expect changes in the activity of the fetal liver to catalyze the biotransformation of xenobiotics. This hypothesis must, however, remain untested since ethical and legal restrictions prohibit the use of fresh fetal tissue obtained by pregnancy termination at times past 20 weeks gestation.

In summary, we have demonstrated that liver obtained from the fetal stumptailed monkey is capable of catalyzing the hydroxylation of benzo[*a*]pyrene and hexobarbital as early as midterm and that the apparent K_m for these reactions is similar to that obtained with human fetal microsomes. Data have also been presented which suggest the possible ontogenesis of different forms of cytochrome P-450-mediated monooxygenases during fetal development. These data further substantiate the use of nonhuman primates as animal models for studying fetal hepatic drug metabolism and the possible role of drug metabolism in human fetal dysfunction, malformation, and transplacental carcinogenesis.

Acknowledgements—The authors wish to thank Ms. Cynthia Severance for her technical assistance and Mrs. Joanne Park for the preparation of this manuscript. The 3-hydroxybenzo[*a*]pyrene was supplied by Dr. David G. Lingfellow of the National Cancer Institute. This work was supported, in part, by the National Institute of Drug Abuse, Grant DA-01180.

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Biochemical Pharmacology, Vol 31, No. 6, pp. 1153–1155, 1982
Printed in Great Britain

0006-2952/82/061153-03 $03.00/0

Activity of various aldehyde-metabolizing enzymes in chemically-induced rat hepatomas*

(*Received* 6 *June* 1981; *accepted* 8 *September* 1981)

Chemically induced rat hepatomas have a unique aldehyde dehydrogenase (EC 1.2.1.3 and 1.2.1.5) phenotype characterized by a several-fold increase in total aldehyde dehydrogenase activity due to the appearance of several new cytosolic isozymes not detectable in normal liver [1–3]. In addition to other properties, the tumor isozymes differ from the normal liver aldehyde dehydrogenases by preferentially oxidizing aromatic aldehydes with NADP as coenzyme. The new isozymes are tumor-specific; they appear concomitant with the appearance of tumors grossly, several months after carcinogen exposure [4].† The phenotypic change is limited to the tumor. Neither morphologically and histologically normal liver directly adjacent to the tumor nor normal lobes of a tumor-bearing liver possess the tumor aldehyde dehydrogenase phenotype [4].†

We are interested in determining whether the tumor-specific aldehyde dehydrogenase phenotype is indicative of an overall alteration of aldehyde metabolism in hepatic tumors or whether the phenotype is due to transformation-associated, stable, genetic changes specific to the expression of the aldehyde dehydrogenases. To this end, we have examined the activities of aldehyde dehydrogenase, aldehyde oxidase, aldehyde reductase and alcohol dehydrogenase in normal rat liver, and in liver tumors induced by the aromatic amine, 2-acetylaminofluorene (2-AAF). In addition to aldehyde dehydrogenase, aldehyde reductase (EC 1.1.1.2) and aldehyde oxidase (EC 1.2.3.1) are directly involved in aldehyde metabolism in mammalian liver (for reviews, see Refs. 5 and 6). Alcohol dehydrogenase (EC 1.1.1.1) may provide a variety of aldehyde substrates for aldehyde dehydrogenase and, under certain conditions, can function in aldehyde reduction [5–7]. In

* Supported in part by a Venture Fund Grant from The University of Alabama to W. K. H. and in part by Grant CA-21103 from the National Institutes of Health to R. L.

† R. Lindahl, S. Evces and W-L. Sheng, *Cancer Res.*, in press.

addition, the activities of these enzymes in the livers of rats treated with 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD), a known inducer of aldehyde dehydrogenase [8] and other drug-metabolizing enzymes, have been examined.

Normal rat livers were obtained from male Sprague–Dawley rats. Hepatomas, induced by the method of Peraino *et al.* [9], were provided by Dr. Carl Peraino of the Argonne National Laboratory. Male Long–Evans rats, treated with TCDD by the method of Deitrich *et al.* [8], were provided by Dr. Richard A. Deitrich of the University of Colorado Medical Center.

For alcohol dehydrogenase, aldehyde reductase and aldehyde oxidase determinations, all tissues were prepared as 10% homogenates in buffers of appropriate composition and pH (see assay systems below). Homogenates were centrifuged at 48,000 *g* for 30 min, and the resultant supernatant fractions were used as enzyme source. For aldehyde dehydrogenase determinations, normal rat livers were prepared as 33% homogenates in 60 mM sodium phosphate buffer containing 1 mM EDTA and 1 mM 2-mercaptoethanol (pH 8.5). Hepatomas and TCDD-treated livers were prepared as 20% homogenates in the same buffer. For aldehyde dehydrogenase, all homogenates were made to 1.0% with Triton X-100 and centrifuged as described [3]. The resultant supernatant fractions were used as enzyme source.

Alcohol dehydrogenase activity was determined at 340 nm according to the method of Koivula *et al.* [10] in 70 mM glycine–NaOH buffer (pH 9.6), using 10 mM ethanol and 0.67 mM NAD, final concentrations. Aldehyde dehydrogenase activities were determined using various substrate (see Table 1) and 5 mM coenzyme final concentrations at 340 nm as described [11]. Aldehyde oxidase activity was measured at 348 nm by the method of Wurzinger and Hartenstein [12] in 50 mM sodium phosphate buffer containing 1 mM EDTA (pH 8.0), using a 30 μM final concentration of 4-hydroxy-3-methoxybenzaldehyde as substrate. Aldehyde reductase activity was assayed at 340 nm in 100 mM potassium phosphate buffer (pH 6.0) according to the method of Felsted *et al.* [13]. The final NADPH concentration was 0.18 mM and the final substrate concentrations were 3.5 mM for 4-carboxy- and 4-nitrobenzaldehyde [13] and 2 mM for succinic semialdehyde [14].

All assays were 3.0 ml final volume and were performed at 23–25°. All appropriate corrections for substrate- and/or enzyme-independent changes in absorbance were made. Proteins were determined by the method Lowry *et al.* [15], using bovine serum albumin as standard.

The activities of the various aldehyde-metabolizing enzymes examined are presented in Table 1. The major difference between normal rat liver and 2-AAF-induced hepatomas was the significantly elevated aldehyde dehydrogenase activity, characterized by high activity with benzaldehyde and its derivatives and NADP. This is consistent with our previous observations [3, 4, 16], as was the elevated aldehyde dehydrogenase activity in TCDD-treated livers [8, 16].

The activity of aldehyde oxidase was also higher in hepatomas than in either normal or TCDD-treated liver. However, in normal rat liver, aldehyde oxidase activity was less than 1% of the aldehyde dehydrogenase activity, using the assay conditions employed here. In hepatomas, aldehyde oxidase activity was approximately 0.5% of the aldehyde dehydrogenase activity.

The major role of aldehyde oxidase in rat liver is believed to be in the metabolism of substituted purines, pyridines and pyrimidines [6, 17–18]. The substrate specificities of aldehyde oxidase and xanthine oxidase are overlapping and, in rat liver, xanthine oxidase activity is several-fold higher than that of aldehyde oxidase [6, 18]. Moreover, there is no evidence of overlapping substrate specificity *in vivo* between aldehyde oxidase and any aldehyde dehydrogenase [18, 19]. These differences indicate that aldehyde oxidase, under our conditions, makes a minimal contribution to aldehyde oxidation in rat liver and, especially, in these hepatomas.

Aldehyde reductase activity, measured with either 4-nitrobenzaldehyde or 4-carboxybenzaldehyde, did not differ significantly between the three tissues. This is especially noteworthy since both 4-nitro- and 4-carboxybenzaldehyde were good substrates for the tumor-specific aldehyde dehydrogenases (see Table 1). Aldehyde reductase activity, measured with succinic semialdehyde, was significantly higher in hepatomas than in either normal or TCDD-treated liver. Succinic semialdehyde was a very poor substrate for aldehyde dehydrogenase with either NAD or NADP as coenzyme (see Table 1).

In rat liver, the occurrence of aldehyde reductases with broad substrate specificities and characteristic responses to inhibitors is well-documented (for reviews, see Refs. 5 and 20). The best substrate for aromatic aldehyde reductases *in vitro* is 4-nitrobenzaldehyde. The activity reported here is very similar to that reported by Felsted and Bachur [20].

Table 1. Activity of aldehyde-metabolizing enzymes in rat liver

Enzyme	Normal liver	Hepatoma	TCDD-treated
	Specific activity*		
Alcohol dehydrogenase	18.6 ± 2.0 (9)	23.4 ± 4.7 (6)	21.7 ± 1.7 (4)
Aldehyde reductase			
4-Nitrobenzaldehyde	105.8 ± 13.5 (12)	110.2 ± 19.5 (12)	90.0 (1)
4-Carboxybenzaldehyde	67.3 ± 11.5 (13)	65.2 ± 11.8 (13)	63.1 (1)
Succinic semialdehyde	101.9 ± 15.5 (13)	202.1 ± 30.0† (13)	71.8 (1)
Aldehyde dehydrogenase‡			
Propionaldehyde/NAD	23.3 ± 2.1 (9)	113.6 ± 28.2† (10)	448.0 ± 40.7† (5)
Benzaldehyde/NADP	7.3 ± 1.0 (9)	342.5 ± 96.2† (10)	1370.0 ± 216.4† (5)
4-Nitrobenzaldehyde/NADP	21.3 ± 1.9 (4)	151.2 ± 49.0† (4)	148.2 ± 60.5† (4)
4-Carboxybenzaldehyde/NADP	1.8 ± 0.4 (4)	63.7 ± 21.2† (4)	64.0 ± 32.3† (4)
Succinic semialdehyde/NADP	1.0 ± 0.5 (4)	2.1 ± 0.9 (4)	4.2 ± 1.1 (4)
4-Hydroxy-3-methoxybenzaldehyde/NADP	0.0 (4)	0.0 (4)	0.0 (4)
Aldehyde oxidase	0.2 ± 0.0 (14)	0.7 ± 0.1† (14)	0.2 ± 0.1 (3)

* Specific activity is the average mIU/mg protein ± S.E.M. for the number of determinations shown in parentheses.

† Activity was significantly greater than in normal liver at at least the $P < 0.05$ level (paired *t*-test).

‡ Final substrate concentrations were as follows: propionaldehyde, 5 mM; benzaldehyde, 1.5 mM; 4-nitrobenzaldehyde, 4-carboxybenzaldehyde and 4-hydroxy-3-methoxybenzaldehyde, 3.5 mM; and succinic semialdehyde, 2 mM.

Aromatic aldehyde reductases are inhibited by certain barbiturates and flavanoids [20]. We could not demonstrate any difference in the sensitivity of normal liver or hepatoma aldehyde reductases to either barbital (approximately 50% inhibition at 1 mM) or rutin (approximately 45% inhibition at 10 μM) with either 4-nitro- or 4-carboxybenzaldehyde. Pyrazole was slightly inhibitory (approximately 20% at 10 mM) in both normal liver and hepatomas.

An aldehyde reductase specific for succinic semialdehyde has been identified in rat brain [21–22]. It functions in the reduction of succinic semialdehyde to 4-hydroxybutyrate in the 4-aminobutyrate shunt. This enzyme is NADPH-dependent and not sensitive to barbiturate inhibition. The succinic semialdehyde reductase activity seen here in both normal liver and hepatomas was inhibited only approximately 20% by either barbital (1 mM) or rutin (10 μM). In this study, the succinic semialdehyde reductase activity of normal liver was somewhat higher than that reported by Felsted *et al.* [13], although the substrate concentrations were different.

The activity of alcohol dehydrogenase did not differ significantly between normal liver, hepatomas and TCDD-treated liver, but the alcohol dehydrogenase activity of tumors was slightly more variable than in the other tissues. Cederbaum *et al.* [23] and Cederbaum and Rubin [24] have reported that some poorly differentiated, transplantable rat hepatocellular carcinomas possess a novel alcohol dehydrogenase with a very high K_m for ethanol (approximately 280 mM). This isozyme is apparently identical to normal rat stomach alcohol dehydrogenase. Although we have not examined alcohol dehydrogenase activity at very high ethanol concentrations, it is unlikely that our tumours possess a novel alcohol dehydrogenase phenotype. The tumors examined in this study were highly differentiated primary neoplasms. Moreover, Feinstein [25] found no evidence for a novel alcohol dehydrogenase isozyme by polyacrylamide gel electrophoresis of similar tumors.

We have concluded that the changes in aldehyde dehydrogenase phenotype observed in 2-AAF-induced hepatomas are indicative of transformation-associated, stable genetic changes specific to the aldehyde dehydrogenases. The increased aldehyde dehydrogenase activity shifts the overall aldehyde-metabolizing capacity of tumors much more toward oxidation from the reduction predominant in normal liver. This is supported by the increase in aldehyde oxidase activity, although this change is probably insignificant for rat liver. Moreover, that there was no change in the activity of aromatic aldehyde reductases suggests that the changes observed were not due to requirements for an overall increase in aldehyde metabolism, regardless of its nature, in these tumors. Finally, except for succinic semialdehyde reductase, identical changes in the activity of aldehyde-metabolizing enzymes occurred in the liver of rats treated with TCDD, an inducer of a normal liver aldehyde dehydrogenase which shares several properties with at least one of the tumor-specific isozymes [4, 8].

The increased activity in hepatomas of an aldehyde reductase preferentially reducing succinic semialdehyde is of interest, especially since succinic semialdehyde oxidation by aldehyde dehydrogenase with NAD or NADP is very poor. We are planning to examine this change in greater detail.

The activities of aldehyde dehydrogenase, aldehyde oxidase, aldehyde reductase and alcohol dehydrogenase have been compared in rat hepatomas induced by 2-acetylaminofluorene, in normal rat liver and in the livers of rats treated with 2,3,7,8-tetrachlorodibenzo-*p*-dioxin. As expected, aldehyde dehydrogenase activity was increased significantly in hepatomas and in tetrachlorodibenzo-*p*-dioxin-treated livers. The only other significant change in any enzyme examined was an elevation of succinic semialdehyde reductase activity in hepatomas. The results indicate that the changes in aldehyde dehydrogenase phenotype observed in these hepatomas are due to transformation-associated, stable genetic changes specific to the aldehyde dehydrogenases, rather than to an overall modification of aldehyde metabolism in these tumors.

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