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LOCALIZATION OF FETAL ALDOLASES DURING EARLY STAGES OF AZO-DYE HEPATOCARCINOGENESIS IN RAT

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Summary : We have looked for the synthesis of fetal aldolases A and C during the early stages of hepatocarcinogenesis induced by 3'-methyl-4-(dimethylamino) azobenzene in the rat. Using indirect immunoperoxidase and immunofluorescence techniques we show that oval and transitional cells are the main cellular sites of fetal aldolases A and C production while hepatocytes only synthesize aldolase B. The synthesis of aldolases A and C was confirmed by electrophoresis analysis. These results indicate that different cell types are involved in fetal aldolase production during the early stages of azo-dye feeding and during regeneration after carbon tetrachloride intoxication where the synthesis of these isozymes is restricted to sinusoïdal cells.

Introduction

Since the work of Schapira et al (1), many reports have shown the resurgence of fetal isozymes in hepatomas (2,3). Considerable experimental evidence suggests that hepatocarcinogenesis involves a series of sequential cellular and metabolic changes. During carcinogenesis induced in rat liver by azo-dyes, the first morphological modifications observed are degeneration of centrolobular hepatocytes and proliferation of so-called oval cells in periportal spaces. The following stages correspond to appearance of hyperplastic foci, then neoplastic nodules and finally the hepatoma (4,5). Production of fetal antigens occur very early, before any morphological

Abbreviations used :

3'-Me-DAB : 3'-methyl-4-(dimethylamino)azobenzene
AFP : α_1-fetoprotein
CCl_4 : carbon tetrachloride

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evidence of tumor development. Thus a net increase in serum AFP was detected as soon as the third week of feeding (6). The synthesis of fetal isozymes such as pyruvate kinase M_2(type III) and aldolase A (Muscle type) - but not aldolase C (brain type) - was also demonstrated on and after the 6th week of feeding (7-8).

It has been suggested that oval cell proliferation corresponds to a compensatory phenomenon of regeneration in response to degeneration of parenchymal cells (9). This differs from the proliferation of remaining parenchymal cells after the necrosis of centrolobular hepatocytes induced by CCl_4 intoxication In this case, as in regeneration after partial hepatectomy, we have recently found that expression of fetal aldolase isozymes A and C is restricted to sinusoïdal cells (10,11). So, in the early stages of 3'-Me-DAB hepatocarcinogenesis in rat, two questions were raised :

a) What is the cell type producing aldolase A ?

b) Is aldolase C also synthesized and if so, in what cell population ?

We have used indirect immunoperoxidase and immunofluorescence techniques as well as electrophoresis to detect and localize fetal aldolases A and C between the 3rd and the 7th week of feeding.

Material and methods

Five-week-old Wistar male rats were fed ad libitum with 3'-Me-DAB(0.1%). Fifteen rats were sacrificed from the 3rd to the 7th week of feeding and 5 normal rats were used as control.

Aldolases A, B and C were purified according to the methods previously described (12-14). Specific antiserum against each type was prepared in rabbits and the purity of antisera was checked by immunodiffusion. Antisera were rendered monospecific by adsorption of each antiserum with rat extracts and normal serum (13).

Immunolocalization of the 3 isozymes of aldolase were performed by indirect immunoperoxidase and immunofluorescence as previously described (11). The 3 aldolases were localized on cryostat sections from paraformaldehyde-picric-acid fixed liver fragments. Control reactions were obtained either by incubating the sections with normal rabbit serum or by incubating the sections with absorbed antisera with the corresponding antigen.

Electrophoresis were performed with Millipore Phoroslide in veronal buffer pH 8.6 for one hour and with 1mA per strip. Aldolase activity was revealed by specific staining (15).

Results

Immunolocalization of aldolase isozymes

Immunoperoxidase and immunofluorescence techniques yielded the same results. The location of the 3 aldolase isozymes was exclusively cytoplasmic in control as well as in 3'-Me-DAB rats.

As previously reported, in normal liver aldolase B was present in parenchymal cells and aldolase A in sinusoïdal cells only. Aldolase C was not detected.

In livers of rat fed with 3'-Me-DAB between the 3rd and the 7th week, aldolase B was observed in normal and megalocytic hepatocytes. No staining was visible within the small cells containing an oval or round nucleus (oval and transient cells). However, some cells which appeared as small hepatocytes showed a weak staining (Fig. 1a).

By contrast, aldolase A (fig. 1b) and aldolase C (fig. 1c) were never found in hepatocytes but only in the small cells (oval and transient cells).

When aldolases A and C were localized in sections of the same liver fragment, the percentage of cells positively stained for aldolase A and the intensity of the staining were always much more important than for aldolase C.

Small amount of aldolase A were also present in sinusoïdal cells. Control reactions were negative (fig. 1d).

Electrophoresis of liver extracts

Electrophoresis of liver extracts after 6 weeks of feeding showed at least three bands : the more cathodic band corresponds to aldolase B and the two others to aldolase A and A-C hybrid (fig. 2).

Discussion

It is now well-admitted that, in early stages of azo-dye hepatocarcinogenesis, so-called oval cells,presumably originating from ductal epithelial cells, are converted to transient cells and hepatocytes (4). The size and the shape of the cells containing aldolases A and C suggest that these cells are mainly oval and transient cells.

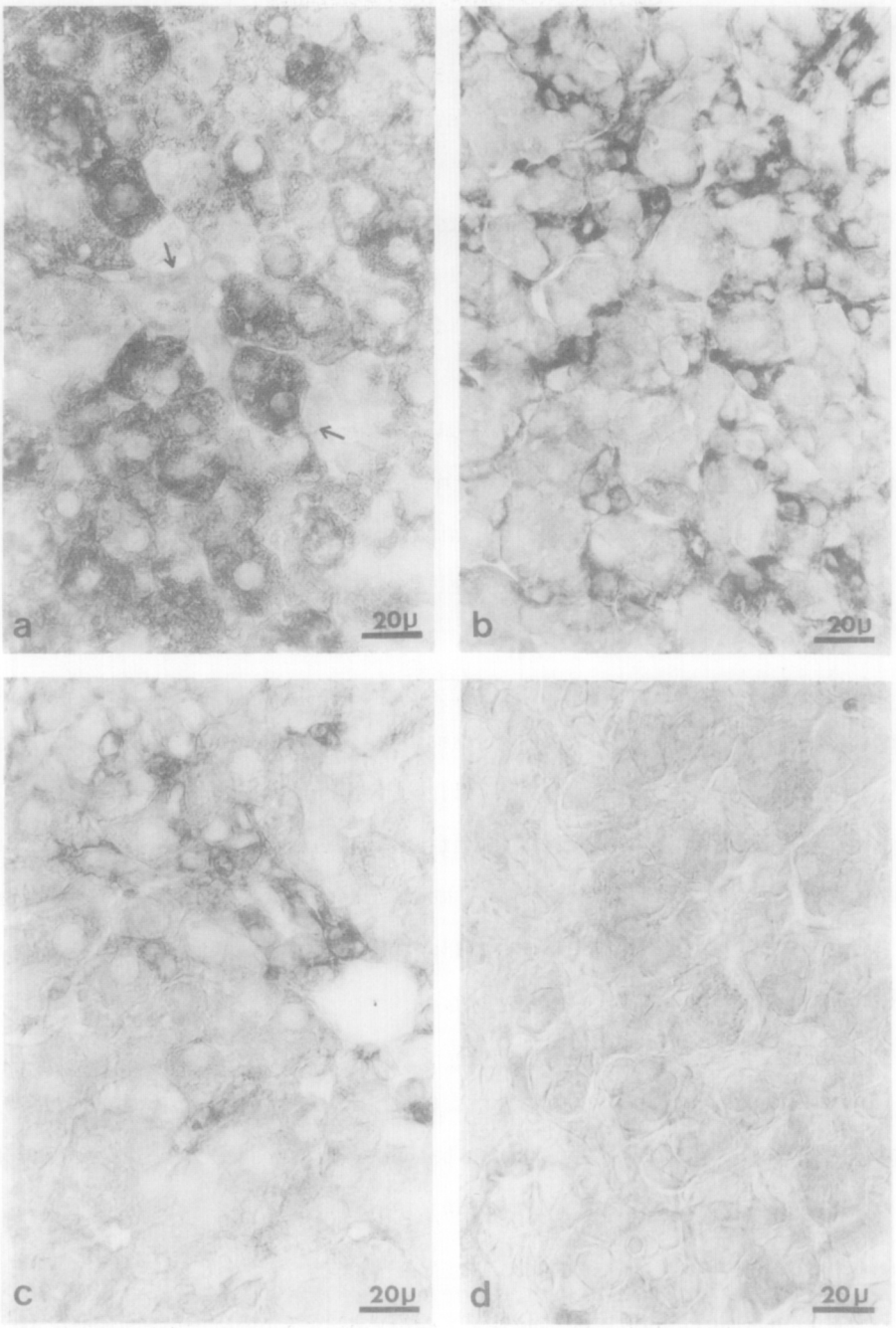

Figure 1 : Light microscopy localization of aldolase isozymes in 3'-Me-DAB fed rat liver after 6 weeks of feeding ; cryostat sections incubated : a) with anti-aldolase B antiserum ; b) with anti-aldolase A antiserum ; c) with anti-aldolase C antiserum ; d) with normal rabbit serum. Aldolase B is visible in hepatocytes of various sizes but not in smaller cells (arrow). Aldolases A and C are located in small cells with an oval and round nucleus. Control reaction is negative (x 512).

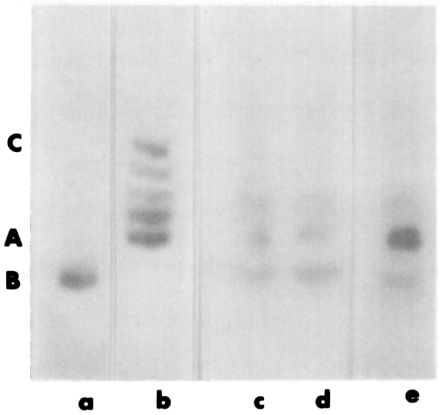

Figure 2 : Electrophoresis of 3'-Me-DAB-fed-rat liver after 6 weeks of feeding, specifically stained for aldolase.
a. normal liver
b. brain
c. 3'-Me-DAB-fed-rat liver } An A-C hybrid is visible
d. idem
e. idem

The difference between these results and those obtained with CCl_4 intoxication can be explained by the different cell types involved in replacement of necrotic hepatocytes. After CCl_4 intoxication, regeneration is promoted by remaining mature hepatocytes which do not express fetal isozymes ; by contrast, during 3'-Me-DAB feeding, only poorly differentiated cells (oval and transient cells) proliferate.

These fetal aldolase synthesizing cells are less differentiated than those producing AFP, which are transient cells and small hepatocytes but not strictly so-called oval cells (16-18). Such a conclusion is in agreement with the findings observed in perinatal rat liver where fetal aldolases (19) disappear earlier than AFP (20). However, fetal hepatocytes are able to express both fetal aldolases (A,C) and adult aldolase B (21). This suggests that, in 3'-Me-DAB-fed rats, where aldolase B begins to appear in small hepatocytes, the synthesis of fetal aldolases occurs in cells which have not reached the level of differentiation of fetal hepatocytes and which therefore would be unable to express aldolase B.

On the other hand, the presence of aldolase A in megalocytic hepatocytes, as reported by Onoe et al (3) was not confirmed. This discrepancy could be related to the different experimental conditions since these authors have localized aldolase A only by a cytochemical method.

The presence of A-C hybrids demonstrated by electrophoresis confirmed our immunomorphological studies and indicates that oval and transient cells can synthesize both aldolases A and C. The small number of aldolase C positive cells could be explained by a synthesis of this isozyme in too low amounts to be detected by our technique and/or during a step of oval cell differentiation more restricted than for aldolase A.

It has been suggested that oval cells could be the precursors of malignant cells (9). Using fetal aldolases as markers, further studies on the subsequent stages of 3'-Me-DAB hepatocarcinogenesis are in progress, in order to verify this hypothesis.

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