The effect of pentoxifylline drug on bax/bcl2 gene dosage expression changes following ischemic reperfusion injury in kidney

Mehrdad Hashemi

Department of Pharmacology and Toxicology, Pharmaceutical sciences branch, Islamic Azad University, Tehran, Iran

Corresponding Author: email address: mhashemi@iautmu.ac.ir (M. Hashemi)

ABSTRACT

Global cerebral ischemia (GCI) and reperfusion induced apoptosis that lead to cell injury and death. The bax and bcl-2 are pro-apoptotic and anti-apoptotic genes, respectively. These genes belong to The B-cell lymphoma-2 (bcl-2) family. In this study; we assessed the effect of pentoxifylline drug on bax/bcl2 gene dosage expression changes following ischemic reperfusion injury in kidney. In this experimental study, 20 male wistar rats were accidently divided them on two tenth group of control and treatment groups. In the control group, celiotomy was performed by ventral midline incision. The left kidney was isolated, and then both the renal artery and vein were obstructed. After 60 minutes of warm ischemia, vessel obstruction resolved and the right kidney was removed. 72 hours after reperfusion, tissue samples were taken from left kidney for histopathology. All these steps in treatment group were exactly repeated after administration of 45 mg/kg/PO pentoxifylline (3 hours before operation) and in this group treatment was continued every 12h until 3 days. In this research quantitative real-time PCR is used for the detection expression Bcl2 and Bax genes in ischemia group and PNT drug group and compared to normal sample. The results showed the gene dosage ratio of bax/bcl2 in PNT group decline than to ischemia group. Therefore, the pentoxyfylline might have a role in control of apoptosis result from Ischemia-reperfusion.

Keywords: bax/bcl2 gene dosage ratio; Real-Time PCR; Ischemia; Kidney.

INTRODUCTION

Acute Renal Failure (ARF) defined as an abrupt decrease in kidney function is a common clinical problem with increasing incidence [1-4]. ARF may be classified as prerenal (functional response of structurally normal kidneys to hypoperfusion), intrinsic renal (involving structural damage to the renal parenchyma) and postrenal (urinary tract obstruction). Intrinsic ARF has emerged as the most common and serious subtype in hospitalized patients [5]. Acute Kidney Injury (AKI) refers to a complex disorder that comprises multiple causative factors and occurs in a variety of settings with varied clinical manifestations [6]. AKI is frequently multifactorial with ischemic, nephrotoxic and septic components and with overlapping pathogenetic mechanisms [7]. Ischemia-Reperfusion is a kind of complex clinical syndrome that in particular situation it might impress different body’s organs, as it is apparent from its name it has one level of reduction or obstruction of perfusion to the tissue and after a while reperfusion to that organ [1]. Reperfusion of the ischemic renal tissue is associated with adramatic inflammatory response leading to TNF-alpha (TNF-a) release, Interleukin-10 (IL-10) induction and subsequent neutrophil-mediated cytotoxic injury [8]. Apoptosis or programmed cell death is in fact a set of predefined cellular events that accomplishes the damage of cells and their contents with full effectiveness. Plenty of proteins and number of genes regulate the apoptosis, in which two pairs of proteins are more important. bcl-2 family of proteins is found in external surface of mitochondria and divided to 3 groups including: antia apoptotic proteins like bcl-2, proapoptotic proteins like bax and the proteins with apoptotic activity like Bik [9]. In this study, we assessed the effect of pentoxifylline drug on bax/bcl2 gene dosage expression changes following ischemic reperfusion injury in kidney.
MATERIALS AND METHODS

Animals
In this experimental study, 20 male wistar rats were accidently divided them on two tenth group of control and treatment groups. In the control group, celiotomy was performed by ventral midline incision. The left kidney was isolated, and then both the renal artery and vein were obstructed. After 60 minutes of warm ischemia, vessel obstruction resolved and the right kidney was removed. 72 hours after reperfusion, tissue samples were taken from left kidney for histopathology. All these steps in treatment group were exactly repeated after administration of 45 mg/kg/PO pentoxifylline (3 hours before operation) and in this group treatment was continued every 12h until 3 days.

Real-time PCR with SYBR green I.
The extracted RNA was purified and high quality RNAs were selected and kept at −80 °C until used for cDNA synthesis. Up to 1 μg RNA was converted to cDNA using Quantitect reverse transcription kit (Qiagen). The primers for Real time PCR of bcl2, bax and Gapdh genes expression were designed and underwent an extensive search using BLAST tool. The characteristics of the primers used in this study are summarized in Table 1. Real-time PCR was carried out in using the following cycling conditions: 95 °C for 10 min, and 40 cycles at 95 °C for 15 s, and 60 °C for 1 min. Each complete amplification stage was followed by a dissociation stage; at 95 °C for 15 s, 60 °C for 30 s, then temperature was ramped up from 60 °C to 95 °C (at the rate of 0.03 °C/s). Melting curve analysis was performed according to the dissociation stage data and reactions.

Data analysis
Quantitative analysis was performed by the measurement of CT values during the exponential phase of amplification. Relative quantity of genes were determined using comparative Ct method and Δ Ct was calculated as the difference between the Ct values of the target gene and the Ct value of Gapdh gene. The data were analyzed using the formula: Gene dosage ratio = 2^(-ΔΔCt). Statistical significances of difference throughout this study were calculated using one-way variance analysis.

RESULTS
Real time PCR were tested for bax and bcl2 genes expression changes in kidney after ischemic/reperfusion with pentoxifylline drug in wistar rat. (Figure 1&2)
Using this method, tested and normal samples were analyzed. There were a significant difference between the tested and normal samples bax and bcl-2 genes expression changes (p<0.05). The results also showed the bax/bcl-2 gene expression was different in control group as compared to experimental groups(Figure 3).

Table 1. Characteristics of the primers used in the real-time PCR assay

<table>
<thead>
<tr>
<th>Gene</th>
<th>Sequence</th>
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<tbody>
<tr>
<td>rat-bcl2-F</td>
<td>ATCGCTCTGTGGATGACTGAGTAC</td>
</tr>
<tr>
<td>rat-bcl2-R</td>
<td>AGAGACAGCCAGGAGAAATCAAAC</td>
</tr>
<tr>
<td>rat-bax-F</td>
<td>AGGGTGCTTGGAAGGCA</td>
</tr>
<tr>
<td>rat-bax-R</td>
<td>TGAGCGAGGCCGTTAGG</td>
</tr>
<tr>
<td>rat-gapdh-F</td>
<td>AAGTTCACGCAGCAGCAAGGG</td>
</tr>
<tr>
<td>rat-gapdh-R</td>
<td>CATACTCAGCAGCAGCATCAC</td>
</tr>
</tbody>
</table>

Figure 1. Results of Real-time PCR analysis for expression of bcl2, bax and gapdh genes.
1. bcl2 gene 2. DNA Size marker 3 bax gene 4. NTC, non-template control. 5 Gapdh gene

Therefore, this study was undertaken to reveal the effect of the pentoxifylline on bax/bcl-2 gene expression changes in kidney after a period of ischemia in rats.
DISCUSSION

One of the important issues in ischemia-reperfusion is cellular injury. Reperfusion induced contradictory cell injury in the tissue. Therefore, in addition to the cells that were irreversibly damaged until the end of ischemia, other cells in the tissue are destroyed [10]. Although, the exact mechanisms of injury are not known, one of the causes of injury (ischemia-reperfusion) stated as follow. Further blood perfusion in ischemia-reperfusion resulted in exacerbation locally absorption of inflammatory cells. These cells release large amounts of oxygen-derived active radicals and promote the membrane destruction process and mitochondrial permeability. Increased permeability of mitochondria and formation of holes in mitochondrial membrane decrease the membrane potential, adenosine triphosphate production and swelling of mitochondria. Increase the permeability of mitochondrial outer membranes causes the releasing initiator inducer of apoptosis, the C cytochrome, into the cell cytosol. This process continued and moves on the proteolytic events and induces the apoptosis [11].

The inflammatory response partially mediates the damage of reperfusion injury. White blood cells carried to the area by the newly returning blood, release inflammatory factors such as interleukins and tumor necrosis factor (TNF-α) [12,13]. TNF-α stimulates the production of bcl-2 family member protein from the cytoplasm to the outer mitochondrial membrane [14-16]. This issue causes mitochondrial swelling and induces apoptosis [17]. Therefore, pharmacological agents could decrease the production of TNF-α in process of ischemia-reperfusion injury that results in reduced reperfusion injury [18-20]. Pentoxifylline (PTX) is a drug that has multiple properties. It decreases oxygen and the production of free radicals [20] and inhibits TNF-α in mononuclear phagocytes. A study indicated that PTX has some protecting effects on remote kidney injury only in the early phase of reperfusion due to ischemia-reperfusion injury [21], but another study indicated that PTX decreases oxidative damage in rat liver after ischemia-reperfusion [22].

In this study, the role of pentoxifylline drug on gene dosage ratio of Bax/bcl2 changes in renal cells was examined. Therefore, the pathway of cell death is going to be started by activation of bax pathway and formation of mitochondrial channels and by facilitating the exit of C cytochrome.

CONCLUSION

In this study showed the gene dosage ratio of Bax/bcl2 in PNT group decline than to ischemia group. Therefore, the pentoxyfylline might have a role in control of apoptosis result from Ischemia-reperfusion.

![Image](image_url)
REFERENCES