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CELL LOCALIZATION OF ALDOLASE FETAL ISOZYMES IN RAT REGENERATING LIVER : DIFFERENCES WITH HEPATOMA

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Summary : Using the indirect immunoperoxidase technique with optical and electron microscopy, we have localized both fetal aldolases A and C from regenerating rat liver in Kupffer and endothelial cells (by contrast with the localization of aldolase B in hepatocytes only). These results have been confirmed by biochemical methods. The localization of the 3 aldolases differs widely from that observed in fast-growing hepatoma and suggests that control mechanisms of gene regulation are different in cancer and in liver regeneration.

Introduction

Isozymic modifications toward a fetal pattern are known to appear in regenerating liver after partial hepatectomy but they are transitory (1) and occur to a lesser extent than in fast-growing hepatoma, although the rate of cell multiplication is comparable. Whereas in fast-growing hepatoma the resurgence of fetal aldolases A and C is an important phenomenon (2, 3), during regeneration, the normal adult B type remains preponderant, aldolase A is increased and aldolase C reappears (4).

Recently our group (5) has shown that the resurgence of fetal aldolases in fast-growing hepatoma was not due to cellular selection but more likely to a disturbance at the gene control level. However, in the case of regeneration the liver cell type responsible for the synthesis of the 3 fetal aldolases isozymes has not yet been determined.

So the question is raised whether the modifications of aldolase isozymic pattern occuring in regenerating liver are due to the same phenomenon which leads to the disturbance of gene expression in fast-growing hepatoma. In an attempt to

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answer this question, we have localized the 3 types of aldolases (A, B and C) in regenerating livers at different times after hepatectomy using the indirect immunoperoxidase technique as well as biochemical methods.

Materials

3-month-old rats of Wistar strain were used. Hepatectomy was performed according to Higgins and Anderson (6) and rats sacrificed 24 h, 48 h, 72 h after the operation.

Isolated hepatocytes were obtained using Seglen's method (7). Population of sinusoïdal cells were obtained using Knook's method (8).

Immunochemical and electrophoretic methods

Aldolase A was prepared from rat muscle using Gracy et al. method (9).

Aldolase B was prepared from rat liver using the same method with modifications (5).

Aldolase C was prepared according to Hatzfeld et al. (10).

The purity of the proteins was checked by SDS polyacrylamide gel electrophoresis.

Rabbit antisera to the three aldolases were prepared as described previously (5). Their purity was checked by Ouchterlony's immunodiffusion method (11) and immunoelectrophoresis. Antisera were rendered mono-specific by absorption using normal rat tissue extracts (12). Anti-aldolase B antiserum was absorbed by normal rat serum and by brain extract ; anti-aldolase A and C antisera were absorbed by normal rat serum and by liver extract. Extracts of normal and regenerating livers as well as purified hepatocytes (and purified Kupffer cells) from normal and regenerating livers were submitted to Millipore Phoroslide electrophoresis in Veronal buffer pH 8.6 for one hour and 1 mA per strip. Aldolases were revealed by specific staining according to Penhoët et al. (13).

Morphological methods

Fixation of livers and liver section labelling procedures for light and electron microscopy were performed as described earlier (5) with the following modifications : before fixation, normal and regenerating rat livers were perfused with Hepes buffer for 30 s, then with the fixative for 30 s (2.75% paraformaldehyde with 0.2% picric acid buffered with 100 mM phosphate buffer pH 7.4 (14). The livers were then cut into fragments which were immersed in the same fixative. Cryostat sections were incubated in A, B or C rabbit aldolase antiserum then with sheep anti-rabbit γ globulin antibodies labelled with peroxidase. After peroxidase staining, some sections (8 µm) were studied by optical microscopy. Others were post fixed in a 1.5% osmium tetroxide solution then dehydrated and embedded in Epon. Ultrathin sections were examined by electron microscope.

Control reactions were obtained by incubating the sections either with normal rabbit serum, then with labelled sheep anti γ globulins antibodies, or with anti-aldolase antiserum absorbed by the corresponding antigen, then with labelled sheep anti-rabbit γ globulin antibodies. Others were incubated in the Graham and Karnovsky solution only (15).

Morphological results

1) Light microscopy.

In normal liver : aldolase B was located only in the cytoplasm (and never

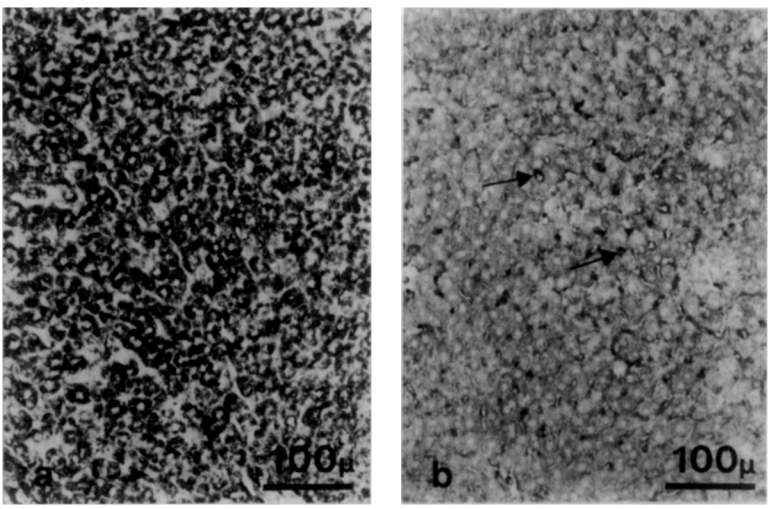

Figure 1. Light microscopy ; normal rat liver ; cryostat section incubated a) with anti-aldolase B antiserum ; b) with anti-aldolase A antiserum. Aldolase B is present only in hepatocytes whereas aldolase A is located in sinusoidal cells (arrows) (x 150).

in the nucleus) of most of the hepatocytes. No reaction was observed in non parenchymal cells (fig. 1a). By contrast, for aldolase A a weak staining was observed in sinusoïdal cells. This aldolase was not detectable in the hepatocytes (fig. 1b). Aldolase C was not visible in any cell type. The control reactions were negative.

In regenerating liver : the reaction indicating the presence of aldolase B was positive in the cytoplasm of almost all the hepatocytes. But the intensity of the staining was weaker than in normal hepatocytes and varied strongly from one cell to another (fig. 2a). The staining corresponding to aldolase A was more intense in the sinusoidal cells than in those of normal liver (fig. 2 b). The presence of aldolase C was also demonstrated in the sinusoidal cells. The hepatocytes were not stained (fig. 2c). The same cellular distribution of the 3 isozymes was seen 24 h, 48 h and 72 h after hepatectomy. All the control reactions were negative (fig. 2d).

2) Electron microscopy.

The distribution of the different aldolase isozymes, both in normal and regenerating liver observed by light microscopy, was confirmed at the ultra-

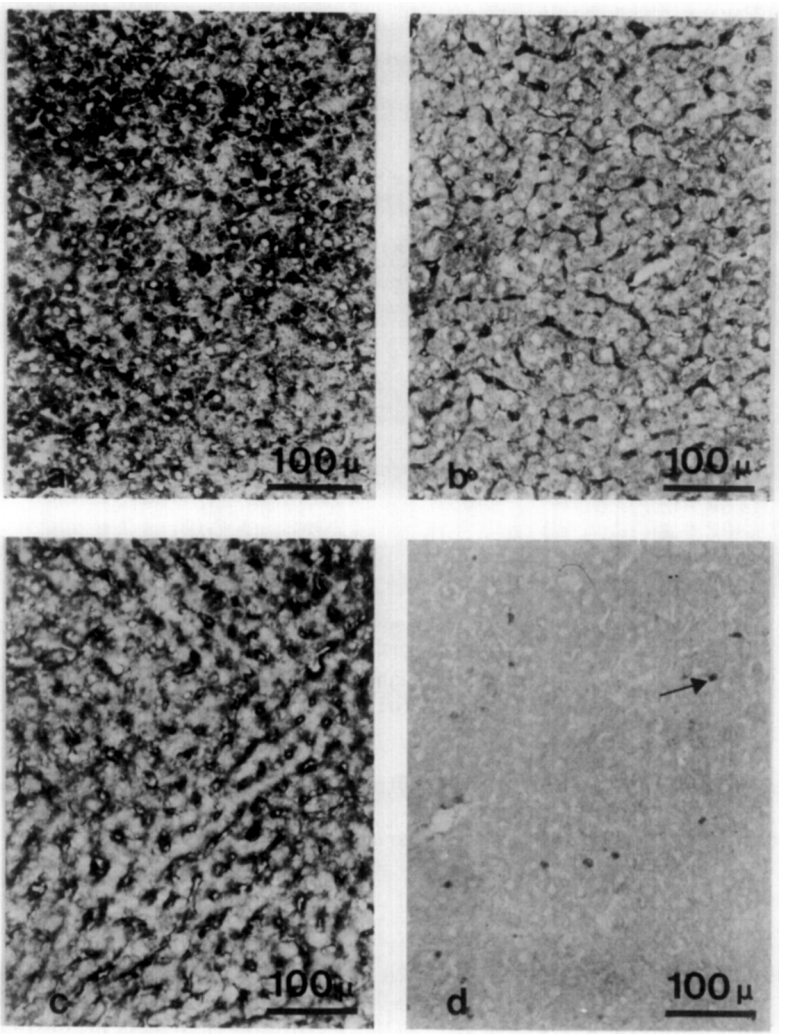

Figure 2. Light microscopy ; rat liver 3 days after partial hepatectomy ; cryostat section incubated : a) with anti-aldolase B antiserum ; b) with anti-aldolase A antiserum ; c) with anti-aldolase C antiserum ; d) with normal rabbit serum. Aldolase B is visible in hepatocytes ; aldolases A and C only in sinusoidal cells. Control reaction is negative except for the non specific staining of red blood cells (arrow) (x 150).

structural level. In normal as well as in regenerating liver, aldolase B was present in the cytoplasm of hepatocytes, and aldolase A in the cytoplasm of both Kupffer and endothelial cells. Aldolase C, which was not detected in normal liver cells, was also present in both Kupffer and endothelial cells (fig. 3). The electron-dense precipitates corresponding to the different types

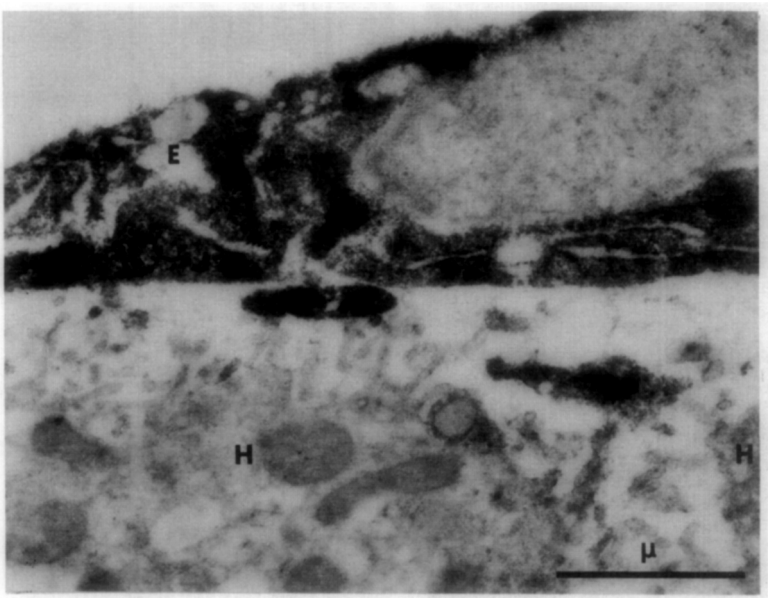

Figure 3. Electron microscopy : rat liver 3 days after partial hepatectomy ; cryostat section incubated with anti-aldolase C antiserum. Aldolase C is dispersed throughout the cytoplasm of an endothelial cell (E). No reaction is visible in hepatocytes (H) (x 31,000).

of aldolase were dispersed throughout the cytoplasm and not in the lumina of the endoplasmic reticulum and the Golgi apparatus.

Electorphoresis results

Microelectrophoresis were performed with extracts of normal livers and regenerating livers (before and after perfusion with Hepes) and with isolated populations of hepatocytes and sinusoidal cells.

In normal livers a strong band corresponding to aldolase B was visible whereas in regenerating livers two bands corresponding to aldolases A and B were present. The pattern was identical after perfusion by Hepes.

With isolated hepatocytes, both normal and regenerating livers showed only aldolase B.

With sinusoidal cells of regenerating livers a band corresponding to the hybrid A_2C_2 was visible indicating the presence of both aldolases A and C. A band of weak intensity corresponding to aldolase B was also present which is very likely due to a slight contamination by hepatocytes.

Discussion

Our results show that, during liver regeneration after partial hepatectomy, the increase of aldolase A and the reexpression of aldolase C takes place in Kupffer and endothelial cells, whereas aldolase B, as in normal liver, occurs only in hepatocytes.

The cell location of the three different isozymes can be ascertained from the following arguments :

- the possibility of serum aldolase diffusion during experimental procedures was avoided since the technique of perfusion used (which could not be used for hepatoma) eliminated red blood cells and serum aldolase.
- the possibility of diffusion of trace amounts of aldolase A from hepatocytes to Kupffer and endothelial cells during Hepes perfusion can be excluded since :

. no aldolase B was detectable in sinusoidal cells (and it was unlikely that one type of aldolase preferentially diffused).

. The electrophoretic pattern of total extracts of regenerating livers, after perfusion with Hepes buffer only, showed that both isozymes were present, whereas only aldolase B was visible in isolated hepatocytes after electrophoresis.

Electron microscopy showed a diffuse distribution of aldolases A and C in the cytoplasm of Kupffer and endothelial cells and of aldolase B in the cytoplasm of the hepatocytes. This is in agreement with the fact that aldolase is not a secretory protein.

The cellular distribution of the three different types of aldolase has been confirmed by immunofluorescence (results not shown). Moreover our immunoperoxidase results are in good agreement with the results obtained by electrophoresis methods using isolated cells.

In the hepatocytes of adult regenerating liver, only type B was found, whereas both A and B were present in total extracts. Purified populations of sinusoidal cells showed a weak band corresponding to hybrids between aldolases A and C. Moreover, isolated hepatocytes from fetal liver synthesized aldo-

lase A (Guguen-Guillouzo, unpublished observation). So it seems that expression of aldolases in physiological conditions depends on the stage of differentiation of hepatocytes. Mature hepatocytes would express only adult aldolase B even in the case of cell multiplication as in regeneration after partial hepatectomy.

These data can be compared to those obtained with fast-growing hepatoma (5), in which the 3 isozymes A, B and C are synthesized by the same cells, derived from hepatocytes. This suggests that control mechanisms are different in cancer and in regeneration. In cancer, abnormal gene expression would correspond to definitively disturbed regulation mechanisms. In regenerating liver, where the rate of cell multiplication is also high, limited division of mature hepatocytes occurs. The cells which express aldolase C are different from hepatocytes (which synthesize only aldolase B). These cells are submitted to normal regulation after a specific step in the regenerating process has been reached and therefore would express fetal isozymes transitorily since mature hepatocytes would be unable to do so.

It remains to be determined why do these sinusoidal cells express fetal isozymes during physiological cell multiplication.

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References.

1. Weber, A. and Schapira, F. (1972) C.R.Acad.Sci. 166, 312-316.
2. Schapira, F., Dreyfus, J.C. and Schapira, G. (1963) Nature 200, 995-997.
3. Schapira, F., Reuber, M.D. and Hatzfeld, A. (1970). Biochem. Biophys. Res. Comm. 40, 321-327.
4. Berges, J., De Néchaud, B. and Uriel, J. (1974) FEBS Letters 48 , 76-78.
5. Hatzfeld, A., Feldmann, G., Guesnon, J., Frayssinet, G. and Schapira, F. (1978) Cancer Res. 38, 16-22.
6. Higgins, C.M. and Anderson, R.M. (1931) Arch. Pathol. 12, 186-
7. Seglen, P.O. (1973) Exp. Cell Res. 76, 25-30.
8. Knook, D.L., Blansjaar, N. and Sleyster, E. Ch. (1977) Exp. Cell Res. 109, 317-329.
9. Gracy, R.W., Lacko, A.G., Brox, L.W., Adelman, R.C. and Horecker, B.L. (1970) Arch. Biochem. Biophys. 136, 480-490.

10. Hatzfeld, A., Elion, J., Mennecier, F. and Schapira, G. (1977) J. Biochem. 77, 37-43.
11. Ouchterlony, O. (1958) Prog. Allergy 5, 1-78.
12. Eisen, H.N. (1974) In : Microbiology. Davis, Dulbecco, Eisen, Ginsberg and Wood Eds. Hagerstown Md., Harper and Row Publishers, 359-405.
13. Penhoët, E.E., Rajkumar, T. and Rutter, W.J. (1966) Proc. Natl. Acad. Sci. USA 72, 659-663.
14. Stefanini, M., De Martins, C. and Zamboni, L. (1967) Nature 216, 173-174.
15. Graham, R.C.Jr. and Karnovsky, M.J. (1966) J. Histochem. Cytochem. 14, 291-302.