CYTOCHROME P-450 CONTENT AND ITS INDUCTION IN PRIMARY RAT LIVER TUMORS

A. Yu. Kolyada, V. I. Gel'shtein, and V. A. Koblyakov

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The enzyme system of microsomal oxygenases, the terminal region of which is cytochrome P-450, plays an important role in the metabolism of chemical carcinogens, drugs, and steroids. The study of the cytochrome P-450 content in hepatomas has shown that it is lower than in normal tissue [4, 6]. Many of these investigations have been undertaken on transplantable strains of hepatomas and, in particular, on "minimal" Morris hepatomas [3, 7, 10], where correlation was found between the level of cytomorphologic differentiation of these hepatomas and their cytochrome P-450 content [10]. Primary tumors in animals are a model that is closer to human tumors than transplantable strains. However, very few investigations have been conducted on primary liver tumors, and most important of all, the samples used in such investigations have been very small [4, 6, 12]. In the investigation described below the content of one form of cytochrome P-450 was studied in primary liver tumors by an immunomorphologic method, using monospecific antibodies [1].

This paper gives data on the content of cytochromes P-450 obtained for a large sample of primary liver tumors in rats, using spectrometric methods.

EXPERIMENTAL METHOD

Hepatomas were induced by diethylnitrosamine, which was given together with the drinking water to male Wistar rats for 12 weeks in a concentration of 10 parts per million. Aroclor 1254,* dissolved in oil, was injected intraperitoneally in a dose of 500 mg/kg body weight into the animals as a single dose 3 days before sacrifice.

The microsomal fraction was obtained from normal liver and from tumors and their surrounding tissue by the standard method [2]. The residue of microsomes obtained at 105,000 g was resuspended in 100 mM pyrophosphate, adjusted with acetic acid to pH 8.0, then incubated for 30 min at 4°C and centrifuged at 105,000 g for 1 h. This procedure is essential if the microsomal fraction obtained from the tumors is to be uncontaminated by membrane-bound hemoglobin. Differential absorption spectra of cytochrome P-450, obtained by the method of Omura and Sato [11], were recorded on a DFS-1 spectrophotometer. Protein was determined by Lowry's method in Hartree's modification [8].

The histologic diagnoses of the tumors were based on recommendations of an international group of experts [9]. The group of trabecular carcinomas was divided according to the level of cytomorphologic differentiation into highly differentiated (HDH), moderately differentiated (MDH) and undifferentiated (UDH) hepatomas [13]. The numerical results were subjected to statistical analysis by the Wilcoxon–Mann–Whitney test.

EXPERIMENTAL RESULTS

BN significant differences were found ($p > 0.05$) between the cytochrome P-450 content in normal liver (1.1 ± 0.2 nmole/mg protein) and the tissue surrounding the tumor (1.8 ± 0.8 nmole/mg protein). The content of cytochrome P-450 in the tumors (0.78 ± 0.3 nmole/mg

*Polychlorinated biphenyl.

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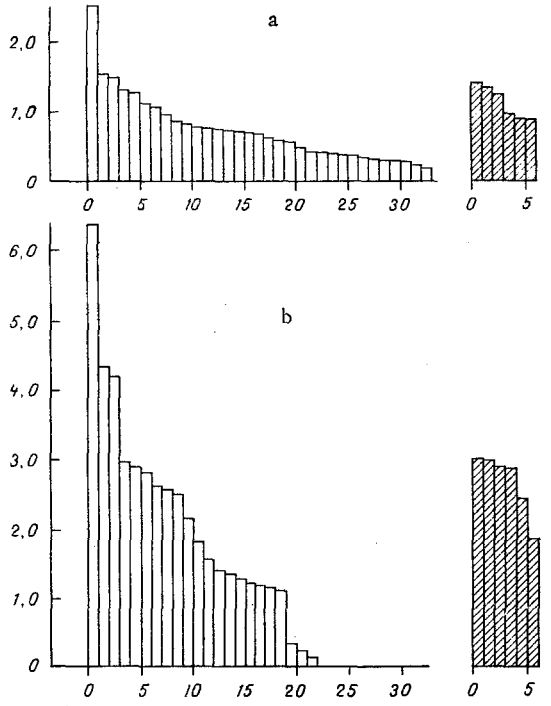

Fig. 1. Cytochrome P-450 content in rat liver tumors (unshaded columns) and in liver of control rats (shaded columns). Abscissa, serial No. of tumors; ordinate, cytochrome P-450 content (nmoles/mg protein). a) Animals not treated with inducing agent (histologic diagnoses: UDH) 4, 5, 6, 7, 8, 11, 15, 21, 22, 25, 27, 32, 33; MDH) 1, 2, 3, 6, 7, 9, 10, 12, 13, 14, 16, 17, 18, 26, 30, 31; HDH) 16, 29; adenocarcinoma) 21; b) after injection of Aroclor 1254 (histologic diagnoses: UDH) 1, 8, 9, 10, 11, 12, 18; MDH) 2, 6, 7, 13, 14, 15, 17, 19; HDH) 3, 4; adenocarcinoma) 5, 21). If the same number is indicated twice, this means either a mixture of two tumors differing in their degree of differentiation, or two forms of differentiation are present in one nodule.

protein) was on average lower than in normal liver ($p < 0.005$) and also in tissue surrounding the tumor ($p < 0.001$). However, within the group of tumors high variability of this parameter was observed (Fig. 1a). In some cases the content of cytochrome P-450 in the tumor could exceed the level of this enzyme in normal liver (Fig. 1a). Variability of the cytochrome P-450 content in the tumors was unconnected with the level of cytomorphologic differentiation of the tumor, for no direct correlation was found between these parameters (Fig. 1).

The tumors were able to induce cytochrome P-450 (Fig. 1). The concentration of cytochrome P-450 in the rat liver after injection of Aroclor 1254 was 2.6 ± 0.3 nmole/mg protein in the control rats and 3.5 ± 1.2 nmole/mg protein in the liver of rats with tumors. The content of cytochrome P-450 in tumors of induced rats (2.0 ± 1.1 nmole/mg protein) was significantly higher than in tumors in uninduced animals ($p < 0.001$), and the degree of induction in the group of tumors was 260%, i.e., not lower than in normal liver, where this parameter was 240%. In a previous article published by the writers [1] on the same material, marked depression of induction of one isoform of cytochrome P-450 in the tumors was demonstrated. This was the isoform P-450Phb, induction of which could not be detected immunomorphologically in the tumors, and is synthesized in response to injection of phenobarbital. We know that induction of cytochrome P-450 by means of phenobarbital is more difficult to achieve in a hepatocyte culture than induction of cytochrome P-450 by means of 3-methylcholanthrene [5]. There is evidence that undifferentiated strains of hepatomas in culture are characterized by induction of isoforms of the 3-methylcholanthrene family, whereas highly differentiated strains are characterized by induction of isoforms of the phenobarbital family [14]. Since the inducing agent used, namely Aroclor 1254, induces both types, it is possible that the tumors which we investigated preserve their ability to induce isoforms of the 3-methylcholanthrene family, and that it is these isoforms which we determined spectrophotometrically,

whereas induction of isoforms of the phenobarbital family was not present in these tumors, as was shown previously by an immunomorpholigic investigation [1]. Another possible explanation may be that induction of isoforms of cytochrome P-450 of families not induced in normal liver may take place in tumors under the influence of Aroclor 1254. Whatever the case it is evident that the set of isoforms of cytochrome P-450 in tumors differs from the set of isoforms in normal liver.

The investigation of a sufficiently large sample of primary liver tumors thus shows that reduction of the total content of cytochrome P-450 or reduction of inducibility are not obligatory features of hepatomas. Disparity between the total content of cytochromes P-450 (spectrophotometric method) in tumors and the content of one form, determined by an immunomorphological method [1], is evidence that the content of individual forms of the enzyme may vary independently. Consequently, analysis of the state of the cytochrome P-450 system in tumors is possible only at the level of individual forms of the enzyme.

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