Sucrose Breath Test

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Non-Invasive Assessment of Intestinal Function

Overview

Introduction

This paper will demonstrate that the ^{13}C-sucrose breath test (^{13}C-SBT) determines the health and function of the small intestine. Damage to the small intestine results in a decrease in the activity of brush border membrane enzymes such as sucrase. The ^{13}C-SBT is a simple, sensitive and specific breath test to quantify sucrase activity in vivo which reflects small bowel function. The test is clinically valuable to screen patients for either known agents that cause gastric and small bowel toxicity (NSAID, alcohol or chemotherapy-induced), with infectious conditions (celiac disease, Crohn's disease, HIV, chronic diarrhea), or intestinal failure (atrophy caused by parenteral nutrition). The ^{13}C-SBT can also be used to screen new drugs and probiotics for treatment of intestinal damage.

Significance

The mucosal lining of the small intestine plays an important role as a barrier to the external environment composed of potentially harmful compounds such as bacteria, toxins and antigens. The mucosal lining is also the major interface for nutrient absorption. The surface area of the brush border is maximized by villi protruding into the gut lumen. Serious health ramifications can result if the functional area of the brush border is significantly reduced by a reduction of the villi absorptive surface area or damage to the enterocytes lining the small intestine.

Applications

The ^{13}C-SBT can be used to assess intestinal function in the following areas:

- Gastric Toxicity caused by NSAID, chronic alcohol or cancer chemotherapeutic agents
- Intestinal damage from GI inflammation such as celiac, Crohn's disease or IBS
- Intestinal failure such as atrophy caused by parenteral nutrition
- Monitoring of Intestinal Healing by Probiotics

Principle of the SBT

The ^{13}C-sucrose breath test uses a naturally-enriched sucrose solution as the test substrate. Sucrose is broken down by sucrase located in the brush border of the small intestinal mucosa. Sucrase splits ^{13}C-sucose into ^{13}C-glucose and ^{13}C-fructose. These sugars are absorbed through the mucosal cells and will be metabolized by first-pass in the liver, releasing $^{13}CO_2$. Labeled bicarbonate is transported by blood to the lungs where it is expelled in the breath. The release of $^{13}CO_2$ in breath correlates with the amount of sucrase

activity in the small intestine. If sucrase activity is diminished, it is a strong indicator that the epithelial cells lining the villi are damaged.

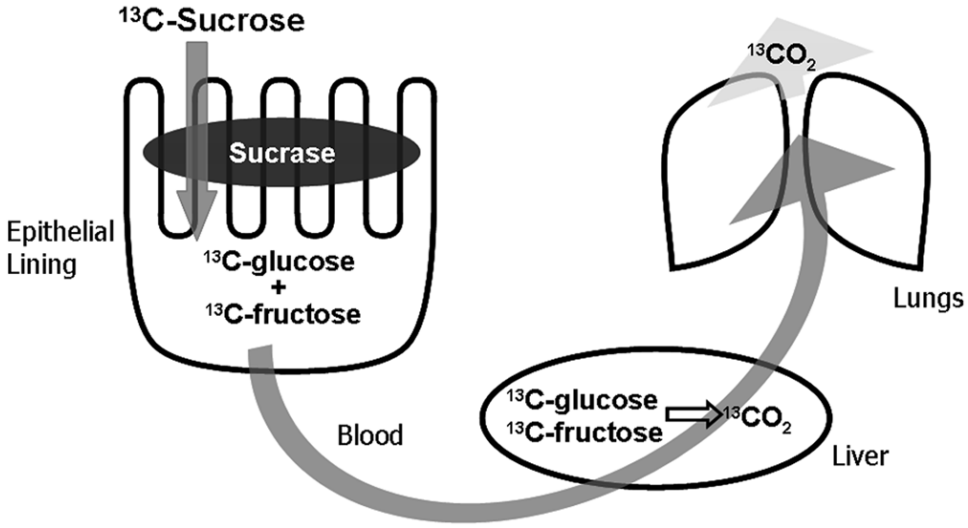

Figure 1. Illustration of the basic principles of the SBT in a healthy individual. The ingested ^{13}C-sucrose is cleaved into glucose and fructose by the brush-border enzyme sucrase. These sugars are then transported to the liver and metabolized, before passing to the lungs where the resultant $^{13}CO_2$ is expired.

SBT Protocol

The SBT can be easily performed at all clinical research facilities. Metabolic Solutions provides a complete administration kit with a pre-measured dose of ^{13}C-sucrose (20 grams), 4 breath tubes and collection device, and a pre-paid and pre-addressed mailer to ship back the samples. The steps to conduct the SBT are:

Step	Action
1	Collect a baseline breath sample.
2	Mix the 20 gram sucrose dose with 200 ml potable water.
3	Administer the ^{13}C-enriched sucrose solution to the subject.
4	Collect first breath sample at 30 minutes post-dosing.
5	Collect two additional breath samples at 60 and 90 minutes post-dosing.
6	Place all breath tubes in mailer and send to Metabolic Solutions.
7	Results of breath test are available the same day received.

Assessment of Small Intestinal Damage

Illustration of Small Intestinal Damage Assesment

Damage to the small intestine results in reduced activity of brush border enzymes such as sucrase and lactase (Gray GM, 1975). Damage to the intestinal mucosa from chemotherapeutics such as methotrexate was evaluated in the rat using the ^{13}C-sucrose breath test (Pelton et al, 2004). Male Sprague Dawley rats received three daily subcutaneous methotrexate (MTX) injections to induce small intestinal damage. Controls received saline injections. After 1 week, a sucrose breath test was performed on all rats. The time course of breath $^{13}CO_2$ levels is shown in figure 2.

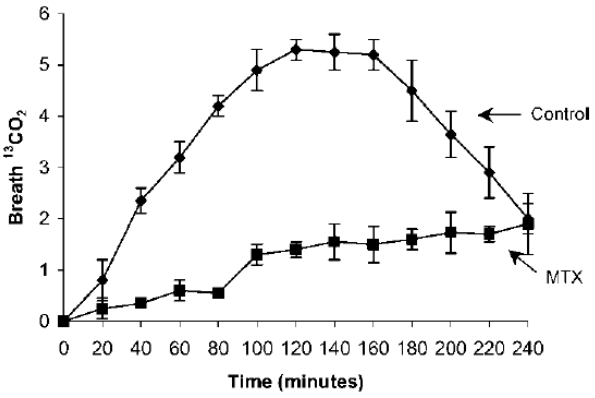

Figure 2: Time course of breath $^{13}CO_2$ exhalation following administration of naturally enriched ^{13}C-sucrose.

Sucrase levels contained in biopsies of the duodenum, jejunum and ileum of methotrexate-treated rats were 15%, 8% and 16% of corresponding gut regions of control rats. Total intestinal sucrase activity assessed biochemically was highly correlated ($r^2 = 0.92$) with sucrase activity assessed by the ^{13}C-sucrose breath test.

Comparison of Methods for Assessing Small Intestinal Function

Small Intestinal Permeability

Small intestinal permeability (SIP) tests have been developed to determine barrier function non-invasively. Intestinal function in many diseased states was first measured by assessment of xylose absorption from the gut (Bjarnason et al, 1995). Xylose is passively absorbed in the jejunum. The xylose test was shown to be variable and was not adopted for routine assessment of small intestinal function. Further advancements have led to using a combination of disaccharide/monosaccharide sugar permeability tests. The substrates that cross the epithelium can be measured in serum or urine. Recent methods have used monosaccharides such as rhamnose and mannitol and disaccharides such as lactulose and sucrose. While these tests are useful in the assessment of barrier function, they do not necessarily provide a clear or sensitive indication of absorptive capacity of the small intestine. The collection of 5-hour urines is often unreliable, tedious and inconvenient for patients.

Hydrogen Breath Tests

Carbohydrate malabsorption of common sugars (lactose, sucrose, glucose and fructose) has been used for detecting gastrointestinal damage. The ingested sugar substrate is degraded and absorbed in the healthy individual. However, individuals deficient in the respective digestive enzyme or required absorption transporters in the small intestine, results in the substrate propelled into the large intestine. Once in the colon, the substrate is metabolized by bacteria to either hydrogen or methane gas. These gases are transported via the bloodstream to the lungs and exhaled in the breath. Significant changes, typically 20 parts per million, above background suggest carbohydrate malabsorption. Unfortunately, up to 20% of the population may not have colonized bacteria capable of metabolism to hydrogen or methane gas.

Serum Citrulline Levels

Citrulline is an amino acid metabolite of glutamine metabolism. Glutamine is predominately metabolized by small intestinal enterocytes. Serum citrulline levels have been identified as a suitable biomarker for small intestinal enterocytes surface area. The principle of the citrulline test is that decreased cell mass in the small intestine results in decreased serum concentrations of citrulline. This measurement has been applied during gut surgery, celiac disease and small intestinal transplant rejection. The disadvantage of the citrulline test is the requirement to analyze serum levels by LC/MS instruments. Levels of citrulline in blood can be influenced by breakdown of ornithine during sample storage.

Summary of Advantages and Disadvantages

Test	Advantages	Disadvantages
Small bowel biopsy	• Gold standard • View of gut health	• Invasive/risky • Requires sedation • Expensive
Sugar Permeability Tests	• Non-invasive • Detects barrier function only	• No absorptive function detected • 5-hr collections
Hydrogen Breath Tests	• Non-invasive • No risk to patient	• Requires H_2 bacteria • Influenced by diet • Indirect test
Serum Citrulline	• Suitable biomarker	• Invasive blood collections • Requires costly equipment
Sucrose Breath Test	• Simple breath test • Assesses small intestinal function	• Requires isotope ratio mass spectrometry

Measurement of Gastric Toxicity

Chemotherapy Injury

Inflammation of the mucus lining of the entire gastrointestinal tract, referred to as mucositis, is a common and debilitating consequence of cancer chemotherapy. The onset and progression of oral mucositis can be determined easily. However, intestinal mucositis is extremely serious with significant morbidity. The detection and progression of intestinal mucositis is not available.

Intestinal mucositis induced by different classes of chemotherapeutic agents were used in a rat model to broaden the application of the sucrose breath test (Howarth et al, 2006). Mucositis was induced in rats by injection of Doxorubicin, Etoposide, Irinotecan, or Cyclophosphamide. The sucrose breath test was performed 72 hours after chemotherapy. Intestinal biopsy was collected for assessment of histology and sucrase activity.

The sucrose breath test was decreased from 30 to 53% of a saline control group. Correlations between the sucrose breath, sucrase activity and histological severity score yielded $r^2 = 0.82$ values. The sucrose breath test detected mucositis and small intestinal dysfunction induced by several different chemotherapy drug classes. These experiments show the sucrose breath test can screen for intestinal side-effects caused by chemotherapeutic drugs.

Assessment of Intestinal Mucositis in Children

The sucrose breath test has been used as a biomarker to detect small intestinal changes in children undergoing cancer chemotherapy (Tooley et al, 2006). Several biomarkers of small intestinal function were assessed in 15 pediatric cancer patients and 26 healthy children. The biomarkers included a small intestinal permeability test using lactulose and rhamnose, a lactulose hydrogen breath test to measure orocecal transit time, and the sucrose breath test.

Clinical mucositis occurred in 28% of the 25 chemotherapy cycles. No differences in the permeability and hydrogen breath testing between the mucositis and non-mucositis groups were found. However, the sucrose breath test results were significantly lower in the mucositis group compared to the unaffected chemotherapy group and controls. In patients who developed mucositis, the sucrose breath test was below the reference range of the controls at all time points. This is the first evidence in humans that the sucrose breath test can non-invasively detect and monitor intestinal damage associated with chemotherapy.

Conclusions

The sucrose breath test is a direct non-invasive assessment of the digestive and absorptive capacity of the small intestine. The level of sucrase activity in the brush border is a measure of small intestinal mucosal health. Sucrase levels are reduced with mucosal injury but are relatively stable by race and throughout life (Welsh et al. 1978). This biomarker has been applied in animal models of chemotherapeutic damage and to childhood cancers. The sucrose breath test is useful to detect and monitor intestinal damage and therapeutic repair.

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