Bioluminescent Analysis of Intensity of Pathological Oxidative Processes in Cells of Perfused Rat Liver after Hyperthermia

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We studied the possibility of evaluation of the intensity of pathological oxidative processes in rat liver using an integral bioluminescent test with controlled perfusion of the isolated organ. The test revealed a significant correlation between the level of TBA-reactive products and bioluminescence intensity.

Key Words: *bioluminescent analysis; controlled perfusion of isolated liver; hyperthermia; oxidative stress*

Evaluation of the intensity of pathological oxidative processes in cells (LPO *etc.*) associated with enhanced production of reactive oxygen species is an actual problem of experimental biology [10]. This problem can be solved with the help of bioluminescent analysis, which is currently used in many fields of experimental biology and medicine [5,8]. Bioluminescent tests are employed for integral assessment of the intensity of LPO and oxidative stress [2]. Bioluminescent methods based on the use of luminescent bacteria or isolated bacterial enzyme systems are rapid, easy, highly sensitive, and require minimal volumes of the examined specimens.

Our aim was to apply bioluminescent methods for evaluation of the intensity of pathological oxidative processes in cells of perfused rat liver. Metabolic changes in the liver were studied during recovery after a short-term *in vivo* hyperthermic exposure. The parameters of the bioluminescent tests were compared with the rate of the release of thiobarbituric acid-reactive substances (TBARS)

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MATERIALS AND METHODS

Experiments were performed on outbred albino rats (males and females, n=80, 1:1) weighing 170-220 g. Each series comprised 20 rats. Hyperthermia was modeled by placing the rats housed in individual cases into a 42°C water bath for 25 min. Before this procedure, the animals were intraperitoneally injected with 0.25% droperidol in a dose of 0.1 ml/100 g body weight to prevent stress reactions.

The liver was perfused *in situ* at the rate of 18-21 ml/min for 90 min with a hemoglobin-free Krebs—Henseleit bicarbonate buffer (pH 7.4) containing 5 mM glucose in a non-recirculation system 1, 6, 18 h after hyperthermia. The perfusate was saturated with O_2 and CO_2 (95 and 5%, respectively) using a bubble oxygenator [9,12]. Before perfusion, the rats were intraperitoneally injected with sodium thiopental (100 mg/kg) and intravenous heparin (10,000 U/kg). The abdominal cavity and the thorax were opened and cannulas were inserted into *v. porta* (influx) and *v. cava caudalis* (efflux) [15]. The control group comprised the rats subjected to the same procedures except hyperthermia.

The state of the perfused organ was assessed by integral bioluminescent test based on the measurement of luminescent reaction catalyzed by coupled enzyme

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system NADH:flavin mononucleotide-oxidoreductase (R):luciferase (L) in the absence and presence of the studied perfusate. Before measurements, L (0.11 mg) and R (0.069 U/ml) were dissolved in 1 ml phosphate buffer (0.1 M, pH 6.8) with dithiothreitol (0.1 mM). Bioluminescence of the coupled enzyme system was measured in a reaction medium containing 50 µl 0.0025% myristic aldehyde, 5-10 µl L+R conditioned solution, 50 µl 0.5 mM flavin mononucleotide, 200 µl phosphate buffer (pH 6.8), 200 µl 0.4 mM NADH. The reaction was triggered by addition of NADH or flavin mononucleotide solutions. After attaining the peak of luminescence, the test solution (20-50 µl) was added to the cuvette, and luminescence was measured again. Luciferase index (LI) was determined as the ratio of bioluminescence intensity measured after addition of the perfusate into the reaction mixture to baseline luminescence [13,14]. Intensity of the coupled enzyme system was measured on a BLM-8801 Bioluminometer (Nauka Engineering Department, Krasnoyarsk Research Center, Siberian Branch of the Russian Academy of Medical Sciences). The enzymes used in bioluminescent analysis were produced in Biotechnology Department of Institute of Biophysics, Siberian Division of the Russian Academy of Sciences.

The concentration of TBARS was determined by their reaction with TBA in an Uvikon-943 spectrophotometer at λ=532-600 nm [11].

The concentration of O_2 in the perfusate was measured by polarography with platinum-lead electrode [1]. The data were analyzed statistically using Pearson's correlation coefficient.

RESULTS

A significant increase in LPO intensity was observed at the end of perfusion under control conditions and after 18-h recovery (Fig. 1, *a)*. During perfusion, the concentration of TBARS 1 and 6 h after hyperthermia remained at a stable low level, whereas in the control and 18 h after hyperthermia the concentration of TBARS was maximum. The rate of oxygen consumption by the liver decreased during perfusion both in the control and after hyperthermia (Fig. 1, *b*).

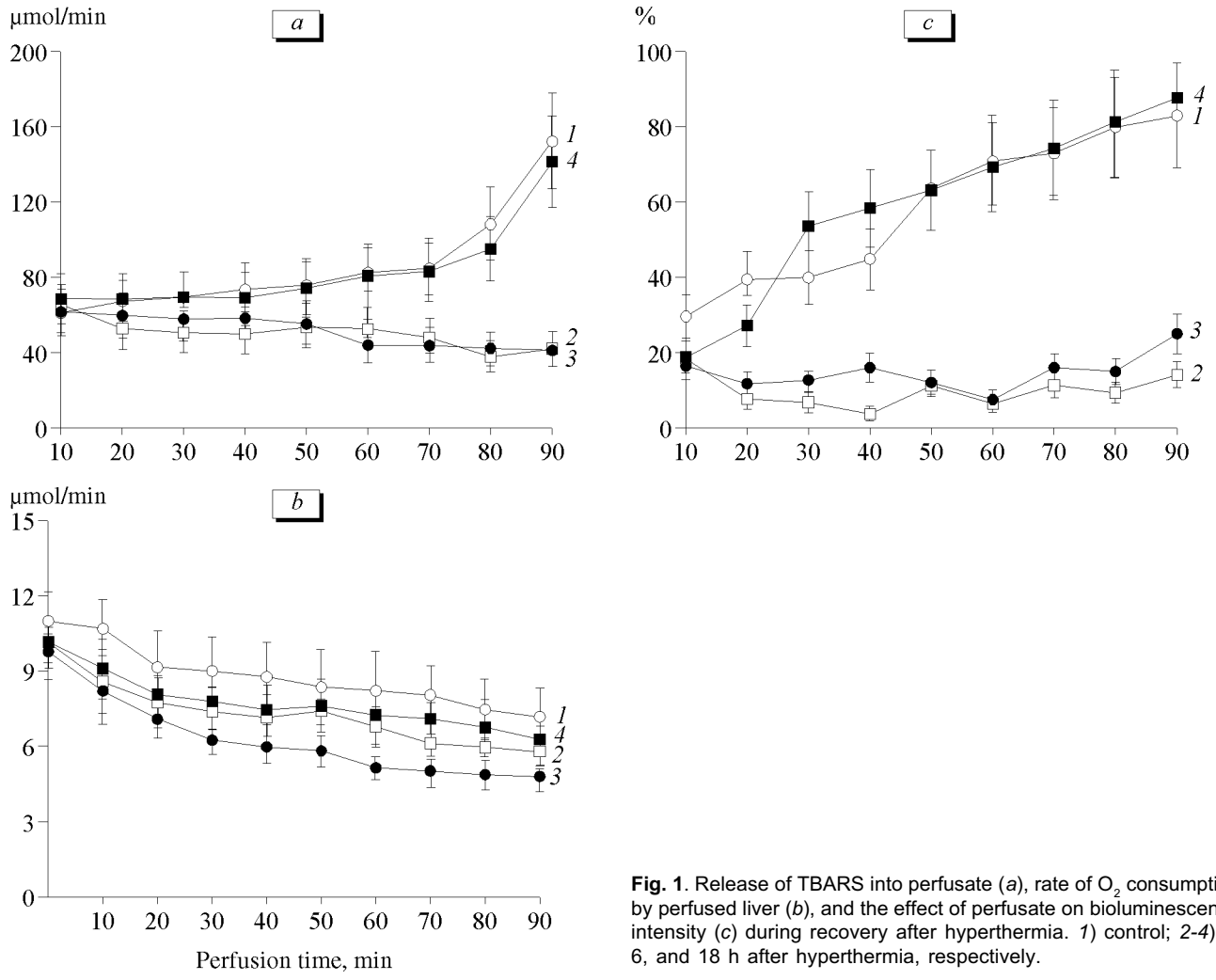

Fig. 1. Release of TBARS into perfusate (*a*), rate of O_2 consumption by perfused liver (*b*), and the effect of perfusate on bioluminescence intensity (*c*) during recovery after hyperthermia. *1*) control; *2-4*) 1, 6, and 18 h after hyperthermia, respectively.

In parallel with the release TBA-active metabolites into the perfusate, LI also increased during perfusion. Similar changes in LI were observed during recovery after hyperthermia. In the control and after 18-h recovery the intensity of bioluminescence was higher than 1 and 6 h after hyperthermia.

Published data suggest that changes in energy metabolism in hepatocytes occur during the first hours after hyperthermia, and manifest in compensatory activation of glycolysis and disturbed regulation of energy exchange [3,4]. Taking into consideration the existence of so-called "acquired thermotolerance" (resistance to the following extreme stimulation [6,7]), we can hypothesize that decreased rate of TBARS release observed 1 and 6 h after hyperthermia results from this phenomenon. On the other hand, oxygen consumption also considerably decreases during this period. This also inhibits production of reactive oxygen forms and LPO processes. By contrast to 18-h recovery, the effect of hyperthermia persists 1 and 6 h after termination of this exposure. Mobilization of adaptation reserves during the recovery period is also a stress factor for hepatocytes. Drastic accumulation of LPO products observed by the end of perfusion period can result from disturbances in physiological adaptation mechanisms, degeneration of cell structures, and hepatocyte cytolysis. Our experiments showed that the rate of the release of TBARS into the perfusate correlates with LI parameters (correlation coefficient 0.84, $p<0.001$).

The revealed relationship between LPO intensity (determined by TBARS content) and intensity of luminescence can provide the basis for integral bioluminescent analysis of the development of pathological oxidative processes in cells of various organs.

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