Oral glutamine supplementation benefits jejunum but not ileum

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GLUTAMINE IS A NONESSENTIAL amino acid, sufficient quantities of which are synthesized by the body in healthy subjects. During surgical stress or critical illness, however, the body's requirement for glutamine frequently exceeds its production (1-4). As well, glutamine is a preferred energy substrate for small intestinal enterocytes (5-8). Therefore, under the stress of a systemic illness or intestinal injury, enterocytes may be unable to meet their requirements for glutamine as a primary metabolic fuel, and exogenous administration of glutamine may, therefore, be necessary.

While glutamine administration has been shown to improve jejunal morphometric measurements in several animal models of enterocolitis (9-13), the effects of glutamine on ileal morphology and functional absorption have not been well studied. Parenteral glutamine administration has been demonstrated to be safe (14) and beneficial (15) for humans, and it is possible that the enteral route may impart additional positive effects to the intestine. It is, therefore, important to know whether the beneficial jejunal effects of orally administered glutamine also occur in the ileum, and whether these beneficial effects occur during typical surgical anastomotic injury or are limited to occasions of experimentally induced enterocolitis or normal tissue.

Key Words: Absorption, Enteral nutrition, Glutamine, Intestine, Transport
Les suppléments oraux de glutamine : avantages pour le jéjunum mais non pour l'iléon

RÉSUMÉ : La glutamine est le principal carburant métabolique du petit intestin. La capacité de la glutamine entérique à supporter l'architecture jéjunale et son métabolisme est bien connue, mais son effet sur la fonction d'absorption intestinale, particulièrement au niveau de l'iléon terminal, reste à déterminer. Le but de cette étude était de mettre au point un modèle fonctionnel d'absorption liquidienne iléale avec lésion chirurgicale et de déterminer si un supplément de glutamine oral pouvait accélérer la cicatrisation et rétablir la fonction. Les effets d'une résection de 1 cm accompagnée d'anastomose termino-terminale iléale ou d'une laparotomie factice sur l'absorption liquidienne in vivo chez le rat ont été mesurés au début de l'étude (jour 0) puis aux jours 1 et 2. Chez les rats ayant subi une laparotomie factice, l'absorption liquidienne n'a pas été modifiée. Par contre, l'absorption liquidienne iléale a été nettement réduite au jour 0 (17,2 ± 4,8 µL/cm/h) et à 1 (31,4 ± 13,6 µL/cm/h), mais est revenue à la normale au jour 2 (71,0 ± 6,2 µL/cm/h) chez les rats anastomosés. Pour examiner les effets de la glutamine dans ce modèle, des rats ont reçu, soit de la glutamine (2,4 g/kg/jour) ou un régime oral élémentaire iso-azote enrichi de glycine durant cinq jours avant leur assignation aux groupes qui allaient subir la laparotomie ou l'anastomose. Cette dose de glutamine a atteint l'iléon et a été complètement absorbée le long du petit intestin. Les rats qui ont reçu de la glutamine n'ont montré aucune différence sur le plan de la récupération pour ce qui est de l'absorption liquidienne iléale in vivo, de la mesure morphométrique des villosités iléales, du ratio mg ADN:mg protéines, du degré d'inflammation ou de l'activité glutaminase. Par contre, la morphométrie des villosités jéjunaux, le ratio mg ADN:mg protéines et l'activité glutaminase se sont élevés chez les rats qui ont reçu de la glutamine (P<0,01), ce qui donne à penser que le jéjunum a répondu au régime enrichi de glutamine. Ces résultats démontrent que la résection iléale et l'anastomose provoquent une altération transitoire de l'absorption liquidienne in vivo et que le supplément oral de glutamine offre un avantage pour la muqueuse intestinale jéjunale mais non iléale. Ces résultats donnent à penser que les effets de la glutamine orale peuvent se limiter à l'intestin proximal.

The purpose of this study was to determine whether supplemental oral glutamine would restore ileal intestinal absorptive function and improve morphometric analysis in a surgical injury model (see part I - ileal end-to-end surgical anastomotic model). In addition, we determined whether oral glutamine supplements conferred benefits, as determined by morphometric analysis, on the jejunum and/or the ileum in a nonsurgical model (see part II - nonsurgical model).

ANIMALS AND METHODS

Animals: Male Sprague-Dawley rats weighing between 230 and 270 g (Biotron) were used in all experiments. The animals were allowed to become acclimatized to the animal facility for at least 48 h before entering the study. Animals were housed in individual cages, on a 12 h light/dark cycle, and allowed water ad libitum.

Chemicals: Glutamine, glycine, alanine, beta-NAD (from yeast, molecular weight 633.4), 2,5- and 3,5-ADP, and glutamic dehydrogenase (type II, from bovine liver in 50% glycerol) were purchased from Sigma Chemical Co (Missouri). L-14C-glutamine (9.5x10⁴ MBq/mmol) was purchased from Amersham. All other chemicals were reagent grade and were purchased from Fisher Scientific.

PART I - ILEAL END-TO-END SURGICAL ANASTOMOTIC MODEL

Feeding and dietary supplementation: Previously Chow-fed animals were randomized to receive either a glutamine-supplemented (treatment group receiving glutamine) or an isonitrogenous glycine-supplemented (untreated group receiving no glutamine) elemental diet. Both groups received the designated diet for five days before experimenta-

PART II - NONSURGICAL MODEL

Feeding and dietary supplementation: To ensure adequate intraluminal ileal concentrations of glutamine in this group, glutamine was administered by oral gavage, 1 g every 12 h (2 g/day, approximately 8 g/kg/day) for five days before experimentation.
Figure 1) Time course of in vivo fluid absorption at the ileal end-to-end anastomotic site. Results are compared with age-matched, sham-operated controls at zero (2 h after anastomosis was performed), and one, two and three days postanastomosis. Ileal anastomosis reduced in vivo fluid absorption on the day of anastomosis (day 0) and one day following anastomosis. Data are mean ± SEM (n=6). *P<0.05 relative to sham-operated controls.

Figure 2) In vivo fluid absorption at the ileal end-to-end anastomotic site one day following anastomosis. Results for glutamine (GLN) – treatment group receiving glutamine – and glycine (GLY) – untreated group receiving no glutamine – dietary supplementation are compared with anastomotic and sham-operated, age-matched controls. Ileal anastomosis lowers in vivo fluid absorption relative to sham-operated controls, and both groups are improved by glutamine dietary supplementation. Data are mean ± SEM (n=6). *P<0.05 relative to sham-operated controls.

Operative procedure: The nonsurgical model received dietary supplementation but did not receive anesthesia or surgical intervention before experimentation.
Table 1

<table>
<thead>
<tr>
<th>Tissue</th>
<th>1 h</th>
<th>3 h</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stomach</td>
<td>49.5±1.9</td>
<td>5.9±0.2</td>
</tr>
<tr>
<td>Intestine</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Segment 1</td>
<td>3.9±2.3</td>
<td>1.6±1.9</td>
</tr>
<tr>
<td>Segment 2</td>
<td>2.9±1.6</td>
<td>2.1±1.5</td>
</tr>
<tr>
<td>Segment 3</td>
<td>3.5±2.2</td>
<td>0.6±0.7</td>
</tr>
<tr>
<td>Segment 4</td>
<td>3.1±3.0</td>
<td>0.7±0.4</td>
</tr>
<tr>
<td>Segment 5</td>
<td>4.3±0.8</td>
<td>1.1±0.8</td>
</tr>
<tr>
<td>Segment 6</td>
<td>2.9±1.4</td>
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</tr>
<tr>
<td>Segment 7</td>
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<td>0.7±1.0</td>
</tr>
<tr>
<td>Segment 8</td>
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</tr>
<tr>
<td>Segment 9</td>
<td>2.0±1.9</td>
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</tr>
<tr>
<td>Segment 10</td>
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<td>1.0±0.9</td>
</tr>
<tr>
<td>Liver</td>
<td>11.0±1.1</td>
<td>4.8±1.6</td>
</tr>
<tr>
<td>Pancreas</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
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<td>0.3±0.3</td>
<td>0.3±0.5</td>
</tr>
<tr>
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</tr>
<tr>
<td>Heart</td>
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<td>0.1±0.4</td>
</tr>
<tr>
<td>Blood</td>
<td>0.4±0.4</td>
<td>0.5±0.6</td>
</tr>
</tbody>
</table>

*Proportion of administered radioactivity recovered from sum of tissues analyzed*
RESULTS: PART II - NONSURGICAL MODEL

Because in vivo fluid absorption, morphometric measurements and glutaminase activity did not benefit from oral glutamine supplementation in the surgical ileal end-to-end anastomotic model despite glutamine being delivered to the ileum, the authors investigated the possibility of a regional difference in the ability of the small intestine to respond to glutamine.

Ileal in vivo fluid absorption: Dietary supplementation with glutamine (treatment group receiving glutamine) or glycine (untreated group receiving no glutamine) did not alter basal jejunal (156±8 µL/cm/h) or ileal (95±7) fluid absorption in the nonsurgical model relative to levels seen in chow-fed animals (149±11 µL/cm/h and 102±12, respectively).

Morphometric assessment: Villus height significantly (P<0.05) increased in the jejunum of glutamine-supplemented animals (Figure 4). In contrast, the ileum demonstrated no increase in villus height with glutamine relative to glycine supplementation.

DNA:protein ratio: To confirm that the increased jejunal villus height was due to an increased number of enterocytes, DNA and protein levels in jejunal and ileal mucosal homogenates were measured. There was a significant (P<0.02) increase in the mg DNA:mg protein ratio in the jejunum of glutamine-supplemented animals. Once again, the ileum did not respond to glutamine supplementation (Figure 5).

Glutaminase activity: As shown in Figure 6, glutaminase activity increased in the jejunum of the glutamine-supplemented group. In contrast, glutaminase activity in the ileum was not enhanced by glutamine.

DISCUSSION

A number of studies have investigated the effects of parenterally and enterally administered glutamine on both normal and injured intestine. The majority of these studies confirmed the beneficial effects on the jejunum (1-8, 10-13,22-24), whereas the effects of glutamine on the ileum have been less well described (9,25). Our study thus examined the effects of oral glutamine supplementation on the ileum in both a surgical (ileal end-to-end anastomosis) and a nonsurgical model. Orally administered glutamine is absorbed in the proximal jejunum, where 85% is transformed before entry into the systemic circulation (26,27). Studies done on rats have shown that the rates of glutamine use by the jejunum are simi-
lar whether glutamine is derived from arterial blood or gut lumen (5). Nevertheless, the importance of an orally administered source of glutamine is suggested by the fact that rats deprived of it develop intestinal hypoplasia (28,29).

Intestinal absorptive function around the ileal end-to-end surgical anastomosis was impaired significantly on days 0 and 1 postanastomosis as assessed by in vivo fluid absorption. While it is not surprising that intestinal fluid and electrolyte absorptive function should be diminished at the site of intestinal anastomosis, we are unaware of this decrease having been previously described. While the mechanism leading to this fluid absorptive dysfunction has not been examined, it likely relates to local ischemia and subsequent free radical or cytokine release. We have previously shown that alteration in in vivo intestinal fluid absorption is a sensitive measure of mucosal injury. Indeed, intestinal transport abnormalities will persist for days after gross macroscopic and morphometric appearance have returned to normal (16).

Our experiment showed that oral glutamine supplementation did not accelerate healing of the fluid absorptive impairment around an ileal end-to-end anastomosis. Furthermore, in this surgical model, glutamine supplementation did not enhance ileal villus morphometric measurements or the level of ileal glutaminase activity; this raises the possibility that adequate amounts of glutamine may not have been presented to the ileum in this model. The total quantity of glutamine (0.96±0.2 g/rat/day) consumed was likely adequate as it was similar to the amount shown to be beneficial in other studies (3,9,13,30). Furthermore, our studies using $^{14}$C-labelled glutamine as a marker confirmed that glutamine was present in the ileal lumen (Table 1).

Although glutamine supplementation has been shown to enhance ileal morphometric measurements, this enhancement occurred in conjunction with a 60% small bowel resection (31) or in normal intestine from rats supplemented with parenteral glutamine (25). These results are unlikely to be a consequence of increased glutamine delivery to the ileum because our study demonstrated no evidence of enhanced ileal morphometric measurements even though equal amounts of glutamine were delivered to the jejunum and ileum following oral administration. It remains to be determined whether a beneficial effect of glutamine on the ileum requires an increased duration of glutamine contact with the intestine or the presence of specific binary or pancreatic products. Incontinuity jejunum in dogs has higher glutaminase activity and glutamine transport levels than those seen in an excluded jejunal limb in the same animals, a difference likely due to the presence of luminal constituents (32).

These observations, and our own negative ileal results, prompted us to study the effects of oral glutamine supplementation in a nonsurgical model to determine whether the ileum is capable of responding to oral glutamine. This part of the study examined the effects of glutamine, compared with glycine (control), in both ileum and jejunum. Our results were similar to those of Salloum et al (24), and demonstrated that glutamine supplementation enhanced jejunal morphometric measurements, DNA content and glutaminase activity. In contrast, glutamine had no positive effect on paired ileal measurements in the same animals. These negative results occurred despite radiolabelled demonstrations that intraluminal glutamine reached the ileum in amounts similar to that seen in jejunum (Table 1). Glutamine absorbed in the proximal small intestine is transformed in the jejunal enterocyte, and this early transformation may alter the distal small bowel response to oral glutamine. An alternate explanation is that the ileum is less sensitive than the jejunum to the effects of glutamine. In support of this hypothesis, Tamada et al (33) recently demonstrated that the parenteral administration of the dipeptide alanyl-glutamine enhanced villus height in the jejunum but not the ileum. The ileum may thus require jejunalization by an enteral nutrient stream to become as sensitive to glutamine as the jejunum.

**CONCLUSIONS**

The beneficial effects of oral glutamine supplementation may be limited to the proximal small intestine. Further studies will be necessary to clarify the mechanisms responsible for the apparent nonresponse of the ileum to oral glutamine.

**Figure 6** Glutaminase activity in jejunum and ileum of the nonsurgical model receiving oral glutamine (GLN) – treatment group receiving glutamine – or glycine (GLY) – untreated group receiving no glutamine – supplementation. Glutaminase activity is increased in the jejunum, but not ileum, of the glutamine-supplemented group. Data are mean±SEM (n=4). *P<0.05 relative to GLY.
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