LOCALIZATION OF CYTOCHROME P-450PB IN THE LIVER AND PRIMARY HEPATIC TUMORS OF RATS OF VARIED HISTOLOGIC STRUCTURE AFTER INJECTION OF THE INDUCER AROCLOR 1254

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The system of microsomal oxygenases, the terminal region of which consists of cytochrome P-450, plays an important role in the metabolism of chemical carcinogens, drugs, and steroids. Many forms of cytochrome P-450 are known [6]. Administration of various substances can give rise to selective induction of the various forms of an enzyme. It is difficult or, in general, impossible to differentiate the different forms of an enzyme in a mixed preparation by spectroscopic methods [10]. The immunochemical method is free from this deficiency and, in addition, it enables systems heterogeneous in their cell composition, such as tumors, to be analyzed. As yet immunohistochemical methods have not been used to study cytochrome P-450 induction in tumors. This process has been studied by spectral methods mainly in transplantable strains of hepatomas. Induction of cytochrome P-450 was not found in Novikoff's [12] and Morris 3924A [5] hepatomas. A marked reduction in the induction of this enzyme compared with that in normal liver [7] was observed in hepatomas of strains Morris 7777 [9], 5123 t.c. (H), 5123 t.c., and 7288 [12], and also in primary hepatomas induced by D,L-ethionine.

In the investigation described below an immunohistochemical method was used to study the localization of one form of cytochrome P-450, that induced by phenobarbital (PB), namely the P-450PB isoform, in cells of primary tumors of the rat liver of different histologic structure. Monospecific antibodies against this form of cytochrome P-450 were obtained previously from rat liver by induction with PB [1].

EXPERIMENTAL METHOD

Experiments were carried out on Wistar rats weighing 150 g. Three groups of healthy animals (six rats in each group) were used: intact, treated with Aroclor 1250, and treated with 3-methylcholanthrene (MCh). Liver tumors were induced by diethylnitrosamine (DENA), synthesized as described previously [2]. DENA, in a concentration of 40 ppm, was given to animals for 15 weeks with their drinking water in dark glass bottles. The rats were killed 25-30 weeks after the beginning of the experiment. To induce cytochrome P-450PB a mixture of polychlorinated biphenyls, Aroclor 1254 (from Monsanto Chemicals Co., USA) was administered in a single dose of 500 mg/kg, intraperitoneally, in oil. Aroclor 1254 is less toxic than PB and is a more powerful inducer of cytochrome P-450PB [4]. As a control of the specificity of induction of cytochrome P-450PB MCh was injected in a dose of 15 mg/kg in oil, intraperitoneally, 1 and 2 h before sacrifice. To block protein synthesis cycloheximide was injected intraperitoneally in a dose of 1 mg/kg 1 h before injection of the inducer.

The cellular localization of cytochrome P-450PB was studied in 51 tumor nodules from the livers of 16 rats; 12 rats (from which 44 nodules were studied) had been treated with Aroclor 1254 before sacrifice, whereas four rats (seven tumors) were untreated. The normal rats were killed 1 and 4 days after injection of Aroclor 1254 and the rats with tumors 4 days after injection. Antibodies against cytochrome P-450PB were obtained, the material fixed, and sections stained by the unlabeled antibodies immunoperoxidase (PAP) method described previously [1].

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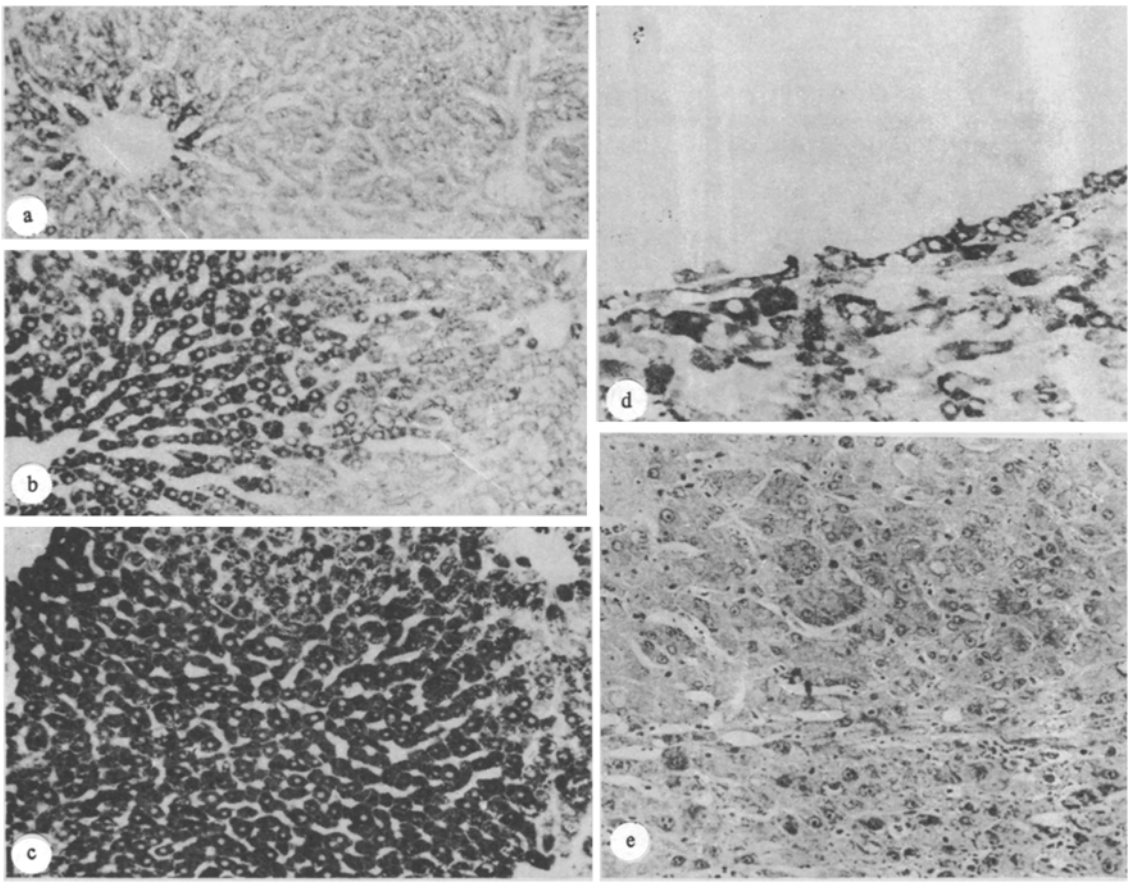

Fig. 1. Localization of cytochrome P-450PB in rat liver (PAP reaction). a) Intact, b, c) 1 and 4 days respectively after injection of Aroclor 1254, d) neoplastic nodule (on right) surrounded by normal tissue (on left), e) serial section of neoplastic nodule stained with hematoxylin-eosin. a, b, c) 180×; d, e) 200×.

EXPERIMENTAL RESULTS

The kinetics of cytochrome P-450PB induction in the rat liver after administration of PB was examined previously [1]. About two-thirds of cells in the hepatic lobule stained intensively 4 days after its administration. Injection of Aroclor 1254 (Fig. 1a-c) was followed by intensive induction in virtually all cells of the hepatic lobule of the normal liver. It was thus anticipated that all cells capable of induction in the tumors would react to injection of the inducer. Staining of liver sections from rats induced with MCh remained the same as that of the intact liver, evidence of the specificity of the antibodies against cytochrome P-450PB. Injection of cycloheximide blocked the appearance of the stain. This shows that synthesis of cytochrome P-450PB takes place de novo in response to injection of the inducer. In all cases only the cytoplasm of the cell was stained and the nuclei remained unstained.

It should be pointed out that the antiserum obtained in these experiments was against the principal form of cytochrome P-450, contained in liver microsomes of rats treated with PB, namely PB-450 [11]. After exhaustion of this serum by microsomes of normal rats antibodies monospecific in Ouchterlony's double diffusion in gel test were obtained [1]. These antibodies were evidently analogous to the monospecific antibodies against cytochrome $P\text{-}450_b$ described in the literature [13]. In that study it was shown by an immunochemical method that the concentration of cytochrome $P\text{-}450_b$ in the microsomal fraction of the liver increases 60 times more than that in the intact liver in response to injection of PB or of Aroclor 1254, but remains unchanged in response to MCh.

Liver tumors induced by DENA were classified in accordance with the recommendations of the International Expert Group [8]. According to this classification tumors in animals treated with Aroclor 1254 could be subdivided as follows: neoplastic nodules four, trabecular carcinomas 34, adenocarcinomas six. The latter were always mixed in structure and contained glandular and trabecular components.

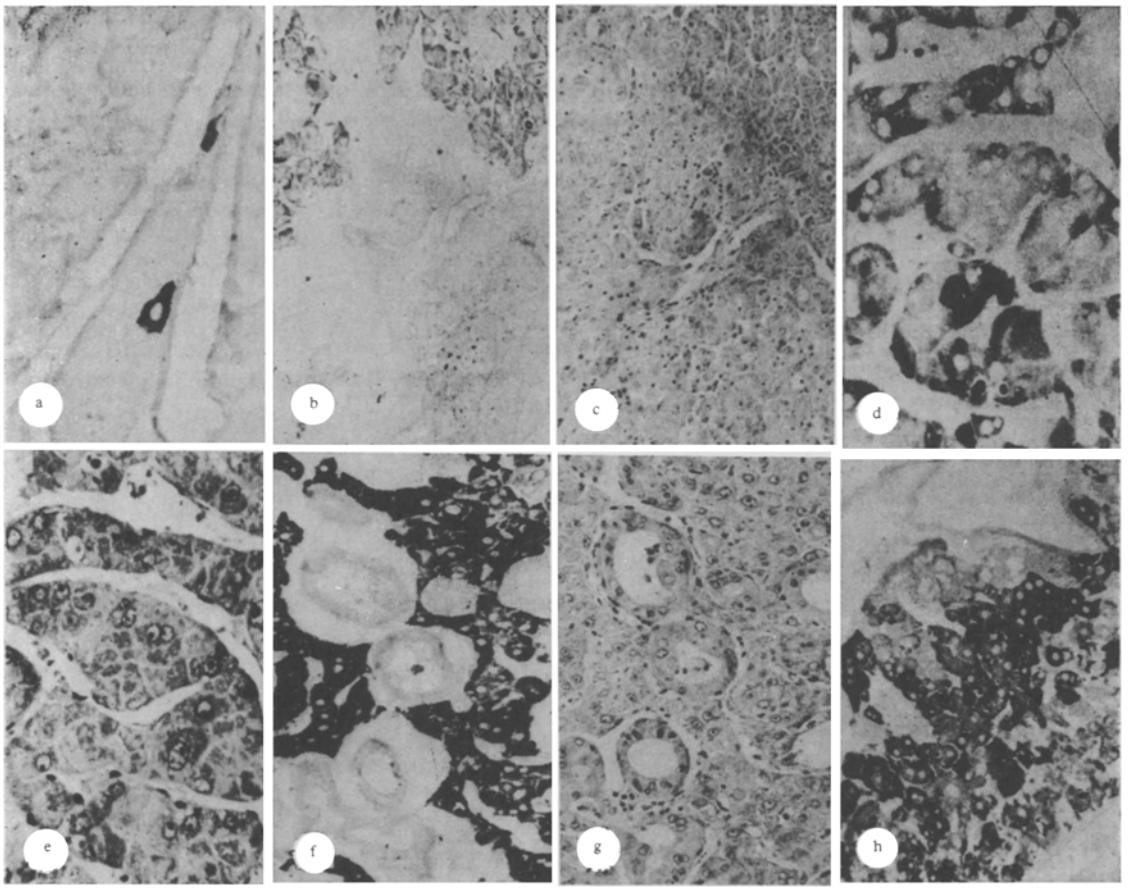

Fig. 2. Localization of cytochrome P-450PB in rat liver tumors: a) staining in individual cell of trabecular carcinoma; b) staining in individual trabeculae of moderately differentiated trabecular carcinoma (c – serial section stained with hematoxylin and eosin); d) staining of mosaic type throughout section of trabecular carcinoma with low differentiation (e – serial section stained with hematoxylin); f) intensive staining in trabecular areas of adenocarcinoma; g) serial section stained with hematoxylin and eosin; h) uneven staining of cells of trabecular area of adenocarcinoma. a, d, e) 300×; b, c, f, g, h) 150×.

Sections through six trabecular carcinomas and one adenocarcinoma in animals not treated with Aroclor 1254 were studied. Positive staining was not obtained in any of these tumors after treatment with antibodies against cytochrome P-450PB.

The following picture was obtained in tumors of rats treated with Aroclor 1254. The neoplastic nodules were not stained by antibodies against cytochrome P-450PB (Fig. 1d, e). Absence of staining was observed in 14 (of 34) trabecular carcinomas. In the remaining 20 tumors of similar structure, weak or uneven staining of the sections was observed. In 10 cases only individual cells were stained (Fig. 2a), in five cases individual trabeculae or groups of cells near blood vessels were stained (Fig. 2b, c), and in five cases mosaic staining of individual cells or small groups throughout the section was observed (Fig. 2d, e). Weak and uneven induction of cytochrome P-450PB was observed equally often in trabecular carcinomas at different levels of cytological differentiation: high, average, or low [3]. In the adenocarcinomas the epithelium of the glandular structures was never stained by antibodies against cytochrome P-450PB, although areas of trabecular carcinoma between them, in all six adenocarcinomas found in three different animals, were often stained just as intensively as normal liver cells, or even more so (Fig. 2f-h). Cells of trabecular areas with the same morphology and within the same adenocarcinoma differed in their degree of staining (Fig. 2b). Even in the most intensely stained tumors individual cells or areas were found not to react. There was no regular arrangement of induced cells and areas, such as is so characteristic of the normal liver. The structural organization of the normal liver is evidently not necessary for induction of cytochrome P-450PB in the individual cell. Staining of cells was observed in the liver tissue surrounding tumor nodules.

Immunohistochemical study of the distribution of cytochrome P-450PB in tumors thus showed that induction of this form of the enzyme is inhibited in the early stages of malignant transformation, namely in neoplastic nodules. Inhibition of induction in tumors does not depend on their level of cytomorphological differentiation. In some tumors induction of cytochrome P-450PB was observed in individual cells and groups of trabeculae, whereas in mixed tumors strong induction was found in cells of the trabecular component. To explain differences in the response of "pure" trabecular carcinomas and of trabeculae in mixed tumors to the inducer, several suggestions may be made. Cells of both "pure" trabecular carcinomas and of trabecular areas in adenocarcinomas may perhaps have equal potential for induction of cytochrome P-450PB, but the epithelium of the glandular component of adenocarcinomas may stimulate the cells of these tumors.

Another possibility is that cells of the trabecular component of adenocarcinomas and of "pure" trabecular carcinomas, although morphologically indistinguishable, possess different sets of expressible genes. This may be connected with differences in the histogenesis of these tumors. The possibility cannot be ruled out that "pure" trabecular carcinomas arise from neoplastic nodules which, in turn, arise from foci of changed hepatocytes, whereas mixed tumors arise as a result of malignant transformation of areas of "oval-cell" proliferation. Cells of these foci of proliferation, unlike foci of changed hepatocytes, evidently do not undergo rigid selection for resistance to the toxic action of carcinogens by virtue of the fact that enzymes of xenobiotic metabolism are not yet expressed in "oval" cells. It can be tentatively suggested that this is why only the progeny of "oval"cells may remain capable of inducing cytochrome P-450.

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