Oxidative Stress in Patients with Primary Pulmonary Hypertension

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We studied the role of oxidative stress in the pathogenesis of primary pulmonary hypertension. In patients with primary pulmonary hypertension the content of malonic dialdehyde in the plasma was higher than in healthy volunteers (5.18±0.46 and 2.95±0.14 nmol/liter, respectively, $p<0.01$). However, glutathione peroxidase activity in the plasma decreased in these patients (0.50±0.17 *vs.* 1.19±0.14 U/ml in the control, $p<0.05$). By contrast, glutathione peroxidase activity in erythrocytes from patients surpassed the control (6.13±0.39 and 4.63±0.45 U/h hemoglobin, $p<0.05$). The increase in malonic dialdehyde content in the plasma and glutathione peroxidase activity in erythrocytes and the decrease in glutathione peroxidase activity in the plasma were most pronounced in patients with severe cardiac insufficiency and pulmonary hypertension. Our results indicate that antioxidant preparations improve the prognosis in patients with primary pulmonary hypertension.

Key Words: *primary pulmonary hypertension; free radical oxidation; malonic dialdehyde; superoxide dismutase; glutathione peroxidase*

Primary pulmonary hypertension (PPH) is a rare and rapidly progressing fatal disease. The pathogenesis and etiology of this disease remain unclear. The diagnosis of PPH is usually made at the late stage, and the life span of patents does not surpass 2-3 years [1]. In patients with PPH small pulmonary vessels are characterized by hypertrophy of the media, proliferation of the endothelium, and thickening of the adventitia. These changes are followed by an increase in the total pulmonary vascular resistance and development of pulmonary hypertension, pulmonary heart disease, and right ventricular cardiac insufficiency [1].

Inflammation is an important etiological factor of PPH. The development of this disease is accompanied by hypoxemia and tissue hypoxia [1]. The data suggest that oxidative stress plays an important role in the progression of pulmonary hypertension [4]. Reactive oxygen species (ROS) are mediators of inflammation. During ischemia and hypoxia the intensity of free radical processes in cells sharply increases [4]. ROS generated by activated macrophages cause dysfunction of the endothelium [4], which underlies the pathogenesis of various cardiovascular diseases [10,13], including PPH [1]. Moreover, ROS are involved in the formation of damages to the left ventricle myocardium and development of cardiac insufficiency [12]. Studies of PPH in animals showed that free radicals and ROS play a role in the progression of pulmonary hypertension [7,9]. However, the involvement of ROS in the pathogenesis of PPH and the formation of damages to the endothelium in pulmonary vessels were assayed only in one clinical trial [11]. The results of this study are ambiguous.

Here we measured the content of lipid peroxidation (LPO) products and activities of antioxidant enzymes in the blood from patients with PPH of different severity and duration.

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MATERIALS AND METHODS

We examined 16 patients with PPH (11 women and 5 men). The age of patients was 16-60 years (35.0±3.3 years). The duration of PPH was 1-220 months (66.0± 16.1 months). The control group included 16 healthy volunteers (12 women and 4 men). The age of volunteers was 18-34 years (27.0±0.9 years). The diagnosis of PPH was made after standard clinical and instrumental examination. The secondary causes of pulmonary hypertension (pulmonary artery thromboembolism, chronic obstructive lung diseases, myocardial diseases, and acquired heart defects) were excluded. According to the classification of the New York Heart Association (NYHA), the patients had PPH of functional classes (FC) I (n=2), II (n=4), III (n=9), and IV (n=1). There were subgroups of patients with PPH and pulmonary artery systolic pressure (PASP) above or below 100 mm Hg. The patients with PPH were also divided into subgroups (PPH history<50 months and >50 months). All patients with PPH were treated with dihydropyridine calcium antagonists. Some patients received anticoagulants.

Activities of superoxide dismutase (SOD), glutathione peroxidase (GSH-Px), and catalase were measured. Whole blood samples (0.1 ml) were mixed with hypotonic 5 mM K,Na-phosphate buffer (pH 7.4, ratio 1:9), hemolyzed, rapidly frozen, and stored at -20°C not more than for 1 month. SOD activity in erythrocytes was measured on a Hitachi 220A spectrophotometer after heme precipitation with the mixture of ethanol and chloroform (ratio 3:5). Superoxide anion radicals were generated using the xanthine-xanthine oxidase system [6]. The amount of SOD inhibiting reduction of nitroblue tetrazolium by 50% was taken as a unit of enzyme activity. GSH-Px activity in erythrocyte lysates and blood plasma was estimated by NADPH oxidation in the coupled glutathione reductase system on a Labsystems Oy FP-900 chemical analyzer using tert-butyl hydroperoxide as the substrate [3]. The amount of GSH-Px that oxidized 1 mmol reduced glutathione was taken as a unit of enzyme activity. Catalase activity was estimated by utilization of H_2O_2 [5]. Malonic dialdehyde (MDA) content was determined in the reaction with 2-thiobarbituric acid on a Hitachi-557 spectrophotometer at 532 nm [2].

The results were analyzed by nonparametric tests, including Spearman correlation test and Mann—Whitney test (for pairwise measurements).

RESULTS

Parameters characterizing the intensity of free radical oxidation in the blood considerably changed in patients with PPH (Table 1). In these patients plasma MDA content 1.6-fold surpassed that in healthy volunteers. It should be emphasized that MDA content increased with the increase in PASP and progression of cardiac insufficiency. However, plasma MDA concentration was lower in patients with long-lasting PPH (Table 1).

GSH-Px activity in the plasma and erythrocytes underwent various changes in patients with PPH. Plasma GSH-Px activity in patients was 1.5 times lower than in the control. However, enzyme activity in erythrocytes from patients 1.3-fold surpassed that in healthy volunteers. Similar changes in GSH-Px activity were observed with the increase in PASP and progression of cardiac insufficiency (Table 1). It should be emphasized that GSH-Px activity in erythrocytes from patients with PPH of longer duration was higher than in other individuals. SOD and catalase activities in erythrocytes from patients with PPH did not differ from the control. However, activities of these enzymes increased in patients with long-lasting PPH. Moreover, catalase activity in erythrocytes was high in patients with PPH of FC III and IV (Table 1). Erythrocyte SOD activity in patients with different severity of pulmonary hypertension was similar. However, Spearman correlation test revealed a correlation between PASP and erythrocyte SOD activity (r=0.61, p=0.036).

Published data show that plasma MDA level increases in patients with various cardiovascular diseases [4], including atherosclerosis, myocardial infarction, and stable coronary heart disease, arterial hypertension [14], and ischemic and non-ischemic cardiac insufficiency [12]. MDA is a secondary product of LPO formed during oxidative destruction of lipid peroxides in biological membranes and circulating plasma lipoproteins [4]. MDA serves as a marker of oxidative stress [12,14]. Inflammation and hypoxemia accompanying PPH contribute to intensification of free radical oxidation. The increase in plasma MDA content indirectly indicates intensification of free radical processes that promote progression of PPH. It was shown that exacerbation of hypoxemia with the progression of cardiac insufficiency and the increase in PASP are accompanied by accumulation of MDA [12]. Our assay produced similar results (Table 1). Clinical observations revealed a correlation between blood MDA level and amount of circulating endotheliocytes appearing after damages to the vascular endothelium [15]. Moreover, MDA concentration increases after exposure of cultured endotheliocytes to hypoxia [8]. The increase in blood MDA content in patients with PPH is an indirect evidence for the progression of endothelial dysfunction.

The decrease in plasma GSH-Px activity in patients with PPH is probably associated with inhibition of this enzyme by free radical oxidation products. This

TABLE 1. Activity of Antioxidant Enzymes and MDA Content in the Blood from Patients with PPH (*M*±*m*)

Examinees	Antioxidant enzyme activity				
	in erythrocytes, per 1 mg Hb			GSH-Px, U/ml plasma	MDA, nmol/ml plasma
	catalase, mmol/min	SOD, U	GSH-Px, U		
Healthy donors (*n*=16)	4.00±0.57	4952±826	5.00±0.45	1.20±0.14	3.00±0.14
Patients with PPH (*n*=16)	5.80±0.72**	6823±1087	6.40±0.39*	0.80±0.17*	4.90±0.46*
FC (NYHA)					
I-II (*n*=6)	5.30±1.59	6703±1748	5.90±0.56	1.20±0.41	4.8±0.8**
III-IV (*n*=10)	6.20±0.69*	6894±1459	6.70±0.53*	0.60±0.08*	5.0±0.6*
PASP, mm Hg					
<100 (*n*=6)	5.80±1.42	6588±2429	6.10±0.59	1.10±0.42	4.4±0.8
>100 (*n*=10)	5.80±0.83**	6963±1084**	6.50±0.54*	0.70±0.12*	5.20±0.58*
Duration of disease, months					
<50 months (*n*=7)	5.3±1.1	4924±1196	6.10±0.71	0.70±0.15**	5.40±0.63*
>50 months (*n*=9)	6.20±0.98*	8299±1574*	6.60±0.45*	0.90±0.29**	4.50±0.67**

Note. *p<0.05 and **0.05<p<0.1 compared to the control.

is confirmed by a negative correlation between GSH-Px activity and plasma MDA content [12]. The increase in GSH-Px activity in erythrocytes from patients with PPH is probably related to intensive enzyme synthesis during adaptation to hypoxemia and high content of LPO products. This assumption is confirmed by published data [14] and our results showing that activity of antioxidant enzymes in erythrocytes increases in patients with PPH of longer duration (Table 1).

Thus, activity of free radical oxidation in patients with PPH increases with progression of the disease. This suggests that antioxidants should be used in combination with standard therapy of these patients. Published data show that synthetic antioxidant probucol produces a positive effect in rats with experimental crotaline-induced PPH [9]. Activation of antioxidant enzymes in erythrocytes from patients with long-lasting PPH probably plays an adaptive role and prolongs patient's life.

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