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Protective effect of Erythropoietin on renal injury induced by acute exhaustive exercise in the rat

Xixiu. Lin¹, Shulin. Qu^{1*}, Minyu. Hu², Chonghe. Jiang^{1,3}

¹ Medical College of Hunan Normal University, Changsha, 410013, China

² Public Health College of Central South University, Changsha 410078, China.

³ Department of Clinical and Experimental Medicine, University of Linköping,
581 85 Linköping, Sweden

Running head: Effect of Erythropoietin on Renal Injury

* To whom correspondence should be addressed:

Prof. Shu-Lin Qu, MD

Medical College of Hunan Normal University

Changsha, Hunan 410013

P. R. China.

Tel & Fax: +86 731 88630333

E-mail: qushulin07@yahoo.com.cn

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Abstract

We investigated the protective effect of Erythropoietin (EPO) analogue rHuEPO on renal injury induced by acute exhaustive exercise in the rat. Rats were randomly allocated to one of 3 groups: normal control (C), exhaustive exercise test (ET) and EPO pre-treatment (rHuEPO 2000 U/ kg) plus ET (EPO+ET). Compared with controls, animals in the ET group had increased serum urea nitrogen, serum creatinine, urine protein, and renal tissue malondialdehyde (MDA) and decreased renal tissue nitric oxide (NO), nitric oxide synthase (NOS) and superoxide dismutase (SOD) activities. There was a severe damage in renal tubular epithelial cells with a lot of cell apoptosis, and TUNEL assay revealed a remarkably high apoptotic index ($p < 0.01$). Changes in renal function and kidney tissue were much less in the EPO+ET group ($p < 0.05$) and the apoptotic index was much lower than in the ET group (18.45 ± 0.32 vs 27.55 ± 0.49 , $p < 0.05$). EPO pretreatment thus significantly prevented renal cell apoptosis, and counteracted high MDA and low NO and NOS renal contents induced by exhaustive exercise. The data point to a potential value of EPO in preventing the acute renal injury after exhaustive exercise.

Introduction

Acute exhaustive exercise is a health risk of physical activity, eventually resulting in reduced functional capacity (19, 20, 27). It also causes organ injury (1, 31), especially due to “tissue ischemia” during the exercise and “ischemia reperfusion” after cessation of the exercise (27, 28).

Acute exhaustive exercise entails particular risks of acute renal injury as demonstrated in athletes and heavy physical workers (22, 23, 29, 31). Blood flow and oxygen supply to kidney will decline rapidly with the increase in exercise intensity, inducing both tissue ischemia, and “ischemia reperfusion” tissue injury (27, 28). Free radical changes and apoptosis in renal tubular epithelial cells have also been described after exhaustive exercise (2, 15).

Recently, erythropoietin (EPO) has been reported to have several important biological effects in addition to the stimulation of erythropoiesis. Recombinant Human Erythropoietin (rHuEPO), a synthetic form of EPO, has been shown to have protective effect against acute ischaemic injury in several organs and tissues (3, 4, 6, 8). In the kidney, the protective effect of EPO has been observed both on acute renal failure (ARF) induced by ischemia reperfusion after experimental arterial occlusion and on damage to tubular cells when cultured under conditions of toxins and hypoxia (7, 34), and appears to be due to inhibition of cell apoptosis, stimulation of cell regeneration, and promoted recovery of renal function (7, 25, 27, 30).

Exhaustive exercise may differ from the above experimental conditions in several respects (no artery occlusion, severe changes in the whole body metabolism) and we were interested in investigating the potential beneficial effects of EPO on kidney in this common situation of effort and exhaustion. In this study we explored the effects of a single dose of EPO pre-treatment on the changes in renal function and renal tissue

induced by acute exhaustive exercise in rats. Special attention was given to monitoring the functional performance of the animals, and to analyzing the degree of cell apoptosis in renal tissue.

Materials and methods

Animals

Twenty-four healthy male Sprague-Dawley rats, aged about two months and weighing 200 ± 20 g were used in this study. They were stratified, with matching body weights, into 3 groups of 8 animals: normal control (C), exhaustive exercise (ET) and exhaustive exercise plus EPO pre-treatment group (EPO+ET). All animals were similarly handled in the animal house of the university, had free access to food and water and were kept at a temperature of $25 \pm 3^\circ\text{C}$, with natural illumination and ventilation. Their general health state and activity were monitored closely during the experiment. The experimental protocol for this study was pre-approved by the Hunan Normal University Institutional Review Board and in accord with the Public Health Service policy on the use of animals for research (10).

Experimental procedure

All animals performed a 15 minutes warming up exercise by running on a horizontal-treadmill (slope gradient 0%, ZH-PT-1 Treadmill, Bio Equipment Co., Ltd. Huaibei, Anhui, China) at a speed of 10 m/min. The animals in group C underwent no more exercise, while those of group ET were further imposed a treadmill running at gradient 10% for 10 minutes and speed of 18 m/min, and then 22 m/min, followed by 26 m/min for 15 minutes, and finally kept at 30 m/min (about 92.3% VO_2max) until exhaustion. In the latter stage, the animals looked tired, their running speed gradually slowed down and movement and posture changed from stride to semi decubitus running with the abdomen occasionally touching the ground, or complete ventral decubitus running. In the last period, rats halted on the runway more than three times without any reaction to various driving manipulations, and exhibited shortness of breath, burnout, lying down, and weak or absent escape response (9, 21). To ensure

the same level of exercise intensity, the animals were forced to keep a consistent running throughout the training by various stimulations such as sound, light, mechanical stimulation, or electrical stimulation of the tail. The same exercise procedure were applied to the EPO+ET group rats, except that they received an intraperitoneal (i.p) injection of rHuEPO (Diao Pharmaceutical, Chendu, China, Cas NO 20050704) 2000 U/kg diluted in 2 ml saline 30 minutes before the training (4).

Samples collection

After 24 hours recovery from exhaustive exercise, or from warming up exercise for control animals, the animals were anaesthetized with Midazolam (0.8 – 1.2 ml/kg, i.p, the Veterinary Institution of Academy of Military Medical Sciences, China) and sacrificed by decapitation. Blood was collected from the heart and centrifuged to obtain the serum, which was stored at -20°C for serum urea nitrogen and creatinine tests. Urine was collected from the urinary bladder for protein test. The left kidney was taken out for morphological and biochemical examinations. The renal cortex was rinsed with ice-cold saline after removing the medulla. About 30% of renal cortex was fixed in 10% formaldehyde solution for histopathology examination; the rest was quickly frozen with liquid nitrogen and stored at -70°C for other related tests.

Determination of NO, MDA, SOD and other parameters of renal function

A 10% homogenate of renal cortex (0.1 g renal cortex and 0.9 ml saline) was prepared using an electric homogenizer. The supernatant was collected after centrifugation for 10 minutes at 2100 x g. NO is very instable with rapid metabolism in vivo, converting into NO₂⁻ and NO₃⁻. The applied method was to revert NO₃⁻ into NO₂⁻ by using nitrate reductase specificity. Thus, the levels of NO were determined photometrically at 550 nm, measuring the color depth and absorbance value of each tube (standard, blank and sample tubes divided as the instruction of kit from Jianche Biological Institution, Nanjing, China). MDA was measured by thiobarbituric acid colorimetry.

SOD was detected using Radioimmunoassay counter (RIA) competitive inhibition

principle of radioimmunoassay: 1) Add different concentrations of SOD standard 100 μ l into standard tubes and rabbit serum 100 μ l to the sample tube, 450 μ l diluent to NSB and 100 μ l to B₀ tubes. 2) Add anti-human SOD serum (1:1 600) 250 μ l into standard, sample and B₀ tubes respectively, vortex and incubate 6 h at 4°C. 3) Add ¹²⁵I-SOD 50 μ l to each tube, vortex and incubate 24 h at 4°C. 4) Add containing 6% PEG goat anti-rabbit IgG (1:32) 100 μ l into standard, sample and B₀ tubes respectively, vortex and incubate 16 h at 4°C. Measurements were done by calculating the total radioactivity of each tube with FJ2003/50 γ immune counter.

NOS was measured by enzymatic test of NADPH diaphorase activity. With NOS catalyzed L-arginine and reaction of molecular oxygen produced NO, colored compounds are formed by the NO and nucleophilic substances. Absorbance was determined at 530nm wavelength and NOS activity was calculated based on the absorbance intensity, defined as 1 nmol NO generated by 1 mg of protein in one minute and expressed as the one unit of enzyme activity (U). Serum urea nitrogen was determined by the technique of 2-acetyl-monoxime colorimetry, serum creatinine by trinitrophenol colorimetry and the content of urine protein by the biuret method. All the above assays were carried out following the instructions of the corresponding kit from Nanjing Jiancheng Bio-engineering Institute, China (<http://www.njjcbio.com>).

Hematoxylin-Eosin (HE) staining

After 24 hours fixation in 4% paraformaldehyde phosphate buffer solution (0.1M, PH = 7.4), the sample of kidney tissue was embedded in paraffin and cut into serial sections of 5 μ m. The sections were dewaxed with dimethylbenzene and stained with HE for evaluation of morphologic changes under light microscope.

Transmission electron microscopy (TEM) sample preparation

1) The renal tissue blocks (1 mm³) were immersed in 2.5% phosphate buffered glutaraldehyde for 2 h and washed three times (each 10 min) with 0.1 mol/L phosphate buffer. 2) 1% Osmium tetroxide fixed for 1.5 h, washed with PBS 3 times

(each 10 min). 3) 50% ethanol dehydrating for 15 min, 70% ethanol 40 min, 90% ethanol 15 min, 90% propanol: 90% ethanol (1:1) overnight, pure embedded medium embedding for 5 h, low viscosity spurr embedded medium embedding overnight at 60°C. 4) Semi-thin sections (1µm) were sliced, and ultrathin sections (500 – 700 Å) prepared after toluidine blue staining and position fixated. 5) The sections were stained with uranyl acetate and lead nitrate, observed under JEM1230 transmission electron microscope (The JEOL institute, Japan).

Assessment of cell apoptosis

Cell apoptosis was visualized by Terminal deoxynucleotidyl Transferase Biotin-dUTP Nick End Labeling (TUNEL), using in situ apoptosis detection kit (Beijing Huamei Biotechnology Company, China). The TUNEL positive cells, i.e. apoptotic cells, were identified by the appearance of brown particles in the nucleus. Two parameters were used as the variables to evaluate the differences between groups: the incidence of apoptosis, which was the rate of apoptotic cells determined by examining six fields per slice in five slices from each kidney sample under high power microscope (x 400); and the apoptotic index (AI), which was the mean number of apoptotic cells defined by the average number of apoptotic positive cells in every 100 cells measured by Simple PCI micro-image software (33).

Statistical analysis

Data were analyzed by using statistical software SPSS (version 11.5 for Windows, SPSS, Chicago, IL, USA). All data are presented as mean ± SD. ANOVA followed by post-hoc test was used for multi-group comparison and Student-Newman-Keul's test for two group comparison. $P < 0.05$ was considered statistically significant.

Results

General condition of the rats after exercise

All the rats of the ET and ET+EPO groups fulfilled the exercise program. The rats of

the ET group appeared exhausted, with obscuration of the eyes, while those of the EPO group looked normal (see Methods). The time until exhaustion was 180.5 ± 41.5 minutes in ET group, and 226.8 ± 64.0 minutes in the ET+EPO group. Although the time until exhaustion was longer in the EPO pre-treated rats, the difference between the two groups did not reach significance.

Changes in renal function

Urine protein content, serum urea nitrogen and serum creatinine were significantly increased in the ET group with respect to control, while only creatinine was increased in the EPO+ET group. The urine protein content and serum creatinine level in ET group were also significantly higher than in the EPO pre-treated group ($p < 0.05$, [Table 1](#)).

Table 1 Levels of urine protein, serum urea nitrogen and creatinine in different groups (mean \pm SD, n = 8)

Group	Urine protein (mg/L)	Serum urea nitrogen (mmol/L)	Serum creatinine (μ mol/L)
Control	18.9 ± 4.7^a	4.6 ± 0.8^a	79.2 ± 48.2^a
ET	$38.5 \pm 13.6^{b*}$	$5.5 \pm 0.9^{b*}$	$199.2 \pm 25.9^{b**}$
ET+EPO	24.9 ± 7.7^a	4.5 ± 0.4^a	123.8 ± 4.8^c

Statistical differences between groups in each column were indicated by different alphabetic superscripts: (b) differs from (a) * $P < 0.05$, ** $P < 0.01$; (b) differs from (c) $P < 0.05$, ANOVA test. ET, exhaustive exercise test; ET+EPO, Erythropoietin pre-treatment plus ET (same for other tables).

Renal MDA, SOD, NO and NOS

Renal MDA concentration was increased in the ET group as compared with control, whereas the cell protective factors, e.g. NO, SOD and NOS activities were all decreased ($p < 0.01$, for NO and SOD; $p < 0.05$ for NOS, [Table 2](#)). The outcome was quite different in the group pre-treated with EPO: parameters in the ET+EPO group exhibited no significant difference with control, except for SOD activity, which was

somewhat decreased ($p < 0.05$, Table 2). Accordingly, there was a significant difference between ET+EPO and ET for all other parameters: MDA, NO and NOS ($p < 0.01$, Table 2).

Table 2 MDA, SOD, NO and NOS activities in the different groups (mean \pm SD, $n = 8$)

Group	MDA (nmol/mg)	SOD (NU/mgprot)	NO (μ mol/mgprot)	NOS (U/mgprot)
Control	24.8 \pm 1.1 ^a	25.8 \pm 0.53 ^a	50.2 \pm 16.4 ^a	1.49 \pm 0.26 ^a
ET	54.4 \pm 1.0 ^{b**}	14.1 \pm 0.8 ^{b**}	21.5 \pm 16.1 ^{b**}	0.85 \pm 0.39 ^{b*}
ET + EPO	28.9 \pm 1.3 ^{a*}	18.9 \pm 0.35 ^{b*}	63.9 \pm 11.6 ^a	1.65 \pm 0.72 ^a

Statistical differences between groups in each column were indicated by different alphabetic superscripts: (b) differs from (a) * or ** vs * $P < 0.05$, ** $P < 0.01$, ANOVA test. MDA, malondialdehyde; SOD, superoxide dismutase; NO, nitric oxide; NOS, nitric oxide synthase.

Morphological changes of the kidney

Representative images of HE stained renal tissue are shown in Figure 1. Normal structure of renal tissue with clear contour of renal glomeruli and tubules was displayed by samples from the control group (Fig. 1A). The pathological changes were obvious in the ET group, manifested as paleness of the renal cortex and congestion of the medulla at the gross inspection (data not shown). At inspection under the light microscope glomeruli appeared remarkably congested and swollen, with leakage of red blood cells and shrunken capsular space. The tubule epithelial cells were collapsed, degenerated and broken down; the lumina were dilated and filled with protein casts and red blood cell casts, the walls were peeled off and broken. A great number of apoptotic cells were identified by the condensed fragmented nuclei and crescent-shaped changes of nuclear membrane (Fig. 1B). Compared with those of ET group, kidneys of the ET+EPO group showed much less morphological abnormalities: there was a mostly intact glomerular basement membrane; less congestion of glomeruli, less degeneration of tubular epithelial cells and less dilatation of tubular lumen were observed; very few shed epithelial cells and villi

were present in the lumen; protein casts and blood cell casts were noticed only occasionally and the peeled off and broken tubular walls were rarely observed (Fig. 1C).

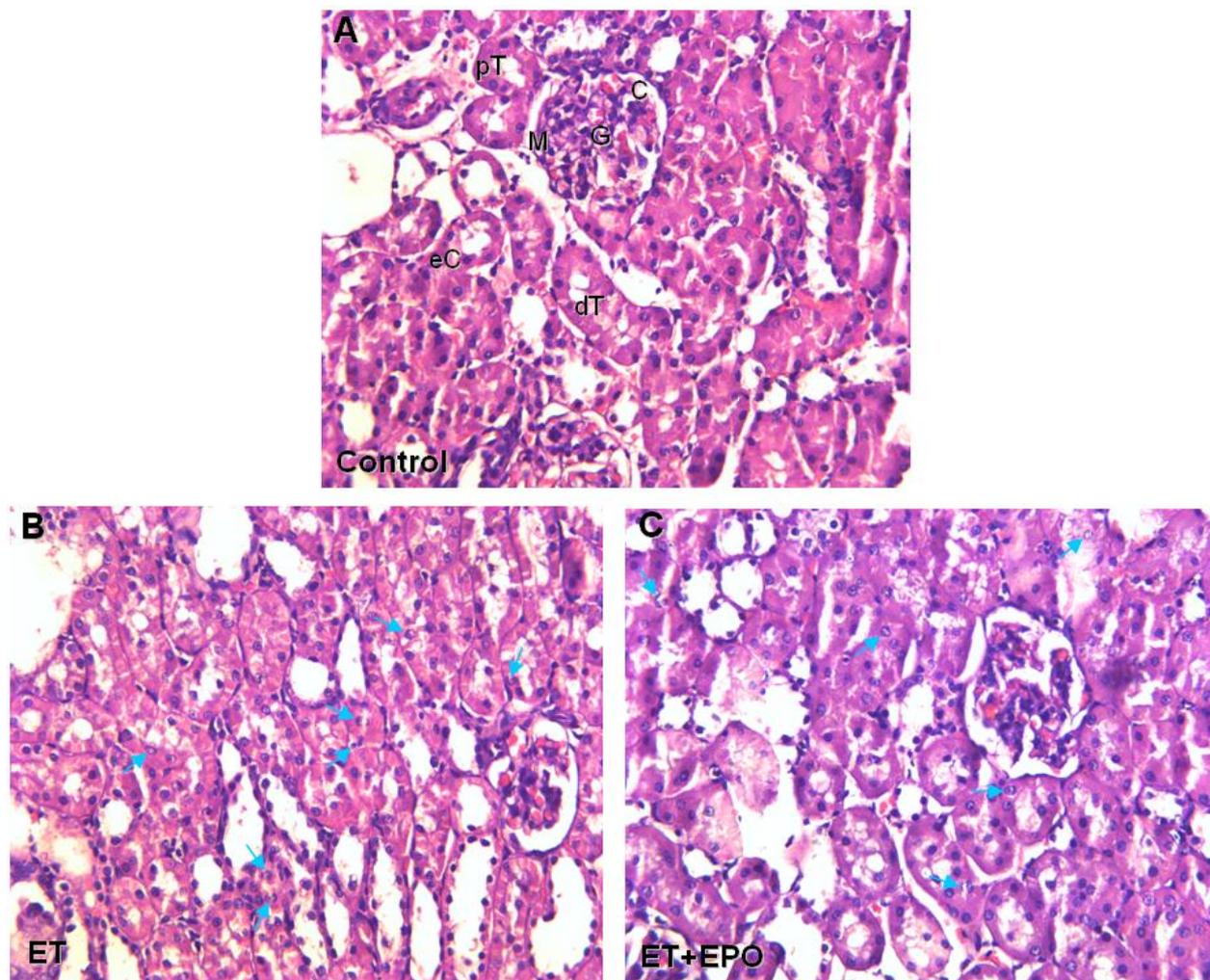


Fig. 1 Representative images of renal tissue stained by HE. A, rat from control group, (C) capsular space, (dT) distal convoluted tubule, (eC) tubule epithelial cell, (G) renal glomerulus, (M) glomerular basement membrane, (pT) proximal convoluted tubule; B, rat from exhaustive exercise group; C, rat from exhaustive exercise plus EPO intervention ($\times 400$). Arrows in B and C point to the nucleus of apoptotic cells.

The ultrastructure of kidney cells in the control group was normal. The filtration membrane was intact; podocyte foot processes were obvious and regularly arranged; capsular space were in focus; there was an intact glomerular basement membrane; Chromatospherite was distinct in the cell nucleus, in which there was abundant well-distributed euchromatin and heterochromatin. (Fig. 2A). In the ET group, the renal tissue was severely damaged: there was an intense intracellular vacuolisation in

the sparse cytoplasm. In the nucleus, the chromatin was broken up and with vacuoles; the filtration membrane was broken; podocyte foot processes were unarranged; capsular space were out of focus; the glomerular basement membrane was foul up, and filtration barriers were markedly diminished compared with those of the control group (Fig. 2B). Compared with those of ET group, kidneys of the EPO+ET group showed much less ultrastructure abnormalities: the glomerular basement membrane was rather intact; podocyte foot processes were less unarranged, capsular spaces less out of focus and filtration barriers less damaged. The structure of the cell nucleus was nearly normal. In a few nuclei, the chromatin had crumbled and was bunched peripherally (Fig. 2C).

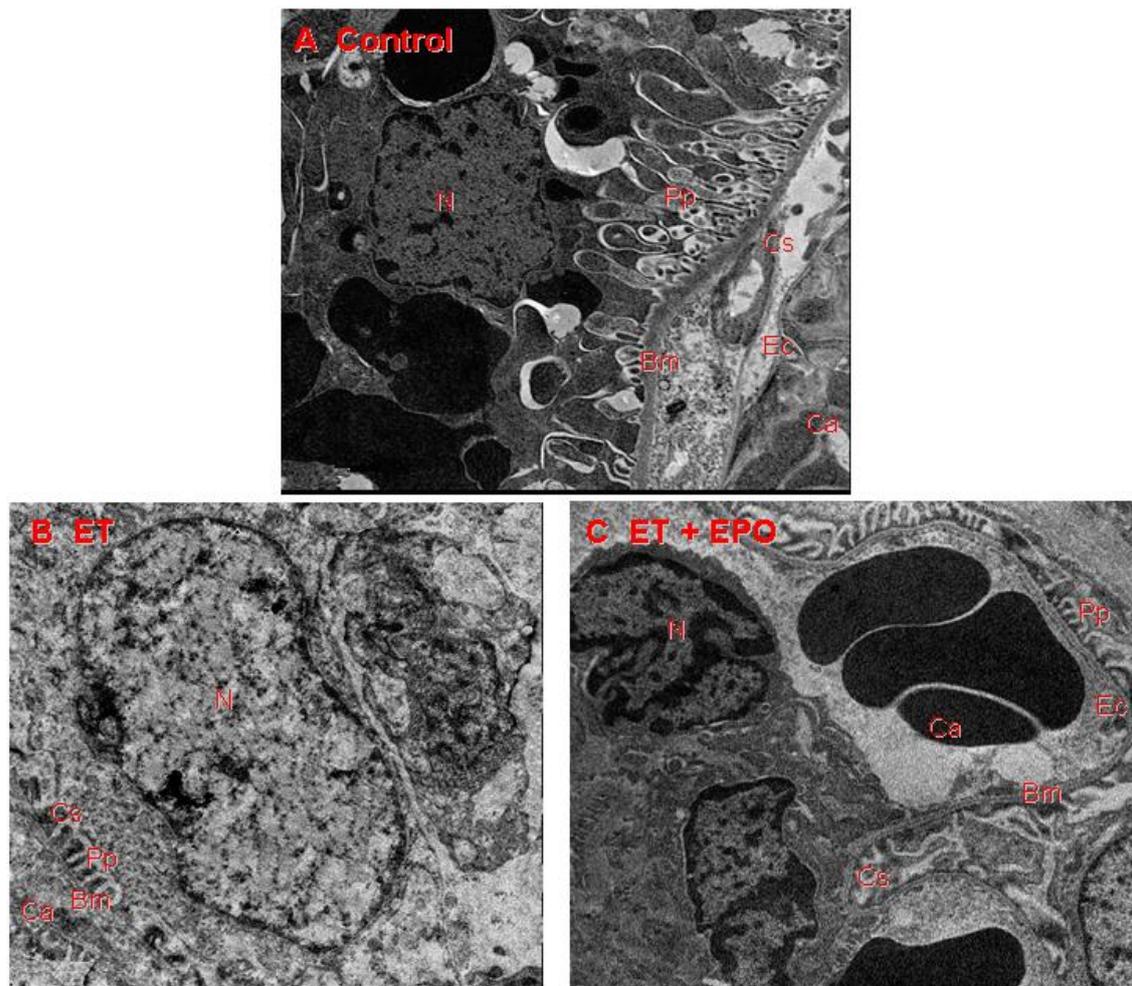


Fig. 2 Representative Transmission Electron Microscopy images of renal tissue. A, rat from control group; (Cs) capsular space, (Ec) tubule epithelial cell, (G) renal glomerulus, (Bm) glomerular basement membrane, (Po) podocyte, (Pp) podocyte foot process, (N) nuclei, (Ca) capillaries; B, rat from exhaustive exercise group; C, rat from exhaustive exercise plus EPO intervention ($\times 10000$).

Outcomes of TUNEL examination

Under the light microscope, the positive apoptotic cells showed brown TUNEL staining in the nuclei (Fig. 3). Some positive TUNEL staining was also present in the cytoplasm due to the leakage of DNA fragments from the nucleus. The nuclei in normal non-apoptotic cells were stained blue, relatively larger with consistent shape and size. Some brownish TUNEL-positive nuclei could be observed in the control group (Fig. 3A), but they were few and found in two rats only. In the ET group, the renal cells were rich in brownish positive nuclei and showed big cell gaps. Such apoptotic positive cells were displayed in all the slices. The same was true in those of the ET+EPO group except that less positive nuclei were seen in the slices (Fig. 3D). Thus, the apoptotic incidence was 100% in both ET and ET+EPO groups comparing with 20% in the control group. The apoptotic *index* however was much higher in the ET than in the ET+EPO group ($p < 0.05$), although both ET and ET+EPO groups had a significantly higher apoptotic index than the control group ($p < 0.01$ and $p < 0.05$, respectively, Table 3).

Table 3 Apoptosis rate and apoptosis index in the renal cortex of the different groups (mean \pm SD, n = 8)

Group	Apoptosis rate (%)	Apoptosis index (%)
Control	20 ^a	5.49 \pm 0.46 ^a
ET	100 ^{b**}	27.55 \pm 0.49 ^{b**}
ET + EPO	100 ^{b**}	18.45 \pm 0.32 ^{c*}

Statistical differences between groups in each column were indicated by different alphabetic superscripts: (b) differs from (a) ** $P < 0.01$; (b) differs from (c) $P < 0.05$; (c) differs from (a) $P < 0.05$, ANOVA test.

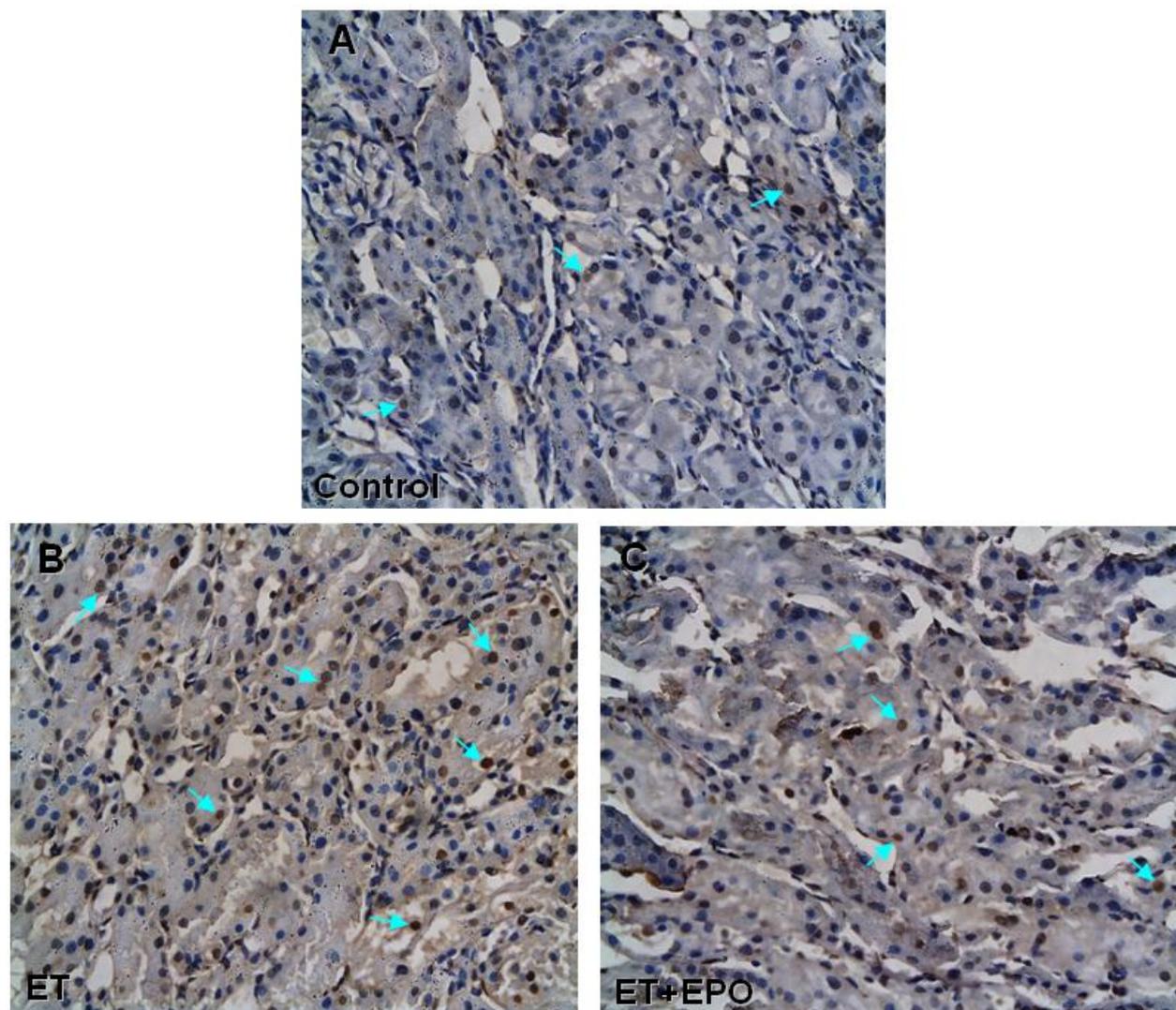


Fig. 3 Representative microscopic images of renal apoptotic cells. (A) rat from control group; (B) rat from exhaustive exercise group; (C) rat from exhaustive exercise plus EPO intervention ($\times 400$). Arrows point to the brown stained nuclei of apoptotic positive cells.

Discussion

The present study showed that, in rats : 1) kidney function and tissue integrity were strongly affected by exhaustive exercise, as manifested by increased urine protein, serum urea nitrogen and serum creatinine, decreased renal SOD, NO and NOS activities, and marked renal cells apoptosis ; 2) that pre-treatment with a single dose of EPO (2000 U/kg) 30 minutes before the beginning of exercise resulted in a better general state of the animal, urine, serum and renal tissue biological markers values

close to control after 24 hours, and a clearly reduced degree of renal cell apoptosis (apoptotic index). EPO pre-treatment thus allowed exhaustive exercise induced renal damage to remain moderate and/or to be of a more reversible kind. A clear protective effect of EPO against exhaustive exercise-induced acute renal injury is thus demonstrated in the rat.

With application of EPO 30 minutes before the training, the general condition of the rat was better after exhaustive exercise. EPO pre-treatment induced conservation of renal tissue and glomerular filtration function might therefore have contributed to raising the rat exercise capability.

Acute high-intensity exhaustive exercise drastically reduces renal blood flow, resulting in incomplete ischemia of the renal tissue. After cessation of the exercise and recovery of blood supply, tissue reperfusion follows. Although essential for the survival of ischemic tissue, the "ischemia-reperfusion" process leads to marked ischemia and hypoxia of the renal cortex (27, 28). Tubular epithelial cells appear particularly vulnerable to this injury. Depending on the severity of cell damage, the pathological process of the tubular epithelial cells may reverse and cell regeneration may occur. However, when cell necrosis takes place no regeneration will occur and the tissue damage will be irreversible (15, 30).

The protective effect of EPO on exhaustive exercise induced kidney damage as found in the present study can be related to several mechanisms. First, EPO is known to inhibit renal cell apoptosis (7, 25). EPO activates Janus tyrosine kinase (JAK2) and protein kinase B (Akt) in tubular cells, initiating the gene transcription of anti-apoptotic factors. These inhibit the activity of caspases, which play a key role in the apoptotic and necrotic processes (7). Uni- and bilateral ischemia-reperfusion injury study showed that apoptosis or necrosis occurred in the epithelial cells of the vasa recta and ascending thick limb of Henle's loop. EPO pre-treatment reduced the degree of renal injury by reducing the cell apoptosis and enhancing the cell

proliferation (30). In agreement with these findings, the present study shows that renal cell apoptosis after exhaustive exercise was significantly reduced by pre-treatment with the EPO analogue rHuEPO. EPO interfering with the apoptotic pathway may be related to the presence of EPO receptors in the renal tubule and the non-haemopoietic roles of EPO in the kidney, such as mitogenesis (13, 32).

A second mechanism of EPO protective effect on kidney can be through NO regulation. Changes in NO content after exhaustive exercise have been described, in the exhaled air after exhaustive running (5), and in the hippocampus together with hippocampal neuronal damage after exhaustive swimming (11). In the present study we found that renal NO, NOS (the key enzyme for NO synthesis) and SOD activities were much decreased by exhaustive exercise, while these activities were largely conserved in rats pre-treated with EPO. This finding is in agreement with the notion that EPO enhances NO (24). Abnormal renal NO contents appear to play an important role in the development of renal injury (23), and may cause damage to renal tissue and function (16-18). The effect of EPO in conserving renal NO during acute exhausting exercise may therefore have significantly contributed to the protection of renal tissue observed concomitantly.

The NO decrease and the MDA increase that we observed in the renal tissue after exhaustive exercise no doubt resulted from renal ischemia and hypoxia. Both these changes were reverted by EPO pre-treatment. Several known effects of EPO may have contributed to this: 1) the severe acidic internal environment was corrected so that the NOS activity for NO synthesis was recovered; 2) the exercise induced hydrolysis of L-arginine was interfered with so that a sufficient substrate was available for the NO synthesis (26); 3) free radicals were eliminated (14, 30); 4) the secretion of vascular endothelin, a strong vasoconstrictor, was decreased, and thus attenuated the ischemia-reperfusion and other pathological changes (12). Therefore, the content of NO would be increased after 24 h recovery from the exhaust exercise as shown by the present experiment.

The fundamental tasks in preventing of acute renal injury are to maintain or improve glomerular filtration function, protect renal cells from injury and promote the recovery from injury of the parenchymal cell function and metabolism. Unfortunately, few reports on this issue could be found in the literature. Application of nutrition supplies lysine was proposed due to its benefit in reducing renal cell apoptosis (35). Yet to date, no effective drugs have been available in the clinical practice for the prevention and treatment of patients with exhaustive exercise-induced acute renal injury. The clear protective effect of rHuEPO against exhaustive exercise-induced acute renal injury demonstrated in the present in vivo study in rat points to the potential interest of EPO in this clinical situation.

Disclosures

No conflicts of interest are declared by the author(s).

References

- 1 *Aslani A, Babae Bigi MA, Moaref AR, Aslani A*. Effect of extreme exercise on myocardial function as assessed by tissue Doppler imaging. *Echocardiography* 2009; 26: 1036-1040.
- 2 *Bonegio R, Lieberthal W*. Role of apoptosis in the pathogenesis of acute renal failure. *Curr Opin Nephrol Hypertens* 2002; 11: 301-308.
- 3 *Burger D, Xenocostas A, Feng QP*. Molecular basis of cardioprotection by erythropoietin. *Curr Mol Pharmacol* 2009; 2: 56-69.
- 4 *Chatterjee PK*. Pleiotropic renal actions of erythropoietin. *Lancet* 2005; 365: 1890-1892.
- 5 *Chirpaz-Oddou MF, Favre-Juvin A, Flore P, Etteradossi J, Delaire M, Grimbert F, Therminarias A*. Nitric oxide response in exhaled air during an incremental exhaustive exercise. *J Appl Physiol* 1997; 82: 1311-1318.
- 6 *Chung H, Lee H, Lamoke F, Hrushesky WJ, Wood PA, Jahng WJ*. Neuroprotective role of erythropoietin by antiapoptosis in the retina. *J Neurosci Res* 2009; 87: 2365-2374.
- 7 *Fishbane S, Ragolia L, Palaia T, Johnson B, Elzein H, Maesaka JK*. Cytoprotection by darbepoetin/epoetin alfa in pig tubular and mouse mesangial cells. *Kidney Int* 2004; 65: 452-458.
- 8 *Grasso G, Graziano F, Sfacteria A, Carletti F, Meli F, Maugeri R, Passalacqua M, Certo F, Fazio M, Buemi M, Iacopino DG*. Neuroprotective effect of erythropoietin and darbepoetin alfa after experimental intracerebral hemorrhage. *Neurosurgery* 2009; 65: 763-769.
- 9 *Hackney AC, Fahrner CL, Gullledge TP*. Basal reproductive hormonal profiles are altered in endurance trained men. *J Sports Med Phys Fitness* 1998; 38: 138-141.
- 10 *Harriss DJ, Atkinson G*. Ethical Standards in Sport and Exercise Science Research. *Int J Sports Med* 2009; 30: 701-702.
- 11 *Hu W, Li X*. Changes of NO and NOS activities in hippocampus of the rats after exhaustive swimming. *Chin J Sports Medicine* 2005; 24: 719-720.
- 12 *Jaquet K, Krause K, Tawakol-Khodai M, Geidel S, Kuck KH*. Erythropoietin and

- VEGF exhibit equal angiogenic potential. *Microvasc Res* 2002; 64: 326-333.
- 13 *Johnson DW, Pat B, Vesey DA, Guan Z, Endre Z, Gobe GC*. Delayed administration of darbepoetin or erythropoietin protects against ischemic acute renal injury and failure. *Kidney Int* 2006; 69: 1806-1813.
- 14 *Juul S*. Erythropoietin in the central nervous system, and its use to prevent hypoxic-ischemic brain damage. *Acta Paediatr Suppl* 2002; 91: 36-42.
- 15 *Kaushal GP, Basnakian AG, Shah SV*. Apoptotic pathways in ischemic acute renal failure. *Kidney Int* 2004; 66: 500-506.
- 16 *Komarietseva IO, Orlova OA, Blahodarenko I*. Vmist oksydu azotu v nyrkakh za aktyvatsii apoptozu. *Ukr Biokhim Zh* 2002; 74: 116-119.
- 17 *Kone BC*. Nitric oxide in renal health and disease. *Am J Kidney Dis* 1997; 30: 311-333.
- 18 *Kone BC, Baylis C*. Biosynthesis and homeostatic roles of nitric oxide in the normal kidney. *Am J Physiol* 1997; 272: F561-F578.
- 19 *Kruger K, Frost S, Most E, Volker K, Pallauf J, Mooren FC*. Exercise affects tissue lymphocyte apoptosis via redox-sensitive and Fas-dependent signaling pathways. *Am J Physiol* 2009; 296: R1518-R1527.
- 20 *Lekhi C, Gupta PH, Singh B*. Influence of exercise on oxidant stress products in elite Indian cyclists. *Br J Sports Med* 2007; 41: 691-693.
- 21 *Li S, Qu S, Tang C*. Research on effects of acute exercise to exhaust on liver apoptosis in rats. *J Beijing Sports University* 2006; 29: 634-636.
- 22 *Mahakkanukrauh A, Sangchan A, Mootsikapun P*. Exertional rhabdomyolysis following excessive exercise of university freshman cheer-training. *J Med Assoc Thai* 2003; 86: 789-792.
- 23 *Manukhina EB, Lapshin AV, Meerson FZ, Mikoian VD, Kubrina LN, Vanin AF*. Vliianie adaptatsii k fizicheskoi nagruzke na endotelioposredovannye reaktsii izolirovannykh sosudov i produktsiiu NO u krys. *Fiziol Zh Im I M Sechenova* 1996; 82: 54-60.
- 24 *Mihov D, Bogdanov N, Grenacher B, Gassmann M, Zund G, Bogdanova A, Tavakoli R*. Erythropoietin protects from reperfusion-induced myocardial injury by enhancing

-
- coronary endothelial nitric oxide production. *Eur J Cardiothorac Surg* 2009; 35: 839-846.
- 25 *Patel NS, Sharples EJ, Cuzzocrea S, Chatterjee PK, Britti D, Yaqoob MM, Thiernemann C*. Pretreatment with EPO reduces the injury and dysfunction caused by ischemia/reperfusion in the mouse kidney in vivo. *Kidney Int* 2004; 66: 983-989.
- 26 *Sakanaka M, Wen TC, Matsuda S, Masuda S, Morishita E, Nagao M, Sasaki R*. In vivo evidence that erythropoietin protects neurons from ischemic damage. *Proc Natl Acad Sci U S A* 1998; 95: 4635-4640.
- 27 *Sharples EJ, Patel N, Brown P, Stewart K, Mota-Philipe H, Sheaff M, Kieswich J, Allen D, Harwood S, Raftery M, Thiernemann C, Yaqoob MM*. Erythropoietin protects the kidney against the injury and dysfunction caused by ischemia-reperfusion. *J Am Soc Nephrol* 2004; 15: 2115-2124.
- 28 *Singbartl K, Green SA, Ley K*. Blocking P-selectin protects from ischemia/reperfusion-induced acute renal failure. *FASEB J* 2000; 14: 48-54.
- 29 *Urhausen A, Kindermann W*. Diagnosis of overtraining: what tools do we have? *Sports Med* 2002; 32: 95-102.
- 30 *Vesey DA, Cheung C, Pat B, Endre Z, Gobe G, Johnson DW*. Erythropoietin protects against ischaemic acute renal injury. *Nephrol Dial Transplant* 2004; 19: 348-355.
- 31 *Wappler F, Fiege M, Steinfath M, Agarwal K, Scholz J, Singh S, Matschke J, Schulte Am Esch J*. Evidence for susceptibility to malignant hyperthermia in patients with exercise-induced rhabdomyolysis. *Anesthesiology* 2001; 94: 95-100.
- 32 *Westenfelder C, Biddle DL, Baranowski RL*. Human, rat, and mouse kidney cells express functional erythropoietin receptors. *Kidney Int* 1999; 55: 808-820.
- 33 *Wijsman JH, Jonker RR, Keijzer R, van de Velde CJ, Cornelisse CJ, van Dierendonck JH*. A new method to detect apoptosis in paraffin sections: in situ end-labeling of fragmented DNA. *J Histochem Cytochem* 1993; 41: 7-12.
- 34 *Yang CW, Li C, Jung JY, Shin SJ, Choi BS, Lim SW, Sun BK, Kim YS, Kim J, Chang YS, Bang BK*. Preconditioning with erythropoietin protects against subsequent ischemia-reperfusion injury in rat kidney. *FASEB J* 2003; 17: 1754-1755.
- 35 *Zhang G, Zhang W*. Effects of supplement of lysine on Bax and Bcl-2 gene

expression in liver and heart of the rat induced by acute exhaustive exercise. *Chin Sports Sci* 2008; 28: 48-52.