Immunocytochemical study of the hepatic innervation in the rat after partial hepatectomy

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Summary
The autonomic nervous system in rats has been assessed by means of indirect immunofluorescence using monospecific antibodies to neuron-specific enolase, neurofilaments, glial fibrillary acidic protein and S-100 protein (10 days after partial (70%) hepatectomy). Different groups of rats were studied:

- group A: 70% resection and normal dual blood supply (n=5);
- group B: 70% resection with only portal blood to the liver remnant (n=5);
- group C: 70% resection with only arterial blood to the liver remnant (n=5);
- group D: sham operated controls (n=5).

All rats of groups A and D showed normal liver/body weight ratios after 10 days in contrast to groups B and C where liver weights were 50-60% of the preresection weight. In group A the regeneration process was histologically normal and associated with a remarkable increase of autonomic innervation patterns in the portal triad. In contrast, livers of animals in groups B and C showed under the light microscope features of hepatocyte degeneration associated with a decreased autonomic innervation compared to the controls. The changes are identical in groups B and C, and are therefore irrespective of the type of blood deprivation (arterial or portal).

These results support the importance of dual blood supply for an optimal regenerative response in liver remnants after liver resection. We suggest that the autonomic nerve supply of the portal triad plays at least an important permissive role in liver regeneration.

Introduction
The liver is unique among mammalian organs in its ability to regenerate. After partial hepatectomy the liver mass will be restored within 7–10 days. Yet, despite great progress in elucidating the morphological and biochemical events, the specific pathophysiological mechanism is still unknown and the discussion about putative hepatotrophic factors still continues. Apart from hepatotrophic factors, changes in liver blood flow and disturbance of the balance between supply of nutrients and demand by the resected liver, many other hypotheses have been advanced (Labrecque, 1985).

However, the possible role of the autonomic nervous system has probably been underestimated (Ashrif et al., 1974; Morley & Royse, 1981; Kato & Shimazu, 1983). Recently it has again been suggested that catecholamines are involved in stimulating DNA synthesis after partial hepatectomy by mediating the increase in the activity of the thymidylate synthesizing enzymes (Nakata et al., 1985).

From the clinical point of view, optimization of liver regeneration after partial hepatectomy is an essential point. The role of adequate blood supply has been stressed. However, if liver resection is performed in livers without dual supply, a great inhibition of liver re-growth occurs (Bengmark & Hafström, 1969).

The aim of this study is to investigate whether changes in the liver blood supply are associated with changes in the autonomic innervation. An immunocytochemical study has been performed using monospecific antisera in order to assess possible changes in nerve supply of the liver in rats after partial hepatectomy under normal conditions, and in the presence of altered blood supply (i.e. portocaval shunt or ligation of the hepatic artery).

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Materials and methods

20 male Wistar rats (weighing 290–320 g), TNO Zeist, fed normal laboratory food and water ad libitum were divided into four groups of five rats:

- group A: partial hepatectomy;
- group B: partial hepatectomy and ligation of the hepatic artery;
- group C: partial hepatectomy and portacaval shunt;
- group D: controls, sham operated, laparotomy and clamping of the portal vein for 15 min.

During surgery the animals were anaesthetized by use of ether anaesthesia and liver resection was a standard procedure embracing about 70% hepatectomy (Waynforth, 1980). Via a midline ventral abdominal wall incision the falciform ligament is cut down to the posterior vena cava. The median and left lateral lobes are moved out of the abdominal cavity. After cutting down two other suspensory ligaments between these lobes and the median blood vessels, the stomach and the dorsal peritoneum, a ligature is tied around these lobes and the blood vessels at their base with a double reefknot. The lobes are severed with pointed scissors above and as near to the ligature as possible. In group B a silk ligature was placed and tied around the hepatic artery close to its origin in the portal hilus.

The resected liver was weighed and all animals were kept alive for 10 days after the operation. On the tenth day the animals were sacrificed by ether inhalation. In group C partial hepatectomy (70%) was performed in rats subjected to an end to side portacaval shunt according to Lee and Fischer (1961); under ether anaesthesia the coronary vein was ligated and the portal vein was then clamped, tied and cut, and subsequently stitched to an incision in the anterior wall of the inferior cava just above the junction with the right renal vein. Continuous sutures using a 7/0 Ethicon silk thread were used. Sampling of the liver tissue was carried out in the hilus, together with the main branches of the hepatic artery and portal vein. The specimens were fixed in a benzoquinone buffered solution (0.4 g/100 ml) for 2 h at room temperature (Bishop et al., 1978). From cryostat blocks, 10 μm sections were cut and collected on poly-L-lysine coated slides (Huang et al., 1983).

Indirect immunofluorescence was performed using antibodies to neuron-specific enolase, a major enzyme in glycolysis, present in central and peripheral neurons (Marangos & Polak, 1982) and a marker for the diffuse endocrine system (Bishop et al., 1985); neurofilaments, as a part of the cytoskeletal network (Osborne & Weber, 1983); S-100 protein and glial fibrillary acidic protein (GFAP), both markers of different types of glial cells (Bishop et al., 1985). All specific antisera were kindly supplied by Professor J. M. Polak (Hammersmith Hospital, London).

Routine controls for the specificity of the immunostaining were carried out using non-immune primary antisera or deletion of one step of the procedure.

Results

At the day of sacrifice groups A and D showed a normal liver weight/body weight ratio, whereas in groups B and C a significant decrease was observed (Table 1). Although the number of liver lobes in group A was decreased they all showed a normal macroscopic appearance. A light microscope picture was also obtained (not shown).

Table 1 shows the results of the immunofluorescence study of nerve fibres and plexus in the liver hilus. A rich network of nerve fibres was found in groups A and D with respect to neuron-specific enolase neurofilaments, S-100 protein and GFAP. Although qualitatively the results were identical in Groups A and D, these parameters of nerve tissue appeared to be more pronounced in regenerating livers than in the controls: in group A the nerve

<table>
<thead>
<tr>
<th>Group</th>
<th>Procedure</th>
<th>Liverweight/bodyweight × 100</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>70% resection, normal blood supply</td>
<td>3.57 ± 0.2</td>
</tr>
<tr>
<td>B</td>
<td>70% resection, portal blood</td>
<td>2.27 ± 0.2*</td>
</tr>
<tr>
<td>C</td>
<td>70% resection, arterial blood</td>
<td>2.36 ± 0.5*</td>
</tr>
<tr>
<td>D</td>
<td>sham operated</td>
<td>3.51 ± 0.1</td>
</tr>
</tbody>
</table>

* p < 0.05, compared to group D.

Table 2. Semi-quantitative assessment of immunocytochemical results.

<table>
<thead>
<tr>
<th>Antisera</th>
<th>Neuron-specific enolase</th>
<th>Neurofilaments</th>
<th>Protein</th>
<th>GFAP</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>++</td>
<td>++</td>
<td>+++</td>
<td>++</td>
</tr>
<tr>
<td>B</td>
<td>-</td>
<td>+</td>
<td>+/−</td>
<td>−</td>
</tr>
<tr>
<td>C</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>D</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

−, Negative immunostaining; +/−, weak equivocal; +, positive staining; ++, intense immunostaining, increased number of nerves; ++++, very intense staining, great amount of nerves.