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To link to this article: https://doi.org/10.1080/08941939.2019.1672841

Published online: 08 Nov 2019.

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\textbf{ABSTRACT}

**Background:** Despite the progress in the treatment of acute kidney injury (AKI), current curative approaches fail to provide adequate treatment. In this study, we aimed to investigate the possible protective effects of thymosin-β-4 (Tβ4) on an ischemic AKI model in rats.

**Methods:** Rats were randomly assigned into four groups (n = 8/group): The control group (sham-operated), the ischemia-reperfusion (I/R) group; renal ischemia (90 min) by infrarenal abdominal aortic occlusion followed by reperfusion (3 h), the Tβ4 + I/R group; treated with Tβ4 before I/R, and the I/R group; treated with Tβ4 just before reperfusion. Besides renal function determination (creatinine [Cr] and blood urea nitrogen [BUN]), histological evaluation was also conducted. Renal tissue caspase-9, matrix metalloproteinase (MMP-9) activities, and hyaluronan levels were measured. Additionally, renal tissue oxidative stress (lipid hydroperoxide, malondialdehyde, superoxide dismutase, glutathione, pro-oxidant-antioxidant balance, ferric reducing antioxidant power, nitric oxide), inflammation (tumor necrosis factor-α, interleukin-1β, interleukin-6, nuclear factor-κB) were evaluated.

**Results:** I/R increased the level of caspase-9, MMP-9 activity, and hyaluronan (p < 0.001) and these were significantly decreased in both Tβ4 groups. Moreover, I/R led to increases in oxidative stress and inflammation parameters (p < 0.001) while the levels of antioxidants were decreased. Nevertheless, Tβ4 in both groups were able to restore oxidative stress and inflammation parameters (p < 0.001). Furthermore, Tβ4 attenuated histologic injury caused by I/R (p < 0.01) and diminished serum urea-creatinine levels (p < 0.001).

**Conclusion:** These results suggest that Tβ4 has significant improving effects in ischemic acute kidney injury. This beneficial effect might be a result of the inhibition of extracellular matrix remodeling and apoptosis cascade via modulation in renal redox status and inflammation.

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**ORIGINAL RESEARCH**

\textbf{JOURNAL OF INVESTIGATIVE SURGERY}

https://doi.org/10.1080/08941939.2019.1672841

\textbf{ARTICLE HISTORY}

Received 15 August 2019
Accepted 23 September 2019

\textbf{KEYWORDS}
thymosin-β-4; acute kidney injury; ischemia

\textbf{INTRODUCTION}

Acute kidney injury (AKI) was defined as "An abrupt reduction in kidney function" by The Acute Kidney Injury Network (AKIN) [1]. Despite the technological advances in therapy and diagnosis, AKI remains to be related to high mortality and morbidity in patients who have been exposed to trauma and surgery [2, 3].

As a pioneer cause of AKI; renal ischemia/reperfusion (I/R) occurs in shock, sepsis, and organ transplantation, and numerous biochemical processes are known to be associated with cellular death; as necrosis, apoptosis, or autophagy [4, 5] by triggering inflammatory cascade and production of reactive oxygen species (ROS) [6]. Several research have shown that inhibition of apoptosis, degradation of extracellular matrix (ECM), and inducing tissue remodeling further after renal I/R injury have promising effects in the context of therapeutic approach. In respect of apoptosis, matricellular protein thrombospondin-1 as an inducer of apoptosis was found to be synthesized in ischemia-injured proximal tubular cells, while it was not synthesized in relevant gene deleted knockout-mice [7]. Additionally, I/R was demonstrated to promote the activation of matrix metalloproteinases (MMPs) which are the key enzymes in degradation of ECM as type IV collagen [8]. Notably, MMP-9 is synthesized following I/R in rats [9]. Also, inhibition of MMP-9 activity was shown to reduce the renal I/R injury [10]. While ischemic deterioration process continues in the ECM, increased production of hyaluronan as a component of ECM could mediate inflammatory response and tissue remodeling via angiogenesis [11].

Thymosin-β4 (Tβ4) which is mostly produced in the white blood cell, is a small, natural peptide [12] and it was...
demonstrated that high level of Tβ4 is present to act in the injured area during the repair process [13]. Due to its small size, Tβ4 can readily diffuse into tissues, triggers angiogenesis, and controls the inflammation cascade [14, 15]. Besides suppressing inflammation, Tβ4 modulates endothelial and epithelial cell migration. Hence, Tβ4 could mediate inhibition of tissue swelling and injury [15–17]. In respect of the mechanistic approach, Tβ4 emerges to have an action in modulating inflammation by inhibiting MMPs, blocking tumor necrosis factor (TNF)-α action and activating nuclear factor (NF)-κB [17, 18]. Consistent with the anti-inflammatory effects of Tβ4, its impacts on the cellular level are reflected in a macroscopic level by decreased infarct size [19].

We, therefore, suggested that apoptosis and ECM degeneration could be the frontline steps for AKI and any drug which modulate these two pathways could be a possible candidate for the protection against renal I/R injury related pathologies. This study aimed to test whether Tβ4 treatment is associated with improved renal function via inhibiting apoptosis and inducing tissue remodeling in a short-term rat model of renal I/R.

Materials and methods

Ethics and animals

All experiments were performed after the approval of the Animal Research Committee of the University of Istanbul (no: 2012/65). Animals were housed in light-dark cycle-controlled and temperature-controlled room (23 ± 1 °C) with free access to food and water. A total of 32 male Sprague-Dawley rats (349 ± 6 g) were used to avoid hormonal interaction.

Surgical protocol

Pentobarbital sodium (60 mg/kg i.p.) was used to anesthetize the rats. A tracheotomy was performed to allow spontaneous breathing. Throughout the experiments, body temperature of the rats was kept at 37 ± 0.5 °C by an external heating pad. Following midline laparotomy the abdominal aorta was gently exposed by deflecting the intestines and other internal organs to the left. After isolation of the infrarenal aorta, an atraumatic microvascular clamp (vascu-statts II, midi straight 1001-532; Scanlan Int. St Paul, MN, USA) was placed on infrarenal abdominal aorta (IAA) for 90 min (ischemia) and reperfusion period started with the removal of the clamp (180 min). The experiment was terminated by withdrawal of the blood via the aorta, and the kidney tissue was sampled.

Experimental groups

Four groups were assigned as control (sham-operated), I/R without any drug, I/R pretreated with Tβ4 (Tβ4 + I/R), and Tβ4 treated just before reperfusion (I/Tβ4/R) groups (n = 8/group). In the control group, midline laparotomy and isolation of IAA is performed without occlusion. In the I/R group, laparotomy was followed by 90 min of ischemia and 180 min of reperfusion without Tβ4 administration. In the Tβ4 + I/R group, a single dose of Tβ4 (10 mg/kg/i.v.) was administered one hour before ischemia via a lateral tail vein. In the I/Tβ4/R group Tβ4 (10 mg/kg, i.v.) was administered to the rats fifteen minutes before reperfusion period. The administration dose of Tβ4 was decided according to Philip and Kleinman [13] and Crockford [20]. Isotonic saline was used as a vehicle for both sham and I/R groups.

Blood and tissue sampling

Obtained blood samples were put into heparinized tubes and centrifugation was performed at 1200 g for 10 min at +4 °C to obtain plasma. Plasma and right kidney samples were stored at −80 °C for biochemical evaluation, whereas left kidney samples were fixed in 10% neutral buffered formalin for the histological evaluations. A small piece of the right kidney tissue sample (around 200 mg) was weighed and diluted 20% wt/vol in 20 mM ice-cold Tris-HCl, pH 7.4 and homogenized. To obtain the supernatant fractions, the homogenate samples were centrifuged at 5000 g for 10 min, and used for biochemical evaluations.

Biochemical analyses

Renal function assessment

The levels of plasma creatinine and urea were measured for the determination of kidney function using routine standard clinical chemical methods.

ELISA measurements in tissue samples

The levels of TNF-α, interleukin (IL)-1β, and IL-6 were determined by ELISA methods using commercially available kits (eBioscience, San Diego, CA, USA). The levels of NF-κB, hyaluronan, caspase-9, MMP-9 and nitric oxide (nitrite/nitrate) were also determined by ELISA methods using commercially available kits (Abcam, Cambridge, MA, USA). ELISA kits were equilibrated at room temperature before use. All measurements were presented by dividing the protein content of the respective sample.

Oxidative stress parameters measurements in tissue samples

Lipoperoxidation of renal tissue was determined according to the method of Buege and Aust [21]. Briefly, the method is based on malondialdehyde (MDA) formation. The spectrophotometric determination of tissue lipid hydroperoxide (LOOH) levels were also performed using the method of ferrous oxidation with xylenol orange version 2 (FOX2) [22].
Ferric reducing antioxidant power (FRAP) assay was used for the nonenzymatic antioxidant level of tissues and the method of Benzie and Strain was used [23]. The method of Alamdari et al. was used for the determination of pro-oxidant-antioxidant balance (PAB) [24].

The enzymatic components of the antioxidant defense system were evaluated with both Cu, Zn-superoxide dismutase (SOD) activities and glutathione levels. The method of Sun et al. was used for the determination of Cu, Zn-SOD activity [25]. For the determination of glutathione (GSH) levels, the method of Beutler et al. was used [26]. A molar absorption coefficient for GSH was accepted as ε = 1.36 × 10^-4 M^-1 cm^-1 at a wavelength λ = 412 nm. All measurements related to oxidative stress were presented by dividing the wet weight of respective tissue weight.

**Histological evaluation**

After the fixation process, all tissue samples were dehydrated and embedded in paraffin. Hematoxylin and eosin (H&E) and periodic acid shift (PAS) staining were performed, and images were acquired using Olympus BX61 and Olympus DP72 (Olympus Corp., Miami, FL, USA) microscope.

**Statistical analysis**

In the histological evaluation, three parameters were combined as total injury a) glomerular damage, b) tubular damage, c) inflammation. The injury level was scored as: 0, normal; 1, mild (<10%); 2, moderate (10–25%); 3, moderate to severe (25–50%); 4, severe (50–75%); and 5, very severe (>75%) [27]. All data sets were presented as mean ± SEM. Data analysis were performed using GraphPad Prism Software (GraphPad Software, San Diego, CA, USA). One-way ANOVA with a Tukey post hoc test was used for comparison. p < 0.05 was considered statistically significant.

**Results**

Tables 1 and 2 show the kidney function (creatinine and urea) and oxidative stress (LOOH, MDA, SOD, GSH, PAB, and FRAP) related variables of the groups. Figures 1 and 2 show the renal inflammation (NF-κB, TNF-α, IL-1β, and IL-6) and injury (MMP-9, caspase-9, hyaluronan, and NO) related markers. The histological injury (as, tubule injury and inflammation) are presented in Figures 3 and 4 and Table 3.

**Renal function parameters**

I/R without Tβ4 administration led to a significant increase in both urea (74.6 ± 4.1 mg/dL; p < 0.001) and creatinine levels (0.6 ± 0.1 mg/dL; p < 0.001) compared to the sham-operated group. Tβ4 administrations as in both treated groups could prevent these increments in urea (57.6 ± 3.1 mg/dL; p < 0.001, 51.3 ± 1.1 mg/dL; p < 0.001) and creatinine (0.4 ± 0.0 mg/dL; p < 0.05, 0.3 ± 0.0 mg/dL; p < 0.001) compared to the I/R group.

**Renal inflammation parameters**

When compared with sham-operated rats, I/R resulted in a significant increase in the tissue levels of pro-inflammatory cytokines (NF-κB; 1.9 ± 0.1 ng/100 μg protein, TNF-α; 928.1 ± 47.3, IL-1β; 1487.0 ± 62.0 pg/100 μg protein and IL-6; 1570.0 ± 76.7 pg/100 μg protein). No significant changes in the levels of NF-κB, TNF-α, IL-1β, and IL-6 were detected in rats assigned to both Tβ4 treatment groups in comparison with sham-operated rats (p > 0.05).

**Specific injury-related markers**

I/R without Tβ4 administration led to a significant increase in MMP-9 (0.8 ± 0.0 ng/100 μg protein, hyaluronan (103.0 ± 9.5 pg/100 μg protein), caspase-9 (1.4 ± 0.1 ng/100 μg protein) and nitric oxide levels (76.6 ± 5.5 μmol/100 μg protein) compared to the sham-operated group (p < 0.001). Tβ4 administrations as in both treated groups could prevent these increments in MMP-9, hyaluronan, caspase-9, and nitric oxide levels compared to respective I/R group (p > 0.05).

**Table 1. Kidney function parameters in the groups.**

<table>
<thead>
<tr>
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<th>Sham (I)</th>
<th>I/R</th>
<th>Tβ4 + I/R</th>
<th>I/Tβ4/R</th>
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</table>
| **Creatinine** (mg/dL) | 0.2 ± 0.0 | 0.6 ± 0.1<sup>a</sup> | 0.4 ± 0.0<sup>b</sup> | 0.3 ± 0.0<sup>b</sup>
| **Urea** (mg/dL)       | 49.1 ± 2.3 | 74.6 ± 4.1<sup>a</sup> | 57.6 ± 3.1<sup>b</sup> | 51.3 ± 1.1<sup>b</sup>
<sup>a</sup>p < 0.001 vs. sham group.
<sup>b</sup>p < 0.05 vs. I/R group.

**Table 2. Oxidative stress parameters in the groups.**

<table>
<thead>
<tr>
<th></th>
<th>Sham (I)</th>
<th>I/R</th>
<th>Tβ4 + I/R</th>
<th>I/Tβ4/R</th>
</tr>
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</table>
| **LOOH** (nmol/wet tissue) | 2.5 ± 0.1 | 3.1 ± 0.1<sup>a</sup> | 2.7 ± 0.1<sup>b</sup> | 2.6 ± 0.1<sup>b</sup>
| **MDA** (μmol/wet tissue) | 65.7 ± 0.7 | 83.8 ± 1.0<sup>b</sup> | 68.4 ± 1.0<sup>bb</sup> | 66.5 ± 1.2<sup>bb</sup>
| **SOD** (U/wet tissue) | 25.8 ± 2.7 | 12.7 ± 1.3<sup>b</sup> | 22.8 ± 2.3<sup>b</sup> | 25.5 ± 2.6<sup>b</sup>
| **GSH** (μmol/wet tissue) | 0.6 ± 0.1 | 0.3 ± 0.0<sup>b</sup> | 0.6 ± 0.0<sup>b</sup> | 0.6 ± 0.0<sup>b</sup>
| **PAB** (H₂O₂ %/wet tissue) | 120.8 ± 2.1 | 163.1 ± 3.0<sup>a</sup> | 128.7 ± 2.3<sup>bb</sup> | 127.0 ± 2.0<sup>bb</sup>
| **FRAP** (mmol uric acid/wet tissue) | 3.1 ± 0.1 | 0.7 ± 0.0<sup>b</sup> | 2.9 ± 0.0<sup>bb</sup> | 2.8 ± 0.0<sup>bb</sup>
<sup>a</sup>p < 0.001 vs. sham group.
<sup>b</sup>p < 0.001.
<sup>a</sup>p < 0.01.
<sup>b</sup>p < 0.05 vs. I/R group.
Figure 1. Proinflammatory cytokine levels in the groups. aaa $p < 0.001$ vs. sham group; bbb $p < 0.001$ vs. I/R group.

Figure 2. Specific injury related markers in the groups. aaa $p < 0.001$ vs. sham group; bbb $p < 0.001$ vs. I/R group.
Renal oxidative stress parameters

I/R injury in the absence of Tβ4 led to a significant increase in tissue LOOH (3.1 ± 0.1 nmol/wet tissue), MDA (83.8 ± 1.0 µmol/wet tissue), and PAB levels (163.1 ± 3.0 H2O2%/wet tissue) while a significant decrease in tissue SOD activity (12.7 ± 1.2 U/wet tissue), GSH (0.3 ± 0.0 µmol/wet tissue), and FRAP levels (0.7 ± 0.0 mmol uric acid/wet tissue) (p < 0.001). Tβ4 administration in both treated groups led to a normalization in tissue LOOH, MDA, PAB, GSH, and FRAP levels and SOD activity (p > 0.05; compared to sham-operated).

Histological results

The sham-operated group showed a good morphology of the tubules and the glomeruli. I/R promoted a significant increase in the histological injury, indicating significant tubular damage (p < 0.001 vs. sham) and intense inflammatory areas (p < 0.001 vs. sham). Inflammatory cell mass around the tubules and the glomeruli and aggregations of the blood cells in the vessels were identified. Moreover, mesangial matrix expansion, microvilli loss, and degenerative tubules were additionally found in the I/R group. In the assessment of blood vessels lumen of the kidney tissues, aggregations of blood cells and adhesions of the blood cells to endothelium were seen in the I/R group. These features were seldom in the other groups. As quantitatively, the tubular damage in the evaluated areas was significantly decreased in the Tβ4 + I/R (p < 0.001; compared to I/R) and I/Tβ4/R groups (p < 0.001; compared to I/R).

Discussion

In this present study, we aimed to evaluate the protective effects of Tβ4 (10 mg/kg i.v.)—which administered in ischemia and reperfusion phases—on renal injury function. Irrespective of the administration time, the key findings were that (1) Tβ4 fully sheltered ECM degeneration and apoptosis and (2) Tβ4 did significantly protect the renal tissue against inflammation and oxidative stress. Moreover, (3) ischemia leads to decreased kidney function. (4) Kidney function was well-kept-up in the Tβ4-treated animals with parallel to the histopathological findings.

As postoperative AKI is a common complication in aortic surgery patients, there are still on-going researches on the efficacy of drug-based strategies to prevent the AKI [28]. Per se, we, as many new types of studies, have previously...
emphasized the prominence of antioxidant and anti-inflammatory agent administration in ischemia-induced AKI [6, 29, 30]. However, it is not clear which mechanism should be taken as the target mechanism to yield the best renal outcome. This controversy includes that I/R injury is a multi-pathway process in which occurs in both intracellular and extracellular compartments and reactive oxygen species/reactive nitrogen species (ROS/RNS) and inflammatory mediators contribute to tissue injury [11, 31]. This complexity makes AKI difficult to treat. Even if it is cured, the risk of chronic kidney disease and recurring AKI is increased following an ischemic attack. Hence, any drug that interrupts the events in both intracellular and extracellular compartments could be a promising candidate for the AKI treatment.

In this study, the rats were subjected to ischemia by abdominal aortic clamping for 90 min. This ischemic period was determined since the irreversible renal damage was stated over the 45-minutes ischemic episode [32]. AKI is defined as a decrease in glomerular filtration rate and decreased glomerular filtration is determined by higher serum creatinine level [33]. In our model, likewise urea, serum creatinine level was found to increase threefold in the ischemic group. Articles published earlier which focused on renal ischemia were stated higher creatinine levels such as 4.8, 7.5-fold compared to control [34, 35]. Hence, our model could be accepted as a severe renal ischemia model, and total functional recovery could be proposed by restoring the serum urea and creatinine levels following Tß4 administration. This functional recovery could be the result of Tß4’s effect on many steps in the multi-pathway AKI process.

Besides intracellular compartments, an ischemic process in kidney tissues includes the changes in ECM components. MMPs which are the member of endopeptidases using Zn²⁺ as a cofactor are mainly responsible for ECM remodeling via breaking down [36]. In this present study, ischemia was found to increase the level of MMP-9 which is a member of MMPs, in renal tissues. This increment agrees with other researches showing degredation of ECM in ischemia [9].

Moreover, in another study, immunohistochemical

Table 3. Quantitative evaluation of tissue injury as tubule damage and inflammation.

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<th>Sham</th>
<th>I/R</th>
<th>Tß4 + I/R</th>
<th>I/Tß4/R</th>
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</thead>
<tbody>
<tr>
<td>Tubule damage</td>
<td>0.20 ± 0.11</td>
<td>1.15 ± 0.21</td>
<td>0.27 ± 0.11</td>
<td>0.75 ± 0.29</td>
</tr>
<tr>
<td>Inflammation</td>
<td>0.29 ± 0.18</td>
<td>1.20 ± 0.40</td>
<td>0.29 ± 0.24</td>
<td>0.58 ± 0.37</td>
</tr>
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</table>

* p < 0.001.
* p < 0.05 vs. sham group.
** p < 0.001.
*** p < 0.05 vs. I/R group.

Figure 4. PAS staining: The sham group as grade 0 (A), the I/R group with mesangial matrix expansion (*), microvilli loosing and degenerative tubules (B) and gradually improvement in the Tß4 + I/R group (C) and I + Tß4 + R group (D).
demonstration of MMP-9 antibody in endothelial cells was performed in the course of focal cerebral ischemia [37]. Besides the ECM components, MMP-9 was shown to break down the tight junctions [38]. Hence, it is suggested that a higher level of MMP-9 could be somewhat responsible for the increased permeability. Recently, a great deal of attention has been focused on the inhibition of MMPs before and after reperfusion. Therefore, inhibition of MMPs could be a possible treatment target for I/R injury. Although the idea of protection against ischemic injury by inhibiting MMP-9 is new, lots of researches have been using several approaches for MMP-9 inhibition, proving to be protective [39]. In this line, Tβ4 may be a selective or nonselective MMP inhibitor, and it may have shown its protective properties in this way.

Furthermore, increased production of hyaluronan was previously shown during ischemia. In our study, we also found an increased level of hyaluronan in renal tissue in I/R subjected rats. As a limitation, we did not identify the cellular localization of hyaluronan by using histological methods. However, it is known that the tissue cells in the ECM promote hyaluronan production during the inflammatory process by released mediators. Fibroblast activity is modulated by inflammatory mediators [40, 41] which are synthesized during I/R. Inflammatory mediators such as cytokines were previously found to induce fibroblast synthesis of hyaluronan [42]. Moreover, hyaluronan is known to have water-binding properties. Hence, taken together, the permeability enhancing the effect of MMP-9 and the water-binding power of hyaluronan accelerate the formation of edema. In our study, the reduction of both MMP-9 and hyaluronan levels in the Tβ4 treated group suggests that Tβ4 has a direct preventing effect on ECM remodeling which could result in edema.

As a trigger mechanism for inflammation, it was proposed that ROS releasing followed by I/R promotes the proinflammatory genes. Both IL-1β and TNF-α are the members of cytokines which act as proinflammatory mediators in the tissue following I/R period [43]. Besides the ROS mediation, as an alternative way, these cytokines are related to cell death and leukocyte infiltration. Additionally, IL-6 and NF-κB also have roles in the pro-inflammatory process [44, 45]. Our results concerning IL-1β, TNF-α, NF-κB and IL-6 show that these cytokines were increased by ischemia and Tβ4 could successfully cope with these alterations. This result could be explained by a direct anti-inflammatory effect of Tβ4 or antioxidant impact via inhibiting ROS releasing or blocking of ECM changes.

Another mechanism proposed to clarify ischemic injury is through oxidative and nitrosative stress during AKI [46], which cause irreversible cellular damage and apoptosis. In ischemic tissue, mitochondrion increases to produce a significant amount of ROS, which is typically produced at a low level. A high amount of ROS leads to impairment of cellular function and cell death [47]. It was previously shown that the precursors of specific MMPs such as proMMP-1, -8, and -9 are activated by ROS [48]. Consequently, two mechanisms could augment the activity of MMP-9 during ischemia. The first is the direct activation of MMP-9, and the second the ROS-mediated activation of proMMP-9 during renal ischemia. In our study, besides the increase in MMP-9, we have additionally presented the increase in oxidative-nitrosative stress associated products in renal ischemia subjected rats. As a consistency, our histological findings are parallel with oxidative stress-related results. However, as a limitation, it could hardly be possible to define that these increments are an independent or dependent process. In this perspective, both MMP-9 and hyaluronan could have the key role of responsibility for ischemic tissue injury. Moreover, it was previously suggested that ischemia could result in cellular damage due to modification in DNA chain nucleophilic sites, leading to apoptosis [49]. Caspases, which are the member of cysteine proteases, are induced by oxidative stress mediators. The oxidant mediators along with the mitochondrial proteins as cytochrome-c lead to caspase-9 activation. Subsequently, caspase-3 is activated by active caspase-9 leading to DNA fragmentation and finally to apoptosis. In respect of apoptosis, Tβ4 could also act as an antiapoptotic agent via the antioxidant effect [50, 51].

The use of antioxidants was proposed against cellular and tissue damage [52, 53]. The present results also imply that Tβ4 reduced renal lipid peroxidation in renal tissue following ischemia and reperfusion as reflected by decreased tissue LOOH and MDA levels. Likewise, it was heretofore presented that Tβ4 administration reduces oxidative injury by increasing oxidative enzymes such as manganese SOD), copper/zinc SOD, catalase. Moreover, it was reported that Tβ4 reduces intracellular ROS thus increasing the cell viability. However, the state of art literature absence evidences the influence of Tβ4 on oxidative injury and the antioxidant defense during and following ischemia in the kidney [50]. In our study, we propose that Tβ4 administration following ischemia and reperfusion could completely restore both enzymatic and nonenzymatic components of redox homeostasis by increasing SOD, GSH, FRAP, and decreasing PAB, which indicate a strengthening of cellular defenses.

Conclusions

To the best of our knowledge, this is the first study evaluating the effects of Tβ4 in the setting of renal I/R injury. Based on our findings, the use of Tβ4 may recover renal dysfunction in AKI. This beneficial effect could be the result of the inhibition of ECM remodeling and apoptosis cascade via modulation in renal redox status and inflammation. A therapeutic approach combining the intracellular and extracellular compartments may be crucial for preventing and treating ischemia-induced AKI. This study points out a new perspective for AKI treatment by targeting the different regions of the kidney cells.

Acknowledgment

Synthetic Tβ4 was gifted by RegeneRx Biopharmaceuticals in Rockville, MD USA. All of the authors have read and approved the manuscript.
Disclosure statement

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the article.

Funding

This study was funded by Scientific Research Projects Coordination Unit of Istanbul University. Project number: 25214.

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