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Review

Mucositis and non-invasive markers of small intestinal function

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Abbreviations: SBT, sucrose breath test; SIP, small intestinal permeability; H_2BT, hydrogen breath test; OCTT, oro-cecal transit time; NSAIDs, non-steroidal anti-inflammatory drugs; MTX, methotrexate; SI, small intestine; GIT, gastrointestinal tract; L/R, lactulose/rhamnose ratio; IRMS, isotope ratio mass spectrometer

Key words: mucositis, small intestine, ^{13}C-sucrose breath test, intestinal permeability, non-invasive assessment

Mucositis is a common and debilitating side effect of chemotherapy that manifests due to the inability of chemotherapy agents to discriminate between normal and neoplastic cells. This results in ulcerating lesions lining the gastrointestinal tract. Moreover, the development of efficacious treatments for small intestinal mucositis has been hindered as the pathobiology of mucositis is still not fully understood. The small intestine is an extensive organ which is largely inaccessible by conventional means. Non-invasive biomarkers such as small intestinal permeability, H_2 breath tests, serum citrulline tests and the ^{13}C-sucrose breath test (SBT) have emerged as potential markers of small intestinal function. The SBT is emerging as the more appropriate biomarker to assess chemotherapy-induced mucositis in cancer patients and animal models, where it measures the decrease in sucrase activity associated with villus blunting and crypt disruption. The SBT has been successfully applied to detect mucositis induced by different classes of chemotherapy agents and has been used successfully to monitor small intestinal function with a range of candidate anti-mucositis treatments. We propose the SBT a superior biomarker of small intestinal function that could be successfully applied in clinical practice for monitoring the development of mucositis in cancer patients undergoing chemotherapy.

Introduction

Mucositis is a common and debilitating side effect of chemotherapy, affecting up to 60% of patients receiving high-dose chemotherapy, and almost 100% of patients undergoing preconditioning chemotherapy regimens for stem cell transplant.[1] Intestinal mucositis is characterized by ulcerating lesions lining the gastrointestinal tract (GIT) and no truly effective therapies are currently available for this distressing disorder. This phenomenon occurs due to the inability of chemotherapy agents, such as methotrexate, irinotecan, etoposide, cyclophosphamide, melphalan and 5-Fluorouracil, to discriminate between normal and neoplastic tissue.[2,3] Cells that divide rapidly such as tumor cells, and those that line all mucosal membranes, are equally sensitive to damage. Many previous studies have been confined to assessing mucositis associated with the oral cavity, primarily due to its accessibility.[4-9] However, lower GIT toxicities are becoming increasingly apparent with the utilization of higher more toxic doses and new agents to maximize tumor kill. The current review discusses the available non-invasive markers available to detect the small intestinal complications of chemotherapy in cancer patients.

In the small intestine, chemotherapy-induced mucositis results in villus blunting, hypoproliferation of crypt cells, and shallow crypts, due primarily to an increase in apoptosis and a decrease in proliferation.[1,10,11] Patients developing intestinal mucositis may experience symptoms ranging from mild nausea, vomiting and abdominal bloating through to painful cramping and diarrhea, with intense abdominal pain (requiring narcotic administration). In its most severe form, bacterial translocation and sepsis, which can be fatal, result from mucositis.[12] Whilst mucositis does not often lead to mortality, the associated symptoms are uncomfortable and painful, and often impair food intake, communication, sleep and mental status.[13] The inability to swallow food, or indeed sometimes liquids, can result in dehydration, malnutrition and in many cases anorexia, where more drastic forms of energy delivery must be utilized such as naso-gastric feeds, or parenteral nutrition. In combination these measures lead to increased hospital stays.[14] In these cases, patient chemotherapy regimens are often postponed or drug doses are reduced, leading to sub-maximal tumor kill. Patients may spend extended or unplanned stays in hospital, often requiring parenteral feeding, and leading to a significantly decreased quality of life.[4,15] Other side-effects of chemotherapy, such as neutropenia and thrombocytopenia, are usually well managed.

The development of effective treatments for small intestinal mucositis has been hindered as the pathobiology of mucositis is

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still not fully understood, resulting in increased costs for the public hospital system. In the United States it has been estimated that each day a cancer patient is admitted for an unplanned hospital visit, it will cost on average US$5,000/day.[4,15] There is therefore a clear need to develop agents to protect the intestine during cancer treatment, potentially enabling the ability to tolerate higher chemotherapy doses.

Methods for Assessing Small Intestinal Function

The small intestine is an extensive organ that is largely inaccessible by conventional endoscopic or colonoscopic means, hence determining its health status has proved difficult.[16] This in turn has limited the development and evaluation of therapeutic interventions. Common techniques for assessing small intestinal function range from surgical exploration, endoscopy or colonoscopy, small bowel biopsy, X-rays and the barium swallow. Whilst endoscopy and colonoscopy procedures are used regularly in clinical practice for diagnosis of gastrointestinal complaints, only the more proximal portions of the small intestine can be routinely assessed, posing a real problem in determining the true functionality of the whole small intestine. The current "gold" standard technique for assessing small intestinal function remains the small bowel biopsy. However, this technique is inadequate for a number of reasons:[17] it is invasive, only assesses the proximal small intestine, requires sedation, is painful and expensive, and importantly only reflects the function of the biopsied fraction of the small intestine.[18-20] Cancer patients not only develop mucositis, but also low platelet and white blood cell counts as a result of the chemotherapy. These additional side-effects increases the risk of utilizing the small bowel biopsy.[21]

Small intestinal permeability. Small intestinal permeability (SIP) tests have been developed to determine barrier function non-invasively.[22] Previously, intestinal function in many diseased states has been measured by absorption of xylose, which is passively absorbed in the jejunum.[23-26] However, this test has been shown to be variable, and has not been adopted for routine assessment of small intestinal function.[24] Further advancements have led to the combination of disaccharide/monosaccharide sugar permeability tests in which man-made sugar probes are utilized. These sugars are metabolized by colonic bacteria and not by intestinal mammalian cells. Substrates that permeate the epithelium can be measured in serum or urine samples utilizing high performance liquid chromatography techniques. More recently, methods have been described utilizing monosaccharides such as L-rhamnose[22,24,27,28] and mannitol,[29,30] and disaccharides such as lactulose,[22,24,30] and more recently, sucrose.[30-33] In general, sugar probes utilized in permeability tests are safe, reproducible, well tolerated and cost effective.

In the healthy gut, two routes are available for passive permeation across the intestinal epithelium: through the enterocyte (transcellular) or between enterocytes (paracellular). The monosaccharide rhamnose and the disaccharide lactulose are non-metabolizable sugars. A reduced urinary rhamnose is thought to be indicative of an altered small intestinal surface area, whilst elevated urinary lactulose levels represents a loss of tight junctions between enterocytes

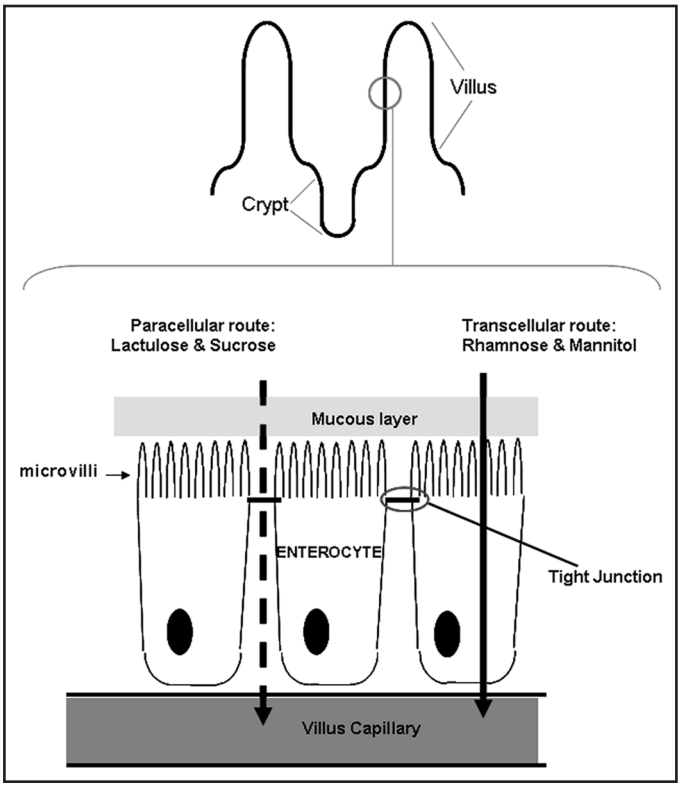

Figure 1. Depiction of the suggested transcellular (through the cell) and paracellular (in between cells) routes of permeation, where the transcellular route is indicative of small intestinal surface area, whilst tight junctions strictly regulate the paracellular pathway.

(Fig. 1). The first reported dual-permeability test was performed by Menzies et al. (1979),[22] examining abnormal sugar permeability in patients with villus atrophy and diagnosed celiac disease. Five-hour urinary excretion of lactulose and L-rhamnose was determined after an overnight fast. These investigators revealed that urinary L-rhamnose excretion was significantly decreased (40%, $p < 0.02$), proposed as a reflection of decreased small intestinal surface area. Lactulose excretion was increased by 340% ($p < 0.01$) possibly due to the small intestinal mucosa becoming "leaky" (more permeable) to larger probe molecules. It was also found that the median value of the lactulose/rhamnose (L/R) ratio was seven times higher in celiac disease patients compared to normals. Previously published studies describe the utilization of dual-sugar permeability tests in different disease settings such as inflammatory bowel disease,[34] non-steroidal anti-inflammatory drugs (NSAIDs),[35-37] diarrheal disease[28] and chemotherapy-induced mucositis.[12,38,39]

Impairment of gut function and small intestinal barrier integrity has previously been described using small intestinal permeability tests in patients with chemotherapy-induced mucositis.[12,38-40] These tests have used a combined monosaccharide and disaccharide sugar drink to determine enteropathy and permeability of tight junctions, respectively. Keefe et al.[12] reported that permeability was significantly altered in adults undergoing high-dose chemotherapy 7 days post-chemotherapy. Whilst this test is useful in the assessment of barrier function, it does not necessarily

provide a clear or sensitive indication of the absorptive capacity of the small intestine or the extent of damage. Additionally, 5-hour urine collection from cancer patients is tedious and often inconvenient for the patient, thereby reinforcing the need for a more suitable non-invasive marker of small intestinal function for implementation in clinical practice.

Hydrogen breath testing. Oro-cecal transit time (OCTT) was first validated by Bond and Levitt[41] by measuring the rise in hydrogen excretion in breath after ingestion of lactulose. The technique is based on the principle that colonic bacteria ferment a synthetic sugar substrate to produce hydrogen, which is then expired in the breath. Lactulose is a synthetic disaccharide, comprising of fructose and galactose, which is not digested by small intestinal brush-border enzymes.[42] Therefore the time between ingesting the substrate and the rise in hydrogen is representative of oro-cecal transit time. OCTT has recently been assessed in children with cancer undergoing chemotherapy, where it was demonstrated that the OCTT of patients who developed mucositis in a cycle of chemotherapy did not differ significantly from patients in whom mucositis was absent.[43] However, it is important to note that cancer patients have a significantly increased OCTT compared to healthy children.[43] Additionally, this test can be used in the detection of small bowel bacterial overgrowth.[44] It should be noted that the sensitivity and specificity of this test is not high as it relies solely on the presence of H_2-producing bacteria.[45] Improvement to the sensitivity of this test has been achieved by its coupling with the $^{14}CO_2$-Xylose breath test.[45]

The Hydrogen breath test (BT) is a similar test in principle to the OCTT, and is the currently employed non-invasive technique for detecting gastrointestinal damage via carbohydrate malabsorption. The test quantifies digestion and absorption of monosaccharides and disaccharides. The ingested sugar substrate, whether it be lactose, sucrose, glucose or fructose (common sugars assessed), is degraded and absorbed in the healthy individual. However, individuals deficient in the respective digestive enzyme, or who do not possess the appropriate transporters in the small intestine, will malabsorb the macro-nutrient. For example, a deficiency in the fructose transporter would result in the remaining luminal substrate being propelled towards the large intestine. The substrate, once in the colon, would be metabolized to form hydrogen due to the presence of hydrogen-producing bacteria. Hydrogen then enters the bloodstream and is transported to the lungs where it is expired. Whilst this breath test is the clinician's test of choice for sugar malabsorption, it does not provide a clear representation of small intestinal damage, and secondly, relies solely on the presence of hydrogen-producing bacteria residing in the colon.[46] Moreover, approximately 20% of the population do not possess these bacteria as a component of their colonic micro-biota, and the growing use of antibiotics further lessens the sensitivity of this test.[46] Additionally, it is well known that changes in the diet,[47,48] including the ingestion of antacids and proton-pump inhibitors,[49] can indeed alter the profile of gut flora. Thus, a negative HBT may be due to a shift from hydrogen to methanogen-producing bacteria. The lactose-HBT has been previously applied in patients, both children[50] and adults,[51] with varying results, but overall it highlights patients who have developed a decreased lactase activity. It is important to note that cancer patients are commonly treated with antibiotics as part of their ongoing treatment, which therefore reduces the sensitivity of the HBT as false negatives may result.

Serum citrulline test. Citrulline is an amino acid by-product of nitrogen glutamine metabolism, which is predominantly metabolized by small intestinal enterocytes.[52-54] Serum citrulline levels have been identified as a suitable biomarker for small intestinal enterocyte mass/surface area as the test measures a product of subsequent cellular metabolism. This test operates on the principle that a decrease in cell mass in the small intestine would results decreased serum concentrations of citrulline. This method has been applied in conditions including surgery,[55] celiac disease,[56] gastroenteritis (viral)[57] and small intestinal transplant rejection.[58] Recently, the serum citrulline test has been utilized to assess epithelial cell loss associated with chemotherapy in the small intestine with relative success.[59-61] Initially, this test was applied in mice receiving small bowel irradiation, where citrullinemia significantly correlated with crypt regeneration and small intestinal surface area.[61] Further application in patients undergoing bone marrow transplantation indicated that alterations in citrulline levels corresponded with the onset of oral mucositis and intestinal permeability changes.[60] A subsequent study confirmed this finding where the authors stated that the citrulline test was more sensitive to SIPT, as citrulline levels returned to normal whilst SIPT changes persisted.[62] However one could argue that this finding could indeed highlight the insensitivity of the citrulline test, as it was not able to detect the persisting damage as identified by the SIPT. Its implementation has been confined to experimental studies in rodents[61] and in patients[59,60] who have undergone total body irradiation or bone marrow transplantation; that is, animals or patients with the anticipated development of severe mucositis. Implementation of the serum test in less severe settings of mucositis has not been addressed, questioning its sensitivity. Furthermore, the serum citrulline test is somewhat invasive as it requires repeated collection of blood specimens which is an added stressor for the patient. It could also be argued that decreases in serum citrulline levels could indeed reflect the decreases in the patient's food intake, as reductions in and withdrawal from food is commonly observed in bone marrow transplant patients.[63] More recently, a study by Boukhettala et al.[64] suggested that a reduction in food intake did not mimic citrulline levels observed in MTX-treated rats. However, this finding has yet to be confirmed in humans, or indeed, in cancer patients.

$^{13}CO_2$ breath test. Mucosal damage in biopsies is commonly accompanied by decreased brush-border enzyme activities.[20] Evaluating disaccharidase activity is a more direct method of assessing gastrointestinal damage than the H_2 breath test. The non-invasive detection of low-intestinal lactase activity in children was studied by Koetse et al.,[46] with the aid of a combined ^{13}C-Lactose $^{13}CO_2/H_2$ breath test. This study found that the combined $^{13}CO_2/H_2$ (lactose) breath test was superior to the H_2 breath test alone for diagnosis of gastrointestinal damage. This suggested that in order to ensure reliable results in the future the combined $^{13}CO_2/H_2$ BT would be a more reliable, accurate and direct method for determining digestive capability of the small intestine. Eighty percent of

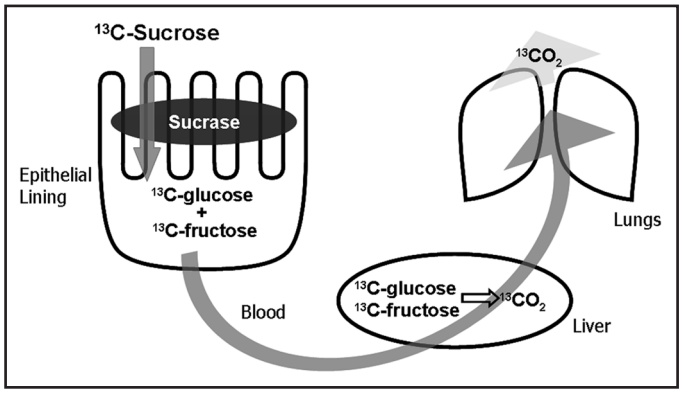

Figure 2. Illustration of the basic principles of the SBT in a healthy individual. The ingested ^{13}C-sucrose is cleaved into glucose and fructose by the brush-border enzyme sucrase. These sugars are then transported to the liver and metabolized, before passing to the lungs where the resultant $^{13}CO_2$ is expired.

non-Caucasians exhibit an age-related low lactase activity,[46] indicating that the ^{13}C-lactose breath test is not a suitable marker of small intestinal damage. In comparison, sucrase levels in the brush-border remain relatively constant throughout life,[65] and only 0.2% of the population present with a genetic sucrase deficiency.[66] Thus, a breath test utilizing ^{13}C-sucrose could provide a more reliable and superior prognostic indicator of mucosal damage. Application of the ^{13}C-lactose breath test has not previously been utilized in children with cancer undergoing chemotherapy.

^{13}C-sucrose breath test. In the healthy individual, sucrose is a disaccharide cleaved into its constituent monosaccharides, fructose and glucose by sucrase, a brush-border enzyme. Subsequent hepatic metabolism of these products produces CO_2, which is excreted in the breath (Fig. 2). Sucrose derived from cane sugar is naturally enriched with ^{13}C; therefore the resultant $^{13}CO_2$ can be detected and measured using isotope ratio mass spectrometer (IRMS) analysis.[46,67,68] ^{13}C-sucrose derived from cane sugar is naturally occurring. The level of ^{13}C enrichment is "selective," as not all carbon ions in the carbohydrate molecule are labeled with ^{13}C. Prior to SBT use, the level of ^{13}C-enrichment must be

Table 1 **Advantages and disadvantages of non-invasive tests with respect to patients with chemotherapy-induced SI damage**

Non-invasive test	Advantages	Disadvantages
Small bowel biopsy	• Gold standard • Visual perspective of gut health	• Assesses only most proximal and distal regions of small intestine. • It is invasive, requires sedation, is painful and expensive • Only reflects the function of the biopsied fraction • Added risk for cancer patients
Small intestinal permeability test	• Simple non-invasive test • Does not cause pain to the patient • Patient ingests a drink containing non-digestible sugars • Assesses barrier function of the SI	• Does not clearly describe the functionality/absorptive capacity of the SI • Five h urine collection is tedious • Requires the patient to drink water throughout testing
Oro-cecal transit time	• Simple breath test • Requires the ingestion of a non-digestible sugar • Can be used to assess the presence of a small bowel bacterial overgrowth when coupled with the radioactive ^{14}C-xylose breath test.	• Dependent on the presence of H_2-producing bacteria in the colon • Results can be influenced by changes in diet and the use of antacids and proton-pump inhibitors. • Does not reflect gut absorptive capacity
H_2BT	• Simple breath test • Indirectly assess small intestinal function • Non radioactive • Poses no risk to the patient	• Dependent on the presence of H_2-producing bacteria in the colon • Results can be influenced by changes in diet and the use of antibiotics • Does not reflect gut absorptive capacity
Serum itrulline	• Suitable biomarker for small intestinal enterocyte mass/surface area	• Invasive and requires multiple time-points of sample collection • Implementation in less severe settings of mucositis has not been addressed
^{13}C-lactose breath test	• Simple breath test • Indirectly assesses small intestinal function • Non radioactive • Poses no risk to the patient	• 80% of non-Caucasians exhibit an age-related low lactase activity • People who are lactose intolerant would be unable to perform this test • ^{13}C-lactose is not readily available
SBT	• Simple and inexpensive breath test • Assesses small intestinal function and absorptive capacity • ^{13}C-sucrose occurs naturally in cane sugar • Only 0.2% of humans have a genetic sucrase deficiency	• Breath collection occurs every 15 min for 2 h • SBT application still in early testing

determined via ^{13}C-combustion, as this is vital for recovered ^{13}C % dose calcuations.[69] The level of $^{13}CO_2$ is detected by measuring the relative enrichment of ^{13}C to ^{12}C in the CO_2 expired after ingestion of ^{13}C-sucrose.[46] In vivo determinations of sucrase activity can be used as an indicator of digestive enzyme activity and brush border integrity and enterocyte differentiation, together providing an indicator of small bowel function.[67,70,71]

As described previously, mucositis results in a decrease in sucrase activity compared to the healthy small intestine.[67,70,71] Initially, the SBT was assessed in a Sprague Dawley rat model of methotrexate (MTX) induced mucositis.[67] MTX-treated rats revealed a significantly decreased cumulative output of $^{13}CO_2$ and diminished small intestinal sucrase activity seven days after treatment. Importantly, these investigations reported a significant correlation between in vitro sucrase activity ($r^2 = 0.85$), and the degree of histological damage. In this initial study, only ^{13}C-sucrose doses of 1 and 2 g/mL were assessed. However, this represents a 100 or 200% saturated solution, which may have induced a hyper-osmotic effect. The ability of lower sucrose doses to detect small intestinal damage remains to be assessed. Additionally, the original SBT studies[67,70] utilized a crude form of data analysis, which have since been improved using ^{13}C gas analyses as described by Koetse et al. (1999).[46] The SBT has been successfully applied to detect mucositis induced by representative drugs from different classes of chemotherapy agents including MTX, 5-Fluorouracil, cyclophosphamide and etoposide, doxorubicin, etoposide and irinotecan.[67,70-75] More recently, the SBT has been used successfully to monitor small intestinal function with a range of candidate anti-mucositis treatments including oral folinic acid,[70] *Streptococcus thermophilus*[71] and Lyprinol[75] in the dark agouti rat receiving chemotherapy.

The SBT has been trialed in a number of novel settings including healthy young adults in a model of sucrase-isomaltase deficiency using the drug Acarbose™. More recently, the SBT has been applied to pediatric cancer patients undergoing chemotherapy.[43] This study demonstrated the ability of the SBT biomarker to non-invasively detect small intestinal changes associated with respect to chemotherapy-induced mucositis. This study was the first to highlight the onset of chemotherapy-induced small intestinal changes (mucositis) before the commonly clinically observed time-point of 7–10 days post-chemotherapy.[3,12,13] In clinical practice it is known that certain chemotherapy agents are likely to cause mucositis. However, there are occasions when patients unexpectedly develop mucositis in a cycle of chemotherapy. This is most likely due to the repeated administration of chemotherapy and/or heightened sensitivity of individual patients. The non-invasive SBT would allow the easy and cost-effective monitoring of small intestinal function in oncology patients to improve clinical management.

Summary

Whilst many advances have been made in the effort to treat chemotherapy-induced small intestinal mucositis, applying these treatments in a clinical setting has been hampered by the absence of a clinical biomarker that sensitively assesses intestinal damage, and indeed the efficacy of proposed new treatment modalities (see Table 1 outlining advantages and disadvantages of reviewed non-invasive tests). The ^{13}C-Sucrose breath is emerging as the most suitable biomarker in this regard. In rodent models, the SBT has proved successful in assessing the effectiveness of potential new anti-mucositis treatments. Moreover, the SBT could be used as the primary endpoint in clinical trials to determine the potential efficacy of novel anti-mucositis agents in humans affected by cancer.

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