Long term exercise preconditioning protects against renal dysfunction after ischemia reperfusion injury in rat kidneys

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Abstract: Background: Acute kidney injury (AKI) has been recognized as one of the most complex clinical complications, and renal ischemia/reperfusion (I/R) injury considered as a main reason of AKI. Aim of the work: This study was planned to identify the possible effect of regular swim exercise on kidney function parameters and oxidative stress in bilateral renal ischemia/reperfusion (I/R) injury model in adult male rats. Material and Methods: Twenty four Male albino rats were randomly divided into three groups of sham control group, ischemia group (I/R), and pre-ischemia exercised group (exercise + I/R). The third group underwent regular swim exercise for 11 consecutive weeks. Ischemic group (I/R), and pre-ischemia exercised groups were subjected to bilateral renal I/R injury. Absolute and relative kidney weights as well as biochemical analysis including serum urea, creatinine & tumor necrosis factor alpha (TNFα) were determined in all groups. In addition, Malondialdehyde (MDA) level, catalase activity & nitrite level were assessed in the renal tissue. Results: The serum urea, creatinine and TNFα levels, as well as absolute kidney weight (KW) and kidney weight to body weight ratio (KW/BW), all were significantly increased in I/R group as compared to the sham control group. Regular swim training decreased levels of serum urea, creatinine and TNFα significantly, in addition to the significant decrease in absolute KW as compared to control group. The renal tissue level of MDA was increased while the catalase activity was decreased in I/R group as compared to the sham control group and both of them nearly normalized in rats that undergo regular swim training before I/R. The renal tissue of nitrite were not significantly different between I/R and sham control groups; however, regular swim training significantly increased the renal level of nitrite. Conclusions: The findings of the current study illustrated that regular exercise seems to be a highly promising way in protecting renal tissue against oxidative damage and in preventing renal dysfunction due to ischemia/reperfusion.

Keywords: Exercise, renal ischemia/reperfusion, oxidative stress, rat.

1- Introduction

Acute kidney injury (AKI) is a common clinical syndrome characterized by rapid disturbance of renal function. Progressive AKI leads to acute renal failure which represents an important clinical problem with a high morbidity and mortality [1]. AKI can be induced by renal ischemia [2,3&4]. Recent studies have demonstrated that AKI was evident in around 20% of patients who died in hospitals and up to 50% of patients in the intensive care unit [5].

Renal ischemia reperfusion (I/R) injury is encountered in many clinical conditions: contrast media-induced nephropathy [6], shock followed by resuscitation in the emergency and intensive care settings [7], sepsis [8], cardiovascular surgery [9], and kidney transplantation [10]. Renal ischemia as a consequence of severe haemorrhagic shock, and organ transplantation is a most common cause of acute renal failure and renal graft rejection [11].

Multiple studies have reported that the acute kidney I/R injury was associated with the generation of oxidative stress and reactive oxygen species (ROS), severe inflammatory reaction, and enhancement of cellular apoptosis after prolonged or even transient I/R injury[12&13]. ROS have many cytotoxic effects, including DNA damage, protein oxidation and, lipid peroxidation, in addition to induction of apoptosis [14].

Experimental studies have further revealed that inhibition of inflammatory reaction and suppression of the generations of pro-inflammatory cytokines and oxidative stress protect the kidney from acute I/R injury [15]. Moreover, I/R injury to the kidney also causes endothelial dysfunction and local inflammatory responses and renal synthesis of pro-inflammatory cytokines such as Interleukin (IL)-1, IL-6, IL-18, and tumor necrosis factor (TNF)-α [16-17]. TNF-α and I/R injury increase nitric oxide synthase activity to synthesize nitric oxide [18]. Nitric oxide production may play several roles in renal pathophysiology, including induction of tubular damage [19].

Jia et al. [20] demonstrated that long-term aerobic exercise could remarkably improve the oxidative stress with hypercholesterolemia. Overall, sport in general applied at moderate loads has

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predominantly positive effect on the health of humans especially concerning cardiovascular and metabolic diseases [21].

Toyama et al. demonstrated that exercise therapy could be an effective clinical strategy to improve renal function [22]. Pechter et al. reported that exercise decreases proteinuria, cystatin C release, and ameliorates glomerular filtration rate in patients with Chronic kidney disease [23]. In addition, long term Treadmill exercises were effective in ameliorating renal cell apoptosis [24].

Exercise training alters the vascular reactivity, enhances endothelium-dependent and independent renal vasodilation [25]. Another beneficial response to exercise is the stimulation of endothelial nitric oxide synthase (eNOS), the main enzyme responsible for vascular NO production which is essential for optimal vascular health [26]. On the other hand, some evidences showing that chronic training from middle age to old age increases blood oxidative damage [27]. Exercise tends to increase oxidative stress as evidenced by stimulated production of MDA and concomitant downregulation of superoxide dismutase (SOD) [28].

Therefore, the aim of the present study to determine whether long term regular exercise pre training decreases or increases vulnerability against I/R-induced AKI.

2- Material and Methods

Animals

The present study was performed on 24 adult Wistar male albino rats weighing initially 120-150g. The rats were purchased from Research Institute of Ophthalmology (Giza) and were maintained in the Physiology Department Animal House under standard conditions of boarding and feeding. The given diet consisted of bread, milk, green vegetables and drinking tap water. The diet was provided ad libitum. Animals were fasted for 12 hours before the experimental procedures. The study was approved by our local ethics committee.

Study design

The rats were randomly assigned to one of the following three experimental groups:

- **Group I**, (sham control): the animals (n=9) in this group were sham operated with exposure of both the renal pedicles, but were not subjected to any I/R.
- **Group II**, ischemic group (I/R): the animals (n=7) in this group were exposed to bilateral I/R. They were subjected to 45 min of bilateral renal pedicle occlusion followed by 24 h of reperfusion.
- **Group III**, preischemia-exercised group (exercise + I/R): animals (n=8) do regular swim training for 11 consecutive weeks prior to surgery and subjected to 45 min of bilateral renal pedicle occlusion followed by 24 hours of reperfusion.

Swimming Exercise Training Protocol

**In the first week**, the pre-swimming exercise for acclimation was started in an experimental swimming pool (30°C, water depth: 44 cm; length: 100 cm; width 70 cm). A gradual progression protocol was applied beginning with swimming for 5 min to 10 min, and then gradually extended to 20, 30, 40, 50 min per day.

**From the second week**, rats were subjected to 60 min-swimming training exercises, 3 days/week for a total period of 11 weeks. During the whole course, the sedentary rats were remained in the cage under the same environmental condition and inspected daily [29].

Renal ischemia reperfusion model

Animals subjected to this procedure are animals of group II and group III (After the end of the period of swim training). Rats were anaesthetized with diethyl ether, the abdominal region was shaved. The abdominal area was prepared with Betadine, and sterile drapes were applied. A midline incision was made and ischaemia was induced by bilateral renal pedicle clamping for 45 min with smooth vascular clamps under sterile conditions. After the clamps were removed, the kidneys were inspected for restoration of blood flow. The abdomen was then closed in two layers and the animals were allowed to recover [30]. Twenty-four hours after reperfusion, the animals were re-anaesthetized with intraperitoneal thiopental sodium (50 mg/kg), blood collected by retro-orbital way [31]. Blood samples were immediately centrifuged and serum samples were stored at −80°C until assayed. After carrying bilateral nephrectomy the left and right kidneys were weighed, then mean weight was calculated. The left one was stored at −80°C until biochemical analysis.

Experimental procedures

1) Animals and kidneys weight analyses:

Animal body weight and mean kidney weight were measured by sensitive balance for different groups. The percent ratio between kidney weight and animal weight was calculated.

2) Biochemical analyses

A) Serum analysis:

**Determination of serum creatinine level:** it was determined by the colorimetric method which was estimated kinetically by the Jaffé reaction [32], using kits supplied by Greiner Diagnostic GmbH (Germany).

**Determination of serum Urea Level:** This was performed colorimetrically according to the modified Urease Berthelot reaction [33], using kits supplied by Greiner Diagnostic GmbH (Germany).

**Estimation of serum TNF-α**

The serum level of TNF-α (tumor necrosis factor-α) was measured Quantikine TNF-α Rat
ELISA kit (R&D Systems, Minneapolis, USA). According to the manufacturer’s instructions. Results were expressed as picograms of TNF-α per milliliter serum (pg/ml) [34].

B) Tissue analysis:

After weighing the tissues, Kidneys were homogenized in 0.2 M sodium phosphate pH 6.25 buffer (1:20, w/v) in a homogenizer fitted with a Teflon pestle. Homogenates were centrifuged at 10,000×g for 1 h and the supernatants were obtained. The supernatant were used for determination of catalase activity, malondialdehyde (MDA) and nitric oxide levels.

Estimation of antioxidant enzymes

Catalase activity was measured by calorimetric method using catalase assay, according to Aebi’s method [35]. Results are expressed as U/gm tissue.

Estimation of malondialdehyde level

Malondialdehyde was measured by colorimetric method according to Satoh [36] using kits supplied by Bio-diagnostic, Egypt. Results were expressed as µmol/0.2 gm tissue.

Estimation of the Nitric Oxide (NO) level

NO was measured after the conversion of nitrate to nitrite by copperized cadmium granules by a spectrophotometer at 545 nm. A standard curve was established with a set of serial dilutions (10–8–10–3 mol/L) of sodium nitrite. The resulting equation was then used to calculate the unknown sample concentrations. Results were expressed as µmol/g wet tissue [37].

3- Results

General characters: as shown in table 1 & figure 1.

Body weight decreased significantly in swim exercise group in comparison to sham and ischemia/reperfusion groups (P < 0.05 for both), absolute kidney weight increased significantly in the I/R group in comparison to the Sham and swim exercise groups (P < 0.05), and kidney/body weight ratio are increased significantly in the I/R group in comparison to the Sham group, but upon exercise this ratio was increased significantly as compared to sham and I/R groups (P < 0.05 for both).

Table 1: Mean ±SEM of Body weight (BW), kidney weight (KW) and kidney weight / body weight ratio in the different experimental groups.

<table>
<thead>
<tr>
<th></th>
<th>Body weight (gm)</th>
<th>kidney weight (gm)</th>
<th>Kidney weight/body weight (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sham</td>
<td>235.00±7.416</td>
<td>0.648±0.012</td>
<td>0.271±0.007</td>
</tr>
<tr>
<td>I/R</td>
<td>217.50±7.416</td>
<td>0.697±0.007</td>
<td>0.320±0.007</td>
</tr>
<tr>
<td>Exercise + I/R</td>
<td>140.16±5.827&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.528±0.025&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.373±0.003&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>a</sup>: significance in comparison to sham control by LSD at P<0.05; <sup>b</sup>: significance in comparison to I/R group by LSD at P<0.05

Figure 1: Body weight (BW), kidney weight (KW) and kidney weight / body weight ratio in the different experimental groups.
Serum urea, Creatinine and TNFα levels;

As shown in table 2 & figure 2, the levels of serum urea, creatinine and TNFα were increased in the I/R group in comparison with the sham group ($P < 0.05$). Upon exercise, the serum concentrations of TNFα and creatinine were lower than I/R group ($P < 0.05$), however serum urea decreased though non-significant.

Table 2: Mean ±SEM of Serum levels of urea, creatinine and TNFα in in the different experimental groups.

<table>
<thead>
<tr>
<th></th>
<th>Urea (mg/dl)</th>
<th>Creatinine (mg/dl)</th>
<th>TNFα (pg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sham control</td>
<td>42.67±2.66</td>
<td>0.586±0.028</td>
<td>2.26±0.282</td>
</tr>
<tr>
<td>I/R</td>
<td>76.04±10.53a</td>
<td>1.067±0.099a</td>
<td>28.77±1.55a</td>
</tr>
<tr>
<td>Exercise +I/R</td>
<td>59.08±2.98b</td>
<td>0.636±0.053b</td>
<td>7.54±1.04b</td>
</tr>
</tbody>
</table>

*a*: significance in comparison to sham control by LSD at $P < 0.05$; *b*: significance in comparison to I/R group by LSD at $P < 0.05$.

Figure 2: Serum levels of urea, creatinine and TNFα in in the different experimental groups.

Biochemical renal tissue analysis.

As shown in table 3 & figure 3, the kidney tissue levels of MDA was elevated in I/R group as compared to sham group, but upon exercise MDA returns to the normal level. However, catalase activity significantly decreased in I/R group as compared to sham group but increased again upon exercise. No significant differences were observed in nitric oxide levels between the I/R and sham groups, but elevated significantly in exercise group as compared to sham and I/R groups.
Table 3: Mean ±SEM of renal tissue levels of MDA, catalase and nitrite, in the different experimental groups

<table>
<thead>
<tr>
<th>Group</th>
<th>MDA (µmol/0.2gm tissue)</th>
<th>Catalase (U/gm tissue)</th>
<th>Nitric oxide (µmol/gm tissue)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sham</td>
<td>2405.59 ±180.18</td>
<td>0.542 ±0.149</td>
<td>4.195 ±0.474</td>
</tr>
<tr>
<td>I/R</td>
<td>3179.00 ±93.49a</td>
<td>0.227 ±0.025a</td>
<td>14.500 ±4.448</td>
</tr>
<tr>
<td>Exercise +I/R</td>
<td>2498.91 ±129.99a</td>
<td>0.351 ±0.028</td>
<td>47.725 ±16.319²</td>
</tr>
</tbody>
</table>

a: significance in comparison to sham control by LSD at P< 0.05
b: significance in comparison to I/R group by LSD at P< 0.05

Figure 3: Renal tissue levels of MDA and nitrite, as well as catalase activity in the different experimental groups.

4- Discussion

The present study demonstrated that, regular exercise before the induction of renal I/R injury significantly reduced renal oxidative stress and improved renal functions. These improvements were associated with reduction of MDA in the kidneys, elevation of antioxidants and decrease of serum TNFα levels. Moreover, exercise significantly increased the survival rate after I/R. In addition, exercise increased nitric oxide concentration in the renal tissue in rats subjected to renal I/R injury.

The current study showed that the renal I/R injury (45 min of ischemia followed by 24 h of reperfusion) impaired glomerular function which detected from the increased serum urea and creatinine levels in rats. This also is in agreement with the previous study that showed that marked disturbances observed in the kidney function parameters following renal I/R injury [38]. It was reported that the acute renal failure induced by I/R is characterized by decreased renal blood flow and glomerular filtration rate, extensive tubular damage, and vasoconstriction and glomerular injury [39]. Moreover, renal I/R injury was initiated by energy depletion and decreased glycogen content due to the lack of oxygen and nutrients at the ischemic stage. At the reperfusion stage, many events cause additional tissue damage, such as cytoskeletal disruption, acidosis, induction of proteolytic and phospholipolytic pathways [15].

In this study, exercise training decreased levels of serum urea and creatinine, so that exercise training can significantly improved the renal dysfunction in I/R rats. This also reported by the other study which suggested that exercise training can significantly improve the renal dysfunction in congestive heart failure rats [40]. It was suggested that exercise could protect the kidney through increased glycogen content and down regulation of enzymes of glycolysis so preserve the glycogen content of kidney. This was in accordance with Mastorakos et al. [41].

The observed increase in absolute kidney weight after I/R compared to other groups in this
study may be explained by renal edema or cell proliferation in the kidney tissue [38].

In this study, the renal tissue levels of MDA increased significantly after renal I/R. Previous studies have reported increased level of MDA after renal I/R [42]. However, in other study no change in the serum level of MDA was reported; probably due to increased activity of super oxidase dismutase [43]. On the other hand, the I/R pre-exercised group showed significantly reduced MDA level and increased catalase activity. Reactive oxygen species (ROS), and increased calcium ions provoked the mitochondrial cytochrome c release from mitochondria to cytosol. Thus, exercise appeared to be beneficial in decreasing renal cell apoptosis in part through the reduction of ROS and prevention of subsequent loss of intra-mitochondrial cytochrome c [24].

Because aerobic exercise training induces higher oxygen consumption in parallel with increased ROS production [44], an antioxidant system is launched during such training to maintain an adequate redox balance [45]. The antioxidant enzymes play an important role in the elimination of H₂O₂, thus, promoting water formation. The perfect balance among the antioxidant enzymes is important for maintaining cellular integrity and preventing cellular damage [46].

One essential finding in the present study is the augmentation the expressions of inflammatory biomarkers as TNFα, in the I/R animals compared to those in the sham control. Accordingly, our findings are consistent with those of previous studies which suggested that I/R elicits tremendous inflammatory response [47]. In addition, the initiation and propagation of inflammatory reaction are major contributors to tissue/organ damage after acute I/R injury [48].

Of importance is the fact that this inflammatory biomarker (TNFα) was markedly suppressed in the I/R animals preconditioned with long term regular exercise. In this way, our findings further reinforce those of previous study that also reported the link between the reduction of inflammatory reaction and the preservation of functional integrity of the kidney after ischemia reperfusion injury [49].

It was reported that an important TNFα, is released during the I/R process is, besides of having a direct cytotoxic effect on the endothelium, stimulates the production of other cytokines, such as the interleukins. These substances interact with the endothelial cells, increasing the pro-coagulating activity and the production of the plasminogen inhibitor, facilitating coagulation and promoting the activation of neutrophils, monocytes and lymphocytes that on their turn, release superoxide anions (O₂⁻) and other ROS that participate in the process of endothelial tissue lesion [50].

Our finding revealed that nitric oxide concentration in the renal tissue was non significantly changed in I/R group compared to control. However it was markedly elevated in the I/R group with regular exercise as compared to other groups. NO has protective effects on cells during I/R injury as it has been demonstrated to inhibit oxidative stress, cytokine release, apoptosis, adhesion and aggregation of neutrophil leukocytes [51]. Some researchers reported that serum and tissue levels of NO were decreased following renal ischemia reperfusion probably may be related to reduced endothelial nitric oxide synthase (eNOS) in ischemic AKI [52].

Exercise training increased NOS activity expression. Upregulation of NOS in the kidney and left ventricle may contribute to the renal and cardiac protective effects of exercise training in cardio-renal syndrome in congestive heart failure rats [53, 54]. It is known that exercise training increases the flow of pulsating blood and the pressure that the blood exerts over the vascular wall and the shearing force over the endothelial cells are powerful stimuli for generating NO in the vascular system. [55]

On the other hand, some researchers reported that, Nitric oxide production may play several roles in renal pathophysiology, including induction of tubular damage [56], and the increased generation of nitric oxide is capable of inducing intracellular oxidizing reaction and cell death [57].

Conclusions

The findings of the current study illustrated that regular exercise seems to be a highly promising way in preparing the renal tissue to withstand high burden of oxidative stress later on.

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