PEROXIDATION OF LIPIDS AND INJURY TO MIXED FUNCTION OXYGENASES IN RAT LIVER ENDOPLASMIC RETICULUM MEMBRANES DURING ISCHEMIA

D. M. Velikhanova, M. V. Bilenko, and V. E. Kagan

UDC 616.36-005.4-092.9-09:[616.36-008.939.15+616.36-008.931

KEY WORDS: ischemia of the liver; cytochrome P-450; peroxidation of lipids.

Two physiologically important mechanisms of modification of biological membranes, produced by changes in their lipid components, are now known: peroxidation of lipids (POL) [7] and hydrolysis of phospholipids by phospholipases. Both these processes are sharply activated during the development of ischemia or anoxia in the tissues [1-4].

The system of mixed function oxygenases, responsible for metabolic activation of hydrophobic xenobiotics (drugs, carcinogens, poisons, etc.) and of many endogenous substrates (steroids, for example), is highly sensitive to the accumulation of POL products in the membranes. Experiments *in vivo* and *in vitro* have shown that the activity of the mixed function oxygenase systems may be regulated through a change in the content of endogenous lipid peroxides in membranes of the endoplasmic reticulum [8].

Injury to the system of mixed function oxygenases in the course of ischemia of the liver was demonstrated previously [9, 11]. Accordingly, the investigation described below was undertaken to study the connection between activity of this system and the content of endogenous POL products in membranes of the microsomal fraction of rat liver in order to estimate the contribution of the POL process to the mechanism of injury to this enzyme system and to determine whether antioxidants can be used to protect the system.

EXPERIMENTAL METHOD

Male Wistar rats weighing 200-250 g were used. During the 12 h before the experiments the animals were kept on a starvation diet. The animals were anesthetized by intraperitoneal injection of a 2% solution of hexobarbital in a dose of 70-80 mg/kg body weight. Ischemia was produced by applying a clip to the vascular pedicle of the left lateral lobe of the liver. The lobe was excised 15, 30, 60, and 120 min later. In the control series a mock operation was performed on the animals. Microsomes were isolated by differential centrifugation in 1.15% KCl solution. The supernatant obtained after centrifugation of the liver homogenate at 10,000g (30 min) was resedimented twice at 105,000g (60 min). Protein was determined by the biuret method. Lipids, isolated by Folch's method, were dissolved in chloroform to measure the concentration of Schiff bases, and in methanol–benzene containing 0.25 M LiCl, to determine the content of lipid hydroperoxides; the concentration of Schiff bases was measured on a fluorometer (Aminco-Bowman, USA) at 365 nm. Before each measurement the instrument was calibrated against quinine sulfate solution (1 mg/ml in 0.1 M H_2SO_4 solution) [12]. The content of lipid hydroperoxides was measured on the LP-7/E polarograph (Czechoslovakia) [5]. The velocity of NADPH-dependent POL was determined by the reaction with 2-thiobarbituric acid (TBA) [13]. The content of cytochrome P-450 was determined by the method in [14], and activity of NADPH-cytochrome c reductase by the method in [15], using an MP-7 spectrophotometer (Shimadzu, Japan); extinction coefficients were taken to be as follows $\varepsilon_{450} = 9.1 \cdot 10^4$ $cm^{-1} \cdot M^{-1}$ and $\varepsilon_{548} = 2.9 \cdot 10^4$ $cm^{-1} \cdot M^{-1}$.

EXPERIMENTAL RESULTS

Table 1 gives the content of endogenous primary (lipid hydroperoxides) and secondary (Schiff bases) POL products in the course of development of ischemia of the liver. These results show that ischemia leads to a significant increase in the content of POL products

Research Institute for Biological Testing of Chemical Compounds, Moscow. Laboratory of Physical Chemistry of Biomembranes, M. V. Lomonosov Moscow University. (Presented by Academician of the Academy of Medical Sciences of the USSR V. V. Kovanov.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 92, No. 7, pp. 50-52, July, 1981. Original article submitted January 27, 1981.

0007-4888/81/9207-0913$07.50

TABLE 1. Effect of Ischemia of the Liver on POL Activity and System of Mixed Function Oxidase in Microsomal Fraction (M ± m)

Experimental conditions	Schiff bases, relative units (n = 5)	Hydroperoxides, nmoles/mg lipid (n = 4)	NADPH-dependent POL, nmoles TBA-active products/min/mg protien (n = 5)	Cytochrome P-450, nmoles/mg protein (n = 5)	NADPH-cytochrome c reductase, nmoles/min/mg protein (n = 4)
Normal	18,6±0,6 (100%)	3,72±0,72 (100%)	1,1±0,17 (100%)	0,706±0,070 (100%)	12,3±2,46 (100%)
Control	17,1±0,8 (91%)	3,32±0,72 (89%)	1,4±0,26 (127%)	0,670±0,034 (96%)	12,0±1,41 (104%)
Ischemia:					
15 min	40,3±2,8† (216%)	6,24±1,08* (167%)	1,45±0,10 (131%)	0,502±0,024* (67%)	7,8±1,50 (64%)
30 min	32,1±1,6† (173%)	7,20±1,32 (192%)	1,42±0,21 (129%)	0,479±0,066* (60%)	8,7±1,74 (70%)
60 min	38,7±2,8† (208%)	8,12±1,68* (217%)	1,33±0,17 (120%)	0,506±0,063* (68%)	7,8±0,51 (64%)
120 min	50,9±5,4† (273%)	25,44±9,20† (682%)	1,38±0,34 (125%)	0,278±0,022† (39%)	7,5±1,23 (61%)

Legend. *P < 0.05, †P < 0.01, n) number of experiments.

after only 15 min, with a subsequent rise in their level during ischemia. After 2 h of ischemia the content of Schiff bases was 273%, and of lipid hydroperoxides 682% of the initial values.

Elevation of the level of POL products in subcellular fractions (mitochondria and microsomes) of the ischemized liver was observed by the writers previously [6]; it was accompanied by a decrease in antioxidant activity of the lipids and in the content of phospholipids with a high percentage of unsaturated fatty acids.

A study of the action of ischemia on the mixed function oxygenase system showed that the content of the terminal component of this polyenzymic complex, cytochrome P-450, was reduced after 15 min of ischemia, it remained at that level until after 1 h of ischemia, and fell sharply until after 2 h of ischemia (39% of its initial level). NADPH-cytochrome c reductase activity fell during the first 15 min and remained at the same level until after 2 h of ischemia, i.e., it was much more resistant to the action of ischemia. In the control series of experiments none of these indices showed any significant changes.

Comparison of the content of endogenous POL products with that of cytochrome P-450 in membranes of the liver microsomal fractions of animals exposed to ischemia for different times showed negative correlation between them: The coefficient of correlation for lipid hydroperoxides and cytochrome P-450 was −0.89 ($P < 0.01$), and that for Schiff bases and cytochrome P-450 was −0.94 ($P < 0.01$). This suggests that POL may be a mechanism for cytochrome P-450 disassembly in the ischemized liver. At the same time, these results also indicate that cytochrome P-450 could hardly be involved in reactions leading to accumulation of POL products in ischemia, unless the initial quantity of cytochrome P-450 is considered to be excessive for POL reactions. Evidence in support of this view is given by the fact that activity of the enzymic NADPH-dependent POL system remained virtually unchanged in the course of ischemia (Table 1), although the content of cytochrome P-450 in the membranes fell steadily. The sharp fall in the cytochrome P-450 content in the period from 1 to 2 h of ischemia took place in the absence of any significant changes in NADPH-cytochrome c reductase activity during that period, evidence of the higher sensitivity of cytochrome P-450 to the inactivating action of POL products. Higher sensitivity of cytochrome P-450 than activity of NADPH-cytochrome c reductase has similarly been observed on many occasions previously in vitro [8].

The experimental data at present available are insufficient to localize the enzymic NADPH-dependent POL process on any one or two components of the electron transfer chain in the microsomes — NADPH-dependent flavoprotein or cytochrome P-450.

The results of the present investigation indicate that a high proportion of the microsomal cytochrome P-450 (61%, or 0.423 nmole/mg protein) does not participate in the induction of POL whether in vivo or in vitro, for the fall in cytochrome P-450 concentration to 0.278 nmole/mg protein did not change the rate of NADPH-dependent POL *in vitro* at a time when endogenous lipid peroxides were accumulating in the membrane (Table 1).

The development of ischemia in the liver was thus accompanied by accumulation of endogenous POL products and by a simultaneous fall in the content of cytochrome P-450, which catalyses the reactions of oxidative metabolism of hydrophobic substrates in membranes of the liver endoplasmic reticulum.

The changes in the two systems are interdependent in time. They appear after 15 min of ischemia of the liver, which coincides with the initial changes in structure and function of the organ described in the literature [10]. In the period from 15 min to 1 h of ischemia both the POL system and the system of mixed function oxygenases undergo little change and are relatively stable. After 2 h of ischemia they change abruptly, to coincide with the appearance of irreversible injuries in the organ [10]. Because of the negative correlation between POL and the system of mixed function oxygenases and also dependence of their state on the duration of ischemia, the study of the trend of changes in these systems can be recommended for use as a criterion of the severity of injury to the ischemized liver and they provide a basis for trials of antioxidants as hepatoprotectors.

LITERATURE CITED

1. A. V. Alesenko, M. V. Bilenko, E. V. Burlakova, et al., Vestn. Akad. Med. Nauk SSSR, No. 8, 61 (1976).
2. M. V. Bilenko, in: Acute Ischemia of Organs and Early Postischemic Disorders [in Russian], Moscow (1978), pp. 51-52.
3. M. V. Bilenko, A. V. Alesenko, E. V. Burlakova, et al., in: Structure, Biosynthesis, and Conversion of Lipids in Animals and Man [in Russian], Moscow (1975), pp. 29-30.
4. Yu. A. Vladimirov, L. G. Korkina, and A. S. Dergunov, in: Acute Ischemia of Organs and Early Postischemic Disorders [in Russian], Moscow (1978), p. 73.
5. V. E. Danilov, M. V. Sitkovskii, V. E. Kagan, et al., Izv. Akad. Nauk SSSR, Ser. Biol., No. 4, 445 (1972).
6. L. B. Ludnik, M. V. Bilenko, A. V. Alesenko, et al., Byull. Éksp. Biol. Med., No. 5, 556 (1980).
7. V. E. Kagan, S. V. Kotelevtsev, and Yu. P. Kozlov, Dokl. Akad. Nauk SSSR, 217, No. 1, 216 (1974).
8. V. E. Kagan, S. V. Kotelevtsev, Yu. P. Kozlov, et al., Stud. Biophys., 47, 133 (1974).
9. T. A. Knyazeva, É. F. Malyugin, S. A. Zarinskaya, et al., Vestn. Akad. Med. Nauk SSSR, No. 12, 3 (1974).
10. Yu. M. Lopukhin and É. M. Kogan, Criteria of Viability of Organs and Tissues before Transplantation [in Russian], Moscow (1975).
11. M. E. Ferrero, R. Orsi, et al., Exp. Mol. Pathol., 28, 256 (1978).
12. J. J. Kohn and M. Liversedge, J. Pharmacol. Exp. Ther., 82, 292 (1944).
13. K. S. Chio and A. L. Tappel, Biochemistry (Washington), 8, 2827 (1969).
14. T. Omura and R. Sato, J. Biol. Chem., 239, 2376 (1964).
15. C. Richter, A. Azzi, V. Wesser, et al., J. Biol. Chem., 252, 5061 (1977).