EXPERIMENTAL METHODS FOR CLINICAL PRACTICE

ATP-Sparing Effect of Histochrome in Acute Myocardial Ischemia in Patients with Coronary Heart Disease

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After *in vitro* ischemia, the content of adenosine triphosphate in myocardial bioptates from patients with heart diseases is reduced. This reduction is more pronounced in patients with coronary heart disease than in patients with rhythm disturbances. Administration of the antioxidant preparation histochrome to patients with coronary heart disease preserves ATP during ischemic exposure.

Key Words: *myocardium; ischemia; ATP; lipid peroxidation; antioxidants*

Surgery, a radical treatment of coronary insufficiency in coronary heart disease (CHD), is associated with risk of some grave complications, especially in early postoperation period [1]. Apart from operation wound, these complications can be caused by reperfusion damage to the myocardium [8]. A prospective way of preventing this damage is the use of antioxidant preparations [5,9]. However, recent *in vitro* experiments have shown that ischemia- and reperfusion-induced disturbances of myocardial contractility, in particular, myocardial contracture are due not only to altered permeability of cell membranes, but also depend on the ATP pool remaining in cardiomyocytes (CMC) before reperfusion [12]. In light of this is was interesting to evaluate the effect of acute ischemia during heart surgery and antioxidant therapy on myocardial ATP content in CHD patients.

MATERIALS AND METHODS

Experiments were carried out on fragments from right auricle obtained during aortocoronary bypass operations in patients with chronic CHD (NYHA functional class II-III) and during maze operation in patients with Wolff—Parkinson—White syndrome (WPW). The mean age of patients was 40 years. The patients were examined and operated at the Department of Cardiovascular Surgery, Institute of Cardiology, Tomsk Research Center.

The fragments were washed from blood with cooled physiological saline and cut into 2 portions: one was immediately frozen in liquid nitrogen, while the other was placed to a humidified chamber (20°C) for 60 min and then frozen in liquid nitrogen. The latter procedure adequately reproduces the conditions of aortocoronary bypass operation.

Some patients with CHD (n=9) were prepared for operation according to routine scheme, while others (n=8) additionally received two intravenous injections of 3% histochrome (HC) in a dose of 1 mg/kg (24 h prior to operation and in the operating room). Histochrome, a novel Russian-manufactured antioxidant, was kindly provided by Dr. A. V. Lebedev (Russian Research and Manufacturing Cardiology Association). Fragments obtained from patients without CHD and operated for cardiac rhythm disturbances (n=6) served as the control.

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The content of ATP in frozen fragments was measured using Sigma kits after preliminary extraction with 6% perchloric acid [7]. The data were processed statistically using the Student's *t* test.

RESULTS

The content of ATP in bioptates immediately after sampling is presented in Fig. 1, *a*. In patients with CHD, myocardial ATP content was 2.5 mmol/g, i.e., 1.63-fold surpassed that in patients with WPW syndrome (relatively normal myocardium). Abnormal myocardial content of ATP in patients with CHD is probably due to activated glycolytic production of ATP and/or inhibited ATP utilization in CMC [13]. Increased myocardial content of ATP in CHD is an adaptive phenomenon. Indeed, animal experiments showed that adaptation to periodical hypoxia is accompanied by activation of tissue respiratory enzymes, the intensity of mitochondrial oxidation being decreased [5,10]. In other words, activation of energy-producing intracellular systems probably occurs at a certain stage of pathological process. The paradoxically high level of ATP in CMC of CHD patients indicates viability of these cells and preserves the ability to respond to various stimuli, for instance, to pharmacological agents. This assumption is based, first, on our data on inotropic capacity of the myocardium from CHD patients [2,3] and, second, on myocardial response to HC injections. Figure 1, *a* shows that the basal level of ATP in the myocardium of CHD patients treated with HC practically does not differ from that in relatively normal myocardium (patients with nonischemic pathology).

The loss of ATP stores may seem to be a too high price for recovery of cell-membrane activity. However, experiments with myocardial fragments exposed to 60-min ischemia argue this assumption. Figure 1, *b* shows a decrease in ATP content in all myocardial fragments exposed to a 60-min ischemia: in relatively normal myocardium it is no more than 51%, while in control CHD patients it is only 12% of the basal level. In fragments from HC-treated patients, 60-min ischemia induced no significant decrease in ATP content: it constituted no less than 80% of the basal value and was considerably higher than in the relatively normal myocardium. Experiments with isolated heart showed that metabolic acidosis of the intracellular medium developed in cardiac ischemia impairs utilization of substrates in the mitochondria and energy supply of CMC [14]. This may account for the decrease in myocardial ATP content in CHD patients. The smaller decrease in the ATP content of ischemized fragments of relatively normal myocardium (49%) can be attributed

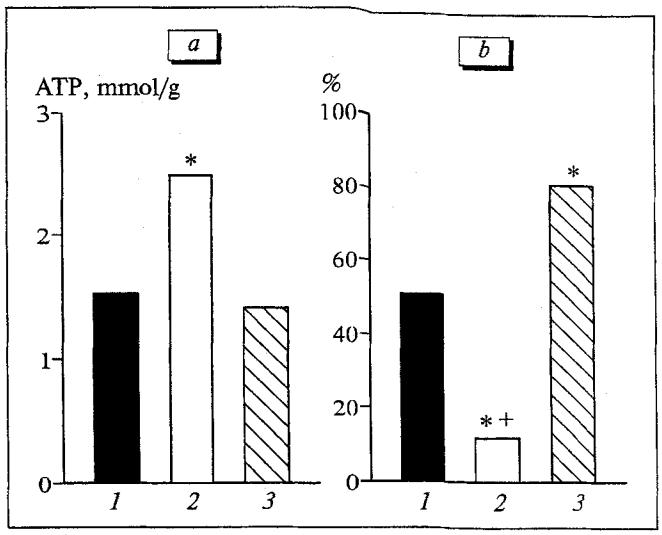

Fig. 1. Effect of chronic coronary heart disease and antioxidant therapy on ATP content in the myocardium of cardiological patients. *a*) basal ATP level in myocardial specimens; *b*) ATP content in the same specimens after 60-min ischemia (% of basal level). *1*) patients with nonischemic pathology; 2) patients with chronic coronary disease; 3) patients with coronary heart disease treated with histochrome. Differences are significant: *compared with *1*; +compared with 3.

to untapped reserves of anaerobic metabolism in intact CMC. Oxygen content even in totally ischemized myocardium far surpassed the capacity of the endogenous antioxidant system [4]. This fact together with our previous data on reduced capacity of antioxidant system in the myocardium on CHD patients [6] attests to the development of uncontrolled lipid peroxidation in ischemized myocardial fragments.

Moreover, it has been previously demonstrated that antioxidants prevent inactivation and stimulate aerobic and anaerobic energy-producing enzymes [13]. In this context, myocardial bioptates from patients treated with antioxidants have some advantages. Pretreatment with HC provides a possibility for its active component to incorporate into cell membrane and protect the lipid bilayer against enhanced free-radical attack in long-term ischemia. Moreover, due to stabilization of CMC membranes, HC can prevent the washout of low-molecular-weight macroergic phosphates from CMC. Despite contradictory data on the relationship between ATP content after ischemia and the efficiency of subsequent reperfusion [11,12], it is obvious that high myocardial level of ATP is a prerequisite for a better recovery during reperfusion.

Thus, acute ischemia during cardiac surgery in CHD patients extremely depletes the basal pool of ATP. Under these conditions HC exerts an ATP-sparing effect, which is an additional mechanism of its protective effect.

REFERENCES

1. V. A. Arkatov and V. A. Novosel'tsev, *Anest. Reanimatol.*, No. 2, 53-54 (1988).
2. S. A. Afanas'ev, E. D. Alekseeva, A. M. Chernyavskii, and V. V. Pekarskii, *Byull. Eksp. Biol. Med.*, **115**, No. 6, 574-576 (1993).
3. S. A. Afanas'ev, S. A. Bogomaz, E. D. Alekseeva, *et al.*, *Ibid.*, **117**, No. 5, 457-459 (1994).
4. M. V. Bilenko, *Biological Aspects of Kidney Transplantation* [in Russian], Moscow (1978).
5. M. V. Bilenko, *Ischemic and Reperfusion Damage to Organs* [in Russian], Moscow (1989).
6. A. V. Lebedev, S. A. Afanas'ev, E. D. Alekseeva, *et al.*, *Byull. Eksp. Biol. Med.*, **119**, No. 6, 584-586 (1995).
7. M. I. Prokhorova (Ed.), *Biochemical Methods (Lipid and Energy Metabolism)* [in Russian], Leningrad (1982), pp. 256-258.
8. E. B. Petukhov and A. A. Korneev, *Grud. Serd.-Sosud. Khir.*, No. 1, 44-46 (1991).
9. A. N. Repin, I. V. Maksimov, V. A. Markov, *et al.*, *Kardiologiya*, **34**, No. 3-4, 4-7 (1994).
10. N. K. Khitrov and V. S. Paukov, *Heart Adaptation to Hypoxia* [in Russian], Moscow (1991).
11. E. D. Lewandowski, M. D. Devous, and R. L. Nunnally, *Am. J. Physiol.*, **253**, No. 5, H1215-H1223 (1987).
12. F. Savabi and S. P. Bessman, *Arch. Biochem. Biophys.*, **248**, No. 1, 151-157 (1986).
13. I. K. De Scheerder, A. M. M. Maas, A. S. Neiukoop, *et al.*, *J. Mol. Cell. Cardiol.*, **21**, No. 4, 69-70 (1989).
14. G. Suleymanlar, H. Z. Zhou, M. McCormack, *et al.*, *Am. J. Physiol.*, **262**, No. 6, Pt. 2, H1818-H1822 (1992).