

Exercise-induced apoptosis in the renal tubular cells of the rat

Marzena Podhorska-Okołów¹, Piotr Dzięgiel¹, Eugenia Murawska-Ciałowicz^{2, 3},
Barbara Krajewska¹, Urszula Ciesielska¹, Zbigniew Jethon², Maciej Zabel^{1, 4}

¹Department of Histology and Embryology, Medical University, Wrocław, Poland

²Department of Physiology, University of Physical Education, Wrocław, Poland

³Department of Hygiene, Medical University, Wrocław, Poland

⁴Department of Histology and Embryology, University of Medical Sciences, Poznań, Poland

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A number of studies have shown that acute physical exercise is associated with the induction of apoptosis not only in skeletal muscle but also in many distant organs. One of the pathogenic agents responsible for exercise-induced damage in many tissues is the generation of oxygen free radicals. The aim of the present study was to examine the influence of exercise-induced oxidative stress on the rat kidney. The analysis was performed on the kidneys of rats subjected to treadmill running until exhaustion. Our results demonstrated that acute exercise led to apoptotic damage of the renal distal tubular cells, although this was not a result of oxidative stress.

Key words: apoptosis, exercise, kidney, oxidative stress

INTRODUCTION

It is well accepted that acute exercise can cause skeletal muscle damage, including apoptosis or necrosis of the myofibres [8]. Excessive physical exercise disturbs the entire homeostasis in the body and leads to haemodynamic and metabolic alterations not only in the skeletal muscles but also in many distant organs. One of the proposed mechanisms responsible for tissue damage after physical effort is oxidative stress [5]. Intense exercise accompanied by a manifold increase in oxygen utilisation has been shown to increase the probability of free radicals of appearance which react with cellular macromolecules and may cause extensive damage to many cellular structures, such as membranes [1]. The most common index of oxidative stress is an increase in oxidative biomarkers, such as lipid peroxidation products. Free radical generation seems to be responsible for exercise-induced

changes not only in the skeletal muscles but also in the heart, cells of the immune system, lungs and liver [2, 5, 7]. Oxidative stress is widely recognised as activating the apoptotic process [7]. Apoptosis is a form of genetically controlled cell death characterised by specific morphological, biochemical and molecular events [10]. Recent studies have shown that apoptosis as well as necrosis contribute to loss of renal tubular cells in response to acute renal failure [9]. Despite the evidence for an increase in oxidative damage biomarkers in many tissues following physical exercise, we still lack a comprehensive study regarding the relationship between exercise-induced oxidative stress and renal tubular cell damage. The present study was designed to elucidate whether intense exercise could cause changes in the rat kidney and whether oxidative stress might be responsible for the tissue damage.

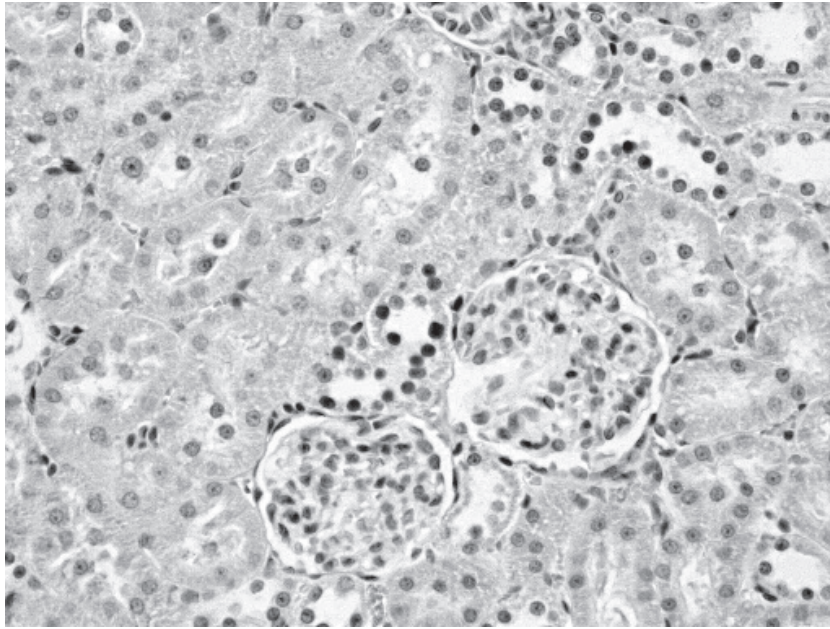


Figure 1. Apoptosis in renal distal tubular cells after acute exercise. TUNEL technique (black nuclei). Cortex of kidney counterstained with haematoxylin. Magnification: $\times 200$.

MATERIAL AND METHODS

18 male Wistar rats, 10–12 weeks of age (200–250 g body weight), formed groups of running ($N = 12$) and non-running animals ($N = 6$). Animals from the exercised group were subjected to running on the treadmill at 1.0 km/h until exhaustion. The mean time to exhaustion was 90 min (range: 60–115 min). After the exercise, the animals returned to their cages and were randomly grouped into animals killed 6 hours ($N = 6$) and 96 hours ($N = 6$) after cessation of the exercise. The control animals ($N = 6$) remained in their cages throughout the experiment.

All the animals were anaesthetized and decapitated. Both kidneys of each rat were excised. The right kidney was divided into two parts. One half was fixed in 4% buffered formaldehyde solution for 24 hours and embedded in paraffin, while the other was fixed according to Karnovsky and prepared for electron microscopy. The left kidney was frozen in liquid nitrogen and stored at -80°C .

Apoptosis was detected in the paraffin sections by the TUNEL technique, using the ApopTag[®] Plus Peroxidase In Situ Apoptosis Detection Kit (INTERGEN, Norcross, USA). The percentage of apoptotic nuclei was evaluated by the number of brownish-labelled cell nuclei detected after scoring 500 cell nuclei per section [3]. The extent of lipid peroxida-

tion in tissue homogenates was calculated by measuring the levels of malondialdehyde and 4-hydroxyalkenals (MDA + 4-HDA) by using the Peroxidation Assay Kit (Calbiochem, La Jolla, CA, USA).

Statistical analysis of the results was conducted by using the Chi-square test and the Statistica 5.1 PL software (StatSoft, Cracow, Poland). The differences were considered significant if $p < 0.05$.

RESULTS AND DISCUSSION

The acute exercise resulted in a significant increase in the number of apoptotic nuclei in the renal tubular cells in comparison to the control animals. Apoptosis was present only in the distal convoluted tubules and in the collecting ducts of the kidney medulla of all the exercised animals (Fig. 1). It is well documented that tubular cells die by necrosis as well as by apoptosis in response to ischaemic or toxic injury [4], and the manner of cell death seems to be dependent upon the type of pathogenic mechanism and on the cell types in the organ. The literature of the subject contains extensive data demonstrating apoptosis in response to transient ischaemia [6, 9], either in the cells of proximal convoluted tubuli or in those of distal convoluted tubuli. In our experiment we observed no apoptotic nuclei in proximal convoluted tubuli. The results obtained were confirmed by the electron microscopy

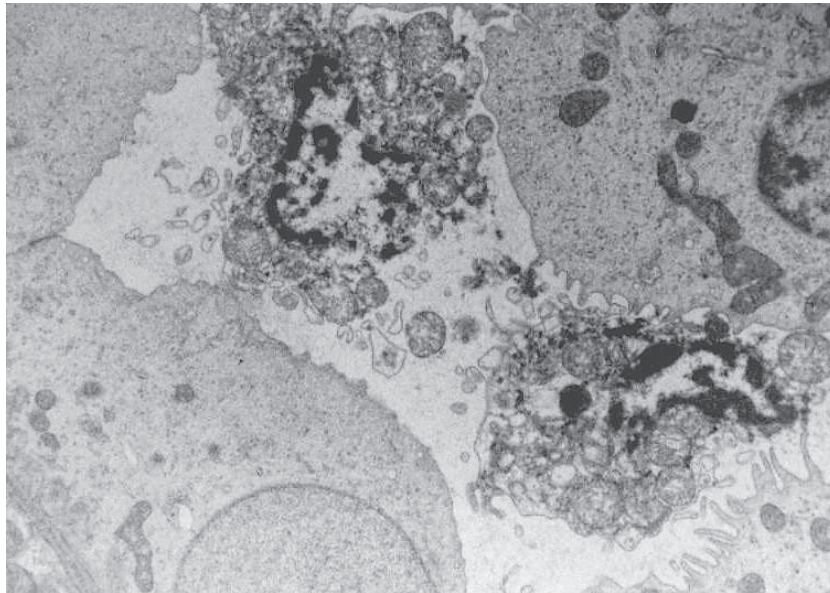


Figure 2. Electron micrograph of two tubular apoptotic cells inside the loop of Henle's lumen. EM × 7500.

observations, which revealed the presence of cells with typical apoptotic cell nuclei only in the distal convoluted tubuli or in the collecting ducts of the medulla in the kidneys of both groups of animals subjected to exercise. Likewise, Oberbauer et al. [6] demonstrated that distal convoluted tubules display suppression of bcl-2 and strong expression of BAX protein, which may provide an explanation for the detection of apoptosis in this study only in the distal convoluted tubuli. Moreover, the electron microscopy observations documented shedding of the apoptotic bodies directly to the lumen of the renal tubule or duct (Fig. 2). This, as we hypothesise, seems to be the simplest way of getting rid of the apoptotic cells instead of their phagocytosis by neighbouring epithelial cells.

There was no significant difference in the appearance of apoptosis between the two exercised groups. The percentages of apoptotic cell nuclei in the kidneys removed 6 hours and 96 hours after the cessation of running were similar, with a slight but statistically insignificant predominance of the latter group (Fig. 3).

As it has been postulated by many authors [1, 2, 5, 7], the generation of oxygen free radicals by exercise-induced lipid peroxidation could be one of the mechanism underlying apoptotic DNA damage. However, in our study the level of lipid peroxidation markers (MDA + 4-HDA) in kidney homogenates 6 hours after exercise was lower as compared to the control group, whereas it returned to

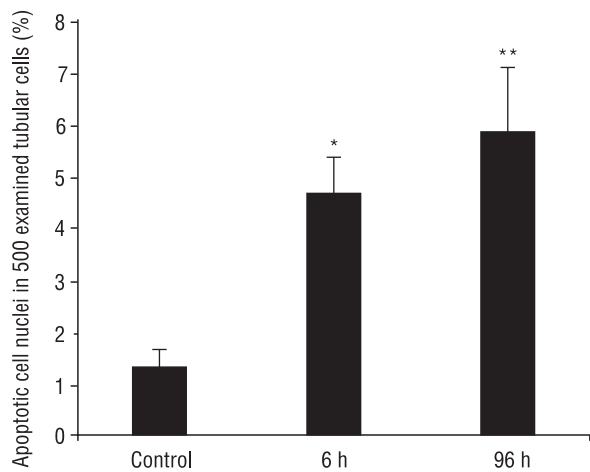


Figure 3. Apoptosis in kidney tubular cells. Control: non-running animals. 6 h: running animals killed after 6 h. 96 h: running animals killed after 96 h. Significant differences: control as compared to 6 h * $p < 0.01$; control as compared to 96 h ** $p < 0.001$.

the control level 96 hours after the exercise (data not shown).

Our results demonstrated that acute exercise could lead to apoptotic damage of the renal distal tubular cells but did not affect the proximal tubuli. The induction of apoptosis in kidney tubular cells is thought not to be associated with oxidative stress. We hypothesise that this process is rather connected with transient ischaemia after exercise, which results in a decrease in glomerular filtration and altered tubular function.

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