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The Effects of Three Months Exercise on Expression of Endothelin-1 in the Lung Tissue

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Abstract: In the present study, the effects of three months exercise training in the expression of ET-1 gene in lungs tissue were investigated. Twenty male Wistar rats (235±27 g) were maintained on a 12:12 h light-dark cycle and received food and water. Animals were randomly divided into two groups (n = 10). Rats in test group ran on a treadmill for 60 min at a speed of 25 m min⁻¹ daily for 3 months. Total RNA was extracted from the lung tissue and the expression of ET-1 mRNA was assessed by RT-PCR. Analysis of results indicated that the expression of ET-1 mRNA in the lung was significantly higher in the exercise rats than in the control rats (p<0.05). The results of this study were shown that three months exercise increased the ET-1 expression in the lungs that could increase the blood flow of lungs via ETB receptor.

Key words: Endothelin-1, treadmill exercise, lung, RT-PCR

INTRODUCTION

ET-1, ET-2 and ET-3 are members of a peptide family that has been the subject of much interest in the past decade (Fagan *et al.*, 2001). ET-1 was found in 1988 from cultured porcine aortic endothelial cells (Yanagisawa *et al.*, 1999). Soon after the discovery of ET-1, two structurally related peptides were identified and termed ET-2 and ET-3 (Inoue *et al.*, 1989). Endothelin-1 is the most abundant isoform and has been best characterized. The lung has the highest levels of ET-1 secreted by endothelium, smooth muscle, airway epithelium and a variety of other cells. ET-1 also circulates in the plasma. In the normal lung, ET-1 mainly localizes to vascular endothelium, airway and vascular smooth muscle cells and to a lesser degree, the epithelium. ET-2 has similar biologic functions as ET-1 and is found in the myocardium, kidney and placental tissues. ET-3 also circulates in plasma and is found in the central nervous system, gastrointestinal tract, lung and kidney although the cellular source is not clear (Arinami *et al.*, 1991). All three endothelins are synthesized as preprohormones and post-translationally processed to active peptides. ET-1 processing has been best characterized and begins with the 212 amino acid peptide (preproET-1), which is then

proteolytically cleaved by endopeptidases to big ET-1 (proET-1). The 39 amino acid proET-1 has 1% of the biologic activity of ET-1 and is cleaved by the metalloendoprotease Endothelin Converting Enzyme (ECE), resulting in the 21 amino acid proteins with potent biologic functions (Fagan *et al.*, 2001). ET-1 is a very potent vasoconstrictor, about ten times more than other vasoconstrictors (Haynes and Webb, 1998). There are currently two distinct human endothelin receptors known, endothelin A (ETA) and endothelin B (ETB) receptors, which are members of the seven transmembrane, G protein-coupled rhodopsin superfamily (Lippton *et al.*, 1993). Activation of both ETA and ETB receptors on smooth muscle cells leads to vasoconstriction whereas activation of ETB receptor located on endothelial cells leads to vasodilation by increasing Nitric Oxide (NO) production (Michael and Markewitz, 1996).

Exercise is an external factor that influenced ET-1 expression and secretion. It has been shown that endurance exercise results in a significant redistribution of tissue blood flow, by which the blood flow is greatly increased in the working muscles, whereas it is decreased in the splanchnic circulation (such as in the kidneys and intestines) and in the nonworking muscle circulation (Armstrong *et al.*, 1987). It has also reported that the

production of ET-1 is increased in the circulation of nonworking muscles by exercise (Seiji *et al.*, 1998a) but the effect of exercise on the ET1 expression in the lung have not been well characterized. Few studies showed that a 4 week exercise did not alter ET-1 expression significantly in the lung (Seiji *et al.*, 1998b). The aim of this study was to evaluate the effect of 3 month exercise on the ET1 expression level in the lung tissues.

MATERIALS AND METHODS

This study was carried out in Drug Applied Research Center of Tabriz University of Medical Science in Iran in 2007.

Animals and exercise protocol: Twenty male Wistar rats (235±27 g) were housed on a 12:12 h light-dark cycle and received food and water. On the day of the experiment the rats were randomly divided into 2 groups. In test group, 10 animals ran on a treadmill (0% grade) for 60 min at a speed of 25 m min⁻¹ for 3 month. The control group consisted of 10 animals remained at rest. Forty eight hours after the last exercise, all animals were anesthetized, killed and the lungs were quickly removed and frozen in liquid nitrogen. Lungs were stored in -70°C for determination of ET-1 mRNA expression by RT-PCR analysis. The expression of β-actin mRNA was also determined as an internal control.

RNA extraction and RT-PCR analysis: Total tissue RNA was extracted by RNX-Plus extraction reagent (Fermentas) according to the manufacturer's instructions. Total RNA (5 μg) was primed with 1 μg oligo (dT) and reverse transcribed by AMV reverse transcriptase using first-strand cDNA synthesis kit (Fermentas). The reaction was performed at 42°C for 60 min. One microliter of cDNA was used for PCR. The sequences of the oligonucleotides were as follows: ET-1 primer (Forward): 5'-CTC TGC TGT TTG TGG CTT TC -3', ET-1 primer (Reverse): 5'-TCG GAC TTC TTT GTC TGC TTG -3', β-actin primer (Forward): 5'-CCC TAA GGC CAA CCG TGA AAA GAT G -3', β-actin primer (Reverse): 5'-GAA CCG CTC ATT GCC GAT AGT GAT G-3'. The cycle profile included denaturation for 60 sec at 94°C, annealing for 45 sec at 57°C and extension for 1 min at 72°C. The amplified PCR products were electrophoresed on 1% agarose gels, stained with ethidium bromide, visualized by a ultraviolet transilluminator and photographed. The photographs were scanned by a scanner and the quantification was performed by computer with scion image software (Scion corporation USA).

Statistics: Values are expressed as mean±SE. To evaluate differences between the control and exercised groups, t-test was used. p<0.05 was considered statistically significant.

RESULTS AND DISCUSSION

The expression of ET-1 mRNA in the lungs of both the control group and the exercise group was evaluated by RT-PCR analysis. The expression of β-actin mRNA was also determined as an internal control. There were no significant differences in body weight between the two groups. The lung wet weight was not differed significantly between two groups. The results of analysis indicated that the level of expression of ET-1 mRNA in the lung was significantly higher in the exercise rats than the control animals (Fig. 1).

In the present study, the expression of ET-1 in the lungs of rats after 3 months exercise was determined. The result of this study indicate that exercise for a period of 3 month resulted in a significant increase of ET-1 mRNA expression in the lungs of rats. The level of ET1 mRNA in the control animals didn't changed significantly. Analysis of results also showed that the level of β-actin in exercise and control animals was not significantly differed (Table 1).

Endogenous ET-1 contributes to the regulation of vascular tone through its direct vasoconstrictive action (Rubanyi and Polokoff, 1994). It has previously reported

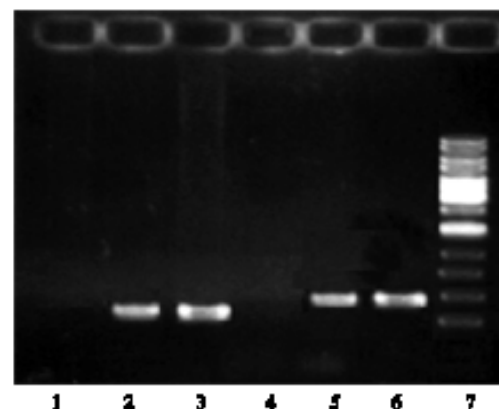


Fig. 1: Expression of ET-1 mRNA in the lungs of exercise (n = 10) and control rats (n = 10). A representative agarose gels of RT-PCR analysis for levels of ET-1 mRNA and β-actin mRNA in the lungs of rats. Lane 1, NO DNA of ET-1; Lane 2, ET-1 expression in control rat; Lane 3, ET-1 mRNA expression in exercised rat; Lane 4, NO DNA of β-actin; Lane 5, β-actin expression in control rat; Lane 6, β-actin expression in exercised rat; Lane 7, DNA size marker

Table 1: Results of statistical analysis of levels of expression of ET1 and β -actin genes in exercised and control rats. Stained agarose gel of PCR products were scanned and ratio of ET-1 mRNA to β -actin mRNA was calculated

ET1/ β -actin ratio	Exercise group	Control group
1	0.95	0.87
2	1.09	0.78
3	0.97	0.87
4	1.01	0.85
5	0.79	0.48
6	0.95	0.78
7	0.97	0.48
8	1.09	0.85
9	0.79	0.78
10	1.01	0.78

Values represented as Means \pm SE, p<0.05 was considered significant

that the circulating plasma concentration of ET-1 is significantly increased by exercise (Maeda *et al.*, 1994). The arteriovenous difference in ET-1 concentration in nonworking muscles was significantly increased after exercise, whereas ET-1 concentration in the working muscles was not significantly different before and after exercise (Maeda *et al.*, 1997). The ET-1 mRNA expression is also increased in internal organs (heart and kidney) by exercise. Earlier findings suggested that the expression of preproET-1 mRNA in the kidneys was markedly higher in the exercise rats than in the sedentary control rats, suggesting that the production of ET-1 was actually increased in the kidney by acute exercise (Seiji *et al.*, 1998a). Few earlier studies shown that ET-1 expression do not differ significantly after a 4 week exercise in the lungs (Seiji *et al.*, 1998a, b). The results of these studies are contradictory with the present study. Reasons for such differences could be attributed to exercise type and duration. It has been shown that both mechanical factors (such as hemodynamic shear stress) and neurohumoral factors (such as angiotensin II, arginine vasopressin and so on) increase the production of ET-1 in cultured vascular endothelial cells (Emori *et al.*, 1991). It was proposed that during long term exercise, these factors affect the ET-1 expression. Incidentally, it has been seen that exercise increased receptor endothelin B (ETB) expression in working muscle (Spier *et al.*, 2004). We supposed that vasodilatation effect of endothelin in lungs was related to increase in ETB expression during exercise that could increase lung's blood flow. As well, Endothelin-1 induces vasodilatation via the endothelial cell ETB receptors through generation of endothelium-derived dilator substances, including Nitric Oxide (NO) (Suzuki *et al.*, 1991). This perhaps acts by physiologically antagonizing ETA receptor-mediated vasoconstriction (De Nucci *et al.*, 1988).

The results of this study indicated that 3 months exercise can result in increased expression of ET-1 gene in the lung tissues.

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