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Chemotherapy Does Not Influence Intestinal Amino Acid Uptake in Children

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ABSTRACT: Chemotherapy will frequently induce intestinal damage (mucositis). Enteral nutrition is then often withheld for fear of impaired intestinal absorption as shown in animal models. There is no clinical evidence, however, that absorption is indeed compromised during chemotherapy-induced mucositis. The aim of this study was to evaluate systemic availability of dietary amino acids (leucine) during chemotherapy-induced mucositis. We studied eight childhood cancer patients (age 1.5–16 y) on 2 d, *i.e.* the day before chemotherapy and 3–5 d after. Chemotherapy-induced oral mucositis and diarrhea were scored on a World Health Organization toxicity scale. Stable isotope tracers were used to measure first-pass splanchnic leucine uptake and whole-body leucine kinetics. Patients showed increased mucositis and/or diarrhea toxicity scores ($p < 0.0001$) after chemotherapy. Systemic availability of enterally administered leucine was not significantly affected by chemotherapy (before 60%, after 90%, $p = 0.46$). Interestingly, five patients already showed a negative leucine balance before chemotherapy. In conclusion, most children receiving chemotherapy are already catabolic before start of a new cycle of chemotherapy. Amino acid transport as measured by leucine uptake in the intestine is not affected by chemotherapy-induced mucositis. **(*Pediatr Res* 62: 195–199, 2007)**

Chemotherapy may severely damage the intestinal mucosa, resulting in a condition referred to as mucositis (1,2). Mucositis is characterized by major morphologic changes of the intestinal epithelium, such as epithelial flattening, villus atrophy, and specific down-regulation of the enterocyte-specific gene expression that is crucial for degradation and absorption of nutrients (3–5). It is unknown how this condition effects digestion and absorption of enteral nutrition.

Normally, the intestine itself metabolizes a substantial part of the nutrients absorbed from the intestinal lumen in first-pass splanchnic uptake (6,7). Animal and human studies have shown that 20–80% of dietary essential amino acids are used within the intestine (8–10). The more nutrients are used by the small intestine, the fewer essential amino acids are systemically available for whole-body energy metabolism and protein synthesis. It is unknown whether chemotherapy-induced mucositis affects first-pass splanchnic amino acid uptake. We hypothesized, first, that chemotherapy-induced mucositis will lead to lower nutrient uptake by the intestine and, second, that it will lead to a higher intestinal utilization rate due to mucosal regeneration. The combined result would be a lower systemic availability of dietary amino acids.

To test these hypotheses we determined first-pass splanchnic uptake of dietary leucine, an essential amino acid, in pediatric patients before and after receiving mucotoxic chemotherapy.

METHODS

Subjects. The ethics review board of the Erasmus MC-Sophia Children's Hospital approved the study. Informed consent was obtained from parents and patients, as appropriate. Eligible subjects were patients aged between 1 and 18 y, admitted for a cycle of chemotherapy in their drug regimen with known high risk of severe intestinal side effects. Patients diagnosed with acute myeloid leukemia (AML) and B cell–non-Hodgkin lymphoma (B-NHL) fulfilled these criteria already at diagnosis. Acute lymphoid leukemia (ALL) patients were eligible if they had developed mucositis during a previous chemotherapy cycle. Exclusion criteria were cow's milk allergy and abdominal radiotherapy.

Protocol. Each subject was studied on 2 d, *i.e.* the day before start of chemotherapy and 3–5 d after chemotherapy. The study protocol was similar on these days, as illustrated in Figure 1. The degree of oral mucositis and diarrhea were scored according to the World Health Organization (WHO) criteria (Table 1).

All patients had a central venous catheter already in place, which was used to infuse stable isotopes. Blood samples were collected by capillary blood puncture. Breath samples of patients younger than 5 y were collected as described before (11). Older patients were asked to exhale into a Vacutainer through a straw. After a 4-h fast, patients who were capable of drinking received a formula diet (Tentrini, Nutricia, Zoetermeer, The Netherlands), every hour for 5 h. Others were continuously fed this formula through a nasogastric tube. Nutrient intake was similar on both study days. Three different stable isotopes were infused. First, a primed, continuous 2-h i.v. infusion [4.7 μmol/kg and 4.7 μmol/(kg/h)] of [^{13}C]sodium bicarbonate (99.0 mol% ^{13}C; Cambridge Isotopes, Woburn, MA) dissolved in sterile saline was administered at a constant rate. This was immediately followed by a primed, continuous i.v. infusion [11.3 μmol/kg prime and 11.3 μmol/(kg/h)] of 1-^{13}C leucine (98.0 mol% ^{13}C; Cambridge Isotopes) and an intragastric infusion [11.0 μmol/kg and 11 μmol/(kg/h)] of $^{2}H_3$ leucine (98.0 mol% $_2H^3$; Cambridge Isotopes), both for 3 h.

Baseline blood and breath samples were collected just before start of tracer infusion. Breath samples were also taken every 15 min during the last 45 min of sodium bicarbonate infusion and during the last hour of leucine infusion. Two blood samples were collected during the last 15 min of leucine infusion (T 285, 300 min.). The heparinized blood was directly put on melting ice, centrifuged, and stored at −80°C until further analysis.

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Abbreviations: KICA, 2-ketoisocaproic acid; **LRP,** leucine release from protein breakdown (measure for protein breakdown); **MPE,** mole percent excess; **NOLD,** non-oxidative leucine disposal (measure for protein synthesis)

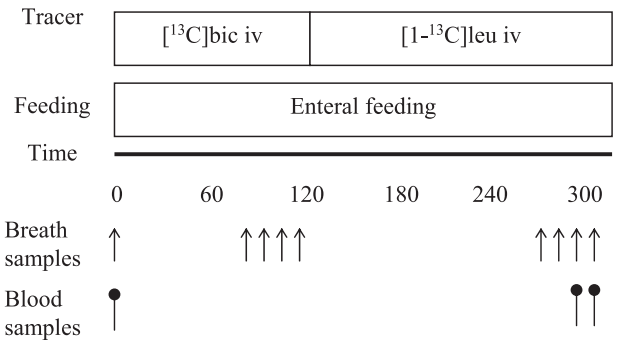

Figure 1. Schematic overview of study day before and after chemotherapy. [^{13}C] bic, [^{13}C] sodium bicarbonate; [1-^{13}C] leu, [1-^{13}C] leucine.

Table 1. *WHO grading scale of oral mucositis and diarrhea*

	Oral mucositis	Diarrhea
0	None	None
1	Soreness/erythema	Transient, <2 d
2	Erythema, ulcers, can eat solids	Tolerable, but >2 d
3	Ulcers require liquid diet only	Intolerable, requires therapy
4	Oral alimentation impossible	Hemorrhagic dehydration

Calculations. The assessment of amino acid kinetics by stable isotope technique is based on the following model (12):

Turnover or Flux (Q) = Intake + Breakdown = Synthesis + Oxidation

Amino acids enter a metabolic pool *via* diet (Intake) and protein breakdown (Breakdown), and are withdrawn from this pool by protein synthesis (Synthesis) or amino acid oxidation (Oxidation). During steady state, tracer enters and leaves the pool at equal rate. This process of replacement or renewal of a given substance is referred to as turnover or flux.

All calculations used were previously described by van der Schoor *et al.* (13).

Equation 1 is the above-mentioned model modified for leucine: leucine entering the pool equals leucine leaving the pool. Leucine enters the pool *via* intake (I) and through leucine release from protein breakdown (LRP) and leaves the pool *via* NOLD, a measure of protein synthesis rate, and through leucine oxidation (OX).

$$Q = I + LRP = NOLD + OX \quad (1)$$

Q, flux of leucine tracer [μmol/(kg/h)]; I, LRP, NOLD, and OX [μmol/(kg/h)].

The rate of leucine flux was calculated by measuring the dilution of its intracellular representation KICA at steady state as modified for stable isotope tracers (14,15).

Equation 2a calculates the leucine flux of the i.v. tracer.

$$Qiv = IL \times [(Ei/Ep)/Ep] \quad (2a)$$

Qiv, flux of intravenous leucine tracer [1-^{13}C] leucine [μmol/(kg/h)]; i_L, leucine infusion rate [μmol/(kg/h)]; Ei and Ep are the enrichments in mole percent excess (MPE) of [1-^{13}C] leucine in the leucine infusate and [1-^{13}C] KICA in plasma during steady state, respectively.

Equation 2b calculates the leucine flux of the intragastric tracer.

$$Qig = IL \times [(Ei/Ep)/Ep] \quad (2b)$$

Qig, flux of intragastric leucine tracer [$^2H_3$] leucine [μmol/(kg/h)]; i_L, leucine infusion rate [μmol/(kg/h)]; Ei and Ep are the enrichments (MPE) of [$^2H_3$] leucine in the leucine infusate and [$^2H_3$] KICA in plasma at steady state, respectively.

After determining both i.v. and intragastric tracer flux, first-pass splanchnic uptake can be calculated.

Equation 3 calculates first-pass up-take fraction (%).

$$\text{First Pass Uptake} = [(Qig - Qiv)/Qig] \quad (3)$$

Equation 4 calculates the absolute first-pass leucine uptake in μmol/(kg/h).

$$\text{Absolute First Pass Uptake} = [(Qig - Qiv)/Qig] \times I \quad (4)$$

I, enteral leucine intake (μmol/(kg/h)).

Equation 5 calculates the fraction of leucine oxidized.

$$\text{Fraction of leucine oxidized} = [IEL \times iB] / [IEB \times iL] \quad (5)$$

IE_L and IE_B, $^{13}CO_2$ enrichment at steady state during i.v. [1-^{13}C] leucine and [^{13}C] sodium bicarbonate infusion, respectively; i_L and i_B, infusion rate [μmol/(kg/h)] of leucine and bicarbonate, respectively, as described previously (16).

Equation 6 is calculated by multiplying the outcome of Equation 5 with the flux of the i.v. leucine tracer as calculated in Equation 2a (although this does not take first-pass oxidation into account).

$$\text{Whole-Body Leucine Oxidation} = [\text{Eq 5.}] \times [Qiv] \quad (6)$$

Equation 7 calculates leucine balance.

$$\text{Balance} = \text{NOLD} - \text{LRP (Leucine Used for Synthesis} - \text{Leucine Used for Breakdown)} \quad (7)$$

Leucine balance [μmol/(kg/h)].

Analytical methods. $^{13}CO_2$ enrichment in the breath samples was measured on an isotope ratio mass spectrometer (ABCA; Europa Scientific, Van Loenen Instruments, Leiden, the Netherlands) (17). Plasma enrichment of [1-^{13}C] and [$^2H_3$] KICA in small aliquots of plasma was determined by gas chromatography/ mass spectrometry (18).

Statistics. All data are expressed in median (25^{th} percentile–75^{th} percentile) values obtained from the breath or blood samples taken at the end of each tracer infusion. The distribution of the differences in direction and magnitude between the two related values (before and after chemotherapy) was compared by Wilcoxon signed-ranks tests. A value of $p < 0.05$ was considered statistically significant.

RESULTS

Patients, treatment, and toxicity. Eight patients participated in the study; age at diagnosis and type of chemotherapy are listed in Table 2. Seven patients were measured both before and after chemotherapy. In one patient, baseline measurements are lacking for logistic reasons. All patients showed good clinical health, with no or only moderate signs of diarrhea

Table 2. *Patient characteristics*

	1	2	3	4	5	6	7	8
Patient	1.8	1.7	2.4	11.9	13.1	6.7	7.0	15.8
Diagnosis	ALL	AML	AML	AML	AML	BNH	BNH	BNH
Cycle	MD-MTX	MAE	ADE	ADE	ADE	COPADM	COPADM	COPADM
Oral mucositis (WHO grade)								
Before chemotherapy	1	0	0	0	0	0	1	0
After chemotherapy	2	0	0	2	0	2	3	1
Diarrhea (WHO grade)								
Before chemotherapy	0	0	0	0	2	0	0	0
After chemotherapy	2	0	1	2	3	3	3	1

Median mucositis and diarrhea scores are shown.

Cycle, chemotherapy cycle; ALL, acute lymphoid leukemia; AML, acute myeloid leukemia; B-NHL, B-non-Hodgkin lymphoma; MD-MTX, medium dose methotrexate; MAE, ara-C, mitoxantrone, and etoposide; ADE, ara-C, daunorubicin, and etoposide; COPADM, vincristine, cyclophosphamide, doxorubicin, and high-dose MTX.

or oral mucositis on the first study day (Table 2). Following chemotherapy, however, the clinical condition of seven patients had deteriorated. They showed increased mucositis and/or diarrhea WHO-toxicity scores ($p < 0.0001$). Two patients demonstrated weight loss and two other patients suffered from severe abdominal pain. Four patients already received tube feeding before the study days, and were given formula through this tube; the other four received a normal diet (although characterized by a reduced intake). Table 3 shows the individual intake of all patients on both study days.

Leucine kinetics. Table 4 shows the $^{13}CO_2$ enrichment expressed in MPE of breath-samples collected during steady state at the end of [^{13}C] bicarbonate and leucine infusion. It also shows the enrichment of [1-^{13}C] and [$^2H_3$] KICA (MPE) of serum collected at steady state. There are no significant differences in enrichment before and after chemotherapy.

The influence of chemotherapy-induced mucositis on first-pass splanchnic uptake and whole-body leucine kinetics is shown in Table 5. Before chemotherapy, there was a first-pass uptake of leucine as shown by the difference in turnover of the enterally and intravenously-administered tracer. Inasmuch as, before chemotherapy, a median of 40% of the dietary leucine was used in first pass or not absorbed, 60% was systemically available. Following chemotherapy, only 10% of the ingested leucine was used in first pass or not absorbed, so that 90% (median) was systemically available. However, this difference in intestinal utilization does not reach statistical significance. Both LRP (an indication of proteolysis) and leucine oxidation increased following chemotherapy. These differences did not reach statistical significance. NOLD (an indication of protein synthesis) was not affected by chemotherapy. Interestingly, five patients were found to be already in negative leucine balance (the equation NOLD – LRP has a negative result) before start of chemotherapy, indicating a catabolic leucine metabolism already before start of chemotherapy. This negative balance almost doubled following chemotherapy, although this difference was not statistically significant.

Table 3. *Calorie (kcal/kg/h) and protein (mg/kg/h) intake during study day 1 and 2*

	Study day 1		Study day 2	
Patient	Calorie	Protein	Calorie	Protein
1	1.5	60	1.5	60
2	2.5	100	2.5	100
3			4.6	184
4	1.1	44	1.4	56
5	0.4	16	0.4	16
6	1.3	52	1.4	56
7	1.5	60	1.8	72
8	1.5	60	1.7	68

Table 4. *MPE CO_2 and leucine plasma isotopic enrichments (%) before and after chemotherapy*

	Breath samples		Serum samples	
Patient	$^{13}CO_2$ enrichment (MPE) (after [^{13}C] bicarbonate i.v.)	$^{13}CO_2$ enrichment (MPE) (after [^{13}C] leucine iv)	1-^{13}C KICA (MPE)	$^2H_3$ KICA (MPE)
Before chemotherapy				
1	0.0149	0.0138	3.59	2.03
2	0.0240	0.0126	3.86	2.35
3				
4	0.0260	0.0136	2.67	3.80
5	0.0161	0.0145	2.65	1.30
6	0.0221	0.0102	3.76	2.37
7	0.0154	0.0882	6.81	3.22
8	0.0326	0.0354	2.78	2.68
Median	0.0161	0.0138	3.59	2.37
p25	0.0149	0.0123	2.67	2.03
p75	0.0240	0.0354	3.86	3.22
After chemotherapy				
1	0.0140	0.0160	2.97	3.20
2	0.0109	0.0140	2.80	2.30
3	0.0157	0.0139	2.54	5.36
4	0.0351	0.0241	2.66	3.03
5	0.0238	0.0235	3.68	1.65
6	0.0224	0.0254	2.44	2.36
7	0.0168	0.0165	4.61	3.15
8	0.0371	0.0319	4.03	0.88
Median	0.0196	0.0200	2.89	2.70
p25	0.0144	0.0145	2.57	1.81
p75	0.0323	0.0251	3.94	3.19
p	0.31	0.50	0.31	0.5

Leu, leucine; p25, 25th percentile; p75, 75th percentile; *p*-value, Wilcoxon signed-rank test.

DISCUSSION

We studied leucine kinetics in children treated with chemotherapy as a means to evaluate the effect of chemotherapy-induced mucositis on intestinal amino acid absorption. Our data demonstrate that childhood cancer patients have at least a similar systemic availability of leucine just before receiving chemotherapy compared with healthy children (19). The systemic availability of leucine after chemotherapy during a period of mucositis did not change significantly, indicating that chemotherapy treatment does not affect amino acid transport in the intestine. On the other hand, in contrast to our hypothesis, it demonstrates that the intestinal mucosa does not use more amino acids during intestinal mucositis. These are unexpected results seeing that almost all patients showed a distinct increase in mucositis toxicity score. We would have expected that leucine availability from the intestinal lumen should be impaired. Surprisingly, too, we found almost all patients to be in negative leucine balance already before start of chemotherapy, representing catabolic metabolism. We would have expected that the children should be in an anabolic state at the start of a new cycle of chemotherapy. We conclude that in our study amino acid (leucine) absorption in children with cancer was not compromised during chemotherapy-induced mucositis.

However, our study may have had some limitations. First, it might have been underpowered to detect possible differences in the systemic availability of leucine: the study group was fairly small and the overall incidence of very severe mucositis (grade 3 or 4) was low. Second, the results on isotopic enrichment and consequent leucine kinetics showed great variability. This could be explained by the heterozygosis of

Table 5. *Leucine kinetics before and after chemotherapy*

	Before chemotherapy		After chemotherapy		
	Median	Percentile (25th–75th)	Median	Percentile (25th–75th)	*p*
Total leucine intake	42.8	(37.9–43.8)	44.9	(34.9–61.5)	0.46
Enteral leucine intake	31.5	(23.6–32.6)	33.7	(23.7–50.3)	0.17
Turnover iv	300.0	(278.0–407.0)	378.0	(272.5–436.8)	0.61
Turnover ig	451.0	(329.0–528.0)	460.5	(339.5–634.5)	0.61
First-pass uptake fraction (%)	40.0	(2.0–50)	10.0	(0.0–50)	0.46
Absolute first-pass uptake	8.6	(0.0–17.0)	4.6	(0.0–11.5)	0.60
Oxidation	88.6	(54.7–149.3)	124.0	(100.3–204.5)	0.31
NOLD	217.3	(150.7–281.2)	215.3	(170.9–275.9)	0.61
LRP	256.4	(214.2–366.3)	323.7	(230.5–401.9)	0.40
Balance	–47.9	(–109.5–3.1)	–92.0	(–150.9–54.7)	0.31

All values are expressed in μmol/(kg/h). *p*-Value, Wilcoxon signed-rank test; ig, intragastric; Oxidation, whole-body leucine oxidation; Balance, NOLD – LRP.

our study group. Patient characteristics showed a wide range in age, diagnosis, and treatment differences, which could cause variability in clinical condition. Third, we are well aware of the limitations of the described amino acid tracer studies. Intestinal absorption of only one essential amino acid was studied and not the entire cascade of digestion and absorption following a protein-containing meal. The transport of amino acids into the cytoplasm and through the basolateral membrane is facilitated by highly regulated transporter systems defined on the kinetic properties of the specific amino acid (20,21). Transport of amino acids such as leucine in the intestinal brush border is regulated by the L and $B^{0.8}$ system (21,22) and is predominantly Na^+-dependent (22). In contrast, dietary components need to be digested before absorption by specific tightly regulated metabolic enzymes expressed in the brush border at the apical membrane of villus enterocytes.

From previous animal studies we know that different kinds of chemotherapy affect intestinal digestion and absorption processes dissimilar. Conflicting reports on protein digestion and amino acid absorption have been published. The expression of peptide transporter 1 (PepT1), involved in absorption of dipeptides and tripeptides formed after digestion of dietary proteins (23), appeared unchanged during 5′-flourouracil induced intestinal mucositis (24), whereas several amino acid transporters (neutral basic transporter and high-affinity glutamate transporter) showed decreased expression (24). On the other hand, glutamine supplementation seems to ameliorate chemotherapy induced toxicity (25–27). Although conflicting results are published, this could indicate that glutamine transport is not affected by chemotherapy. *In vivo* data available for glutamine transporters are lacking. Other macronutrients such as carbohydrates might be less well absorbed. Expression of sucrase isomaltase and lactase, two glycohydrolases responsible for degradation of complex carbohydrates into absorbable monosaccharide, is strongly down-regulated during mucositis (5,28,29). Also, the monosaccharide transporters sodium glucose co-transporter 1 and glucose transporter 5, harbored at the apical enterocyte membrane and glucose transporter 2 at the basolateral membrane, are distinctly down-regulated during mucositis (5). Although not clinically tested, these findings suggest that carbohydrates might be less properly absorbed during mucositis. Only a few data are available on the third macronutrient in the diet, lipids. Transport of fatty acid in the enterocyte by fatty acid binding protein seems to be less affected during severe mucositis (5).

So, in contrast to leucine absorption, we don't know whether the digestion of whole proteins in a regular meal is disturbed during a period of mucositis. An intrinsically labeled protein diet could be used to investigate whether digestion is impaired in children with mucositis following chemotherapy. Our data on leucine absorption and findings from previous animal studies (24) suggest a role for an elementary diet consisting of small peptides and free amino acids during mucotoxic treatment. Considering that most of our patients already showed catabolic state before start of chemotherapy, there is a definite need for the development of such elementary feeding. The more so since a major positive effect of proper nutrition in critically ill patients was demonstrated in recent years (30–32).

In conclusion, we found that after mucotoxic chemotherapy in pediatric patients the intestinal mucosa is still capable of absorbing leucine efficiently. Additionally, most children receiving chemotherapy are already catabolic before start of a new cycle of chemotherapy. Therefore, all efforts should be directed at initiating enteral feeding even before start of chemotherapy to reduce catabolic state. Moreover, our data imply that this might be accomplished best by hydrolyzed formula.

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