SHORT COMMUNICATION

The effect of 1/3 partial hepatectomy on the growth of glutathione S-transferase positive foci

Aroon Yusuf, Ezio Lacconi1, Prema M.Rao, Srinivasan Rajalakshmi and Dittakavi S.R.Sarma2

Department of Laboratory Medicine and Pathobiology, University of Toronto, Medical Sciences Building, Toronto, Ontario M5S 1A8, Canada and 1Ospedale Oncologico ‘A. Businco’ and Istituto di Patologia Sperimentale Universita di Cagliari, Cagliari, Italy

2To whom correspondence should be addressed
Email: sarma.dittakavi@utoronto.ca

Our previous studies indicated that glutathione S-transferase 7-7 (GST 7-7) positive foci induced after initiation have a lower threshold towards proliferative stimuli compared with surrounding hepatocytes. This observation would predict that persistent growth stimuli of low intensity could be very effective in promoting the emergence of focal lesions. To test this possibility, the present study was designed to determine the effect of 1/3 partial hepatectomy (PH) on the incidence and growth of foci in initiated rat liver. The rationale for using a 1/3 PH was that it is known to induce a proliferative response which is less intense but more prolonged compared with that elicited by 2/3 PH. Male Fischer 344 rats (110–120 g) were initiated with diethylnitrosamine (200 mg/kg, i.p.). Three weeks later 1/3 PH (median lobe), 2/3 PH (median and left lobes) or sham operation (SH) was performed. An additional group of initiated animals had the median lobe and the left lobe of the liver removed sequentially (1/3 + 1/3 PH), 3 weeks apart. All rats were killed 8 weeks after carcinogen administration. The results indicated that the number of GST 7-7 positive foci was similar in all groups; however, the percent area occupied by foci was increased in rats receiving 2/3 PH compared with SH (0.01 ± 0.08 versus 0.09 ± 0.03). Interestingly, 1/3 PH was nearly as effective as 2/3 PH in stimulating the growth of foci (percent area 0.18 ± 0.06 versus 0.21 ± 0.08), although the magnitude of the stimulus is only half for the former group compared with the latter; peak labeling index was 19 ± 6 with 1/3 PH compared with 40 ± 2 with 2/3 PH. Moreover, the maximum increase in the size of foci (percent area 0.37 ± 0.12) was achieved when the median and left lobes were removed sequentially, three weeks apart. These results indicate that persistent growth stimuli of low intensity can be very effective in promoting the growth of focal lesions.

Initiated hepatocytes are characterized by their ability to form focal proliferations when exposed to tumor promoting regimens (1,2). One phenotypic attribute of biological relevance to initiated cells is their resistance to the cytotoxic effects of several agents (1,3–8). Such a resistance can provide the basis for their selective growth under conditions that are non-permissive for the surrounding, non-initiated cells. However, growth of initiated hepatocytes also occurs, albeit at low level, in initiated rat liver without exposure to any exogenous promoting regimen. Thus, there may be other phenotypic properties, in addition to ‘resistance’, which may explain how focal growth takes place during the carcinogenic process in the absence of exogenous tumor promoter. In fact there have been reports indicating an enhanced proliferative response to direct mitogenic agents such as phenobarbital, α-hexachlorocyclohexane, cyproterone acetate and nafenopin in enzyme-altered foci appearing early during rat liver carcinogenesis (9,10). Although these studies generated important information, the possibility that the increased response of enzyme-altered hepatocytes could be related to differences in the metabolism of the mitogenic chemicals was not ruled out. To address this issue, we examined the kinetics of the response of enzyme-altered foci following 2/3 partial hepatectomy (PH). The results obtained by us (11) as well as those of others (12) indicated that enzyme-altered foci have an earlier response towards the growth stimulus induced by 2/3 PH, compared with surrounding parenchyma.

Based on this phenotypic property of initiated cells, one can postulate that prolonged proliferative stimuli of relatively low intensity may be very effective in stimulating the emergence of enzyme-altered foci. This consideration is important in explaining focal proliferation of initiated hepatocytes in the absence of any exogenous tumor promoting regimens. If in fact the initiated cell population can preferentially respond to the growth stimulus, this will result in selective expansion of the initiated hepatocytes as long as the stimulus persists. To test this possibility, in the present investigation we compared the effect of 1/3 versus 2/3 PH in stimulating the growth of enzyme-altered foci in initiated rat liver. The rationale for comparing these two treatments is that the response pattern of the liver is remarkably different in these two instances. One-third PH is in fact known to induce a proliferative response that is much less intense but more prolonged compared with that elicited by 2/3 PH (13–15).

We first examined the kinetics of liver regeneration following 1/3 or 2/3 PH under the experimental conditions of the present study. Two-month-old male Fischer 344 rats were subjected to either a 1/3 (median lobe removed) or 2/3 PH (median and left lobes removed) and killed at different time points thereafter. Tritiated thymidine was given to each animal 2 h before killing. Liver samples were processed for standard histological analysis and for autoradiography. Results are reported in Figure 1. As expected, rats given a 2/3 PH exhibited a peak labeling index at 24 h (40 ± 2%), declining rapidly within 48 h (7 ± 1%) and returning close to baseline levels by 4 days (1 ± 0.5%). In contrast, the proliferative response to 1/3 PH was undetectable at 24 h, was still low at 48 h (11 ± 3%), peaked at day 3 (19 ± 6%) and remained well above baseline for at least 5 days (15 ± 4%). However, the peak labeling index in response to 1/3 PH was <50% of that seen with 2/3 PH. These data are in agreement with previous reports in the literature (13–16). They clearly indicate that the kinetics of

Abbreviations: GST, glutathione S-transferase; DEN, diethylnitrosamine; PH, partial hepatectomy; SH, sham hepatectomy.

© Oxford University Press
Fig. 1. The kinetics of liver regeneration after 1/3 or 2/3 PH. Two-month-old male Fischer 344 rats (110–120 g) were subjected to either a 1/3 or 2/3 PH and killed at different time points. A single injection of 3H-labeled thymidine (100 μCi/rat, sp. act. 80.9 Ci/mmol; New England Nuclear, Montreal, Quebec) was given to each animal 2 h before killing. Rats were killed under mild anesthesia. Liver samples were processed for standard histological analysis and for autoradiography (17). Labeled nuclei were counted and expressed as percent of the total hepatocytes. At least 3000 nuclei were scored for each sample. Data represent means ± SD of five rats.

<table>
<thead>
<tr>
<th>Group</th>
<th>mg DNA/100 g liver</th>
<th>mg DNA/liver</th>
<th>mg DNA/liver/100 g body wt</th>
</tr>
</thead>
<tbody>
<tr>
<td>SH</td>
<td>2.13 ± 0.10</td>
<td>21.49 ± 1.39</td>
<td>6.96 ± 0.42</td>
</tr>
<tr>
<td>2/3 PH</td>
<td>2.28 ± 0.15</td>
<td>19.99 ± 1.72</td>
<td>6.64 ± 0.46</td>
</tr>
<tr>
<td>1/3 PH</td>
<td>2.25 ± 0.13</td>
<td>19.97 ± 2.14</td>
<td>6.69 ± 0.47</td>
</tr>
<tr>
<td>1/3 + 1/3 PH</td>
<td>2.29 ± 0.13*</td>
<td>17.19 ± 1.78***</td>
<td>6.05 ± 0.30***</td>
</tr>
</tbody>
</table>

Data represent means ± SD of 3–5 animals. Liver samples derived from the experiment described in Table I were used for the measurement of liver DNA. Total DNA content was measured in the PCA precipitable fraction of the liver homogenate (17).

Table I. The effect of 1/3, 2/3 or 1/3 + 1/3 PH on number and mean foci area 8 weeks post-initiation.

<table>
<thead>
<tr>
<th>Partial hepatectomy GST 7-7 positive foci</th>
<th>No./cm²</th>
<th>Mean focus area</th>
<th>Percent focus area</th>
</tr>
</thead>
<tbody>
<tr>
<td>SH</td>
<td>5 ± 1.19</td>
<td>0.017 ± 0.002*</td>
<td>0.09 ± 0.026***</td>
</tr>
<tr>
<td>2/3 PH</td>
<td>5 ± 1.16</td>
<td>0.042 ± 0.013*</td>
<td>0.21 ± 0.075***</td>
</tr>
<tr>
<td>1/3</td>
<td>6 ± 1.23</td>
<td>0.030 ± 0.005*</td>
<td>0.18 ± 0.056****</td>
</tr>
<tr>
<td>1/3 + 1/3</td>
<td>8 ± 2.79</td>
<td>0.049 ± 0.009**</td>
<td>0.37 ± 0.115****</td>
</tr>
</tbody>
</table>

Data represent means ± SD of 3–5 animals. See text for details of experimental procedure.

*P < 0.005, all groups versus SH; **P < 0.001, 1/3 + 1/3 PH versus 1/3 PH; ***P < 0.01, all groups versus SH; ****P < 0.001, 1/3 + 1/3 PH versus 1/3 PH or P < 0.003, 1/3 + 1/3 PH versus 2/3 PH.

response to either 1/3 or 2/3 PH are remarkably different with respect to the time of maximum hepatocyte DNA synthesis, the level of the peak response and the duration of the proliferative stimulus.

The next study was performed to compare the effect of 1/3 and 2/3 PH on the growth of enzyme-altered foci in initiated liver. Male Fischer 344 rats were injected with diethylnitrosamine (DEN; 200 mg/Kg, i.p.) and 3 weeks later they were subjected to sham hepatectomy (SH), 1/3 or 2/3 PH; an additional group of animals underwent removal of the median lobe first, and 3 weeks later the left lobe was removed (1/3 + 1/3 PH). All rats were killed 8 weeks after DEN administration. Liver samples were processed for glutathione S-transferase (GST) 7-7 positive foci and for DNA content (17).

The results presented in Table I show that there was no difference in the number foci/cm² among the different groups. However, the mean focus area was significantly higher in all groups of animals receiving PH as compared with sham operation. This pattern was further substantiated by the results on percent area occupied by enzyme-altered foci (Table I). Of greater significance was the finding that the percent area occupied by the foci was very similar in the 1/3 and 2/3 PH groups, although the magnitude of the total stimulus is clearly different in these two cases. Moreover, rats undergoing sequential removal of the median and the left lobes showed the highest value in percent area occupied by foci, and yet the magnitude of the total stimulus was roughly comparable with that of 2/3 PH. Furthermore, the observation that 1/3 PH promoted the growth of initiated hepatocytes is in itself interesting in view of the fact that only half of the remaining 2/3 of hepatocytes need to respond to proliferate following 1/3 PH.

Levels of total liver DNA in different groups and the amount of liver DNA per 100 g body weight at the end of 8 weeks are reported in Table II. No significant decrease in this parameter was observed between the 1/3 (6.69 ± 0.47 mg) and 2/3 (6.64 ± 0.46 mg) PH groups and the SH controls (6.96 ± 0.42 mg), while liver DNA content was significantly lower in animals which had the median and left lobes removed 3 weeks apart (6.05 ± 0.30 mg/100 g body wt) compared with SH group (P < 0.002). These results indicate that liver regeneration is largely complete in all experimental groups at the time point considered, although a slight delay in the response seems to be present and being more accentuated in the group having the median and left lobes removed sequentially.

Taken together, the above results suggest that the duration more than the intensity of a proliferative stimulus may be an important determinant for its overall effect on the selective growth of focal lesions. This conclusion is also consistent with the findings indicating that initiated hepatocytes are more efficient in their response to various growth stimuli, including the one(s) elicited by PH (11,12). This could be attributed to at least two reasons: (i) an inherent increased responsiveness of initiated cells to proliferative signals (9–12) and/or (ii) a primary defect in the surrounding non-initiated cells, such that their response to growth stimuli is delayed. Such a possibility is not unreasonable since most carcinogens, including DEN, are known to inhibit cell proliferation (18,19). Interestingly, liver DNA content was still low in rats given DEN followed by removal of median and left lobe and killed 3 weeks after the last operation. Thus, in summary, these results indicate that persistent growth stimuli of low intensity can be very effective in promoting the growth of focal lesions.
Acknowledgements

This work was supported in part by USPHS grant CA 37077 and funds from National Cancer Institute Canada. A.Y. was supported by the Canadian Liver Foundation.

References


Received December 11, 1998; accepted March 1, 1999

Effect of 1/3 PH and growth of GST positive foci