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 (involvming 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: antiapoptotic 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 CTvalues 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=$2^{-\Delta\Delta Ct}$ 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. (Figure1&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).

Table1. Characteristics of the primers used in the real-time PCR assay

Gene	Sequence
rat-*bcl2*-F	ATCGCTCTGTGGATGACTGAGTAC
rat-*bcl2*-R	AGAGACAGCCAGGAGAAATCAAAC
rat-bax-F	AGGGTGGCTGGGAAGGC
rat-bax-R	TGAGCGAGGCGGTGAGG
rat-gapdh-F	AAGTTCAACGGCACAGTCAAGG
rat-gapdh-R	CATACTCAGCACCAGCATCACC

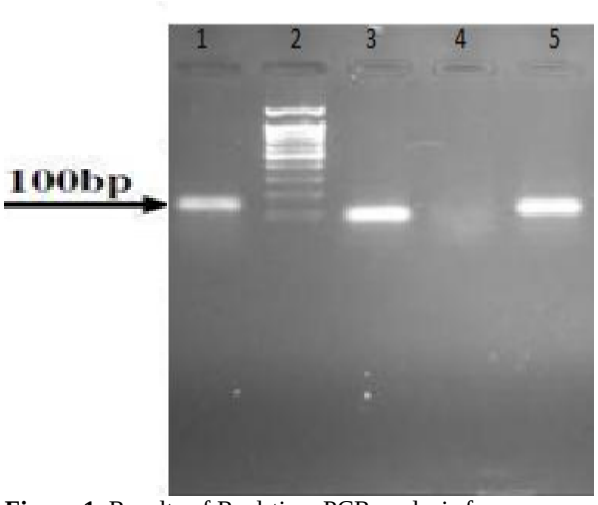

Figure 1. Results of Real-time PCR analysis for expression of bcl2 , *bax* and gapdh genes.
1,bcl2 gene 2, DNA Size marker 3bax gene 4.NTC, non-template control. 5 Gapdh gene

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.

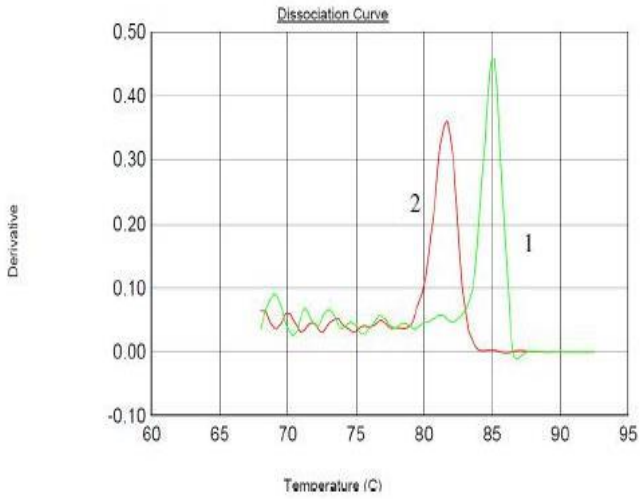

Figure 2. Melting curve analysis of the bcl2 and bax genes 1,bcl2 gene 2bax gene

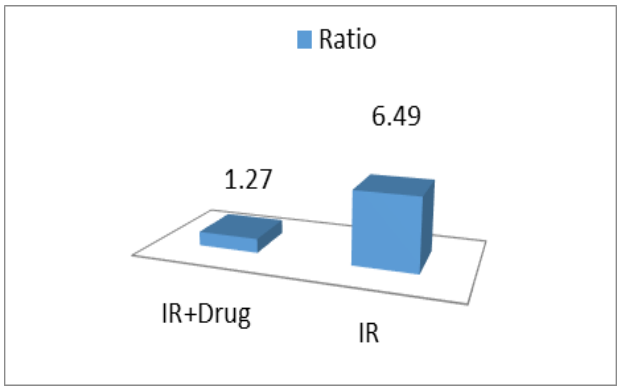

Figure 3. The gene dosage ratio of Bax/bcl2 in PNT and ischemia groups

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

REFERENCES

1. Hashemi M, Doustar Y, Hashem Dabaghian F, Goushegir SA. Effects of grape seed on renal ischemic reperfusion in rats.Journal of animal and veterinary advances. 2012; 11(14):2529-2532.
2. Yu Du, Hua uifamg Guo, Hangxiang Lou. Grape seed polyphenols cardiac cells from apoptosis via induction of endogenous antioxidant enzymes. J Agri Food Chem 2007; 55:1695-1701.
3.Sheridan AM, Bonventre JV. Pathophysiology of acute renal failure. Contrib Nephrol 2001; 132: 7–21
4.Juul S. grap seed in the central nervous system, and its use to prevent hypoxic-ischemi**c** brain damage. Acta Paediatr 2002; 438: 36–42
5.Patel NSA, Sharples EJ, Cuzzocrea S et al. Pretreatment with EPO reduces the injury and dysfunction caused by ischemia/reperfusion in the mouse kidney in vivo. Kidney Int 2004; 66: 983–989.
6.Vesey DA, Cheung C, Pat B, Endre Z, Gobe G, Johnston DW. Grap seed protects against ischaemic acute renal injury. Nephrol Dial Transpl 2004; 19: 348.
7.Pinent M, Blay M, Bladé MC, *et al*. Grape seed-derived procyanidins have an antihyperglycemic effect in streptozotocin-induced diabetic rats and insulinomimetic activity in insulin-sensitive cell lines. Endocrin 2004; 145(11):4985-90.
8.Irina C .Chis. Antioxidant effects of grape seed extractin a rat model of diabetes mellitus. Diabetes and vascular research. 2009; 3: 200-204.
9.Hashemi M., Mahdian R., Entezari M.,Kamyab A. Application of Multiplex Real-Time PCR Assay Using TaqMan MGB Probes on Amniocyte Samples for Prenatal Diagnosis of Trisomy 21.Advanced Studies in Biology. 2012;4(1):11-17.
10. Hashemi M. The Study of pentoxifylline drug effects on renal apoptosis and bcl2 gene expression changes following ischemic reperfusion injury in rat. Iranian Journal of Pharmaceutical Research. 2014; 13 (1): 181-189.
11.Khazaei Koohpar Z, Hashemi M, Mahdian R, Parivar K. The Effect of Pentoxifylline on *bcl-2* Gene Expression Changes in Hippocampus after Long-term use of Ecstasy in Wistar Rats. Iranian Journal of Pharmaceutical Research 2013; 12 (3): 521-527.
12.Hu X, Zhang Y, Zeng F. Effects of sodium B-aescin on expression of adhesion molecules and migration of neutrophils after middle cerebral artery occlusion in rats. Acta Pharmacol Sin 2004 ; 25 (7): 869-875
13.Devarajan P. Cellular and molecular derangements in acute tubular necrosis. Curr Opin Pediatr 2005; 17: 193–99.
14.Francischetti I, Moreno JB, Scholz M, Yoshida WB. Leukocytes and the inflammatory response in ischemia-reperfusion injury. Rev Bras Cir Cardiovasc 2010;25(4):575-84.
15.Harukuni I, Bhardwaj A. Mechanisms of brain injury after global cerebral ischemia. Neurol Clin. 2006 ;24(1):1-21.
16.Vanden Berghe T, Vanlangenakker N, Parthoens E, Deckers W, Devos M, Festjens N, Guerin CJ, Brunk UT, Declercq W, Vandenabeele P. Necroptosis, necrosis and secondary necrosis converge on similar cellular disintegration features. *Cell Death and Differentiation* 2010;17: 922–930
17.Friedewald JJ, Rabb H. Inflammatory cells in ischemic acute renal failure. Kidney Int 2004; 66: 486–90.
18.Chen Q, Moghaddas S, Hoppel CL, Lesnefsky EJ. Ischemic defects in the electron transport chain increase the production of reactive oxygen species from isolated rat heart mitochondria. Am J Physiol Cell Physiol. 2008 ;294(2):C460-6.
19.Lakhan Sh, Kirchgessner A, Hofer M. Inflammatory mechanisms in ischemic stroke: therapeutic approaches .J Transl Med. 2009; 7: 97.
20.Mallick IH, Yang W, Winslet MC, Seifalian AM. Ischemia-reperfusion injury of the intestine and protective strategies against injury. Dig Dis Sci. 2004 Sep;49(9):1359-77.
21.Teruya R, Fagundes DJ, Oshima CT, Brasileiro JL, Marks G, Ynouye CM, Simões MJ. The effects of pentoxifylline into the kidneys of rats in a model of unilateral hindlimb ischemia/reperfusion injury. Acta Cir Bras. 2008 ;23(1):29-35.
22.Inal M,Kanbak G,Adali M.prevention of ischemia – reperfusion –induced oxidative injury in liver by Allopurinol and pentoxifylline . Transplantationsmedizin 2006;18:29-32.