ANTIOXIDANTS AS CYTOCHROME P-450 STABILIZERS IN HEPATOCYTES

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The technique of hemoperfusion has recently begun to be introduced into clinical practice, and for detoxication it is customary to use hepatocytes, which contain a highly effective system of mixed-function mono-oxygenases, whose catalytically active component is cytochrome P-450 (terminal oxygenase) [1, 2, 7]. This raises the urgent task of obtaining native hepatocytes, developing methods of their long-term culture and maintenance of high mixed-function oxygenase activity (in other words, a high level of cytochrome P-450).

Lipid peroxidation (LPO) is known to play an essential role in the mechanism of injury to cytochrome P-450 [4, 5]. This suggests that antioxidants, inhibitors of free-radical oxidation, may behave as stabilizers of cytochrome P-450 in hepatocyte cultures.

This paper describes the study of the action of two antioxidants — water-soluble 2-ethyl-6-methyl-3-hydroxypyridine (OP-6) and fat-soluble 4-methyl-2,6-di-tert-butylphenol (ionol) — on cytochrome P-450 in rat hepatocytes.

EXPERIMENTAL METHOD

Intact noninbred albino rats weighing 150-180 g were used. Hepatocytes were isolated by the method in [10] with some modifications. To isolate the cells Tyrode's solution of the following composition was used: 125 mM NaCl, 3 mM KCl, 1 mM $MgCl_2$, 1.2 mM NaH_2PO_4, 2 mM $CaCl_2$, 40 mM Tris, pH 7.4 (37°C). After isolation the liver was perfused with this solution but without Ca^{++} and the perfusate was aerated with carbogen (95% O_2 + 5% CO_2). For recirculating perfusion a solution containing Ca^{++} and 0.3% collagenase (from Sigma, USA) was used. After dispersion of the liver tissue a suspension of hepatocytes was obtained by triple centrifugation (100g, 1-2 min) in Tyrode's solution containing 1.5% purified albumin. The hepatocyte suspension was incubated in the same solution at 37°C, with shaking once per second. The number of cells was counted in a Goryaev's chamber with trypan blue to estimate the number of viable hepatocytes. The cytochrome P-450 concentration was determined by the method in [9]. LPO was induced in the hepatocyte suspension by a system of Fe^{++}-ADP (5×10^{-5} M) + NADPH (10^{-4} M). Accumulation of malonic dialdehyde (MDA), a secondary product of LPO, was determined by the reaction with 2-thiobarbituric acid (TBA) [6]. Ionol was added to the hepatocyte suspension in ethyl alcohol so that the concentration of alcohol did not exceed 1%, and OP-6 was dissolved in Tyrode's medium. The final concentration of the antioxidants was 10^{-4} M. The protein concentration was determined by the biuret reaction, using bovine serum albumin as the standard. The results were subjected to statistical analysis by Student's t test and by nonparametric methods.

EXPERIMENTAL RESULTS

The hepatocyte suspension contained 1×10^6-2×10^6 cells/ml (10-20 mg protein/ml), of which 60-90% were viable. The cytochrome P-450 concentration in the native hepatocytes was 0.053 ± 0.006 nmole/mg protein, in agreement with data in the literature [8]. During incubation of the hepatocyte suspension spontaneous destruction of cytochrome P-450 took place in the cells (Fig. 1a); the concentration fell by 50% during 3 h of incubation. Destruction of

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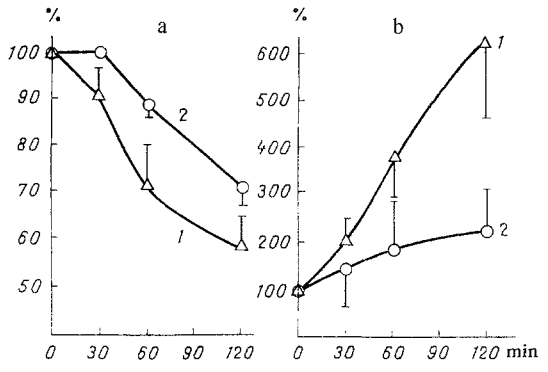

Fig. 1

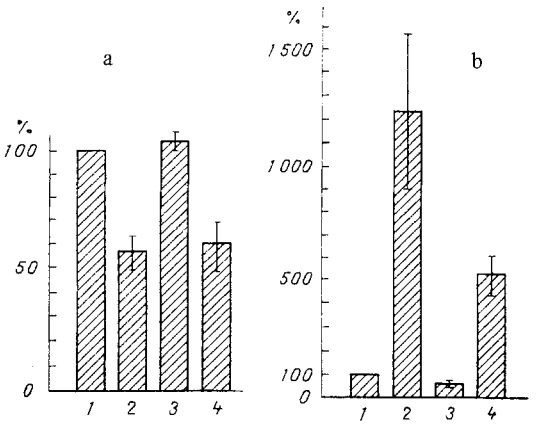

Fig. 2

Fig. 1. Effect of OP-6 on spontaneous degradation of cytochrome P-450 (a) and MDA accumulation (b) in isolated hepatocytes. 1) Control; 2) OP-6 (10^{-4} M). Abscissa, incubation time; ordinate, concentrations of: a) cytochrome P-450, b) MDA.

Fig. 2. Effect of antioxidants on cytochrome P-450 degradation (a) and MDA concentration (b) in isolated hepatocytes during activation of enzymic LPO in them. 1) Control; 2) hepatocytes + Fe^{++}-ADP + NADPH; 3) the same + ionol (10^{-4} M); 4) the same + OP-6 (10^{-4} M). Incubation time 1 h. Ordinate, concentration of: a) cytochrome P-450, b) MDA.

TABLE 1. Rate of Degradation of Cytochrome P-450 and of MDA Accumulation in Rat Hepatocytes (M ± m)

Experimental conditions	Rate of degradation of cytochrome P-450, nmole/mg protein/30 min	Rate of MDA accumulation, nmole/mg protein/30 min
Hepatocytes	0,007±0,001	0,070±0,026
Hepatocytes + Fe^{++} + ADP + NADPH	0,015±0,002	1,81±0,78

cytochrome was accompanied by accumulation of LPO products (MDA; Fig. 1b). The cytochrome P-450 and MDA concentration in the hepatocytes varied from one experiment to another, due to individual differences between animals (cells for each experiment were isolated from the liver of one rat), seasonal changes, and so on, and for that reason the dynamics of accumulation and destruction of these components is expressed in percentages.

Considering the well-known destructive action of LPO on cytochrome P-450 in the microsomal fraction of liver *in vitro* [3, 4], spontaneous degradation of P-450 may perhaps be due to accumulation of LPO products in the hepatocytes (coefficient of negative correlation r = −0.98). If this is true, LPO activation ought to lead to more rapid destruction of cytochrome P-450, whereas inhibition of LPO, on the other hand, should lead to stabilization of cytochrome P-450.

Incubation of hepatocytes in medium containing the LPO initiation system (Fe^{++}-ADP + NADPH) in fact led to more rapid destruction of cytochrome P-450: the concentration of cytochrome P-450 fell by 50% in this case after incubation for 1 h. In the presence of the Fe^{++}-ADP + NADPH system, accumulation of LPO products took place in 30 min, however, i.e., 30 times faster than in the absence of initiators (Table 1). This may mean that initiated degradation of cytochrome P-450 is associated mainly with LPO activation, in the same way as its spontaneous destruction.

Direct experimental proof of this fact was obtained by studying the action of inhibitors of free-radical lipid oxidation on destruction of cytochrome P-450 (Figs. 1 and 2). Addition

of the fat-soluble antioxidant ionol and the water-soluble OP-6 to the incubation medium caused inhibition of LPO, on the one hand, and stabilization of cytochrome P-450 on the other hand. It must be noted that ionol is essentially a more effective inhibitor of LPO in hepatocytes and a correspondingly more effective protector of cytochrome P-450 against degradation than the water-soluble OP-6.

It can thus be concluded from these results that antioxidants are highly effective stabilizers of cytochrome P-450 in hepatocytes.

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PHYLOGENETICALLY AND ONTOGENETICALLY PREDETERMINED MECHANISM OF DISTRIBUTION OF LYSOSOMAL ACID PHOSPHATASE ACTIVITY ALONG THE ALIMENTARY TRACT

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KEY WORDS: lysosome; acid phosphatase; alimentary tract; phylogeny; ontogeny.

Electron-microscopic [1, 11] and biochemical [2, 5] research has yielded evidence that the ultrastructure of cellular lysosomes of the intestinal mucosa and the activity of their enzymes undergo considerable changes depending on the animals' dietary conditions. However, this evidence has been obtained only in rats. In young rats during the period of milk feeding, for instance, lysosomes in the small intestine were found to be larger and more "active" than in mature rats. On the basis of facts such as these it has been postulated [3, 11] that lysosomes can participate in the digestion only of milk proteins in early postnatal development. Accordingly, the possible role of lysosomes (or of their enzymes) in systemic digestion must be regarded as a temporary, adaptive phenomenon, taking place only at an early age, and confined to mammals.

This paper gives the results of a study of the functional organization of lysosomes of the alimentary tract.

EXPERIMENTAL METHOD

Experiments were carried out on cartilaginous and bony fishes (beluga sturgeon, crucian carp), frogs, water snakes, pigeons, rats, newborn puppies, 20- and 28-day-old rabbit fetuses, and 40-day-old cat fetuses. The animals were decapitated (the fetuses were decapitated after

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