Effect of Ramipril on Endothelin-1 Expression in Myocardial Tissue at Wistar Rats Induced Myocardial Infarction

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Abstract

BACKGROUND: Acute myocardial infarction occurs due to a sudden decrease in coronary blood flow caused by coronary artery embolism, coronary dissection, or coronary vasospasm. The Endothelin-1 (ET-1) is the most potent endogenous vasoconstrictor; it is synthesized and released from vascular and endocardial endothelial cells and myocytes. The action of ET-1 induces endothelial dysfunction in the coronary circulation through several mechanisms, such as reduced NO pathway activity, increased oxidative stress and inflammation, and interference with glucose and lipid metabolism. Ramipril is one of the angiotensin-converting enzyme inhibitors (ACE-I) that can reduce the formation of ET-1 by enhancing the NO expression. NO can down-regulate the ET-1 secretion through soluble guanylate cyclase activation and increased cellular generation of cGMP.

AIM: This study aimed to investigate the effect of Ramipril on ET-1 expression in rats-induced myocardial infarction.

METHODS: Six-week-old male Wistar rats were randomly allocated into three groups: negative control, positive control was given NaCl 0.9% and treatment group treated with ramipril 3 mg/kg/day orally for 7 days. Myocardial infarction was induced in positive and treatment group by subcutaneous injection of isoproterenol, and 24 h after the last administration, rats were sacrificed to evaluate the relative expression of ET-1 using the real-time polymerase chain reaction and $2-\Delta\Delta Ct$ method.

RESULTS: The average expression for the negative control was 0.0098, positive control was 0.0136 and treatment group was 0.0118, with $p = 0.210$ ($p > 0.05$).

CONCLUSION: Our data suggest that there is no difference between groups for the relative expression of ET-1.

Introduction

Acute myocardial infarction occurs due to a sudden decrease in coronary blood flow. Availability of oxygen cannot supply the oxygen demand in the heart, which causes cardiac ischemia. Many factors cause decreased coronary blood flow, such as coronary artery embolism, coronary dissection, and coronary vasospasm [1]. The Endothelin-1 (ET-1) is the most potent endogenous vasoconstrictor, and it is synthesized and released from vascular and endocardial endothelial cells and myocytes. ET-1 affects coronary and peripheral vascular tone regulation by activating the contractile ETA and relaxant ETB receptors. ETA receptors primarily mediate the vasoconstrictor response to ET-1 in vascular smooth muscle cells (VSMCs), and the vasodilator effect is mediated by ETB receptors located in the endothelium [2].

Endothelin interacts with the sympathetic nervous system during acute myocardial infarction. The actions of ET-1 include arterial and venous vasoconstriction, direct positive inotropic effects on cardiomyocytes, growth-promoting effects on VSMCs, and proliferative actions on fibroblasts. The ETA receptor mediates most of these effects; by contrast, endothelial ETB receptor stimulation releases vasodilators (NO and prostaglandins) and results in ET-1 clearance principally in the lung, where 80% of circulating ET-1 is cleared. Furthermore, ET-1 induces endothelial dysfunction in the coronary circulation through several mechanisms, such as reduced NO pathway activity, increased oxidative stress and inflammation, and interference with glucose and lipid metabolism [3].

Endothelial dysfunction is a prognostic indicators of cardiovascular events, NO play important role to protect cardiovascular including relaxation of media smooth muscle cells, prevention of leukocyte adhesion and migration into the arterial wall, and prevention of muscle cell proliferation.

Angiotensin-converting enzyme inhibitors (ACE-I) have been shown to normalize endothelial function in coronary artery diseases and reduce the formation of ET-1, which is associated with lower ET-1 gene expression and increases the production of nitric oxide (NO) in human vascular endothelial cells (HUVECs). NO decreases ET-1 secretion through soluble guanylate cyclase activation and increased cellular generation of cGMP, which decreases the

release of ET-1 and pre pro-ET-1 mRNA [4]. ACE-inhibitors have been shown to reduce cardiac remodeling and mortality after myocardial infarction by decreasing afterload and preload, reducing sympathetic nerve stimulation, balancing oxygen demand and supply, and inhibiting the degradation of bradykinin, a vasodilator [5], [6].

Ramipril is an ACE inhibitor [7]. Recent studies have shown that pretreatment Ramipril 3 mg/kg BB before induced myocardial infarction prevents increased serum BNP, which is an indicator of left ventricular systolic function compared to the group without Ramipril [8]. Ramipril has proven to affect the state of post-myocardial infarction patients. However, the study of pretreatment of Ramipril before induction of acute myocardial infarction was limited.

Materials and Methods

Experimental animal and study groups

This research is a true experimental design research model and a post test-only control design to determine the effect of Ramipril on ET-1 expression at rats induced myocardial infarction. There were three groups of experimental animals in this study: negative control, positive control, negative control, positive control, and treatment group. The study was approved by the Ethics Committee of Faculty of Medicine Andalas University (No. 483/KEP/FK/2019) and was conducted in line with the Declaration of Helsinki.

A total of 24 Six-week-old male Wistar rats, weighing approximately 200 g, purchased from the Experimental Animal Center, Faculty of Pharmacy, Universitas Andalas, Padang, Indonesia, were used. Animals were housed in diurnal lighting conditions (12 h/12 h) and allowed free food and water for 7 days before the experiment. The rats were grouped as follows: negative control (n = 8), received no treatment, positive control (n = 8), which was given NaCl 0.9% orally for 7 days, treatment group (n = 8), Ramipril 3 mg/kg/day orally for 7 days. Acute myocardial infarction was induced in the positive control and treatment group by subcutaneous injection of isoproterenol (ISO) (85 mg/kg/day) for 2 consecutive days. Twenty-four hours after the final administration of ISO, rats were anesthetized and sacrificed by intraperitoneal injection 80 mg/kg ketamine HCl (Ketamine-Hameln® 50 mg vial, Combiphar, Indonesia). Previously conducted studies selected the drug doses administered in the present study. After exposure of the heart through a median sternotomy, the heart was excised, weighed, and immersed in ice-cold saline. Myocardium tissues in the left ventricle of sacrificed rats (approximately 2 mm in thickness) were removed. Precooling PBS (Rnase free) was used to wash the tissues. The left ventricle was isolated, snap-frozen in liquid nitrogen, and stored at −80°C.

Histologic examination

Hematoxylin and eosin staining was performed using 4 μm tissue sections fixed in 10% formalin and embedded in paraffin for examination under an Olympus BX 51 light microscope. Microscopic examination and photography were performed with a Beta 3.1 MP Sony Exmor. The BetaView program was used for histologic examination.

Total RNA Isolation and complementary DNA synthesis

Total RNA was isolated from LV myocardial tissue using TRIzol Reagent, followed by treatment with RNase-free DNase. Sample RNA concentration was determined using a NanoDrop ND-1000 Spectrophotometer (NanoDrop Technologies, Wilmington, DE, USA). Isolated RNA was stored at −80°C until used. Complementary deoxyribonucleic acids (cDNAs) were synthesized by 1 μg of total RNA according to the manufacturer's protocol (High Capacity RNA-to-cDNA kit, Applied Biosystems, USA).

Measuring ET-1 expression

The data were obtained by measuring the expression of ET-1 myocardial tissue in experimental animals using real-time polymerase chain reaction (PCR). Quantitative real-time PCR analysis was performed using the CFX96TM Real-Time System (C1000TM Thermal Cycler, Bio-Rad). All reactions were carried out using SensiFAST SYBR N0-ROX Kit in a 10 μL total sample volume (5.0 μL of 2 × SensiFAST SYBR N0-ROX Mix, gene-specific forward and reverse primers, 1.0 μL of cDNA, 3.2 ddH2O). For analysis of each gene, an (no reverse transcription control) and (no template control) were also performed. The relative gene expression levels were conducted using the $2^{-\triangle\triangle CT}$ method. Forward and Reverse Primer were designed using Primer3Plus software and primary specifications are analyzed with the Basic Local Alignment Search Tool (Table 1).

Reactions were carried out as follows: after an initial denaturation-activation step at 95°C for 2 min,

Table 1: Primers used for quantitative RT-PCR

Gene symbol	Forward	Tm	Reverse	Tm
ET-1	*GAACATCTGTCCGGCTTCTAC*	57.9°C	*TATGGAATCTCCTGGCTCTCT*	57.9°C
GAPDH	*GGCACAGTCAAGGCTGAGAATG*	57°C	*TCTCGCTCCTGGAAGATGGTGA*	57°C

ET-1: Endothelin-1, GAPDH: Glyceraldehyde-3-phosphate dehydrogenase, RT-PCR: Real-time polymerase chain reaction.

amplifications consisted of 40 cycles of denaturation at 95°C for 5 s, annealing at Tm for 10 s, and measurement of fluorescence at 72°C for 1 s. Ct values of each gene amplification were detected. $2^{-\Delta\Delta Ct}$ value method was used to calculate the relative expression levels of target gene: the average value of three parallel repeated experiments was calculated as the Ct value of each sample. $\Delta Ct = Ct$ (Target Gene) - Ct (GAPDH), $\Delta\Delta Ct = \Delta Ct$ (sample) $- \Delta Ct$ (contro1). For statistical analysis, the calculation of the relative expression of the genes of each sample uses the $2^{-\Delta Ct}$ formula which is a variant of the Livak method which could correct the efficiency of each sample based on mathematical operations that calculate the efficiency for each fluorescence curve.

Statistical analysis

Results were expressed as the mean. The normality test used the Shapiro Wilk test and the homogeneity test using the Levene Statistic test. Statistical test results using One-way ANOVA test, and p < 0.05 was considered statistically significant. Statistical Package for the Social Sciences (SPSS) 12.0 for Windows was used for all statistical.

Results

Ramipril attenuate ET-1 expression

Based on the analysis of the relative expression of ET-1 myocardial tissue using the $2^{-\Delta Ct}$ formula. The variation of Livak Schmittgen formula ($2^{-\Delta\Delta Ct}$), the relative expression of ET-1 gene in the treatment group with Ramipril was lower than the positive control group (Table 2 and Figure 1).

Table 2: ET-1 expression

Group	Average relative expression of ET-1	p-value
Negative control	0.0098 ± 0.0027	
Positive control	0.0136 ± 0.0041	0.210
Treatment	0.0118 ± 0.0037	

ET-1: Endothelin-1.

The normality test using the Shapiro Wilk test with the analysis results obtained a significance value of each group of more than 0.05, so it can be concluded that the data are normally distributed. The homogeneity test of data using the Levene Statistic test with the analysis results obtained a significance value that is p = 0.811 (p > 0.05), so it can be concluded that the data is homogeneous. Statistical test results using One Way ANOVA test, p = 0.210 (p > 0.05). Based on these results, it can be concluded that there was no significant difference between groups for the relative expression of ET-1.
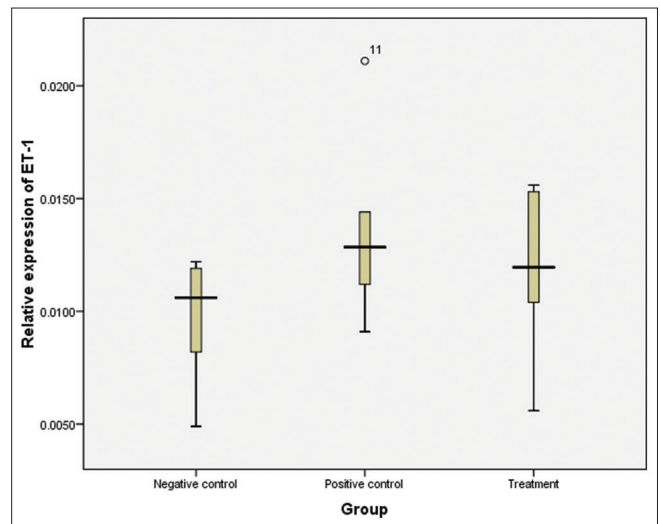

Figure 1: Error bars relative expression of ET-1

Histologic examination of myocardial infarction

In the histopathological examination, ISO-induced experimental animal groups, edema spread over most of the myocardium, the interstitial area that appears swollen, dilated capillaries, and pollen of inflammatory cells consisting of inflammatory cells of lymphocytes, neutrophils, and histiocytes. In some muscle cells, visible signs of degeneration were characterized by granular and paler changes in the cytoplasm (Figure 2).

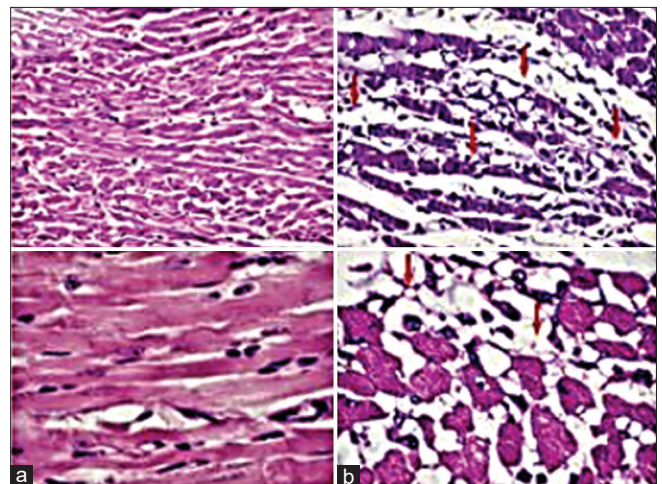

Figure 2: (a) normal tissue, (b) infarcted tissue. Arrows show edema areas scattered between the myocardium

Discussion

Based on Table 2, the relative expression of the ET-1 gene in the treatment group with Ramipril was lower compared to the positive control group because

Ramipril was given before rats were induced with ISO. ACE-I was able to reduce the formation of the potent vasoconstrictor ET-1 and increase NO bioavailability in HUVECs. In turn NO represents a barrier against oxidants such as unscavenged superoxide anion, decrease generation of reactive oxygen species induced by TNFα in HUVECs and decrease LDL susceptibility to oxidation [9], [10], [11]. ACE-I completely prevented NO-deficient that responsible for vasodilative, antiagregative, and antiproliferative action to prevent the acute myocardial infarction [12]. Recent studies show a significant risk reduction of myocardial infarction in the ACE-inhibitor-treated group compared to the placebo group [13].

In this experiment, the induction of infarction using ISO which is a synthetic catecholamine and beta-adrenergic agonist resulted in a significant decrease in tissue antioxidants and an increase in the levels of total, ester and free cholesterol, triglycerides, free fatty acids, and glycoprotein components in plasma and heart. The phospholipid content showed an increasement in plasma and a simultaneous decrease in the heart tissue, while the Na+/K+ ATPase activity decreased and Ca2+ ATPase and Mg2+ ATPase activities increased, resulting in destabilization of the membranes [14]. NO can also contribute to attenuate the β-adrenergic-mediated increase in inotropic and chronotropic thus protecting the heart against excessive stimulation by catecholamines, which is ISO induction [15]. NO plays an important role in the progression of the myocardial infarction induced by ISO. The increased generation of NO was evidenced from 0,5 h until 12 h of ISO administration these likely resulted from an increase in the activity of both eNOS and iNOS. The increase in eNOS is responsible for ISO's hypotensive effect, decreasing the systolic and diastolic pressure since the first 2 min of its administration. Hypoperfusion induced energy unbalance possibly due to the chronotropic and inotropic effect of ISO [16]. Recent studies have shown that administration of pretreatment Dicarboxylic-containing ACE inhibitors, Ramipril 3 mg/KgBB before induced myocardial infarction caused a higher NO production and decreased the level of 8-OHGua, which is an indicator of DNA damage level compared to the group without Ramipril [17], [18].

ET-1 and NO act as mutual antagonists in determining many processes including vascular tone, atherosclerosis, platelet activity, as well as leukocyte chemotaxis [19]. ET-1 and NO function in negative feedback loops for each other, each acting to limit the action of the other. These effects are exerted through actions at multiple levels, including reduced transcription and modulation of enzyme activity [20]. Similarly, the addition of NO donors has been shown to reduce the increasement in ET-1 dependent hypoxia in human umbilical vein endothelial cells [4].

Conclusions

ET-1 expression was lower in the Ramipril group than the positive control group. Nevertheless, there was no significant difference between groups for relative expression of ET-1.

Acknowledgments

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