Quantitative liver function and morphology after paracetamol administration to rats

H. E. POULSEN, P. PETERSEN & H. VILSTRUP, Rigshospitalet, Division of Hepatology and Experimental Pathology, Copenhagen, Denmark

Received 26 August 1980 and in revised form 10 December 1980

Abstract. The functional status of the liver after paracetamol administration to rats was dissociated with regard to cytosolic and microsomal functions. The cytosolic function, measured as galactose elimination capacity, was unchanged even after high doses of paracetamol. The microsomal function, measured as the prothrombin time, was reversibly decreased to a minimum 12 h after paracetamol administration and showed dose dependence. The structural changes of the liver cells were centrilobular necroses and dilatation of the endoplasmic reticulum. They were most marked 36 h after paracetamol and were not correlated to the functional impairment. We conclude that during acute paracetamol induced liver damage to rats different functions of the liver are unequally influenced and that the structural changes occur later than the functional ones. The paracetamol intoxicated rat is a promising model for further investigation of dissociation of subcellular functions during acute liver damage.

Key words. Quantitative liver function, paracetamol, ultrastructure.

Introduction

The galactose elimination capacity (GEC) [1] and the prothrombin index (PP, coagulation factors II, VII, and X) [2] have been claimed to be quantitative measures of 'the functional liver mass' in liver failure. Though representing predominantly cytosolic and microsomal functions, respectively, the two measures are usually found to be 'associated', i.e. impaired to the same degree, in liver disease [3].

We have recently reported a dissociation of the time course of GEC and PP in a patient with paracetamol hepatitis [4].

In normal doses paracetamol is metabolized in the liver mainly by glucuronidation and sulphatation, while a few per cent is oxidized by microsomal P-450 mixed function oxidases to a toxic arylating electrophilic metabolite which is inactivated by conjugation to hepatic glutathione. By overdose the liver is depleted of glutathione and the P-450 metabolite destroys intracellular macromolecules leading to cell death [5].

The aim of the present study was to investigate whether we could reproduce experimentally the dissociation described in the patient and to develop a model for further investigations of different subcellular functions during acute liver failure.

Materials and Methods

Female Wistar rats fed Rostock standard pellets and tap water ad libitum were given paracetamol suspended in 0-2% tragacant gum (300 mg paracetamol/ml) by gastric tube. Control animals were given a corresponding volume of the vehicle. Food was withheld for 12-16 h prior to and 2 h after the administration in order to control the reported feeding dependent susceptibility [6] to paracetamol.

The dose mortality relation was examined by giving different doses of paracetamol to groups of nine to twelve rats. LD_{10} and LD_{90} were calculated from the regression of mortality on dose.

The LD_{10}, 4-25 g/kg, was used in the subsequent time course experiment where rats were divided into three groups, one was used to examine the time course of GEC, one the time course of PP, and the last was evaluated histologically. The groups were divided into subgroups which were examined at 0 h (N=8), 6 h (N=5), 12 h (N=6), 24 h (N=5), 36 h (N=6), 48 h (N=7), 72 h (N=5) and 96 h (N=6) after administration. The dose response of GEC was investigated 24 h after administration of 0 (N=6), 3-25 (N=4), 5-5 (N=6), 7-0 (N=4) and 9-0 g/kg rat (N=5). The 24 h period was chosen because the GEC showed a nadir at this time. The dose response of PP was investigated at 12 h after the administration (N=5, 4, 5, 6, 4 at the stated doses).

9% of the animals died after paracetamol (4 25 g/kg) before the animals were investigated, blood sampling failed in 2%, the mortality of the operation prior to GEC determination was 2%, and 3% of the animals were excluded due to technical failures.

Animals were anaesthetized with thiopental i/p. Galactose was given intravenously as a priming dose (200 μmol/100 g body weight) followed by a con-
tunuous infusion of 1·5–2·5 μmol/min for 1 h. The GEC was calculated as GEC = \( \frac{de}{dt} \times 0.4 \times \text{body weight} \), where \( \frac{de}{dt} \) is the infusion rate, \( \text{body weight} \) is the estimated volume of distribution of galactose [7].

The coagulation factors II, VII and X were determined in aortic blood diluted 1:1 by isotonic saline using Owren’s method [8], values are given as arbitrary units (au%, of control).

Biopsies were obtained from the median lobe of the liver with a 1·8 mm Menghini needle after opening the abdomen. The tissue was washed a few seconds in isotonic saline to remove blood and cut to 2 mm long cylinders. The cylinders were immediately fixed in 1% osmium tetroxide, dehydrated and embedded in Epon. Semi thin (1 μm) sections stained with toluidine blue were examined by conventional light microscopy. The shortest distance from the central vein to the border of necrosis and to the border of hepatocytes with visible fat droplets was measured in μm for all central veins in the section (mean 2·7 veins per section, range 1–8). The shortest distance was used in order to minimize overestimates due to oblique cutting of the central vein. One section was omitted as it did not contain a central vein. Dilatation of endoplasmic reticulum was graded + (1–5 hepatocytes per lobulus with dilated endoplasmic reticulum), ++ (6–20) and +++ (more than 20). Ultra thin sections (50–80 nm) were examined in a Zeiss EM-9S-Z transmission electron microscope. Sections were obtained from the optimally fixed depth (30–200 μm) of the biopsy [9]. All readings were done blindly.

Difference between groups was tested statistically by Kruskal-Wallis one-way analysis of variance and correlation by Spearman rank correlation coefficient [10]. P values less than 0·05 were considered significant.

Results

Fig. 1 shows the dose–mortality relation. \( \text{LD}_{50} \) was 7·0 g/kg ± 1·3 (SD). Death occurred between 5 and 18 h after administration, except in one animal which died after 24 h.

![Figure 1. Relation between dose of paracetamol and mortality in female rats fasted 12–16 h prior to intragastric administration (\( \text{LD}_{50} = 7·0 \text{ g/kg} \pm 1·3 \text{ (SD)} \)).](image)

Fig. 2 shows the time course of GEC (μmol per min and 100 g rat) and PP (fraction of control, arbitrary units, au, after \( \text{LD}_{50} \)). The lowest value of GEC, 78% of control value observed after 24 h, was not statistically different from control value (\( P = 0·13 \)). The lowest PP was observed after 12 h when it was 0·45 (\( P = 2 \times 10^{-4} \)).

The relation at 24 h between dose and GEC, and at 12 h between dose and PP, are shown in Fig. 3. The GEC was unchanged after doses up to 9·0 g/kg (\( \text{LD}_{50} \)). Doses up to 7·0 g/kg reduced the PP linearly to 0·19 ± 0·028 (SEM) but no further reduction was seen at the higher dose.

The examination of semi-thin sections showed sharply demarcated centrilobular necrosis, dilatation of the endoplasmic reticulum, and accumulation of fat droplets in hepatocytes surrounding the necrosis or the central veins.

The extent of necrosis and steatosis 12 h after administration, measured in μm from the central vein, was correlated with PP (\( r = -0·67, \ P = 0·01 \)) (Fig. 4). There was no correlation between GEC or PP and the extent of necrosis in the dose experiments.

![Figure 2. Time course of the galactose elimination capacity (GEC) (○) and prothrombin index (PP) (●) after intragastric administration of 4·25 g paracetamol per kg rat. Symbols and bars indicate mean and standard error of the mean, respectively (\( N = 5–8 \)).](image)

![Figure 3. Relation between the galactose elimination capacity (GEC) (○) and dose of paracetamol given intragastrically 24 h earlier, and between prothrombin index (PP) (●) and paracetamol given intragastrically 12 h earlier. Symbols and bars indicate mean and standard error of the mean, respectively (\( N = 4–8 \)).](image)
Figure 4. Relation between extent of necrosis + steatosis in liver biopsies and PP from rats 12 h after paracetamol given intragastrically in doses from 0 to 9.0 g/kg. The Spearman rank correlation coefficient r = -0.67, P=0.01. Necrosis + steatosis were measured in μm, PP in arbitrary units (au, % of control).

Table 1 shows the light microscopic findings in control liver biopsies and biopsies obtained 6–96 h after paracetamol. 4.25 g/kg. Centrilobular necrosis was most marked 36 h after paracetamol, both as to number and as to extent. Also centrilobular steatosis or steatosis surrounding the centrilobular necrosis was most marked 36 h after paracetamol (Table 1). The findings by examination of semi-thin sections were verified by electron microscopy.

Discussion

This study supports our earlier report [4] of a dissociation of hepatic subcellular functions after paracetamol induced liver necrosis. After 12 h the PP was reduced to a minimum and after 76 h it had returned to normal. The GEC, however, was not significantly changed with time. Examination of the liver biopsies showed centrilobular necrosis and steatosis. The predominant ultrastructural change in the damaged liver cells was dilatation of the endoplasmic reticulum. Both phenomena occurred after 24 h and were at maximum after 36 h. The reduction of PP was dose dependent, while GEC was unchanged after all doses.

Paracetamol caused a dose dependent mortality. The LD₅₀ obtained from our experiments, however, differs from that of other reports [11]. This may be due to differences in strain, sex, or duration of fasting prior to administration [6]. We chose a dose of 4.25 g/kg, corresponding to a mortality of 10%, for the time course study, as we wanted a potentially lethal liver damage without too much bias from examining only surviving animals.

The rapid fall in PP can be caused by decreased synthesis of coagulation factors, which are formed in the microsomes [12–14], or by an enhanced peripheral degradation. Since the half life of the PP proteins in plasma is 6–8 h [15] and because of the linear dose dependence of PP we interpret the fall as a result of decreased synthesis in the microsomes. This interpretation is supported by the finding of normal titres of fibrin degradation products in man during paracetamol induced liver damage [16]. Hence the damage to the endoplasmic reticulum must have taken place within the first few hours after administration of paracetamol.

The ultrastructural changes appeared 24–36 h after the maximum decrease of PP and thus do not reflect the current functional status of the cell. This is emphasized by the observation that the function was already undergoing restoration when the most marked structural changes were seen.

The unchanged GEC could be due to extrahepatic galactose elimination; the capacities we have found are, however, of the same magnitude as in the isolated

<table>
<thead>
<tr>
<th>No. of animals with necrosis/No. of animals examined</th>
<th>Extent of centrilobular necrosis, m x 10⁻⁶ mean (range)</th>
<th>Dilatation of ER in the damaged hepatocytes</th>
<th>No. of animals with necrosis and/or steatosis/No. of animals examined</th>
<th>Extent of centrilobular necrosis and steatosis, m x 10⁻⁶ mean (range)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control*</td>
<td>0</td>
<td>0</td>
<td>0/7</td>
<td>0</td>
</tr>
<tr>
<td>6 h</td>
<td>1/6</td>
<td>4 (0–25)</td>
<td>++</td>
<td>1/6</td>
</tr>
<tr>
<td>12 h</td>
<td>0/6</td>
<td>0</td>
<td>0</td>
<td>2/6</td>
</tr>
<tr>
<td>24 h</td>
<td>2/7</td>
<td>41 (0–250)</td>
<td>++</td>
<td>4/7</td>
</tr>
<tr>
<td>36 h</td>
<td>3/6</td>
<td>96 (0–187)</td>
<td>++ ++ + + +</td>
<td>5/7</td>
</tr>
<tr>
<td>48 h</td>
<td>4/7</td>
<td>78 (0–312)</td>
<td>++ ++ + + +</td>
<td>4/7</td>
</tr>
<tr>
<td>72 h</td>
<td>5/6</td>
<td>77 (0–312)</td>
<td>+ +</td>
<td>5/6</td>
</tr>
<tr>
<td>96 h</td>
<td>3/6</td>
<td>22 (0–112)</td>
<td>+ +</td>
<td>4/6</td>
</tr>
</tbody>
</table>

Grading of dilatation of ER: + (1–5 hepatocytes per lobulus with dilated ER), ++ (6–20) and +++ (more than 20).

* Only vehicle administered.
liver [17]. Neither is the unchanged GEC an expression of lack of reactivity of the GEC during experimental liver injury, since CCl₄ reduces GEC to 30% [7].

The dissociation of the microsomal function (PP) and the cytosolar function (GEC) was more pronounced in the rat than in man [4] as GEC was not influenced at all. After partial hepatectomy, however, the cytosolar enzymes metabolizing galactose show an early and pronounced increase in activity [18]. A similar phenomenon may exist after paracetamol induced liver cell necrosis. Such an induction or very early regenerative response would preserve the total galactose elimination capacity and other cytosolar functions until recovered and regenerated cells were able to maintain the function.

A marked enhancement is seen in microsomal lipid peroxidation measured as diene conjugation [19] 12 h after paracetamol administration, in contrast to indicators of smooth endoplasmic reticulum functions, i.e. N-demethylation of ethylmorphine and ω-hydroxylation of aniline, showing a maximal decrease later than 24 h [19, 20].

Our observations show that liver functions can be chronologically dissociated from each other and from the changes in morphology. The influence of a particular function depends on the type of damage, the half life of the proteins involved, and the regeneration rate of the enzymes. Paracetamol induced liver damage in rats is a promising model for further investigation of the significance of different subcellular functions for the survival of the cell.

Acknowledgments

HEP is a Michelsen Foundation Research Fellow. Supported by Managing Director Jacob Madsen and wife Olga Madsen Foundation. The Central Laboratory of Rigshospitalet kindly provided the PP analyses.

References