REAPPEARANCE OF ALDOLASE C IN RAT LIVER DURING COMPENSATORY REGENERATION AFTER PARTIAL HEPATECTOMY

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1. Introduction

The absence of noticeable modifications in the distribution of aldolase isozymes (I.E.C. 4.1.2.13) after partial hepatectomy as compared to normal liver has been described by Rutter and Weber [1], Farina et al. [2] and more recently by Weber and Schapira [3] and by Walker and Potter [4].

Only a slight decrease of aldolase B (liver type isozyme), the major constituent of adult liver and, at times, a weak increase of aldolase A (muscle type isozyme) have been observed in regenerating liver.

The results presented here confirm these observations, but in addition, they show by electrophoretic and immunologic methods the transitory reappearance of aldolase C (brain type isozyme), absent from the normal normal adult liver. Several authors have reported the presence of this isozyme in certain primary hepatomas and considered its resurgence as being associated with the neoplastic transformation of the liver [5,6].

2. Materials and methods

2.1. *Animals*

Four lots of Wistar rats (two lots of males and two lots of females) were divided into two age groups: the first composed of 39-day-old rats and the second 5-month-old rats.

2.2. *Isozymes*

The technique of Penhoet, Kochman and Rutter [7] was used for the purification of the three isozymes A, B and C of aldolase from extracts of muscle, liver and brain respectively.

2.3. *Antiserum: anti-aldolase C*

A preparation of Aldolase C was used to immunize the rabbits. They were injected in the foot-pads with a mixture of 0.6 ml of the aldolase C preparation (4 mg/ml) and 0.6 ml of Freund's adjuvant. One and two months after, they received 2 ml of the same mixture by subcutaneous injection distributed on the dorsal region. The animals were bled 15 days after the last injection.

The antiserum was made monospecific by immunoadsorption on polymers prepared from extracts of liver and muscle from adult rats according to Avrameas and Ternynck (8). The specific anti-alsolase C antiserum did not cross-react, as demonstrated by immunodiffusion techniques, with either alsolase A or B preparations.

2.4. *Hepatectomy*

Two-thirds hepatectomy (excision of the left and median lobes) was performed according to Higgins and Anderson [9]. One-third hepatectomies were made by a single excision of the median lobe after ligation of its vascular hilum.

2.5. *Extraction*

The animals were exsanguinated under ether anesthesia 24, 48 and 72 hr after hepatectomy. Immediately after, the livers were cut in small pieces and suspended in three volumes of 0.01M Tris buffer, pH 7.5, containing 0.2% Triton X-100 and then ground for 20 sec in an Ultraturax homogenizer. The homogenate was centrifuged for 60 min at 100 000 *g* in a Spinco model L ultracentrifuge. The supernatant was removed, filtered through a 0.8 μ millipore membrane filter and stored at -80°C until use.

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2.6. *Electrophoresis and electroimmunodiffusion*

Electrophoretic separations were made on a mixed polyacrylamide agarose gel (3.5% acrylamide and 0.8% agarose) in a Tris–glycine buffer at pH 8.7 [10]. After electrophoresis, the aldolasic activity was determined by the colorimetric method of Penhoet, Rajmukar and Rutter [11]. The immunologic quantitation of aldolase C was made by electroimmunodiffusion [12].

2.7. *Histological examination*

The liver specimen were fixed, paraffin embedded, and prepared for light microscopy according to standard techniques.

3. Results and discussion

3.1. *Electrophoretic modifications of the aldolase isozymes*

Fig. 1 shows the distribution of the aldolase isozymes in the livers removed at various times after hepatectomy. The results are shown for two groups of male rats of different ages (39-day-old young adults, and 5-month-old adults).

The reappearance of aldolase C and its hybrids with aldolase A was observed 24 hr after one-third or two-thirds hepatectomy in both the young and the adults. Aldolase C and its AC hybrids were always present 48 hr after one-third or two-thirds hepatectomy in young rats. At the same time only AC hybrids were observed in the 5-month-old animals, the aldolase C having disappeared. After 72 hr, aldolase C and its AC hybrids were still present in the young rats after excision of a third of the liver, but only the hybrids remained after a two-thirds excision. In 5-month-old animals, aldolase C was always absent 72 hr after hepatectomy and, at this time, the whole pattern of aldolase isozymes become identical to that of the control animals. The same results were obtained in female animals.

In the same figure, the variations in isozymes A and B as already noted by other authors can be seen: a slight transitory decrease in type B aldolase and a very light transitory increase in type A aldolase 24 hr after hepatectomy on all the lots of animals studied

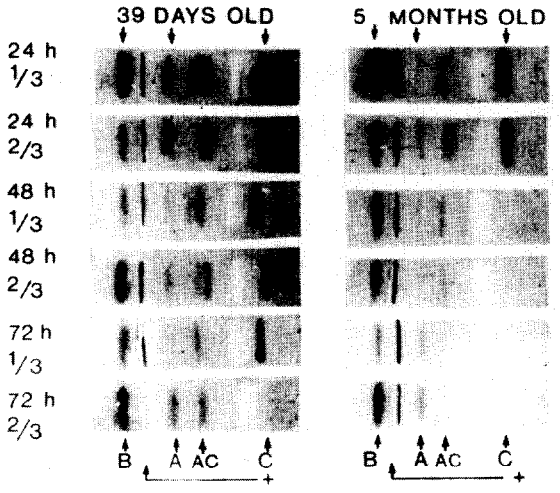

Fig. 1. Electrophoretic patterns of aldolase in soluble extracts of liver after partial hepatectomy in young and old rats. In front of each electrophoretic pattern: time in hours after partial hepatectomy of either 1/3 or 2/3 of the liver. A, B, C: muscle, liver and brain type aldolases respectively; AC: muscle-brain hybrids.

3.2. *Immunological characterization*

The immunoelectrodiffusion diagram of liver extracts with specific anti-aldolase C antiserum (fig. 2), shows precipitation peaks of variable heights which correspond to different concentrations of aldolase C in young adults after hepatectomy. Three dilutions, 1/8, 1/6 and 1/32 of each liver extract were studied. The extract with the largest quantity of aldolase C corresponds to an extract obtained 24 hr after one-third

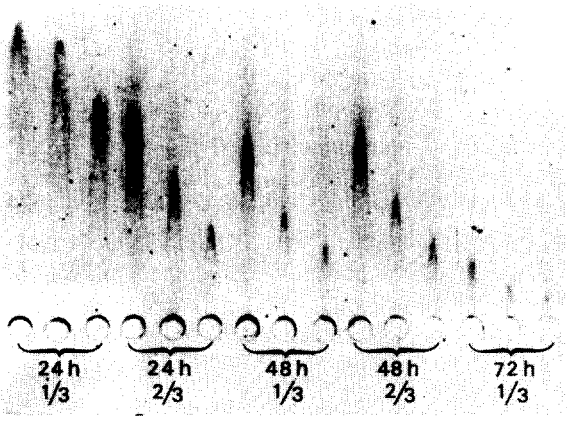

Fig. 2. Electroimmunodiffusion patterns of liver extracts from young rats at various times (hr) after partial hepatectomy of either 1/3 or 2/3 of the liver. Three dilutions (1/8; 1/16; 1/32) of each extract have been analysed.

hepatectomy. The results in fig. 2 correlate with those obtained by electrophoresis.

3.3. *Histological signs of hepatic regeneration*

In the young adults 24 hr after one- or two-thirds hepatectomy, an intense regeneration was observed for 48 hr afterwards. At the end of 72 hr, discrete signs of regeneration were observed in the animals that had one third of their liver excised, the livers after two-thirds hepatectomy had become practically normal. 24 hr after hepatectomy, the livers of 5-month-old adults showed the same signs of regeneration as seen in the young; whereas, 48 hr after two-third hepatectomy, regeneration seemed already terminated while a few discreet signs of regeneration persisted in the case of one-third hepatectomy. As was expected, the liver was quite normal 72 hr after whatever type of hepatectomy. From these results, a close correlation appears between the kinetics of the reappearance of the type C aldolase and the histological signs of liver regeneration.

The presence of three aldolase isozymes in fetal liver from the 15th day of gestation is now well established [5, 13]. As the fetal and postnatal liver develops, isozymes A and C decrease rapidly until they are no longer detectable three days after birth, while during the same interval, isozyme B increase until it attains the level found in adult liver. The reappearance of isozymes A and C in primary hepatoma induced by chemical carcinogens was first reported by Schapira et al. [5,14]. Several authors [1–4] have failed to demonstrate the resurgence of rat fetal isozyme C in liver after partial hepatectomy. By contrast, the evidence presented here shows that isozyme C and its AC hybrids reappear transitorily in analogous circumstances. An explanation of this contradiction seems to be either the utilization of different methods than ours, or where similar methods have been employed, the use of much too diluted liver extracts [3] and the absence of detergents in the extraction medium. We noticed by electrophoretic analysis that, the yield of aldolase isozymes extraction was considerably increased in regenerating adult liver by buffers containing Triton X-100, while little or no modifications were observed in fetal livers under analogous treatment.

Our results approach those obtained by studying rat α-fetoprotein (αFP), a fetal antigen of liver origin which is associated with primary liver cancer [15,16] and also capable of reappearing during the compensatory liver regeneration provoked by partial hepatectomy [17] or by hepatic toxins [18].

Regenerating liver is the seat of deep changes in cell population-dynamics. Iwasaki et al. [19] and Uriel et al. [20] advanced the hypothesis that αFP may be synthesized by 'transitional' hepatic cells that can differentiate toward hepatocyte or cholangiocyte. These cells would reappear during liver regeneration subsequent to hepatic injuries of neoplastic or non neoplastic origin [21]. Given the close correlation found between the resurgence of aldolase C and the signs of hepatic regeneration, it is not impossible that 'transitional' cells of an analogous type may be implicated in the resurgence of aldolase isozymes.

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