Role of Lipoproteins Transporting Lipophilic Xenobiotics in the Induction of Microsomal Monooxygenases

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Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 133, No. 3, pp. 289-291, March, 2002
Original article submitted November 19, 2001

We studied the effects of lipopolysaccharide on activity of liver microsomal enzymes against the background of xenobiotics treatment. Against the background of lipopolysaccharide stimulation of macrophages we observed *in vivo* activation of cytochromes P-450 1A subfamily in liver microsomes with Arochlor 1254, but not induction of cytochrome P-450 2B subfamily with phenobarbital.

Key Words: *lipoproteins; phenobarbital; Arochlor 1254; cytochrome P-450; lipopolysaccharide*

Some aspects of xenobiotic metabolism, in particular, their transfer into the liver are poorly understood. Previous studies showed that various plasma lipoproteins (LP) play an important role in binding and transport of lipophilic compounds, including fat-soluble vitamins, steroid hormones (hydrocortisone, corticosterone, pregnenolone, and deoxycorticosterone) [4], xenobiotics (benzoapyrene, benzanthracene, benzylpenicillin, and cytochalasin) [7]. Resident macrophages, in particular Kupffer cells in the liver play a key role in LP metabolism. Stimulation of these cells promotes binding, internalization, and metabolism of LP [5].

Here we studied the role of LP as the xenobiotic transporting system in induction of enzymes metabolizing these compounds.

MATERIALS AND METHODS

Experiments were performed on male Wistar rats weighing 180-220 g. *Serratia marcencens* lipopolysaccharide (LPS, Sigma) was injected intraperitoneally in a dose of 0.25 mg/kg. Microsomal monooxygenases were induced by intraperitoneal injection of Arochlor 1254 (Sigma) and phenobarbital in single doses of 300 and 80 mg/kg, respectively. Microsomal fractions of the liver were isolated by differential centrifugation 24 and 48 h after induction. Protein content was measured by the method of Lowry. Cytochrome P-450 concentration in liver microsomes was estimated as described elsewhere [13]. Activity of xenobiotic-metabolizing enzymes was determined by the rate of 7-alkoxyresorufin O-dealkylation [10]. The reaction of 7-alkoxyresorufin O-dealkylation is specifically catalyzed by various forms of cytochrome P-450: CYP1A1 for 7-ethoxyresorufin, CYP1A2 for 7-methoxyresorufin, CYP2C6 for 7-benzoxyresorufin, and CYP2B1 for 7-pentoxyresorufin (PROD activity).

The results were analyzed by the nonparametric Mann—Whitney test using Statistica 4.5 software.

RESULTS

Lipophilic xenobiotic Arochlor 1254 acts as an inductor for cytochromes P-450 of both 1A and 2B subfamilies. Activity of liver microsomal enzymes markedly increased 24 h after intraperitoneal injection of this agent (Table 1). Stimulation of macrophages with LPS was accompanied by 2-fold activation of CYP1A1 and CYP1A2, slight activation of CYP2B1 and CYP2C6 24 h after treatment. However, LPS did not change the total content of cytochrome P-450 in the liver (Table 1). Forty-eight hours after combined treatment with Arochlor 1254 and LPS, only activity of CYP1A differed from that observed after induction with Arochlor

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0007-4888/02/1333-0246$27.00

1254 alone, but this difference was less pronounced, than 24 h after treatment.

Thus, inducibility of CYP1A increased more than 2-fold over the first day after stimulation of Kupffer cells.

It should be emphasized that *in vitro* experiments with cocultured macrophages and hepatocytes revealed no cytochrome P-450 activation under similar conditions [12], probably, because *in vitro* experiments exclude the LP-mediated transport of lipophilic compounds. This assumption is confirmed by published data that LP promote transfer of oligonucleotides and drugs into the nucleus [11].

Biotransformation of xenobiotics includes 2 stages: oxidation (stage I) and formation of water-soluble conjugates (stage II). Cytochromes P-450 occupy a special place among stage I enzymes. These enzymes catalyze oxidation of various xenobiotics, steroid hormones, vitamins, and bile acids. The content of cytochrome P-450-containing monooxygenases is maximum in the liver, where detoxification of xenobiotics primarily proceeds. Published data suggest that cytochrome P-450 activity is regulated at different levels (from gene transcription and mRNA translation to posttranslational regulation of enzyme activities) [1,8,9].

Previous studies showed that LP are involved in the transport of xenobiotics. Activation of resident macrophages with LPS is accompanied by intensive binding and internalization of plasma LP [5]. There are functional relationships between Kupffer cells and hepatocytes metabolizing steroid hormones [3]. Kupffer cells probably bind LP and regulate metabolism of xenobiotics in hepatocytes.

Stimulation of resident macrophages with LPS did not modulate CYP2B and CYP2C activities and the total cytochrome P-450 content 24 and 48 h after phenobarbital administration (Table 1). Probably, plasma LP do not play a role in phenobarbital transport. Intensive binding of LP by macrophages does not change the rate of xenobiotic oxidation by subfamily 2B cytochromes P-450.

The mechanism of the stimulatory effect of macrophages on xenobiotic metabolism in the liver remains unclear. Previous studies showed that LP are involved in binding and transport of various lipophilic compounds. Probably, the effects of Kupffer cells on xenobiotic metabolism in hepatocytes are realized via binding of LP and lipophilic substances from the plasma. This implies not only intensive binding of LP by macrophages, but also their transformation and generation of active forms. It was shown that macrophages recognize galactose regions in transferrin and ferritin molecules, which promotes their binding by hepatocytes [14].

Our results show that treatment with LPS is accompanied by functional changes in hepatocytes. Stimulation of macrophages with LPS promotes activation of cytochrome P-450 1A subfamily enzymes in the liver lipophilic xenobiotic Arochlor 1254, but has

TABLE 1. *In Vivo* Effects of LPS on O-Dealkylation of Alkoxyresorufins (pmol/mg Protein) and Cytochrome P-450 Concentration (nmol/mg Protein) after Treatment with Arochlor 1254 and Phenobarbital (*M*±*m*)

Parameter	Control	LPS	Arochlor	Arochlor+LPS	Phenobarbital	Phenobarbital+ LPS
CYP1A1	118±42					
24 h		58±39	1682±396*	3639±558+	35±18	30±15
48 h		76±46	3490±1795*	4464±998*	37±24	36±18
CYP1A2	81±24					
24 h		36±22	399±103*	1804±605+	38±19	42±28
48 h		63±43	1389±730*	1701±603*	25±10	48±39
CYP2C6	57±16					
24 h		17±6	289±86*	562±281*	526±140*	600±139*
48 h		47±29	610±379*	515±333*	760±116*	827±130*
CYP2B1	46±10					
24 h		5±3	196±44*	377±99*	603±194*	717±200*
48 h		32±28	358±41*	389±235*	750±71*	810±109*
P-450	0.7±0.1					
24 h		0.7±0.1	1.2±0.3*	1.2±0.2	1.00±0.28	0.96±0.20
48 h		1.1±0.2	1.9±0.6*	1.9±0.7*	1.98±0.10*	1.8±0.4*

Note. $p<0.05$: *compared to the control, +compared to Arochlor.

no effect on induction of cytochrome P-450 2B subfamily with hydrophilic xenobiotic phenobarbital.

We hypothesize that LP transporting lipophilic xenobiotics enhance inducibility of enzymes metabolizing these compounds in the liver.

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