Effect of high-dose chemotherapy on intestinal permeability in humans

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1. Mucositis is a common side-effect of chemotherapy which is difficult to assess except by invasive means such as upper gastrointestinal endoscopy. Differential absorption of mono- and di-saccharides, such as rhamnose and lactulose, is a non-invasive measure of intestinal damage.

2. The purpose of the study was to assess the duration and severity of intestinal damage in patients undergoing high-dose chemotherapy and autologous blood stem-cell transplantation for malignant disease.

3. Thirty-five patients were studied before treatment and at 7, 28, 60 and 90 days after treatment.

4. The median lactulose/rhamnose ratios before treatment and at 7 and 90 days post-treatment were 0.09, 0.62 and 0.06 respectively. Altered permeability was due to both increased lactulose permeation and decreased rhamnose absorption. These abnormalities suggest a defect in tight-junction integrity as well as a decrease in surface area of small bowel.

5. We conclude that chemotherapy given for malignant disease is associated with a transient abnormality in intestinal sugar permeability, which peaks at 7 days after treatment and is composed of both mono- and di-saccharide absorption abnormalities.

INTRODUCTION

Mucositis is a clinical term describing a syndrome defined by mucosal ulceration and gastrointestinal symptoms [1], which is a common side-effect of many forms of cancer chemotherapy. It is uncertain whether inflammation is present. Most of the limited research carried out on mucositis has been confined to the oral mucosa, as examination is easy and results of treatment are evident. Despite its common occurrence in patients having chemotherapy, the exact mechanism is unclear and there is no definitive treatment. Mucositis has become the main factor that limits higher doses of chemotherapy, as bone-marrow toxicity is reduced and recovery improved by the use of colony stimulating factors. Some symptoms, such as nausea, abdominal pain and particularly diarrhoea, would suggest an intestinal origin.

Intestinal function has been measured traditionally by xylose absorption. This monosaccharide sugar undergoes passive mediated absorption in the jejunum [2-4]. An alternative monosaccharide test sugar is rhamnose which is passively absorbed throughout the whole of the small intestine. In this respect, it is a better measure of intestinal function. A further development has been to combine rhamnose with the disaccharide sugar lactulose. The ratio of lactulose to rhamnose is termed 'sugar permeability'.

By using the ratio of the two sugars, effects such as altered gastrointestinal transit time or mild renal impairment are cancelled out. Neither sugar is metabolized except by bacteria. Rhamnose is believed to be absorbed transepithelially by passive diffusion across enterocytes with little intestinal reserve, and thus absorption reflects total intestinal absorptive capacity [3]. Thus rhamnose is a better measure of carbohydrate absorption than xylose. There is, however, some controversy about the exact path of the monosaccharide absorption [5]. Lactulose is normally excluded (<2% absorbed), with any absorption that does occur being paracellular and presumed to involve leakiness of tight epithelial junctions between enterocytes. The advantages of this double sugar test are that it is well-tolerated, non-invasive and that it may be repeated sequentially. In the future it could be used to assess possible treatments of mucositis.

Our aims in this study were to assess the severity and time-course of changes in intestinal permeability after high-dose chemotherapy and autologous blood stem-cell transplantation, and to assess ease of administration and patient tolerance of the test.

METHODS

Subjects

All patients receiving high-dose chemotherapy and autologous stem-cell transplantation at The...
Queen Elizabeth Hospital were eligible, and the study was approved by the Ethics of Human Research Committee at The Queen Elizabeth Hospital. The study was carried out in accordance with the Declaration of Helsinki. Informed consent was obtained from each patient before enrolment in the study. Patients were excluded if they had pre-existing small bowel disease (such as coeliac disease, inflammatory bowel disease or small bowel malignancy), or were unwilling to participate in the study. The characteristics of the 35 patients enrolled in the study are given in Table 1. Four patients underwent the procedure twice. The chemotherapy involved combinations of drugs in marrow ablative doses given over several days. Most patients received combinations of drugs such as busulphan, cyclophosphamide, epirubicin and melphalan (Table 2). Patients were studied before receiving chemotherapy and at 7, 28, 60 and 90 days after chemotherapy.

Assessment of mucositis

*Intestinal permeability.* After an overnight fast (water was allowed throughout), the patient was given a solution to drink comprising 5 g of lactulose, 1 g of rhamnose and 22.6 g of glucose in 100 ml of water. The glucose acts as an osmotic stressor on the tight junctions. All urine was collected for the next 5 h, the total volume was recorded and a 10 ml aliquot was stored for later analysis by high performance TLC [2].

Rhamnose was measured in urine using a method originally designed for plasma, but with a modification to correct for urinary urea which co-elutes with rhamnose in this system [2]. A quadratic equation was derived for rhamnose versus urea concentrations which had been previously established in the laboratory. Standards were regularly applied to the chromatograph plates in the present analysis. Intestinal permeability was expressed as the milligram ratio of urinary lactulose to rhamnose, with each expressed as the percentage of ingested dose.

The National Cancer Institute common toxicity criteria were used for assessing oral mucositis, diarrhoea, nausea and vomiting. Briefly, toxicities are graded from 1 to 4, following the convention that 1 = mild, 2 = moderate, 3 = severe and 4 = life-threatening [6].

**Statistics**

Results were analysed using Peritz' F test, which is a robust measure of differences in the group means, where the group sizes are not identical [7]. Permeability ratios and both lactulose and rhamnose absorption values were transformed to log(10)(x+1) to normalize the data and stabilize the variance before analysis. A P value of <0.05 was used across Peritz' analysis-for-significance testing, but an adjusted P value was calculated for pair-wise comparison. For ease of presentation, median values were also calculated as the measure of central tendency.

**RESULTS**

Forty-four courses of high-dose chemotherapy and autologous blood stem-cell transplantation were performed in 40 patients. Two patients declined to be enrolled, one patient was withdrawn for medical reasons, and two were excluded because they only had the test before treatment. Thus 35 patients were available for the study, with four undergoing the treatment twice, giving 39 treatment episodes. Their characteristics are shown in Table 1. The female preponderance was due to a higher proportion of...
patients being treated for breast cancer. Thirty-four of the patients received chemotherapy as priming before stem-cell harvesting from peripheral blood. Only one patient did not have chemotherapy before enrolment.

**Patient acceptability**

Four patients found the test sugar solution unacceptably sweet and were unable to swallow the solution on day 7. They were able to swallow it by day 9. No other adverse effects were experienced from the test.

Oral mucositis occurred in all patients, with toxicity reaching common toxicity criteria grade 3–4 in 50%. Diarrhoea with a grading of 3–4 occurred in 41% and grade 3–4 nausea and vomiting in 16%. These symptoms peaked at 7 days after chemotherapy.

**Intestinal permeability**

Intestinal permeability is given in Fig. 1. The median lactulose to rhamnose milligram excretion ratio before high-dose chemotherapy was 0.09 ($n = 39$). Thirteen patients had a mild abnormality before treatment. There was a 6.8-fold increase to a peak of 0.62 ($n = 36$) on day 7, and this decreased thereafter to 0.12 by day 28 ($n = 27$), to 0.08 by day 60 ($n = 17$) and to 0.06 by day 90 ($n = 13$). The percentage absorptions of rhamnose and lactulose are given in Figs. 2 and 3. Median rhamnose absorption was 5.53% before treatment, decreased 6.1-fold to 0.90% on day 7, and improved thereafter to pretreatment levels. This indicated a reduction in monosaccharide absorption following high-dose chemotherapy. Median lactulose absorption was 0.42% before treatment. It increased to 0.68% on
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day 7, and subsequently decreased again to pretreatment levels.

DISCUSSION

The gastrointestinal tract is particularly vulnerable to the side-effects of chemotherapy, presumably because of high physiological cell proliferation and turnover [8, 9]. Intestinal mucosal damage is traditionally documented by biopsy either by upper gastrointestinal endoscopy or by a Crosby capsule [10]. Both of these procedures may be hazardous in patients after chemotherapy and autologous blood stem-cell transplantation because of low platelet and leucocyte counts. However, Forbes et al. [11] were able to safely perform upper endoscopy at 30 days after transplantation, and found a large number of unsuspected gastrointestinal lesions. This and another study [12] investigated allogeneic bone-marrow transplantation, but graft versus host disease or irradiation may confound interpretation of any findings. In the present study we were interested in applying the non-invasive test of sugar permeability as a way of assessing intestinal damage in patients receiving high-dose chemotherapy and autologous blood stem-cell transplantation.

We showed that the non-invasive intestinal sugar-permeability test was well tolerated. The only problem was a transient taste aversion in some patients. In part this could have been due to the hyperosmolar test solution containing glucose. We are presently studying an iso-osmolar sugar test solution to avoid this problem. A mild abnormality of intestinal permeability was present in some patients before high-dose chemotherapy. This could be due to the effects of the malignancy, subclinical malnutrition, or to prior chemotherapy.

One patient had high-dose chemotherapy as first ever chemotherapy, and interestingly she had normal permeability before treatment. The other patients had all had at least one cycle of chemotherapy before the present high-dose treatment.

The maximum sugar-permeability abnormality occurred at 7 days after treatment, and returned to normal by 28 days after chemotherapy. The ratio continued to decline until day 90. This abnormality corresponded with the period that patients were unwell from anorexia, nausea and other gastrointestinal symptoms.

Analysis of the two components of the sugar-permeability test elucidated the cause of the heightened permeability. One reason was a 62% increase in median lactulose permeability, presumably through loss of integrity of the mucosal barrier constituted by tight junctions between epithelial cells. However, the second reason for increased permeability was an 84% decrease in median rhamnose permeation, implying a lowered intestinal surface area for nutrient absorption. As permeability is the ratio of lactulose to rhamnose absorption, it will be increased by either an increased lactulose absorption or a decreased rhamnose absorption.

The prolonged period of increased permeability suggested that the damage was not purely due to a direct toxic effect of the chemotherapy on the mucosa, but rather that there was also an indirect, prolonged component. Direct enterocyte damage would be expected to be present for the life-cycle of one cohort of enterocytes, and thus should have resolved by 48-72 h. Studies in the rat after methotrexate treatment have shown that there is a direct toxic effect resulting in mucosal hypoplasia, but also that this is followed by a rebound hyperplasia [13], indicating an indirect component in this animal model. Studies of the effects of methotrexate on the human small intestine by Trier [14], showed a transient reduction in crypt cell mitoses for 48 h after methotrexate administration, followed by a return to baseline or higher levels by 96 h. Smith et al. [10] also showed a transient reduction in crypt cell mitoses after chemotherapy using various cytotoxic drugs. The abnormal permeability lasts for longer than these changes.

The severity of the permeability defect was similar to that seen in untreated coeliac disease, where the increased lactulose/rhamnose milligram excretion ratio correlates with villous atrophy on duodenal biopsy [15]. This suggests that there may be a similar abnormality of villous atrophy, at least transiently, in the small intestine after chemotherapy.

The flow rate of fluid through the small intestinal mucosa may also be important in determining the permeability, as a high fluid flow rate leads to a reduced permeability ratio [16]. Chemotherapy could have transiently decreased fluid flow through the small intestinal mucosa. More work is planned to determine the morphological correlates of the altered permeability and to discover the cause of the pre-treatment abnormality.

The advantages of the sugar-permeability test are that it is non-invasive, well-tolerated and can be repeated sequentially. It is an objective measure of small intestinal permeability and could be used to test the efficacy of future interventions.

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REFERENCES

Intestinal permeability after chemotherapy


