Effects of Anthracycline Therapy on Intestinal Absorption in Patients with Advanced Breast Cancer

Gianpaolo Parrilli, Rosario V. Iaffaioli, Marco Martorano, Rosario Cuomo, Salvatore Tafuto, Maria G. Zampino, Gabriele Budillon, and A. Raffaele Bianco

Division of Hepatology and Gastroenterology, Department of Systematic Pathology, and Division of Medical Oncology [R. V. I., S. T., M. G. Z., A. R. B.], University of Naples Medical School II, Italy

ABSTRACT

Although cytotoxic chemotherapy for human cancer has been reported to induce alterations in intestinal permeability, its effects on the absorptive process are still controversial. We have studied mediated and nonmediated absorption in 10 patients with metastatic breast cancer before and after treatment with Adriamycin by the use of specific test sugars given orally and their subsequent urinary recovery, as measured by chromatography. Mediated absorption was investigated by the use of D-xyllose and 3-O-methylglucose, while lactulose and L-rhamnose were used to study nonmediated permeation. Lactulose is considered a marker of unmediated paracellular (tight junction) permeation, while L-rhamnose explores passage across cell membranes. The test was performed on patients before and on the second and the eighth days after Adriamycin administration, and only once in 22 age-matched healthy women.

Under basal conditions, as well as 2 and 8 days after chemotherapy, D-xyllose and 3-O-methylglucose absorption was 35% lower in patients than in controls (P < 0.001). Lactulose absorption was significantly higher in patients than in controls under basal conditions (P < 0.001); it reached levels three times higher the second day after chemotherapy, and returned to basal levels by the eighth day. The data suggest an early reversible effect of Adriamycin on cellular tight junctions with resulting increased permeabilization. This effect seems of a toxic nature rather than due to an increase in cell loss. It is interesting that both nonmediated absorption and mediated absorption were already altered before chemotherapy in cancer patients, suggesting a preexisting functional damage of the intestine. The significance of this alteration as a potential mechanism of cancer cachexia is discussed.

INTRODUCTION

Interactions between cancer and absorptive functions of the small intestine may be of three types: (a) the intestine might be the port of entry of ingested carcinogens; (b) the tumor might release substances altering intestinal mucosa; (c) cytotoxic drugs used for cancer treatment might damage intestinal mucosa and alter intestinal absorption.

Many of the side-effects of cytotoxic drugs used in cancer chemotherapy are the result of changes induced in normal tissues of the host, particularly those with a high proliferative rate, including the epithelia of the gastrointestinal tract (1). Epithelial cells of intestinal mucosa proliferate exclusively in the crypts and migrate to the apex of the villi during their maturation process, the life cycle of the enterocyte having a duration of 5 to 7 days. The absorptive function of the intestine is dependent on a normal turnover of mucosal cells.

Three intestinal absorptive mechanisms have been identified: (a) an active process, carrier mediated and ATP-dependent, involving amino acids, glucose, 3-omG, and galactose; (b) a facilitated mechanism, carrier mediated, not requiring energy consumption, responsible for the absorption of fructose and D-xyl; (c) a passive mechanism, due to diffusion along a concentration gradient, and taking place through either cell pores (e.g., L-rh) or intercellular junctions and/or cell extrusion zones (e.g., LacI) (2). By measuring the mucosal transfer of different probe molecules, specific for individual absorption pathways, a simultaneous evaluation can be made of the various absorptive mechanisms (3–5).

Previous studies of intestinal absorption in cancer patients have demonstrated an increase in passive permeation following cytotoxic chemotherapy (6, 7), while data on active absorption are contradictory (8, 9).

The present study was designed to better define the changes in the absorptive function of the small intestine in cancer patients before and after cytotoxic therapy. The study population was a group of breast cancer patients with metastatic disease, and a group of age-matched healthy subjects. Intestinal absorption was assessed by recovery in blood and urine of four test sugars administered as a single oral load before and after Adriamycin treatment. Test sugars used were 3-omG and D-xyl which selectively measure the two types of carrier-mediated absorption, and L-rh and Lac which measure passive permeation.

PATIENTS AND METHODS

Ten female patients with breast cancer and 22 healthy women volunteered to participate in the study, giving informed consent. The control subjects had a median age of 48 (range, 29–60). All breast cancer patients had metastatic infiltrating duct carcinoma of the mammary gland, and were aged 55 years (range, 35–63). All had been subjected to previous radical mastectomy, and had a median disease free interval of 2 years (range, 1–3). None of them had been subjected to prior cytotoxic drug chemotherapy. All patients received one cycle of chemotherapy consisting of Adriamycin, 60 mg/m², administered as i.v. bolus on Day 0.

None of the women included in the study showed evidence of altered renal function or of malnutrition or malabsorption, as confirmed by anthropometric values and biochemical data including fecal fat, prothrombin time, and blood level of albumin. The oral load of test sugars, given after an overnight fast, consisted of 2.5 g 3-omG (M.W. 194.2), 5 g D-xyl (M.W. 150.1), 1 g L-rh (M.W. 164), 5 g Lac (M.W. 342) dissolved in 250 ml water. The final osmolality of the solution was 340 mOsm/liter. None of the subjects showed intolerance to the sugar load.

The test was carried out only once in the normal volunteers, and on Days −2, 2, and 8 of chemotherapy in breast cancer patients. Heparinized blood was obtained 10 min before and 30, 60, 90, and 120 min after the sugar load, and plasma samples were stored at −20°C until assayed. Complete 5-h urine was collected following the oral load and an aliquot was kept frozen with merthiolate (10 mg/100 ml). Concentration of monosaccharides in plasma and urine were determined by quantitative thin layer chromatography (10).

Sugar separation was achieved by multiple development on half plates (10 x 20 cm) of 1500 plastic-backed silica gel (Schleicher and Schull, Dassel, FRG). For sugars in plasma (D-xyl, 3-omG) three consecutive ascending runs (8.5 cm each) were made, one with solution

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A (Butan-1-ol/ethanol/acetic acid/water; 60:50:10:10) and two with solution B (Butan-1-ol/ethyl acetate/pyridine/acetic acid/water; 57:15:10:10). For sugars in urine (d-xyl, 3-omG, L-rh) three consecutive ascending runs (8.5 cm each) were made with solution B. The precision of each method was 7%, recovery above 90%, and minimum detection level below 0.1 mmol/l for the three sugars tested.

Lac in urine was estimated by gas-liquid chromatography according to Laker (11) with modifications to the gas carrier and its flow rate. The chromatograph used was a Fractovap 4002, Carlo Erba, Italy. Operative conditions were: oven 250°C, injector 275°C, detector (ionization flame 305°C, gas carrier N₂, flow rate 35 ml/min, attenuation input 1:100 and output 1:2, back-out 1:100. The column was a 3-m OV 17 (8% phases of gaschrom Q 80–100 mesh). The lactulose retention time relative to that of turanose (internal marker, 10 mg/100 ml) was 0.73. The variation coefficient was above 8% and the minimum concentration value of Lacl was 80 μg/100 ml.

Values representing timed d-xyl and 3-omG concentrations in plasma for each test were processed (Sperry Univac personal computer) calculating second to tenth degree equations with their integrals. The fourth degree equation, according to correlation coefficients, most accurately expressed the kinetics of the two sugars in plasma. This was used to calculate the area under the 0–120-min plasma curves for each patient and control subject. Statistical analysis included Student's t and Wilcoxon's tests.

RESULTS

Table 1 shows that d-xyl and 3-omG plasma concentration at 1 h, as well as the area below the 0–120-min curve, are significantly lower in cancer patients than in controls (P < 0.001) for both sugars, with a ratio of d-xyl to 3-omG concentrations similar in patients and controls. No significant variations between pre- and postchemotherapy sugar plasma levels were noted in the cancer patients. These data suggest that mediated intestinal absorption is impaired in cancer patients before chemotherapy. Table 2 shows the urinary sugar recovery, as percentage of the administered dose, during the 5-h period following the oral load, in cancer patients before and after therapy, as well as in controls. d-xyl, 3-omG, and L-rh concentrations were significantly lower in the patients as compared to the controls, both before and after chemotherapy; the d-xyl:3-omG ratio was similar in both groups. Lacl recovery was higher in patients before chemotherapy than in controls, and a further significant increase was seen two days after Adriamycin administration. On the eighth day after treatment the urinary recovery of Lacl was similar to that observed before therapy (Fig. 1). A similar pattern was seen in the Lacl:L-rh ratio. These data confirm the existence of an impaired absorption of d-xyl and 3-omG and indicate a similar alteration of the L-rh permeation process. The increased absorption and excretion of lactulose also indicate the existence of an altered intestine barrier function, which is further, although temporarily, impaired after chemotherapy.

DISCUSSION

The data show a marked increase in passive intestinal permeation of Lacl in cancer patients on the second day after ADM administration. By the eighth day Lacl permeation had returned to basal values, which were within the normal range, although higher than those of controls. This finding, which is supported by previous observations (7), would indicate a transient and reversible derangement of intestinal barrier function, possibly a consequence of a toxic effect of ADM on intercellular structures of the small intestine, rather than a direct blocking effect on intestinal cell proliferation. Altered intestinal permeability has been suggested as a potential cause of infection, by back-diffusion of intestinal contents, in cancer patients (6). However, none of our patients showed evidence of gastroenteric or systemic infection in the 3-week period following ADM administration; this confirms the observation that infections of gastroenteric origin can develop only when intestinal mucosal lesions are associated with granulocytopenia (6), which was absent in our patients.

ADM had no appreciable effect on the small intestine's absorptive function: neither plasma nor urine concentration of the three test monosaccharides (d-xyl, 3-omG, L-rh) showed significant variations after chemotherapy.

### Table 1 Plasma values of sugars after combined oral loads

<table>
<thead>
<tr>
<th></th>
<th>Controls (n = 22)</th>
<th>Cancer patients* (n = 10)</th>
<th>P valuesa</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>A</td>
<td>B</td>
<td>C</td>
</tr>
<tr>
<td>d-xyl</td>
<td>12.6 ± 1.9</td>
<td>8.71 ± 3.4</td>
<td>8.11 ± 1.8</td>
</tr>
<tr>
<td>3-omG</td>
<td>11.2 ± 1.9</td>
<td>8.6 ± 2.7</td>
<td>8.23 ± 1.75</td>
</tr>
<tr>
<td>d-xyl/3-omG</td>
<td>0.81 ± 0.18</td>
<td>1.1 ± 0.38</td>
<td>1.0 ± 0.2</td>
</tr>
<tr>
<td>PTCA d-xyl</td>
<td>1119 ± 233</td>
<td>704 ± 285</td>
<td>745 ± 853</td>
</tr>
<tr>
<td>PTCA 3-omG</td>
<td>1036 ± 308</td>
<td>640 ± 145</td>
<td>740 ± 214</td>
</tr>
</tbody>
</table>

* A, before; B, 2 days after; C, 8 days after ADM; PTCA = plasma concentration/time curve (mg-min).

a Wilcoxon's test; NA, N vs. A; NB, N vs. B; etc.

### Table 2 Urine recovery (% of oral dose) after combined oral loads of test sugars

<table>
<thead>
<tr>
<th></th>
<th>Controls (n = 22)</th>
<th>Cancer patients* (n = 10)</th>
<th>P valuesa</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>A</td>
<td>B</td>
<td>C</td>
</tr>
<tr>
<td>d-xyl</td>
<td>34.0 ± 6.3</td>
<td>22.1 ± 4.1</td>
<td>25.2 ± 6.3</td>
</tr>
<tr>
<td>3-omG</td>
<td>51.0 ± 6.4</td>
<td>27.2 ± 14.2</td>
<td>32.3 ± 13.8</td>
</tr>
<tr>
<td>d-xyl/3-omG</td>
<td>0.66 ± 0.09</td>
<td>0.88 ± 0.21</td>
<td>0.87 ± 0.46</td>
</tr>
<tr>
<td>L-rh</td>
<td>10.2 ± 3.0</td>
<td>4.95 ± 3.60</td>
<td>6.06 ± 3.50</td>
</tr>
<tr>
<td>L-acid</td>
<td>0.24 ± 0.10</td>
<td>0.44 ± 0.16</td>
<td>1.35 ± 0.44</td>
</tr>
<tr>
<td>Lac/L-rh</td>
<td>0.023 ± 0.010</td>
<td>0.14 ± 0.09</td>
<td>0.32 ± 0.15</td>
</tr>
</tbody>
</table>

* A, before; B, 2 days after; C, 8 days after ADM.

a Wilcoxon's test; NS = not significant; NA, N vs. A; NB, N vs. B; etc.
The impaired D-xyl, 3-omG, and L-rh absorption in patients before chemotherapy was an unexpected finding. Low plasma and/or urine concentrations of all three test monosaccharides are observed after oral sugar load tests in states of obvious malabsorption, usually associated with steatorrhea, such as celiac disease, and featuring a marked reduction of the intestine’s absorptive area and villous atrophy (12). However, under these conditions the D-xyl to 3-omG ratio is also reduced since the active absorption pathway for 3-omG is much less extensively damaged than that for D-xyl (12). There is no reason to believe that in patients with extraintestinal cancer, and without malnutrition or diarrhea, there should be intestinal alteration like that of celiac disease. Samples of intestinal tissue for histological examination were not obtained in any of our patients, since none of them had signs of malabsorption or body wasting to justify biopsy. However, studies comparing pre- and postchemotherapy histological samples of the intestinal mucosa in various tumors have confirmed the existence of mucosal changes only after therapy (13).

The data suggest the existence of a subtle intestinal absorption defect in cancer patients, in the absence of or preceding the appearance of morphological changes, such as those produced by ionizing radiations (14–15). It is widely accepted that morphological changes are not necessarily correlated with derangement in intestinal function.

The observed abnormality might be confined to the monosaccharides employed in the present study, and could depend on a quantitative and/or qualitative defect of the specific carrier molecules resulting from the host-tumor interrelationship. The tumor’s influence on specific organ functions, possibly through the release of substances interfering with intestinal absorption such as cachectins, might also play a role (16). In this respect anorexia, which is so frequent in cancer patients, has been related to anorexigenic peptides produced by the tumor (17).

Another effect of the tumor is the catabolic drive known to occur in cancer patients (18), which causes increased utilization of absorbed substances by the tumor. The low blood and urine levels of two of the test sugars observed in our patients, with the exception of 3-omG which is not utilized by human cells (19), could be at least in part a result of this process.

In conclusion, our study confirms the existence of an acute effect of cytotoxic drugs on intestinal passive permeability to disaccharides, with restoration of normal permeation within a week after drug administration. However, our data rule out a direct effect of ADM on active absorption and on the absorptive mass of the intestine. They also demonstrate the existence of reduced monosaccharide absorption and of increased passive permeation in cancer patients, independent of chemotherapy. Whether this finding is related to the enigma of the development of cancer cachexia remains to be proven. This possibility is currently being investigated.

REFERENCES

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