Free Radical Oxidation and Antioxidant Activity in Blood Plasma and Myocardium during Long-Term Dipyridamole Treatment

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Activation of free radical oxidation and inhibition of antioxidant activity in mouse myocardium after long-term dipyridamole treatment were demonstrated using chemiluminescent techniques. At the same time, dipyridamole 10-fold inhibited free radical oxidation and slightly increased antioxidant activity in the plasma. Dipyridamole-induced platelet disaggregation was accompanied by an increase in platelet count.

Key Words: *myocardium; plasma; platelets; free radical oxidation; antioxidant activity*

Recent studies demonstrated the important role of free radical oxidation (FRO) and antioxidant protective systems in the myocardium in the pathogenesis of cardiac insufficiency [5-7,11].

Free radical processes occur continuously in cells. Under physiological conditions these processes are regulated by the system maintaining oxidative homeostasis. The imbalance between FRO and antioxidant activity (AOA) caused by different stress factors leads to impairment of cell membrane permeability and integrity, dysfunction of membrane-bound enzymes, structural changes in DNA, and disturbances in mitotic cell division accompanied by pathological processes in tissues and organs [1,8,13].

Published data show that destructive processes resulting from changes in FRO and AOA in cardiomyocytes lead to the development of cardiovascular diseases [7,11]. We first showed that dipyridamole-induced long-term platelet disaggregation is accompanied by destruction of bone marrow megakaryocytes, which is related to the growth and accumulation of giant lipid drops in their cytoplasm and changes in the fatty acid composition of bone marrow lipids [2].

The regulation of relationships between FRO and AOA in myocardial tissues is of considerable interest. Here we studied changes in FRO and AOA in mouse myocardial tissues and blood plasma during dipyridamole-induced long-term platelet disaggregation.

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MATERIALS AND METHODS

Experiments were performed on 20 adult male (C57Bl×CBA)F_1 mice weighing 18-20 g. The animals were divided into 2 groups. Experimental mice received daily intraperitoneal injections of 30 mg/kg dipyridamole in isotonic NaCl (0.2 ml) for 30 days. Control animals were injected with physiological saline. The mice were decapitated on day 30 of the experiment. Blood smears were prepared routinely and stained by the method of Romanovsky. Platelets were counted routinely.

The blood was centrifuged at 1500 rpm for 15 min, and the plasma was stored in liquid nitrogen for 5 days.

Specimens of the myocardium isolated from the control and experimental groups were used for evaluation of FRO and AOA. The hearts were washed with physiological saline, dried with paper filter, and stored in liquid nitrogen for 5 days.

Myocardial samples were washed with cold physiological saline and homogenized in ice-cold phosphate buffer (pH 7.4, 1:10 w/v). The homogenate was centrifuged at 10,000 rpm and 4°C for 5 min.

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The intensity of FRO and AOA in the plasma and myocardial tissues were evaluated by chemiluminescence measured on an Emilite-1105 chemiluminometer.

Chemiluminescence was measured in reaction mixtures containing 700 ml phosphate buffer (pH 7.4), 50 ml 1.1 mM luminol, and 50 µl plasma or 700 ml buffer (60 mM K_2HPO_4 and 105 mM KCl, pH 7.4), 50 µl 10 M luminol, and 50 µl supernatant of homogenized myocardial tissue. Chemiluminescence induced by adding H_2O_2 (200 µl, 20 mM) was measured at 37°C for 2 min and expressed in arb. units/mg protein.

Total AOA was evaluated by the intensity of chemiluminescence in the reaction of riboflavin with H_2O_2 in the presence of Fe^{2+} [3,12]. Protein content was measured by the method of Lowry]9].

The correlations between FRO and AOA were analyzed by Statistica 5.0 software.

RESULTS

In control mice the intensity of FRO and AOA in the myocardium 5-fold surpassed those in the plasma (Fig. 1). Long-term dipyridamole treatment impaired the balance between FRO and AOA in the myocardium and plasma. The intensity of FRO in the myocardium increased 2-fold, while AOA was below the control (Fig. 1, *a*). The intensity of FRO in the plasma of dipyridamole-treated mice decreased 10-fold compared to the control, while plasma AOA in these animals was significantly higher than in controls.

Correlation analysis showed that FRO and AOA in myocardial tissues and plasma from dipyridamole-treated and control mice underwent opposite changes. No direct correlations between these parameters were found.

Thus, oxidative homeostasis in the myocardium and plasma is regulated by oppositely directed FRO and AOA. Therefore, the mechanisms maintaining oxidative homeostasis in cellular and cell-free biological systems are different.

Platelet count in mice receiving daily injections of dipyridamole for 30 days was higher than in controls ($331.6\pm10.2\times10^9$ and $218.1\pm15.5\times10^9$ cells/liter, respectively, $p<0.01$). Plasma AOA in dipyridamole-treated mice increased from 1.35±0.02 to 1.81±0.02 arb. units/mg protein ($p<0.01$). The coefficient of correlation between platelet count and plasma AOA in these mice was 1.0 (*vs*. 0.78 in the control, $p<0.01$).

Studies of the cholesterol and lipoprotein metabolism in mice during aging showed that treatment with platelets attenuates the severity of hypercholesterolemia in old animals [10]. Our previous experiments demonstrated the appearance of pathologically changed and destructed megakaryocytes in mouse bone marrow after heterotopic bone marrow transplantation combined with long-term platelet disaggregation treatment with dipyridamole [2]. Destruction of these bone marrow cells was associated with intensive lipid synthesis and accumulation of giant lipid drops in their cytoplasm. Destruction of the plasma membrane was accompanied by the release of cytoplasmic elements into the intercellular space.

Cardiospecific fatty acids-binding protein is also involved in the inhibition of cell growth and differentiation. This cytoplasmic protein plays a role in intracellular transport of long-chain fatty acids [3]. Previous studies showed that dipyridamole increases the content of these substances in mouse bone marrow [2].

Destructive processes in the myocardial tissue accompanied by structural changes and impairment of cell membrane permeability are associated with an

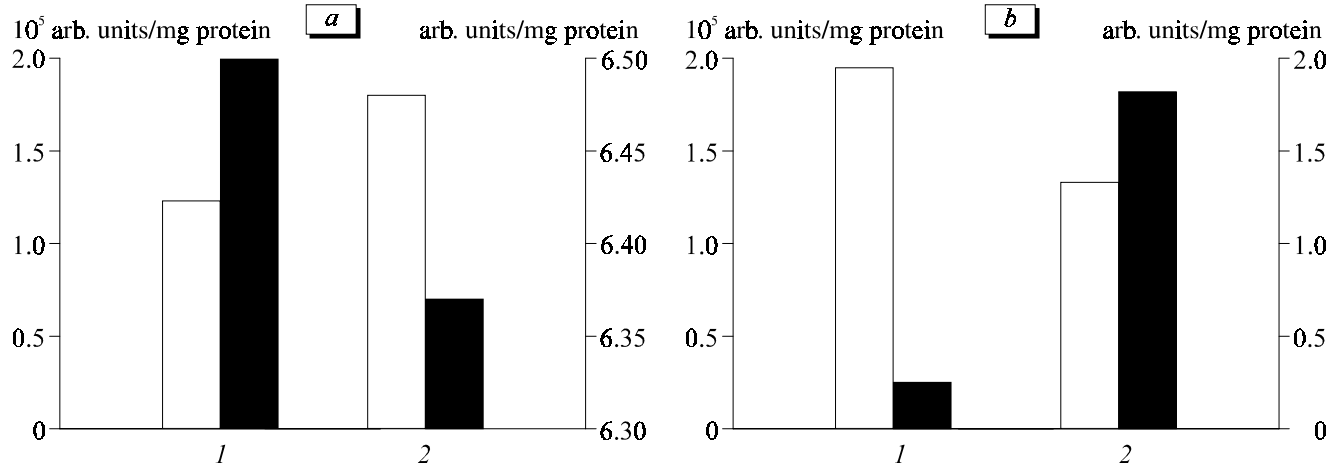

Fig. 1. Free radical oxidation (*1*, left ordinate) and antioxidant activity (*2*, right ordinate) in the myocardial tissue (*a*) and blood plasma (*b*) from mice after dipyridamole-induced long-term platelet disaggregation (30 days). Ordinate: chemiluminescence intensity. Light bars: control; dark bars: dipyridamole. *$p<0.01$ compared to the control.

imbalance between FRO and antioxidant protective systems [11]. Dipyridamole-induced changes in the content of lipids, phospholipids, and humoral factors probably shift the balance between FRO and AOA in the blood. These changes, in turn, can be a response to impaired antioxidant homeostasis.

Recent studies showed that structural changes in DNA and disturbances in mitotic cell division can result from oxidative stress [5,8]. It was unclear why long-term dipyridamole treatment arrests bone marrow myelokaryocytes in the G_2 phase of the cell cycle [1]. In our experiments, long-term dipyridamole treatment increased plasma AOA and inhibited FRO. These results indicate that blockade of cell passage through G_2 phase of the cell cycle is probably related to changes in plasma oxidative homeostasis. The apoptosis marker p53 is responsible for the arrest of cultured human large-cell cancer H-1229 cells in the G_2 phase [13]. Apoptosis causing dysfunction of cardiomyocytes and endothelial cells via oxidative stress promotes the development of cardiac insufficiency [5].

Our results indicate that FRO and AOA in myocardial tissues and plasma change during oxidative stress caused by long-term platelet disaggregation treatment with dipyridamole. Thus, oxidative homeostasis in cellular (myocardial tissue) and cell-free systems (blood plasma) during long-term treatment with dipyridamole is maintained by oppositely directed AOA and FRO.

Under conditions of long-term platelet disaggregation oxidative homeostasis in the plasma is maintained via various mechanisms. Our results show that stimulation of AOA and inhibition of FRO in the plasma are the major mechanisms maintaining oxidative homeostasis in mice. Dipyridamole stimulates mouse plasma AOA, which is probably related to an increase in platelet count.

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