Bolus Oral Glutamine Protects Rats against CPT-11-Induced Diarrhea and Differentially Activates Cytoprotective Mechanisms in Host Intestine but Not Tumor

Hongyu Xue, Michael B. Sawyer, Catherine J. Field, Levinus A. Dieleman, David Murray, and Vickie E. Baracos

Abstract

Dietary glutamine has been suggested to preserve structural and functional integrity of the gut and high dose bolus glutamine has been hypothesized to protect against potentially fatal endotoxic shock, hyperthermic stress, and side effects of chemotherapy. In this study, we aimed to relate the ability of high dose oral bolus glutamine to mitigate the severe diarrhea induced by 7-ethyl-10-[4-(1-piperidino)-1-piperidino]carbonyloxy-camptothecin (CPT-11) chemotherapy to specific cytoprotective mechanisms [heat shock response, glutathione (GSH)] in gut and tumor tissues. Female rats bearing Ward colon tumor received CPT-11 (125 mg·kg⁻¹·d⁻¹×3 d) with or without an oral glutamine bolus (0.75 g/kg) administered 30 min prior to each CPT-11 dose. Glutamine reduced incidence and severity of late-onset diarrhea following CPT-11 treatment (P < 0.05) and was associated with potentially beneficial and protective responses in the colon: 1) a 3.1- to 7.2-fold increase of heat shock protein (Hsp)25,-70, and -90; 2) increased reduced GSH (rGSH):oxidized GSH ratio (P < 0.05); 3) prevention of upregulated activity of a key bacterial enzyme (β-glucuronidase) in the cecal content that mediates CPT-11 intestinal toxicity (P < 0.05); and 4) increased proportions of CD3⁺CD8⁺ lymphocytes and memory CD8⁺ subset in mesenteric lymph nodes following CPT-11 therapy. By contrast, glutamine treatment did not alter CPT-11’s antitumor activity, the amino acid concentrations, Hsp expression, or the ratio of rGSH:oxidized GSH in the tumor. Our data demonstrate a striking dichotomy in the response of tumor and host to oral glutamine administration, concurring with the concept that this nutrient may favorably alter the balance between the host and tumor.

Introduction

7-Ethyl-10-[4-(1-piperidino)-1-piperidino]carbonyloxy-camptothecin (CPT-11) is used to treat colorectal and other cancers (1). CPT-11 is metabolized to SN-38, a metabolite responsible for both the dose-limiting toxicity (severe diarrhea) and antitumor activity (2). SN-38 is metabolized to a nontoxic glucuronide derivative (SN38G) prior to biliary excretion; however, β-glucuronidase produced by intestinal microflora regenerates SN38 and is thought to play a critical role in mediating the gut toxicity (2). Severe diarrhea is prevalent (3) and can be life threatening for ~8% of colorectal cancer patients treated with regimens containing CPT-11 and 5-fluorouracil (4).

Glutamine protects the gut during a variety of stress conditions, including trauma, sepsis, burns, and several cancer chemotherapies (5-fluorouracil, methotrexate, doxorubicin) (5–9). Several potential mechanisms for this protection are proposed. Glutamine preserves glutathione (GSH) in the gut during oxidative stress during methotrexate chemotherapy and endotoxin challenge (10,11). Glutamine may also preserve gut barrier integrity and gut-associated and systemic immune competence (12). Glutamine (0.3–2 g·kg⁻¹·d⁻¹) is usually provided continuously in the diet in parenteral nutrition or mixed with enteral feed (7–9,11). Wischmeyer et al. (13,14) recently explored administration of glutamine as a single i.v. or oral bolus and a dose of 0.75 g/kg was optimally protective from lethal endotoxin shock or hyperthermia in rats (13,14). Wischmeyer et al. (13,14) suggest that additional protective mechanisms not seen with continuous feeding occur after bolus doses of glutamine, particularly the heat shock response, an inherent cellular cytoprotective response. Our recent work (15) provides the first direct evidence, to our knowledge, that high-dose oral bolus glutamine limits the development of severe diarrhea in rats treated with CPT-11.
There has been concern expressed that provision of glutamine may promote tumor growth, based on the prevalent notion that tumors have a propensity to use glutamine as a source of energy and of nitrogen for biosynthesis (16,17). The utility of nutritional treatments to reduce chemotherapy toxicity would depend upon a differential effect on the host and tumor, such that host tissues would be protected from injury and there would be no protection, or even sensitization, of tumor tissues to anti-neoplastic treatments. Although such differential effects on host and tumor would be ideal, it could equally be predicted that nutritional support might invoke cellular protective mechanisms in the tumor and may cause resistance to therapy. Based on these considerations, we designed a study to examine the effects of an oral bolus glutamine in tumor-bearing rats treated with CPT-11. The objective of our work was to determine the effect of this treatment on specific cytoprotective effectors, including heat shock protein (Hsp) and GSH, in the host and tumor.

Materials and Methods

Rats and treatments
Animal use was approved by the Institutional Animal Care Committee and conducted in accordance with the Guidelines of the Canadian Council on Animal Care. Female Fisher 344 rats (body weight, 150–180 g), 11–12 wk old, were obtained from Charles River. Rats were housed 2 per cage in a temperature- (22°C) and light-controlled (12 h light) room and consume water and food ad libitum. One week before chemotherapy, rats were separated into individual housing in wire-bottom cages. The Ward colorectal carcinoma, which was originally chemically induced by azoxymethane in its host strain (Fisher rat) and is transplantable within that rat strain, was provided by Dr. Y. Rustum, Roswell Park Institute (18). Tumor pieces (0.05 g) were transplanted subcutaneously on the flank via trocar under slight isoflurane anesthesia. Tumor volume was estimated (cm³) = 0.5 × length (cm) × width (cm) × height (cm), as described (15). CPT-11 was provided by Pfizer as a ready-to-use clinical formulation. Atropine (0.6 g/L) was a clinically injectable formulation.

Diet

The diets used in this study are described in detail elsewhere (15). Briefly, semipurified diet was based on AIN-76 basal diet, with a modified fat component similar to a North American dietary pattern (40% of energy, polyunsaturated-saturated fat ratio of 0.35). Rats were initially fed Rodent Laboratory Chow (Harlan Teklad). During the adaptation period, this nonpurified diet was mixed with study diet (50:50 wt:wt) for 1 wk, followed by transition to a 100% semipurified diet for 2 wk before tumor implantation.

Experimental design

When tumor volume reached ~2 cm³, 3 consecutive daily CPT-11 i.v. injections at 125 mg/kg⁻¹ d⁻¹, the maximal tolerated dose (15), were initiated. Atropine (1 mg/kg subcutaneously) was administered immediately before each CPT-11 injection to alleviate early-onset cholinergic symptoms (15).

Glutamine was administered by oral gavage (0.75 g·kg⁻¹·d⁻¹) 30 min before each daily CPT-11 injection. The sham treatment group was gavaged with isosmotic sterile water. Glutamine (Sigma) was made as a 3% (w/v) solution immediately before use and filtered with a 0.45-μm filter. The first day of CPT-11 administration was designated d 0. Sham- and glutamine-treated rats (n = 6 per treatment) were killed on d 3, 6 h after the last injection of CPT-11 (~6.5 h after last glutamine gavage). This time point was selected to capture early responses to glutamine treatment, especially Hsp expression. Additional sham- and glutamine-treated rats (n = 12 per treatment) were followed until d 9 for tumor response to CPT-11 treatment and to evaluate diarrhea. β-Glucuronidase activity was assayed in cecal content collected from glutamine- and sham-treated rats killed at these 2 time points (d 3 and d 9) and additionally before chemotherapy (d 0) (n = 6 per treatment).

Outcome measures

Diarrhea. A clinically comparable 3-point scale was adopted in grading the diarrhea (19); these assessments were conducted by a researcher unaware of the study treatments. Incidence of grade 3 diarrhea was calculated for each animal by counting observations of a particular score(s) out of the total 8 observations between d 3 and d 7 when diarrhea developed to its full severity (19). Area under curve of diarrhea score was calculated between d 3 and d 7 (15).

Sample collection and assays. Rats were killed by CO₂ asphyxiation. Immediately after rats became unconscious, whole blood was collected via cardiac puncture into heparinized tubes. Cecal digesta content was collected under aseptic conditions. The full length of the colon was washed and longitudinally cut into halves, 1 of which was mounted on a wax strip and fixed in 10% (v/v) neutral buffered formalin and the mucosa was gently scraped off the other half and immediately frozen in liquid nitrogen. Tumor was excised intact, blotted, and weighed before the nonneocrotic portions were collected and frozen in liquid nitrogen.

Apoptosis in situ via terminal transferase-mediated dUTP nick end labeling. Apoptotic cells in colonic mucosa were identified with the DeadEnd Colorimetric terminal transferase-mediated dUTP nick end labeling (TUNEL) System (Promega) according to the manufacturer’s instruction. In each section, 20–25 areas of colonic mucosa, each of which contained at least 10 crypts, were randomly selected to count TUNEL-positive cells under 400× magnification. The analysis was performed by a researcher who was unaware of the dietary treatment. Hsp were detected by Western blot (13) using antibodies to Hsp70 (1:4000), Hsp90a (1:4000), Hsp25 (1:12,000), and corresponding horseradish peroxidase-conjugated secondary antibodies (Stressgen). Hsp were detected by enhanced chemiluminescence (Amersham Pharmacia Biotech) and analyzed by autoradiography using a Typhoon 8600 Variable Mode Imager (Amersham Biosciences). The densitometry determination was performed using ImageQuant 3.2 software (Molecular Dynamics). Data shown are relative densitometric ratios against the value for heat shock cognate70 (1:5000, Stressgen), the cognate constitutively expressed form of Hsp70 used as the protein loading control.

Reduced GSH (rGSH) and GSH disulfide (GSSG) were determined with Oxford rGSH/GSSG kit (product no. GT-30), which features 1-methyl-2-vinyl-pyridinium trifluoromethane sulfonate as a scavenger of rGSH. Free amino acid concentrations were determined by HPLC after conversion to their p-hydroxyphenylalanine (20). Cysteine and proline are not detected by this method. We determined β-glucuronidase activity by a modified method of Freeman as described (15). The β-glucuronidase activity is expressed as U/g protein. One unit is defined as 1.0 μg of phenolphthalein liberated from phenolphthalein glucuro- nide per hour.

Mesenteric lymph node cell phenotype. Immune cells were isolated from the mesenteric lymph nodes (MLN) (21) and cells (200,000 cells per well) were phenotyped using 2 color direct immunofluorescence (22), using antibodies to CD3, CD4, CD8, CD25, CD45RA (BD Bioscience), and OX12 (Cedarlane). Streptavidin QR (Sigma) was added to all biotin-labeled antibodies. The percentage of cells expressing each of the antibody markers was determined by flow cytometry (FacScan, Becton Dickinson) (22). We attempted to perform every phenotype assay on each rat, but this was not always possible due to limited lymph node size; the actual number of rats utilized for each assay is indicated in tables.

Statistical analysis

Data are expressed as means ± SEM. The effect of glutamine on tumor growth following CPT-11 treatment was tested using 2-way ANOVA followed by Bonferroni post hoc tests (GraphPad Prism, GraphPad). Treatment differences in apoptotic index and immune phenotype were analyzed using 1-way ANOVA followed by post hoc Tukey’s test (SPSS 12.0). For the other measures, the effects of glutamine were analyzed using the unpaired 2-tailed Student’s t test for independent samples. P < 0.05 was considered significant.

Glutamine and CPT-11 chemotherapy
Results
Bolus glutamine decreased diarrhea without altering anti-tumor efficacy of CPT-11. CPT-11 therapy resulted in a clinically comparable diarrhea profile and mortality of <5% (15). Two sham-treated rats and no glutamine-treated rats died. A reduction of tumor volume compared with baseline initially followed CPT-11 treatment, with the maximal reduction at d 5 of −44.5 ± 4.4% (sham) and −45.5 ± 1.5% (glutamine). Tumors regrew thereafter to 91.5 ± 8.1% (sham) and 83.2 ± 3.8% (glutamine) of tumor size at the start of treatment by d 9 (data not shown). Bolus glutamine treatment decreased the incidence of severe (grade 3) diarrhea (sham-treated, 53.8 ± 4.2% vs. glutamine, 34.1 ± 4.7%; P < 0.005) and area under curve of diarrhea score (sham-treated, 18.8 ± 0.5 vs. glutamine, 16.5 ± 1.0; P < 0.05). CPT-11 caused reduced feed intake and a rebound-like recovery thereafter; these changes did not differ between groups. For the sham- and glutamine-treated groups, respectively, the daily relative feed intake dropped to its nadir on d 4 by 66 ± 9% and 69 ± 4% (P = 0.8) and the daily relative feed intake increased to its top value on d 8 by 110 ± 14% and 100 ± 12% (P = 0.7).

Bolus glutamine administration differentially induces cytoprotective mechanisms in host tissues following CPT-11 treatment. Amino acids were determined in samples taken −6.5 h after the last glutamine bolus. This time point was selected for detection of the Hsp response and would likely be past the peak glutamine concentrations; however, at this time, concentrations of glutamine, glutamate, and several other amino acids were raised in either plasma or colonic mucosa tissue (P < 0.05) (Table 1). Ornithine and citrulline were not detectable in colon or tumor, and in plasma their concentrations (ornithine, 35.8 ± 7.4 μmol/L; citrulline, 16.5 ± 0.8 μmol/L) were not affected by glutamine. Despite increased plasma concentrations, none of the amino acid concentrations in tumor tissue were altered by glutamine treatment.

In CPT-11-treated rats, bolus glutamine increased the accumulation of the stress-inducible Hsps, namely Hsp70, 25, and 90α, in the colonic mucosa by 3.1-, 7.2-, and 3.8-fold, respectively, compared with sham treatment (Fig. 1). However, the abundance of these inducible Hsp in tumor tissue was not affected by glutamine treatment (Hsp25 relative density: 0.67 ± 0.20 for sham vs. 0.72 ± 0.16 for glutamine; Hsp90α relative density: 1.10 ± 0.08 for sham vs. 1.02 ± 0.12 for glutamine). Tumors did not contain any detectable Hsp70.

GSH concentration was analyzed in colonic mucosa and tumor at 6 h after the 3rd CPT-11 treatment. Glutamine significantly enhanced the rGSH:GSSG ratio in colonic mucosa compared with sham-treated rats, mainly through an increasing trend in rGSH (Table 2). By contrast, glutamine treatment significantly decreased the reduced and total GSH concentrations in the tumor, resulting in a trend of increased tumor rGSH:GSSG ratio.

Previous studies (23) suggested that apoptosis in colon peaked ~6 h after CPT-11 treatments, so we also selected this time point. CPT-11 increased apoptotic index in both sham- (20.9 ± 2.1/mm2; P < 0.0005) and glutamine-treated (16.7 ± 2.8/mm2; P < 0.005) rats compared with healthy reference rats (2.3 ± 0.4/mm2) (Fig. 2). The sham- and glutamine-treated rats did not differ (P = 0.2).

We analyzed the activity of β-glucuronidase in the cecal digesta before chemotherapy, 6 h after the last dose of CPT-11, when the diarrhea started, and 7 d after the last dose (d 9) when late diarrhea resolved spontaneously. In sham-treated rats, β-glucuronidase activity increased by 3.8-fold 6 h after completion

### TABLE 1
Concentrations of free amino acids in rat plasma, colonic mucosa, and tumor tissue after CPT-11 chemotherapy with or without glutamine treatment1,2

<table>
<thead>
<tr>
<th>Amino acid</th>
<th>Plasma</th>
<th>Colon</th>
<th>Tumor</th>
</tr>
</thead>
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<tr>
<td></td>
<td>Sham</td>
<td>Glutamine</td>
<td>Sham</td>
</tr>
<tr>
<td>Aspartate</td>
<td>8.9 ± 1.1</td>
<td>11.5 ± 1.6</td>
<td>0.428 ± 0.054</td>
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<tr>
<td>Glutamate</td>
<td>55.0 ± 10.3</td>
<td>63.3 ± 3.0</td>
<td>1.62 ± 0.19</td>
</tr>
<tr>
<td>Asparagine</td>
<td>36.9 ± 2.7</td>
<td>40.9 ± 4.2</td>
<td>0.136 ± 0.019</td>
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<tr>
<td>Serine</td>
<td>164 ± 5</td>
<td>166 ± 9</td>
<td>0.759 ± 0.149</td>
</tr>
<tr>
<td>Glutamine</td>
<td>560 ± 10</td>
<td>666 ± 40*</td>
<td>2.59 ± 0.50</td>
</tr>
<tr>
<td>Histidine</td>
<td>68.6 ± 5.9</td>
<td>83.7 ± 5.1</td>
<td>ND2</td>
</tr>
<tr>
<td>Glycine</td>
<td>148 ± 3</td>
<td>168 ± 9</td>
<td>0.491 ± 0.111</td>
</tr>
<tr>
<td>Threonine</td>
<td>204 ± 14</td>
<td>196 ± 6</td>
<td>0.506 ± 0.053</td>
</tr>
<tr>
<td>Arginine</td>
<td>65.4 ± 7.8</td>
<td>92.1 ± 4.1*</td>
<td>0.075 ± 0.014</td>
</tr>
<tr>
<td>Tyrosine</td>
<td>49.2 ± 2.7</td>
<td>69.1 ± 18*</td>
<td>0.628 ± 0.008</td>
</tr>
<tr>
<td>Tryptophan</td>
<td>86 ± 5</td>
<td>104 ± 2*</td>
<td>0.076 ± 0.012</td>
</tr>
<tr>
<td>Methionine</td>
<td>37.5 ± 1.7</td>
<td>45.7 ± 4.3</td>
<td>0.080 ± 0.010</td>
</tr>
<tr>
<td>Valine</td>
<td>207 ± 10</td>
<td>261 ± 15*</td>
<td>0.296 ± 0.041</td>
</tr>
<tr>
<td>Phenylalanine</td>
<td>74.2 ± 3.8</td>
<td>92.2 ± 18*</td>
<td>0.099 ± 0.016</td>
</tr>
<tr>
<td>Isoleucine</td>
<td>92 ± 6</td>
<td>106 ± 6</td>
<td>0.196 ± 0.032</td>
</tr>
<tr>
<td>Leucine</td>
<td>157 ± 10</td>
<td>194 ± 12*</td>
<td>0.229 ± 0.026</td>
</tr>
<tr>
<td>Lysine</td>
<td>372 ± 23</td>
<td>387 ± 29</td>
<td>0.298 ± 0.058</td>
</tr>
<tr>
<td>Alanine</td>
<td>ND3</td>
<td>–</td>
<td>–</td>
</tr>
</tbody>
</table>

1 Data are means ± SEM; n = 5 (sham) or 6 (glutamine). *Different from Sham, P < 0.05.
2 Tumor-bearing rats received a bolus glutamine or isovolemic sham treatment 30 min before each CPT-11 injection and concentrations of free amino acids in plasma, colon, and tumor tissues were analyzed 6 h after the last CPT-11 injection.
3 ND, Not detectable.
4 For plasma and colonic tissue, alanine was not separated from taurine at the same run as the other amino acids.
of CPT-11 treatment compared with baseline (P < 0.002) and returned to baseline by d 9. Oral bolus glutamine treatment counteracted the immediate rise of cecal β-glucuronidase 6 h after the treatment, with the concentration in glutamine-treated rats (0.14 ± 0.07 U/g protein) less than in sham-treated rats (0.64 ± 0.12 U/g protein) (P < 0.01).

Phenotype of mesenteric lymph node cells was analyzed 7 d after the completion of CPT-11 chemotherapy (Table 3). There were numerous effects of CPT-11 treatment compared with rats that had not received chemotherapy. CPT-11 decreased the proportion of CD3+T cells (primarily CD3+CD4+ cells, as suggested by the reduced CD3+CD4+:CD3+CD8+ ratio) and, accordingly, an increased proportion of B cells (OX12+) in MLN, irrespective of glutamine treatment. Although only a small proportion of cells expressed the IL-2 receptor, the proportion in CPT-11-treated rats was ~30% lower due to proportionately less CD8+CD25+ cells (P < 0.05). CPT-11 also decreased the proportions of cytotoxic T cells (CD3+CD8+) cells and antigen mature CD8+ cells (CD45RA−) as after CPT-11 compared with healthy controls (P < 0.05). Glutamine normalized the relative abundance of CD3+CD8+ cells and memory/antigen mature CD8+ cells (CD45RA−) to levels in healthy controls and did not affect the CD3+CD4+CD8+ ratio or the proportion of CD8+CD25+ cells.

### TABLE 2

<table>
<thead>
<tr>
<th></th>
<th>Total GSH</th>
<th>GSSG</th>
<th>rGSH</th>
<th>GSH:GSSG ratio</th>
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<tr>
<td><strong>Colonic mucosa</strong></td>
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<td></td>
</tr>
<tr>
<td>CPT-11+sham</td>
<td>1.04 ± 0.16</td>
<td>0.027 ± 0.003</td>
<td>0.99 ± 0.16</td>
<td>41.9 ± 11.8</td>
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<tr>
<td>CPT-11+glutamine</td>
<td>1.45 ± 0.18</td>
<td>0.021 ± 0.004</td>
<td>1.41 ± 0.17</td>
<td>19.7 ± 9.0*</td>
</tr>
<tr>
<td><strong>Tumor</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CPT-11+sham</td>
<td>1.39 ± 0.12</td>
<td>0.024 ± 0.004</td>
<td>1.34 ± 0.12</td>
<td>66.3 ± 14.9</td>
</tr>
<tr>
<td>CPT-11+glutamine</td>
<td>1.02 ± 0.10*</td>
<td>0.025 ± 0.003</td>
<td>0.97 ± 0.10*</td>
<td>40.6 ± 5.9</td>
</tr>
</tbody>
</table>

1 Data are means ± SEM; n = 5 (CPT-11+sham) or 6 (CPT-11+glutamine). *Different from CPT-11+sham, P < 0.05.

### Discussion

A dichotomy in tumor and host's response to glutamine treatment. We observed a striking dichotomy in the response of a tumor and of the tumor-bearing host to oral glutamine administration. In addition to symptomatic relief of severe diarrhea, several potentially beneficial protective mechanisms were invoked in host tissues: 1) increased proportion of rGSH; 2) heat shock response; 3) suppression of colonic β-glucuronidase activity; and 4) increased proportions of cytotoxic T (CD3+CD8+) cells in MLN, especially memory CD8+ T cells. By contrast, cytoprotective pathways were not induced in Ward colon tumor and tumor response to CPT-11 was unaffected by glutamine treatment. These data concur with the concept that a nutrient may alter the balance between the host and the tumor in a manner that favors the host overall.

The potential for purified nutrients at high doses to “feed the tumor” or to confer tumor protection against cytotoxic therapy remains a prevalent notion. Tumors are said to be glutamine traps, and glutaminolysis has been proposed as a major energy-producing process in tumor cells (16,17). In tissue culture, tumor cells rely on glutamine for cell growth as an essential energy source and biosynthetic precursor (24,25). In animal models, however, the size, protein synthetic rate, and DNA content of a tumor and of the tumor-bearing host to oral glutamine supplementation. Of CPT-11 chemotherapy with or without glutamine treatment. Tumor-bearing rats received a bolus glutamine or sham treatment 30 min before each CPT-11 injection and accumulation of stress-inducible Hsp (Hsp70, 25, and 90α) in colonic mucosa was examined by Western blot 6 h following the last CPT-11 injection. Each lane represents the sample from an individual animal. Relative densitometry is expressed as means ± SEM. Samples left of the central black line are from the rats receiving CPT-11+sham treatment (n = 5); samples on the right are from rats receiving CPT-11+glutamine (n = 6). Asterisks indicate different from CPT-11+sham, *P < 0.05, **P < 0.001.

**FIGURE 1** Hsp induction in rat colonic mucosa after CPT-11 chemotherapy with or without glutamine treatment. Tumor-bearing rats received a bolus glutamine or sham treatment 30 min before each CPT-11 injection and accumulation of stress-inducible Hsp (Hsp70, 25, and 90α) in colonic mucosa was examined by Western blot 6 h following the last CPT-11 injection. Each lane represents the sample from an individual animal. Relative densitometry is expressed as means ± SEM. Samples left of the central black line are from the rats receiving CPT-11+sham treatment (n = 5); samples on the right are from rats receiving CPT-11+glutamine (n = 6). Asterisks indicate different from CPT-11+sham, *P < 0.05, **P < 0.001.
A multiplicity of glutamine actions in the tumor-bearing host. Glutamine bolus significantly improved CPT-11-induced diarrhea and we are currently pursuing a clinical investigation of this potential benefit of glutamine therapy. Because transient anorexia after CPT-11 chemotherapy was identical in sham- and glutamine-treated rats, feed intake-related effects on the examined endpoints could be excluded.

We know of no other published data on the effects of tumor and concurrent CPT-11 chemotherapy on plasma and tissue amino acids and our results suggest notably low levels of arginine and citrulline compared with published results for normal rats. There are suggestions in the literature that hypocitrullinemia could be a potential quantitative marker for intestinal damage caused by intense chemotherapeutic agents (33,34). Diminished plasma citrulline could limit arginine synthesis through the intestinal-renal axis, resulting in decreased arginine concentration (35,36).

Glutamine administered orally is subject to extensive first pass metabolism in the small intestine and liver (37) and its actions may be due as much to secondary metabolites as to glutamine per se. The wide range of changes in plasma and colon amino acid concentrations (Table 1) reflects the sophisticated metabolic pathways of glutamine in vivo. Some products derived from glutamine metabolism, such as arginine, are important modulators of gut physiology and immunity (38). Consistent with previous findings, our glutamine bolus increased arginine concentration systemically and in the colon (39). T-cell depletion has been considered as the primary contributor to immunodeficiency related to high-dose chemotherapy (40) and both glutamine and arginine promote lymphocyte proliferation and activation (11). Our oral glutamine treatment attenuated the relative depletion of cytotoxic T cell, especially memory CD8+ cells in MLN associated with CPT-11 treatment. This may add to the competence of gut-associated immunity against invasion of pathogens present in the gut lumen.

CPT-11 is associated with oxidative stress in normal tissues with diminished GSH store (41). GSH synthesis may play a pivotal role in protecting from CPT-11-related toxicity (42). Glutamine (via glutamate) is a precursor for GSH synthesis and has been suggested to be limiting for GSH synthesis during stress (43). Glutamine treatment raised the ratio of rGSH:oxidized GSH in the colon tissue but at the same time lowered the reduced and total GSH stores in tumor. Previous work examining tumor-bearing rats treated with methotrexate suggests that glutamine decreased GSH content in tumor but increased it generally in host organs including heart, kidney, and intestine (10,44). These authors conjectured that the acidic tumor environment blocks GSH regeneration and ultimately depletes the GSH store in the tumor (10).

Glutamine increased Hsp25, 70, and 90α in colon mucosa, the 3 key inducible Hsp playing a central role in protecting cells by preserving the structure of normal proteins and removing damaged ones. This response varied somewhat between individual animals; however, this variability remains largely unexplained. Wischmeyer et al. (13) had also observed greater variability in the Hsp response of colon and ileum compared with lung and heart. The action of glutamine to induce Hsp may be direct, because there is evidence that Gln induces Hsp in cells in culture (45,46). Induction of Hsp preserves the functional integrity of epithelia, i.e. electrolyte absorption/secretion, intestinal myoelectric activity, and mucosal barrier (47–49) and reduces production of pro-inflammatory mediators (48,50), which may in turn be involved in triggering diarrhea (19).

The protective ability of glutamine treatment may or may not have derived from alteration of apoptosis in colonic epithelium and our results on this point were equivocal. The improvement of diarrhea outcomes independent of gut apoptosis has been

### TABLE 3

<table>
<thead>
<tr>
<th>Subpopulations</th>
<th>Healthy control</th>
<th>CPT-11+sham</th>
<th>CPT-11+glutamine</th>
</tr>
</thead>
<tbody>
<tr>
<td>B cell + (OX12)</td>
<td>19.1 ± 0.6 (4)</td>
<td>32.5 ± 2.9 (6)</td>
<td>31.2 ± 2.5 (7)</td>
</tr>
<tr>
<td>CD3+</td>
<td>73.0 ± 2.1 (5)</td>
<td>57.8 ± 2.1 (7)</td>
<td>59.6 ± 1.4 (7)</td>
</tr>
<tr>
<td>CD3+ + CD8+</td>
<td>22.2 ± 1.2 (5)</td>
<td>18.0 ± 0.4 (6)</td>
<td>21.1 ± 1.1 (7)</td>
</tr>
<tr>
<td>CD25 + CD8+</td>
<td>1.8 ± 0.1 (5)</td>
<td>0.9 ± 0.1 (7)</td>
<td>0.9 ± 0.1 (7)</td>
</tr>
<tr>
<td>CD8+ + CD45RA−</td>
<td>25.2 ± 1.3 (5)</td>
<td>20.3 ± 0.6 (6)</td>
<td>23.4 ± 0.4 (5)</td>
</tr>
<tr>
<td>CD3 + CD4+</td>
<td>48.7 ± 0.7 (5)</td>
<td>38.0 ± 2.6 (7)</td>
<td>36.2 ± 1.3 (4)</td>
</tr>
<tr>
<td>CD25 + CD4+</td>
<td>2.7 ± 0.4 (4)</td>
<td>2.3 ± 0.3 (7)</td>
<td>2.0 ± 0.3 (7)</td>
</tr>
<tr>
<td>CD4 + CD45RA−</td>
<td>43.5 ± 3.4 (5)</td>
<td>38.9 ± 4.3 (5)</td>
<td>40.2 ± 2.0 (7)</td>
</tr>
<tr>
<td>CD4 + CD68RA−</td>
<td>18.1 ± 1.1 (5)</td>
<td>3.1 ± 1.0 (6)</td>
<td>5.2 ± 0.9 (5)</td>
</tr>
<tr>
<td>CD3CD4 + CD23CD8</td>
<td>2.2 ± 0.6 (5)</td>
<td>1.9 ± 0.2 (5)</td>
<td>1.8 ± 0.2 (4)</td>
</tr>
<tr>
<td>CD25+</td>
<td>3.5 ± 0.2 (5)</td>
<td>2.4 ± 0.2 (6)</td>
<td>2.4 ± 0.2 (7)</td>
</tr>
</tbody>
</table>

1 Values are means ± SEM (n); means in a row with superscripts without a common letter differ, P < 0.05.

2 Tumor-bearing rats received an oral bolus glutamine or sham treatment 30 min before each CPT-11 injection and GSH concentration was analyzed in the colonic mucosa and tumor tissue 6 h after the last CPT-11 injection.
observed by other authors using keratinocyte growth factors (51,52). Glutamine could exert its benefit on CPT-11-induced diarrhea largely through other mechanisms that may more predominantly correct intestinal absorptive/secretory function.

Local deconjugation of SN-38 glucuronide catalyzed by β-glucuronidase produced by colonic microflora intensifies the epithelial exposure to SN-38 (53). We provide direct evidence that administration of CPT-11 affects colonic β-glucuronidase; a marked increase of the enzyme was detected 6 h after CPT-11 treatment. CPT-11 can increase levels of several β-glucuronidase-producing flora (Escherichia, Clostridium, and Staphylococcus species) within 1–6 h following treatment (54). The post-CPT-11 increase of the enzyme activity was abolished with glutamine treatment in a manner that might alleviate the exposure of intestinal epithelium to SN-38.

Glutamine induced Hsp and favorably altered GSH stores and β-glucuronidase activity in colon. The early induction of these effects suggests that they have the potential to contribute to the subsequent mitigation of severe diarrhea, which was most evident on d 4–7. It may be that 1 of these effects is primary; however, it is also possible that their simultaneous occurrence may be important. As suggested by Wischmeyer et al. (13,14), bolus glutamine may represent a novel therapeutic paradigm for preventing chemotherapy-related injury via boosting the inherent stress response of tissues vulnerable to the drug. A future objective of research in this context will be to further clarify the dose, schedule of administration, and additional dietary elements to optimize the protective response.

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