RELATIONSHIP BETWEEN OXYGEN ANION-RADICAL GENERATION AND LIPID PEROXIDATION IN LIVER MICROSOMES

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Determination of the O_2 consumption and accumulation of malondialdehyde, induced by phenobarbital and 3-methylcholanthrene in rat or rabbit liver microsomes revealed inhibition of lipid peroxidation but a relatively high level of O_2^--radical generation. It is postulated that the absence of direct correlation between lipid peroxidation activity and O_2^- generation in microsomes depends on the antioxidant level in the microsomal membrane.

KEY WORDS: O_2^--radical generation; induction; liver microsomes; peroxidation of lipids.

It has recently been shown that oxygen anion radicals (O_2^-) formed on the flavoprotein region of the microsomal electron-transport chain [1] can inhibit the reaction of lipid peroxidation (LP) in artificial systems [4, 8]. It is suggested that initiation of enzymic LP in whole microsomes is linked with the function of an NADP·H_2-specific flavoprotein supplying the O_2^- [1].

The object of this investigation was to examine correlation between LP and O_2^- generation in the liver microsomes. The model used for this purpose was induction of a microsomal monooxygenase system by xenobiotics, namely phenobarbital (PB) and 3-methylcholanthrene (3-MC), which induce changes in activity of microsomal enzymes [3, 10]; induction by PB, moreover, is accompanied by increased O_2^- generation by microsomes [1].

EXPERIMENTAL METHOD

Microsomes were isolated from the liver of intact rats induced by PB (80 mg/kg body weight, 4 days) and 3-MC (50 mg/kg body weight, 4 days) and from the liver of intact rabbits by differential centrifugation [7]. LP was determined in the microsomes from the accumulation of malondialdehyde (MDA) and the O_2^- assimilation [12]. Oxidation of adrenalin into adrenochrome was determined by the method of Aust et al. [1]. NADP · H_2—cytochrome c reductase was determined by the reduction of cytochrome c, as described earlier [9].

Nitro-BT was reduced in 0.15 M phosphate buffer, pH 8.0, in the presence of 10^{-4} M EDTA, microsomal protein concentration 0.5 mg, nitro-BT 50 μM, NADP · H_2 10 μM, volume of cell 3 ml, wavelength 550 nm, molecular extinction 28,600 M · cm^{-1} [11]. Protein was determined by the biuret method [6].

EXPERIMENTAL RESULTS AND DISCUSSION

Induction by PB and 3-MC was found to be accompanied by definite inhibition of LP of both enzymic (NADP ·H_2-dependent) and nonenzymic (activated by ascorbate) nature (Table 1). The decrease in LP was recorded by two independent methods: measurement of the O_2^- assimi-

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TABLE 1. LP Level in Liver Microsomes of Control Rats Induced by PB and 3-MC

Group of animals	LP measured as O_2 in natoms /min/ mg protein		LP measured as MDA in	
	NADP · H_2	ascorbate	NADP · H_2	ascorbate
Control	194	145	42	37
Induced by PB	69	39	12	9
Induced by 3-MC	120	53	28	9

TABLE 2. NADP · H_2-Ferricytochrome Oxidoreductase Activity in Preparations of Liver Microsomes

Group of animals	Cytochrome reductase*	Adrenalin oxidase†	Nitro-BT reductase‡		
			total	O_2 dependent	%
Control	76	12,4	37,0	7,4	20
Induced by PB	175	21,0	58,3	9,3	16
Induced by 3-MC	62	10,3	34,7	4,5	13

*In nmoles reduced cytochrome/min/mg protein.
†In nmoles adrenochrome formed/min/mg protein.
‡In nmoles reduced nitro-BT/min/mg protein.

lation and measurement of accumulation of MDA, a product of peroxide breakdown of phospholipids.

No inhibitory effect on LP was found by the action of 3-MC *in vitro* in concentrations of up to 2 μmole/mg microsomal protein which, according to the data of Fujita et al. [5], can be found in liver microsomes if it is administered *in vivo* by the same scheme. Wills [13] has also shown that PB does not inhibit LP reactions of microsomes *in vitro*, so that the decrease in LP cannot be explained by the direct action of the xenobiotics.

The results in Table 2 show that induction of the liver microsomal system by xenobiotics is accompanied either by increased NADP · H_2-ferricytochrome oxidoreductase activity in experiments with PB or by preservation of the activity of this enzyme at the control level in the experiments with 3-MC. The rate of adrenalin conversion into adrenochrome, reflecting O_2^- generation increases significantly during induction of microsomal enzymes of the rat liver by PB and is comparable in preparations of liver microsomes from intact rats and rats induced with 3-MC. Nitro-BT is known to be reducible with the aid of O_2^- [2] and also to receive electrons directly from other donors [13].

It will be clear from Table 2 that reduction of nitro-BT by intact liver microsomes takes place in both ways; the O_2^--dependent pathway accounts for about 20% (the degree of inhibition by an excess of O_2^- dismutase). The ratio between the O_2^--dependent and O_2^--independent pathways of nitro-BT reduction is reduced during induction by PB, but the absolute rate of O_2^--dependent nitro-BT reduction increases.

It will be clear from these results that during induction by PB and 3-MC there is no direct correlation between the enzymic LP activity and O_2^- generation effected by NADP · H_2-dependent flavoprotein.

Rabbit liver microsomes, in which LP is substantially lower than in liver microsomes of intact rats, also were investigated (Table 3).

However, O_2^- generation by rabbit liver microsomes was at the same level as that obtained for liver microsomes of intact rats (Table 2). In this case also, no correlation was thus found between enzymic LP and O_2^- production by microsomes.

Meanwhile, the decrease in both enzymic and nonenzymic LP activity in the rat liver microsomes after induction by xenobiotics and the low LP level in the liver microsomes of intact rabbits are evidence that the inhibition of these reactions is not entirely attributable to changes in the activity of the microsomal enzymes. Inhibition of enzymic and nonenzymic LP in the situations studied may be connected with an increase in the antioxidative capacity of the microsomal lipids.

TABLE 3. LP and NADP · H_2-Ferricytochrome Oxidoreductase Activity in Rabbit Liver Microsomes

LP activity				Activity of NADP · H_2-ferricytochrome reductase				
NADP · H_2	ascorbate	· NADP H_2	ascorbate	cytochrome reductase*	adrenalin oxidase†	nitro-BT reductase‡		
						total	O_2^--dependent	%
17	60	1,3	6,0	71	13,2	38,6	7,3	19

*In nmoles reduced cytochrome c/min/mg protein.
†In nmoles adrenochrome formed/min/mg protein.
‡In nmoles reduced nitro-BT/min/mg protein.

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