Retention of acetaminophen in an in vitro model of solid-phase gastric emptying of animals

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Objective—To apply an in vitro model for assessment of the solid-phase binding capacity of acetaminophen and thus assess the reliability of this marker for evaluation of solid-phase gastric emptying in vivo in animals.

Sample Population—4 test meals.

Procedures—A spectrophotometric method for detection of acetaminophen was validated and applied for assessment of the percentage retention of acetaminophen in the solid phase of 4 test meals. The gastric milieu was simulated by incubating each meal in artificial gastric juice for 2 hours in a shaking water bath maintained at 37°C. Solid-phase retention was then assessed 3 times by measuring the amount of acetaminophen that had leaked into the liquid phase.

Results—Acetaminophen was poorly retained in the solid phase of all the test meals examined in the study. There was also a large degree of variability in the percentage retention for each meal when the experiment was repeated 3 times.

Conclusions and Clinical Relevance—Analysis of the results of this in vitro study confirmed that acetaminophen may not be an appropriate marker of solid-phase gastric emptying. The acetaminophen gastric emptying test should be applied only for the assessment of liquid-phase emptying in animals. (Am J Vet Res 2007;68:895–898)

The criterion-referenced standard for detection of the rate of solid-phase gastric emptying in humans and other animals is radioscintigraphy, but this method involves a radiation risk and requires access to a nuclear medicine facility. Carbon 13–gastric emptying breath tests have been applied in veterinary medicine and have the advantage of being nonradioactive and applicable at the point of care. However, these tests are expensive and are not yet widely available in veterinary clinical practice. An inexpensive and simple test for assessment of solid-phase gastric emptying would be a useful investigative tool for application in small animal and equine clinical practice.

The acetaminophen gastric emptying test has been validated for assessment of liquid-phase gastric emptying in humans and horses. This method has been used to detect gastroparesis in diabetic dogs and to investigate the effects of prokinetic drugs, sedatives, and atropine on the rate of gastric emptying in horses. The principle of this test is that acetaminophen is poorly absorbed in the stomach but is rapidly and completely absorbed in the small intestines so that the rate of detection of acetaminophen in serum can be used as an indication of the rate of gastric emptying. The test assumes normal small intestinal function; therefore, conditions that affect gastrointestinal permeability may affect the reliability of this method.

The advantages of the acetaminophen gastric emptying test are that the test substrate is inexpensive and freely available and that acetaminophen can be easily measured in serum by use of a simple spectrophotometric method. Although acetaminophen has been validated as a marker of liquid-phase gastric emptying, it has been suggested in several studies that acetaminophen ingested in food may remain bound or partially bound in the solid phase.

The reliability of chemical markers of solid-phase gastric emptying is largely dependent on their ability to remain bound to the solid phase of ingesta in the stomach, and this can be easily assessed in a laboratory. To our knowledge, the solid-phase binding capacity of acetaminophen has not been reported, although this marker has been administered in food for assessment of gastric emptying in dogs and humans, presumably on the supposition that the marker would be retained in the solid phase. The objective of the study reported here was to investigate the solid-phase binding capacity of acetaminophen in an in vitro model of the physical and chemical properties of the stomach and thus assess the reliability of acetaminophen as a marker.
of solid-phase gastric emptying. The hypothesis tested was that acetaminophen would not be retained in the solid phase of an in vitro system designed to simulate solid-phase gastric emptying in vivo.

**Materials and Methods**

**Test meals**—Four test meals were used in the study. The meals were scrambled egg yolk, canned food formulated for dogs, a proprietary pelleted feed formulated for horses, and a pasta meal prepared as described in another study. At 3 times, 20 mg of acetaminophen dissolved in 100 µL of ethanol (to aid solubility) was incorporated into each test meal and solid-phase binding was assessed. The dose of acetaminophen added to each meal was designed to replicate the dose used in other studies of the acetaminophen gastric emptying test in humans and other animals.

**Assessment of solid-phase binding**—To facilitate comparison of our results with results of other studies, solid-phase binding was assessed by use of a method described for investigation of binding of radiolabelled substrates. Gastric emptying was simulated in vitro by incubating a portion (5 g) of each test meal with the substrate (20 mg of acetaminophen) in a 50-mL sample tube in a water bath at 37°C. Blank tubes that contained 5 g of each test meal but not acetaminophen were included in all experiments. Control samples to represent maximal dissolution of acetaminophen into the liquid phase were prepared by adding 20 mg of acetaminophen to 10 mL of artificial gastric juice.

Artificial gastric juice was prepared in accordance with published recommendations. Briefly, 2 g of sodium chloride was dissolved in distilled water with 3.2 g of purified pepsin derived from porcine stomach mucosa (activity of 800 to 2,500 U/mg of protein). Then, 7 mL of hydrochloric acid was added, and a sufficient quantity of distilled water was added to achieve a final volume of 1 L (0.08M HCl; pH, 1.2).

The sample tubes were gently shaken in the water bath by a mechanical shaker to simulate gastric peristalsis. After incubation in the water bath for 2 hours, the tubes were centrifuged at 1,320 X g for 20 minutes. Supernatant was decanted for spectrophotometric analysis of acetaminophen concentration. The solid-phase binding capacity of each test meal was assessed by use of this method 3 separate times.

**Analysis of acetaminophen concentrations**—Acetaminophen was measured by use of a colorimetric assay. The assay was based on the reaction between acetaminophen and nitrous acid that results in the dissolution of acetaminophen into the liquid phase. The measured mean ± SEM concentration of an equivalent dose of acetaminophen in 10 mL of artificial gastric juice. Linear regression analysis was used to investigate the association between acetaminophen concentration and absorbance at 430 nm. A single-factor ANOVA was used to investigate whether the degree of solid-phase binding differed among test meals. Data are reported as mean ± SEM. Significance was defined at values of P < 0.05.

**Results**

**Validation of acetaminophen assay**—A strong linear relationship (R² = 0.9998; P < 0.001) was detected between the acetaminophen concentration for the range of interest (1 to 100 mg/dL) and absorbance at 430 nm. Inter assay CVs were 30.0%, 3.6%, 2.0%, 0.8%, and 1.7% for acetaminophen concentrations of 1, 5, 25, 50, and 100 mg/dL, respectively. Intra-assay CVs were 22.0%, 3.7%, 1.1%, 0.8%, and 0.8% for acetaminophen concentrations of 1, 5, 25, 50, and 100 mg/dL, respectively.

**Solid-phase binding tests**—Retention of acetaminophen in the solid phase was examined by measuring the concentration of acetaminophen in the supernatant of the tubes. The measured mean ± SEM concentration of acetaminophen in the supernatant of the control tubes was 161.8 ± 13.1 g/L. Mean solid-phase retention of acetaminophen for each of the test meals was calculated (Table 1). Acetaminophen was poorly retained in the solid phase of all the test meals examined 3 separate times, and there was no significant (P = 0.9) difference in retention among the 4 meals. Retention in the solid phase was variable with CVs for the 3 test days of 19%, 37%, 63%, and 64% for the pelleted food formulated for horses, canned food formulated for dogs, pasta, and egg test meals, respectively.
Table 1—Mean, SEM, and CV* for the percentage of solid-phase retention of acetaminophen in 4 test meals investigated by use of an in vitro model of gastric emptying in animals.

<table>
<thead>
<tr>
<th>Test meal</th>
<th>Mean (%)</th>
<th>SEM (%)</th>
<th>CV (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pelleted food formulated for horses</td>
<td>30.3</td>
<td>3.4</td>
<td>19.4</td>
</tr>
<tr>
<td>Canned food formulated for dogs</td>
<td>27.1</td>
<td>5.7</td>
<td>26.6</td>
</tr>
<tr>
<td>Pasta</td>
<td>27.8</td>
<td>10.2</td>
<td>63.2</td>
</tr>
<tr>
<td>Scrambled egg yolk</td>
<td>29.3</td>
<td>10.8</td>
<td>63.8</td>
</tr>
</tbody>
</table>

Data represent results for 3 experiments; no retention was considered to be the concentration of acetaminophen measured in a control tube that did not contain a solid phase. Retention did not differ significantly (P = 0.9) among the 4 meals.

*The CV was calculated as the SD divided by the mean.

Discussion

Analysis of the results of the study reported here revealed that acetaminophen was poorly retained in the solid phase of an in vitro model of the physical and chemical conditions in the stomach. This suggests that acetaminophen will not be a reliable marker of solid-phase gastric emptying in vivo.

The test meals used in the study were typical of those commonly used in the assessment of gastric emptying in animals. The pasta meal was included because this meal has been applied in the assessment of solid-phase gastric emptying with acetaminophen in human medicine. There was only minimal solid-phase retention of acetaminophen in each of the 4 test meals, and this retention was highly variable when the experiment was repeated. Considered in combination, these findings confirm that acetaminophen is a poor marker of solid-phase gastric emptying for the test meals examined in this study. This is in agreement with results of other in vivo studies in which investigators determined that there was no correlation between the rate of gastric emptying of solids measured by use of acetaminophen and radioscintigraphy.

On the basis of the poor retention found in the study reported here, it is unlikely that acetaminophen would bind to the solid phase of other food materials. Investigators in a study described the preparation of a novel solid matrix composed of albumin and arabic gum; sulfamethizole was incorporated into the matrix, which allowed the serum concentration of that drug to reflect gastric emptying. That solid matrix was designed to simulate digestible food and permit the use of a chemical marker (sulfamethizole) for assessment of solid-phase gastric emptying. Retention of sulfamethizole in the solid phase of that matrix was detected by use of a protocol similar to that used in the study reported here. Additional studies are necessary to establish whether acetaminophen may be similarly retained in an artificially prepared solid matrix.

The in vitro model used in the study reported here is commonly used in physiologic and pharmacologic studies to investigate the dissolution of tablets and other solids in the stomach. However, it must be considered that centrifugation cannot truly represent gastric peristalsis, and the possibility that the markers may be differentially retained in vivo cannot be discounted. Nevertheless, the poor retention of acetaminophen in our study (27% to 30%) is in contrast to the reported retention (determined by use of similar in vitro simulations) for radiolabels of 78% to 98% in food formulated for dogs, 80% to 99% in food formulated for cats, and > 90% in cooked liver. Similarly, high values (> 90%) for solid-phase retention were reported for C-octanoate in egg yolk, which is a test meal commonly used in gastric emptying breath tests.

Because acetaminophen is poorly retained in the solid phase, it is likely that this marker will reflect liquid-phase emptying, even when ingested in a solid meal. Liquids empty from the stomach in an exponential pattern, whereas solids empty in a slower, more linear pattern that is often preceded by a lag phase that reflects gastric trituration. Different physiologic mechanisms dictate the rate of gastric emptying of liquids and solids, and ingestion of liquids during feeding does not affect the rate of solid-phase gastric emptying in dogs. Gastric emptying of digestible food is dependent on particle size, energy density, and (to a lesser extent) meal volume. Gastric emptying of liquids is controlled largely by volume but also by energy density and osmolarity. Gastric emptying of solid food is reliant on the triturating or grinding forces of the stomach, whereas gastric emptying of nonnutrient liquids is essentially controlled by the pressure gradient maintained between the stomach and duodenum.

Because gastric emptying of liquids and solids is controlled by different physiologic mechanisms, it is important that putative markers remain bound to the phase of gastric emptying that is being investigated. Analysis of the results of the study reported here suggests that the gastric emptying of digestible food cannot be reliably assessed by use of the acetaminophen gastric emptying test and supports the contention that the only application of this test is for the assessment of liquid-phase emptying in animals.

References

6. Takeda M, Mizutani Y, Yamano M, et al. Gastric emptying in...