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ORIGINAL ARTICLE

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Alterations in intestinal permeability following the intensified polydrug-chemotherapy IFADIC (ifosfamide, Adriamycin, dacarbazine)

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Abstract *Purpose:* The aim of this study was to investigate the severity and time-course of alterations in gastroduodenal and intestinal permeability in relation to nausea/emesis following administration of the highly emetogenic polydrug regimen IFADIC (ifosfamide, Adriamycin, dacarbazine) using a differential lactulose/mannitol absorption (SLM) test. We also assessed the ease of administration and patients' tolerance of the SLM test. *Methods:* The SLM test was performed in seven patients with soft tissue sarcomas on days 1, 3 and 14 of cycle I and cycle III of chemotherapy; seven healthy volunteers served as controls. The degree of correlation between the clinical grade of nausea/emesis according to WHO criteria and gastroduodenal permeability, expressed in terms of urinary sucrose excretion, and intestinal permeability, expressed in terms of the permeability index (urinary lactulose to mannitol permeability ratio), was also assessed. *Results:* The permeability index values were significantly different ($P \leq 0.01$) on days 1, 3 and 14 during both cycles of chemotherapy. The median permeability index on day 3 was higher ($P \leq 0.01$) in patients with nausea/emesis than in those without symptoms. Additionally, the permeability index when nausea was present (day 3) was higher ($P \leq 0.01$) than when nausea/emesis was absent (days 1 and 14). In 59% of patients the increased permeability index on day 3 was accompanied by nausea/emesis of WHO grade 3. Gastroduodenal permeability did not alter consistently following chemotherapy. *Conclusions:* Our study confirms an acute, transient increase in intestinal permeability following the polydrug regimen IFADIC, accompanied by nausea/emesis of WHO grade 3 in the majority of patients. Normal intestinal permeability was achieved on day 14 in all patients, thus allowing intensified 2-weekly treatment administration. The SLM test may be recommended as a feasible test for the objective assessment of alterations in intestinal permeability following chemotherapy administration.

Keywords Intestinal permeability · Polydrug-chemotherapy · IFADIC · Ifosfamide · Adriamycin · Dacarbazine

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Introduction

Since the introduction of colony-stimulating growth factors, dose-intensified polydrug chemotherapies are no longer limited by haematologic toxicity. Instead, toxic effects, especially of the gastrointestinal tract, with symptoms of nausea/emesis still remain an unresolved problem for many patients. Clinically, the course of chemotherapy-induced nausea/emesis can be divided into an acute phase occurring within 2 h of chemotherapy administration and a delayed phase of nausea/emesis starting after 16 h and lasting for up to 96 h [5].

Currently, the extent of nausea/emesis is evaluated clinically according to the WHO or NCI-CTC criteria

[7, 8]. Although there are several reliable noninvasive methods for assessment of gastroduodenal and/or intestinal permeability, and alterations in permeability are an accepted indication of gut damage, no objective method is routinely used as a complement in clinical trials [1, 16, 17].

The aim of this study was to investigate the severity and time-course of gastroduodenal and intestinal permeability in relation to nausea/emesis during administration of the highly emetogenic polydrug regimen IFADIC (ifosfamide, Adriamycin, dacarbazine). We also assessed the ease of administration and patients' tolerance of the test.

Patients and methods

Patients and control subjects

Seven consecutive patients (male/female 6/1) with histologically proven soft tissue sarcoma with a median age of 56 years (range 41–75 years) were enrolled into the study after providing written informed consent. Seven healthy volunteers with a median age of 40 years (range 29–52 years) served as normal controls for the SLM test.

Inclusion criteria

Patients with histologically proven soft tissue sarcoma eligible for the intensified polydrug regimen IFADIC (ifosfamide 1500 mg/m^2 days 1–4, Adriamycin 25 mg/m^2 days 1–2, dacarbazine 200 mg/m^2 days 1–4) were included. Patients were not allowed to have received any previous chemotherapy. All substances were administered intravenously at 2-weekly intervals. The colony-stimulating growth factor G-CSF (30 MU/day) was given subcutaneously from day 5 to day 10 of each therapy cycle. All patients were given ranitidine intravenously twice-daily for stress ulcer prophylaxis.

Patients and control subjects had to be free from any sign of systemic infection, were not allowed to have taken antibiotics or to have consumed alcohol for at least 1 week before testing, and were not allowed to have taken nonsteroidal antiinflammatory drugs during the 2 weeks before and during permeability testing.

None of the patients showed evidence of altered renal function (creatinine and blood urea nitrogen within normal institutional limits) or malnutrition as confirmed by prothrombin time and blood level of albumin. Written informed consent was obtained from each patient before study enrolment.

Permeability testing

Permeability was determined using a sucrose/lactulose/mannitol absorption (SLM) test. The kit consisted of a small (250 ml) and a large plastic collection vessel (1.5 l), and one bottle of test solution (100 ml of an aqueous solution containing 20 g sucrose, 10 g lactulose and 5 g mannitol). Sodium azide as a preservative was added to both urine collection vessels. All patients were also given detailed written instructions for performance of the test.

Permeation of lactulose, a synthetic disaccharide (fructose, galactose) is considered a marker of unmediated paracellular passive permeation through tight junctions and is less than 1% in a healthy intestinum. It is increased in mucosal damage. Mannitol is a small molecule and therefore approximately 20% permeates the healthy intestinal surface. Sucrose is nearly completely digested and absorbed in the small bowel of healthy individuals. In patients with gastroduodenal lesions, it may pass the gastroduodenal mucosa and would be excreted in the urine.

After an overnight fast (8 h), each subject collected a pretest urine sample in the small plastic vessel to test for any endogenous mannitol. The subjects drank the test solution, and the urine passed during the next 5 h was collected in the large plastic bottle. The subjects were not allowed to eat any food for the duration of the test, but they were allowed to drink tap-water during the last 3 h. The volume of the 5-h urine was recorded and 10-ml samples of the pretest and the 5-h urine were stored at –20°C until analysis.

For determination of gastroduodenal permeability the sucrose concentrations in the 5-h urine samples were determined by first desalting and declorizing each sample with Microionex (Laevosan, Linz, Austria). Sucrose in the supernatant was then determined indirectly by reaction with β-fructosidase to form one mole of D-glucose and one mole of D-fructose. Subsequently, D-glucose was measured using the hexokinase procedure with measurement of NAPDH at 340 nm (Test Combination Sucrose/D-Glucose; Boehringer Mannheim, Germany). All supernatants were analysed for D-glucose before the addition of β-fructosidase to determine the amount of free D-glucose in the urine. Gastroduodenal permeability was expressed as the percentage recovery of the ingested dose of sucrose (%S) in the 5-h urine.

For intestinal permeability lactulose and mannitol levels in the urine samples were determined by HPLC. Intestinal permeability was expressed as the ratio of the percentage recovery of the ingested dose of lactulose (%L) relative to that of mannitol (%M) in the 5-h urine (%L/%M).

Study design

The SLM test was performed on 3 days during cycle I and cycle III of chemotherapy. The timing of the SLM tests was as follows. On day 1 the test was performed before the start of chemotherapy and served as the patients' baseline data. The next test was started on day 3, i.e. 45 h after the start of chemotherapy, and took place over the period 45–51 h after the start of chemotherapy during which the highest incidence and severity of delayed nausea/emesis associated with this chemotherapy regimen would be expected. Chemotherapy administration on day 3 was started after performing the SLM test. The next test was performed on day 14 when complete recovery from the gastrointestinal side effects would be expected.

Antiemetic treatment

In all patients antiemetic therapy consisted of metoclopramide 3×10 mg intravenously from days 1 to 5, ondansetron 2×8 mg intravenously from days 1 to 5 and dexamethasone 8 mg intravenously every morning from days 1 to 5. Additional antiemetic treatment was given when clinically indicated (metoclopramide or ondansetron).

Reporting severity of nausea/emesis

Reporting of nausea/emesis was performed according to the WHO criteria [7]. The degree of nausea/emesis was recorded by the patients themselves using documentation sheets provided as well as by one responsible physician on the basis of three daily visits. Patients and physicians were unaware of the permeability data. Nausea/emesis requiring antiemetic therapy in addition to the above-listed standardized antiemetic treatment was regarded as grade 3 or 4 toxicity according to the WHO criteria.

Normal values of gastroduodenal and intestinal permeability

Normal gastroduodenal permeability was defined as sucrose excretion in the urine lower than 43 mg according to the institutional standard [16]. Normal intestinal permeability was defined in terms of a permeability index (PI) lower than 0.03 according to the institutional standard [17].

Statistics

Data are expressed as medians and quartiles and for statistical evaluation the SPSS for Windows, release 10.0.5, software package was used. The Friedman test was used for evaluation of the significance of changes in the PI and sucrose excretion with time, the Mann-Whitney *U*-test was used for calculation of the significance of differences in the median PI/sucrose excretion in urine between patients with and without nausea, and the Wilcoxon test was used for determination of the significance of differences in the values of PI/sucrose excretion in urine between time-points with nausea/emesis and time-points without nausea. *P*-values ≤ 0.01 were considered statistically significant.

Results

The SLM test was performed during a total of 14 cycles of IFADIC chemotherapy in seven patients. In all of them the test was performed on days 1, 3 and 14. Values determined on day 1 during cycle I served as the patients' baseline data.

Healthy controls

All healthy controls had normal gastroduodenal as well as intestinal permeability with a median sucrose excretion in the urine of 13 mg (range 5–28 mg) and a median PI of 0.015 (range 0.008–0.026).

Patients' gastroduodenal permeability

Alterations in gastroduodenal permeability, expressed in terms of sucrose excretion in urine (median and quartiles), are illustrated in Fig. 1. Sucrose excretion did not change significantly with time. The median sucrose excretion was within the normal range on all study days during cycles I and III. In single patients the values of sucrose excretion were inconsistent. Unexpectedly, sucrose excretion was elevated by more than 70% above the normal value in two patients on day 1 of cycle I and in one of these two patients also on day 1 of cycle III. The elevated values were confirmed by remeasurement of the samples. In the first patient it was possibly due to the erroneous intake of acetyl salicylic acid (500 mg). In the other patient, suffering from an unresectable soft tissue sarcoma, no explanation for the elevated sucrose excretion could be found. Neither patient suffered from nausea/emesis or from any other gastroduodenal symptom. However, because clinically unapparent mucosal damage cannot be excluded with this noninvasive method, the patients were not excluded from the analysis.

On day 14, sucrose excretion was pathological in only one patient during cycle I (137 mg) and normal in all patients during cycle III (range 1–30 mg).

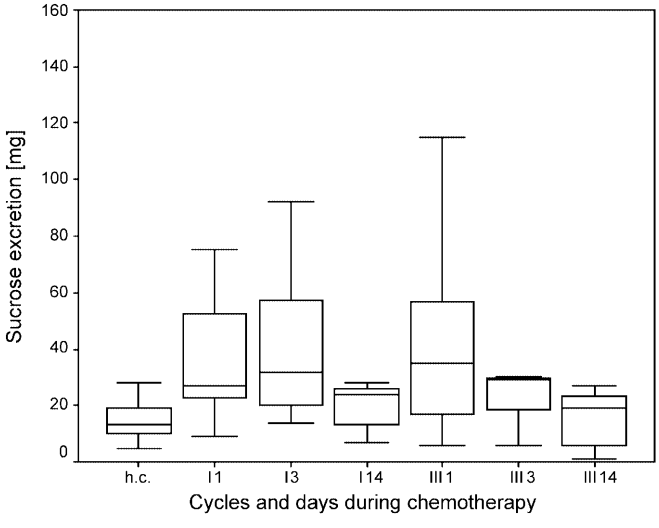

Fig. 1 Urinary sucrose excretion in patients with soft tissue sarcoma receiving the 2-weekly intensified polydrug regimen IFADIC. Urinary sucrose excretion was measured on days 1, 3 and 14 during cycle I and cycle III of chemotherapy. The data are presented as medians and quartiles (*h.c.* healthy controls)

Patients' intestinal permeability

The influence of IFADIC on patients' intestinal permeability during cycles I and III, expressed in terms of the PI (median and range), is illustrated in Fig. 2. The PI changed significantly with time ($P \leq 0.01$, Friedman-test) during both cycles of chemotherapy. Values of the PI at time points with nausea (day 3) were significantly increased ($P \leq 0.01$, Wilcoxon test) as compared to time points without nausea/emesis (days 1 and 14). On day 3, an increase in PI was observed in 7 of 14 chemotherapy administrations. In 59% of patients these increased

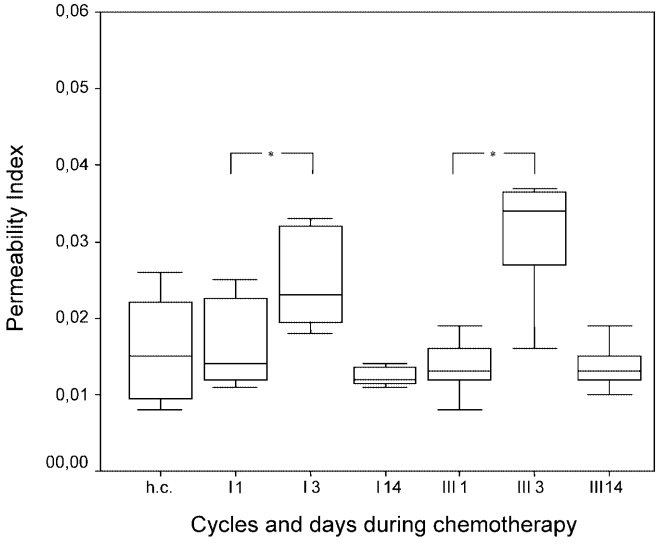

Fig. 2 Urinary lactulose to mannitol permeability ratio in patients with soft tissue sarcoma receiving the 2-weekly intensified polydrug regimen IFADIC. The permeability ratios were measured on days 1, 3 and 14 during cycle I and cycle III of chemotherapy. The data are presented as medians and quartiles (*h.c.* healthy controls). $*P \leq 0.01$, day 1 vs day 3

values of PI were accompanied by nausea/emesis of WHO grade 3 despite standardized antiemetic treatment. On days 14, the PI values were again within the normal range. In the patient who showed elevated sucrose excretion, the PI on day 1 of cycle I was above the normal value, possibly due to the erroneous intake of acetyl salicylic acid (500 mg).

The median PI values on day 3 were significantly increased ($P \leq 0.01$, Mann-Whitney U-test) in patients with nausea/emesis (0.024) and in those without nausea/emesis (0.034). The median PI values on day 1 were normal during cycles I (0.013) and III (0.014). On day 14, the median PI values were also within the normal range (0.012) and showed no differences between cycle I and cycle III.

Furthermore, on day 3 of cycle III the median PI for all patients (0.023) was higher than the median PI on day 3 (0.034) of cycle I, but the difference did not reach significance.

Nausea/emesis and patients' acceptance

In two patients nausea/emesis of WHO grade 3 was recorded from day 3 to day 5 during cycle I and cycle III. The remaining five patients did not experience any nausea/emesis.

Two patients experienced the SLM test solution as extremely sweet but were able to perform the test according to the protocol. However, these patients did not experience either nausea or emesis. No other side effects from the test solution were experienced.

Discussion

The main finding in this study was the demonstration of statistically significant alteration in intestinal permeability on day 3 following the administration of the polydrug regimen IFADIC, suggesting that the small intestine was primarily affected by the cytotoxic agents administered. Restoration of normal intestinal permeability had been achieved by day 14. Unexpectedly, gastroduodenal permeability remained unaltered following chemotherapy administration, possibly as a result of increased gastric motility due to administration of metoclopramide for antiemetic treatment.

The increase in intestinal permeability on day 3 was interpreted as an acute toxic effect and did not seem to be associated with a hyporegenerative mechanism. Epithelial hyporegenerative damage would not have resulted in such an early increase in intestinal permeability because cytotoxic drugs block the epithelium at the crypt level, and cell migration from the crypt floor to the apex of the intestinal villi is known to take 5–7 days [2]. Indeed, an early increase in intestinal permeability with a maximum on day 2 has also been observed following administration of Adriamycin and the polychemotherapy regimens MOPP (mustargen, vincristine, procarbazine, prednisone) and CVP (cyclophosphamide, vincristine, prednisone) with the permeability returning to basal levels between day 8 and day 10 [9, 10]. Additionally, in a paediatric population suffering from acute lymphocytic leukaemia, the administration of methotrexate resulted in a significant increase in transmucosal movement [6]. Unfortunately, in these studies the degree of correlation between increased intestinal permeability and clinical symptoms of nausea/emesis was not evaluated.

Although Sartori et al. have observed abnormalities in microvilli on about day 8 after chemotherapy administration by light microscopy and transmission electron microscopy on the one hand, and mucosal injury on gastroduodenoscopy on the other, these findings need not necessarily be accompanied by alterations in gastrointestinal permeability. Permeability investigations were not performed in these studies [11, 12, 13].

Interestingly, increases in intestinal permeability with a maximum on day 8 have been seen following 5-fluorouracil administration [15]. Clinically, this was not accompanied by nausea/emesis but by stomatitis, a typical side effect of 5-fluorouracil. Consequently, different time courses of the maximal increase in intestinal permeability may be the reason for the different side effects of cytotoxic drugs.

Recently, Johansson and Ekman [3], using the ^{51}Cr-EDTA absorption test, found a significant intestinal barrier dysfunction 2 days after conditioning therapy preceding clinical signs of gastrointestinal toxicity. However, later during the transplantation course, clinical toxicity caught up with the intestinal barrier dysfunction. Thus, the early increase in permeability may be one of the initiating factors responsible for the development of clinical gastrointestinal side effects.

The 59% correlation between increased values of PI on day 3 and nausea/emesis of WHO grade 3 led us to speculate that this early increase in intestinal permeability may have been one of the initiating factors responsible for the development of intestinal injury and that this may have contributed to the symptoms of delayed emesis. Additionally, the tendency towards increasing median PI on day 3 from cycle I to cycle III suggests that the effect on intestinal permeability may increase with further chemotherapy administrations.

Unfortunately, the PI cannot be used as a negative predictive parameter for the degree of nausea/emesis to be expected following chemotherapy administration because values of PI were within the normal range before the start of chemotherapy. Thus, the SLM test cannot be used as a screening test to select patients who are at risk of experiencing a higher degree of nausea/emesis necessitating an intensified prophylactic antiemetic treatment regimen.

Our study demonstrated that the SLM test may be an objective method for routine use in further intervention trials dealing with chemotherapy-induced alterations in intestinal permeability as a complement to clinical evaluation of chemotherapy-related intestinal toxicity.

This is in accordance with a recent study by Keefe et al. [4] in which the median lactulose/rhamnose ratios were determined in patients undergoing high-dose chemotherapy and autologous blood stem-cell transplantation. In contrast to gastroduodenoscopy for evaluation of mucosal injury [14], sugar-permeability tests are non-invasive, well tolerated, well-accepted by patients and can be repeated sequentially without substantial burden on the patient. The SLM test may be recommended for further objective assessment of the efficacy of treatments aimed at reducing gastrointestinal toxicity of cytotoxic drugs, but a larger series of SLM tests under such conditions are needed.

In conclusion, our study confirmed the existence of statistically significant acute and transient alterations in intestinal permeability following the administration of IFADIC correlating with nausea/emesis of WHO grade 3 in 59% of patients.

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