CYTOCHROME P-450 AND ARYL HYDROXYLASE ACTIVITY IN TUMOR CELLS DURING LONG-TERM TRANSPLANTATION

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Of all the particular features of malignant tumors there is great interest in the low activity of enzymes of the endoplasmic reticulum – the microsomal mono-oxygenases (MMO) – found in their cells. This feature, which is linked both with the mechanisms of malignant transformation and with the pathways of tumor progression, bestows on tumor cells a number of new properties and, in particular, resistance to the action of compounds activated in the cytochrome P-450 system. These compounds include many indirect-acting carcinogens (procarcinogens) as well as some chemotherapeutic antitumor preparations [1, 2].

The main investigations in this field have been conducted on hepatomas. Features of the microsomal oxidation system in other tumors have received much less study, probably because of technical difficulties connected with the lower cytochrome P-450 content in their cells.

The test object in this investigation was tumor MCh-II, maintained by passage through mice for 27 years [6]. The basal level of activity and content of cytochrome P-450 and their changes during induction and the action of inhibitors were investigated in this extreme variant of a disdifferentiated tumor.

EXPERIMENTAL METHOD

Male C57BL/6j mice weighing 23-26 g were used and each group contained 10 to 15 animals. 3-Methylcholanthrene (3-MCh) was injected intraperitoneally in a dose of 50 mg/kg for 3 days before sacrifice. Arochlor 1254 was injected intraperitoneally in a dose of 50 and 500 mg/kg once during the 5 days before sacrifice. The two inducers were injected in olive oil. Microsomes from both tumor and liver were isolated at 40°C in 1.15% KCl, 0.05 M KH_2PO_4, and 1 mM EDTA (pH 7.4). By means of a Potter's homogenizer a 20% tissue homogenate was obtained and then centrifuged at 9000g for 20 min and again at 105,000g for 1 h. The residue was resuspended in 0.1 M $Na_4P_2O_7$ and 1 mM EDTA (pH 7.4) and centrifuged again for 1 h at 105,000g. Microsomes were isolated from the solid tumor on the 11th day after subcutaneous injection of 1×10^6 ascites cells. Ascites cells were taken on the 7th-8th day after intraperitoneal injection of 1×10^6 MCh-II ascites cells. The content of cytochrome P-450 was determined by the method in [11] in the presence of 2 mM NaCN, and also by the method in [9, 10] in the presence of Na succinate [8]. Aryl hydroxylase activity was determined by the method in [7], with incubation at 37°C for 30 min. The concentration of microsomal protein was 0.1 mg/ml for liver and 1.5 mg/ml for the MCh-II tumor. The calibration curve was plotted over the range from 5 to 500 pmoles 3-OH-benz(α)pyrene (3-OH-BP). To determine the effect of inhibitors, α-naphthoflavone (up to 1×10^{-5} M), SKF 525-A, and metyrapone (up to 1×10^{-4} M) were added to the incubation medium. To determine protein, a modified Lowry's method was used. The results were subjected to statistical analysis by Student's t test.

EXPERIMENTAL RESULTS

The experiments showed that the MCh-II tumor contains a functionally active mono-oxygenase system. The presence of CO-binding hemoprotein was demonstrated in the microsomal fraction of the tumor. Determina-

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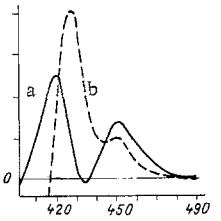

Fig. 1

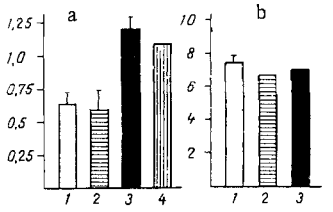

Fig. 2

Fig. 1. Differential spectra of microsomal hemoproteins of tumor MCh-II. a) CO-difference spectrum of microsomes reduced with dithionite (protein concentration 4.4 mg/ml) in 0.1 M Tris-HCl, pH 7.4, with 2 mM NaCN; b) dithioniate difference spectrum of microsomes incubated with 5 mM Na succinate in the presence of CO (protein concentration 4.6 mg/ml, pH 7.4). Abscissa, wavelength (in nm); ordinate, optical density (value of scale division for (a) 0.002 unit, for (b) 0.005 unit).

Fig. 2. Effect of 3-MCh and arochlor 1254 on microsomal BP-hydroxylase activity of tumor MCh-II. 1) Uninduced MCh-II microsomes: a) BP hydroxylase activity (0.58 ± 0.10 pmole/min/mg protein), b) concentration of cytochrome P-450 (6.8 ± 0.8 pmoles/mg protein); 2) microsomes induced by arochlor 1254: a) BP hydroxylase activity (0.57 ± 0.10 pmoles/min/mg protein), b) cytochrome P-450 concentration (6.5 pmoles/mg protein); 3) microsomes induced by 3-MCh: a) BP hydroxylase activity (1.22 ± 0.07 pmoles/min/mg protein), b) cytochrome P-450 concentration (6.6 pmoles/mg protein); 4) microsomes induced by arochlor 1254 in a dose of 500 mg/kg, BP hydroxylase activity (1.1 ± 0.1 pmoles/min/mg protein). Ordinate: a) pmoles 3-OH-BP/min/mg protein, b) pmoles P-450/mg protein.

tion of the concentration of cytochrome P-450 was made difficult by its low concentrations and by the presence of contaminating hemoproteins: hemoglobin and methemoglobin, as well as cytochromes of the respiratory chain. The latter contamination was due to the vigorous homogenization used during work with the solid tissues, and it showed itself as a long-wave shift of the absorption maximum of cytochrome P-450 in the differential spectra of the microsomes [13]. To reduce to a minimum the effect of the above components, repeated reprecipitation of the tumor microsomes was used and cytochrome P-450 was determined in the presence of NaCN.

We found that the concentration of cytochrome P-450 in the tumor microsomes by the method of Omura and Sato was 6.8 pmoles/mg protein (Fig. 1a). The second method of determination of cytochrome P-450 [9, 10], which we used after preliminary incubation of the microsomes with 5 mM Na succinate to abolish the effect of cytochrome oxidase (Fig. 1b), gave a somewhat higher value: 9 pmoles/mg protein. The concentration of the hemoprotein in the tumor microsomes (7-9 pmoles/mg protein) is one of the lowest values, 33-50% lower than the cytochrome level in microsomes from pig aorta [14].

Aryl hydroxylase activity can be considered as a functional characteristic of the mono-oxygenase system. It was shown by the use of benz(α)-pyrene (BP) as substrate that 3-OH-BP is formed in the presence of tumor homogenate and NADPH. The study of the distribution of BP hydroxylase activity among subcellular fractions showed that the latter is concentrated chiefly in the microsomal fraction, it is very low in mitochondrial and nuclear fractions (3% of activity in microsomes), and virtually absent in the cytosol.

For the next experiments microsomes of the MCh-II tumor were used. The quantity of BP metabolite formed was shown to be a linear function of the quantity of microsomal protein within the limits of 0.5-5 mg/ml and of the duration of incubation up to 1 h. The mean level of BP hydroxylase activity in tumor microsomes was 0.58 pmole/min/mg protein, which is about 0.2% of the BP hydroxylase activity in mouse liver.

Inducibility is a characteristic property of the MMO system. In the present experiments aryl hydroxylase activity was more than doubled after injection of 3-MCh into the animals (Fig. 2a). The cytochrome P-450 level

TABLE 1. Effect of MMO Inhibitors on BP Hydroxylase Activity of Tumor MCh-II

Microsomes	Residual activity, % of control			
	α-naphthoflavone		SKF 525-A ($1 \cdot 10^{-4}$ M)	metyrapone ($1 \cdot 10^{-4}$ M)
	$1 \cdot 10^{-5}$ M	$5 \cdot 10^{-5}$ M		
Uninduced	39	24	117	102
Induced by 3-MCh	20	9	92	—
Induced by arochlor 1254, 50 mg/kg	65	31	—	109
500 mg/kg	—	18	—	97

showed no appreciable change under these circumstances (Fig. 2b). Arochlor 1254, an inducer belonging to the polychlorinated biphenyl group, in a dose of 50 mg/kg had no effect on activity of the enzyme, or on the cytochrome P-450 content in the tumor (Fig. 2), whereas induction in the liver in these experiments was the same as under the influence of 3-MCh, namely about 500%. When a larger dose of inducer (500 mg/kg) was used aryl hydroxylase activity in the tumor was increased by 190%, and in the liver of these animals by 18.7 times. Data in the literature show that arochlor 1254 is a powerful inducer of BP hydroxylase and cytochrome P-450 in various mammalian tissues [4, 5] and in liver tumors [3]; it induces a broad spectrum of isoforms of cytochrome P-450, including forms inducible by 3-MCh [12]. The present experiments thus showed that the ability of the microsomal mono-oxygenase system to induce is not lost in tumor MCh-II. Both inducers in the case of the tumor were about equally effective, whereas for the liver arochlor 1254 proved to be much stronger than 3-MCh. These facts are evidence of the essentially different tissue specificity of action of modifiers of the MMO system.

For a correct interpretation of these results it was deemed important to identify forms of cytochromes P-450 contained in the MCh-II tumor. For this purpose the action of MMO inhibitors (α-naphthoflavone, SKF 525-A, and metyrapone) was studied. The results showed that SKF 525-A and metyrapone, in a concentration of 1×10^{-4} M, had only a weak effect on BP hydroxylase activity (Table 1). By contrast α-naphthoflavone, in a concentration an order of magnitude lower, inhibited BP hydroxylase activity by more than 50%. α-Naphthoflavone is known to be a specific inhibitor of forms of cytochrome P-450 inducible by 3-MCh [15]. In agreement with this, potentiation of inhibition of BP hydroxylase by α-naphthoflavone also was observed in the present experiments in microsomes of tumor MCh-II induced by 3-MCh (Table 1).

The results of this investigation thus demonstrate that tumor MCh-I contains a hemoprotein similar in its properties to cytochrome P_1-450.

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