Serial sections (4µm) were realized for routine Haematoxylin and Eosin (H&E) staining. The kidney sections were examined using an Olympus BX 51 microscope and the images were taken with Olympus UC 30 digital camera and processed using an image acquisition and processing program Olympus Stream Basic.

RESULTS AND DISCUSSION

In both groups all individuals presented marked elevations in blood urea and creatinine concentrations at the moment of euthanasia (day 3 for group 1 and day 9 for group 2). Increased urinary NAG index activity indicates acute tubular injury, which was also confirmed by renal histology analysis.

Control group urea, creatinine and NAG index values

The control group recorded an average of 17.9375 urea in mg/dl, with a standard deviation (SD) of 1.2806 mg/dl (Fig. 3 & 5). The variation within the group is reduced, the group is homogeneous in terms of this parameter, the coefficient of variation was below 10%:

Fig.5. Renal ischemia influence on NAG index

Fig.6. Gentamicine Nephrotoxicity influence on urea

Fig.7. Gentamicine nephrotoxicity influence on creatinine

C.V% = 7.14. Creatinine recorded in the control group varies between 0.8 and 1.02 g/dL, with an average of 0.88125 mg/dL and a standard deviation of 0.0844 mg/

dL. The control group is homogeneous, coefficient of variation of creatinine has value 9.58% (Figure 4 & 7).

Fig.8. Gentamicine nephrotoxicity influence on NAG Index

NAG index has averaged 5.71125 U/g in the control group, with a standard deviation of 0.4848 and a low variation within the batch. C.V.% = 8.49 (Fig. 5 & 8).

Lot average was 81.225 U/g, with a standard deviation of 10.73 U/g and a variation in average values: C.V. = 3.21%.

Renal ischemia/reperfusion influence on urea, creatinine and NAG index values

Following induction of renal ischemia, urea values risen over 15 times, between 258.3 mg/dL and 334.5 mg/dL. Average urea value is 298.0625 mg/dL with a standard deviation of 28.1525 mg/dL. Variation of urea in the renal ischemia/reperfusion rat group is reduced: C.V.% = 9.45 (Fig. 3)

Renal ischemia/reperfusion injury produces a increase of about 9 times in creatinine concentration so that creatinine values recorded in the group have an average of 7.99875 mg/dL. the mean standard deviation of 0.9137 mg/dL (Fig. 4).

NAG index increases by an average of 15 due to renal ischemia/reperfusion injury (Fig. 5) NAG index values recorded where within the range of 75.1-112.8 U/g. Group average is 88.22857 U/g, with a standard deviation of 16.84 U / g and an average variation values: C.V = 19.09%.

The influence of gentamicine nephrotoxicity on urea, cratinine and NAG index values

Following gentamicin administration urea values risen by nearly 20 times, between 298.6 mg/dL and 389.7 mg/dL. Urea lot average was 340.55 mg/dL, with a standard deviation of 34.6272 mg/dL. Urea variation in the rat group with gentamicine nephrotoxicity is low to medium: C.V% = 10.17 (Fig. 6).

Creatinine increases nearly 10 times, the average value for the group of rats with gentamicin nephrotoxicity is 9.095 mg/dL, with a standard deviation of 0.7958 mg/dL and a low variation: C.V = 9.58% (Fig. 7).

NAG index increases by an average of 14 times due to gentamicine nephrotoxicity (Fig. 8) so that in the gentamicine nephrotoxicity group NAG index values were found to be between 64.9 and 95.7 U/g.

Histological studies

Group 1

Grossly, areas of congestion and necrosis were observed in the cortex of the kidney. Microscopically, the renal morphological alterations were represented by necrosis and mineralization of the renal tubules and of some glomeruli from the cortex, without any inflammatory infiltrate. The epithelial cells of the proximal and distal convoluted tubules were detached from the basement membrane, with pyknotic nuclei and mineralization. No signs of regeneration were observed (fig. 9).

Group 2

All animals showed massive tubular necrosis with the presence of granular eosinophilic material in the tubular lumen. In most of the cases even the basement membrane was destroyed. Some tubules were still intact, with granular eosinophilic material within the tubular epithelial cells (hyaline). Hyaline casts were also observed in some sections. No signs of mineralization were present. Slight mononuclear inflammatory infiltrate was observed in all cases (Friedewald *et al.*, 2004) (fig.10).

CONCLUSION

Our study demonstrates that both induction of warm renal ischemia/reperfusion injury by bilateral renal pedicle surgical occlusion and acute kidney injury by administration of nephrotoxic doses of gentamicin are feasible methods for obtaining a proper *in vivo* model of acute kidney injury. Both experimental models demonstrate a increase in urinary NAG values which are reflected by the histological alterations induced by renal ischemia/reperfusion respectively gentamicin nephrotoxicity. The renal extent injuries were also

Fig.9. Severe necrosis (black arrow) and mineralization (red arrow) of the renal tubules, no signs of regeneration were observed HEx200.

Fig.10. Massive renal tubular necrosis (black arrows), mononuclear inflammatory infiltrate (blue arrow), renal hyalinos (red arrow) HEx200.

proved by significant elevation of serum creatinine and urea in both experimental groups.

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