Taurocholate induced gastric mucosal injuries in experimental portal hypertension

W J Angerson, J G Geraghty, D C Carter

Abstract
The susceptibility of the gastric mucosa to injury by topical sodium taurocholate (40 mmol/l) in hydrochloric acid (150 mmol/l) was studied in prehepatic and cirrhotic rat models of portal hypertension. Portal venous pressure was increased in rats who had undergone partial portal vein ligation compared with rats that had undergone sham operation on days 3, 7, and 28 after operation (20-6/0-9, 14-8/0-8, and 11±3/0-5 mm Hg v 7±3/0-7, 7±3/0-6, and 8-2/0-2 mm Hg respectively (mean (SEM))). At day 3 gastric mucosal injuries were increased in rats with partial portal vein ligation compared with sham operated rats (55±3/9-4 v 22±3 (10-5) mm², p=0-006), but at the later time intervals there was no significant difference in injuries between the two groups. In rats with carbon tetrachloride induced hepatic cirrhosis, portal pressure was increased (15-6 (1-0) v 6-7 (0-6) mm Hg), but again there was no significant difference in mucosal injuries relative to control animals. We conclude that gastric mucosal defence mechanisms are impaired in acute but not chronic experimental portal hypertension.

There is increasing interest in the effects of portal hypertension on the gastric mucosa. According to a recent review, an average of 30% of portal hypertensive patients with upper gastrointestinal bleeding are found on endoscopy to have haemorrhagic gastric mucosal lesions. Histologically, these are more consistently found to be associated with vascular and microvascular oedema than with the inflammatory changes typical of classic chronic gastritis, and this led McCormack et al to propose the descriptive term 'congestive gastropathy.' Little is known, however, about the pathogenesis of gastric mucosal lesions in portal hypertension and the factors that precipitate bleeding. In particular, there is considerable uncertainty about the respective roles of portal hypertension per se, liver damage, and reduced mucosal blood flow and oxygenation.

It has been shown experimentally that the gastric mucosa in portal hypertensive rats has impaired resistance to damage by several ulcerogenic agents, but this evidence relates to a model of acute prehepatic portal hypertension in which it is difficult to differentiate between the effects of increased portal venous pressure and reduced mucosal blood flow. We have previously shown that the induction of prehepatic portal hypertension in rats initially precipitates a reduction in gastric mucosal blood flow, but that perfusion subsequently returns to a normal or above normal level despite the maintenance of high portal venous pressures. The present study was designed to elucidate the effect of these haemodynamic changes on gastric mucosal susceptibility to injury and to determine whether hepatic cirrhosis modifies the effect of portal hypertension alone.

Methods
Male Sprague-Dawley rats (Bantin and Kingman, Hull, UK) were used in all experiments. They were housed in a controlled environment with a 12 hour light/dark cycle and were fed on standard rat diet with water freely available.

PREHEPATIC PORTAL HYPERTENSION
Portal hypertension was produced in animals weighing 320-360 g by creating a calibrated portal vein stenosis. Under light ether anaesthesia, the abdomen was opened via an upper midline incision and a 21 gauge needle was placed alongside the mobilised portal vein. A 3-0 silk ligature was tied around both the needle and portal vein; the needle was then removed resulting in a localised resistance to portal venous flow. In sham operated control rats, the portal vein was mobilised but not stenosed. After closure of the abdomen, animals were returned to their cages and allowed to recover from anaesthesia.

HEPATIC CIRRHOSIS
Hepatic cirrhosis was produced by weekly gavage with carbon tetrachloride (CCl₄) in animals with an initial weight of 130–170 g. They were given phenobarbitone (50 mg/100 ml) in their drinking water, starting 10 days before the first dose of CCl₄ was administered, to induce cytochrome P450 and increase the sensitivity of the liver to CCl₄. Animals were gavaged under light halothane anaesthesia with an initial dose of 0·15 ml CCl₄, and were weighed daily to assess their response, which varied considerably between individual animals. The loss of weight which followed each dose was used to estimate the subsequent dose. CCl₄ administration was stopped when ascites developed (as signalled by a rapid increase in body weight); this occurred after 8–12 weeks (mean 10 weeks). Control animals underwent identical procedures including weekly orogastric intubation under anaesthesia, but did not receive CCl₄.

PORTAL VENOUS PRESSURE MEASUREMENTS
Portal venous pressure was measured in separate groups of animals on days 3, 7, and 28 after portal vein ligation or, in the cirrhotic model,
two to three weeks after administration of the last dose of CCl₄ or stopping control procedures. Animals were fasted overnight in individual, wire bottomed steel cages, but were allowed free access to water until one hour before the experiment. Anaesthesia was induced with 4% halothane and maintained with 0.5–0.75% halothane in a 2:1 nitrous oxide/oxygen mixture, using artificial ventilation (Harvard Rodent Respirator, model no 680). A femoral artery and an ileocolic tributary of the portal vein were cannulated for measurement of arterial pressure and portal venous pressure respectively, using a strain gauge transducer and a Gould 8000 series dual recorder. Arterial blood gas measurements (ABL, Radiometer) showed that all animals had a PaO₂ over 100 mm Hg, a PaCO₂ in the range 35–42 mm Hg, and an arterial pH over 7·30. Core temperature was measured with a rectal thermometer and was kept at 37±0.5°C by means of a heat lamp. Animals were given heparin (130 U/100 g) to prevent clotting within the canulae, and portal venous pressure measurements were accepted only when a respiratory pattern could be obtained on portal venous tracings. Definitive measurements of mean arterial and portal venous pressures were performed simultaneously when animals were haemodynamically stable, at least 30 minutes after the end of surgery.

At the end of the experiment, the stomach was opened and assessed macroscopically for spontaneous mucosal injuries. Tissue samples of the stomach were fixed and stained with haematoxylin and eosin for histological assessment. When cirrhotic animals and their controls were being studied, liver samples were also assessed histologically. Blood samples were taken for measurement of serum concentration of bilirubin, total protein, albumin, alanine aminotransferase, aspartate aminotransaminase, and alkaline phosphatase.

GAstric mucosal injury studies

Studies were performed in separate groups of fasted animals at the same time intervals as specified above. To compensate for time or batch related changes in mucosal susceptibility to injury, groups comprising approximately equal numbers of portal hypertensive animals and their corresponding controls were studied in random order on any given experimental day.

Under light ether anaesthesia, the abdomen was opened and the pylorus ligated with 3/0 silk. The abdomen was closed and 3 ml of a solution of 40 mmol/l sodium taurocholate in 150 mmol/l hydrochloric acid were administered via an orogastric tube. Animals were returned to their cages for two hours, and were then killed by ether overdose. The stomach was removed immediately, opened along the greater curvature, and rinsed clean in water, revealing discrete, clearly delineated mucosal lesions that were generally linear in form. An injury score was derived by multiplying the length of individual lesions by their maximum width (in mm) and summing these products for the whole stomach.¹ The scoring was performed by a single observer who was unaware of the experimental group to which each stomach belonged. Previous assessment of the reproducibility of scoring by this technique, as determined by repeated measurements on a separate series of 24 stomachs presented in random order on two different days, showed an intraobserver coefficient of variation of 7%.

**STANSTICAL ANALYSIS**

The Mann-Whitney U test was used for all statistical comparisons. Differences were considered significant if the probability of their arising by chance was less than 5%.

**Results**

In the rats that had undergone portal vein ligation, portal venous pressure was raised by 14·3, 7·5, and 3·1 mm Hg relative to control values on days 3, 7, and 28 after operation respectively (Table I). There were no significant differences in mean arterial pressures between any of the experimental groups in the prehepatic model.

All animals that received CCl₄ had histological evidence of micronodular cirrhosis. Relative to control animals, they showed an increase in portal venous pressure of 8·9 mm Hg, reduced mean arterial pressure, hypoalbuminaemia, and raised plasma bilirubin and liver enzyme values (Table II).

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**Table I** Pressure measurements in a prehepatic portal hypertensive model

<table>
<thead>
<tr>
<th>Surgical procedure</th>
<th>PVL</th>
<th>SO</th>
<th>PVL</th>
<th>SO</th>
<th>PVL</th>
<th>SO</th>
</tr>
</thead>
<tbody>
<tr>
<td>No of animals</td>
<td>3</td>
<td>9</td>
<td>7</td>
<td>10</td>
<td>6</td>
<td>10</td>
</tr>
<tr>
<td>Mean (SEM) arterial pressure (mm Hg)</td>
<td>102±2 (4-4)</td>
<td>102±6</td>
<td>100±4 (4-2)</td>
<td>102±5</td>
<td>102±5</td>
<td>100±3 (2-4)</td>
</tr>
<tr>
<td>Mean (SEM) portal venous pressure (mm Hg)</td>
<td>20±6 (5-3)</td>
<td>14±6 (3-3)</td>
<td>14±6 (5-3)</td>
<td>11±6 (6-3)</td>
<td>8±2 (2-6)</td>
<td>0±6 (0-5)</td>
</tr>
</tbody>
</table>

PVL = portal vein ligation; SO = sham operation.

*p<0.01, **p<0.001 for comparison of portal hypertensive and control groups; otherwise p>0.05.

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**Table II** Pressure/biochemical measurements in a cirrhotic model. (Values, mean (SEM))

<table>
<thead>
<tr>
<th>Arterial pressure (mm Hg)</th>
<th>Control rats</th>
<th>Cirrhotic rats</th>
</tr>
</thead>
<tbody>
<tr>
<td>Portal venous pressure (mm Hg)</td>
<td>106±2 (4-4)</td>
<td>120±0 (5-6) ***</td>
</tr>
<tr>
<td>Bilirubin (mmol/l)</td>
<td>14±5 (2-0)</td>
<td>6±0 (6-6) ***</td>
</tr>
<tr>
<td>Total protein (g/l)</td>
<td>49±2 (3-2)</td>
<td>63±1 (1-0) ***</td>
</tr>
<tr>
<td>Albumin (g/l)</td>
<td>19±1 (6-6)</td>
<td>29±1 (6-6) **</td>
</tr>
<tr>
<td>Alanine aminotransferase (u/l)</td>
<td>100±14 (5-4)</td>
<td>56±4 (5-4) **</td>
</tr>
<tr>
<td>Aspartate aminotransaminase (u/l)</td>
<td>221±31 (11-3)</td>
<td>114±3 (3-3) **</td>
</tr>
<tr>
<td>Alkaline phosphatase (u/l)</td>
<td>356±45 (204-30)</td>
<td>204±30 (204-30) **</td>
</tr>
</tbody>
</table>

*p<0.01, **p<0.001, ***p<0.001.

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**Table III** Gastric mucosal injury scores

<table>
<thead>
<tr>
<th>Experimental group</th>
<th>No</th>
<th>Median score</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Day 3: PVL</td>
<td>12</td>
<td>44**</td>
<td>9–118</td>
</tr>
<tr>
<td>SO</td>
<td>11</td>
<td>6</td>
<td>0–121</td>
</tr>
<tr>
<td>Day 7: PVL</td>
<td>13</td>
<td>2</td>
<td>0–101</td>
</tr>
<tr>
<td>SO</td>
<td>13</td>
<td>10</td>
<td>0–65</td>
</tr>
<tr>
<td>Day 28: PVL</td>
<td>10</td>
<td>21</td>
<td>5–89</td>
</tr>
<tr>
<td>SO</td>
<td>11</td>
<td>18</td>
<td>2–104</td>
</tr>
<tr>
<td>Cirrhotic</td>
<td>15</td>
<td>20</td>
<td>0–83</td>
</tr>
<tr>
<td>Control</td>
<td>12</td>
<td>40</td>
<td>0–164</td>
</tr>
</tbody>
</table>

PVL = portal vein ligation; SO = sham operation.

*p<0.01 portal hypertensive relative to corresponding control group; otherwise p>0.05.
No gastric mucosal lesions were observed macroscopically or microscopically in animals that did not receive intragastric taurocholate. There was no qualitative evidence of mucosal or submucosal vascular congestion or oedema in any portal hypertensive or control group rats.

Gastric mucosal injury scores after taurocholate administration are shown in Table III and Figures 1 and 2. Haemorrhagic lesions were confined to gastric corpus mucosa, the antrum showing no macroscopically visible damage. Injury scores were increased in portal vein ligated relative to control rats on day 3 after operation, but did not differ significantly between portal hypertensive and control animals at later time intervals in the prehepatic model. Similarly, there was no statistically significant difference in injuries in cirrhotic compared with control rats.

Discussion

Previous studies of the effects of experimental portal hypertension on drug induced gastric mucosal injuries have generally focused on the acute period after portal vein ligation. Sarfeh et al., who studied a rat model in which the portal vein was first constricted and three days later completely occluded, reported that 48 hours after the second procedure the gastric mucosa showed an increased susceptibility to injury by alcohol, taurocholate, and aspirin, together with other evidence of functional changes, including a reduced transmucosal potential difference and increased net hydrogen ion back diffusion. After three weeks, the impairment in mucosal resistance to injury had resolved, but portal venous pressure had also returned to control values and it was therefore not possible to assess the effect of chronic portal hypertension.
The present study confirms that mucosal defences are impaired in acute prehepatic portal hypertension, but shows that a chronic increase portal venous pressure by up to 9 mm Hg above control value, with or without liver damage, is not associated with increased mucosal injuries.

There are a number of possible mechanisms that could be responsible for the greater severity of injuries in acute prehepatic portal hypertension. Submucosal oedema was a prominent feature of Sarfeh’s model, and it was proposed that this could disrupt the gastric mucosal barrier by imposing greater distances for nutrient transport or causing separation of tight junctions. This mechanism does not seem to have been important in the present study, in which we found no evidence of appreciable submucosal oedema in any of the experimental groups. Alternatively, it is possible that a high portal venous pressure has a direct effect on local protective factors such as prostaglandin synthesis and mucus and bicarbonate secretion. If so, this study implies that only the very high pressures transiently attained in the acute period after portal vein ligation are sufficient to influence these factors significantly.

It was shown by Sarfeh et al. that the increased susceptibility to injury of the gastric mucosa in acutely portal hypertensive rats was associated with reduced mucosal oxygenation. This is consistent with studies of the haemodynamic consequences of portal vein ligation in the rat, which have shown that the obstruction to portal venous outflow precipitates an immediate reduction in splanchnic blood flow. This reduction is temporary, however, and as portasystemic collateral channels open to bypass the obstruction, splanchnic blood flow increases and ultimately exceeds the control value. A hyperdynamic splanchnic circulation has also been shown to occur in cirrhotic rats. In previous experiments, we found that blood flow in gastric corpus mucosa was reduced by 35% relative to the control value three days after portal vein ligation, but at the later time intervals in the prehepatic model there was no mucosal diffusion in mucosal blood flow between portal hypertensive and sham operated animals. The results of the present study, therefore, support the hypothesis that reduced blood flow and oxygenation are disruptive to the gastric mucosal barrier in portal hypertension. However, they call into question the view that a high portal venous pressure in the presence of unimpaired mucosal perfusion has an important effect on mucosal integrity.

Studies in portal hypertensive patients with hepatic cirrhosis have provided evidence of adverse changes in several factors closely related to gastric mucosal barrier function, including reduced scintigraphic portal venous transit time, increased hydrogen ion back diffusion, and reduced intramucosal prostaglandin levels. If it is assumed that these changes imply an increased susceptibility to mucosal injury, the present study suggests that portal hypertension per se is not their immediate cause. This is consistent with the fact that no relation has been found between portal venous pressure and the presence or absence of gastric mucosal lesions in patients. Observations that the presence and severity of gastric mucosal lesions seem to be unrelated to Child’s grade or the cause of portal hypertension are also consistent with the fact that hepatic cirrhosis did not exacerbate mucosal damage in the present study.

These considerations do not exclude the possibility that portal hypertension influences the functional status of the gastric mucosa in patients indirectly, through the medium of haemodynamic changes. The question of whether gastric mucosal blood flow is altered in portal hypertensive patients is not completely resolved, but a number of reports suggest that it is reduced. It is not clear why the effects of chronic portal hypertension on gastric mucosal haemodynamics should differ between patients and experimental animals, but a differential propensity to develop submucosal arteriovenous shunts would be a possible explanation. Some authors have reported that gastric mucosal blood flow is lower in portal hypertensive patients with lesions than in those with endoscopically normal gastric mucosa, while others have found no relation between blood flow and the presence of mucosal lesions. Because of the likelihood that mucosal damage alters blood flow, as well as the presence of chronic increase of gastric mucosal injury alterations, it is difficult to draw conclusions about causal relations from comparisons of this type.

It is important to recognize that the most prominent histological feature of congestive gastropathy - e.g., of mucosal and submucosal blood vessels - is not fully reproduced even in acute experimental models of portal hypertension. It is at present unknown whether this morphological change is related to changes in mucosal blood flow and barrier function, but it is closely associated with the presence of clinically severe bleeding. It is possible that structural changes in mucosal blood vessels provide focal points for the precipitation of bleeding in the presence of what would otherwise be a subclinical disruption of the functional integrity of the gastric mucosa. The present study supports the concept that the functional status of the gastric mucosa is related to its haemodynamic status, and suggests that further attention should be paid to the role of haemodynamic factors in the pathogenesis of gastric mucosal lesions.

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