Sucrose Urinary Excretion in the Rat Measured Using a Simple Assay: A Model of Gastroduodenal Permeability

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Purpose. To develop a non-invasive animal model suitable for studies of altered gastroduodenal (GD) permeability, which is suggested to indicate GD damage; to validate a low cost and convenient assay for sucrose in urine, a permeability marker of GD.

Methods. Control (n = 87) and treated male Sprague-Dawley rats were dosed orally with 1 g of sucrose. Urinary excretion of the sucrose (0–8 h) was measured indirectly by cleavage to glucose and subsequent measurement of glucose in urine using a calorimetric assay. Treated rats were administered single oral doses of 10 and 20 mg/kg indomethacin, or 42 mg/kg aspirin alone or with 0.5 mL 50% ethanol (n = 7 in each group).

Results. The assay was linear within the examined range of 10–100 ug/mL sucrose. The inter and intraday variations were 7.63% and 6.89%, respectively. The urinary excretion of sucrose was complete in 8 h. In control rats the urinary excretion of sucrose exhibited a left skewed frequency distribution curve with a mean of 0.6 ± 0.14% of the dose excreted. All treatment, with the exception of 10 mg/kg indomethacin significantly increased the GD permeability. The GD effect was found to be dose dependent and parallels those reported for humans.

Conclusions. The rat is a suitable model for studies of GD permeability. Combined use of sucrose and ^{51}Cr-EDTA, a marker of intestinal permeability, allows for non-invasive examination of abnormalities of the entire gut. The sucrose assay is convenient and cost effective. The rat model may be useful in the preclinical screening of NSAID formulations and also in the detection of other GI abnormalities.

KEY WORDS: gastroduodenal permeability; ulceration; sucrose; NSAIDs.

INTRODUCTION

The main side effect of non-steroidal antiinflammatory drugs (NSAIDs) are gastrointestinal (GI) disturbances, due to their potent cyclooxygenase inhibitory effect (1–2). An inherent problem with examining adverse effects of NSAIDs is that diagnosis is difficult and invasive, and is usually only demonstrated after, at least, one of the complications (e.g., bleeding, perforation, or hemorrhage) becomes clinically apparent (3). There is often a poor correlation between patient reported symptoms of upper GI distress and endoscopically proven gastropathy (4).

Recently, NSAID-induced GI permeability increases, that precede ulceration, have received close attention (5–6). The enhanced permeability of the GI epithelium are postulated to be a prerequisite for the mucosal inflammation and the gross toxicological manifestations seen with NSAID use (5,7,8). Markers of permeability, therefore, offer surrogate measurements of GI abnormalities.

We have previously reported the suitability of the rat as a model for non-invasive detection of NSAID induced small intestinal permeability using ^{51}Cr-EDTA (9). The data generated after administration to the rat of various NSAIDs alone or with cytoprotective agents resembled those of human results. The model, therefore, was found useful for prediction of NSAID-induced intestinal abnormalities. To further improve the model so that it could be used for studies of the entire GI tract, we undertook the present study to examine whether the gastroduodenal (GD) permeability changes in the rat also parallel those reported in humans. Such an animal model can be used to provide quantitative assessment of the relative safety profile of various NSAIDs with different pharmacokinetic or release characteristics.

Sucrose as a permeability probe in humans (5,6) and rabbits (5), has been suggested to be a suitable marker for upper GI side-effects of NSAIDs. The sugar has very limited permeability across a healthy GD tract, and since it is rapidly hydrolyzed in the small intestine, its detection in urine after oral doses indicates permeability at the GD level. The suggested methods for determination of urinary sucrose, however, are costly and inconvenient. They include HPLC with electrochemical detection or refractometers which involve time-consuming extraction steps during sample preparation and offer only a moderate degree of sensitivity (5,10). Another approach to sucrose analysis is by enzymatic or chemical assay of monosugars formed following hydrolysis of sucrose (Boehringer Mannheim Biochemica, Laval Canada). However, the costs of the commercially available reagents and lengthy multiple step procedures make these methods inconvenient.

The present work was initiated to 1) examine the suitability of the rat as an animal model of GD permeability studies and 2) to develop and validate a convenient method for assay of sucrose in urine.

MATERIALS AND METHODS

Materials

Indomethacin, sucrose and Trinder's Reagent (glucose oxidase, peroxidase, 4-aminoantipyrine and p-hydroxybenzene in pH 7 buffer) were purchased from Sigma (St. Louis., MO, USA). Aspirin was purchased from Mallinckrodt Chemical Works (St. Louis, MO. U.S.A.). D-glucose, methylcellulose, and ethanol were purchased from BDH chemicals (Toronto, Canada). ELISA assay plates were purchased from Fisher Scientific (Edmonton, Canada).

Animals

The study was carried out according to the Principles of Laboratory Animal Care. Male Sprague-Dawley rats (300–370 g) were housed at ambient temperature and humidity in individual metabolic cages with wire mesh floors allowing for selective quantitative collection of urine and feces. Animals were fasted overnight then were allowed free access to

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food (standard rat chow) three hours post-NSAID. Water was supplied for the duration of the experiment.

Study Design

To test baseline GD permeability, 1.0 ml of a solution containing 1.0 g/ml of sucrose was administered to each rat (n = 87) orally through an 18 gauge 5 cm curved feeding needle attached to a 1 ml syringe. Urine was collected 0 to 8 h following the administration of the sucrose solution.

To examine the effect of NSAIDs, each drug (indomethacin 10 and 20 mg/kg, aspirin 42mg/kg, and aspirin 42 mg/kg with 0.5 ml of 50% ethonol) was suspended in 2% methylcellulose and administered orally to each group of rats (n = 6–7) at the same time of day (9 a.m.) 1 hour prior to the sucrose solution. The above NSAIDs doses are equal to those used previously by us to examine intestinal permeability changes (9). Sucrose dosing and urine collection were performed as for the baseline study. Each rat served as its own control with at least a 48 h wash-out period between sucrose doses.

Relative permeability was determined by calculating the sucrose present in each urine sample as a percent of the administered dose after correcting for baseline levels of glucose and sucrose present in urine for each individual rat.

Assay

Standard Solutions

Stock solutions of sucrose (1.9 mg/mL), glucose (1.0 mg/mL), and Trinder's Reagent were prepared in Sorensons phosphate buffer (pH = 7.0). All solutions were stored at 5°C.

Sample Preparation

To a 100 μl aliquot of rat urine were added volumes of either glucose or sucrose for calibration curves with final concentrations of 0, 10, 20, 40, 50, 80 and 100 μg/mL. The constituents were then vortex-mixed for 30 s. Since sucrose is not reactive with the Trinder's Reagent, it was completely cleaved to glucose and fructose using 25 μl of 2M H_2SO_4 per 100 μl urine sample followed by a brief vortex-mix and 10 min in a boiling water bath. Forty μl of 2M NaOH was then added followed by Sorensons phophate buffer q.s. 0.5mL. In preliminary work, glucose standard curves prepared with acid hydrolysis and heating showed no significant change in absorbance from those of sucrose prepared with acid hydrolysis. One mL of Trinder's Reagent was then added and the entire mixture was gently vortex-mixed and allowed to stand for 18 min before reading absorbance. Following preparation of the samples 100 μl of sample were plated into well bottom ELISA plates and absorbance was determined on an ELISA plate reader (Cayman Chemical, Ann Arbor, MI. U.S.A.) with a 492 nm lens.

Statistical Analysis

Differences between pre and post dose GD permeability were determined by paired t-test at $\alpha = 0.05$. Data are presented as mean ± standard deviation.

RESULTS

Linear relationships ($r > 0.99$) were observed for cleaved sucrose and glucose curves (10–100 μg/mL) which was typically described as Absorbance = 0.004×-0.00024. Sucrose standard curves constructed without acid hydrolysis and heating showed no U.V. absorbance above baseline at 492 nm. Addition of acid (2M H_2SO_4) without heating did not result in detectable cleavage of sucrose. The interday and intraday coefficient of variance were 7.63 and 6.89%, respectively. The minimal quantifiable concentration was 10 μg/mL.

The mean baseline permeability (percent of the sucrose dose excreted in urine from 0–8 h) in untreated male rats (n = 87) after oral administration was determined to be 0.6 ± 0.14% (Fig. 3). The baseline permeability values in male rats from 0–8 h are positively skewed with a range of 0 to 0.77. The wide range of values found in the baseline population is indicative of the degree of variability inherent in measurement of GD permeability. Negligible amounts of sucrose were found in urine samples collected in a 8–24 h collection period in all treated and untreated animals.

Aspirin (42 mg/kg) in the presence and absence of alcohol caused significant increase in the urinary excretion of sucrose by 1.24 ± 0.56% and 0.79 ± 0.51% of administered dose respectively (Fig. 2). The effect of indomethacin appeared to be dose dependent as 10 mg/kg doses had little effect while 20 mg/kg doses significantly increased sucrose permeability by 0.59 ± 0.39% of dose administered (Fig. 3).

DISCUSSION

Tests of permeability have been employed for many applications in the investigation of gastrointestinal diseases (5,7). These tests are safe, well tolerated, reproducible, and easy to perform. Their non-invasive nature allows for easy application to diagnostic screening and research, complementing currently available techniques of GI imaging (11). Another advantage of permeability tests is that they reflect the functional integrity over an area, whereas biopsy and endoscopy may miss damage caused in inaccessible zones.

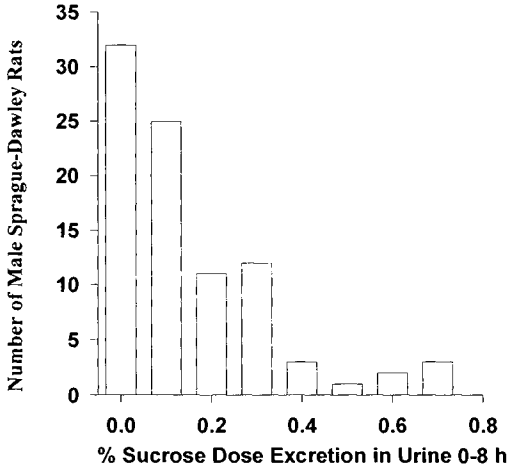

Fig. 1. Frequency distribution histogram of baseline sucrose urinary excretion 0–8 h in control male Sprague-Dawley rats after oral administration of 1 mL of a solution containing 1 g/mL of the probe.

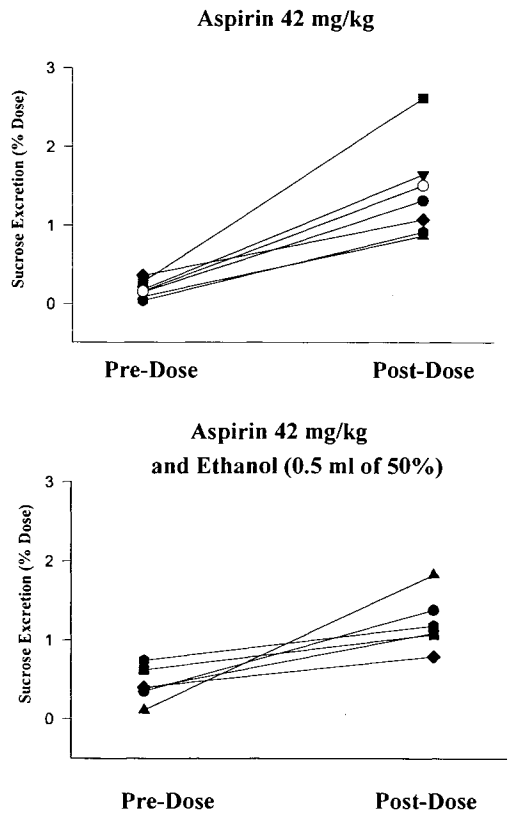

Fig. 2. Gastroduodenal permeability measured as % urinary excretion of sucrose excretion 0–8 h following oral administration of single doses of 42 mg/kg aspirin with or without 0.5 mL, 50% ethanol. All treatments are significantly different from baseline. Each line represents one rat.

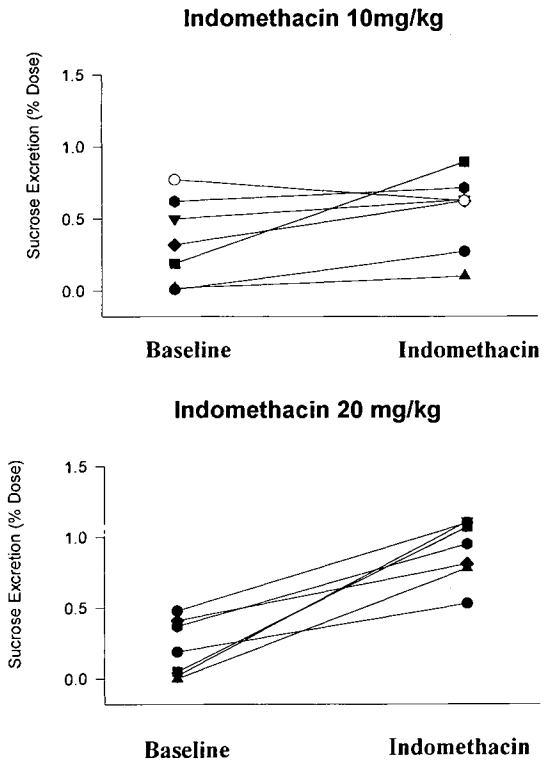

Fig. 3. Gastroduodenal permeability measured as % urinary excretion of sucrose excretion 0–8 h following oral administration of single doses of 10 and 20 mg/kg Indomethacin. 20 mg/kg treatment significantly above baseline. Each line represents one rat.

Sucrose (5,6) and ^{51}Cr-EDTA (7,12) have limited GI permeation through a healthy GI tract. It has been shown, however, that ingestion of NSAIDs causes increased GI permeability to these molecules (9,14): Passive flow of molecules through the intestinal barrier takes place *via* two routes: The polarised epithelial cells (transcellular) and intercellular spaces (paracellular). The major pathways for passive crossing of ions is the paracellular route and flow *via* the transcellular pathway is negligible unless cell damage occurs *e.g.*, in ulceration. The paracellular space is bounded on the luminal side by the tight junction. Upon stimulation, polymorphonuclear leucocytes migrate out of capillaries into the lamina propia and across the intestinal epithelium to form crypt abscesses. The results of this migration is a substantial but reversible increase in permeability and consequently passage of macromolecules (12).

The entire GI tract is prone to increased permeability. The effect, however, appears to be NSAID dependent. For example, despite significant upper GI toxicity, regular release aspirin formulations have minimal effect on the distal intestinal permeability in both humans (7) and rats (9). Previously, we hypothesized (9) that this is due to the almost complete absorption of the drug from the upper GI and its rapid metabolism to salicylic acid (13). Hence, despite its potent cyclooxygenase inhibitory effect, aspirin does not reach the lower intestinal epithelium in sufficient concentrations either during absorption or *via* systemic distribution. In contrast to aspirin, indomethacin permeability enhancing effect appears to be concentrated in the lower GI since the drug increased the sucrose urinary excretion only after 20 mg/kg doses (Fig. 3). At this dose level, indomethacin elicits its maximum effect on the intestinal permeability as measured using ^{51}Cr-EDTA. The possibility of a tissue-selective gut effect highlights the importance of considering the entire GI tract and the usefulness of site specific probes such as sucrose and ^{51}Cr-EDTA in studies of NSAID side effects. This may be even more important when modified released NSAID formulations are examined for their GI toxicities.

The use of sucrose over other markers offers unique advantages. An increased sucrose permeability appears to reflect clinical gastrophathy (6,14). Sucrose is inexpensive, non-toxic, and is specific due to its cleavage after passing through the stomach into the intestine. Once absorbed, sucrose is rapidly cleared intact *via* the renal route (15). Hence, its urinary excretion reflects the amount which has entered the systemic circulation. The use of a calorimetric assay to quantify sucrose in rat urine does not require extensive sample preparation. The cleavage of sucrose with acid and heat appears to be complete and without degradation of the resultant monosugars. Samples can be processed using economical commercially available reagents without the need for expensive HPLC equipment.

The findings reported here for baseline GD permeability of sucrose in rats are similar to those reported for humans and rabbits. In rabbits, GD permeability of sucrose was previously determined to be approximately 0.7% of the administered sucrose dose over 24 h following intragastric admin-

istration (5). The mean percent of an oral sucrose dose excreted in urine of healthy controls (0–5 hours) has previously been reported to be 0.11 ± 0.005% (5). The rat results (Fig. 2) are consistent both qualitatively and quantitatively with previously reported results in humans.

Similarly, the findings reported here for GD permeability after treatment with NSAIDs parallel those seen in other species. GD permeability of sucrose in humans shows almost a four fold increase with 600 mg of aspirin and 50 mL of vodka or aspirin alone (5,14). Our results in rats (Fig. 2) are similar to these findings. The effect of 20 mg/kg indomethacin on rabbit GD permeability has been demonstrated to be up to 5 fold above baseline (5). Similarly, in the rat a 5–10 fold increase in GD permeability above baseline was demonstrated at the 20 mg/kg dose of indomethacin. (Fig. 3).

The rat appears to be a suitable and useful investigational animal model of sucrose GD permeability studies. Combined use of sucrose and ^{51}Cr-EDTA may allow for non-invasive examination of abnormalities of the entire gastrointestinal tract.

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