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Moderate aerobic exercise on the recovery phase of gentamicin-induced acute kidney injury in rats

C.S. Oliveira [a], A.M. Rodrigues [b], G.B. Nogueira [b], M.A. Nascimento [a], G.R. Punaro [b], E.M.S. Higa [a,b,c,*]

[a] Translational Medicine, Universidade Federal de Sao Paulo, UNIFESP-EPM, Brazil
[b] Nephrology, Universidade Federal de Sao Paulo, UNIFESP-EPM, Brazil
[c] Emergency Division, Universidade Federal de Sao Paulo, UNIFESP-EPM, Brazil

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ABSTRACT

Introduction: Acute kidney injury is a serious public health problem, especially in intensive care units, where patients may require dialysis support, resulting in 50% mortality.
Aim: To evaluate the effects of moderate aerobic exercise on the recovery phase of acute kidney injury induced by gentamicin in rats.
Main methods: Male adult Wistar rats were allocated into 4 groups: W10 + R30, G10 + R30, W10 + EX30 and G10 + EX30; W10 received water (gentamicin vehicle) and G10 received gentamicin for 10 days; R30 remained resting and EX30 made exercise for 30 days after gentamicin suspension. Training was performed on treadmill. Blood, 24 h urine and kidneys were collected for renal function and oxidative stress, antioxidant, TGF-β and histological analysis.
Key findings: Gentamicin treatment caused decreased renal function significant oxidative stress, reduced urinary nitric oxide and increased TGF-β. G10 + R30 presented partial recovery of metabolic data, renal function and lipoperoxidation levels, although they were still altered compared to W10 + R30. Besides, we observed the presence of lymphomononuclear infiltrate in the kidneys of G10 + R30. G10 + EX30 vs G10 + R30 showed additional improvement of all the mentioned parameters, showing at histology, regeneration of the tubule epithelium.
Significance: Our data suggest that moderate exercises could help in the recovery of metabolic parameters, renal function and structure on gentamicin-induced AKI, perhaps due to restoration of redox balance. This could protect the kidneys from further insults like challenges with nephrotoxic drugs or the aging per se.

1. Introduction

Acute kidney injury (AKI) is a serious public health problem, especially in intensive care units, where patients may require dialysis support, resulting in 50% mortality. Several factors can cause AKI, including the low renal perfusion and septicemia, the use of radiocontrast agents or antibiotics such as the aminoglycosides, like gentamicin (G), which should be avoided mainly in established AKI [1].

G is an aminoglycoside used alone or in combination with other antibiotic for the treatment of serious infections caused by Gram negative bacteria [2]; however, it has several side effects such as the nephrotoxicity. It is estimated that up to 30% of patients treated with G for 7 or more days show signs of AKI, and uncritical use of this drug is associated with increase in hospital morbidity and mortality [3].

G nephrotoxicity starts with its absorption by megalin, a membrane receptor of tubular cell. Once inside the cell, G can follow two ways: it can be captured by the lysosome and cause damage in the cell membrane by phospholipidosis; for the other hand it can act on mitochondria and induce oxidative stress, inducing necrosis and apoptosis. The tubular injury is the major effect of G, with loss of the brush border of the epithelial cells, progressing to acute tubular necrosis [4].

According to Rivas-Cabanero et al. [5] G increased the synthesis of nitric oxide (NO) in cell culture, and this increase was blocked by L-nitroarginine methyl ester (L-NAME, a NO inhibitor); furthermore, the contractile effect caused by G in the planar surface of mesangial cells was diminished by this inhibition. However, NO synthesis by G is controversial, since other studies showed a reduction of that molecule both in rats and cells treated with G [6].

Other mechanisms seem to be involved in G-induced nephrotoxicity including the increase in endothelin-1 and infiltration of monocytes-macrophages [7], lipid peroxidation and reduction in antioxidant defense [8]. Another study showed that rats treated with G had increased lipid peroxidation and reduced glutathione in kidney tissue [9].

In recent decades, surveys have been applied in an attempt to reverse the kidney damage from different etiologies, and the physical exercises showed beneficial effects for example, to slow the progression of

* Corresponding author at: Universidade Federal de Sao Paulo, Department of Medicine, Rua Botucatu, #740, Vila Clementino, 04023-900 Sao Paulo, SP, Brazil.
E-mail address: emshiga@unifesp.br (E.M.S. Higa).

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diabetic nephropathy [10]. In this study, it was described a relationship between NO and training, since the rats submitted to exercises on treadmill had recovered the production of NO in the kidneys; at the same time, training partially blunted the progression of diabetic nephropathy. Moreover, in other research, it was found that exercise increased the extracellular superoxide dismutase activity by a mechanism dependent of NO [11].

Besides, there are few studies about exercise and acute kidney disease [12,13], with no data reporting exercises in the recovery phase. The aim of the present study was to evaluate the effects of moderate aerobic training on the recovery phase of G induced AKI in rats.

2. Materials and methods

2.1. Animals

Male Wistar rats weighing in average 280 g were obtained from the Central Animal Housing of Escola Paulista de Medicina. The rats were kept at a controlled temperature of 22 ± 2 °C, in an environment with regular period of light and dark cycle of 12:12 h and with standard chow and water ad libitum. The protocol was approved by the Ethics Committee in Research of Universidade Federal de Sao Paulo, protocol #424014.

2.2. First protocol – induction of AKI

A dose of 100 mg/kg/day ip of G (Gentatec, Sao Paulo, Brazil) was applied for 10 days. The control group received the same amount of vehicle (distilled water) [6].

2.3. Second protocol – aerobic training

The exercise protocol began with the G discontinuation, and all animals were pre-selected for running; this is because there are animals that do not adapt to a treadmill exercise routine, and these ones were allocated to the resting group. The exercise consisted of a moderate running on treadmill at 16 m/min during 60 min/day, 5 days/week, during 30 days, with no inclination (0%). The training program was preceded by 1 week period of adaptation; this adaptation was made in periods of 10, 20 and 30 min with a speed of 10 m/min, with 2 min of interval each time. After the adaptation week, the running speed was increased gradually every day, until the rats started to run at the standard speed of 16 m/min [14]. From this point, they were randomly assigned to the groups with 6–8 animals each:

a) W10 + R30: they received G vehicle and remained resting.
b) W10 + EX30: they received G vehicle and were submitted to aerobic training.
c) G10 + R30: they received G and remained resting.
d) G10 + EX30: they received G and were submitted to aerobic training.

2.4. Metabolic profile

All animals were placed in metabolic cages (Tecniplast, Italy) for 24 h, receiving water and chow ad libitum, at the end of the first and second protocols; diuresis, water and food intake were recorded. At the end of both protocols, we collected samples of 24 h' urine and a small aliquot of blood from the retro-orbital plexus under anesthesia, after 3 h of fasting (same day); the urine and plasma samples were stored at −20 °C for further analysis.

2.5. Renal function

Plasma and urinary levels of creatinine were measured by colorimetric assay using a Labtest Creatinine kit (Centerlab Ltda, Sao Paulo, Brazil). The plasma and urinary levels of urea were measured using a Labtest Urea CE kit (Centerlab Ltda, Sao Paulo, Brazil). The proteinuria was measured by Sensiprot Labtest kit (Centerlab Ltda, Sao Paulo, Brazil).

2.6. NO measurement

The NO was measured in the plasma, urine and renal cortex samples by chemiluminescence using the Nitric Oxide Analyzer (NOA™280, Sievers Instruments Inc., CO, USA), a high-sensitivity detector for measuring NO, which is based on the gas-phase chemiluminescent reaction between NO and ozone. The emission of a photon from electrically excited nitrogen dioxide is in the red and near-infrared region of the spectrum, and it is detected by a thermoelectrically cooled red-sensitive photomultiplier tube. The sensitivity for measurement of NO and its reaction products in liquid samples is ~1 picomol [15].

2.7. Estimation of lipid peroxidation

Lipid peroxidation was estimated by the thiobarbituric acid reactive substances (TBARS) method, with a molar extinction coefficient of 1.56×10^5 cm/mol, in plasma, urine and kidney [16,17].

2.8. Antioxidant

The antioxidant profile was determined in the kidneys: superoxide dismutase (SOD) was analyzed by Superoxide Dismutase Assay Kit (ESOD-100) and catalase (CAT) was determined by Catalase Assay Kit (ECAT-100), both from BioAssay Systems, EnzyChrom™ (Hayward, USA); total glutathione (GSH) was determined by Colorimetric Glutathione Assay Kit from Biovision (Boulevard, USA).

2.9. Euthanasia

At the end of the second protocol, the animals were euthanized with a high dose of anesthetic (ketamine chloridrate at 90 mg/kg and xylazine chloridrate at 18 mg/kg, both i.m.), an incision was made in the abdominal region and one of the kidneys of each animal was removed and stored in the freezer at temperature of −80 °C for NO, TBARS, SOD, CAT and GSH analysis; while the other kidney was prepared for histological analysis.

2.10. Histological analysis

The kidneys were fixed in 10% formaldehyde and embedded in paraffin, sectioned at 4 mm thickness and stained with hematoxylin-eosin (HE) and periodic acid Schiff (PAS). The analysis was carried out at a magnification of ×200 and ×400 respectively, analyzed by a pathologist, expert in kidneys, under blinded conditions.

Table 1
Metabolic profile, renal function, NO and TBARS after 10 days of G treatment in rats.

	W10	G10
Diuresis (mL/24 h)	11.5 ± 0.6	26.3 ± 3.0 [a]
Water intake (mL/24 h)	27.1 ± 1.5	46.9 ± 5.3[a]
Food intake (g/24 h)	17.4 ± 0.7	12.4 ± 1.4[a]
Body mass (g)	323.3 ± 0.7	324.1 ± 6.9
Plasma creatinine (mg/dL)	0.6 ± 0.1	2.6 ± 0.6[a]
Creatinine cl. (mL/min)	1.1 ± 0.1	0.5 ± 0.1[a]
Plasma urea (mg/dL)	37.8 ± 1.4	228 ± 49.0[a]
Proteinuria (mg/24 h)	12.6 ± 0.1	36.5 ± 4.0[a]
NO excretion (μmol/24 h)	2282 ± 198.4	1228 ± 336.9[a]
Plasma TBARS (nmol/mL)	6.5 ± 0.3	8.0 ± 0.7
TBARS excretion (nmol/24 h)	58.0 ± 5.7	203.0 ± 16.4[a]

Values expressed as mean ± SEM, unpaired student *t*-test; N = 18 for both groups. W10, water for 10 days; G10, G for 10 days; G, gentamicin; NO, nitric oxide; TBARS, thiobarbituric acid reactive substances. Cl, clearance. $p < 0.05$: [a]vs W10.