ORIGINAL ARTICLE

Biomarkers for Assessing Mucosal Barrier Dysfunction Induced by Chemotherapy: Identifying a Rapid and Simple Biomarker

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SUMMARY

Background: Chemotherapy-induced mucosal barrier dysfunction is of clinical interest. However, the assessment of mucosal barrier dysfunction still poses challenges. In this study, we compared several biomarkers with the dual sugar gut permeability test for assessing mucosal barrier dysfunction during chemotherapy.

Methods: Forty-two patients with gastric or colorectal cancer underwent chemotherapy, including FAM or FOLFOX4 regimens. Patients were asked to grade and record their symptoms of gastrointestinal toxicity daily. The urinary lactulose-mannitol ratio was measured to assess the intestinal permeability. Plasma levels of citruline, diamine oxidase (DAO), D-lactic acid, and endotoxin were also measured. Intestinal permeability was observed in the subgroup of patients with diarrhea or constipation.

Results: The urinary lactulose-mannitol ratio and plasma citrulline levels increased on the third and sixth post-chemotherapy days, respectively. There were no significant differences in the plasma levels of D-lactic acid, endotoxin or DAO activity compared to their levels before chemotherapy. The urinary lactulose-mannitol ratio in diarrhea patients was significantly higher than in constipation patients.

Conclusions: These results indicate that the urinary lactulose-mannitol ratio and plasma citrulline level are appropriate biomarkers for assessing mucosal barrier dysfunction in patients receiving chemotherapy. Mucosal barrier dysfunction in diarrhea patients was greater than in constipation patients.


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KEY WORDS

Citrulline, biomarkers, intestinal mucosa, chemotherapy, lactulose, mannitol

INTRODUCTION

The intestinal epithelium comprises an important barrier between the intraluminal environment and the host [1, 2]. Impaired integrity of the mucosal barrier induced by anticancer therapy is thought to promote the translocation of microorganisms from the lumen of the digestive tract to the bloodstream, which results in bacteremia [1]. Anticancer therapy can affect all or some particular regions of the gastrointestinal tract. Mucositis is the clinical manifestation of mucosal barrier injury. Oral mucositis is easy to recognize, whereas the detection of intes-
intestinal mucosal injury relies on nonspecific symptoms [2-4]. Measures of intestinal mucosal barrier integrity using orally administered saccharide or radiolabeled probes have been used in studies of gastrointestinal mucositis [5,6]. Dual sugar permeability testing is cumbersome and wholly dependent on patient compliance. Many factors, including gastric emptying, renal function, and diarrhea, impact the results [1,7]. By contrast, lactulose is a substrate for fermentation by colonic bacteria and is degraded upon incubation with stool [8]. Therefore, lactulose can be used as a marker of small intestinal permeability [9,10]. However, because mucositis may affect any part of the gastrointestinal tract, lactulose is of little use for assessing colonic barrier function. Although some biomarkers for assessing intestinal permeability, such as the plasma citrulline level, urinary sucrose, breath analysis of sucrose and fructose, and the D-xylose level, have emerged as potential markers of intestinal permeability, there is no easy, cost-effective, noninvasive method for monitoring small intestine function in oncology patients. In this study, we compared some biomarkers with dual sugar permeability testing to investigate surrogate biomarkers for the assessment of intestinal permeability.

MATERIALS AND METHODS

Study Design
This study was approved by the Ethics Committee of Nanjing University and was conducted in accordance with the Helsinki Declaration of 1975 (revised in 1983). All statistical analyses and interpretation as well as the preparation of the manuscript were independent and were performed by the authors.

Patient eligibility and treatment
Patients who required postoperative chemotherapy for gastrointestinal cancer received chemotherapy, including FAM (5-FU 600 mg/m² IV, d1-d5; doxorubicin 30 mg/m² IV, d1; and mitomycin 10 mg/m² IV, d1) in gastric cancer patients (n = 23) and FOLFOX-4 (oxaliplatin 85 mg/m², d1; FA 200 mg/m², d1, 2; and 5-FU 400 mg/m² bolus + 600 mg/m² infusion over 22 hours, d1, 2) in colorectal cancer patients (n = 19). Forty-two patients (twenty-eight men and fourteen women) with a mean age of 58.3 (range, 34 - 65 years) were enrolled in this study, and their characteristics are provided in Table 1. Patients were asked to grade and record symptoms of nausea, vomiting, diarrhea, and constipation daily from the first day to the sixth day of chemotherapy.

Intestinal permeability
After an overnight fast, the sugars (10 g of lactulose and 5 g of mannitol dissolved in 100 mL of water) were ingested. Urine that passed within 6 hours of the beginning of the test was collected, and 2 aliquots were stored immediately at -80°C. Urinary lactulose and mannitol were determined using the HPLC method [4]. The ratio between the levels of lactulose and mannitol was calculated.

Plasma citrulline level
Blood samples were collected and stored at -80°C until analysis. For determination of the citrulline level, 250 µL of plasma was deproteinized and serum citrulline concentrations were determined using the HPLC method as previously described [11].

Plasma diamine oxidase (DAO) activity
Plasma DAO activity was assayed according to the modified method published by Suzuki et al. [12].

Plasma D-lactic acid level
The plasma D-lactic acid level was measured according to the method described in the literature with minor modifications [13]. D-Lactic acid concentrations were estimated as the UV absorption spectra of NADH measured at 340 nm with a DV800 spectrophotometer (Beckman Coulter, United States).

Plasma endotoxin level
The plasma endotoxin level was measured as described previously [14].

Statistical analysis
Measurements were averaged and expressed as the mean ± standard deviations. The data were entered into a computerized database (SPSS Inc., and Minitab). The paired t-test was used to evaluate parametric data, and the Mann-Whitney test was used for nonparametric variables; data from the two phases of the study were compared. Statistical significance was accepted at the p < 0.05 level.

RESULTS

Intestinal permeability
Chemotherapy induced significant worsening of intestinal permeability (Figure 1). The urinary lactulose-mannitol ratio increased on the 3rd and 6th post-chemotherapy days (0.0424 ± 0.008% vs. 0.0854 ± 0.006%; p < 0.05). The ratio decreased on the 6th day, but it was not significantly different compared to the ratio on the 3rd day (0.0854 ± 0.006% vs. 0.0673 ± 0.004%; p > 0.05).

We compared the intestinal permeability in the subgroup of patients with diarrhea or constipation (Figure 2). The urinary lactulose-mannitol ratio in diarrhea patients was significantly higher than in constipation patients on the third and sixth post-chemotherapy days (p < 0.05).
Table 1. Baseline characteristics of the patients (N = 42).

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<td>Gender (M/F)</td>
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<td>Age (years) (median, range)</td>
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<tr>
<td>Time of initiation of adjuvant therapy (days) (median, range)</td>
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Figure 1. The urine lactulose-mannitol ratio.

The urinary lactulose-mannitol ratio increased on the 3rd and 6th post-chemotherapy days (* p < 0.05). The data are presented as the medians and 10th/90th percentiles.
Figure 2. The urine lactulose-mannitol ratio in the subgroup of patients with diarrhea or constipation.

The urinary lactulose-mannitol ratio in constipation patients was significantly higher than in diarrhea patients on the 3rd and 6th post-chemotherapy days (\(^* \ p < 0.05\)).

Figure 3. Plasma citrulline levels.

The plasma citrulline level decreased on the 3rd post-chemotherapy day, but there was no significant difference compared with pre-chemotherapy. On the 6th post-chemotherapy day, the plasma citrulline level decreased significantly (\(^* \ p < 0.05\)). The data are presented as the medians and 10th/90th percentiles.

**Plasma citrulline level**

There was no significant difference between the levels of plasma citrulline measured before chemotherapy and on the 3rd post-chemotherapy day (Figure 3, 15.31 ± 2.72 μmol/L vs. 17.08 ± 1.87 μmol/L; \(p > 0.05\)). On the 6th post-chemotherapy day, the plasma citrulline level decreased significantly compared to that measured before chemotherapy (9.59 ± 2.36 μmol/L vs. 17.08 ± 1.87 μmol/L; \(p < 0.05\)).
**Plasma DAO activity**

There was no significant difference between the pre-chemotherapy and post-chemotherapy plasma DAO activity (Figure 4, 0.674 ± 0.128 U/mL on the pre-chemotherapy day vs. 0.721 ± 0.178 U/mL on the 3rd day and 0.784 ± 0.183 U/mL on the 6th day; p > 0.05).

**Plasma D-lactic acid level**

The plasma D-lactic acid level was not significantly different between pre-chemotherapy and on the 3rd and 6th post-chemotherapy days (17.08 ± 4.51 μmol/L on the pre-chemotherapy day vs. 19.31 ± 5.73 μmol/L on the 3rd day and 18.59 ± 4.10 μmol/L on the 6th day; p > 0.05, Figure 5).

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Figure 4. Plasma DAO activity.

There was no significant difference between the pre-chemotherapy and post-chemotherapy activity (p > 0.05). The data are presented as the medians and 10th/90th percentiles.

Figure 5. Plasma D-lactic acid levels.

The plasma D-lactic acid level increased after chemotherapy; however, there was no significant difference between pre-chemotherapy and post-chemotherapy (p > 0.05). The data are presented as the medians and 10th/90th percentiles.
Figure 6. Plasma endotoxin levels.

The plasma endotoxin level increased after chemotherapy; there was no significant difference between pre-chemotherapy and post-chemotherapy (p > 0.05). The data are presented as the medians and 10th/90th percentiles.

**Plasma endotoxin level**

Figure 6 shows the plasma endotoxin levels. The plasma endotoxin level before chemotherapy was not significantly different from that measured on the 3rd and 6th post-chemotherapy days (1050.89 ± 321.87 µg/L before chemotherapy vs. 1110.05 ± 438.59 µg/L on the 3rd day and 1130.9 ± 471.08 µg/L on the 6th day; p > 0.05).

**DISCUSSION**

Mucosal barrier dysfunction induced by chemotherapy alone or in combination with radiation therapy is of clinical interest. Mucosal damage increases the risk of bleeding, the duration of antibiotic therapy and narcotic drug use, need for parenteral nutrition, and the total number of hospitalization days [1,3,15-17]. It can affect all or particular regions of the gastrointestinal tract. Mucosal damage in the oral cavity can be easily assessed by direct inspection. However, the assessment of intestinal mucosal barrier dysfunction is challenging due to the lack of standard diagnostic criteria [3]. Symptoms of gastrointestinal toxicity, including nausea, vomiting, diarrhea, and abdominal complaints, affect nearly every patient immediately following treatment with high-dose chemotherapy. Mucositis occurs in approximately 40% of patients undergoing standard dose chemotherapy and in almost all patients undergoing high-dose chemotherapy and stem cell or bone marrow transplantation [16, 17]. These symptoms are often difficult to detect because many patients receive antiemetics to manage their nausea and vomiting, and narcotic analgesics are frequently given to relieve the pain [1,2,18]. In this study, the urinary lactulose-mannitol ratio increased on the third post-chemotherapy day (p < 0.05). Our results were similar to those of another study in animals [19]. Leblond et al. found that MTX induces mucosal damage and increases intestinal permeability (7-fold) and the mucosal concentration of interleukin (IL)-1b and IL-6 (4- to 6-fold) at day 4 [19]. A number of cytokines, chemokines, and growth factors are involved in chemotherapy-induced epithelial damage [20]. Disorders in the function of the intestinal barrier may be defined by altered permeability to different substances.

51Cr-EDTA, polyvinyl pyrrolidone, and tobramycin were used to study intestinal permeability in early studies [9,10,18,21]. Currently, dual sugar permeability testing is the most common method for assessing intestinal permeability [1,18,21]. The lactulose/mannitol ratio has been reported to have the highest diagnostic value for assessing intestinal permeability [4,6,7,18]. Many factors, including gastric emptying, renal function, and diarrhea, impact the results [8-10]. Lactulose may be fermented by colonic bacteria; consequently, this method cannot be used to assess colonic permeability, which could also be affected by chemotherapy [8,22,23]. The lactulose-mannitol ratio reflects the status of intestinal tight junctions. Several papers have described changes in tight junctions following chemotherapy administration [1]. Therefore, the lactulose-mannitol ratio is a good surrogate for the assessment of chemotherapy-induced mucosal barrier dysfunction.

Both diarrhea and constipation are well-recognized side effects of cancer treatment. The mechanism of diarrhea...
is extensive, complex and likely to be the result of a number of mechanisms. Direct damage by anticancer agents to the basal epithelial cell layer leads to loss of the renewal capacity of the epithelium and has been linked to other nonmucosal toxicities of anticancer therapy, such as fatigue, malnutrition, and nausea. Constipation is not as well recognized, and very little is known about its mechanisms. To the best of our knowledge, there have been no reports on the difference in the impact of chemotherapy on the intestinal mucosal barrier in patients with either diarrhea or constipation induced by chemotherapy. We found that the urinary lactulose-mannitol ratio in diarrhea patients was significantly higher than in constipation patients ($p < 0.05$). Although the mechanisms are very poorly defined, patients with diarrhea may have more serious mucosal barrier injury. Future research is required to investigate the difference in the mucosal barrier injury induced by chemotherapy in patients with diarrhea or constipation. An ideal marker of intestinal injury should be simple to use, rapid, inexpensive, reproducible, lightly impacted by other factors, and able to identify individuals who are at risk for developing the disease [9,22,23]. For patients receiving chemotherapy, the biomarker should be useful for monitoring and guiding treatment [1-3,17]; therefore, many noninvasive methods of monitoring small intestinal function, such as evaluating the plasma citrulline level and urinary sucrose, the sucrose breath test, and measuring the D-xylene level, have emerged as potential markers of intestinal permeability [22-24]. Citrulline, a nitrogenous end-product of glutamine metabolism in enterocytes, is a reliable biomarker of small bowel enterocyte masses [22-25]. Serum citrulline levels are significantly decreased in patients receiving intensive chemotherapy [23]. Gut damage assessed with the citrulline assay has been observed 1 - 2 weeks earlier than gut damage assessed via the sugar permeability test [24]. Recently, van Vliet et al. demonstrated that plasma citrulline might be a good parameter for mucosal barrier injury. In our study, the plasma citrulline level decreased significantly following chemotherapy; however, the decline was later than that observed with the sugar permeability test [26]. On the third post-chemotherapy day, the lactulose-mannitol ratio increased, and the plasma citrulline level decreased on the sixth post-chemotherapy day. Cytotoxic agents alter the ecological balance in the alimentary tract, allowing some of the resident flora to initiate a pathogenic process [25]. Patients with mucositis induced by chemotherapy often harbor more yeasts, Gram-negative bacilli, and Gram-positive cocci [27-29]. Breakdown of the gut mucosal barrier function and changes in intestinal flora could result in systemic effects, contributing to the development of chemotherapy-induced mucositis [25,30,31]. DAO is an intracellular enzyme with a high level of activity in the upper layer of intestinal villi. Plasma DAO activity is a good biomarker for assessing the structure and function of the intestinal mucosa [32]. Lactate-fermenting bacteria in the colon increase the synthesis of the D-isomer of lactate, which is absorbed by the intestinal epithelial cells. This lactate isomer has been used as an index of increased intestinal permeability [33]. In this study, the plasma levels of D-lactic acid, DAO, and endotoxin increased after chemotherapy; however, these effects were not significant. Therefore, plasma levels of D-lactic acid, DAO, and endotoxin are not adequate surrogate biomarkers for assessing intestinal permeability. Chemotherapy-induced increases in intestinal permeability can be evaluated with the dual sugar test and plasma citrulline level. The increase in the dual sugar test occurs earlier than the increase in the plasma citrulline level. The plasma levels of D-lactic acid, DAO, and endotoxin, however, do not change during and after chemotherapy. In the future, the mechanism underlying increased intestinal permeability caused by chemotherapy should be investigated.

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**Declaration of Interest:** No potential conflicts of interest relevant to this article are reported.

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