Activity and Inducibility of Cytochrome P-450-1A in the Liver of Mice with Different Sensitivity to Hepatocarcinogenic Effect of o-Aminoazotoluene

L. F. Gulyaeva, L. Yu. Zakharova, V. V. Lyakhovich, T. S. Morozkova*, and V. I. Kaledin*

Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny,* Vol. 126, No. 8, pp. 201-203, August, 1998
Original article submitted December 1, 1997

Specific activity of cytochrome P-450-1a is detected in the liver of 7 inbred mouse strains sensitive (SWR, C3HA, DD, and CBA) and resistant (AKR, CC57BR, and C57BL) to o-aminoazotoluene-induced hepatocarcinogenesis: 7-ethoxyresorufin-O-diethylase and 7-methoxyresorufin-O-dimethylase, induced by benzo(a)pyrene and o-aminoazotoluene. Mice sensitive (CC57BR and C57BL) and resistant (AKR) to P-450-1a induction were resistant to induction of liver tumor and, similarly, mice both resistant (SWR and DD) and sensitive (C3HA and CBA) to P-450-1a induction were sensitive to hepatic tumor induction. Therefore, there was no correlation between inducibility of cytochrome P-450-1a and sensitivity to o-aminoazotoluene-induced hepatocarcinogenesis at the initial stages of chemical carcinogenesis.

Key Words: *inbred mice; o-aminoazotoluene; induction; cytochrome P-450-1a*

Azobenzenes widely used as dyes are carcinogenic for animals and probably for man. Carcinogenic effect of these compounds is realized through formation of highly active metabolites covalently binding to DNA and causing mutations [5]. Aminoazobenzenes are activated by cytochrome P-450-1a in N-hydroxylation reaction [6]. Enzymes of this subfamily are induced in animal and human liver by many compounds, including some azobenzenes [4], the liver being the primary target of carcinogenic action of the majority of azocompounds. Increased content of cytochromes P-45o-1a stimulates the production of genotoxic intermediates. Therefore, the inducibility and activity of cytochrome P-450-1a can be important factors in the initiation of carcinogenic processes.

Mice of different strains are characterized by different sensitivity to hepatocarcinogenic effect of o-aminoazotoluene (OAT). This compound induces tumors in 100% DD, SWR, CBA, and other mice but not in CC57BR or AKR [2]. A 30% incidence of spontaneous hepatomas in male CC57BR mice and resistance to OAT-induced carcinogenesis and a high incidence of liver tumor induction in females of the same strain [1] indicate that the genetic mechanisms of neoplastic transformation are different. Carcinogenic effect is produced by activated OAT metabolites but not by OAT; activated reactions in an organism are paralleled by reactions aimed at detoxification and discharge of the carcinogen. Therefore, sensitivity or resistance to tumor induction by OAT is apparently determined at the level of its metabolism, which is different in dif-

Institute of Molecular Pathology and Ecological Biochemistry, Siberian Division of Russian Academy of Medical Sciences; *Institute of Cytology and Genetics, Siberian Division of Russian Academy of Sciences, Novosibirsk

0007-4888/98/0008-0820$20.00

ferent mouse strains: activating reactions predominate over inactivating in sensitive and vice versa in resistant strains.

We studied the activity and inducibility of cytochrome P-450-1a that is responsible for primary oxidation of aminoazo dyes in the liver of inbred male mice with different sensitivity to the inductors of liver tumors [2].

MATERIALS AND METHODS

Male SWR, AKR, DD, C57BL, CC57BR, C3HA, and CBA mice from the Breeding Center of the Institute of Cytology and Genetics were used.

The microsomal monooxygenases were induced by intraperitoneal injection of benzo(a)pyrene (BP) in a dose of 100 mg/kg or by OAT in a dose of 200 mg/kg. The animals were fed standard diets, water *ad libitum,* and were sacrificed after a 24-h fast. The microsomal fraction of the liver was isolated routinely by differential centrifugation 3 days after the inductor injection. Protein concentration in microsomes was measured routinely [7] and that of cytochrome P-450 by a previously described method [9]. The rate of O-dealkylation of 7-ethoxy- and 7-methoxy-resorufins was determined as described elsewhere [3].

The results were processed using Statgraphics software.

RESULTS

In order to evaluate the inducing effect of OAT on liver monooxygenases in mice of different strains, induction of BP was carried out, whose effect on mouse microsomal monooxygenase system Ah^bAh^b (BP inducibility genotype) and Ah^dAh^d (BP induction resistance genotype) is well known [8]. SWR, DD, and AKR mice not sensitive to BP were not sensitive to OAT, as evidenced by low activities of 7-ethoxyresorufin-O-diethylase and 7-methoxyresorufin-O-dimethylase, which practically did not increase in comparison with the control (Table 1). The total content of P-450 in liver microsomes did

TABLE 1. Content and Activity of Cytochrome P-450 in the Liver of Inbred Mice Induced with BP and OAT (*M±m*)

Strain	Sensitivity to OAT-induced hepatocarcinogenesis	Sensitivity to P-450-1a cytochrome induction	Exposure	Content of cytochrome P-450, nmol/mg protein	O-dealkylase activity, πmole resorufin in min/nmol P-450	
					7-ethoxy-resorufin-O-diethylase	7-methoxy-resorufin-O-dimethylase
SWR	+	—	Control	0.47±0.04	126±25	109±15
			BP	0.45±0.03	173±30	148±22
			OAT	0.42±0.08	178±19	165±30
C3HA	+	+	Control	0.8±0.05	105±30	82±11
			BP	1.2±0.15	1060±205	608±151
			OAT	1.4±0.22	716±119	515±98
CBA	+	+	Control	0.62±0.05	250±32	170±21
			BP	0.91±0.01	1545±91	1040±120
			OAT	1.11±0.04	1400±102	828±60
DD	+	—	Control	0.58±0.05	144±14	182±25
			BP	0.50±0.11	185±70	220±41
			OAT	0.50±0.10	252±62	430±16
AKR	—	—	Control	0.6±0.04	89±25	100±19
			BP	0.58±0.10	75±28	115±14
			OAT	0.55±0.12	69±15	125±21
CC57BR	—	+	Control	0.78±0.01	186±82	261±52
			BP	1.50±0.07	1178±54	1419±115
			OAT	1.36±0.04	2390±152	2685±205
CC57Bl	—	+	Control	0.66±0.10	153±20	144±34
			BP	0.93±0.13	2472±190	3682±301
			OAT	0.73±0.03	2911±580	3261±428

not change either. BP-inducible strains C3HA, CBA, CC57BR, and C57BL were sensitive to OAT induction: a 1.5-2-fold increase in the content of cytochrome P-450 was observed in the first three of these strains and its negligible increase in C57BL mice. An increase in the content of this hemoprotein in the liver of four studied strains was paralleled by a marked increase in specific activity of P-450-1a. The most pronounced effect of OAT was observed in CC57BR and C57BL mice: the above-mentioned activities were increased 10-20 times in the liver of these animals. In C3HA and CBA a smaller increase was observed (5-6-fold). Basal activity of P-450-1a was virtually the same in all strains.

There is no relationship between P-450-1a inducibility genotype and sensitivity to OAT-induced hepatocarcinogenesis (Table 1). Among mice with Ah^bAh^b genotype, C57BL and CC57BR were resistant and C3HA and CBA were sensitive to tumor induction. SWR mice with genotype Ah^dAh^d are sensitive to carcinogenic effect of OAT, while AKR mice are resistant to BP and OAT induction and to tumors. Studies of the inductor effect of azobenzenes including OAT on rat liver monooxygenases showed a positive correlation between the carcinogenicity and mutagenicity of these compounds and inducibility of cytochrome P-450-1a [4]. We observed no correlation of this kind in inbred mice. This may be explained by many factors, including genetic differences in the spectrum of cytochrome P-450-1a forms differently metabolizing OAT. Another probable factor is genetic differences in the activities of enzymes of the second phase of metabolism of xenobiotics inactivating the reactive metabolites. More detailed study of this carcinogen metabolism in mouse liver will help elucidate the role of enzyme systems of cytochrome P-450 and transferases at the initial stages of chemical carcinogenesis.

The study was partially supported by the Russian Foundation for Basic Research (grant No. 97-04-49434).

REFERENCES

1. V. I. Kaledin, I. A. Serova, and L. A. Semenova, *Eksp. Onkol.*, **12**, 28-30 (1990).
2. V. I. Kaledin, I. A. Serova, Yu. G. Tsellarius, and L. A. Semenova, *Ibid.*, **7**, 23-26 (1985).
3. M. D. Burke, R. T. Mayer, and R. E. Kouri, *Cancer Res.*, **37**, 460-464 (1977).
4. Y.-L. Cheung, S. M. Puddicombe, T. J. B. Gray, and C. Ioannides, *Carcinogenesis*, **15**, 1257-1263 (1994).
5. F. P. Guengerich, *Cancer Res.*, **48**, 2946-2954 (1988).
6. E. L. Loechler, *Carcinogenesis*, **17**, 895-902 (1996).
7. O. H. Lowry, N. J. Rosenbrough, A. L. Farr, and R. J. Randalls, *J. Biol. Chem.*, **193**, 265-275 (1951).
8. D. W. Nebert, *CRC Crit. Rev. Toxicol.*, **20**, 153-174 (1989).
9. T. Omura and R. Sato, *J. Biol. Chem.*, **239**, 2379-2385 (1964).