Iron Content and Parameters of Blood Antioxidant Activity in Rats with Hereditary Arterial Hypertension during Experimental Myocardial Infarction

M. G. Yakobson, A. R. Antonov, A. V. Golovatyuk, A. V. Efremov, A. L. Markel', and G. S. Yakobson

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Plasma, lymph and myocardial Fe^{2+} contents and blood antioxidant activity were studied in hypertensive NISAG and normotensive Wistar rats with experimental myocardial infarction. In hypertensive rats, iron levels in plasma, lymph and myocardial tissue were increased throughout the experiment. In both strains iron content in the plasma during experimental myocardial infarction negatively correlated with blood antioxidant activity. In hypertensive NISAG rats this correlation persisted also during recovery. This attests to prooxidant effect of Fe^{2+} aggravating the course of myocardial infarction in NISAG rats.

Key Words: *trace elements*; *arterial hypertension*; *myocardial infarction*

Essential hypertension and ischemic heart disease rank first among cardiovascular diseases in Russia and other countries [2,6]. Recent progress in medical sciences opened new aspects in the etiology and pathology of arterial hypertension and myocardial infarction (MI). Considerable attention was attracted to the role of trace elements in the pathogenesis of cardiovascular diseases. It was established that iron, copper, zinc, manganese, and selenium, cofactors of various enzymes, produce considerable effects on the course of arterial hypertension and MI. Main targets for trace elements are pro- and antioxidant enzyme systems [8]. Mechanisms of these effects are now intensively studied, however some aspects of this problem are still unclear. Thus, little is known about metabolism of trace elements in damaged myocardium during arterial hypertension. Arterial hypertension in combination with MI represents a complex severe systemic disease. Unfortunately, our knowledge about intrinsic mechanism of this pathology is still fragmentary. On the one hand, enhanced production of reactive oxygen species (ROS) and intensification of lipid peroxidation (LPO) are the major factors leading to myocardial injury. On the other hand, trace elements are the immanent part of pro- and antioxidant enzymes. Thus, it is interesting to evaluate the dynamics of plasma antioxidant activity (AOA), an integral characteristic of antioxidant enzyme activity, during myocardial infarction. Here we studied iron distribution in the plasma, lymph, and myocardium in normo- and hypertensive rats with experimental myocardial infarction. A correlation between plasma antioxidant activity and iron levels was analyzed.

MATERIALS AND METHODS

Experiments were carried out on 116 male Wistar (normotensive) and NISAG (hereditary stress-induced arterial hypertension, Institute of Cytology and Genetics, Novosibirsk) rats weighing 180-200 g. Basal blood pressure in hypertensive rats was 170±2 mm Hg, during stress it reached 205±2 mm Hg. Myocardial infarction was induced in rats of both groups by subcutaneous injection of 0.1% epinephrine (0.2 mg/100

Institute of Physiology, Institute of Cytology and Genetics, Siberian Division of the Russian Academy of Medical Sciences, Novosibirsk; Novosibirsk State Medical Academy

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g) and verified by ECG. The rats were decapitated under ether narcosis on day 1, 2, 3, 7, 14, and 21 of experimental MI and the blood from the decapitation wound was collected. The lymph was collected as follows: the rats were intraperitoneally narcotized with Hexenal, the skin was cut (1 cm) parallel to *m. erector spinae* near its intersection with the rib, and after transection of the external oblique muscle and blunt dissection of the retroperitoneal adipose tissue, the cisterna chyli of the thoracic duct was exposed and punctured. The lymph was pumped out with an aspiration pump. About 1 ml fresh lymph was usually obtained from an adult rat by this method. The lymph was centrifuged at 900*g* (3000 rpm) for 10 min and stored at -20°C before assay. The hearts were removed immediately after decapitation, ventricles (300±10-mg specimens) were dried in a thermostat at 105°C for 48 h, powdered in a porcelain mortar, and kept at room temperature until measurements.

Iron content in the plasma, lymph, and myocardium was measured on an Unicam-939 atomic absorption spectrophotometer. AOA was evaluated by chemiluminescence technique. Plasma-to-lymph iron index (PLI) was calculated as the ratio of iron contents in the plasma and lymph.

RESULTS

Iron content in the plasma, lymph and myocardial tissue in hypertensive rats during MI surpassed that in Wistar rats (Fig. 1, *a*, *b*). Moreover, iron concentration in hypertensive NISAG rats and normotensive Wistar rats during the acute MI underwent opposite changes. These findings agree with previous clinical and experimental

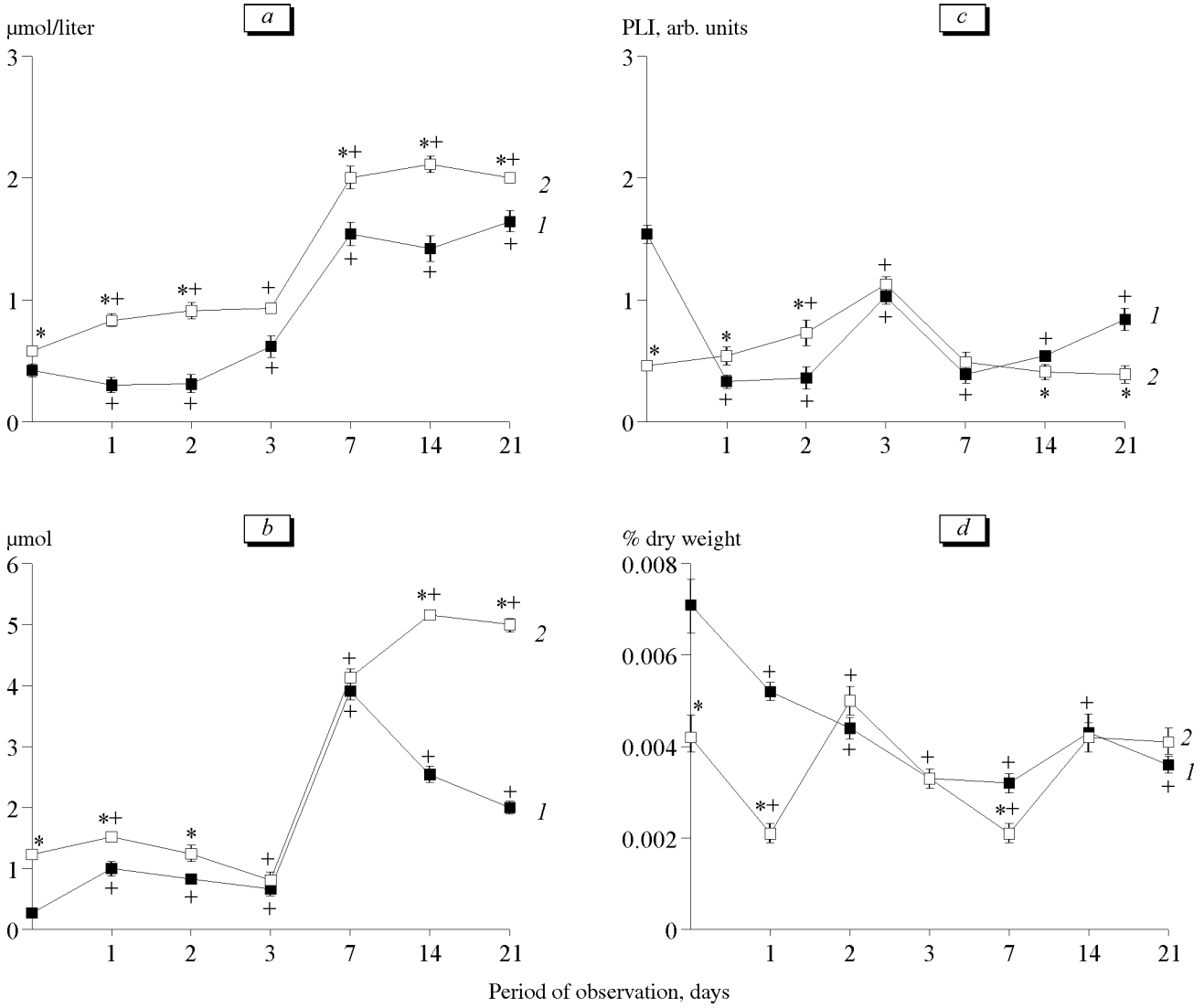

Fig. 1. Content of iron ions in plasma (*a*), lymph (*b*), myocardium (*d*), and plasma-to-lymph iron index (PLI, *c*) in Wistar rats (*1*) and NISAG rats (*2*) during experimental myocardial infarction. Here and on Fig. 2: **p*<0.05 compared to Wistar rats; +*p*<0.05 compared to the baseline (zero).

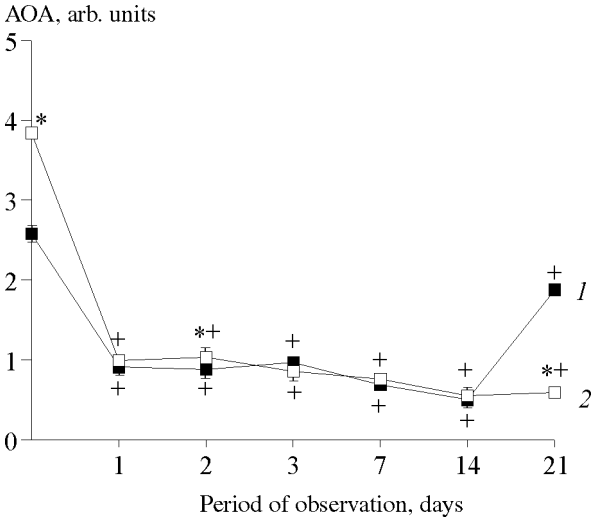

Fig. 2. Plasma antioxidant activity (AOA) in Wistar rats (*1*) and NISAG rats (*2*) during experimental myocardial infarction.

data on high iron content is a potent risk factor of ischemic heart disease and MI [15]. A close correlation was found between iron content and risk of fatal MI [12].

This negative effect of iron can be explained by activation of lipid peroxidation. The increase in plasma iron content leads to sharp activation of LPO in the myocardium, liver, spleen, and plasma [11]. Fe^{2+} is necessary for ROS production in mitochondrial, microsomal, xanthine oxidase, and other oxidative systems and in the Fenton and Haber—Weiss reactions yielding hydroxyl radical ($^{\bullet}OH$). Moreover, iron autooxidation can initiate free-radical lipid oxidation without ROS. Iron-catalyzed splitting of lipid hydroperoxide radicals (ROOH) and formation of alkoxyl radicals ($RO^{\bullet}$) resulting in chain branching also intensity LPO processes. This mechanism is similar to the role playing by iron in the Fenton reaction. Iron ions in high concentration inhibit glutathione peroxidase, a major antioxidant enzyme. Iron is present in the active center of 5-lipooxygenase enzyme catalyzing conversion of arachidonic acid into leukotrienes (potent proinflammatory substances) accompanied by ROS generation. On the other hand, iron is an essential part of catalase, an antioxidant enzyme. However, catalase is absent in the myocardium. Moreover, H_2O_2 neutralization in rats is performed by glutathione peroxidase rather than catalase. Thus, damaged myocardium provides favorable conditions for realization of iron prooxidant activity. It cannot be excluded that the negative effect of iron ions in MI can be realized through some unknown mechanisms [13].

Thus, high concentration of iron ions in the plasma and lymph of NISAG rats aggravates the development of MI.

In Wistar rats, PLI continuously decreased during MI and was 2-3 times below the initial value in the late period (Fig. 1, *c*), which attested to its redistribution to the lymph. This can be a defense reaction to high plasma content of Fe^{2+}. In NISAG rats no redistribution of iron ions between the plasma and lymph was found. Moreover, plasma pool of iron in hypertensive rats increased during acute MI, which also can aggravate this condition. The content of iron ions in the myocardium of Wistar rats decreased during MI, which probably represented an adaptive reaction protecting the myocardium from their damaged effects. No stable decrease in Fe^{2+} was observed in the myocardium of hypertensive NISAG rats (Fig. 1, *d*).

In Wistar rats, the increase in plasma-to-myocardium iron index was more pronounced and lasted for a longer time period compared to Wistar rats. These findings suggest that iron redistribution from the myocardium to the plasma in hypertensive rats was less pronounced.

A strong negative correlation was found between plasma concentration of iron ions and plasma antioxidant activity in both rat strains during experimental MI (Fig. 2). During recovery this correlation became weaker in normotensive rats but remained strong in hypertensive rats. These data also confirm the hypothesis on more pronounced prooxidant activity of iron ions in hypertensive NISAG rats.

Thus, iron metabolism in hypertensive NISAG rats during MI differs from that in Wistar rats. In Wistar rats, iron content in the myocardium decreased predominantly due to its redistribution to the lymph, while in NISAG rats this decrease in myocardial levels of iron ions was unstable, and no significant exchange of iron ions between tissues was observed. Taking into account the fact that experimental MI in hypertensive rats develops against the background of high concentration of iron ions in the plasma and lymph, the aggravating effect of iron ions in these animals is more pronounced, because potent prooxidant effects of excessive iron ions are associated with stimulation of LPO processes and production of ROS.

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