QUANTITATIVE CHANGES IN LIVER DEHYDROGENASE ACTIVITY DURING AND AFTER TEMPORARY ISCHEMIA OF THE LIMBS

O. D. Mishnev, A. P. Raksha,
A. V. Zhukotskii, and A. I. Shchegolev

UDC 617.57/.58-005.4-07:616.37-008.931:577.152.1

KEY WORDS: ischemia of the limbs; postischemic syndrome; liver; dehydrogenases; histophotometry.

In modern vascular surgery aid for patients with acute arterial obstruction is one of the most important problems. Embolism, especially of large arteries, leads to sudden disturbances of the peripheral and central hemodynamics, which arise in response to the appearance of mechanical obstruction in the blood vessel. Disturbances of correlation between metabolism and the formation of toxic substances not normally present in the body, during ischemia have a considerable harmful action on the microcirculatory system and on tissue structures of the internal organs and may cause death. This intricate symptom-complex, characterized by definite clinical, morphological, and biochemical changes in the damaged tissues and in the body as a whole, is known in the literature as the revascularization syndrome or the postischemic syndrome (PIS), and it develops in 20-50% of patients with occlusion of the aorto-iliac zone (the mortality in these cases is 25-40%) [3, 6, 7]. One form of manifesta-

TABLE 1. Dehydrogenase and Diaphorase Activity in the Liver during Temporary Ischemia of the Limbs and in the Postischemic Period

Name of enzyme and control value	Control	Ischemia of limbs			Ischemia of limbs followed by revascularization for 2 h		
		3 h	6 h	12 h	3 h	6 h	12 h
LDH	0,1968±0,0042	0,2057±0,0021 (+5%)	0,1929±0,0019 (−2%)	0,1566±0,0022 (−20%)	0,2004±0,0066 (+2%)	0,1639±0,0042 (−17%)	0,1392±0,0045 (−29%)
P		>0,05	<0,05	<0,01	>0,05	<0,05	<0,05
					>0,05	<0,01*	<0,01*
G-6-PDH	0,0626±0,0062	0,0951±0,0027 (+52%)	0,0554±0,0026 (−12%)	0,0341±0,0023 (−45%)	0,0917±0,0100 (+46%)	0,2048±0,0025 (−60%)	0,0142±0,0030 (−77%)
P		<0,01	>0,05	<0,01	<0,05	<0,01	<0,01
					>0,05*	<0,01*	<0,01*
SDH	0,0956±0,0038	0,1153±0,0019 (+21%)	0,1031±0,0026 (+8%)	0,0759±0,0019 (−21%)	0,1092±0,0031 (+14%)	0,0802±0,0035 (−16%)	0,0615±0,0038 (−36%)
P		<0,01	>0,05	<0,01	<0,05	<0,05	<0,05
					>0,05*	<0,01*	<0,01*
GDH	0,2238±0,0056	0,2536±0,0024 (+13%)	0,2169±1,0021 (−3%)	0,1635±0,0020 (−27%)	0,2331±0,0095 (+4%)	0,1772±0,0102 (−21%)	0,1440±0,0092 (−36%)
P		<0,05	>0,05	<0,01	>0,05	<0,05	<0,05
					<0,05*	<0,01*	<0,05*
NAD	0,1503±0,0056	0,1943±0,0022 (+29%)	0,1633±0,0026 (+9%)	0,1275±0,0023 (−15%)	0,1887±0,0071 (−26%)	0,1236±0,0031 (−18%)	0,925±0,0063 (−38%)
P		<0,01	<0,05	<0,05	<0,01	<0,01	<0,05
					>0,05*	<0,01*	<0,1*
NADP	0,2236±0,0056	0,2447±0,0024 (+9%)	0,2098±0,0032 (−6%)	0,1566±0,0019 (−30%)	0,2350±0,0024 (+5%)	0,1502±0,0110 (−33%)	0,1316±0,0097 (−41%)
P		<0,05	>0,05	<0,01	<0,05	<0,01	<0,01
					<0,05*	<0,01*	<0,05*

Legend. P) Significance of differences compared with control.
*Differences between data for ischemia and for ischemia followed by revascularization are significant.

Department of Pathological Anatomy, Faculty of Internal Medicine, and Laboratory of Quantitative Microscopy, Central Research Laboratory, N. I. Pirogov Second Moscow Medical Institute. (Presented by Academician of the Academy of Medical Sciences of the USSR V. V. Kupriyanov.) Translated by Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 99, No. 1, pp. 22-24, January, 1985. Original article submitted February 17, 1984.

0007-4888/85/9901-0027$09.50

tion of PIS is acute hepatorenal failure, due to disturbance of the capillary circulation and organid damage to the parenchymatous cells of these organs [2]. Since the liver participates actively in the maintenance of dynamic equilibrium between the constants of the internal medium, a close study of hepatocyte metabolism must be of great interest and help to the understanding of the pathogenesis of PIS. Many workers have described the important role of the liver in occlusion of the bifurcation of the aorta and states of similar pathogenesis [1, 2]. However, the enzyme histochemical characteristics of the hepatocytes in acute disturbances of the arterial circulation in the lower limbs and in the early stages after revascularization have not previously been studied.

The aim of this investigation was a quantitative analysis of changes in dehydrogenase activity in the hepatocytes during temporary ischemia caused by acute occlusion of the main arteries of the limbs, and in the postischemic period.

EXPERIMENTAL METHODS

Experiments were carried out on 36 mongrel dogs of both sexes weighing 13-18 kg. A model of acute occlusion of the trifurcation of the aorta, by the method described in [3], was used. The duration of ischemia of the limbs was 3, 6, and 12 h. In the next series of experiments, these same periods of ischemia were followed by revascularization in the course of 2 h. To assess the dynamics of enzyme activity we chose as tests a series of six enzymes, involved in different pathways of metabolism: succinate dehydrogenase (SDH), an indicator of the intensity of oxidative processes in the Krebs' cycle, lactate dehydrogenase (LDH), associated with anaerobic glycolysis, glucose-6-phosphate dehydrogenase (G6PDH), a marker of the pentose shunt of glucose oxidation, and glutamate dehydrogenase (GDH), connected with amino-acid and protein metabolism. As integral parameter of the energy potential, NAD-diaphorase, was investigated. To assess the state of the energy supply for synthesis, NADP-diaphorase was used. The enzyme-histochemical tests were carried out on frozen sections 10 μ thick by the usual methods [5]. For the quantitative analysis, a Microvideomat television system (Opton, West Germany), controlled by a Wang 720C (USA) computer, by a specially written program of photometric analysis of histological preparations [14], was used. The parameter chosen for study, the mean optical density, is proportional to enzyme activity in the tissues. The significance of differences was estimated by Student's test.

EXPERIMENTAL RESULTS

In the control group the enzyme histochemical profile of the hepatocytes was established, and described as 100% (Table 1). In experiments in which the terminal part of the aorta was occluded, the level of enzyme activity of the hepatocytes was higher than in the control. The principal changes concerned the pentose shunt of glucose oxidation, as the shortest pathway and the one yielding the greatest energy, and NAD-diaphorase, signifying an increase in energy potential of the cells and activation of glycolysis, and which also gives some evidence of an increase in the functional load on the hepatocytes. If the period of ischemia was lengthened to 6 h, the levels of enzyme activity fell. The course of this period was characterized in particular by relative normalization of the metabolic pathways and approximation of levels of enzyme activity to the control values. After ischemia for 12 h a dramatic decline in the activity of the dehydrogenases was observed, as shown by the appearance of large grains of diformazan in the hepatocytes, evidence of damage to the intracellular structures. In experiments in which ischemia of the hind limbs for 3 h was followed by revascularization in the course of 2 h, increased enzyme activity also was found compared with the control. Parameters obtained during ischemia with revascularization had values rather lower than those obtained during ischemia, specifically, 3% lower for LDH, 7% for SDH, 6% for G6PDH, 4% for NADP-diaphorase, 3% for NAD-diaphorase, and 9% for GDH. In experiments with ischemia of the hind limbs for 6 and 12 h, followed by revascularization for 2 h, a progressive decline in enzyme activity was observed. In these cases, just as after ischemia for 3 h, the levels after revascularization were lower than those obtained after ischemia of the limbs alone. The difference for the two periods, respectively, was 15 and 9% for LDH, 24 and 15% for SDH, 48 and 32% for G6PDH, 27 and 11% for NADP-diaphorase, 18 and 11% for GDH, and 27 and 23% for NADP-diaphorase.

The quantitative study of levels of activity of certain liver dehydrogenases during temporary ischemia of the hind limbs and ischemia followed by revascularization revealed definite patterns of changes in the enzymologic homeostasis of the liver and changes in the levels of enzyme activity were found to be periodic in character. The metabolic system of the

hepatocytes was shown to react regularly to changes taking place in the body during temporary ischemia of the limbs, and the response depended on the duration of ischemia. Changes in hepatocyte dehydrogenase activity took the form of an increase in the early stages of the experiments, followed by a marked decrease.

In the postischemic period levels of hepatocyte dehydrogenase activity exceeded the control values only on restoration of the blood flow after ischemia of the limbs for 3 h. Later restoration of the blood flow in the limbs led to a decrease in hepatocyte dehydrogenase activity by a greater degree compared with the corresponding ischemic period.

LITERATURE CITED

1. N. P. Aleksandrova, E. B. Pastukhov, M. P. Isaev, et al., in: Acute Ischemia of the Organs and Early Postischemic Disorders [in Russian], Moscow (1978), pp. 236-237.
2. P. P. Alekseev, in: Acute Ischemia of Organs and Methods of Management of Postischemic Disorders [in Russian], Moscow (1973), pp. 199-200.
3. I. I. Zatevakhin, V. M. Koshkin, N. P. Istomin, et al., Eksp. Khir., No. 3, 16 (1976).
4. L. M. Nemirovskii et al., Transactions of the N. I. Pirogov Second Moscow Medical Institute [in Russian], Moscow (1980), pp. 65-68.
5. A. G. Pearse, Histochemistry: Theoretical and Applied, 3rd edn., Williams and Wilkins (1969).
6. V. S. Savel'ev, in: Current Problems in Vascular Surgery [in Russian], Part 3, Moscow (1976), pp. 41-45.
7. H. Haimovici, J. Cardiovasc. Surg., 14, 589 (1973).

SCOPE FOR SOME ENZYMIC TESTS AND FOR RADIONUCLIDE HEPATOGRAPHY IN THE DIAGNOSIS OF EARLY CHANGES IN LIVER FUNCTION

N. Vlakhov, P. Georgiev, G. Angelov, and T. Zhelyazkova

UDC 616.36-008.1-036.18-073.961 +616.36--008.1-036.18-07:616.36-008.931-074

KEY WORDS: Serum enzymes; liver; tetrachloromethane; hepatogram

The writers showed previously [3] that liver scanning after tetrachloromethane poisoning in rabbits is not the most suitable method of detecting mild and early parenchymatous changes.

TABLE 1. Activity of Serum Enzymes (in I.U./ml) ChE, AlP, Ald, and LAP in Rabbits before and after Tetrachloromethane Poisoning (M ± m)

Exptl. conditions	CLE	AIP	Ald	LAP
Control (normal rabbits)	1342±269	33,0±3	142±22	16,5±1,6
48 h after CCl_4 poisoning	418±43	44,0±7,3	248±42,7	15,0±2,2
15 days after CCl_4 poisoning	287±32	41,0±4,2	42±7,7	13,0±2,1

Radionuclide Center, Regional Cancer Dispensary, and Department of Pathophysiology, Postgraduate Medical Faculty, Stara Zagora, Bulgaria.

(Presented by Academician of the Academy of Medical Sciences of the USSR A. D. Ado.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 99, No. 1, pp. 25-26, January, 1985. Original article submitted February 26, 1984.

0007-4888/85/9901-0029$09.50