SUPEROXIDE RADICAL—SUPEROXIDE DISMUTASE SYSTEM DYSFUNCTION IN THE RAT LIVER DURING ISCHEMIA

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KEY WORDS: ischemia of the liver; superoxide radical; superoxide dismutase; xanthine oxidase; reoxygenation.

It was shown previously that during ischemia of various organs lipid peroxidation (LPO) is intensified [2, 4]. Simultaneously with this, a significant decrease is observed in the antioxidative activity of lipids and activity of protective enzymes in the cytosol: glutathione peroxidase and superoxide dismutase (Cu,Zn-SOD). It was concluded from these data that in ischemia the systems which normally maintain a low steady-state concentration of LPO products are thrown out of balance [6]. We know that disturbance of balance in the $O_2^{\cdot-}$—SOD system is not specific for any particular pathology, as also in intensification of LPO. Maintenance of the balance is an essential condition for normal functioning of the organism.

A sharp drop in (Cu,Zn-SOD activity during ischemia by itself does not imply any obligatory increase in the steady-state concentration of $O_2^{\cdot-}$ radicals. The absence of any data on the functioning, during ischemia, of systems generating superoxide radicals in different intercellular organelles, and also on activity of mitochondrial Mn-containing SOD (Mn-SOD) does not enable the state of the $O_2^{\cdot-}$—SOD system to be judged either during ischemia or during subsequent reoxygenation.

EXPERIMENTAL METHOD

Experiments were carried out on Wistar rats. Ischemia was produced by application of microforceps to the vascular pedicle of the central and left lateral lobes of the liver for 30 min and 2 h. In the reperfusion experiments (for 2, 4, and 24 h, after 2 h of ischemia), the nonischemic lobes (about 30%) were resected. The subcellular fractions were isolated by differential centrifugation [1]. The subcellular fractions were isolated by differential centrifugation [1]. SOD activity in the cytosol was determined by the method in [9] in the modification of [4]; SOD activity in the mitochondria was determined in a riboflavin—methionine system [9]. Total xanthine dehydrogenase + xanthine oxidase (XDA + XOA) activity was determined by the method in [10]. Samples of cytosol were passed first through a column filled with Sephadex G-25 gel. The rate of $O_2^{\cdot-}$ generation by the microsomal NADPH-dependent electron transfer chain was determined by oxidation of 2,2,6,6-tetramethylpiperidine to the corresponding stable nitroxyl radical [8]. LPO products reacting with 2-thiobarbituric acid (TBA) were determined by the method in [7].

EXPERIMENTAL RESULTS

On the basis of data on survival of animals after ischemia of the liver, two periods of ischemia were chosen: 30 min, when disturbances of liver function were reversible, and 2 h, the critical period of ischemia.

During ischemia of the liver for 30 min or 2 h a sharp decrease in Cu,Zn-SOD activity was observed in the cytosol (Fig. 1), in agreement with results obtained previously [6]. During reperfusion after ischemia for 2 h, some increase in SOD activity took place, although not up to the normal level (Fig. 1). Cu, Zn-SOD activity for intact animals was 145 ± 15 conventional units/min/mg protein. As will be clear from Fig. 2, the rate of regeneration of superoxide radicals ($V_{O_2^{\cdot-}}$) also fell during ischemia, and in the next 24 h of

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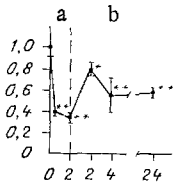

Fig. 1

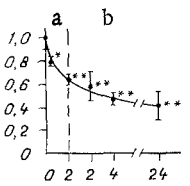

Fig. 2

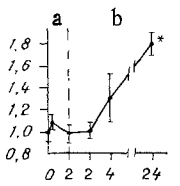

Fig. 3

Fig. 1. Changes in cytosol Cu,Zn-SOD activity during ischemia (a) and reperfusion (b) of rat liver. Abscissa, time (in h); ordinate, Cu,Zn-SOD activity (in relative units compared with control, taken as 1). Here and in Fig. 2: *P < 0.05, **P < 0.01 compared with control.

Fig. 2. Changes in rate of $O_2^{\cdot}$ generation in microsomes during ischemia (a) and reperfusion (b) of rat liver. Abscissa, time (in h); ordinate, $V_{O_2^{\cdot}}$ (in relative units compared with control, taken as 1).

Fig. 3. Changes in Mn-SOD activity during ischemia (a) and reperfusion (b) of rat liver (M ± m). Abscissa, time (in h); ordinate, Mn-SOD activity (in relative units compared with control, taken as 1). *P < 0.01 compared with control.

reoxygenation $V_{O_2^{\cdot}}$ for intact animals was 1.98 ± 0.15 nmole $O_2^{\cdot}$ /min/mg protein. Judging by this parameter, the microsomal electron transfer chain was not restored throughout the early reperfusion period and it continued to function at a lowered level. The authors of [3], who observed a fall in the cytochrome P-450 concentration during ischemia and in the early period of reperfusion, reached the same conclusion. Thus during ischemia $V_{O_2^{\cdot}}$ in the microsomes and SOD activity in the cytosol both fall. However, comparison of $V_{O_2^{\cdot}}$ of the microsomes with SOD activity in the cytosol shows (Table 1) that during ischemia the ratio of $V_{O_2^{\cdot}}$ to SOD activity increases to twice its normal value. This may lead to an increase in the steady-state concentration of superoxide radicals in the cytosol during ischemia. During reoxygenation the ratio of $V_{O_2^{\cdot}}$ in the microsomal membranes to SOD activity in the cytosol becomes close to normal. It will also be clear from Table 1 that in the total homogenate and microsomal fraction of the liver there was a rapid rise in the level of TBA-active products during ischemia and a fall virtually to normal afer 24 h of reperfusion. This may be evidence of the important role of the level of the steady-state concentration of $O_2^{\cdot}$ in the hepatocyte cytosol in the mechanism of LPO processes during ischemia and reperfusion of the liver, both in the hepatocyte as a whole and in membranes of the endoplasmic reticulum.

Among the potential sources of $O_2^{\cdot}$ radicals in the cytosol known at the present time, an important role is played by the xanthine oxidase system. In the normal rat liver this enzyme functions as a dehydrogenase, using NAD^+ as the acceptor. It has been shown [11] that XDA *in vitro* may undergo partial conversion into the oxidase, capable of single-electron reduction of oxygen, with the formation of O_2^- radicals. In ischemia the transition from XDA to XOA is found [11]. However, there are no data on changes in total xanthine-oxidizing enzyme activity. The present experiments showed that XDA + XOA after 2 h of ischemia was 0.65 ± 0.10 (P < 0.01), but after 24 h of reperfusion it was 0.93 ± 0.05 of the corresponding normal values, taken as 1. XDA + XOA for intact animals was 0.41 ± 0.03 mmole uric acid/min/mg protein. It is clear that ischemia causes a decrease in total activity of the enzyme, but this returns to normal during reperfusion. Since after only 30 min of ischemia of the liver about 50% of XDA is converted into XOA [11], it can be concluded that during ischemia a powerful potential source of $O_2^{\cdot}$ appears in the cytosol, and this may become a real source during postischemic reoxygenation.

By contrast with Cu,Zn-SOD activity, Mn-SOD, located in the mitochondrial matrix, did not significantly change its activity during ischemia (Fig. 3). During postischemic reoxygenation, Mn-SOD activity increased, and after 24 h exceeded its normal value by 1.8 times (Fig. 3). Mn-SOD activity in the intact animals was 33 ± 3 conventional units/min/mg protein. It can be tentatively suggested that activation of Mn-SOD is linked with oxygen-induced synthesis of the enzyme *de novo*. Since no adequate methods of determination of $V_{O_2^{\cdot}}$ in submitochondrial particles of the liver cells exist, it is not yet possible to compare $V_{O_2^{\cdot}}$ and Mn-

TABLE 1. Changes in Ratio of $V_{O_2^{\cdot}}$ to Cu, Zn-SOD Activity (in relative unit compared with control, taken as 1) and Concentration of TBA-Active Products during Ischemia and Reperfusion of Rat Liver (M ± m)

Experimental conditions	$V_{O_2^{\cdot}}$/Cu, Zn-SOD	TBA-active products	
		microsomes, nmoles/mg protein	homogenate, nmoles/g tissue
(Control (intact rats)	1,0	0,97±0,28	18,4±1,9
30 min	2,0	—	—
2 h of ischemia	1,97	10,71±1,25**	31,1±2,2*
2 h of ischemia + 2 h of reperfusion	0,80	—	—
2 h of ischemia + 4 h of reperfusion	0,90	—	—
2 h of ischemia + 24 h of reperfusion	0,74	2,36±0,32	20,4±1,5

Note. *P < 0.05; **P < 0.01 compared with control.

SOD activity in this object. The observed increase in SOD activity is evidently insufficient, for the intensity of LPO processes in the mitochondrial membranes during postischemic reoxygenation was higher than in other cell organelles [5].

The results of the present investigation, together with data in the literature, show on the whole that during ischemia substantial disturbances in the regulation of the $O_2^{\cdot}$—SOD system may take place on account of the relatively high rate of generation of $O_2^{\cdot}$ radicals in the microsomal membranes, compared with Zu,Zn-SOD activity in the cytosol. During postischemic reoxygenation the main contribution toward disturbance of regulation may be made by the xanthine oxidase system of $O_2^{\cdot}$ generation in the cytosol, against the background of depressed Cu, Zn-SOD activity.

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*[9] missing in Russian original — Publisher.