Continuous Enteral Administration Can Enable Normal Amino Acid Absorption in Rats with Methotrexate-Induced Gastrointestinal Mucositis

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Abstract

It is unknown what feeding strategy to use during chemotherapy-induced gastrointestinal mucositis, which causes weight loss and possibly malabsorption. To study the absorptive capacity of amino acids during mucositis, we determined the plasma availability of enterally administered amino acids (AA), their utilization for protein synthesis, and the preferential side of the intestine for AA uptake in rats with and without methotrexate (MTX)-induced mucositis. Four days after injection with MTX (60 mg/kg) or saline (controls), rats received a primed, continuous dual-isotope infusion (intraduodenal and intravenous) of labeled L-leucine, L-lysine, L-phenylalanine, L-threonine, and L-methionine. We collected blood samples, assessed jejunal histology, and determined labeled AA incorporation in proximal and distal small intestinal mucosa, plasma albumin, liver, and thigh muscle. MTX-induced mucositis was confirmed by histology. The median systemic availability of all AA except for leucine was similar in MTX-treated rats and in controls. However, the individual availability of all AA differed substantially within the group of MTX-treated rats, ranging from severely reduced (<10% of intake) to not different from controls (>40% of intake in 5 of 9 rats). More AA originating from basolateral uptake than those originating from apical uptake were used for intestinal protein synthesis in MTX-treated rats (420% more, P < 0.05).


Introduction

Gastrointestinal mucositis (hereafter referred to as mucositis) is one of the most severe and debilitating side effects of anticancer treatment, causing small intestinal villus atrophy and loss of enterocytes (1). Patients with mucositis suffer from anorexia, nausea, diarrhea, and weight loss (2). It is unknown how to optimally feed patients with mucositis, although nutritional support might improve the nutritional state, accelerate recuperation, and increase survival of mucositis patients (3–6). Normally, enteral nutrition, which is the physiologic way of feeding, is preferred to total parenteral nutrition because the latter carries a high risk of infection and, upon prolonged administration, may cause liver disease (7,8). However, when the absorptive function of the intestine is compromised, total parenteral nutrition offers a useful feeding alternative.

We developed a methotrexate (MTX)9-induced mucositis rat model to determine nutrient digestion and absorption during mucositis and to ultimately design a rational feeding strategy for mucositis patients (9). In this model, we showed that trace amounts of glucose are absorbed normally during mucositis (9). Because there are indications that intestinal absorption of amino acids (AA) might be intact during mucositis (10), in contrast to absorption of di- and tripeptides (11), we here aimed to determine the capacity to absorb enterally administered AA during mucositis.

1 Supported by an unrestricted research grant from KiKa KinderenKankerVrij.
3 Supplemental Methods and Supplemental Figure 1 are available from the “Online Supporting Material” link in the online posting of the article and from the same link in the online table of contents at http://jn.nutrition.org.
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† Abbreviations used: AA, amino acid; ASR, absolute synthesis rate; FSR, fractional synthesis rate; i.d., intraduodenal; MTX, methotrexate.
AA serve several important functions in the human body (12), particularly during periods of growth (13), and play an important role in mucosal homeostasis (14,15). Normally, the intestine itself metabolizes a substantial part (∼80%) of nutrients after absorption from the intestinal lumen before nutrients become systemically available—a process called “first-pass splanchnic utilization” (12,16–19). When more nutrients are used for first-pass utilization, fewer nutrients are systemically available for whole-body energy metabolism and peripheral tissue synthesis (12). A unique feature of intestinal enterocytes is that they do not absorb only AA directly from the lumen by their apical membrane, they can also take up AA from the mesenteric arterial circulation by their basolateral membrane after becoming systemically available (12,20–22). It is not well known how mucositis affects the first-pass splanchnic uptake and the resulting systemic availability of AA, or to what extent systemically available AA are used for protein synthesis in diverse tissues. Knowledge about these processes is needed to determine the absorptive capacity of enterally administered AA, and whether the gut can be used for uptake of AA during mucositis. Furthermore, we hypothesized that there could be a preferential side of the intestine for AA uptake to synthesize proteins during mucositis.

To study the absorptive capacity of AA during mucositis, we determined the plasma availability of 5 enterally administered, essential AA (to indirectly test the function of different AA transporter systems), their utilization for protein synthesis, and the preferential side of the intestine for AA uptake in rats with and without MTX-induced mucositis. We determined absorption of a physiologically relevant amount of AA (i.e., a normal hourly AA intake, instead of a trace amount of AA) when continuously administered by intraduodenal (i.d.) infusion, because continuous enteral nutrient administration has been shown to improve absorption of another nutrient during mucositis in the rat—i.e., glucose (23).

**Materials and Methods**

**Rats and housing**

Male Wistar outbred rats (4 wk old, 65–75 g, Specific Pathogen Free) were obtained from Charles River. 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-h light/12-h dark cycle (lights on 0700–1900 h). Water and purified diet [AIN-93G (24); Research Diet Services B.V.] were available ad libitum unless otherwise stated. The experimental period, based on the mean daily AA intake in control rats [i.e., 5/24 × mean daily AA intake in controls, calculated from their mean daily feed deprivation (2300 h on d 3 to 0800 h on d 4) to reach a steady state (26)]. Infusion rates and doses of the i.d. and i.v. infusates (Table 1) were based on results from pilot studies that we had executed earlier (M. Fijlstra, H. Schierbeek, G. Voortman, K. Y. Dorst, J.B. van Goudoever, E. H. H. M. Rings, and W. J. E. Tissing, unpublished data). Unlabeled AA were added to stable isotope–labeled AA in the i.d. infusate to reach a normal intake of each AA during the experimental period, based on the mean daily AA intake in control rats [i.e., 5/24 × mean daily AA intake in controls, calculated from their mean daily feed deprivation, which is ± 20 g AIN93G per 230 g body weight (9)], to study physiologic AA absorption instead of studying a tracer effect. Blood samples were obtained at baseline and in steady state [at 4, 4.5, and 5 h after the start of the dual-isotope infusion, based on pilot studies and as done previously (26)] for MS analyses. After the 5-h AA infusion protocol, rats were killed under general anesthesia by an intravenous injection of pentobarbital (i.e., 5 mg/50 g). Blood samples were centrifuged immediately (10 min at 2000 × g), and collected plasma was stored at −80°C until further analysis.

**Tissue collection.** Immediately after rats were killed, the abdomen was opened via a midline incision, and the small intestine, liver, and a sample from the thigh muscle were quickly removed. After the small intestine was flushed with ice-cold PBS, a small part of the jejunum (anatomic middle of the small intestine) was collected for histology and fixed in formalin (1 cm) or 2% paraformaldehyde (1 cm) dissolved in PBS, dehydrated, and embedded in paraffin according to standard procedures for histology. Tissue samples were collected and stored at −80°C until further analysis. Liver and thigh muscle were weighed (wet weights), freeze-clamped, pulverized in liquid nitrogen, and stored at −80°C until further analysis.

**Experimental procedures**

**AA infusion protocol in the mucositis rat model.** One week after arrival at the animal facility, rats were equipped with permanent catheters in the duodenum and jugular vein as described previously (25). One week after surgery, rats (6 wk old, 205–250 g) were injected once i.v. in the tail vein with MTX (60 mg/kg, n = 9) to induce mucositis or with saline (0.9%; controls, n = 7) under general anesthesia (9). The assignment of rats to one of the treatment groups was randomly performed by the researcher (M.E.). Intake of food and water, body weight, and the presence of diarrhea [present as watery diarrhea or absent (9)] were recorded daily at −0800 h. Four days after injection, when histologic and clinical symptoms of MTX-mucositis were most severe (9), the AA absorption experiment was performed. A primed (once the hourly dose), continuous i.d. and i.v. infusion of AA in distilled water was started in unanesthetized rats for 5 h after an overnight feed deprivation (2300 h on d 3 to 0800 h on d 4) to reach a steady state (26). Infusion rates and doses of the i.d. and i.v. infusates (Table 1) were based on results from pilot studies that we had executed earlier (M. Fijlstra, H. Schierbeek, G. Voortman, K. Y. Dorst, J.B. van Goudoever, E. H. H. M. Rings, and W. J. E. Tissing, unpublished data). Unlabeled AA were added to stable isotope–labeled AA in the i.d. infusate to reach a normal intake of each AA during the experimental period, based on the mean daily AA intake in control rats [i.e., 5/24 × mean daily AA intake in controls, calculated from their mean daily feed deprivation, which is ± 20 g AIN93G per 230 g body weight (9)], to study physiologic AA absorption instead of studying a tracer effect. Blood samples were obtained at baseline and in steady state [at 4, 4.5, and 5 h after the start of the dual-isotope infusion, based on pilot studies and as done previously (26)] for MS analyses. After the 5-h AA infusion protocol, rats were killed under general anesthesia by an intravenous injection of pentobarbital (i.e., 5 mg/50 g). Blood samples were centrifuged immediately (10 min at 2000 × g), and collected plasma was stored at −80°C until further analysis.

**Materials**

MTX was obtained from Pharmachemie Holding B.V. Stable isotope–labeled AA of 88–99% isotopic purity for i.d. and i.v. infusion were purchased from CortecNet. Unlabeled AA for i.d. infusion were purchased from Sigma-Aldrich Chemie GmbH (Table 1).

**Table 1**

<table>
<thead>
<tr>
<th>Infusion rate</th>
<th>µmol · kg⁻¹ · h⁻¹</th>
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<tbody>
<tr>
<td><strong>Intraduodenal</strong></td>
<td></td>
</tr>
<tr>
<td>Stable isotope–labeled</td>
<td></td>
</tr>
<tr>
<td>L-[2,13C]leucine</td>
<td>117</td>
</tr>
<tr>
<td>L-[15N]lysine-HCl</td>
<td>107</td>
</tr>
<tr>
<td>L-[15N]threonine</td>
<td>73</td>
</tr>
<tr>
<td>L-[2H5]phenylalanine</td>
<td>46</td>
</tr>
<tr>
<td>L-[13C5,15N]methionine</td>
<td>26</td>
</tr>
<tr>
<td>Unlabeled</td>
<td></td>
</tr>
<tr>
<td>L-leucine</td>
<td>262</td>
</tr>
<tr>
<td>L-lysine-HCl</td>
<td>218</td>
</tr>
<tr>
<td>L-phenylalanine</td>
<td>86</td>
</tr>
<tr>
<td>L-threonine</td>
<td>153</td>
</tr>
<tr>
<td>L-methionine</td>
<td>68</td>
</tr>
<tr>
<td><strong>Intravenous</strong></td>
<td></td>
</tr>
<tr>
<td>Stable isotope–labeled</td>
<td></td>
</tr>
<tr>
<td>L-[5,5,2-H]leucine</td>
<td>228</td>
</tr>
<tr>
<td>L-[15N]lysine-HCl</td>
<td>181</td>
</tr>
<tr>
<td>L-[1]phenylalanine</td>
<td>75</td>
</tr>
<tr>
<td>L-[15N]threonine</td>
<td>146</td>
</tr>
<tr>
<td>L-[2H]methionine</td>
<td>56</td>
</tr>
</tbody>
</table>

1 Values are absolute. For individual rats, values were adjusted to their individual body weight on d 4 (range: 185–250 g, n = 16. MTX, methotrexate.

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Analytic methods
Hematoxylin-and-eosin staining of formalin- and paraformaldehyde-fixed jejunal segments to assess histology, as well as their morphometric analysis was carried out as described previously (9). Plasma citrulline concentrations [indicating functional enterocyte mass (27)] were measured as described previously (9). Plasma albumin concentrations were measured in 150 μL plasma via the bromocresol green method, which is a calorimetric assay, as described by the manufacturer (Roche Diagnostics GmbH). Tissue samples (±50 mg) of proximal and distal small intestinal mucosa, liver, and thigh muscle were homogenized in distilled water (±500 μL) to measure tissue isotopic enrichment of all AA (see “Tissue enrichment analyses” below). An aliquot of 50 μL was taken to measure tissue protein concentrations according to Lowry et al. (28).

Mass spectrometry
Plasma enrichment analysis. Plasma samples (30 μL) were prepared to determine isotopic enrichment of all AA by gas chromatography/MS (MSD 5975C; Agilent Technologies), as described previously (26,29–31). Instead of plasma leucine enrichment, plasma enrichment of its keto-analog α-ketoisocaproic acid was measured to correct for intracellular transamination of leucine, as done before by us and others (10,32). The enrichment of all AA in steady state was calculated by using the mean enrichment between 4 and 5 h after continuous dual-isotope infusion, corrected for the enrichment at baseline, as described previously (33). Enrichment was expressed in mole percent excess.

Tissue enrichment analysis. Aliquots of 200 μL homogenized tissue (see “Analytic methods” above) were taken to measure isotopic enrichment of all free (unbound) and protein-bound AA. The protein fraction was isolated and analyzed as previously described (30). In short, proteins were precipitated and the supernatant was collected and used for enrichment analysis of free AA. The washed, precipitated protein pellets were hydrolyzed by adding 1 mL of 6 mol/L HCl and incubated at 110°C for 20 h. An aliquot was dried at room temperature in a speedvac (GeneVac mVac; GeneVac Ltd.), and the residue was dissolved in 0.2 mL milliQ. AA were isolated by cation exchange separation. To measure the enrichment of AA in the protein-bound tissue pool, hydrolyzed samples were derivatized to form acetyl-ethoxycarbonyl-ethylsters. The 13C:12C ratio of AA in protein isolates were measured by using a gas chromatograph/combustion/isotope ratio MS (Delta XP; Thermo Fisher) according to the method used in previous work (26,31). Isotopic enrichment of protein-bound 2H- or 13N-labeled AA, and of all free AA in the supernatants, was determined by gas chromatography/MS analysis of their acetyl-ethoxycarbonyl-ethylsters by using electron impact ionization, as described for the plasma samples. Enrichment was expressed in mole percent excess.

Plasma albumin analysis. Albumin was isolated from collected plasma at 4, 4.5, and 5 h after the start of the dual-isotopically labeled AA infusions. After hydrolysis, the isotopic enrichment of enterally administered AA was measured by using gas chromatograph/combustion/isotope ratio MS to determine the synthesis rate of plasma albumin as described previously (34,35).

Values obtained for isotopic enrichment of all AA (including α-ketoisocaproic acid) were corrected for the contribution of natural abundance on the measured fragments, as well as for the contribution of administered tracers to the measured fragments.

Calculations
The equations used to obtain the results are detailed in the Supplemental Methods.

Statistical analysis
Statistical analysis of data for the MTX-treated rats versus controls (i.e., rat characteristics, AA kinetics, and protein synthesis) was performed by using the Mann-Whitney U test (SPSS 16.0 for Windows; SPSS, Inc.). Analysis of data on basolateral versus apical AA uptake for protein synthesis in MTX-treated rats or in controls (Supplemental Methods) was performed by using the Wilcoxon signed-rank test. Data are presented as absolute values (Table 1) and median and range (Tables 2–5) or as data for individual rats (Supplemental Fig. 1) for the indicated number of rats (n) per group. Correlations are expressed as nonparametric Spearman correlation coefficients. For significant correlations, optimal curve fitting was performed by using nonlinear regression with a polynomial model (Supplemental Fig. 1). P < 0.05 was considered significant.

Results
The mucositis rat model
We studied the capacity to absorb enterally administered AA during mucositis in a previously established MTX-induced mucositis rat model (9). As seen in previous studies by us and others (9,36–41), MTX-treated rats showed typical histologic and clinical symptoms of mucositis (i.e., villus atrophy, a reduced plasma citrulline concentration, a reduced intake, weight loss, and watery diarrhea), in contrast to controls (Table 2). Although symptoms of mucositis varied from mild to severe in individual rats, most MTX-treated rats (7 of 9) suffered from severe mucositis [i.e., villus length <300 μm and plasma citrulline concentration <30 μmol/L (9)] (Supplemental Fig. 1).

Plasma kinetics of i.v.- and i.d.-infused AA
As shown in Table 3, median plasma AA fluxes, based on i.v.-infused tracers and on i.d.-infused tracers, were similar in MTX-treated rats and in controls for all AA except for leucine. However, MTX-treated rats showed substantial interindividual differences in AA fluxes based on i.d.-infused tracers, with maximal values 500–1400% higher than maximal values in controls. This was due to a large variability in individual plasma enrichment of i.d.-infused tracers in MTX-treated rats (ranging from severely reduced to normal; data not shown).

Median first-pass splanchnic utilization, and resulting systemic availability, of all i.d.-infused AA except for leucine did not differ between MTX-treated rats and controls. In contrast to controls, MTX-treated rats showed substantial interindividual differences (Table 3). Maximal first-pass splanchnic utilization of all AA was >90% of intake in MTX-treated rats, whereas it was ≤60% of intake in controls. As a result, minimal systemic availability of all AA was <10% of intake in MTX-treated rats, whereas it was ≥40% of intake in controls. The availability of all AA varied from severely reduced (<10% of intake) to not different from controls among individual MTX-treated rats with symptoms of severe mucositis (7 of 9 rats; Supplemental Fig. 1).

Although the systemic availability of enterally administered leucine in all rats correlated with villus length (r = 0.80, P < 0.05), that of lysine, phenylalanine, threonine, and methionine did not. Similarly, the systemic availability of enterally administered leucine and threonine in all rats correlated with plasma citrulline (r = 0.64–0.81, P < 0.05), but that of lysine, phenylalanine and methionine did not.

**TABLE 2** Characteristics of control and MTX-treated rats

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control</th>
<th>MTX</th>
</tr>
</thead>
<tbody>
<tr>
<td>Villus length (μm)</td>
<td>408</td>
<td>262</td>
</tr>
<tr>
<td>Citrulline (μmol/L)</td>
<td>69</td>
<td>15</td>
</tr>
<tr>
<td>Food intake (g/d)</td>
<td>11</td>
<td>0</td>
</tr>
<tr>
<td>Body weight (g)</td>
<td>108</td>
<td>91</td>
</tr>
<tr>
<td>Diarrhea (%)</td>
<td>0</td>
<td>67</td>
</tr>
</tbody>
</table>

1 Values are medians and range, except for diarrhea for which values are absolute, n = 7–9. *Different from control, P < 0.01. MTX, methotrexate.

2 Body weight was related to weight at d 0 (day of MTX or saline injection), which was arbitrarily set at 100%.

3 Diarrhea was present as watery diarrhea or completely absent.
Protein breakdown in MTX-treated rats, based on i.d.-infused tracers, varied according to AA and was higher than (leucine and methionine, \(P < 0.05\)), lower than (threonine, \(P < 0.05\)), or similar to controls (lysine and phenylalanine; Table 3).

**Tissue protein and albumin synthesis with i.d.-infused AA**

The utilization of enterally administered AA for protein synthesis was expressed by the fractional and absolute synthesis rates of protein (FSR and ASR, respectively), indicating the relative and absolute need for AA for protein synthesis, respectively.

**Small intestinal mucosa.** The FSR with systemically available, enterally administered AA (FSR\textsubscript{basolateral}) was \(\geq 20\%\) higher in MTX-treated rats than in controls, depending on the specific AA (proximal and distal mucosa, \(P < 0.05\); Table 4). However, because the total amounts of mucosa (proximal and distal mucosa \(\geq 49\%\) lower, \(P < 0.05\)) and/or the protein concentration of mucosa (proximal mucosa 16\% lower, \(P < 0.05\)) were lower in MTX-treated rats than in controls, the ASR (ASR\textsubscript{basolateral}) was lower in MTX-treated rats than in controls (proximal mucosa, \(P < 0.05\)) or similar to controls (distal mucosa). In both MTX-treated rats and in controls, protein synthesis in proximal and distal small intestinal mucosa was higher with enterally administered AA taken up from the systemic side (FSR\textsubscript{basolateral} and ASR\textsubscript{basolateral}) than with AA taken up from the luminal side (FSR\textsubscript{apical} and ASR\textsubscript{apical}, \(P < 0.05\)). These differences between basolateral and apical AA uptake seemed to be more pronounced for MTX-treated rats than for controls both in proximal and distal mucosa. The enrichment of methionine was too low to measure.

**Albumin.** The FSR with systemically available, enterally administered AA was \(\geq 60\%\) higher in MTX-treated rats than in controls, depending on the specific AA (\(P < 0.05\); Table 5). However, because plasma albumin concentration was lower in MTX-treated rats than in controls (24\% lower, \(P < 0.05\)), the ASR was similar in MTX-treated rats and in controls. The enrichment of threonine and methionine was too low to measure.

**Liver.** The FSR with systemically available, enterally administered AA was 20\% and 30\% higher in MTX-treated rats than in controls for leucine and lysine, respectively (\(P < 0.05\); Table 5), or similar to controls for phenylalanine. Both the total amounts of liver and the protein concentrations in liver were similar in MTX-treated rats and in controls, and therefore the ASR was also 20\% and 30\% higher in MTX-treated rats than in controls for leucine and lysine, respectively (\(P < 0.05\)), or similar to controls for phenylalanine. The enrichment of threonine and methionine was too low to measure.

**Thigh muscle.** The FSR with systemically available, enterally administered AA was similar in MTX-treated rats and in controls (Table 5). The protein concentration of thigh muscle was similar in MTX-treated rats and in controls, and therefore the ASR (per kg muscle) was also similar in both groups. The enrichment of threonine and methionine was too low to measure.

**Discussion**

We aimed to determine the absorptive capacity of AA in rats with and without MTX-induced mucositis. Our data indicate that continuous enteral administration can enable normal AA
### TABLE 4  Amount, protein concentration, FSR, and ASR of small intestinal mucosa using intraduodenally infused, stable isotope–labeled amino acids in control and MTX-treated rats

<table>
<thead>
<tr>
<th></th>
<th>Leucine</th>
<th>Lysine</th>
<th>Phenylalanine</th>
<th>Threonine</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>MTX</td>
<td>Control</td>
<td>MTX</td>
</tr>
<tr>
<td></td>
<td>Total amount of mucosa, g</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td>Mucosal protein concentration, g/kg</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td>Total amount of mucosal protein, g/kg BW</td>
<td>7.6 (5.0–8.1)</td>
<td>2.4 (0.9–4.6)*</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td>FSRbasolateral, %/d</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td>ASRbasolateral, g · kg BW⁻¹ · d⁻¹</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td>FSRapical, %/d</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td>ASRapical, g · kg BW⁻¹ · d⁻¹</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
</tbody>
</table>

**Leucine**

- **Proximal mucosa**
  - Total amount of mucosa, g: 16.1 (8.7–17.2) vs. 6.3 (2.0–7.7)*
  - Mucosal protein concentration, g/kg: 108 (99–141) vs. 91 (71–111)*
  - Total amount of mucosal protein, g/kg BW: 7.6 (5.0–8.1) vs. 2.4 (0.9–4.6)*

**Lysine**

- **Proximal mucosa**
  - Total amount of mucosa, g: 14.3 (11.1–19.3) vs. 7.3 (3.0–13.4)*
  - Mucosal protein concentration, g/kg: 96 (65–110) vs. 86 (62–107)
  - Total amount of mucosal protein, g/kg BW: 53 (38–7.9) vs. 36 (12–4.9)*

**Phenylalanine**

- **Proximal mucosa**
  - Total amount of mucosa, g: 16.1 (8.7–17.2) vs. 6.3 (2.0–7.7)*
  - Mucosal protein concentration, g/kg: 108 (99–141) vs. 91 (71–111)*
  - Total amount of mucosal protein, g/kg BW: 7.6 (5.0–8.1) vs. 2.4 (0.9–4.6)*

**Threonine**

- **Proximal mucosa**
  - Total amount of mucosa, g: 16.1 (8.7–17.2) vs. 6.3 (2.0–7.7)*
  - Mucosal protein concentration, g/kg: 108 (99–141) vs. 91 (71–111)*
  - Total amount of mucosal protein, g/kg BW: 7.6 (5.0–8.1) vs. 2.4 (0.9–4.6)*

**Distal mucosa**

- Total amount of mucosa, g: 14.3 (11.1–19.3) vs. 7.3 (3.0–13.4)*
- Mucosal protein concentration, g/kg: 96 (65–110) vs. 86 (62–107)
- Total amount of mucosal protein, g/kg BW: 53 (38–7.9) vs. 36 (12–4.9)*

**Notes:**

1. Values are medians and range, n = 7–9. *Different from control, P < 0.05; # different from apical FSR or apical ASR, P < 0.05. ASR, absolute synthesis rate; BW, body weight; FSR, fractional synthesis rate; MTX, methotrexate; ND, not detectable.

2. FSR and ASR with intraduodenally infused amino acids via basolateral uptake (from the systemic side).

3. FSR and ASR with intraduodenally infused amino acids via apical uptake (from the luminal side).
shown to be a useful surrogate marker for mucositis and for citrulline concentrations. Although plasma citrulline was earlier linked to tight junctions could also have been possible because mucositis paracellular absorption (44). Leakage of AA through damaged transporters on damaged epithelial membrane (9), and/or via (1)

**TABLE 5** Amount, protein concentration, FSR, and ASR of albumin, liver, and thigh muscle using intraduodenally infused, stable isotope–labeled amino acids in control and MTX-treated rats1

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>MTX</th>
<th>Control</th>
<th>MTX</th>
<th>Control</th>
<th>MTX</th>
<th>Control</th>
<th>MTX</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Plasma albumin</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Albumin concentration, g/L plasma</td>
<td>37 (28–39)</td>
<td>28 (25–33)*</td>
<td>25 (16–30)</td>
<td>49 (11–102)*</td>
<td>19 (15–27)</td>
<td>30 (14–70)*</td>
<td>60 (14–91)</td>
<td>112 (33–198)*</td>
</tr>
<tr>
<td>FSR, %/d</td>
<td>9.3 (4.6–11.2)</td>
<td>12.2 (3.5–25.5)</td>
<td>6.8 (4.1–10.0)</td>
<td>8.4 (4.5–17.4)</td>
<td>22.1 (5.2–33.6)</td>
<td>28.1 (10.6–49.6)</td>
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<tr>
<td>ASR, g - L⁻¹.d⁻¹</td>
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<tr>
<td><strong>Liver</strong></td>
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<tr>
<td>Total amount of liver, g</td>
<td>8.9 (7.8–9.7)</td>
<td>8.2 (7.0–9.3)</td>
<td>42 (42–50)</td>
<td>51 (43–77)*</td>
<td>35 (28–39)</td>
<td>45 (31–76)*</td>
<td>86 (76–97)</td>
<td>84 (72–115)</td>
</tr>
<tr>
<td>Liver protein concentration, g/kg</td>
<td>228 (208–240)</td>
<td>215 (194–235)</td>
<td>3.8 (3.4–4.2)</td>
<td>4.5 (3.8–7.1)*</td>
<td>2.9 (2.3–3.3)</td>
<td>3.9 (2.7–6.7)*</td>
<td>7.5 (6.0–8.2)</td>
<td>7.5 (6.2–10.5)</td>
</tr>
<tr>
<td>Total amount of liver protein, g/kg BW</td>
<td>8.5 (7.7–9.0)</td>
<td>8.6 (8.2–10.5)</td>
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<tr>
<td>FSR, %/d</td>
<td>42 (42–50)</td>
<td>51 (43–77)*</td>
<td>35 (28–39)</td>
<td>45 (31–76)*</td>
<td>86 (76–97)</td>
<td>84 (72–115)</td>
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<tr>
<td>ASR, g - kg BW⁻¹.d⁻¹</td>
<td>3.8 (3.4–4.2)</td>
<td>4.5 (3.8–7.1)*</td>
<td>2.9 (2.3–3.3)</td>
<td>3.9 (2.7–6.7)*</td>
<td>7.5 (6.0–8.2)</td>
<td>7.5 (6.2–10.5)</td>
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<tr>
<td><strong>Thigh muscle</strong></td>
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<tr>
<td>Muscle protein concentration, g/kg</td>
<td>42 (33–47)</td>
<td>45 (31–50)</td>
<td>7.2 (4.5–9.5)</td>
<td>4.9 (0.8–9.5)</td>
<td>5.4 (2.9–7.6)</td>
<td>3.5 (0.8–6.1)</td>
<td>12.6 (7.4–18.1)</td>
<td>8.1 (5.0–15.7)</td>
</tr>
<tr>
<td>FSR, %/d</td>
<td>2.9 (2.0–3.7)</td>
<td>2.1 (0.3–4.4)</td>
<td>2.2 (1.3–3.0)</td>
<td>1.1 (0.4–3.6)</td>
<td>5.4 (2.2–7.1)</td>
<td>3.5 (1.8–7.2)</td>
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<tr>
<td>ASR, g - kg muscle⁻¹.d⁻¹</td>
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1 Values are medians and range, n = 7–9. *Different from control, P < 0.05. ASR, absolute synthesis rate; BW, body weight; FSR, fractional synthesis rate; MTX, methotrexate.

absorption in rats with MTX-induced mucositis. The intestine prefers basolateral AA uptake to meet its need for AA for protein synthesis during mucositis.

The absorptive capacity of AA was determined in a previously established MTX-induced mucositis rat model (9). We chose 5 different essential AA that are absorbed by different transporter systems, which are normally present on the apical and basolateral membrane of intestinal enterocytes (42,43), to indirectly test the function of all these systems during mucositis. In earlier studies, only the absorption of leucine was studied in small intestinal mucosa in the rat (10). AA were administered by continuous i.d. infusion, because continuous administration of enteral nutrition during intestinal failure is thought to enhance enteral absorption by maximizing saturation of the (residual) carrier proteins, thereby increasing intestinal function (8). Furthermore, we previously showed that continuous enteral glucose absorption in rats with MTX-induced mucositis. The intestine seems to be in a catabolic state during mucositis as can be concluded from weight loss and increased protein breakdown (containing leucine and methionine), as found previously (10).

In contrast to absolute synthesis, fractional synthesis of intestinal proteins and plasma albumin with enterally administered leucine, lysine, and phenylalanine for tissue protein and albumin synthesis was mostly lower or similar in MTX-treated rats, compared with controls, reduced systemic AA availability in some AA, we measured their utilization for absolute protein synthesis during mucositis. Absorption of continuously administered AA in 4 of 9 rats; Supplemental Fig. 1) was probably caused by AA malabsorption. The enrichment of threonine and/or methionine was too low to measure.

In contrast to absolute synthesis, fractional synthesis of intestinal proteins and plasma albumin with enterally administered leucine, lysine, and phenylalanine was higher in MTX-treated rats than in controls. A relatively increased AA utilization for intestinal protein synthesis during mucositis might indicate an increased renewal of the intestinal mucosa, which seems plausible after initial chemotherapy-induced intestinal damage (47). However, intestinal inflammation during mucositis might also cause a decreased synthesis of inflammatory proteins, such as myeloperoxidase (9). Albinin synthesis might be increased during mucositis to compensate for the reduced albumin concentrations that we measured. Reduced albumin concentrations during mucositis were probably caused by increased losses via the intestine as seen with other gastroenteropathies such as colitis ulcerosa (48). In general, the rat seems to be in a catabolic state during mucositis as can be concluded from weight loss and increased protein breakdown (containing leucine and methionine), as found previously (10).
rats, compared with controls, but could not be calculated because the total amount of muscle per rat was unknown.

We hypothesized that there could be a preferential side of the intestine for AA uptake during mucositis. In MTX-treated rats, protein synthesis in proximal and distal small intestinal mucosa with enterally administered leucine, lysine, phenylalanine, and threonine was higher when taken up basolaterally than when taken up apically, indicating preferred AA uptake from the systemic side for intestinal protein synthesis during mucositis. Others have shown that enterocytes near the crypt-villus junction prefer systemically available AA, whereas enterocytes at villus tips prefer AA at the luminal side for protein synthesis (22). Therefore, AA absorption by the intestine during mucositis was probably mainly performed by enterocytes at the crypt-villus junction, which is compatible with the observation that villi of MTX-treated rats were atrophied and damaged whereas crypts were already regenerating [as shown in this study and seen previously by us and others (9,47,49)]. Also, in controls, AA uptake for intestinal protein synthesis was preferred with AA originating from the systemic side, as found by others (12,17), although differences seemed less pronounced than in MTX-treated rats, especially in the proximal mucosa. Normally, intestinal AA absorption is very efficient (50); the proximal small intestine (i.e., duodenum and proximal jejunum) absorbs almost all intraluminal AA, thereby leaving few AA to be absorbed in the distal small intestine (i.e., distal jejunum and ileum). The distal small intestine is therefore mainly dependent on systemically available AA, except for some AA at the luminal side that become available by recycling (proteinolysis and reabsorption) of intestinal proteins (12).

If we extrapolate our findings to the clinic, they imply that enteral AA administration by continuous infusion could be useful for a substantial portion of mucositis patients to improve their nutritional state, recuperation, and survival (4–6,51). Furthermore, enteral nutrition could possibly accelerate intestinal recovery because intraluminal nutrients have a stimulatory effect on intestinal epithelial cells and the production of trophic hormones (52–54). Our results show that AA are important for mucositis patients so that they can meet their need for AA for intestinal protein synthesis and albumin synthesis.

We determined AA absorption during mucositis at d 4 after injection with MTX or saline. However, symptoms of mucositis are actually present during a longer period of time—from d 2 until d 5 after injection with MTX (9). We do not know whether the observed AA malabsorption in a portion of mucositis rats is structural (present on all days during mucositis) or temporal (only present on d 4). When AA malabsorption is structural, indeed only a portion of mucositis patients would benefit from continuous enteral AA administration. However, if AA malabsorption is temporal, all mucositis patients might benefit from continuous enteral AA administration during mucositis. Future studies should focus on studying the effects of continuous enteral AA administration in mucositis patients. If only a portion of patients benefit from continuous enteral AA, a marker that distinguishes between mucositis patients with a good or poor AA absorptive capacity would be highly desirable to anticipate which patients would benefit the most from continuous enteral AA administration. For now, parenteral AA administration might be a rational alternative for enteral AA administration to guarantee optimal AA availability in all patients with mucositis.

In conclusion, we showed that continuous enteral administration can enable normal AA absorption in rats with chemotherapy-induced gastrointestinal mucositis. The intestine prefers basolateral AA uptake to meet its need for AA for protein synthesis during mucositis. So, although the gut might be usable for AA uptake in at least a portion of mucositis patients, when enterally administered continuously, parenteral AA administration might be a rational alternative to guarantee optimal AA availability in all patients with mucositis.

Acknowledgments

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Literature Cited


Normal amino acid absorption during mucositis 1989


