Abstract

The objective of this work was to study the gastric emptying (GE) of liquids in fasted and sucrose-fed rats with toxic hepatitis induced by acetaminophen. The GE of three test meals (saline, glucose and mayonnaise) was evaluated in Wistar rats. For each meal, the animals were divided into two groups (N = 24 each). Group I was fed a sucrose diet throughout the experiment (66 h) while group II was fasted. Forty-two hours after the start of the experiment, each group was divided into two subgroups (N = 12 each). Subgroup A received a placebo and subgroup B was given acetaminophen (1 g/kg). Twenty-four hours later, the GE of the three test meals was assessed and blood samples were collected to measure the serum levels of alanine aminotransferase (ALT), aspartate aminotransferase (AST) and acetaminophen. In group IIB, the mean AST and ALT values were 515 and 263 IU/l, respectively, while for group IIB they were 4014 and 2472 IU/l, respectively. The mean serum acetaminophen levels were higher in group IIB (120 µg/ml) than in group IIB (87 µg/ml). The gastric retention values were significantly higher in group IIB than in group IIA for all three test meals: saline, 51 vs 35%; glucose, 52 vs 38% and mayonnaise, 51 vs 29% (median values). The correlation between gastric retention and AST levels was significant (P<0.05) for group IIB for the three test meals: r = 0.73, 0.67 and 0.68 for saline, glucose and mayonnaise, respectively. We conclude that GE is altered in rats with hepatic lesions induced by acetaminophen, and that these alterations may be related to the liver cell necrosis caused by the drug.

Introduction

Complaints of nausea and vomiting are relatively frequent in patients with acute hepatic lesions caused by a virus or drugs (1,2). Although complications related to acute hepatitis, such as hemorrhage and encephalopathy, have been extensively studied, little attention has been paid to the possible effects of hepatic injury on the motor activity of the stomach. In adult patients with chronic hepatic disease, the gastric emptying (GE) of liquids and solids is delayed (3), but changes in GE following acute hepatic injury, both in humans and in animals, have not previously been reported. In the present study, we have evaluated the GE of liquids in fasted and sucrose-fed rats suffering from hepatic lesion induced by acetaminophen.
Material and Methods

Animals

The study was conducted on 144 male Wistar rats (8-10 weeks old, 220-280 g) supplied by the University’s Animal House. The animals were acclimatized to the laboratory for at least three days before the experiments. During this time, they received a standard rat chow (Labina, Purina) and water ad libitum. All of the animals were weighed at the beginning of the study and daily thereafter until the end of the protocol. On the day of acetaminophen or placebo infusion and on the day of the GE test, weighing was done approximately one hour before administration.

Study protocol

The study consisted of three groups of rats (N = 48 each) with the GE of only one test meal (saline, glucose or mayonnaise) being studied in each group. Prior to assessing the GE, the animals in each test-meal group were either fed cubes of sucrose for 66 h (N = 24) or were fasted for the same length of time (N = 24). Water was provided ad libitum to all the animals. Forty-two hours later, each of these groups was further subdivided, with one half (N = 12) receiving a placebo (0.5 ml/100 g body weight, Johnson & Johnson) via an orogastric catheter, and the other half (N = 12) receiving acetaminophen (infant Tylenol 200 mg/ml, Johnson & Johnson, New Brunswick, NJ) orogastrically (1 g/kg body weight) in the same volume as the placebo. Twenty-four hours after these infusions (the time at which acetaminophen produces the highest level of liver toxicity (4)), the GE of the desired meal was determined and blood samples were drawn for laboratory analyses (see below).

Test meals

Three test meals (a 0.9% (w/v) NaCl solution, a 5% (w/v) glucose solution, and a 2.5% (w/v) mayonnaise solution) were studied. The mayonnaise solution (Goodie mayonnaise, SANBRA, São Paulo, SP) had the following composition: 28.7 mmol/l Na⁺, traces of K⁺, 25.1 mmol/l Cl⁻, 0.9 mmol/l titrative acidity and 1.45% fat (v/v). All of the meals were labeled with phenol red (final concentration, 6 mg/dl). The osmolalities of the solutions were determined using an Advanced Instruments Laboratories Osmometer (Medkam Heights, MA) with the following results: 305 mOsm/kg saline, 309 mOsm/kg glucose, and 39.8 mOsm/kg mayonnaise. The test meals were warmed to room temperature prior to administration at a dose of 2 ml/100 g body weight. The mayonnaise was continuously stirred at low speed prior to administration.

Gastric emptying

The techniques for infusion of the test meals and for the determination of gastric retention have already been described (5,6). Gastric retention was assessed 10 min after infusion of the saline meal and 20 min after infusion of the glucose and mayonnaise meals.

Laboratory analyses

The Na⁺ and K⁺ content of the mayonnaise meal was determined by flame photometry using a Micronal photometer (São Paulo, SP). Chloride (Cl⁻) was measured by a titration method using a Merckotest (Merck, Darmstadt, Germany). The titrative acidity was determined by titration using 0.1 N NaOH with thymol blue (0.1%) as the indicator. The level of fat was quantified in a Gerber butter meter calibrated for concentrations up to 2% (v/v) (7).

The serum activities of aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were assayed using commercial kits (Miles Laboratories of AMES Division, São Paulo, SP). Acetaminophen levels
were measured by the TDx method using a kit from Abbott Laboratories (Irving, TX).

Statistical analysis

Analysis of variance (8) was employed to compare the serum concentrations of ALT, AST and acetaminophen. When necessary, the Tukey test (8) was also applied. For comparison of the gastric retention values, the Kruskal-Wallis test (9) was used with an alpha value of 0.10 for a two-tailed test being applied. When a significant difference was detected by this approach, a multiple comparative test (9) was also applied. In this case, the alpha value was obtained by dividing 0.10 by the number of possible comparisons. The correlation between variables was analyzed by determining the Spearman rank correlation coefficient (10), for which an alpha value of 0.05 for a unilateral test was applied.

The data in the figures are reported as boxplots (11). In each plot, the upper and lower short horizontal lines indicate the maximum and minimum gastric retention values observed, respectively. The median, first and third quartiles of the gastric retention values for each subgroup are represented, respectively, by the intermediate, lower and upper horizontal lines used to construct each rectangle.

Results

Serum aminotransferase and acetaminophen levels

The serum aminotransferase levels are shown in Table 1. The highest values were obtained in fasted animals that received acetaminophen (subgroup IIB). These values were significantly different from the corresponding placebo group (subgroup IIA). The serum levels of acetaminophen were significantly higher in the fasted subgroup IIB than those obtained in the corresponding sucrose-fed subgroup IB.

Gastric emptying

The gastric retention values for the three meals were significantly higher in both sucrose-fed and fasted animals that received acetaminophen (subgroups IB and IIB) than...
in the corresponding placebo subgroups (IA and IIA; Figures 1-3). With the saline and mayonnaise test meals, there was no significant difference between the gastric retention values of fasted animals that received acetaminophen (subgroup IIB) and those that received the drug but were protected by ingesting sucrose (subgroup IB). However, when a glucose test meal was employed, the fasted animals that received acetaminophen (subgroup IIB) showed significantly higher gastric retention values than animals fed with sucrose and which received the drug (subgroup IB). With this same test meal, fasted animals given the placebo (subgroup IIA) had significantly higher gastric retention values than those fed sucrose and given the placebo (subgroup IA; Figure 2). This finding was not observed with the other test meals.

**The correlation between gastric retention and serum acetaminophen and aminotransferase levels**

A significant positive correlation between gastric retention and the serum levels of acetaminophen was observed only in fasted animals which received acetaminophen prior to the saline and mayonnaise test meals (subgroups IB and IIB, respectively) and in animals fed sucrose and given acetaminophen (subgroup IIA; Table 2). A significant correlation between gastric retention and the serum levels of AST occurred with the three test meals in fasted animals which were given acetaminophen.

**Discussion**

The present results confirm the observations of Martinelli et al. (12) that the administration of a high dose of acetaminophen (1 g/kg) to fasted rats produces hepatic lesions within 24 h and that this damage can be attenuated by the ingestion of sucrose. They also agree with our previous report (13) on the macro- and microscopic acetaminophen-induced hepatic lesions in the absence and presence of sucrose ingestion.

The serum aminotransferase levels were higher in fasted rats which received acetaminophen than in animals initially protected by the ingestion of sucrose. The wide variation in the serum enzyme levels in each
group/subgroup most likely reflects different degrees of hepatic necrosis. It is also possible that variations in the efficiency of detoxification pathways and/or individual differences in the gastrointestinal absorption of the drug could account for some of the variability seen (14,15). The significant difference in serum acetaminophen levels between fasted rats given acetaminophen and animals protected by ingesting sucrose may reflect an overall decrease in the metabolism of the drug as a consequence of intense hepatic cellular necrosis in the former group (16,17).

While the above results point to greater hepatic damage by acetaminophen in fasted rats, this situation was not reflected on the GE which was retarded for the three meals in animals receiving acetaminophen compared to the respective controls. Overall, there was no significant difference in the GE for the saline and mayonnaise meals between the fasted rats which received acetaminophen and those which were given sucrose and the drug. With the glucose test meal, gastric retention was more marked in fasted animals receiving acetaminophen compared to those protected with sucrose. Interpretation of the GE data for the glucose test meal in intoxicated animals in the absence or presence of sucrose is hampered because of the higher GE observed in the fasted control animals compared to the control animals that received sucrose. This finding may reflect a decrease in the sensitivity of the glucose uptake mechanisms as a result of having ingested sucrose. As a result, a greater quantity of the monosaccharide reaches the lower small intestine. This situation results in a reduction in the number of glucose receptors exposed in the latter region which in turn attenuates the mechanism for retarding GE. In fasted animals which received a placebo, the rapid absorption of glucose may promote the transfer of water from the lateral intercellular space to the internal environment which consequently reduces the size of this space and enhances GE (21).

While acetaminophen has toxic effects on the liver (22,23), its effect on GE has not been previously reported. Although no acetaminophen-induced macroscopic or microscopic alterations have been observed in the stomach (24), it is possible that the drug may, nevertheless, have an effect on gastric motility. Since the motor regulation of the stomach is complex (25), various mechanisms could be involved. Hepatic cellular necrosis ought to be considered as an important factor in view of the increase in serum aminotransferase levels and of the significant correlation with the GE retardation. Mezzacappa and Collares (26) observed a similar effect on GE in rats with chemical pneumonia induced by a petroleum derivative. These authors attributed the retardation

<table>
<thead>
<tr>
<th>Test meal</th>
<th>Subgroup</th>
<th>GR vs Acetaminophen</th>
<th>GR vs AST</th>
<th>GR vs ALT</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saline (I)</td>
<td>Sucrose-fed (A)</td>
<td>-0.04</td>
<td>-0.49</td>
<td>-0.19</td>
</tr>
<tr>
<td></td>
<td>Fasted (B)</td>
<td>0.61*</td>
<td>0.73*</td>
<td>0.51*</td>
</tr>
<tr>
<td>Glucose (II)</td>
<td>Sucrose-fed (A)</td>
<td>0.47</td>
<td>0.09</td>
<td>-0.11</td>
</tr>
<tr>
<td></td>
<td>Fasted (B)</td>
<td>0.33</td>
<td>0.67*</td>
<td>0.52*</td>
</tr>
<tr>
<td>Mayonnaise (III)</td>
<td>Sucrose-fed (A)</td>
<td>0.67*</td>
<td>0.67*</td>
<td>0.65*</td>
</tr>
<tr>
<td></td>
<td>Fasted (B)</td>
<td>0.61*</td>
<td>0.68*</td>
<td>0.04</td>
</tr>
</tbody>
</table>

Note that all of the subgroups in this table were treated with acetaminophen prior to administration of the test meal. *P<0.05 indicates the significance of the Spearman’s correlation coefficient for each comparison shown below.
in GE to an inflammatory process. Since nitric oxide (NO) is an endogenous mediator released at or near the site of inflammation by various stimuli (27), it is possible that this mediator may somehow participate in the observed GE effect, particularly since NO relaxes both the inferior esophageal sphincter (28) and the gastric fundus (29) and also influences duodental motility (30).

We conclude that acute hepatic injury provoked by acetaminophen in rats retards GE. Further studies will be required to elucidate the precise nature and pathophysiology of the mechanisms involved.

Acknowledgments

We thank Dr. Eliana M. Barison for technical assistance and Dr. Stephen Hyslop for reviewing the English of the manuscript.

References


25. Hunt JN (1985). Regulation of gastric emptying by neurohumoral factors and by gastric and duodenal receptors. In: Dubois A & Castell DO (Editors), Esophageal and