Effects of aspirin and *Helicobacter pylori* on the gastroduodenal mucosal permeability to sucrose

A A Rabassa, R Goodgame, F M Sutton, C N Ou, C Rognerud, D Y Graham

Abstract

Background—A non-invasive marker is needed to identify patients with significant gastrointestinal injury due to non-steroidal anti-inflammatory drugs. Gastrointestinal permeability to sucrose has been suggested as such a test.

Aims—To assess the utility of sucrose permeability as a marker of gastroduodenal mucosal injury after single and multiple doses of aspirin, to identify the site of increased sucrose permeability, to explore the relation between sucrose permeability and endoscopic findings, and to evaluate whether *Helicobacter pylori* infection influenced gastroduodenal sucrose permeability.

Methods—After a fasting urine was obtained, 500 ml of a solution containing 100 g of sucrose was ingested. Urine was collected for five hours and assayed for sucrose by high performance liquid chromatography. Sucrose permeability was also assessed 20 minutes after ingestion of 650 mg of aspirin and eight to 12 hours after a 72 hour course of 650 mg aspirin four times a day. The site of increased permeability was identified after pyloric occlusion with a double balloon tube.

Results—Thirty seven healthy volunteers participated. Sucrose permeability (mean (SEM)) increased after both single (195·2 (27) mg and multiple (196·4 (31) mg) doses of aspirin compared with baseline (53·7 (10) mg; p<0·0005). Balloon pyloric occlusion confirmed that the site of increased sucrose permeability was the stomach. The effect of aspirin on sucrose permeability was similar in those with and without *H pylori* infection.

Conclusion—These results confirm the use of sucrose permeability as a marker of aspirin induced gastroduodenal mucosal injury and identify the stomach as the major site of increased permeability. *H pylori* infection does not seem to change gastric mucosal sucrose permeability either at baseline or after ingestion of aspirin.

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Keywords: aspirin, *Helicobacter pylori*, sucrose, sucrose permeability, non-steroidal inflammatory drugs, gastric permeability, intestinal permeability.

Department of Medicine, Baylor College of Medicine, Texas, USA
A A Rabassa
R Goodgame
F M Sutton
C N Ou
C Rognerud
D Y Graham

Department of Medicine, VA Medical Center, Houston, Texas, USA
D Y Graham

Department of Pathology, Texas Children's Hospital, Texas, USA
C N Ou
C Rognerud

Correspondence to:
Dr D Y Graham, Veterans Affairs Medical Center, 2002 Holcombe Blvd, Houston, TX 77030-4211, USA.

Non-steroidal anti-inflammatory drug (NSAID) use is a common cause of gastrointestinal morbidity and mortality. Endoscopy is the most accurate method for the evaluation of gastrointestinal damage related to the use of NSAIDs but is an impractical tool for screening. Only 50% of NSAID users with dyspepsia are found to have clinically significant mucosal injury and many longterm NSAID users with life threatening gastrointestinal complications have no antecedent history of ulcer symptoms.[1 2] A non-invasive marker of mucosal injury may help identify those patients with serious NSAID induced mucosal damage as well as those most likely to benefit from prophylaxis or treatment.

Macromolecules such as disaccharides are relatively impermeable to healthy gastrointestinal mucosa. Recently, Meddings *et al*[3] showed that gastrointestinal sucrose absorption increased after acute gastrointestinal mucosal injury caused by the administration of aspirin and alcohol. Their experiments were based on the fact that, upon entering the duodenum, sucrose is rapidly degraded into glucose and fructose. They attempted to correlate gastrointestinal sucrose permeability with gastric mucosal damage represented by the amount of sucrose appearing in the urine. Urinary sucrose can be used as an indirect measure of sucrose permeability because, once absorbed, sucrose is efficiently filtered by the kidneys and excreted in the urine.

We began our studies with the notion that the published conclusion possibly erred as they examined sucrose permeability 20 minutes after aspirin ingestion, a time when the gastric mucosal potential difference is considerably depressed and severe surface damage is present.[4–9] Control experiments with multiple doses of aspirin administration at a time when the mucosal potential difference is normal but the mucosa is visibly damaged were not presented. Experiments two or more hours after aspirin administration were important controls to confirm that the abnormalities in permeability were not simply due to acute surface damage. With longterm aspirin administration, the gastric potential difference still falls immediately after aspirin administration despite the fact that the gastric mucosa becomes resistant to the development of endoscopically grossly visible lesions.[9–11] Because NSAID damage extends into the proximal small intestine, it was also possible that the site of increased permeability was not the stomach but rather the proximal small intestine. We therefore set out to confirm their original findings and extend those observations to multiple dose aspirin administration as well as to test whether the stomach was the major site of increased sucrose permeability. Finally, as they had not taken into account the possible confounding effect of gastric infection with

Helicobacter pylori, we also evaluated the effect of *H pylori* infection on sucrose permeability in the presence and absence of aspirin.

Methods

Thirty seven asymptomatic healthy volunteers participated in one or more phases of the study. All were over 18 years of age, and none had previous gastrointestinal surgery, diabetes, symptomatic gastro-oesophageal reflux disease, renal insufficiency, any use of non-steroidal anti-inflammatory agents, steroids, potassium, antibiotics, histamine 2 antagonists, proton pump inhibitors, bismuth or excess alcohol consumption during the previous 30 days. Written informed consent was obtained. The protocol of this study was approved by the local Human Subjects Review Committees.

Gastroduodenal sucrose permeability

Gastroduodenal permeability was assessed by the gastroduodenal sucrose permeability test. The first morning voided urine specimen was collected from all patients (fasting) prior to testing. The subjects then ingested 500 ml of a solution containing 100 g of sucrose in water. Urine was collected for five hours. During the collection subjects were encouraged to drink water but to abstain from drinking coffee or anything with sugar. Only water was permitted during the first two hours of the collection. Urine samples were immediately refrigerated and frozen at $-20°C$ within 24 hours. Urine sucrose was measured by high performance liquid chromatography.

Gastric sucrose permeability

The gastric contribution to sucrose permeability was assessed by the gastric sucrose permeability test utilising a double balloon tube for pyloric occlusion. Endoscopy was performed after an overnight fast using only topical oropharyngeal anaesthesia. The gastroduodenal mucosa was evaluated with attention to the location and number of epithelial haemorrhages, erosions or ulcers, or all three. A 400 cm length flexible tipped piano guidewire (Microvasive, Natick, MA) was introduced into the working channel of the endoscope and advanced into the second portion of duodenum. The endoscope was withdrawn. A multilumen, double balloon orogastric tube was passed over the wire. Under endoscopic view, and assistance, the distal balloon was positioned in the duodenal bulb, and inflated. The proximal balloon was then inflated forming a gastric outlet seal by apposition of the balloons. The endoscope was withdrawn and an 18 French Salem Sump orogastric tube (Argyle, St Louis, MO) was passed orally into the stomach.

Five hundred millilitres of a sucrose solution containing 100 g of sucrose was instilled in the stomach through the orogastric tube. After 30 minutes, the solution was removed and the volume recovered was measured to assess adequate recovery, defined as greater than 90%. The stomach was then rinsed three times with 500 ml of room temperature tap water. In four patients, an aliquot of the total collection was obtained, refrigerated, and frozen at $-20°C$ to determine the amount of sucrose recovered. Urine was collected for the following five hours as previously described.

Aspirin and H pylori induced injury

Mucosal injury was evaluated 20 minutes after the oral administration of 650 mg of aspirin (single dose). As the greatest degree of endoscopically visible mucosal injury has been shown to occur after three days of continuous aspirin administration,[9] aspirin was continued at the dose of 650 mg four times daily for three days. Mucosal injury was assessed eight to 12 hours after the final dose of aspirin (multiple dose). No biopsy specimens were taken to assess histology or to confirm *H pylori* status because of possible effects on sucrose permeability.

H pylori status was determined by serum ELISA (HM-CAP, EPI, Westbury, NY) in 28 subjects who received aspirin to assess whether the presence of *H pylori* infection influenced sucrose permeability. This assay has been shown to have a sensitivity of greater than 95% for detecting *H pylori* infection in patients without history of antibiotic or proton pump inhibitor use. The endoscopist was blinded to the *H pylori* infection status of the subjects.

Statistics

Statistical analysis was performed by the Sigma Stat computer program (Jandel Corp, San Rafael, CA) using the paired and unpaired Student's *t* test. The Mann-Whitney rank sum test was used when data were not normally distributed. Linear regression was performed to evaluate the relation between sucrose permeability and the number of antral erosions. A p value of less than 0·05 was considered statistically significant. The data are presented as mean (SEM).

Results

A total of 37 subjects (27 men and 10 women) with a mean age of 32·2 years (range 20 to 53) participated in one or more experiments.

Gastroduodenal permeability after single and multiple dose aspirin administration

In the first experiment the gastroduodenal sucrose permeability test was sequentially performed in 19 subjects (a) in the absence of aspirin, (b) after single dose aspirin administration, and (c) after multiple dose aspirin ingestion to assess the utility of the test as a marker of gastroduodenal mucosal injury. Fasting (pre-sucrose), baseline (after sucrose but no aspirin), single dose injury (20 minutes after the first dose of aspirin), and multiple dose injury (eight to 12 hours after the final dose of aspirin) urine samples were collected. The degree of gastroduodenal mucosal injury
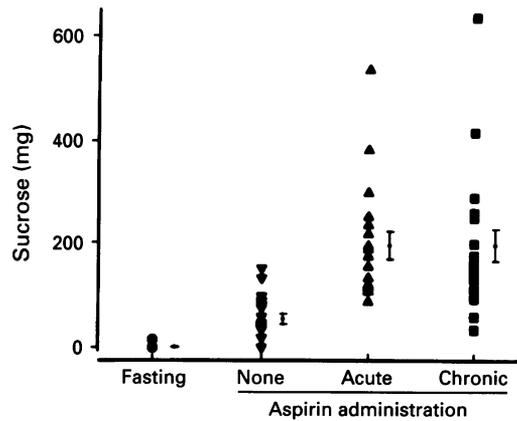
Figure 1: Gastroduodenal sucrose permeability was measured three times in 19 subjects. No sucrose was detected in the fasting (pre-sucrose) urine samples. Urinary sucrose recovery increased after single (195·2 (27·1) mg) (mean (SEM)) and multiple (196·4 (31·3) mg) dose aspirin administration compared with no aspirin (53·7 (10·1) mg) (p<0·0005). Single and multiple dose aspirin administration resulted in similar mean urine sucrose recoveries.
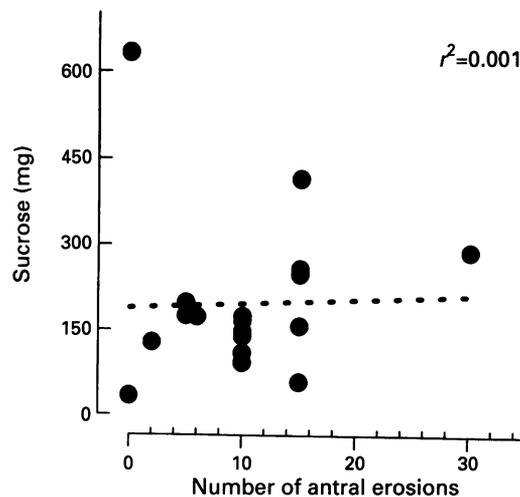

Figure 2: Relation between the number of antral erosions and five hour mean urinary sucrose recovery eight to 12 hours after the final dose of a 72 hour course of 650 mg aspirin four times a day (multiple dose injury) ($r^2=0·001$). There was no relation between the presence, absence, or degree of acute macroscopic injury and the degree of sucrose permeability.

was evaluated at endoscopy immediately prior to the last sucrose permeability test One person failed to collect the urine sample following acute aspirin mucosal injury and his data are not shown for that time point. There was no detectable sucrose in the fasting urine samples of any subject. The mean urinary sucrose recovery after single and multiple dose administration of aspirin was higher than at baseline (p<0·0005) (Fig 1). Single and multiple dose aspirin exposure resulted in similar mean urinary sucrose recoveries. When the entire group is analysed together, aspirin induced a threefold increase in sucrose permeability. However, there was a broad range of individual response to aspirin. Some patients had almost no change while others showed a fivefold increase.

Gastrointestinal mucosal injury was evaluated by endoscopy after the final dose of aspirin to assess the relation between sucrose permeability and endoscopic findings. Sucrose recovery did not correlate with the number of

epithelial haemorrhages or antral erosions (Fig 2).
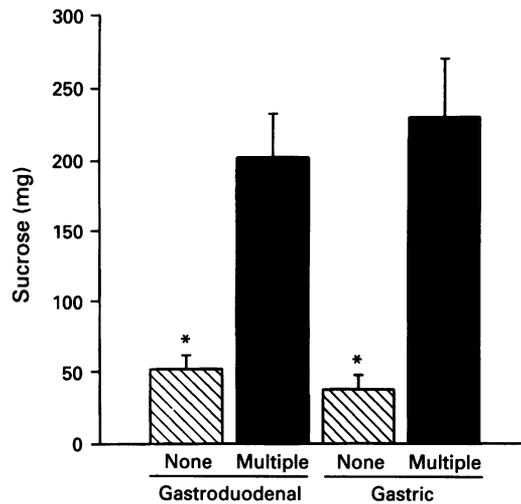
Gastric sucrose permeability

Preliminary studies were performed to assess the effectiveness of pyloric occlusion technique in preventing sucrose from entering the duodenum and to assess the effect of balloon occlusion itself on sucrose permeability. The effectiveness of the pyloric occlusion technique in preventing sucrose from entering the duodenum was confirmed by measuring the volume and total amount of sucrose recovered from the stomach after the exposure period. Seventeen of 18 subjects who completed the gastric sucrose test had greater than 450 ml (90%) recovery at the end of the 30 minutes sucrose exposure period. Accurate measurement of the amount of sucrose solution remaining in the stomach of one person was not possible because he experienced emesis at the completion of the 30 minute sucrose exposure period. A mean of 89·6 g (range 85·5 to 93·7 g) of sucrose was recovered from the stomachs of four subjects in whom measurements were obtained.

The effect of balloon occlusion itself on sucrose permeability was assessed in a group of eight subjects (six men and two women, mean age of 33·5 years, range 26–42). Sucrose permeability was assessed without balloon occlusion of the pylorus (gastroduodenal permeability) and with balloon occlusion (gastric permeability). In both cases there was no sucrose in the fasting urine samples. Mean urinary sucrose recovery after the gastric sucrose test was approximately twice that seen after the gastroduodenal sucrose test but the difference was not statistically significant (p=0·06).

In a group of 10 subjects who had already completed gastroduodenal sucrose permeability testing with and without aspirin, gastric sucrose permeability testing was performed eight to 12 hours after the final dose of a 72 hour course of 650 mg aspirin four times a day. There was no detectable sucrose in the fasting urine samples. The effect of multiple doses of aspirin on sucrose permeability seemed the same whether the gastroduodenal or gastric permeability (balloon occlusion) was assessed (Fig 3).

Effect of H pylori infection

To assess the contribution of *H pylori* to changes in gastric mucosal integrity, the gastroduodenal sucrose test was performed in a group of 28 subjects (18 without *H pylori* infection) without aspirin (baseline) and following single dose aspirin. Endoscopic assessment of mucosal injury and the gastroduodenal sucrose test was also performed in 19 subjects (12 without *H pylori* infection) after multiple dose aspirin. No sucrose was detected in fasting urine samples. Baseline values were similar between the *H pylori* infected and uninfected volunteers (Fig 4). Mean urinary sucrose recovery increased after single and

Figure 3: Effect of balloon occlusion technique on sucrose permeability after multiple dose aspirin administration was studied in 10 subjects. With the balloon occlusion technique the five hour mean urinary sucrose recovery (gastric sucrose permeability) increased after multiple dose aspirin administration (229·7 (40·1) mg) compared with no aspirin (p<0·005)*. The increase in urinary sucrose recovery was similar to that seen after the gastroduodenal sucrose testing in the initial group of 19 subjects (196·4 (31·3) mg) (p<0·005)*. There was no significant difference in sucrose recovery between gastroduodenal sucrose permeability and gastric sucrose permeability (balloon occlusion) after multiple dose aspirin administration (p=0·1).
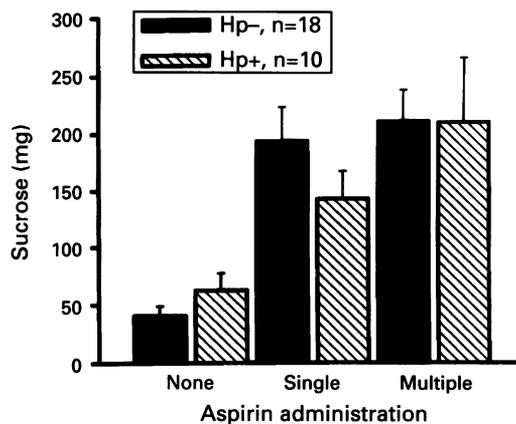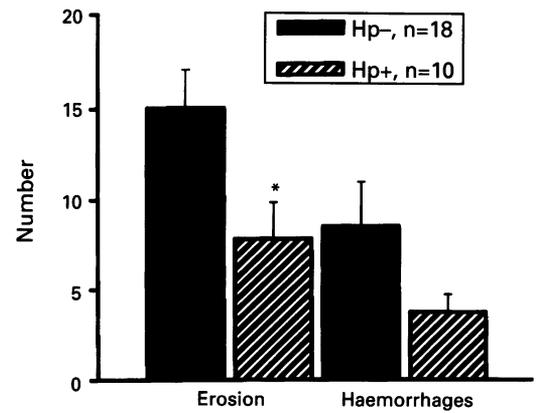

Figure 4: Effect of aspirin on gastroduodenal sucrose permeability was studied in H pylori infected and uninfected subjects. H pylori infected subjects had similar gastroduodenal sucrose permeability at baseline (62·1(15·17) v 40·2 (8·42) mg, p=0·2) and after single (142·0 (24·5) v 193·4 (29·8) mg, p=0·3) and multiple (209 (55·6) v 210·1 (27·4) mg, p=0·6) dose administration of aspirin. Gastroduodenal sucrose permeability increased after the single and multiple dose ingestion of aspirin compared with baseline, irrespective of H pylori status.

Figure 5: The effect of H pylori infection on antral mucosal injury in the setting of multiple dose aspirin administration was assessed by endoscopy. Compared with uninfected subjects, H pylori infected subjects had fewer erosions (15 (2·0) v 7·8 (2·0)) (p<0·05) and epithelial haemorrhages (8·5 (2·5) v 3·7 (1·0)) (p=0·2) than uninfected subjects.

multiple dose aspirin compared with baseline values irrespective of the *H pylori* status (Fig 4). Mean urinary sucrose recovery after single and multiple dose aspirin administration was similar in both groups. *H pylori* infected subjects had fewer number of erosions (p<0·03) and epithelial haemorrhages (p=0·2) when compared with uninfected (Fig 5).

Discussion

We began with the hypothesis that the conclusions from the previous experiments in humans were possibly incorrect.[3] Instead, we confirmed that gastrointestinal sucrose per-

meability increased in association with aspirin induced mucosal injury. In our population, the mean urinary sucrose recovery in the absence of aspirin was less than previously reported (53·7 mg compared with 110 mg).[3] Nevertheless, a fourfold increase in sucrose recovery after aspirin administration was seen in both studies. We also extended the previous findings to three days of aspirin administration.

We confirmed that the increase in sucrose permeability was site specific. We hypothesised the increase in aspirin induced sucrose permeability was probably the result of small intestinal sucrose absorption and that obstruction of the stomach with a balloon would abolish the increase in sucrose absorption. However, we found aspirin induced a fourfold increase in sucrose permeability even when the duodenum was occluded suggesting that the stomach is the primary site of increased intestinal permeability. It is unknown whether the trauma associated with placement of the pyloric occlusion balloon also increased mucosal injury and contributed to the increased sucrose recovery. As sucrose empties from the stomach during the standard sucrose permeability test but does not during balloon pyloric occlusion, and the balloon displaces the mass of sucrose solution, the mucosal surface area exposed to sucrose was likely to have been much greater during the balloon test. It is therefore not clear whether increased surface area or longer duration of contact is the most important factor related to increased sucrose absorption during balloon pyloric occlusion. Tests with higher concentrations of sucrose and larger volumes of solution are needed to tackle this question.

Sucrose recovery after single and multiple dose aspirin ingestion were similarly increased suggesting that the increase in sucrose permeability was not due to generalised leakiness of the gastric mucosa associated with the pronounced fall in mucosal potential difference and widespread surface damage seen immediately after injury with aspirin and alcohol (Fig 1). The variability in the aspirin induced changes among subjects suggest that some people may be more sensitive to the affects of aspirin.

The increase in sucrose permeability after multiple dose aspirin administration did not correlate with the number of erosions and haemorrhages. This finding in a group of healthy volunteers suggests the increase in sucrose permeability after a 72 hour course of aspirin is related to microscopic, and not macroscopic changes. Prospective, randomised trials evaluating sucrose permeability in a group of longterm NSAID users is needed to determine the clinical utility of this test.

H pylori infection did not seem to affect gastric mucosal permeability to sucrose either at baseline or after the administration of single or multiple doses of aspirin. Furthermore, as previously reported, the gastric mucosa of our group of asymptomatic *H pylori* infected people seemed less susceptible to mucosal injury.[12-16] Despite fewer number of erosions and epithelial haemorrhages, the *H pylori* group had similar mean sucrose recovery after aspirin ingestion than the uninfected group.

Recently, the sucrose test was suggested as being able to predict endoscopic mucosal injury.[17] In a group of 187 patients requiring endoscopic evaluation, or taking NSAIDs, increased sucrose permeability was associated with severe gastritis and gastric ulcers but not with mild gastritis or duodenal disease. In that study, the sensitivity for detecting mild gastritis, duodenitis, severe gastritis, and gastric ulcers was 16%, 29%, and 84%, respectively. The specificity for predicting an abnormal endoscopy was 96%. That analysis included patients with previous ulcer history and those requiring urgent endoscopy. Of interest, our study used the same endoscopic scoring system and we found that the sensitivity of sucrose permeability for mild gastritis (n=12), duodenitis (n=14), and severe gastritis (n=6) was similar; 17%, 29%, and 67%, respectively. No ulcers were seen in our population.

In summary, our data confirm the use of sucrose permeability as a marker of acute aspirin induced gastroduodenal mucosal injury and extends its usefulness to multiple dose. Furthermore, we have shown that multiple dose administration of aspirin increases gastric (compared with gastroduodenal) permeability to sucrose. *H pylori* infection does not seem to

change gastric permeability nor macroscopic mucosal injury as baseline or after single and multiple doses of aspirin. Larger clinical trials evaluating sucrose permeability in a variety of clinical conditions will be necessary to further define the role of this novel marker as a tool in the evaluation of patients with upper gastrointestinal symptoms or patients taking NSAIDs.

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