The role of atenolol in the modulation of the expression of genes encoding pro- (caspase-1) and anti- (Bcl2L1) apoptotic proteins in endothelial cells exposed to intestinal ischemia and reperfusion in rats[1]

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Abstract

Purpose: To investigate the role of atenolol in the gene expression of caspase 1 (Casp1) and Bcl2L1 on vascular endothelium of rat intestine after ischemia and reperfusion (IR).
Methods: Eighteen adult male Wistar rats were randomly divided into 3 groups (n=6): SG (Sham group): no clamping of the superior mesenteric artery; IRG: IR plus saline group: IRG+At: IR plus Atenolol group. Rats from IRG and IRG+At were subjected to 60 min of intestinal ischemia and 120 min of reperfusion. Atenolol (2mg/kg) or saline were injected in the femoral vein 5 min before ischemia, 5 min and 55 min after reperfusion. Thereafter, intestinal segments were appropriately removed and processed for Endothelial Cell Biology Rat RT2 Profiler PCR Array.
Results: the anti-apoptotic Bcl2L1 gene expression was significantly down-regulated (-1.10) in the IRG and significantly up-regulated in the IRG+At (+14.15). Meanwhile, despite Casp1 gene expression was upregulated in both groups, it was significantly higher in the IRG (+35.06) than the IRG+At (+6.68).
Conclusions: Atenolol presents antiapoptotic effects on rat intestine subjected to IR partly by the up-regulation of the anti-apoptotic Bcl2L1 gene expression. Moreover, atenolol can mitigate the pro-apoptotic and pro-inflammatory effects of Casp1 gene on rat intestine after IR.
Key words: Mesenteric Ischemia. Atenolol. Gene Expression. Ischemia. Reperfusion. Apoptosis. Rats.

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■ Introduction

Ischemia/reperfusion (I/R) injury is a frequent phenomenon that is associated to high morbidity and mortality[1-4]. It constitutes as part of pathophysiology of diseases such as intestinal obstruction, acute mesenteric ischaemia, hypovolemic and septic shock and necrotizing colitis. Moreover, I/R occurs in several daily medical situations including major operations and organ transplantations[1,2,4].

It is known that blood reperfusion is essential to the ischemic tissue, in order to prevent irreversible cell damage. However, the reoxygenation may worst cell damage by leading to the formation of reactive oxygen species (ROS), which initiates a complex cascade of events that culminate in additional tissue injury, promoting immediate cell death (necrosis), programed cell death (apoptosis) and alteration of cellular phenotype[5-7].

Several drugs, such as beta blockers, have been proposed as modulating agents to minimize I/R injury. It was demonstrated that β1 adrenergic receptors are activated during I/R by catecholamines. Increased ROS production and apoptosis are thought to be mediated by the β1 adrenergic receptors, both in-vitro and in-vivo[8].

Previous studies showed that atenolol, a selective β1 sympathetic receptor blocker, used to treat cardiovascular diseases, has cytoprotective effects when administered intravenously and attenuates intestinal injury caused by I/R[9]. However, the cytoprotective mechanisms of atenolol against I/R are not well understood.

Therefore, this experimental study was designed to evaluate the effects of atenolol on gene expression of rat intestine tissue submitted to I/R.

■ Methods

The study was designed as a randomized controlled trial with a blinded assessment of the outcome and was approved by the Ethics Committee in Research, Universidade Federal de São Paulo (UNIFESP). All animal procedures were carried out according to the recommendations of the international legislations on animal protection.

Eighteen adult male Wistar-EPM1 rats with body weight of 250g to 300g were provided by the Center for the Development of Experimental Models for Medicine and Biology – (CEDEME-UNIFESP). The animals were housed under controlled temperature and light condition (22ºC, 12h light/dark cycle) with free access to water and standard pellet chow until 6h prior to the surgical procedures.

Anaesthesia and surgical procedures

Under intramuscular anaesthesia (80 $mg.kg^{-1}$ ketamine and 10 mg.kg-1 xylazine), a median laparotomy was performed in order to exposure the superior mesenteric vessels. The animals were then randomly assigned into the following groups: Sham group (SG, n=10): without clamping of superior mesenteric artery; Ischemia and reperfusion group (IRG, n=10): clamping of superior mesenteric vessels plus saline solution; Ischemia and reperfusion plus Atenolol group (IRG+At, n=10): clamping of superior mesenteric vessels plus 2mg/kg of Atenolol. The rats from IRG and IRG+At were subjected to 60 min of intestinal ischemia followed by 120 min of reperfusion. Atenolol or saline solution (0.5 ml) was injected in the femoral vein 5 min before ischemia, 5 min and 55 min after reperfusion.

Intestinal samples and gene expression procedures

Intestinal segments (3 cm) were removed at 20 cm from the duodenum-jejunum flexure, opened longitudinally, gently washed in saline solution, wrapped in

aluminium foil, and immediately flash-frozen in liquid nitrogen. The tissue samples were submitted to the Endothelial Cell Biology Rat RT² Profiler™ PCR microarray method (SA Biosciences - Qiagen Co, USA). Briefly, total RNA was extracted from tissues using Trizol reagent (Life Technologies, USA) and purified using an RNeasy MiniKit (SA Biosciences - Qiagen Co, USA). Concentration of each total RNA sample was determined by spectrophotometry, and the quality was assessed by electrophoresis on 2% agarose gels. The mRNA of each sample was converted into cDNA using the RT² First Strand Kit (SA Biosciences - Qiagen Co, USA). Equal amounts of cDNA and the Master Mix SYBR® Green qPCR Mastermix (SA Biosciences - Qiagen Co, USA) were added to each well of the PCR microarray plate. This 96-well plate contains five housekeeping genes (ACTB, Gapdh, Hsp90ab1, Hprt1, and Gusb), three positive PCR controls used to determine the efficiency of reaction, three reverse transcriptase controls and one negative control to detect the presence of genomic DNA contamination. For each PCR reaction, the instrument's software (MxPro Equipment Real Time Systems, Stratagene, GE, Co, USA) Data normalisation was based on correcting all threshold cycle (Ct) values for the average Ct values of the average of housekeeping genes and the comparisons was made by calculating fold changes in gene expression from the raw Ct data using the ΔΔCt method. Gene expression data for each sample was evaluated in triplicate. The gene expression results are presented as positive/up-regulation expression (IRG>GC) or negative/down-regulation expression (IRG<CG). The numbers represent how many times each gene was expressed above [plus sign (+)] or below [minus sign (-)]. The software established that the results three-fold above (over expression) or three-fold below (hypo expression) the threshold allowed by the algorithm [2^(ΔΔCt)] are biologically relevant. The same procedure was performed between IRG+At and SG[10-12]. The method used is contained in the spreadsheet for PCR Array Data Analysis v3.3 (SA Biosciences - Qiagen Co, USA)[10].

Genes of choice

From 84 genes allowed by the Endothelial Cell Biology Rat RT² Profiler™ PCR microarray method, the genes that encode the proteins Bcl2L1 and Caspase 1 (Casp1) were selected. The choice criterium considered the statistical significance of the genic expression between the IRG group and IRG+At group in comparison to IR group.

Statistical analysis

Gene expression data for each sample was evaluated in triplicate. Analysis of variance (ANOVA) and the Tukey *post hoc* test were performed to validate the homogeneity of the reaction of expression of each gene ($p<0.05$).

■ Results

Our results of the two selected genes (Bcl2L1 and Casp1) showed a statistical significance between the group submitted only to the ischemia /reperfusion (IRG), in comparison to the IRG+At (Table 1). The anti-apoptotic Bcl2L1 gene expression was significantly ($p<0.05$) down-regulated in the IRG (-1.10) and significantly ($p<0.05$) up-regulated in the IRG+At (+14.15). On the other hand, despite the apoptotic CASP1 gene expression was up-regulated in both groups, it was significantly ($p<0.05$) higher in the IRG (+35.06) when compared to IRG+At (+6.68) (Table 1).

Table 1 - Gene expression of Bcl2L1 and Casp1 from sham (SG), ischemia and reperfusion (IRG) and ischemic and reperfusion plus Atenolol (IRG+At) groups. Significant values of fold up (+) or down (-) regulation was marked in bold [2^ (- Delta Ct].

			GROUPS		
	Bank	**Symbol**	**IRG**	**IRG+At**	***P value***
A09	NM_031535	Bcl2L1	-1.10	+14.15*	0.00014
A12	NM_012762	Casp1	+35.06	+6.68*	0.00005

(*)p<0.05

■ Discussion

The mechanisms involving intestinal I/R are not entirely clear. However, it is known to be activated by many cellular and extracellular factors[14]. On the other hand, assuming that gene expression is quantitatively related to the need for proteins encoded by specific genes, the aim of this study was to investigate the expression of genes encoding pro and anti-apoptotic proteins in rat intestines after I/R, as well as the effects of Atenolol as a modulator of these genes.

We hypothesized that during the intestinal ischemia/reperfusion, ROS causes cell changes including cell membrane damages that can activate genes encoding anti- and pro-apoptotic proteins such as Bcl2L1 and caspase 1, respectively. In addition, it is expected that Atenolol can promote modulating effects on the gene expression that encode these two proteins[13].

The anti-apoptotic Bcl2L1 protein, for instance, is a potent inhibitor of cell death by means of preventing activation of caspases. This protein has been shown to regulate cell death by binding and blocking the voltage-dependent anion channel (VDAC), thereby preventing the release of the caspase activator cytochrome C1 (CYC1), from the mitochondrial membrane[14]. Decreased of Bcl2L1 can trigger a caspase cascade that ultimately leads to apoptosis, whereas increased Bcl2L1 can inhibit apoptosis[15]. The gene expression of Bcl2L1 was significantly up regulated in IRG+Atenolol group (+14,15) in comparison to IRG (-1,10), which indicates that Atenolol presents antiapoptotic effects that could mitigate the intestine damages caused by ischemia and reperfusion.

The Casp1 gene encodes a protein that is a member of the cysteine-aspartic acid protease family (known as caspase family). It was identified by its ability to proteolytically cleave and activate the inactive precursor of interleukin-1, a cytokine involved in several processes such as inflammation, septic shock, and wound healing. It also plays a pivotal role in the cascade that leads to apoptosis in several organs and tissues, including the epithelial cells of intestine. Once activated, the inflammatory response increases expression of Caspase-1 by a positive feedback that worse the response[16].

Despite we observed that Casp1 gene expression was up-regulated in both IRG and IRG+At groups, it was significantly higher in the IRG when compared to IRG+At. These results indicate that Atenolol could alleviate the intestine damages after ischemia and reperfusion, by reducing both inflammation and apoptosis.

■ Conclusions

Our results demonstrate that exogenous atenolol is associated with antiapoptotic effects on the rat intestine subjected to IR by, in part, the up-regulation of Bcl2L1 gene expression. In addition, Atenolol can mitigate

the pro-apoptotic and inflammatory effects of CASP1 gene in rat intestine after ischemia and reperfusion.

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