Lactose maldigestion during methotrexate-induced gastrointestinal mucositis in a rat model

M. Fijlstra,1,2 E. H. H. M. Rings,2 H. J. Verkade,2 T. H. van Dijk,2 W. A. Kamps,1 and W. J. E. Tissing1

1Pediatric Oncology and 2Pediatric Gastroenterology and Hepatology, Department of Pediatrics, Beatrix Children’s Hospital, Groningen University Institute for Drug Exploration, Center for Liver, Digestive and Metabolic Diseases, University of Groningen, University Medical Center Groningen, the Netherlands

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Fijlstra M, Rings EH, Verkade HJ, van Dijk TH, Kamps WA, Tissing WJ. Lactose maldigestion during methotrexate-induced gastrointestinal mucositis in a rat model. Am J Physiol Gastrointest Liver Physiol 300: G283–G291, 2011. First published November 18, 2010; doi:10.1152/ajpgi.00462.2010.—Patients with chemotherapy-induced gastrointestinal mucositis suffer from anorexia, diarrhea, and stomach pain, often causing weight loss and malnutrition. When the intestinal function during mucositis would be known, a rational feeding strategy might improve the nutritional state, accelerate recuperation, and increase survival of mucositis patients. We developed a methotrexate (MTX)-induced mucositis rat model to study nutrient digestion and absorption. To determine lactose digestion and absorption of its derivative glucose during mucositis, we injected Wistar rats intravenously with MTX (60 mg/kg) or 0.9% NaCl (controls). Four days later, we orally administered trace amounts of [1-13C]lactose and [U-13C]glucose and quantified the appearance of labeled glucose in the blood for 3 h. Finally, we determined plasma citrulline level and harvested the small intestine to assess histology, myeloperoxidase activity, glycohydrolase activity, immunohistochemical protein, and mRNA expression. MTX-treated rats showed profound villus atrophy and epithelial damage. During the experimental period, the absorption of lactose-derived [1-13C]glucose was 4.2-fold decreased in MTX-treated rats compared with controls (P < 0.01). Lactose-derived [1-13C]glucose absorption correlated strongly with villus length (ρ = 0.86, P < 0.001) and with plasma citrulline level (ρ = 0.81, P < 0.001). MTX treatment decreased jejunal lactase activity (19.5-fold, P < 0.01) and immunohistochemical protein and mRNA expression (39.7-fold, P < 0.01) compared with controls. Interestingly, MTX treatment did not affect the absorption of [U-13C]glucose during the experimental period. We conclude that lactose digestion is severely decreased during mucositis while glucose absorption is still intact, when supplied in trace amounts. Plasma citrulline level might be a useful objective, noninvasive marker for lactose maldigestion during mucositis in clinic.

carbohydrate digestion; absorption; chemotherapy; radiotherapy; citrulline

GASTROINTESTINAL MUCOSITIS (further referred to as “mucositis”) is a severe and debilitating side effect of chemotherapy, especially in children (23, 30). Mucositis is a transient condition that consists of different stages of inflammation and loss of enterocytes, ending with spontaneous healing of the mucosa (28, 29). Because accurate evaluation of mucositis by intestinal biopsies is problematic in patients, mucositis is primarily diagnosed by more subjective symptoms in clinic (30). With chemotherapy, 40–100% of patients report symptoms of mucositis, depending on the chemotherapeutic agent that is used and the given dose per cycle (30). In children with acute myeloid leukemia, who receive multiple high doses of different chemotherapeutic agents, mucositis was found to be present in 55% of chemotherapy cycles (38). There is a lack of objective, noninvasive markers for mucositis (30, 38), albeit recently, we and others suggested plasma citrulline level to be a good marker (3, 21, 22, 38). Citrulline is a nonprotein amino acid made by enterocytes. Because plasma citrulline represents functional enterocyte mass, reduced citrulline levels during mucositis represent reduced enterocyte mass (6).

Patients with mucositis suffer from anorexia, diarrhea, and stomach pain, often leading to weight loss and malnutrition (16). These complications of mucositis are associated with an increased use of injectable analgesics, nutritional problems, and longer hospitalizations (30). Moreover, because mucositis and its associated complications lead to a dose reduction of chemotherapy, mucositis compromises overall survival in cancer patients (9).

Mucositis is histologically characterized by villus atrophy, enterocyte damage, and infiltration of inflammatory cells (30–32). Although these histological changes suggest loss of epithelial function, the digestive and absorptive capacity of enterocytes during mucositis is still not known. A number of studies showed that protein and mRNA expression of enzymes and transporters involved in nutrient absorption are decreased during mucositis, indicating maldigestion and malabsorption (8, 31, 40). However, only a few functional digestion and absorption studies during mucositis have been performed (14). Up to now, there is still no rational feeding strategy for mucositis patients. Directed nutritional support might actually improve the nutritional state, accelerate recuperation, and increase survival of mucositis patients (2, 17, 24, 27).

We chose to determine nutrient digestion and absorption in a methotrexate (MTX)-induced mucositis rat model. Our ultimate objective is to design a more rational feeding strategy for mucositis patients. We focus on carbohydrate digestion and absorption because of its major role in dietary energy supply, and started with lactose. Lactose is an important carbohydrate in Western pediatric diets and formulas (26). It is a disaccharide that has to be digested by the glycohydrolizing enzyme lactase into the monosaccharides glucose and galactose before absorption of these monosaccharides takes place (33). Absorption of glucose and galactose occurs by active and passive transport across the epithelial border by sodium-dependent glucose transporter 1 (SGLT1) and glucose transporter 2 (GLUT2), respectively (33). Both the enzyme lactase and the transporters SGLT1 and GLUT2 are normally present in the brush border of enterocytes.
In this study, we aim to determine lactose digestion and absorption of its derivative glucose in our mucositis rat model by using stable isotope-labeled [1-13C]lactose and [U-13C]glucose. We hypothesize that both digestion and absorption of these carbohydrates is decreased during mucositis.

MATERIALS AND METHODS

Rats and Housing

Male Wistar outbred rats (4 wk old, 95–105 g) were obtained from Harlan (Horst, The Netherlands). Rats were individually housed in Plexiglas cages (42.5 × 26.6 × 18.5 cm) on a layer of wood shavings under controlled temperature (21 ± 1°C) with a relative humidity of 55 ± 10% and a 12:12-h light-dark cycle (lights on 7:00 A.M.–7:00 P.M.). Water and chow (AIN-93G; Harlan Laboratories, Madison, WI) were available ad libitum unless otherwise stated. The experimental protocol was approved by the Ethics Committee for Animal Experiments, Faculty of Medical Sciences, University of Groningen, The Netherlands.

Materials

MTX was obtained from Pharmachemie Holding (Haarlem, The Netherlands). [1-13C]lactose (kindly donated by Dr. R. J. Vonk) and [U-13C]glucose of 99% isotopic purity were purchased from Isotec (Miamisburg, OH).

Experimental Procedures

The mucositis rat model. We developed a MTX-induced mucositis rat model to determine nutrient digestion and absorption during mucositis. To find the optimal dosage of MTX and the time interval to study digestion and absorption during mucositis, we did some pilot experiments. Pilot experiments were done with different dosages of MTX (30, 45, 60, 90, 120, and 150 mg/kg) via a single intravenous injection in the tail vein under general anesthesia (day 0), based upon other mucositis rat and mouse models (8, 39, 40). Clinical findings were recorded daily, and rats were killed at several days postinjection (days 2, 4, 6, and 10) to study small intestinal damage. Based on results from our pilot studies (see results), digestion and absorption experiments were performed with 60 mg/kg MTX 4 days after injection. Jejunal histology was used as a representative for small intestinal damage.

The lactose digestion and glucose absorption test. Two weeks after arrival at the animal facility, rats (6 wk old, 184–215 g) were injected once intravenously with MTX (60 mg/kg, n = 14) or 0.9% NaCl (controls, n = 7). Intake of food and water and body weight was recorded daily at 8:00 A.M. Four days after injection, after an overnight fast (11:00 P.M. day 3 to 8:00 A.M. day 4), rats received a bolus with trace amounts of [1-13C]lactose (40 mg/rat) and [U-13C]glucose (20 mg/rat) in 600 μL PBS by oral gavage to study both lactose digestion and glucose absorption. Before and at time points 7.5, 15, 30, 45, 60, 90, 120, and 180 min after bolus administration, blood samples were obtained by blood spot technique from the tail tip to measure blood glucose levels and to quantify blood enrichment of lactose-derived [1-13C]glucose and of [U-13C]glucose (35). Before and at the end of the test, we obtained additional blood samples to measure plasma insulin levels. Samples were centrifuged immediately (10 min at 3,000 rpm), and collected plasma was stored at −80°C until further analysis.

Euthanization. At the end of the digestion/absorption test (3 h after bolus administration), rats were euthanized under general anesthesia by obtaining a large blood sample through cardiac puncture for determination of plasma citrulline levels. Blood samples were centrifuged immediately (10 min at 3,000 rpm), and collected plasma was stored at −80°C until further analysis. Next, the abdomen was opened via a midline incision, and the small intestine was excised, flushed with ice-cold PBS, and divided into three segments of similar size (4.5 cm) [duodenum (proximal small intestine), jejunum (anatomic middle of the small intestine), and ileum (1/6 part proximal from the cecum)]. Smaller parts from each intestinal segment were harvested for assessment of histology and immunohistochemical protein expression (2.5 cm), myeloperoxidase (MPO) levels (0.5 cm), glycohydrolase activity (1.0 cm), and mRNA expression (0.5 cm). Small intestinal parts for histology and protein expression were fixed in formalin (1 cm) or 2% formaldehyde (PFA, 1 cm), dissolved in PBS, dehydrated, and embedded in paraffin according to standard procedures. Morphometric analysis was carried out as described previously (20). Villus and crypt length were measured manually in well-oriented sections (10 measurements/rat) from digitized images that were evaluated at ×10 magnification (1 pixel = 0.397 μm) using a calibrated image analysis system (Qwin V3.0; Leica Microsystems). Goblet cell distribution was analyzed by Alcian-Blue staining of 3-μm-thick sections of PFA-fixed material according to standard procedures.

Mucosal MPO levels. Mucosa of frozen jejunal sections was scraped on ice to make tissue homogenates in lysis buffer. Homogenates were 5–50 times diluted in dilution buffer before MPO levels were quantitatively measured via a solid bound antibody against MPO as described by the manufacturer (rat MPO ELISA kit; Hyctul Biotech, Uden, The Netherlands).

Plasma citrulline levels. Plasma citrulline levels were measured in 30 μL plasma at room temperature by using automated ion exchange chromatography as described previously (37, 38).

Blood glucose and plasma insulin levels. Blood glucose levels were measured with a Lifescan EuroFlash glucose meter (Lifescan, Middelburg, The Netherlands). Plasma insulin levels were measured in 25 μL plasma via a solid bound antibody against insulin as described by the manufacturer (Rat Insulin Ultrasensitive EIA; Alpcp Diagnostics, Salem, NH).

[1-13C]- and [U-13C]glucose absorption. After bolus administration with trace amounts of [1-13C]lactose and [U-13C]glucose, blood samples were obtained to quantify blood enrichment of lactose-derived [1-13C]glucose and of [U-13C]glucose. The quantification of lactose-derived [1-13C]glucose and [U-13C]glucose enrichment in blood from blood spots was performed according to Van Dijk et al. (35) by gas chromatography-mass spectrometry (Agilent 5957C Series GC/MSD; Agilent Technologies, Amstelveen, The Netherlands) as has been done previously (20). The calculations for blood glucose kinetics were described recently by Van Dijk (34) and Laskewitz (18).

In short, a single-pool, first-order kinetic model was assumed for this test. The mole percent enrichments of mass isotopomers M1 and Mn, due to administered [1-13C]lactose and [U-13C]glucose, respectively, were used to calculate the first-order absorption process in a one-compartment model using SAAM-II software (version 1.2.1; SAAM Institute, University of Washington, Seattle, WA) (36). Absorption of lactose-derived [1-13C]glucose and [U-13C]glucose during the experimental period was calculated as area under the curve of [1-13C]glucose and [U-13C]glucose concentration (time 0–180 min), respectively.

Mucosal glycohydrolase activity. Mucosa of frozen duodenal, jejunal, and ileal sections was scraped on ice to make tissue homogenates in distilled water. Homogenates were 100–400 times diluted before glycohydrolase activity levels were measured of lactase, sucrase, isomaltase, and maltase as described previously (7, 20). Activ
ity levels were normalized to protein levels that were measured by the BCA method as described by the manufacturer (BCA protein assay kit; Thermo Fischer Scientific, Rockford, IL).

**Immunohistochemical protein expression.** Jejunal protein expression of lactase, sucrase-isomaltase (SI), and SGLT1 was detected using immunohistochemistry according to standard procedures. Lactase was visualized on frozen material (4-μm slides) using a monoclonal mouse anti-rat lactase antibody (kindly donated by Dr. A. Quaroni) (25, 40), dilution 1:500, as described previously (12). After incubation with the first antibody (30 min), endogenous peroxidase activity was blocked, and slides were incubated with the peroxidase-conjugated secondary (rabbit anti-mouse) and tertiary (goat anti-rabbit) antibodies (Dako North America, Carpinteria, CA). SGLT1 was also visualized on frozen material (4-μm slides) using a commercially available polyclonal goat anti-mouse antibody (sc-20584; Santa Cruz Biotechnology, Santa Cruz, CA), dilution 1:20, with a slightly adapted protocol for immunofluorescent staining. After incubation with the SGLT1 antibody (overnight), slides were incubated with fluorescent secondary (donkey anti-goat) antibody (Alexa Fluor 488; Invitrogen, Carlsbad, CA). Slides were covered with fluorescent mounting medium (Dako North America, Carpinteria, CA). SI was visualized on formalin-fixed material (3-μm slides) using a polyclonal rabbit anti-rat SI antibody (kindly donated by Dr. K. Y. Yeh) (42), dilution 1:600, as described previously (40).

**Mucosal mRNA expression.** Mucosa of frozen duodenal, jejunal, and ileal sections was scraped on ice to isolate RNA, synthesize cDNA, and subsequently measure mRNA expression of glycohydrolases lactase (Lct) and SI (Si) and glucose transporters SGLT1 (Slc5a1), GLUT2 (Slc2a2), and glucose transporter 5 (GLUT5 or Slc2a5). mRNA expression was measured by real-time PCR as described previously (1). Integrity of isolated RNA was checked via gel electrophoresis, and disintegrated samples were not included for PCR analysis. PCR results were normalized to β-actin (Actb) mRNA levels. Sequences of the primers and probes are listed in the Supplementary data [Supplemental Table S1 (Supplemental data for this article can be found on the American Journal of Physiology: Gastrointestinal and Liver Physiology website.)].

**Statistical Analysis**

Statistical analysis was performed using the Mann-Whitney U-test (SPSS 16.0 for Windows, Chicago, IL). Values represent medians and first to third quartiles (Figs. 1–4) or ranges (Tables 1 and 2) for the indicated number of rats per group. All correlations are expressed as nonparametric Spearman correlation coefficient. P values were considered statistically significant if P < 0.05. NS means “not significant.”

**RESULTS**

**Pilot Studies**

To find the optimal dosage of MTX and time interval to study nutrient digestion and absorption during mucositis, we did some pilot experiments. At day 2, MTX-treated rats (≥60 mg/kg) showed crypt loss and atrophy while villi still appeared normal. Typical histological signs of mucositis like villus atrophy and blunting, enterocyte damage, and infiltration of inflammatory cells were present in most MTX-treated rats (≥60 mg/kg) at day 4. By this time, crypts tended to be elongated, a sign of crypt regeneration. From day 6 on, villi of...
MTX-treated rats started to recover (results not shown). Histological signs of mucositis were basically the same in the duodenum, jejunum, and ileum. Typical clinical signs of mucositis, such as a decreased food intake, weight loss, and diarrhea, were present in most MTX-treated rats (≥60 mg/kg) from day 2 until day 5, after which rats started to recover (results not shown). Histological and clinical signs of mucositis differed substantially between MTX-treated rats, dependent on the dosage of MTX. When lower MTX dosages were used (30–60 mg/kg), some rats developed only mild signs of mucositis. When higher dosages were used (90–150 mg/kg), all rats developed severe signs of mucositis, but mortality increased. For our experiments, we chose the MTX dosage of 60 mg/kg since this caused pronounced mucositis in most rats, without causing mortality.

The Mucositis Rat Model During the Present Experiment

Histological findings. We analyzed jejunal sections by H&E staining to demonstrate that MTX-treated rats developed histological signs of mucositis (Fig. 1, a and b). Most MTX-treated rats showed profound villus atrophy and blunting with irregular, sometimes even vacuolized, enterocytes (Fig. 1b). Furthermore, there was an influx of inflammatory cells in the stroma of villi (Fig. 1b). However, individual signs of mucositis varied between MTX-treated rats, with some of them only showing scattered cuboidal-shaped enterocytes. Villus length of MTX-treated rats was 1.8-fold decreased (P < 0.01, Fig. 2A) while crypt length was 1.3-fold increased (P < 0.01, Fig. 2B) compared with controls. Goblet cells were evenly distributed along the crypt-villus axis in controls (Alcian-Blue staining, Fig. 1c). In contrast, Goblet cells were restricted along the villus or solely present on villus tops of MTX-treated rats (Fig. 1d). Our findings indicate that MTX-treated rats developed histological signs of mucositis, varying from mild to severe.

Mucosal MPO levels. We measured MPO levels in scraped mucosa of the jejunum to quantify intestinal inflammation during mucositis (Fig. 1b). MPO levels were 20.3-fold increased in MTX-treated rats compared with controls (P < 0.01, Fig. 2C), indicating significant infiltration of neutrophils in the small intestine during mucositis.

Plasma citrulline levels. We measured plasma citrulline levels to estimate the level of functional enterocyte mass during mucositis (6) and to see whether plasma citrulline can serve as a noninvasive marker for mucositis, as has been suggested previously (3, 21, 22, 38). Citrulline levels were 3.6-fold decreased in MTX-treated rats compared with controls (P < 0.01, Fig. 2D), corresponding with significant loss of functional, citrulline-producing small intestinal enterocytes during mucositis. Plasma citrulline level correlated with the severity of mucositis as measured by villus length (rho = 0.90, P < 0.001, Supplemental Fig. S1).

Clinical findings. We recorded the intake of food and water and body weight daily after injection with NaCl or MTX to see if MTX-treated rats developed clinical signs of mucositis. Food intake in MTX-treated rats was decreased on all days postinjection (day 0) with a maximum of 1.5-fold on both day 2 and 3 compared with controls (P < 0.01, Fig. 3A). On day 3, food intake of all rats was decreased since rats were fasted before the digestion and absorption test at day 4. Water intake of MTX-treated rats was decreased from day 2 on with a maximum of 1.9-fold on day 3 compared with controls (P < 0.01, Fig. 3B). Body weight was decreased in MTX-treated rats from day 1 on with a maximum on day 4 compared with controls (P < 0.05, Fig. 4C). Compared with the day of injection, MTX-treated rats lost 2% of initial body weight at day 4 while, in contrast, controls gained 9% of initial body weight by this time (Fig. 4C). Other clinical signs of mucositis, like a sick appearance in general and watery diarrhea, were present in MTX-treated rats from day 3 on. Our findings indicate that MTX-treated rats developed clinical signs of mucositis.

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**Fig. 2.** Morphometric analysis and myeloperoxidase (MPO) and citrulline levels in the mucositis rat model. Jejunal villus (A) and crypt length (B), mucosal MPO levels (C), and plasma citrulline levels (D) in NaCl (○, n = 7)- and MTX (●, n = 14)-treated rats. Dots represent data of individual rats; horizontal lines represent medians of groups. *P < 0.01 for NaCl- vs. MTX-treated rats.
after bolus administration (t_{max}) and was 0.34 mmol/l. In MTX-treated rats, [1^{-13}C]glucose appearance was significantly delayed and decreased compared with controls (Fig. 4A and Table 1). During the experimental period, the absorption of [1^{-13}C]glucose was 4.2-fold decreased in MTX-treated rats compared with controls (P < 0.01, Table 1). Absorption correlated with villus length (rho = 0.86, P < 0.001, Fig. 4B) and with plasma citrulline level (rho = 0.81, P < 0.001, Fig. 4C). Our findings indicate that lactose digestion and/or absorption of its derivative glucose is severely decreased during mucositis.

[U^{-13}C]glucose was determined over a 3-h period after bolus administration. In controls, [U^{-13}C]glucose entered the glucose pool 5 min after bolus administration (t_{lag}, Fig. 4D and Table 1). Maximal [1^{-13}C]glucose concentration (c_{max}) was reached at 33 min after bolus administration (t_{max}) and was 0.33 mmol/l. Interestingly, [U^{-13}C]glucose appearance was slightly delayed (P < 0.05) but not decreased in MTX-treated rats compared with controls (Fig. 4D and Table 1). MTX treatment did not affect the absorption of [U^{-13}C]glucose during the experimental period (Table 1). Absorption correlated with the level of mucositis as measured by villus length (rho = 0.48, P = 0.027, Fig. 4E) but did not correlate with plasma citrulline level (rho = 0.36, NS, Fig. 4F). Our findings indicate that glucose absorption is still intact during mucositis, when given in trace amounts. Therefore, decreased absorption of lactose-derived [1^{-13}C]glucose during mucositis indicates disturbed lactose digestion instead of glucose malabsorption, since [U^{-13}C]glucose is absorbed normally.

Mucosal Glycohydrolase Activity

We measured mucosal glycohydrolase activity of lactase to investigate whether disturbed lactose digestion during mucositis can be explained by a decreased lactase activity. We also studied activity of glycohydrolases sucrase, isomaltase, and maltase. Lactase activity was most abundant in the jejunal mucosa of controls and was 19.5-fold decreased in MTX-treated rats compared with controls (P < 0.01, Table 2). As with lactase activity, a decreased jejunal activity of sucrase (13.9-fold, P < 0.05), isomaltase (17.0-fold, P < 0.01), and maltase (9.1-fold, P < 0.01) was found in MTX-treated rats compared with controls (Table 2). Our findings indicate that the hydrolyzing activities of lactase, sucrase, isomaltase, and maltase are all severely decreased during mucositis. Disturbed lactose digestion during mucositis can therefore be explained by a decreased lactase activity.

Immunohistochemical Protein Expression

We studied jejunal immunohistochemical protein expression of lactase and SGLT1 to investigate whether a decreased activity of these glycohydrolases during mucositis can be explained by a decreased protein expression. Immunohistochemical protein expression of SGLT1 was studied to investigate whether intact glucose absorption during mucositis can be explained by an intact protein expression of this glucose transporter. Protein expression of lactase (Fig. 1, e and f), SGLT1 (Fig. 1, g and h), and SGLT1 (Fig. 1, i and j) was normally present along the brush border of villi in control rats. In contrast, expression was merely present in the remaining villus tops of MTX-treated rats. Therefore, a decreased lactase protein expression during mucositis is most likely not explained by a decreased protein expression.
Our findings indicate that decreased lactase, sucrase, and isomaltase activity during mucositis can be explained by a decreased lactase and SI protein expression. However, intact glucose absorption during mucositis cannot be explained by the protein expression of SGLT1, since this was severely decreased.

**Mucosal mRNA Expression**

We measured mRNA expression of lactase, SI, and SGLT1 to investigate whether a decreased protein expression of these glycohydrolases and glucose transporter during mucositis can be explained by a decreased mRNA expression. We also studied expression of GLUT2 and GLUT5. All mRNA expression profiles were most abundant in the jejunum of controls (Table 2). Jejunal expression of lactase and SI was decreased 39.7- and 9.4-fold, respectively, in MTX-treated rats compared with controls (P < 0.01 and P < 0.05, respectively). Jejunal mRNA expression of SGLT1, GLUT2, and GLUT5 was decreased 9.6-, 10.1-, and 9.5-fold, respectively, in MTX-treated rats compared with controls (both P < 0.01). Our findings indicate that a decreased lactase, SI, and SGLT1 protein expression during mucositis can be explained by a decreased mRNA expression.

**DISCUSSION**

In this study, we aimed to determine lactose digestion and absorption of its derivative glucose during mucositis. We hypothesized that both digestion and absorption of these carbohydrates is decreased during mucositis. Our results show that lactose digestion is severely decreased during mucositis. Interestingly, the absorption of glucose is still intact during mucositis, at least, when supplied in trace amounts.

We used a MTX-induced mucositis rat model to determine lactose digestion and absorption of its derivative glucose. Histology and mucosal MPO level (indicating infiltration of neutrophils) were studied to show that the model really represented mucositis. MTX-treated rats showed typical histological characteristics of mucositis like blunting of villi with irregular or even vacuolized enterocytes. Goblet cells were depleted and accumulated at villus tops. Crypts of MTX-treated rats tended to be elongated, which is a sign of crypt regeneration via hyperproliferation and hyperplasia after initial crypt damage.
caused by MTX (39, 40). Also, we saw an influx of inflammatory cells in villus stroma and increased mucosal MPO levels during MTX treatment. Besides typical gastrointestinal characteristics of mucositis, MTX-treated rats also showed typical clinical characteristics like a decreased intake of food and water, weight loss, and diarrhea. These characteristics of mucositis were also found by others (8, 19, 31, 32, 40). Histological and clinical signs of mucositis differed substantially between MTX-treated rats. Out of 14 MTX-treated rats, 3 rats showed minimal histological and clinical signs of mucositis, normal MPO and citrulline levels (Fig. 2), and a normal glucose absorption test (Fig. 4, C). The variance in observed individual signs of mucositis could be a result of genetic variability between outbred Wistar rats (30). Also, our mucositis model is based upon a single intravenous injection of MTX, leaving the period of epithelial crypt cell establishment of a mucositis rat model in our laboratory using a single injection with MTX.

Here, we prove that “lactose malabsorption” during mucositis is a result of defective lactose hydrolysis instead of defective absorption of its derivative glucose. Our findings implicate that lactose should be omitted from the diet of mucositis patients since it cannot be used as a source of energy. Lactose maldigestion might even exaggerate diarrhea and stomach pain, which often is already present during mucositis (11, 26, 41). We also found a decreased enzyme activity of other glycohydrolases, such as sucrase, isomaltase, and maltase, in MTX-treated rats compared with controls. These findings indicate that all disaccharides, as well as polysaccharides, will probably not be hydrolyzed and its derivatives not be absorbed by MTX shorter than in models where multiple MTX injections are used (4, 10, 13, 28, 29). The amount of subsequent crypt loss by apoptosis, crypt atrophy, and ultimately villus atrophy (39) therefore differs per MTX-injected rat.

During the experimental period, the absorption of lactose-derived [1-13C]glucose was severely decreased in MTX-treated rats compared with controls. In contrast, the absorption of [U-13C]glucose was still intact in MTX-treated rats. We therefore concluded that decreased absorption of lactose-derived glucose during mucositis is a result of disturbed lactose digestion instead of glucose malabsorption. The hydrolysis of the disaccharide lactose in the monosaccharides glucose and galactose by the enzyme lactase must be defective during mucositis. A decreased in vitro lactase enzyme activity and a decreased immunohistochemical protein and mRNA expression of lactase in MTX-treated rats compared with controls further supported this conclusion. Although others already showed a decreased lactase breath test, lactase activity, and lactase protein and mRNA expression during mucositis (8, 14, 31, 40), we are the first to functionally demonstrate that lactose is indeed maldigested during mucositis. Furthermore, the fact that we could confirm lactase activity and expression profiles found in other mucositis studies demonstrates the correct establishment of a mucositis rat model in our laboratory using a single injection with MTX.

Table 1. Blood appearance of lactose-derived [1-13C]glucose and of [U-13C]glucose during the lactose digestion/glucose absorption test

<table>
<thead>
<tr>
<th></th>
<th>NaCl (n = 7)</th>
<th>MTX (n = 14)</th>
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<tbody>
<tr>
<td>Lactose-[1-13C]glucose appearance</td>
<td></td>
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</tr>
<tr>
<td>tlag, min</td>
<td>11 (5–13)</td>
<td>31 (6–52)*</td>
</tr>
<tr>
<td>tmax, min</td>
<td>56 (54–82)</td>
<td>144 (63–175)*</td>
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<tr>
<td>cmax, mmol/l</td>
<td>0.34 (0.30–0.38)</td>
<td>0.09 (0.02–0.46)*</td>
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<tr>
<td>AUC, mmol · 1^{-1} · min^{-1}</td>
<td>45 (35–50)</td>
<td>11 (2–58)*</td>
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[U-13C]glucose appearance

<table>
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<th></th>
<th>NaCl (n = 7)</th>
<th>MTX (n = 14)</th>
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<tr>
<td>tlag, min</td>
<td>5 (3–6)</td>
<td>6 (3–20)</td>
</tr>
<tr>
<td>tmax, min</td>
<td>33 (28–48)</td>
<td>45 (30–61)#</td>
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<tr>
<td>cmax, mmol/l</td>
<td>0.33 (0.26–0.37)</td>
<td>0.28 (0.19–0.41)</td>
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<tr>
<td>AUC, mmol · 1^{-1} · min^{-1}</td>
<td>29 (23–33)</td>
<td>30 (23–38)</td>
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Data indicate medians of groups, and ranges are in parentheses; n, no. of rats. Appearance is shown after administration of the [1-13C]lactose/[U-13C]glucose bolus in NaCl- and methotrexate (MTX)-treated rats. Data belong to the curves that are plotted in Fig. 4, A and D. tlag, Point of time where the label enters the glucose pool; tmax, point of time where the concentration of the label is maximal; cmax, the concentration of the label that is reached at tmax. AUC, area under the curve. #P < 0.05 and *P < 0.01 for NaCl- vs. MTX-treated rats.

Table 2. Glycohydrolase activity and relative mRNA expression of glycohydrolases and glucose transporters in the mucositis rat model

<table>
<thead>
<tr>
<th></th>
<th>Duodenum</th>
<th>MTX</th>
<th>Jejunum</th>
<th>MTX</th>
<th>Ileum</th>
<th>MTX</th>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lactase</td>
<td>0.1 (0.1–0.2)</td>
<td>0.0 (0.0–0.2)*</td>
<td>1.3 (0.9–1.6)</td>
<td>0.1 (0.0–1.1)*</td>
<td>0.2 (0.0–0.4)</td>
<td>0.0 (0.0–0.4)</td>
</tr>
<tr>
<td>Sucrase</td>
<td>7 (6–9)</td>
<td>0 (0–8)*</td>
<td>8 (6–10)</td>
<td>1 (0–11)#</td>
<td>1 (1–2)</td>
<td>1 (0–3)</td>
</tr>
<tr>
<td>Isomaltase</td>
<td>18 (15–22)</td>
<td>0 (0–20)*</td>
<td>41 (32–49)</td>
<td>2 (0–38)*</td>
<td>14 (10–21)</td>
<td>11 (0–36)</td>
</tr>
<tr>
<td>Maltase</td>
<td>63 (51–82)</td>
<td>0.5 (73)*</td>
<td>102 (80–113)</td>
<td>11 (2–103)*</td>
<td>31 (26–45)</td>
<td>30 (0–65)</td>
</tr>
</tbody>
</table>

Relative mRNA expression (normalized to β-actin)

<table>
<thead>
<tr>
<th></th>
<th>Duodenum</th>
<th>MTX</th>
<th>Jejunum</th>
<th>MTX</th>
<th>Ileum</th>
<th>MTX</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>6</td>
<td>13</td>
<td>7</td>
<td>13</td>
<td>7</td>
<td>13</td>
</tr>
<tr>
<td>Lactase</td>
<td>0.0 (0.0–0.0)</td>
<td>0.0 (0.0–0.0)</td>
<td>8.5 (5.9–12.6)</td>
<td>0.2 (0.0–7.3)*</td>
<td>2.0 (0.6–2.9)</td>
<td>0.4 (0.2–7.3)#</td>
</tr>
<tr>
<td>SI</td>
<td>0.8 (0.5–1.1)</td>
<td>0.8 (0.1–1.4)</td>
<td>2.2 (1.4–2.5)</td>
<td>0.2 (0.2–4.2)#</td>
<td>1.4 (0.9–1.9)</td>
<td>0.9 (0.1–1.6)#</td>
</tr>
<tr>
<td>SGLT1</td>
<td>1.3 (0.7–1.6)</td>
<td>1.0 (0.3–1.9)</td>
<td>2.8 (2.3–3.2)</td>
<td>0.3 (0.1–2.7)*</td>
<td>0.7 (0.5–1.2)</td>
<td>0.3 (0.1–1.4)*</td>
</tr>
<tr>
<td>GLUT2</td>
<td>1.8 (0.9–2.1)</td>
<td>1.1 (0.3–1.8)</td>
<td>2.4 (2.1–2.8)</td>
<td>0.2 (0.0–2.6)*</td>
<td>0.8 (0.5–1.1)</td>
<td>0.4 (0.0–1.1)#</td>
</tr>
<tr>
<td>GLUT5</td>
<td>1.0 (0.5–1.1)</td>
<td>0.9 (0.1–1.6)</td>
<td>2.2 (1.7–2.9)</td>
<td>0.2 (0.0–2.4)*</td>
<td>1.1 (1.0–2.0)</td>
<td>0.7 (0.0–1.4)*</td>
</tr>
</tbody>
</table>

Data indicate medians of groups, and ranges are in parentheses; n, no. of rats. Activity- and/or mRNA expression profiles of lactase, sucrase, isomaltase, maltase, sodium-dependent glucose transporter 1 (SGLT1), glucose transporter 2 (GLUT2), and glucose transporter 5 (GLUT5) of NaCl- and MTX-treated rats are shown. SI, sucrase-isomaltase. #P < 0.05 and *P < 0.01 for NaCl- vs. MTX-treated rats.
during mucositis. It therefore seems wise to omit disaccharides and polysaccharides from the diet of patients with mucositis. Because plasma citrulline level was earlier suggested to be a good, noninvasive marker for mucositis (3, 21, 22, 38), we measured plasma citrulline levels in NaCl- and MTX-treated rats. Levels of plasma citrulline, a nonprotein amino acid, were severely decreased in MTX-treated rats compared with controls, corresponding with loss of functional enterocyte mass (6). In individual rats, plasma citrulline level strongly correlated with the level of mucositis as measured by villus length and with lactose digestion during mucositis. Plasma citrulline level might therefore not only be an objective, noninvasive marker for the level of mucositis but, more important, for lactose maldigestion during mucositis. It could be a better alternative for the currently used, more subjective “National Cancer Institute Common Toxicity Criteria” (30, 38), as a parameter for gastrointestinal mucositis. Furthermore, plasma citrulline level could be easily used in clinic to adapt the (feeding) therapy of mucositis patients.

Because the absorption of [U-13C]glucose was still intact in MTX-treated rats, we conclude that glucose transport across the epithelial border must, at least to some extent, still be intact during mucositis. However, immunohistochemical protein and/or mRNA expression of glucose transporters SGLT1 and GLUT2 was decreased in MTX-treated rats compared with controls, as was found by others (8, 31, 40). It should be noted that the given bolus contained only trace amounts of [U-13C]glucose and [1-13C]lactose. Minimal glucose absorption might therefore have been possible via residual transporters on the damaged epithelial membrane, maybe in combination with leakage through damaged tight junctions since mucositis often leads to an increased gut permeability (5, 15). Whether glucose can be an appropriate source of dietary energy for mucositis patients should be further studied by glucose absorption studies using relevant amounts of glucose.

In conclusion, our study shows that lactose digestion is severely decreased during mucositis while glucose absorption is still intact, when supplied in trace amounts. We recommend to omit lactose from the diet of mucositis patients to prevent possible negative side effects of lactose maldigestion, like lactose intolerance. Plasma citrulline level might be a useful objective, noninvasive marker for lactose maldigestion during mucositis in clinic.

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GRANTS

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DISCLOSURES

We have nothing to declare.

REFERENCES


