Cancer Chemother Pharmacol (2010) 65:913–921
DOI 10.1007/s00280-009-1098-2

ORIGINAL ARTICLE

Optimization of the non-invasive ^{13}C-sucrose breath test in a rat model of methotrexate-induced mucositis

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Received: 15 February 2009 / Accepted: 26 July 2009 / Published online: 19 August 2009

Abstract
Purpose In order to determine the sensitivity and specificity of the test and to optimize experimental conditions utilizing the SBT in a rat model of chemotherapy-induced small intestinal damage.
Methods Initially, a ^{13}C-sucrose dose-response study was performed in rats to determine an optimal sucrose concentration for the SBT; then applied to assess chemotherapy-induced intestinal damage. A further study was conducted to establish a SBT time-course of methotrexate-induced small intestinal damage and repair. Animals were killed at 96 or 144 h.
Results A sucrose concentration of 0.25 g/ml was optimal (20% CV) for reproducibility and detection of intestinal damage. Maximal damage occurred at 72 h, small intestinal repair was initiated by 96 h and continued at 144 h post-MTX, as determined by the SBT and confirmed by biochemical analyses. Levels of sensitivity and specificity for the SBT were 98 and 94%, respectively.
Conclusions The SBT is a reliable non-invasive marker of small intestinal health and damage with a high degree of sensitivity and specificity.

Keywords Sucrose breath test · Reproducibility · Specificity · Mucositis · Time-course · Chemotherapy · Methotrexate

Abbreviations

MTX	Methotrexate
SBT	Sucrose breath test
SI	Small intestine
CV	Coefficient of variation
DAR	Dark agouti rat
ROC	Receiver operator curve

Introduction

Compromised small intestinal function can arise from a range of endogenous and exogenous causes. However, defining physiological function of the small intestine in real-time has previously been difficult due to its inaccessibility [13], which in turn has limited the development of therapeutic interventions. Currently, small bowel biopsy is the recognized a standard technique for determining small intestinal function. However, this technique is inadequate for a number of reasons [17]; the small bowel biopsy is invasive, painful, and expensive, requires sedation, does not quantify the functional/absorptive capacity of the small intestine, and only assesses the biopsied fraction of the small intestine. The small intestinal damage and dysfunction typical of Crohn's disease, celiac disease, and chemotherapy-induced mucositis, can cause epithelial atrophy, apoptosis, ulceration, inflammation, and decreases in

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brush-border enzymes. In such instances, the availability of a rapid, simple, and non-invasive test would be highly beneficial as an indicator of small intestinal function. Specifically, mucositis can affect all regions of the gastrointestinal tract, and is a common manifestation in cancer patients undergoing chemotherapy [16, 20]. Mucositis is characterized by the development of ulcerating lesions. In the small intestine, patients experience symptoms, such as abdominal pain and bloating, distension, diarrhea, vomiting and in its most severe form, bowel perforation, and sepsis, which can be fatal [3, 21].

As a consequence of the deficiencies and risks associated with applying the small bowel biopsy, the non-invasive ^{13}C-sucrose breath test (SBT) has been developed to aid in determining/monitoring small intestinal function and assessing small intestinal toxicity related to current or novel chemotherapies [7–9]. Pelton et al. [18] demonstrated that SBT results were depressed in rats treated with methotrexate (MTX) compared to controls 7 days post-treatment. The SBT result correlated significantly with in vitro sucrase activity ($r^2 = 0.85$). It has been demonstrated previously that the SBT is simple to perform and cost-effective as it enables assessment of the overall functional and absorptive capacity of the small intestine [1, 18], and it can be applied in both animals and humans [1, 15, 18, 24, 25].

Original rat studies employing the SBT rats utilized a 1 ml of 1 g/ml concentration of sucrose [1, 18], however, at that time, a dose-response study of sucrose was not conducted to optimize sensitivity of the SBT. The primary aim of the current study was to assess the sensitivity and specificity of the SBT using sucrose concentrations below 1 g/ml utilizing new mathematical methods of data analysis, and second, to establish a time-course of the SBT following methotrexate-induced small intestinal damage utilizing the optimal concentration of ^{13}C-sucrose.

Materials and methods

Animals

Female Dark Agouti rats (DAR) were provided by the Institute of Medical and Veterinary Science (IMVS), Gilles Plains, Adelaide, South Australia. Animals were housed in standard rat cages with an environmental temperature of 25°C with a 12 h light:dark cycle for the duration of the study. Animals were injected with either saline (control) or MTX (acute small intestinal damage), and in the final phase of testing each animal was housed individually in Tecniplast® metabolism cages. Rats consumed an 18% casein-based diet [23] (containing sucrose) for a minimum period of 4 days prior to any SBT being performed. The study was approved by the Animal Ethics Committees of the Women's and Children's Hospital and the University of Adelaide, Australia, and complied with the National Health and Medical Research Council (Australia) Code of Practice for Animal Care in Research and Teaching (2004).

Dose-response study experimental design

Female DAR ($n = 16$), mean weight 144 ± 2 g, undertook a sucrose dose-response study to determine the optimal ^{13}C-sucrose dose for use in the SBT. Each rat was orogastrically administered a 1 ml dose of 0.1, 0.25, 0.75, 1.0, or 1.5 g/ml of sucrose for the SBT. SBT dose-responses studies commenced with the lowest dose and increased for each subsequent test and were performed at least 3 days apart to ensure no carry-over effect. Rats were not killed, and were used for sensitivity and reproducibility studies using ^{13}C-sucrose doses of 0.25 and 0.75 g/ml, with a 4-day clean-out period between each test. After a wash-out period of 7 days, following the previous study, rats were injected with 1.5 mg/kg MTX (Pharmacia Corporation, Peapack, New Jersey, USA) intramuscularly (i.m.) at 0 and 24 h, to induce mucositis as described previously [9]. Small intestinal function was assessed by the SBT at −24, 24, and 96 h. Rats were killed at 96 h following the first MTX injection.

MTX time-course trial design

Female DAR ($n = 15$) with a starting weight of 136 ± 2 g were allocated to two groups: group 1: −24 to 96 h time-course ($n = 7$) and group 2: −24 to 144 h time-course ($n = 8$). Daily indices of bodyweight, food intake, fluid intake, and fecal and urine output were recorded. Rats were injected with MTX as described previously and compared to saline-treated, weight-matched animals. Group 1: the SBT was performed at −24 (baseline), 24, and 96 h following the initial MTX injection and rats were killed immediately after cessation of the 96 h SBT. Group 2: the SBT was performed at −24, 72, and 144 h after the initial MTX injection and rats killed at 144 h.

^{13}C-Sucrose breath test

Following an overnight fast, rats were gavaged with a single concentration of ^{13}C-sucrose dissolved in water. This sucrose was naturally and selectively enriched with ^{13}C, with selective enrichment of ^{13}C defined as percentage of ^{13}C molecules determined in 1 g of sucrose derived from cane-sugar in comparison to the percentage of ^{12}C molecules. It was selective, as only a small percentage of the naturally occurring ^{13}C molecules are present in sucrose derived from cane-sugar. The SBT was performed every 3–5 days, a total of five tests, for dose-response and reproducibility studies. Breath tests for the time-course of

MTX-damage were divided between the two treatment groups to minimize effects of fasting and sucrose overload.

Breath collection was performed as described previously [24]. Breath collection is simple and cost-effective, and multiple rodents can be assessed simultaneously. Rats were oro-gastrically gavaged with 1 ml of the respective dose of sucrose (AnalaR, BDH, MERCK, Pty. Ltd., Victoria, Australia) solution [18]. Breath samples were collected every 15 min for 120 min. Breath $^{13}CO_2$ samples were analyzed using an isotope ratio mass spectrometer equipped with a V410 data collection system; expressed as a delta value, representative of the $^{13}C/^{12}C$ ratio in the sample relative to the internal standard of calcium carbonate (Pee Dee Belemnite Limestone, South Carolina, USA). Raw data was expressed as parts per thousand. $^{13}CO_2$ data were previously expressed as delta over baseline, or area under the curve [18]. Recent literature for ^{13}C breath tests expresses $^{13}CO_2$ data as the percentage of ^{13}C dose/h recovered or the percentage cumulative dose of ^{13}C (% CD) [8]. The complete $^{13}CO_2$ analysis considers the contribution of bodyweight, the amount of ^{13}C dose recovered over 90 min [12], and CO_2 production rate which was approximated as 17 ml/kg/min for an adult female DAR [22]. The cumulative breath $^{13}CO_2$ analysis up to 90 min was used for the SBT as small intestinal transit of the substrate was complete (% CD_{90}).

Kill procedure

Rats were killed by CO_2 overdose and cervical dislocation at the respective time-point. The abdomen was opened via midline incision and the stomach, duodenum, small and large intestine, liver, heart, lungs, kidney spleen, and thymus were removed. The jejunum was separated from the duodenum by cutting at the ligament of Treitz and intestinal segments were determined as previously described [2, 14]. At each intestinal site, 2 and 4 cm sections were removed for histology and biochemical analyses, respectively. Histological samples were immediately placed in formalin. Intestinal segments (4 cm) were placed into pre-weighed sterile tubes, weighed, frozen in liquid nitrogen, and then stored at −70°C. As, we have described this previously, detailed histological interpretation of this particular time-course was not carried out for the purpose of this study [1].

In vitro sucrase activity

Tissue samples (4 cm) of the duodenum, proximal jejunum (10%), and distal ileum (90%) were thawed on ice in 1.5 ml of 10 mM PBS (pH 6.1), homogenized (mechanical), aliquoted into 200 µl samples and stored at −70°C until sucrase activity analysis was performed as described previously [24]. Briefly, addition of the substrate sucrose was added to intestinal homogenates, incubated at 37°C, resultant glucose liberation was detected by the addition of horseradish peroxidase and glucose oxidase solution. Absorbance was read at 490 nm using a plate reader and sucrase activity was expressed as nmol glucose/min/cm liberated from the homogenates. An average of the three analyzed sections was calculated, reflective of the total sucrase activity of the small intestine and an average of the duodenum and jejunum homogenates was also calculated.

Data and statistical analyses

All data were expressed as mean ± SEM. Previously determined data in weight-matched saline-treated DAR were used as a reference for normal data of DAR of equivalent age. Daily data indices were analyzed using a repeated measure one-way ANOVA with a Tukey's post-hoc test. All other data were analyzed using a one-way ANOVA with a Tukey's post-hoc test. Significance was determined when $P < 0.05$. All data and statistical analyses were performed using GraphPad Prism version 4.01 for Windows® (GraphPad Software, San Diego, California, USA) or Microsoft Office 2003 Excel® for Microsoft Windows XP.

Results

Reproducibility

Percentage CD_{90} levels for 0.1 and 0.25 g/ml sucrose were significantly ($P < 0.001$) higher compared to sucrose concentrations of 0.75, 1.0, and 1.5 g/ml. Additionally, the % CD_{90} for 1.0 and 1.5 g/ml sucrose were significantly (Table 1; $P < 0.05$) lower compared to the 0.75 g/ml concentration. SBT reproducibility of three tests performed 4 days apart revealed that SBT results for a sucrose concentration of 0.25 g/ml did not differ significantly from the other sucrose doses (Fig. 1a). In contrast, test 1 and 2 SBT results were not significantly different for a sucrose dose of

Table 1 Reproducibility effects of increasing ^{13}C-sucrose concentration (g/ml) on the SBT (% CD_{90}) in rats ($n = 16$)

Sucrose concentration (g/ml)	% CD_{90}	CV (%)
0.1	13.8 ± 1.1	32
0.25	14.1 ± 0.7	20
0.75	$6.3 \pm 0.3^{\alpha\beta}$	19
1.0	$3.4 \pm 0.2^{\alpha\beta\delta}$	25
1.5	$2.6 \pm 0.2^{\alpha\beta\delta}$	26

Data expressed as mean ± SEM and the coefficient of variation percentage (CV %)

Significance denoted by α where $P < 0.001$ compared to 0.1 g/ml sucrose; β where $P < 0.001$ compared to 0.25 g/ml; and δ where $P < 0.05$ compared to 0.75 g/ml sucrose

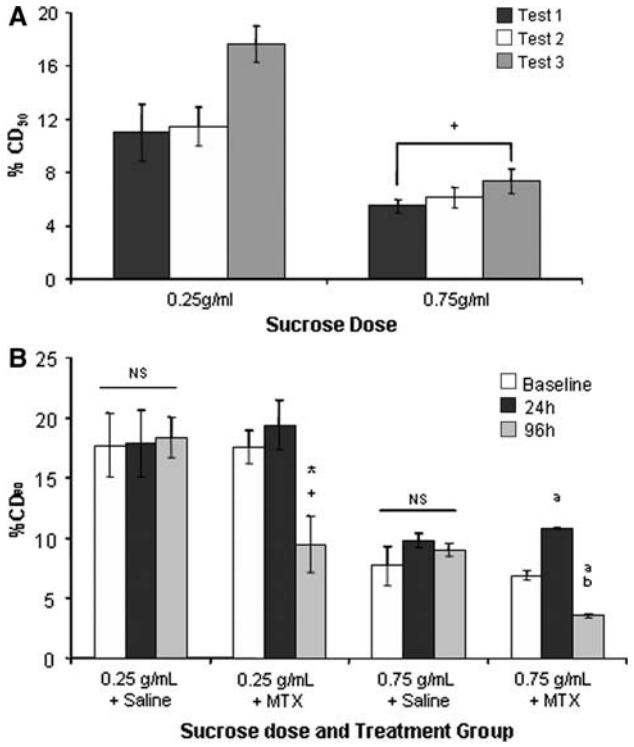

Fig. 1 **a** SBT reproducibility utilizing sucrose doses of 0.25 and 0.75 g/ml ($n = 8$/group). Breath tests performed 4 days apart: test 1 (*black-fill*), test 2 (*white-fill*) and test 3 (*gray-fill*). **b** SBT time-course of saline/MTX-treated rats, receiving 0.25 or 0.75 g/ml of sucrose, performed at baseline, 24 and 96 h. Data expressed as mean ± SEM. Significance between 0.25 g/ml + MTX at 96 h compared to baseline and 24 h ($P < 0.05$) denoted by * and +, respectively. Significance between 0.75 g/ml + MTX: compared to baseline, denoted by *a*, where $P < 0.001$; compared to 24 h, denoted by *b*, where $P < 0.001$

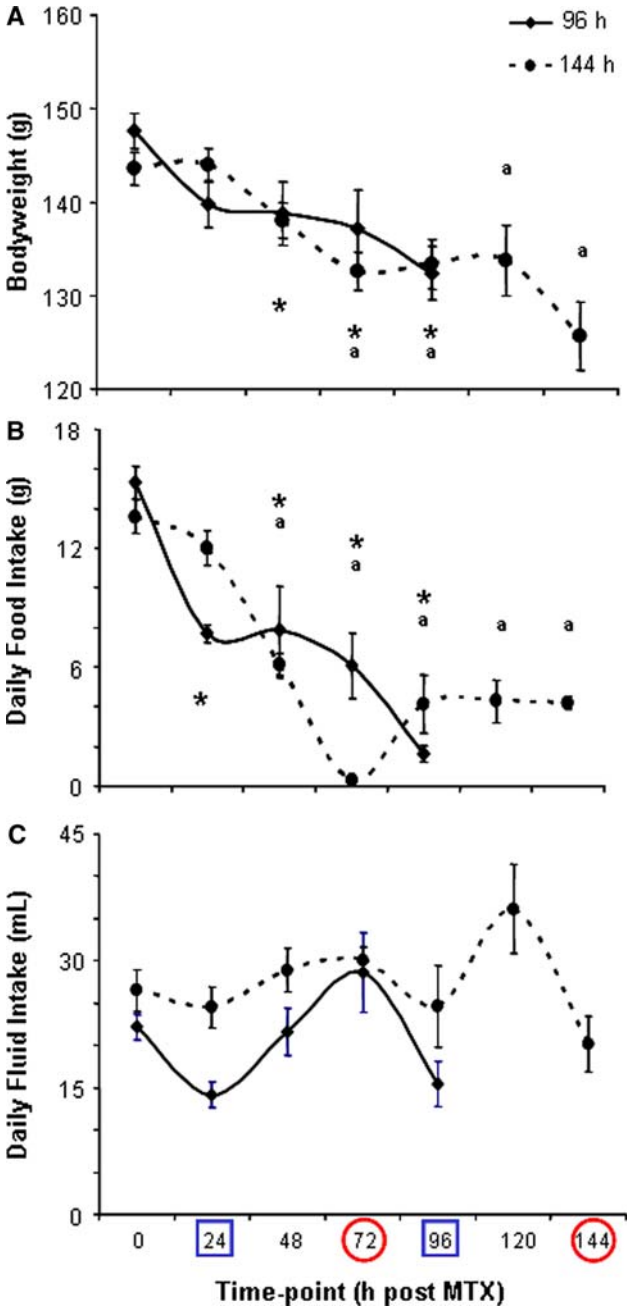

Fig. 2 **a** Bodyweight (g), **b** Food intake (g), and **c** Fluid intake (ml) daily indices throughout a time-course of MTX-treatment in rats. Over-night fasts for short and long time-course denoted by *squares* and *circles*, respectively. Data expressed as mean ± SEM. Significant differences in the short time-course compared to baseline is denoted by *, where $P < 0.01$. Significant differences in the long time-course compared to baseline is denoted by *a*, where $P < 0.001$

0.75 g/ml, although the SBT result for test 3 was significantly ($P < 0.05$) higher compared to test 1 (Fig. 1a). A significantly decreased SBT result was evident in MTX-treated rats at 96 h compared to baseline and 24 h ($P < 0.05$) using a sucrose concentration of 0.25 g/ml. SBT values in MTX-treated rats receiving 0.75 g/ml sucrose were significantly higher at 24 h compared to baseline levels ($P < 0.001$), and were significantly lower compared to both baseline and 24 h SBT levels at 96 h post-injection ($P < 0.001$; Fig. 1b). Sucrose concentrations of 0.25 or 0.75 g/ml at the 96 h time-point yielded a significantly lower SBT result (48 and 61% decrease, respectively) compared to their equivalent sucrose concentration in saline-matched rats ($P < 0.02$; Fig. 1b).

Small intestinal damage time-course effects

Daily indices

Bodyweights in group 1 were significantly lower at 48, 72, and 96 h post-MTX injection compared to their baseline weight (Fig. 2a; $P < 0.01$). Bodyweights from group 2 (0–144 h; longer time-course) were significantly lower at 72, 96, 120, and 144 h post-MTX compared to baseline weights ($P < 0.001$). The greatest impact of MTX on food intake was observed at all time-points after 0 h in group 1 ($P < 0.01$) and at 48–144 h post-MTX compared to baseline

Table 2 Fractional weights (g weight/kg bodyweight) of visceral organs from rats 96 and 144 h post-MTX compared to weight-matched saline-treated controls

Organ Weights (g wt/kg bwt)	Controls	96 h	144 h
Heart	4.28 ± 0.13	4.22 ± 0.09	4.30 ± 0.11
Lungs	6.17 ± 0.16	6.58 ± 0.23	7.67 ± 0.31*,**
Thymus	1.70 ± 0.16	0.97 ± 0.08*	1.24 ± 0.13
Left kidney	3.87 ± 0.08	3.99 ± 0.05	4.28 ± 0.08*,**
Right kidney	4.13 ± 0.12	4.05 ± 0.03	4.50 ± 0.11*,**
Liver	29.7 ± 1.0	31.4 ± 0.8	35.8 ± 1.0*,**
Spleen	2.06 ± 0.06	1.91 ± 0.05	2.23 ± 0.04*,**

Data are expressed as mean ± SEM

* Significance compared to controls ($P < 0.05$)

** Significance compared to 96 h ($P < 0.05$)

Table 3 Fractional weights (wt g/kg bwt) and lengths (cm) of intestinal regions in rats 96 and 144 h post-MTX, compared to weight-matched saline-treated controls

Gut tissue	Controls	96 h	144 h
Total gut wt	44.8 ± 1.0	47.9 ± 2.4	49.6 ± 1.9
Stomach wt	7.0 ± 0.2	7.6 ± 0.6	7.4 ± 0.2
SI wt	30.7 ± 1.2	33.3 ± 2.0	36.7 ± 1.8
SI length	75.6 ± 1.0	74.7 ± 1.4	91.1 ± 0.9*,**
Colon wt	7.2 ± 0.3	7.0 ± 0.6	5.4 ± 0.2*,**
Colon length	13.5 ± 0.4	11.4 ± 0.5	13.0 ± 0.2

Data are expressed as: weight (weight g/kg bodyweight); length (cm); mean ± SEM

* Significance compared to controls ($P < 0.05$)

** Significance compared to 96 h ($P < 0.05$)

for group 2 ($P < 0.001$; Fig. 2b). Fractional weights of visceral organs are depicted in Table 2. A significant decrease in small intestinal length was observed in rats at 96 and 144 h post-MTX compared to saline controls ($P < 0.05$). Additionally, at 144 h, colon weights were significantly lower compared to saline controls and 96 h post-MTX ($P < 0.05$; Table 3).

Breath test and biochemical sucrase activity

Twenty-four hours after the initial MTX injection, SBT results were significantly elevated compared to baseline levels (39% increase; $P < 0.05$; Fig. 3). SBT levels were 54 and 33% lower at 72 and 96 h post-MTX, respectively, compared to baseline levels. SBT levels at 144 h after the initial MTX injection were not significantly different compared to baseline ($P > 0.05$). Duodenal sucrase activity was significantly reduced at 96 and 144 h post-MTX ($P < 0.001$; Fig. 4) compared to saline controls. Proximal

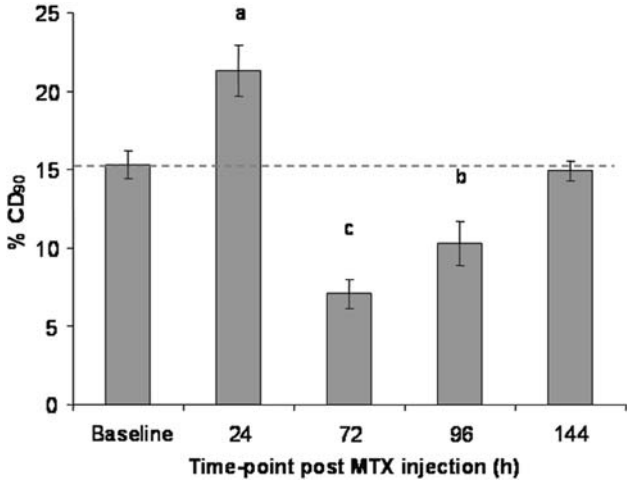

Fig. 3 SBT (percentage ^{13}C cumulative dose 0–90 min; $\%CD_{90}$) time-course of rats injected with MTX (expressed as h post-MTX injection). Data are expressed as mean ± SEM; *a*, *b*, and *c* denotes significant difference compared to baseline SBT, where $P < 0.05$, 0.01 and 0.001, respectively

jejunal sucrase activity was significantly lower in MTX-treated animals at 96 h compared to saline controls and at 144 h post-MTX ($P < 0.001$). Sucrase activity in rats at 144 h post-MTX was not significantly different compared to saline controls in proximal jejunum homogenates ($P > 0.05$; Fig. 4). Ileal sucrase activity was significantly elevated in rats at 144 h post-MTX injection (approximately 280%) compared to both saline controls and rats killed at 96 h after MTX injection ($P < 0.001$). Net sucrase activity from pooled small intestinal homogenates was significantly lower in animals at 96 h post-MTX compared to both saline controls and at 144 h post-MTX ($P < 0.001$;

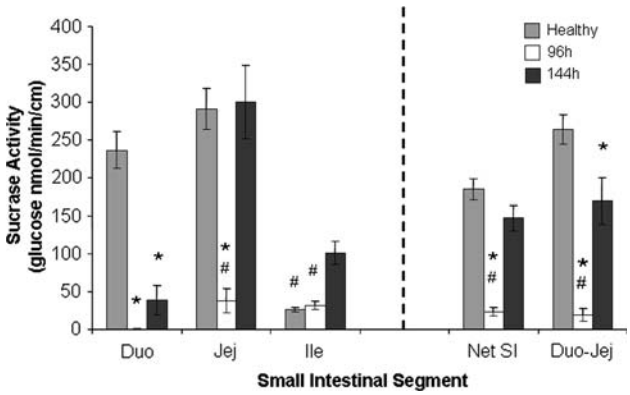

Fig. 4 Biochemical determination of sucrase activity (glucose released nmol/min/cm) in rats at 96 and 144 h post-MTX injection in the duodenum, proximal jejunum (10%), and distal ileum (90%), compared to saline-matched controls. Sucrase activity also represented as: the average of the three segments (Net SI); duodenum–jejunum average (Duo–Jej). Data are expressed as mean ± SEM; *significance between saline-matched controls for each respective segment ($P < 0.001$); #significance compared to 144 h ($P < 0.001$)

Fig. 4). Similarly, duodenal–jejunal sucrase activity average was significantly decreased in rats at 96 h post-MTX compared to both saline controls and rats killed at 144 h post-MTX (Fig. 4). Additionally, duodenal–jejunal (average) sucrase activity was significantly lower in rats killed at 144 h post-MTX compared to saline-treated matched controls (Fig. 4).

Correlations

Duodenal and jejunal sucrase activity correlated significantly with SBT levels ($r = 0.74$ and $r = 0.71$, respectively). Ileal sucrase activity, as determined biochemically, did not correlate significantly with SBT levels (data not shown). Combinations of different small intestinal regions were assessed [11]: (1) total small intestinal sucrase activity average (Fig. 5a), and (2) duodenum–jejunum average were calculated (Fig. 5b). Average small intestinal and duodenum–jejunum sucrase activity correlated significantly with SBT results ($r = 0.83$; Fig. 5).

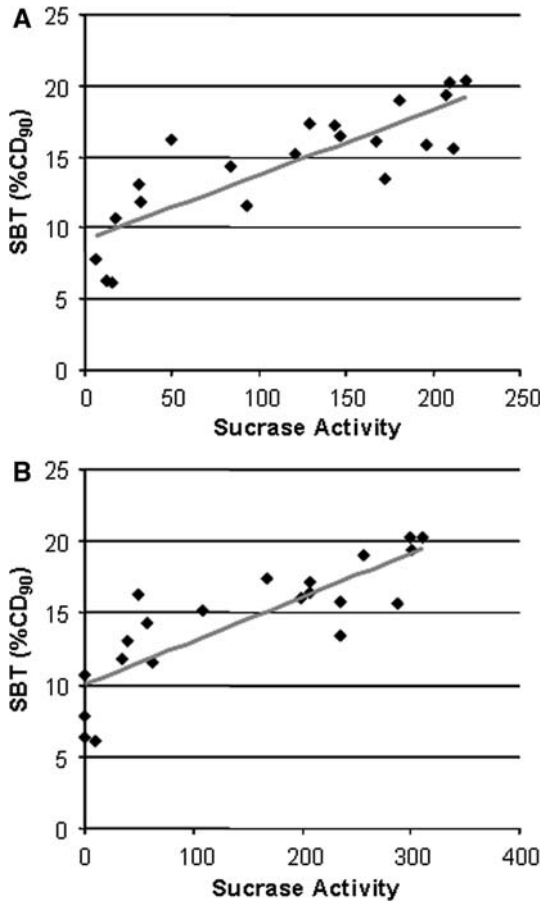

Fig. 5 Correlation between SBT (%CD_{90}) and **a** total small intestinal sucrase activity ($r = 0.83$) and **b** duodenal–jejunal sucrase activity ($r = 0.83$), determined biochemically (nmol glucose/min/cm), at 96 and 144 h after MTX administration

Sensitivity and specificity

Data for SBT analyses performed in rats derived from several chemotherapy-induced [15, 24] studies (including the current investigation and those published previously) were collated to determine a receiver operator curve (ROC), outlining the sensitivity and specificity of the SBT detecting small intestinal damage. The SBT results from different chemotherapy agents were combined to achieve a population with impaired digestive function. The chemotherapy agents used included: MTX, etoposide, cyclophosphamide + etoposide, doxorubicin, and irinotecan. Additionally, SBT results from healthy rodents were collated (Fig. 6). The sensitivity and specificity for the SBT at the designated cut-off of 13.5% CD_{90} were 98 and 94%, respectively, with a false-positive rate in healthy controls of 6%.

Discussion

The current study demonstrated that a sucrose dose of 0.25 g/ml revealed the greatest sensitivity for the SBT at detecting MTX-induced small intestinal damage, and this dose was best able to detect small intestinal changes attributed to MTX-induced damage over time. Specifically, the present dose-response and reproducibility data revealed that a sucrose concentration of 0.25 g/ml produced a reliable coefficient of variation, was capable of detecting small intestinal damage associated with MTX, and was less

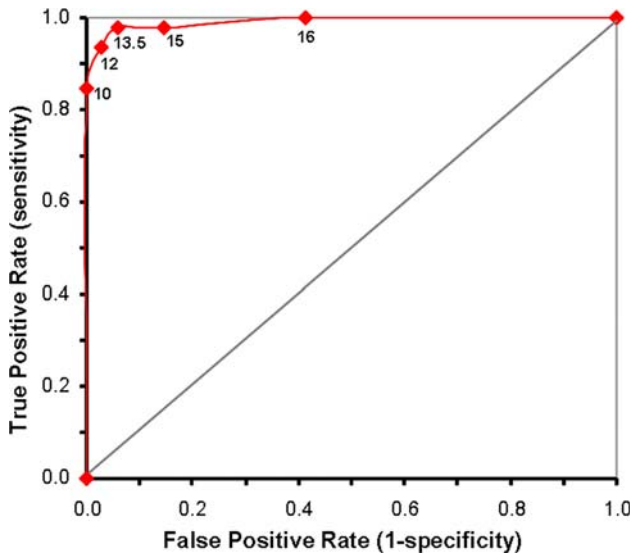

Fig. 6 Receiver operating curve of the non-invasive SBT used from multiple models of chemotherapy-induced mucositis (diseased state) compared to their healthy, saline-treated, counterparts. Cut-off points of ≤10, 12, 13.5, 15, and 16% CD_{90} were implemented to identify truly positive animals exhibiting damaged associated with small intestinal chemotherapy-induced mucositis

variable compared to all sucrose concentrations assessed. Accordingly, a sucrose concentration of 0.25 g/ml was proposed as the optimal dose for future SBT studies in rats. Moreover, the solution was less viscous compared to the higher (1 g/ml) dose utilized in previous studies, ensuring easier and safer animal handling. Most importantly, the current study revealed the sensitivity and specificity of the non-invasive biomarker to be high demonstrating that the SBT is a reliable, sensitive, and cost-effective breath test for detecting small intestinal damage.

The brush-border enzyme sucrase is inducible in the rodent [10, 11, 26]. In order to reduce variability of the SBT, animals must consume a diet-containing sucrose to account for the apparent time-lag required to up-regulate sucrase-isomaltase mRNA (sucrase) and result in its expression on the villus brush-border [5]. Ferraris and colleagues [4] have suggested that this is due to the inability of mature enterocytes to re-program the density of the enzyme sucrase and glucose transporters. Instead, the presence of luminal sucrose signals to crypt cells to re-program the expression of the respective mRNA. The ongoing presence of luminal carbohydrate would therefore ensure that appropriate enzymes and transporters are expressed as the cell migrates to the tip of the villus [4, 6, 7]. Specifically, this illustrates the requirement for rodents to consume a diet-containing sucrose for 72–96 h prior to undergoing SBT procedures to ensure adequate villus sucrase activity.

Sucrase activity in rodents is localized predominantly to the proximal small intestine, and declines gradually in concentration along the small intestine, with the lowest levels situated in the distal ileum [11]. This is consistent with the decreasing gradient in luminal carbohydrate levels (glucose, fructose, and sucrose) along the small intestine [24]. In the present study, MTX small intestinal damage SBT time-course studies revealed that the time-point of maximal damage to be 72 h after the initial MTX injection, and the SBT was capable of detecting the early phase of mucosal repair at 96 h post-MTX [1]. A return to SBT baseline levels at 144 h indicated that the small intestine was undergoing the repair process. In the current study, while the SBT was unable to discriminate between the region of damage and adaptation at 144 h post-MTX, it clearly indicated that villus health and its absorptive capacity, as indicated by sucrase activity, was being restored.

Biochemical sucrase analyses, in the current study, revealed that the return to baseline SBT levels at 144 h after the initial MTX injection was indeed attributed to an increase in jejunal and ileal sucrase activity. Specifically, jejunal sucrase activity at 144 h post-MTX injection had returned to normal levels. In contrast, duodenal sucrase activity was not restored, and a compensatory increase in ileal sucrase activity was observed. To the best of our knowledge, this "regional adaptation" has not been reported previously. Since specific RNA and immuno-histochemical analyses were not performed, it could only be hypothesized that the changes in regional expression of sucrase activity may have been due to continued consumption of a diet-containing sucrose, thus up-regulating regional sucrase activity. Damage to the proximal small intestine alters the luminal carbohydrate concentration, such that the increased presence of sucrose in the ileum would lead to a compensatory expression of sucrase activity [5] and its associated glucose transporter [6, 7].

In the current study, sensitivity and specificity of the SBT, as demonstrated by a receiver operator curve, was encouraging, where a SBT cut-off of 13.5% CD_{90} was determined to discriminate between healthy animals with normal or damaged small intestinal function. Importantly, it was demonstrated that the SBT at this desired cut-off only identified 6% false-positives of all healthy animals displaying compromised absorptive function. Additionally, it was shown that the biochemical sucrase activity at 144 h confirmed that the SBT reflected the entire small intestinal digestive/absorptive capacity, as combined biochemical results from the proximal jejunum and the distal ileum significantly correlated with SBT. These results also confirmed that cessation of breath collection after 90 min was appropriate to ensure that the breath test truly represented the digestive function of the entire small intestine.

While the 48 h post-MTX time-point was not assessed in this study, the results from this time-course of damage could be applied to the recently proposed 5-phase model of mucositis [19], which outlines specific modalities of damage incurred by chemotherapy. Figure 7 illustrates the incorporation of the SBT damage time-course of MTX-treated rats with respect to the 5-phases of mucositis [19]. From the current study, it is hypothesized that: (1) the initiation phase occurs from 0 to 20 h, (2) the phases of up-regulation and message generation occurs at 21–35 h, (3) signaling and amplification overlaps with the previous phase (30–71 h post-MTX), (4) maximal damage due to inflammation and ulceration occurs from 72 to 95 h, and (5) healing has commenced by 96 h post-MTX (Fig. 7). It is important to note that the final time-point (144 h post-MTX) assessed in this time-course illustrated that the small intestine was still undergoing healing and restitution and should be extrapolated further to ascertain this time-point.

In conclusion, a sucrose concentration of 0.25 g/ml is proposed as being appropriate for the optimal performance of the SBT in healthy rodents and in rodents, with compromised small intestinal digestive function. Additionally, the assessment of the double MTX-injection model in the rat highlights the time-points of the initial phase of repair (96 h) and healing (144 h) as determined by the SBT and in vitro determinations of sucrase activity. The SBT together with biochemical sucrase measurement could, at specific

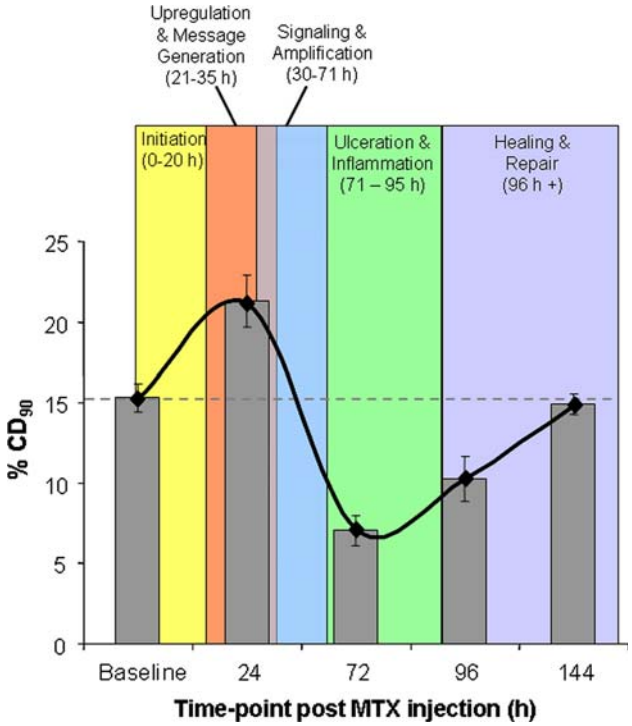

Fig. 7 Representation of the 5-phase mucositis concept model incorporated with the current study's SBT time-course results in MTX-treated rats

time-points, identify an adaptation of the functional response to damage. Finally, the sensitivity and specificity of the SBT in multiple animal models of mucositis is high, producing minimal detection of false-positives, clearly identifying the SBT as a reliable marker of small intestinal damage or health.

Acknowledgments The authors would like to thank Esther Burt and Betty Zacharakis for analysis of breath samples by isotope ratio mass spectrometer. Associate Professor Gordon Howarth is supported by a Cancer Council South Australia Research Fellowship.

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