Effect of Partial Hepatectomy on Tyzzer’s Disease of Mice

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ABSTRACT. Effect of partial hepatectomy on experimental Tyzzer’s disease was studied. When the 2/3-hepatectomy was performed shortly after infection, severer hepatic lesions were produced in operated mice than sham-operated ones. No effect was observed when mice were hepatectomized three days before infection. Hepatic lesions after the 2/3-hepatectomy were severer as compared with the 1/3-hepatectomy. The results suggested that hepatocellular activation after hepatectomy might enhance the growth of organisms.—KEY WORDS: mouse, partial hepatectomy, Tyzzer’s disease.

Tyzzer’s disease in mice is characterized by multiple focal necroses in the liver. Histopathologically, the organisms are detectable mainly in the cytoplasm of liver cells surrounding the necrotic foci [3, 5, 17].

In our pervious reports [6, 9, 15] the importance of the hepatocellular activities in the growth of organisms was suggested. The formation of liver lesions was markedly enhanced by cortisone [6] or CCl4 [15] treatments, whereas it was markedly reduced by fasting or feeding with low protein diet after inoculation [9]. During infection after CCl4 administration, necrotic foci with intracellular organisms were observed mostly at the intermediate zone rather than at the central zone of hepatic lobuli, suggesting that organisms preferably proliferate in activated hepatocytes at the intermediate zone covering the decreased or lost activity of the necrotized centrilobular zone due to CCl4 administration.

Since the rapid hepatic regeneration after partial hepatectomy was studied by many authors [1, 8, 16, 18, 19, 21], we attempted to see the effects of partial hepatectomy on the propagation of organisms in the regenerating liver.

MATERIALS AND METHODS

Animals: Four- to five-week-old female ICR mice (Charles River Japan, Atsugi, Kanagawa) weighing 17 to 21 g were used. They were housed in aluminium cages with a filter cap (Sanki-Kagaku, Tokyo) and were given commercial pellets (Oriental Yeast, Tokyo) and water ad libitum.

Partial hepatectomy: The 2/3-hepatectomy was performed with a slight modification by the method of Higgins and Anderson [8]. Animals were anesthetized with ether and the left lateral, left median, and the quadrate lobes of the liver were ligated with surgical No. 2 silk thread (Shirokawa-Shigyo, Tokyo) and excised. In case of the 1/3-hepatectomy, the left median and quadrate lobes were removed. In sham-operated controls, the skin and peritoneum were incised and the liver was gently pulled out of and put back to the abdominal cavity.

Inoculation: Tissues of the mouse liver infected with the MSK strain of Tyzzer’s organisms [4], having been stored at −80°C, were emulsified in Trypticase Soy Broth (TSB-B; BBL, Cockeysville, Maryland), pH 7.3, and 0.2 ml of a 1:100 emulsion was
Table 1. Time of partial hepatectomy and disease indices

<table>
<thead>
<tr>
<th></th>
<th>Hepatectomy on Day p.i.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>-7 -4 -3 -2 0 1 2</td>
</tr>
<tr>
<td>Exp. I</td>
<td>1.7(10) 1.8(9) 2.3(9) 1.7(7) 2.4(8) 2.0(9)</td>
</tr>
<tr>
<td>Exp. II</td>
<td>3.7(9) 3.3(8) 4.3(7) 3.4(9) 3.1(9)</td>
</tr>
</tbody>
</table>

a) Inoculated with $5 \times 10^5$ organisms.
b) Inoculated with 1.7 to $1.9 \times 10^6$ organisms.
c) In parenthesis Number of test animals.

Table 2. Disease index in 2/3-hepatectomized and sham-operated mice

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Number of animals</th>
<th>Disease index (Range)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Day -3</td>
<td>Hepatectomized</td>
<td>3.1±1.4 (2-6)</td>
</tr>
<tr>
<td></td>
<td>Sham-operated</td>
<td>2.6±0.8 (2-4)</td>
</tr>
<tr>
<td>Day 0</td>
<td>Hepatectomized</td>
<td>4.4±2.2 (2-7)*</td>
</tr>
<tr>
<td></td>
<td>Sham-operated</td>
<td>2.3±1.1 (1-4)*</td>
</tr>
</tbody>
</table>

a) Mice were inoculated with 2.2 to $3.2 \times 10^6$ organisms.
b) Mean±SD.
* Significant difference of p<0.05 between the two groups.

inoculated intravenously into mice. The number of organisms was counted as previously reported [14].

Disease index: Infected animals were euthanized by chloroform at 7 days postinoculation (p.i.) and the livers were graded from score 1 to 4 according to its severity of lesions [6]. Mice dead on Day 7 p.i. were given score 5, and those dead 'n' days before Day 7 p.i. were given a score of (5+n). The mean of scores in each group was calculated and used as a disease index representing the severity of disease. The statistical difference between groups was examined by Student's t test.

Histopathology: Tissue samples from the liver were fixed in 10% neutral buffered formalin and embedded in paraffin by a routine procedure. Sections 2 μm thick were stained with hematoxylin-eosin (HE) and Gomori's methenamine silver (GMS) method [7].

RESULTS

The 2/3-hepatectomy was performed on Days -7 to -2 p.i. and shortly after inoculation (Day 0) with $5 \times 10^5$ organisms. The operated and infected mice were examined for hepatic lesions on Day 7 p.i. As shown in Table 1, in the experiment I (Exp. I) no significant difference in the disease index was seen between the groups, although the indices of groups hepatectomized on Day -3 and Day 0 were slightly higher than those of others.

In the next experiment (Exp. II), the 2/3-hepatectomy was done on Day -2 to Day 2 p.i. with 1.7 to $1.9 \times 10^6$ organisms, and hepatic lesions on Day 7 p.i. were observed. No significant difference was seen between the groups, while hepatic lesions of groups operated on Day -3 and Day 0 were slightly higher than those of others (Table 1).

In the next experiment, comparison of hepatic lesions were made between the 2/3-hepatectomized mice and those sham-operated on Day -3 or Day 0 p.i. with 2.2 to $3.2 \times 10^6$ organisms. As shown in Table 2, on Day 7 p.i., Day 0 hepatectomized mice had severer hepatic lesions than Day 0 sham-operated mice (p<0.05). In case of mice hepatecto-
Fig. 1. Mitosis in liver cells in non-affected areas. (a) Frequent metaphase and anaphase, 2 days after 2/3-hepatectomy. (b) Still frequent metaphase and two separate daughter cells (arrow), 3 days after 2/3-hepatectomy. HE stain, ×660.
Fig. 2. Disease index curves after inoculation with 1.3 to $1.9 \times 10^6$ organisms.

Fig. 3. Organisms within hepatocytes near necrotic foci in 2/3-hepatectomized (a), sham-operated (b), and non-operated (c) mice. Day 4 p.i. GMS-HE stain, $\times 660$. 
mized on Day –3, however, there was no difference from Day –3 sham-operated group. According to those results, the partial hepatectomy was done on Day 0 in the subsequent experiments.

The next experiment was done to see changes of the disease scores with time after inoculation. Eighty mice were inoculated with 1.3 to 1.9 × 10⁶ organisms and they were divided into three groups. Shortly after inoculation, the first group was subjected to the 2/3-hepatectomy, while the second was sham-operated. The third was non-operated. Three or four mice of each group were killed every day for 7 days and examined for their hepatic lesions. In the hepatectomized mice mitotic figures were observed most frequently on Days 2 and 3 but less frequently thereafter (Fig. 1). The hepatectomized mice had liver cells as well as their nucleus larger than those of other groups of mice.

On Day 2 p.i., all mice examined were shown to have score 1 lesions in the liver (Fig. 2). Hepatectomized and sham-operated mice showed similar disease scores on Days 3 and 4. Later, however, hepatectomized mice showed higher scores than sham-operated mice. On Day 4, many organisms were present in hepatocytes surrounding necrotic foci of the liver in each group. Intracellular growth of organisms was much more frequently encountered in the hepatectomized mice (Fig. 3). Organisms were present in seemingly intact or slightly degenerated hepatocytes but not in those under mitosis.

In the following experiment, effects of fasting after inoculation were examined in 2/3-hepatectomized and sham-operated mice. Forty-two mice were inoculated with 1.5 × 10⁶ organisms and they were divided into three groups just as in the foregoing experiment. A half of each group was deprived of pellets for 72 hr after inoculation while the other half was fed ad libitum. As shown in Table 3, without fasting, sham-operated mice (2.0) showed a significantly smaller disease index than those of hepatectomized (4.3, p<0.02) or non-fasted (4.0, p<0.01) mice, as already shown in the foregoing experiments. Hepatectomized mice were shown to have a lower grade lesion when they were fasted for 72 hr after infection (p<0.1). In sham-operated mice, however, there was no significant difference between non-fasted and fasted mice.

Since the lower grade of lesions in sham-operated mice might result from decrease in food consumption after operation, food con-
Table 4. Effect of 24 hr fasting on food consumption

<table>
<thead>
<tr>
<th>Treatment a)</th>
<th>Number of animals</th>
<th>Daily food consumption per mouse (g)</th>
<th>Disease index</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1d</td>
<td>2d</td>
<td>3d</td>
</tr>
<tr>
<td>2/3-Hepatectomized</td>
<td>7</td>
<td>0.63</td>
<td>1.13</td>
</tr>
<tr>
<td>(fasted for 24 hr)</td>
<td>7</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sham-operated</td>
<td>8</td>
<td>1.88</td>
<td>2.13</td>
</tr>
<tr>
<td>(fasted for 24 hr)</td>
<td>8</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Non-operated</td>
<td>8</td>
<td>2.50</td>
<td>3.63</td>
</tr>
<tr>
<td>(fasted for 24 hr)</td>
<td>8</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

a) Shortly after inoculation with $7.6 \times 10^9$ organisms.

* Significant difference of $p<0.01$ between the two groups.

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Table 5. Disease index and food consumption in 2/3- and 1/3-hepatectomized mice

<table>
<thead>
<tr>
<th>Treatment a)</th>
<th>Number of animals</th>
<th>Daily food consumption per mouse (g)</th>
<th>Disease index</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1d</td>
<td>2d</td>
<td>3d</td>
</tr>
<tr>
<td>Hepatectomized</td>
<td>2/3</td>
<td>8</td>
<td>0.36</td>
</tr>
<tr>
<td>1/3</td>
<td>11</td>
<td>0.64</td>
<td>0.64</td>
</tr>
<tr>
<td>Sham-operated</td>
<td>11</td>
<td>1.09</td>
<td>1.18</td>
</tr>
<tr>
<td>Non-operated</td>
<td>11</td>
<td>2.00</td>
<td>3.18</td>
</tr>
</tbody>
</table>

a) Shortly after inoculation with $8 \times 10^9$ organisms.

* Significant difference of $p<0.05$ between the two groups.

** Significant difference of $p<0.01$ between the two groups.

Consumption was checked in both 2/3-hepatectomized and sham-operated mice which were infected and fasted for 24 hr p.i. Forty-six mice were inoculated with $7.6 \times 10^5$ organisms and shortly after inoculation, fourteen of them were hepatectomized while sixteen sham-operated. The remaining sixteen were non-operated. A half of each group was fasted for 24 hr p.i. and the other half was fed *ad libitum*. Food consumption in each group was measured every day for 7 days p.i. On Day 7 p.i. mice were killed and examined for hepatic lesions (Table 4).

On Day 2, the food consumption of mice fasted for 24 hr was greater than that of mice fed *ad libitum* in sham- and non-operated groups. Daily food consumption per mouse was not greatly different between these two groups. In hepatectomized mice, however, the consumption was about 1/2~1/3 as compared with other two groups. There was no difference in the disease index between mice fed *ad libitum* and those fasted for 24 hr in each group. When mice were fed *ad libitum*, the disease index of hepatectomized mice was significantly higher than that of sham-operated mice ($p<0.01$), as already indicated. In this experiment, the disease index of hepatectomized mice was greater than that of non-operated mice.

Next, the disease index was compared between groups with 1/3- or 2/3-hepatectomy. Forty-two mice were inoculated with $8 \times 10^5$ organisms and they were divided into four groups. Shortly after inoculation, the first group was subjected to 2/3-hepatectomy,
while the second was to 1/3-hepatectomy. The third was sham-operated and the fourth was non-operated. Daily food consumption in each group was measured and disease scores on Day 7 p.i. were recorded. The mean weights of excised liver tissues taken from 2/3- and 1/3-hepatectomized mice at operation were 0.91 g (0.83–1.14 g) and 0.4 g (0.34–0.50 g), respectively.

As shown in Table 5, the disease index of 2/3-hepatectomized group was significantly greater than those of 1/3-hepatectomized (p<0.05) or sham-operated (p<0.01) groups. The 1/3-hepatectomized mice showed a slightly greater disease index than sham-operated ones. In this experiment, there was no great difference in food consumption between the four groups.

DISCUSSION

In this study, a significant difference in disease indices was observed between the 2/3-hepatectomy group and sham-operated group, suggesting the propagation of Tyzzer’s organisms depending upon metabolic activation of hepatocytes.

When operation was performed 3 days before inoculation, no significant difference in disease indices was seen between hepatectomized group and sham-operated one. In this case, metabolic activation of hepatocytes had been peaking at inoculation [2, 19, 21], and organisms might be unable to grow under already decreasing metabolic activities of host hepatocytes. In contrast, when inoculation and partial hepatectomy were performed on the same day, the increased activities of hepatocellular metabolism might remarkably benefit the proliferation of organisms.

On Day 4 p.i., the number of organisms in the liver cells of hepatectomized mice was greater than that of sham-operated mice and there was difference in disease scores between both groups, indicating that a maximum intracellular growth of organisms might occur concomitantly with the enlargement of hepatocyte cytoplasm after mitosis following the partial hepatectomy [16, 19].

Without fasting, sham-operated mice had smaller disease indices as compared with non-operated ones, suggesting that some factor(s) of metabolism or defence might be activated due to sham-operation. In hepatectomized mice, a favorable state for microbial growth resulting from hepatocellular activation might overcome these factor(s).

The 2/3-hepatectomized mice showed a significantly higher disease index than the 1/3-hepatectomized or sham-operated mice. In 1/3-hepatectomized mice, metabolic activities of hepatocytes during regeneration following operation might be lower than those in 2/3-hepatectomized ones [1], and the propagation of organisms in the liver seemed not different from that in sham-operated mice.

On the other hand, hepatic lesions in mice fasted for 72 hr p.i. were less remarkable than those in fed control both in hepatectomized and non-operated mice. However, such effect was not observed in sham-operated mice, probably because of the involvement of some factor(s) of nutrition or defence. There was no difference in disease indices between mice fasted 24 hr and non-fasted mice, indicating that the hepatic lesions in operated mice might not be affected by decrease in food consumption on the first day after operation. Daily food consumption of sham-operated mice either non-fasted or 24 hr-fasted was not different from that of non-fasted and non-operated mice, whereas disease indices of sham-operated mice were lower than those of non-operated ones. These results suggested that the difference might be due to some factor(s) of defence but not to those of metabolism, while the effect of 72 hr-fasting in sham-operated mice might have resulted from decrease in food consumption during the first three days.

The mean daily food consumption of hepatectomized mice was only 1/2~1/3 as com-
pared with sham-operated mice, suggesting difference in surgical stress between partial hepatectomy and sham-operation. It is conceivable that surgical stress due to partial hepatectomy was strong enough to produce more severe hepatic lesions. However, hepatectomized mice and non-operated mice showed similar hepatic lesions, indicating that the stress effect of partial hepatectomy was not equivalent to that induced by hydrocortisone or cyclophosphamide [6, 12]. It was reported that not only partial hepatectomy but also acute stresses induced the increase of protein synthesis in the liver [10]. On the other hand, the immune system has been shown to be activated during liver regeneration after partial hepatectomy [11, 13, 20].

REFERENCES


要約

マウス Tyzzer 病における肝部分切除の効果：二井愛介・中山裕之・藤原公策（東京大学農学部家畜病理学教室）——ICR マウスに MSK 株接種直後に肝 2/3 部分切除を行なうことにより，Tyzzer 病は増悪された。接種前 3 日の肝切除では，この効果はみられなかった。また肝 2/3 切除群では，1/3 切除群よりも肝病変は重度であった。これらの成績から，肝切除後の肝細胞代謝活性の変化が，菌増殖を促進することが示唆された。