CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER:

21-667

CLINICAL PHARMACOLOGY AND BIOPHARMACEUTICS REVIEW(S)

CLINICAL PHARMACOLOGY AND BIOPHARMACEUTICS REVIEW

NDA: 21-667	
Submission Date(s)	8/8/2003; 10/14/2003
Brand Name	Nutrestore• •
Generic Name	L-Glutamine
Reviewer	Sue-Chih Lee, Ph.D.
Team Leader	Suresh Doddapaneni, Ph.D.
OCPB Division	Division of Pharmaceutical Evaluation II
OND division	Division of Gastrointestinal & Coagulation Drug Products
Sponsor	Nutritional Restart Pharmaceutical, L.P.
Relevant IND(s)	54,284; 48,750
Submission Type; Code	NME; 1S
Formulation; Strength(s)	Powder; 5-gram packet
Proposed Dosing Regimen	30 g/day (5 grams, 6 times daily) for 16 weeks to 3 years
Proposed Indication	for patients with short bowel syndrome

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1 EXECUTIVE SUMMARY

1.1 Recommendations

From the Clinical Pharmacology and Biopharmaceutics standpoint, the application is acceptable provided that a mutually satisfactory agreement can be reached between the sponsor and Agency regarding the language in package insert.

1.2 Phase IV Commitments

None

1.3 Summary of Clinical Pharmacology and Biopharmaceutics Findings

In this submission, the sponsor is seeking approval of Nutrestore• •(L-glutamine for oral use) as a cotherapy with recombinant human growth hormone (rhGH) · in patients with short bowel syndrome (SBS). Due to the low occurrence rate of SBS, drugs for this indication are qualified as orphan drugs. rhGH used alone (Zorbtive•) was approved by the Agency in 2003 for the same indication.

This NDA is a 505(b)(2) application. The sponsor relies upon data from published literature to fulfill nonclinical and part of clinical requirements. For clinical pharmacology and biopharmaceutics information, the sponsor provided 20 peer-reviewed articles. A literature search by this reviewer turned up similar articles. Some information cited in this review is based on other reference books.

As an endogenous compound, L-glutamine (hereafter called glutamine) is predominantly synthesized in skeletal muscle from glutamate. It is considered a conditionally essential amino acid since dietary supplementation is thought to be necessary during severe illness. Currently, it is available on the market as a dietary supplement or as a component of medical foods. For the indication of interest here, glutamine will be a prescription item.

To support the safety and efficacy of the cotherapy, the sponsor provided data from one clinical trial that had three treatment arms (glutamine alone, rhGH alone, and rhGH+glutamine). This clinical study was also used to support the approval of rhGH alone. The package insert for Zorbtive• •includes a table that summarizes the efficacy results of all three arms. Under the "INDICATION AND USAGE" section of the label, it is stated that "Nutritional supplements may be added according to the discretion of the treating physician." It is conceivable that some patients being treated with Zorbtive• •are also receiving glutamine as a nutritional supplement. Although the Zorbtive• •label does not specify the dosing regimen for glutamine, the description of the clinical trial indicates that glutamine was given at 30 g/day.

Following oral administration, glutamine absorption involves active transport process. In a study in 6 healthy male subjects, mean peak blood glutamine concentration following a single oral

dose of 0.1 g/kg was 1028±97 • M (or 150±14 • g/mL), occurring approximately 30 minutes after dosing. The bioavailability of glutamine for the proposed dosing regimen cannot be accurately assessed based on the literature information. From various literature sources, however, it is believed that absorption in healthy subjects is efficient but first-pass effect is high, resulting in an oral bioavailability of <50%. The first-pass effect occurs in both the intestine and liver.

Because active transport is involved in the uptake of glutamine, nonlinear kinetics is a possibility. There is some information to indicate that Cmax is nonlinear at three times of the therapeutic dose. However, there is no information on the linearity for Cmax at other lower doses or AUC at any doses.

Glutamine that enters the systemic circulation is distributed to various tissues. From an IV bolus study in three healthy subjects, the volume of distribution was estimated to be 210±20 mL/kg (or 14.7±1.4 L/70 kg) with a distribution half-life of 12±2 min. These numbers were given in one article with no plots or any other means for verification.

Although glutamine is eliminated by glomerular filtration, it is almost completely reabsorbed by the renal tubules. Glutamine participates in various metabolic activities, including the formation of glutamate, and synthesis of proteins, nucleotides and amino sugars. Based on the IV bolus study cited above (N=3), the terminal half-life of glutamine was estimated to be 67±11 min. In an IV infusion study in 9 healthy subjects, steady state was reached within 1-2 hours.

Glutamine powder is to be dissolved in water before ingestion and, therefore, there are no bioequivalence issues and there is no need for a dissolution test. Food effect was not characterized. The proposed label indicates that glutamine is to be taken with meals or snacks which is consistent with the conduct of the clinical trial used to support this NDA.

There is no information on the effect of intrinsic/extrinsic factors on the pharmacokinetics of glutamine.

Pharmacokinetics of glutamine in patients with SBS following oral administration has not been characterized. These patients usually have more efficient bowel per unit length through adaptation following resection. However, the very fact of having a short bowel decreases their overall efficiency in absorption. Following cotherapy of rhGH and glutamine, absorption will improve, however, the plasma glutamine concentrations in these patients are generally expected to be lower than those observed in healthy subjects. The plasma concentrations in these patients can be highly variable due to differences in length, segment, and presence/absence of ileal-cecal valve for the remnant bowel. It is noteworthy that the efficacy of oral glutamine may be derived partly from the local effect.

According to Drs. Hugo Gallo-Torres and Gary Della' Zanna, Medical Team Leader and Medical Officer of HFD-180, there are no special safety concerns for the cotherapy compared to rhGH alone.

It is apparent that the clinical pharmacology and biopharmaceutics information as submitted in the application is scant. Because of the above reasons, however, we will not request for more information.

2 QUESTION BASED REVIEW

2.1 General Attributes

2.1.1 What regulatory background or history information contributes to the assessment of the clinical pharmacology and biopharmaceutics of this drug?

Current NDA:

- Orphan drug designation:
  - Incidence rate of SBS: ~ 2 to 4 per million, according to the review of NDA 21-597 for Zorbtive• •by Dr. Hugo Gallo-Torres, Medical Team Leader of HFD-180
- Subpart E drug:
  - SBS is a life-threatening and severely-debilitating illness (for risk/benefit consideration).
- 505(b)(2) submission:
  - Reference literature data: non-clinical and some clinical (PK and some safety and efficacy) information.
  - Note: Clinical data submitted include one double-blind and two open-label studies.

rhGH:

rhGH used alone was approved in 2003 for the same indication (NDA 21-597; Zorbtive•).

- Data from only one double-blind efficacy and safety trial with 41 patients were submitted to support NDA 21-597. This trial included three arms: rhGH alone, glutamine alone, and coadministration of rhGH and glutamine. (Note: The same clinical study was submitted to support the current NDA.)
- The approved label for rhGH includes a table that summarizes the efficacy data of all three arms. Under the "INDICATION AND USAGE" section, it states that nutritional supplements may be added according to the discretion of the treating physician but it does not specifically mention glutamine and/or the dosage regimen for glutamine
- The approved dosing regimen for rhGH is "approximately 0.1 mg/kg subcutaneously daily to a maximum of 8 mg daily."

Glutamine:

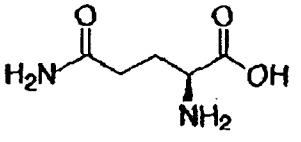
- Currently, glutamine is available on the market as a dietary supplement and as a component of medical foods (e.g. Vivonex Plus: 10 g glutamine/L).
- The typical dietary intake of L-glutamine as derived from animal and plant protein in a normal diet may range 5-10 grams/day.

- As a medical food, typical doses of L-glutamine for those with cancer, AIDS, trauma, burns, infections may range 4-21 grams/day. As an ergogenic aid, supplemental L-glutamine may be taken 1.5-4.5 g/day (Reference: "PDR for Nutritional Supplements").

2.1.2 What are the highlights of the chemistry and physico-chemical properties of the drug substance, and the formulations of the drug product?

Drug Substance:

Structure:



(L-Glutamine)

Empirical formula:	$C_5H_{10}N_2O_3$
Molecular weight:	146.15
Chemical name:	(S)-2-aminoglutaramic acid, L-glutamic acid 5-amide, (S)-2,5-diamino-5-oxopentanoic acid, or L-glutamine
PKa:	
Solubility:	

Formulation: L-Glutamine powder, 5-gram packets (Dissolve in 8 oz of water before use.)

2.1.3 What is the proposed mechanism of action, therapeutic indication and dosage recommendations for oral glutamine?

Mechanism of Action:

- Glutamine reportedly has important functions in regulation of gastrointestinal cell growth, function, and regeneration. It is not regarded as an essential amino acid because it can be readily synthesized by various tissues in healthy subjects. However, the role of and nutritional requirement for glutamine may differ significantly for certain illnesses (e.g., catabolic illness, trauma, and infection) with manifestations of reduced glutamine concentrations[18] and increased tissue glutamine metabolism.

- Nonclinical studies indicated that villous height, bowel growth, plasma insulin-like growth factor I, and body weight were significantly higher when glutamine was administered in combination with rhGH to rats compared to those in animals given either glutamine or rhGH alone.

- It is thought that cotherapy of glutamine with rhGH promotes mucosal regeneration and improves absorption of nutrients from the remnant bowel in patients with short bowel syndrome.

Proposed Indication:

- As a cotherapy with rhGH in patients with short bowel syndrome. The sponsor considers that cotherapy of rhGH and oral glutamine is significantly better in efficacy than either product alone.

Proposed Dosage Recommendation:

- 5 grams, 6 times daily (30 g/day) as a cotherapy with rhGH.
- Dissolve the powder in 8 oz of water immediately before use.
- Consume with meals or snacks.

2.2 General Clinical Pharmacology

2.2.1 What are the design features of the pivotal clinical trials?

Only one pivotal trial was conducted to support this NDA.

- It is a randomized, double-blind efficacy and safety trial.
- Forty-one SBS patients participated and completed the study. According to Dr. Hugo Gallo-Torres, Medical Team Leader of HFD-180, this sample size is large enough for SBS trials.
- The trial included three arms: rhGH alone, glutamine alone, and coadministration of rhGH and glutamine.
- All patients were on a specialized oral diet (high protein; high dietary fiber: 55-60% and low-fat: 20-25%).

2.2.2 What are the response endpoints and how are they measured in clinical pharmacology and clinical studies?

- Primary efficacy endpoint: change in weekly total volume of intravenous parenteral nutrition (IPN) from Week 2 to Week 6. Treatment started at the beginning of Week 3. Total IPN volume is defined as the sum of the volumes of intravenous parenteral nutrition, supplemental lipid emulsion (SLE), and intravenous hydration fluid.
- Secondary efficacy endpoints: (a) change in total IPN calories; (b) change in IPN or lipid frequency.

2.2.3 Are the active moieties in the plasma appropriately identified and measured to assess pharmacokinetic parameters and exposure response relationship?

The sponsor referenced PK studies published in peer-reviewed journals. The authors did not indicate the assay validation results in these articles.

2.2.4 What are the characteristics of the exposure response relationships for safety or efficacy?

The exposure-response relationship for safety or efficacy has not been established. Only one dose of glutamine (5 grams, six times daily) was used in the clinical trial. Plasma concentrations in these patients were not determined.

2.2.5 From clinical pharmacology and biopharmaceutics standpoint, what are the special considerations for this drug?

It is plausible that the efficacy is derived from local effects more than systemic effects following oral administration of glutamine. Therefore, the plasma glutamine concentration may not be a good measure of efficacy.

2.2.6 What are the basic pharmacokinetic characteristics of glutamine in healthy young subjects?

The primary PK information was obtained from the studies described below which is followed by the information on the pharmacokinetic characteristics of glutamine.

Study A[1]: Single oral dose
Six healthy male subjects (age: 33 ± 5 yrs, wt: 74 ± 7 kg) received under fasting condition glutamine solution orally at 3 dose levels (0, 0.1 and 0.3 g/kg; mean dose: 0, 7.4, and 22.2 g) in a randomized cross-over fashion with at least a 1-week washout between periods. Serial blood samples and urine samples were collected up to 4 h postdose.

Study B: Single IV bolus
In a study in 3 healthy subjects (2 males & 1 female; age: 33±1 yr; wt: 70±5 kg), IV injections (over 2 min) of glutamine solution at 0.05 g/kg was administered under fasting conditions and blood samples were collected up to 2 h postdose for assay of glutamine and glutamate.

Study C: IV infusion
In a study in 9 healthy subjects (5 males & 4 females; age: 28±3 yrs; wt: 68±4 kg), each subject received under fasting condition IV infusion of glutamine over 4 hrs at 3 dose levels (0, 0.0125, and 0.025 g/kg/hr; mean dose: 0, 3.5, and 7.0 g) in a randomized cross-over fashion with at least a 2-week washout between periods. Serial blood samples and urine samples were collected up to 4 hrs postdose.

Study D[7]: Multiple Oral Doses
Three healthy volunteers (2 males & 1 female; mean age: 40 yr; mean wt: 64.6 kg) participated in the study. Subjects first adapted to a standard diet (2.5 kcal/d; protein: 16.3%; carbohydrate: 60%; fat: 23.7%). Subsequently, all subjects received supplemental oral glutamine at 0.3 g/kg/day (as a slurry in water) with meals for 10 days. Blood samples were collected every 4 hours for 24 hours while the subjects were on the standard diet alone and again when they were receiving supplemental glutamine. Assay was performed using a method involving glutaminase and glutamate dehydrogenase.

2.2.6.1 What are the single dose and multiple dose PK parameters in healthy young volunteers?

The blood glutamine concentration-time profiles for the 3 dose levels in Study A are presented in Figure 1 and the mean parameter values in Table 1. Table 1 also lists parameter values estimated from Study B (single IV bolus) and Study D (multiple dose study).

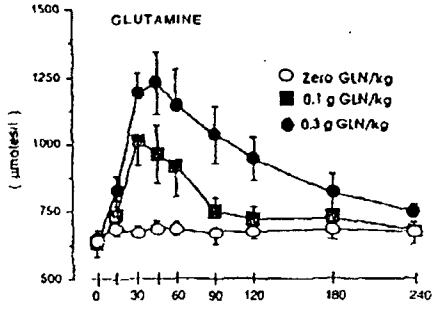


Figure 1. Blood glutamine concentration-time profile following single oral dose of glutamine at 3 dose levels (placebo, 0.1 g/kg and 0.3 g/kg) (Study A)

Table 1. Mean (±SD) Glutamine PK Parameters in healthy subjects

Single Dose						
Dose	**N**	**Cmax* (• M)**	**Tmax* (h)**	**AUC_{0-4h}** (• M x 10^{-3})**	**Vd (mL/kg)**	**Elimination T1/2 (min)**
Placebo P.O.	6	721 ± 25	-	~~4.4±5.2~~	-	-
0.1 g/kg P.O.	6	1028 ± 97	0.5	~~36.3±6.8~~	~~512±63~~	106±11
0.3 g/kg P.O.	6	1328 ± 99	0.75	~~72.6±12.7~~	~~1254±84~~	117±17
0.05 g/kg IV bolus	3	-	-	-	210±20	67±11

Multiple Dose			
Dose	**N**	**Glutamine*** (• M)**	**Glutamate*** (• M)**
Placebo P.O.	3	521 ± 44.8	72 ± 16
0.3 g/kg/day P.O.	3	629 ± 49.6	157 ± 44

*Under fasting conditions.

**The AUC values given in the article do not agree with Figure 1.

***Mean of 3 subjects. Each subject had plasma concentration measurements at 6 time points.

Reviewer's Comments:

- Regarding Study A, the AUC values provided in the article appear to be in error as they are inconsistent with Figure 1. Mean baseline blood glutamine concentration was approximately 680 • M as estimated by visual inspection of Figure 1. Based on the variability in AUC for

the placebo, it appears that the variability in baseline glutamine concentrations would be greater than what is reflected from Figure 1.

- Regarding the multiple dose study, the dosage regimen for glutamine is unclear. It was indicated that glutamine was given after each meal at 0.3 g/kg/day but how the daily dose was divided was unclear. (Subjects had 3 meals and 3 snacks a day.) This study also had a small sample size (N=3).

2.2.6.2 How does the PK of the drug in healthy volunteers compare to that in patients?

There are no PK data in the target patient population. Patients with short bowel syndrome have reduced capability in absorbing nutrients, including glutamine. It should be noted that, compared to healthy subjects, these patients generally have more efficient bowels per unit length following bowel resection. Following treatment with rhGH and glutamine, absorption in these patients will improve but the overall absorption is expected to be less than that observed in healthy subjects. Plasma glutamine concentrations in these patients are expected to be variable depending on the length, segment and presence/absence of ileal-cecal valve in the remnant bowel.

2.2.6.3 What are the general pharmacokinetic characteristics of glutamine following oral administration in healthy young subjects?

Absorption:
Based on data obtained in Study A, peak blood glutamine concentrations occurred 0.5-0.75 hr after oral administration of glutamine solution at the dose levels of 0.1 g/kg and 0.3 g/kg under fasting conditions[1]. There is no information on dose proportionality in the vicinity of the therapeutic dose. Using data from the oral and IV studies, the mean bioavailability of glutamine solution was estimated to be approximately 30-40%. (In one published article[6], enterally delivered glutamine was found to have a bioavailability of 47% when administered at 0.125 g/kg/h. Other authors reported a bioavailability of 26-47% for enterally delivered glutamine[3-5].)

Distribution:
Authors for Study B indicated that the volume of distribution based on this single IV bolus study was estimated to be 210±20 mL/kg (equivalent to 14.7±1.4 L/70 kg).

2.2.6.4 Is renal or hepatic pathway the major route of elimination?

Elimination
Metabolism is the major route of elimination for glutamine. Although glutamine is eliminated by glomerular filtration, it is almost completely reabsorbed by the renal tubules.

The blood glutamine concentrations following a 4-hr IV infusion (Study C) is presented in Figure 2. Steady state was reached within 1-2 hrs. Urinary excretion of glutamine was <0.3% of the administered dose, indicating that renal excretion is a minor route of glutamine elimination.

Based on Figure 2, this reviewer made a crude estimate of mean clearance of glutamine from the whole blood as 0.7-0.9 L/kg/hr. In a separate study with IV bolus administration (Study B), blood glutamine concentrations declined in a biphasic manner with the distribution half-life estimated to be 12±2 min and terminal elimination half-life estimated to be 67±11 min.

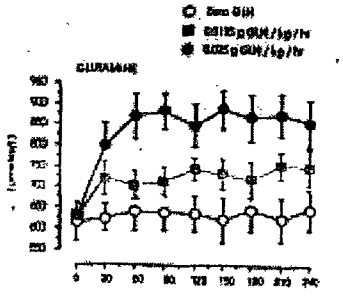
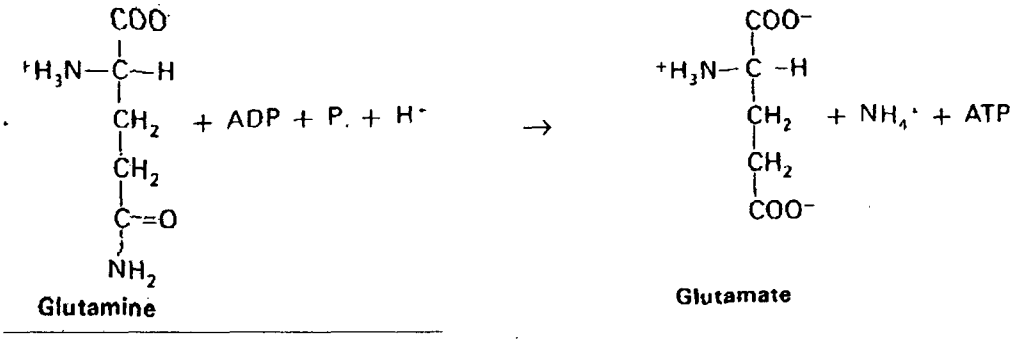


Figure 2. Blood glutamine concentration-time profile following a 4-hr IV infusion of glutamine at 3 dose levels (placebo, 0.0125, and 0.025 g/kg/hr)

2.2.6.5 What are the characteristics of drug metabolism?

Metabolism[a]:
Endogenous glutamine is predominantly synthesized in the skeletal muscle from glutamate, which is catalyzed by glutamine synthetase. The reversed reaction (hydrolysis of glutamine to form glutamate and ammonia) is catalyzed by glutaminase as shown below.



Glutamine

Glutamate

(Note: When ammonia is limiting, glutamine can serve as a hydrogen donor along with α-ketoglutarate to produce glutamate with glutamate synthase as the catalyst.)

[a]Reference: "Biochemistry" 2nd ed., edited by Lubert Stryer, W.H. Freeman and Company, New York (1981)

Metabolite concentrations:
Glutamate and ammonia are two metabolites monitored in Study A because high concentrations of these metabolites can cause toxicities.

Glutamate:
Based on Figure 3, mean blood glutamate concentrations at the dose level of 0.1 g/kg were similar to those observed for placebo (i.e., baseline values) while the concentrations were higher at 0.3 g/kg. However, the authors indicated that mean glutamate AUC increased with dose (see Table 2).

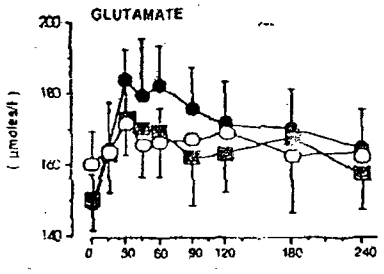


Figure 3. Mean blood glutamate concentration-time profile following single oral dose of glutamine at 3 dose levels (placebo, 0.1 g/kg and 0.3 g/kg)

Ammonia:
There is no difference in mean Cmax values for ammonia at various dose levels following oral administration based on Study A (Table 2).

Table 2. Mean (±SD) PK Parameters for g lutamate and ammonia following oral administration of glutamine

Single Dose (g/kg)	Cmax (• M)	AUC_{0-4h} (• M x 10^{-2})	Cmax (• g/mL)
	Glutamate		Ammonia
0	181±12	1.3 ± 1.3	3.3 ± 0.4
0.1	182±10	4.0 ± 1.5 (?)	3.2 ± 0.2
0.3	195±10	5.5 ± 1.6	3.4 ± 0.3

2.2.6.6 Based on PK parameters, what is the degree of linearity or nonlinearity in the dose concentration relationship?

- The available data suggest glutamine Cmax is less than dose proportional between glutamine doses of 0.1 g/kg and 0.3 g/kg (or between 5 g/50 kg and 15 g/50 kg).
- However, it is unclear whether glutamine AUC is linear.
- Note that the sponsor's proposed dose is 5 grams, 6 times daily.

2.2.6.7 How do the PK parameters change with time following chronic dosing?

Absorption of nutrients including glutamine is expected to improve in patients with SBS following cotherapy of rhGH and glutamine. There are no data to assess whether other PK parameters would change upon chronic dosing.

In one study[13], 23 patients were randomly allocated to receive for 8-10 days either glutamine dipeptide-enriched TPN (GTPN; glutamine: 0.21 g/kg/day) or standard (no glutamine) TPN

(STPN). These patients, nutritionally depleted but with no renal or hepatic insufficiency, were admitted for GI surgery and scheduled to receive TPN preoperatively. Before and after 8-10 days of TPN, patients were administered a 6-h IV infusion of L-[5-^{15}N]glutamine and L-[1-^{13}C]leucine. Blood samples were drawn for up to 6 hours after the start of the radiolabeled dose. It was found that GTPN increased the total appearance rate of plasma glutamine (GTPN group: 281±16 → 361±34 • mol/kg/h for N=; STPN group: 250±8 → 267±14 • mol/kg/h).

2.3 Intrinsic & Extrinsic Factors

- There are no studies to assess the effect of intrinsic or extrinsic factors on glutamine pharmacokinetics.
- No dosage adjustment is proposed by the sponsor for any subpopulation or particular situations.
- Renal or hepatic impairment: Glutamine is eliminated primarily through metabolism which can occur in various tissues (e.g., gut, kidney and immune system) besides liver. How various degrees of hepatic impairment impact the glutamine pharmacokinetics, especially in patients with SBS, is unclear. Although glomerular filtration does not contribute significantly to the elimination of glutamine, it is not known whether renal impairment can have an impact on the elimination of glutamine through other mechanisms.
- Without knowledge of safety implications in renal or hepatic impairment patients, the sponsor's proposed label states that caution should be exercised when glutamine is administered to patients with renal or hepatic impairment and close monitoring of these patients' kidney or liver functions is advisable .
- DDI: Metabolism of glutamine is mediated via non-CYP enzymes. Therefore, glutamine pharmacokintics are unlikely to be affected by other agents through CYP enzyme inhibition or induction.

2.3.1 Does the label specify co-administration of another drug and, if so, has the interaction potential between these drugs been evaluated?

The drug will be coadministererd with rhGH. There are no studies to assess PK interactions between the two drugs. According to Drs. Hugo Gallo-Torres and Gary Della 'Zanna, Medical Team Leader and Medical Officer of HFD-180, the clinical trial showed better efficacy for the combination without any additional safety concern when compared to rhGH alone.

2.4 General Biopharmaceutics

The drug has high solubility (5 grams [redacted]). Literature tends to describe absorption into enterocytes as efficient. However, permeability may be dose related because active transport process is involved. Glutamine powder is dissolved in water before ingestion and, therefore, there are no bioequivalence issues and there is no need for a dissolution test. Food effect was not characterized. The proposed label indicates that glutamine is to be taken with meals or snacks which is consistent with the conduct of the clinical trial that demonstrated the efficacy of the trial.

2.5 REFERENCES

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11 page(s) of revised draft labeling has been redacted from this portion of the review.

4 APPENDIX

4.1 Reference Article #1

Safety and Metabolic Effects of L-Glutamine Administration in Humans

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ABSTRACT. A series of dose-response studies was conducted to evaluate the clinical safety, pharmacokinetics, and metabolic effects of L-glutamine administered to humans. Initial studies in normal individuals evaluated the short-term response to oral loads of glutamine at doses of 0, 0.1, and 0.3 g/kg. A dose-related increase in blood glutamine occurred after oral loading and elevation of amino acids known to be end products of glutamine metabolism occurred (including alanine, citrulline, and arginine). No evidence of clinical toxicity or generation of toxic metabolites (ammonia and glutamine) was observed. Glutamine was infused intravenously in normal subjects over 4 hr at doses of 0.0125 and 0.035 g/kg/hr. In addition, glutamine was evaluated as a component of parenteral nutrition solutions (0.285 and 0.570 g/kg/day) administered for 5 days to normal subjects. Intravenous administration of glutamine was well tolerated without untoward clinical or biochemical effects. Subsequent studies in patients receiving glutamine-enriched parenteral nutrition for several weeks confirmed the clinical safety of this approach in a catabolic patient population. In addition, nitrogen retention appeared to be enhanced when glutamine was administered at a dose of 0.570 g/kg/day in a balanced nutritional solution providing adequate calories (145% of basal) and protein (1.5 g/kg/day). Nitrogen balance in patients receiving lower doses of glutamine (0.285 g/kg/day) was similar to that in patients receiving standard formulations. Further controlled clinical trials of the metabolic efficacy, tolerance, and dose response of glutamine in other patient groups are necessary to determine the appropriate use of glutamine enrichment of nutrient solutions. (*Journal of Parenteral and Enteral Nutrition* 14:137S–146S, 1990)

Glutamine (Gln) has long been classified as a nonessential amino acid. During catabolic illness, muscle and plasma Gln concentrations fall markedly and a large portion of circulating Gln has been shown in animals to be extracted by gut-associated tissues.[1-3] Recent investigations in animals have demonstrated that Gln-enriched enteral or parenteral nutrition improves growth and repair of the small bowel and colonic mucosa[4,5] and attenuates the pancreatic atrophy,[6] hepatic steatosis,[7] and loss of intestinal immune function[8] associated with standard nutrient solutions. These effects appear to contribute to improved survival following high-dose chemotherapy or radiation therapy in animals receiving Gln-supplemented diets.[9,10]

These studies suggest that Gln may serve as a conditionally essential nutrient during severe illness. If the Gln requirement in critically ill patients is not satisfied by dietary provision, a deficiency state could develop. Such a condition may be characterized by reduced concentration of Gln in intracellular and plasma pools and altered structure and function of tissues that utilize Gln. Under these conditions, net body protein catabolism (particularly of skeletal muscle) would increase in order to provide increasing quantities of Gln. If this hypothesis is correct, dietary supplementation of Gln should reverse these sequelae.

Before one can appropriately incorporate Gln as a component of nutrient solutions, the safety and clinical efficacy of this amino acid must be determined. The purpose of these studies was to evaluate the short- and long-term metabolic effects and clinical safety of Gln in humans. Studies in normal volunteers were performed first, followed by the administration of Gln-containing parenteral nutrition for up to 4 weeks in a catabolic patient population.

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MATERIALS AND METHODS

All research protocols were approved by the Committee for the Protection of Human Subjects from Research Risks, Brigham and Women's Hospital, Boston, MA. Studies in normal volunteers were performed in the Clinical Research Center and patients studies were performed in the Bone Marrow Transplant Unit of Brigham and Women's Hospital. Informed consent was obtained from all subjects prior to study.

Oral Gln Administration

Six healthy male subjects (age 33 ± 5 years, range 18–50; weight 74 ± 7 kg, range 60–105; mean ± SEM) were studied. Each subject was evaluated prior to entry and found to be in good health, as judged by a complete medical history, physical examination, body weight, blood chemistry profile, complete blood count, and urinalysis. The subjects were not taking medications and had no family history of endocrinopathy or other metabolic disease.

To evaluate dose-related responses, each subject was studied on three occasions in random order, during which one of three Gln doses was administered orally. The

individual study periods were separated by at least 1 week, during which subjects continued their normal oral diet; the subjects were unaware of which dose of Gln they received. After an overnight fast, the subjects were admitted to the Clinical Research Center at 0800 hr. After the subject had voided, an intravenous catheter was inserted in a dorsal hand vein and used for intermittent blood sampling. Patency was maintained by filling the catheter with heparin. The hand was placed in a heating pad to "arterialize" the venous blood.[11]

The subjects rested undisturbed for at least 30 min, and then basal blood samples were drawn (time 0). Immediately following this initial blood sample, the subject drank the test solution over 1 min. The solution contained either 150 ml distilled water (0 Gln dose), 150 ml distilled water containing 0.1 g of Gln/kg body weight (BW) (low Gln dose), or 150 ml distilled water containing 0.3 g/kg BW (high Gln dose). The powdered L-Gln (Ajinomoto USA, Inc, Raleigh, NC) was dissolved in water immediately prior to Gln administration. The average dose of Gln administered was 0, 7.4, and 22.2 g in the three study periods, respectively.

Additional blood samples were obtained at 15, 30, 45, 60, 90, 120, 180, and 240 min after Gln administration, and urine was also collected throughout the 4-hr study period. The subjects were observed for clinical evidence of toxicity and asked to report any subjective mental or physical symptoms. The subjects took nothing by mouth during the study period.

Blood was analyzed for Gln, glutamate (Glu), and other amino acids (AA) at all time points. Whole blood was immediately deproteinized with ice-cold 10% perchloric acid (w/v) and centrifuged at 3000 rpm for 20 min at 4°C. An aliquot was adjusted to pH 4.75–4.90 and stored at −20°C for subsequent batch analysis. Gln and Glu were measured by an enzymatic method[12] or by automated reverse-phase high-performance liquid chromatography after O-phthaldialdehyde precolumn derivatization.[13] Previous studies have determined that both methodologies yield similar results. Other AA were measured on a Beckman model 6300 Amino Acid Analyzer (Beckman Instruments, Fullerton, CA). Samples were also analyzed for whole blood ammonia, plasma glucose and glucagon, and serum growth hormone and insulin by standard techniques.[11] Areas under the concentration-time curves (as blood concentration × hr) were calculated by standard techniques for AA and hormones, and these values were utilized to express integrated response over time. Urinary concentrations of creatinine, ammonia, urea, and total nitrogen in the 4-hr urine sample were determined by standard methods, and excretion of these substances was calculated.

Short-term Gln infusion

Nine healthy volunteers were studied (five male, four female; age 25 ± 3 years, range 18–41; weight 62 ± 4 kg, range 55–83; mean ± SEM). The subjects were found to be healthy prior to study. No medications were taken by the subjects, although two females were taking oral contraceptive agents. Each subject was studied on three occasions in random order (determined by the research pharmacist), during which one of three Gln doses was administered as a constant intravenous infusion over 4 hr. Individual study periods were separated by at least 2 weeks and the subjects were blind to the Gln dose administered.

The study solutions were manufactured in the hospital pharmacy within 24 hr of administration. The Gln-containing solutions were prepared by mixing Gln powder in 500 ml of 0.2% saline solution at a concentration of 6% (2 g Gln/100 ml 0.2% saline). The control solution consisted of 0.2% saline solution alone. The test solutions were cold sterilized by use of a 0.22-μ filter (Millipore Inc, Bedford, MA) and stored at 4°C.

After an overnight fast, subjects were admitted to the Research Center at 0800 hr. A dorsal hand vein catheter for blood sampling was inserted and the hand was placed in a heating pad. An automated pulse and blood pressure monitor was placed on the upper arm (Dinamap Critikon, Inc, Tampa, FL). An infusion catheter was inserted percutaneously in the contralateral antecubital vein, its patency maintained by infusion of 0.2% saline solution at 25 ml/hr through a calibrated infusion pump (I-med Inc, San Diego, CA).

Following a 30-min equilibration period, the patient voided and a 4-hr urine collection was initiated. Basal (time 0) blood samples were obtained for determination of AA, ammonia, glucose, insulin, glucagon, and growth hormone. The test solution was infused through a Y connector by use of a second pump. The administration rate of the test solution was calculated to deliver either 0 (control), 0.0125 g Gln/kg/hr, or 0.025 g Gln/kg/hr during individual studies. The administration rate of the 0.2% saline was simultaneously adjusted so that the total fluid administered was 125 ml/hr during the 4-hr infusion period. The average dose of Gln infused in these subjects was 0, 3.5, and 7.0 g/4 hr, respectively.

Blood samples from the dorsal vein catheter were obtained at 30, 60, 90, 120, 150, 180, 210, and 240 min. Urine collected at the end of the study was analyzed for ammonia, urea, total nitrogen, Gln, and Glu. Subjects were observed for clinical and subjective evidence of toxicity, and vital signs and temperature were recorded at times 0, 60, 180, and 240 min. The subject took nothing by mouth during the study period. At the end of the infusion period, the residual volume of the test solution was measured to confirm actual volume delivered.

Long-term Gln infusion

Studies were also performed to evaluate the safety of Gln added to total parenteral nutrition (TPN).[14] Briefly, seven normal volunteers (age 33 ± 2 years, weight 77 ± 4 kg, range 63–95 kg) received three isonitrogenous, isocaloric solutions by intravenous infusion for 5 consecutive days in studies separated by at least 2 weeks. Each subject maintained a normal oral diet during the interval between studies.

The intravenous diet provided maintenance calorie requirements[15] and 1.5 g protein/kg/day. Nonprotein calories were supplied as lipid emulsion (82%) and dextrose (18%). Three AA solutions were manufactured in the research pharmacy by adding various amounts of

Gln, alanine, and glycine (Ajinomoto USA, Inc) to a constant amount of a commercially available base AA solution (Reanamine, Baxter Healthcare Corp, McGaw Park, IL). These solutions were sterilized by membrane filtration and stored for up to 8 days at 4°C. The solutions provided either 0 Gln, 0.285 g Gln/kg/day, or 0.570 g Gln/kg/day. The subjects were allowed only distilled water by mouth during the infusion period. Subjects were monitored daily for clinical or subjective side effects, and received mental status examinations and continuous performance testing six times/5 days to evaluate perceptual status and motor skills.[17] Daily nitrogen balance studies were performed and blood was drawn three times/week for determination of plasma and whole blood AA and ammonia, serum hormones, standard clinical chemistries, and complete blood counts.

Gln-Supplemented Intravenous Diets in Patients

To study initial safety and metabolic effects of Gln-enriched TPN administered in a patient population, a dose-escalation trial was performed in patients undergoing bone marrow transplantation (BMT). This patient population was chosen because they usually require intravenous feeding for 3–4 weeks to maintain nutritional status because of gastrointestinal toxicity following combined total body irradiation and chemotherapy.

Eight consecutive cancer patients treated according to an identical allogeneic BMT protocol were studied (Table 1). All patients were within their ideal body weight range prior to entry into the BMT protocol. They had normal mental status, hepatic function (total bilirubin <3 mg/dl), renal function (creatinine <1.2 mg/dl), and glucose tolerance (blood glucose <200 mg/dl); had no history of diabetes; and otherwise were without acute illness.

The patients received a 6-day conditioning period that included total body irradiation and high-dose cyclophosphamide and cytosine arabinoside prior to BMT from an HLA-matched relative.

On day 0, the allogeneic marrow was administered to the patient. The intravenous nutrient solutions were started on the next day. A TPN solution designed to provide 40% of the calculated energy requirements was administered on day 1; the volume of TPN was advanced the next day to provide full maintenance energy, protein, and micronutrient requirements.[18] Energy needs were calculated as 1.45 times basal requirements; these were provided as dextrose [70% of nonprotein energy] and fat emulsion (20% Intralipid, KabiVitrum, Inc, Alameda, CA, 30% of nonprotein energy). Protein intake was calculated to provide 1.5 g/kg/day.

The first two patients received a standard AA solution that does not contain Gln (Novamine, Baxter Healthcare Corp); the subsequent two patients received an AA solution providing 0.285 g Gln/kg/day (low dose), and the next two patients received a solution providing 0.570 g Gln/kg/day (high dose). After these initial six studies, two additional patients received the low Gln dose TPN solution. AA solutions were prepared by the research pharmacist by adding various amounts of Gln, alanine, and glycine to Reanamine (Baxter Healthcare Corp) to formulate the low-dose or high-dose Gln-containing AA solutions. Thus, all AA solutions contained adequate amounts of essential AA for stressed patients[18]; however, the two Gln-containing solutions contained slightly less essential AA than did the control solution (33% vs 43%) and, thus, proportionately more nonessential AA, including Gln. All nutrient solutions were isocaloric and isonitrogenous (see Table 2).

The Gln-containing solutions were sterilized by membrane filtration and stored for up to 8 days at 4°C prior to daily mixing of the AA with the prescribed amounts of dextrose, fat emulsion, electrolytes, vitamins, and minerals, as described.[18] The electrolyte and mineral composition of the TPN solution was adjusted to maintain normal serum concentrations. Protocol criteria were established to facilitate responses to potential complications, including alteration in mental status, organ failure, metabolic abnormalities (eg, hyperglycemia and hypertriglyceridemia), fluid restrictions, and violations of the dedicated TPN infusion catheter.

All patients were followed daily by the investigators from the conditioning period until hospital discharge. Clinical end points, including vital signs, mental status, medications and antibiotics, infections, organ dysfunction, transfusion requirements, fluid intake and output, and nutrient intake, were recorded. Standardized mental status examinations were administered to each patient weekly by one of the investigators, including questions assessing orientation, attention, calculation, and language.[17] The patients were also evaluated for subjective

TABLE 1
Patient characteristics: Phase I bone marrow transplant trial

Patient no.	Age (years)	Sex	Weight (kg)	BSA (m^2)	Underlying diagnosis	Total Req (kcal/kg)	Protein Req (g/day)	TPN days (no.)
1	41	F	54	1.54	CML	2318	110	21
2	37	M	81	2.12	CML	2986	122	35
3	34	F	64	1.70	Myelodysplasia	2142	90	20
4	41	M	68	1.83	AML	2158	92	21
5	38	M	73	1.86	Myelodysplasia	2302	120	26
6	19	F	56	1.54	Lymphoma	2205	84	20
7	21	F	62	1.68	CML	2158	98	35
8	41	F	66	1.44	CML	2026	90	31
Mean	34		63	1.72		2244	100	30
SEM	3		3	0.07		68	4	2

Patients 1 and 2 received TPN with zero Gln/kg/day; patients 3–6 received TPN with 0.285 g Gln/kg/day; and patients 7 and 8 received TPN with 0.570 g Gln/kg/day. Req, calculated nutrient requirements; BSA, body surface area; CML, chronic myelogenous leukemia; AML, acute myelogenous leukemia.

TABLE II
Composition of study amino acid formulas

AA (mg/100 ml)	Control diet (non-Gln)*	Gln-free II (0.338 g Gln/kg)†	Gln-free III (0.675 g Gln/kg)†
Leucine	790	791	791
Isoleucine	670	670	670
Valine	730	413	413
Methionine	670	670	670
Lysine	900	343	343
Threonine	670	268	268
Phenylalanine	790	268	268
Tryptophan	150	68	68
Histidine	600	227	227
Arginine	1120	340	340
Proline	600	169	169
Alanine	1650	770	770
Glycine	750	450	152
Serine	450	152	152
Tyrosine	30	22	22
Aspartate	230	0	0
Glutamate	870	0	0
Glutamine	0	1436	2727
Total mg/100 ml	11410	6356	8237
% AA/100 ml	11.41	6.36	8.24

* Novamine.
† Reconstituted AA solution.

impressions several times weekly by the investigators. All blood studies obtained for clinical monitoring by the primary physicians were recorded; additional blood was obtained prior to the initiation of TPN and then weekly for determination of plasma ammonia and AA. Daily nitrogen balance studies were performed by subtracting all nitrogen losses in urine, stool, and emesis from intake during days 3 to 10 after the initiation of TPN. The study protocol is shown in Figure 1.

Pharmacokinetic Analysis

Pharmacokinetic modeling techniques[9–11] were utilized to analyze the data from the oral studies. Volume of distribution (V_d), elimination rate constant (k_e), elimination half-time ($t_{1/2}$), and clearance (C_l to Cl) of Gln were determined. This analysis assumed that endogenous Gln production and release remained constant throughout the 4-hr study period. The calculated V_d from the analysis of the high Gln dose was greater than body mass, suggesting loss or splanchnic uptake of Gln during the oral study. To determine Gln V_d, three additional healthy volunteers (two male, one female, age 33 ± 1 years, weight 70 ± 6 kg) were studied. A solution was prepared by mixing 0.05 g Gln/kg BW in 150 ml 0.2% saline on the morning of study (mean Gln dose was =3.5 g). After an overnight fast, two baseline arterialized blood samples were drawn between 0800 and 0900 hr (times = =30 min and time 0), followed by rapid intravenous injection of the Gln solution over 2 min. Blood samples

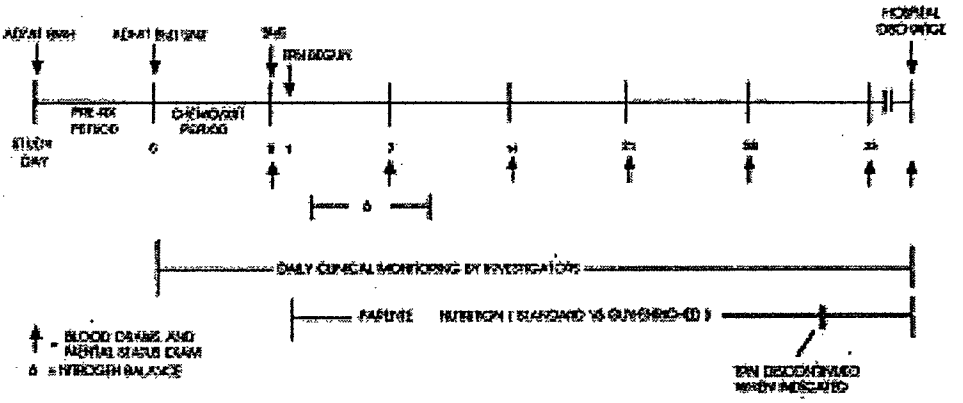

FIG. 1. Study protocol for phase I trial of Gln-enriched TPN in BMT patients. XRT, irradiation.

were then obtained at 5, 10, 20, 30, 40, 50, 60, 75, 90, and 120 min after injection and analyzed for Gln and Glu. V_d (determined from the intravenous Gln bolus studies) was utilized to calculate the portion of the oral test dose that was trapped by the splanchnic bed.

Statistical Analysis

Repeated measures analysis of variance (ANOVA) and linear regression techniques were utilized to compare dose-response data obtained in the oral Gln and 4-hr Gln infusion studies. Repeated measures and multifactorial ANOVA were performed on data obtained in the 5-day Gln infusion studies. Statistical analysis was not utilized to compare responses in the BMT studies, given the small number of subjects in each diet group. Data are reported as mean ± SEM, except as noted. Calculations were performed on a personal computer (Macintosh SE, Apple Computer, Inc., Cupertino, CA) using statistical software packages (Cricket Graph, Cricket, Inc., Philadelphia, PA, and Statview 512+, Brainpower, Inc., Calabasas, CA). A p value of <0.05 between means was considered statistically significant.

RESULTS

Short-term Studies in Normal Subjects

All subjects tolerated the oral Gln and the 4-hr Gln infusions without objective or subjective evidence of toxicity. Subjects could not distinguish when they received Gln parenterally, nor the Gln dose when given enterally. Vital signs remained stable throughout the short-term studies (data not shown).

Whole blood concentrations of Gln rose in proportion to the administered oral Gln load (Fig. 2). Blood Gln levels peaked 30–45 min after Gln ingestion and then declined steadily to the normal range within 90–120 min (low dose) or 180–240 min (high dose). Gln levels peaked at a mean concentration of 1028 ± 97 μM and 1828 ± 93 μM after 0.1 and 0.3 g Gln/kg, respectively ($p < 0.05$ vs. control), representing increments of ≈60% and ≈100% above the normal range. Whole blood levels of Glu and ammonia tended to rise in proportion to Gln dose (NS; Fig. 2). The integrated area described by the Gln concentration curve rose in a nonlinear fashion (Table III) and the responses for Glu and ammonia also tended to rise (NS). However, peak concentrations of these potentially toxic metabolites could not be distinguished from basal values (Table IV).

Administration of oral Gln resulted in a significant increase in the concentrations of AA that are known end products of Gln metabolism, including alanine, citrulline, and arginine (Table III). These alterations were related to the dose of Gln administered. Blood histidine concentration also rose significantly (−245 ± 652 μM × 4 hr vs 926 ± 274 vs 1935 ± 728; $p < 0.006$). In contrast, the integrated area for total branched-chain amino acids (BCAA) fell as the dose of Gln increased ($p < 0.02$; Table III), primarily because of a decrease in leucine concentrations (Table III). Levels of glycine, methionine, and phenylalanine also fell slightly ($p < 0.05$), most markedly in the high-dose study. The concentrations of isoleucine, valine, taurine, aspartate, threonine, serine, asparagine, proline, cystine, tyrosine, ornithine, and lysine were unaltered following the oral Gln load. The integrated response of Gln was positively related to that of several other AA such as glutamate ($r = 0.881$), arginine ($r = 0.821$), citrulline ($r = 0.877$), alanine ($r = 0.617$), and total nonessential AA excluding Gln ($r = 0.907$; all $p < 0.01$). Integrated responses for Cit and Arg were also highly correlated ($r = 0.697$, $p < 0.001$). No AA alterations were observed when subjects took only water.

Integrated responses for plasma glucose, glucagon, serum insulin, growth hormone (GH), and the insulin/

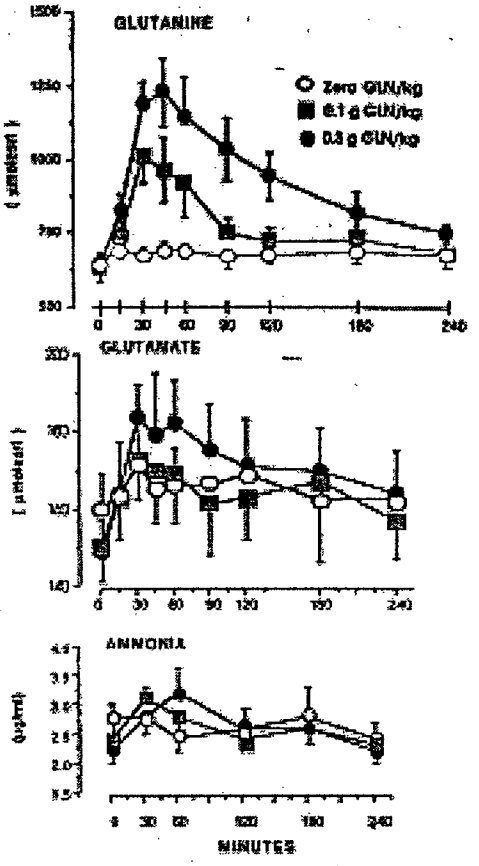

FIG. 2. Response over time for whole blood Gln, Glu, and ammonia following oral L-Gln loading in healthy humans. Although concentrations of Gln rose in proportion to the administered dose, Gln was rapidly cleared from the bloodstream. Levels of Glu and ammonia tended to rise as a function of Gln dose; however, integrated responses over time were not significantly different than when water alone was ingested.

TABLE III
Integrated responses for selected whole blood amino acids: Oral study

Gln dose	Glu	Gly	Ala	Cit	Arg	Leu	BCAA
0 g/kg	4.4 ± 2.3	1.5 ± 1.3	−2.2 ± 2.1	−1.1 ± 0.4	−0.1 ± 0.2	0.1 ± 0.5	0.4 ± 1.1
0.1 g/kg	35.3 ± 6.3	4.0 ± 1.3	2.5 ± 4.1	1.1 ± 0.3	1.5 ± 0.3	−1.0 ± 0.5	−1.5 ± 1.2
0.3 g/kg	22.4 ± 12.7	5.5 ± 1.6	5.0 ± 1.3	1.3 ± 0.4	2.1 ± 0.2	−2.3 ± 0.4	−4.8 ± 1.3
r value	0.811	[illegible]	0.287	0.702	0.851	0.656	0.687
p value	0.001	0.023	0.285	0.003	0.000	0.003	0.013

Results are given as μM × 4 hr × 10^3, mean ± SEM.

TABLE IV
Peak responses after oral Gln feeding

Gln dose	Gln (μM)	Glu (μM)	NH_3 (μg/dl)
0 g/kg	721 ± 32	166 ± 12	[illegible]
0.1 g/kg	926 ± 67	162 ± 10	[illegible]
0.3 g/kg	1358 ± 64	186 ± 10	[illegible]
r value	0.809	0.130	0.058
p value	0.001	0.548	0.576

glucagon ratio were not significantly altered during the investigations, although there was a trend for GH to rise with increasing Gln dosage. The peak insulin responses at 30 min after Gln ingestion tended to increase with dose (zero Gln, 5.4 ± 0.4 μU/ml; low dose, 9.8 ± 1.7; high dose, 8.4 ± 1.5; $p = 0.147$), as did GH at 120 min (zero Gln, 11.1 ± 4.2 ng/ml; low dose, 14.3 ± 5.6; high dose, 25.2 ± 7.5; $p = 0.084$). The basal to peak concentrations of glucagon were positively correlated with the Gln dose ($r = 0.560$, $p < 0.01$) and the integrated glucagon response was also related to dose ($r = 0.835$, $p < 0.006$). Urinary excretion of creatinine and ammonia was similar in the three studies. Excretion of urea (1.6 ± 0.1 g/4 hr vs 2.1 ± 0.3 vs 2.7 ± 0.2) and of total nitrogen (1.9 ± 0.1 g/4 hr vs 2.4 ± 0.2 vs 2.9 ± 0.3) increased with the Gln dose (both $p < 0.03$).

With the short-term infusions, blood Gln concentrations were elevated within 30 to 90 min and then plateaued (Fig. 3). Gln levels rose in proportion to the dose, from a basal level of 634 ± 31 μM to 725 ± 36 μM at 30 min with infusion of 0.0125 g/kg/hr (≈10% increase) and from a basal level of 627 ± 28 μM to 886 ± 44 μM at 90 min with infusion of 0.025 g/kg/hr (≈40% increase). Blood glutamic acid and ammonia did change significantly during Gln infusion (Fig. 3). A decrease (10–15%) in blood concentrations of alanine, leucine, isoleucine, valine, phenylalanine, ornithine, and glycine occurred, but these responses were not significantly related to Gln dose. Blood concentrations of GH, insulin, glucagon, or glucose did not change. Urinary excretion of creatinine, ammonia, urea, and total nitrogen was similar during the three infusion studies. Urinary excretion of Gln was <0.5% of the administered Gln dose in the infusion studies.

Pharmacokinetic Studies

Following the intravenous bolus administration of Gln, the blood concentration fell in two phases, elimination compatible with a two-compartment elimination model. A rapid initial phase ($t_{1/2}$ = 18 ± 2 min), was followed by a terminal disappearance slope ($t_{1/2}$ = 67 ± 11 min). The apparent V_d was similar to distribution within the extracellular fluid compartment (Table V). The K_e of the terminal slope was 0.110 ± 0.008 min^{-1}. This estimated V_d following intravenous administration (280 ± 30 ml/kg) was significantly less than the V_d derived from the oral studies (512 ± 83 ml/kg and 1254 ± 84 ml/kg in the low- and high-dose studies, respectively). Gln $t_{1/2}$ derived from these studies was 106 ± 11 min and 117 ± 17 min, respectively (Fig. 2). The estimated splanchnic uptake of oral Gln in the high-dose study (0.3-g/kg) was ≈84% of the oral load and ≈57% of the 0.1-g/kg dose.

Studies of Gln-Enriched Parenteral Nutrition in Normal Subjects

During the 5-day infusion studies, vital signs, serial mental status examinations, and continuous performance testing scores remained normal regardless of the Gln dose infused.[23] Blood chemistry values and hormonal concentrations were not significantly altered by Gln-TPN; however, elevations in plasma Gln of ≈30% were observed with TPN providing 0.285 g Gln/kg/day or 0.570 g Gln/kg/day. No significant change in circulating ammonia or Glu levels occurred. Nitrogen balance did not vary significantly between the intravenous diets. Solution stability studies demonstrated no appreciable ammonia generation when the Gln-TPN was stored for up to 10 days at 4°C. Degradation of Gln to ammonia and Glu in these TPN solutions was <0.1% at 22°C for up to 24 hr.

Studies in BMT Patients

All patients completed their prescribed chemotherapy/radiation protocol. They received at least 80% of their prescribed caloric and protein requirements as the study TPN solutions, which were administered for an average of 30 ± 2 days (Table I). No patient developed objective or subjective evidence of clinical toxicity to the Gln-enriched TPN. Results of serial tests of mental status remained within normal limits for patients undergoing the BMT regimen. One patient (patient 3) developed generalized myalgias and arthralgias during the first post-BMT week. These symptoms resolved after elimination of fat emulsion and recurred after rechallenge with this product. Thereafter, fat emulsion was discontinued and nonprotein calories were maintained by increasing the quantity of dextrose provided; protein intake was unchanged. All patients were eventually discharged to home after satisfactory engraftment of the transplanted marrow (total time in the BMT unit was 42 ± 3 days).

Plasma Gln levels were within the normal range in all eight patients prior to TPN, but fell slightly in the

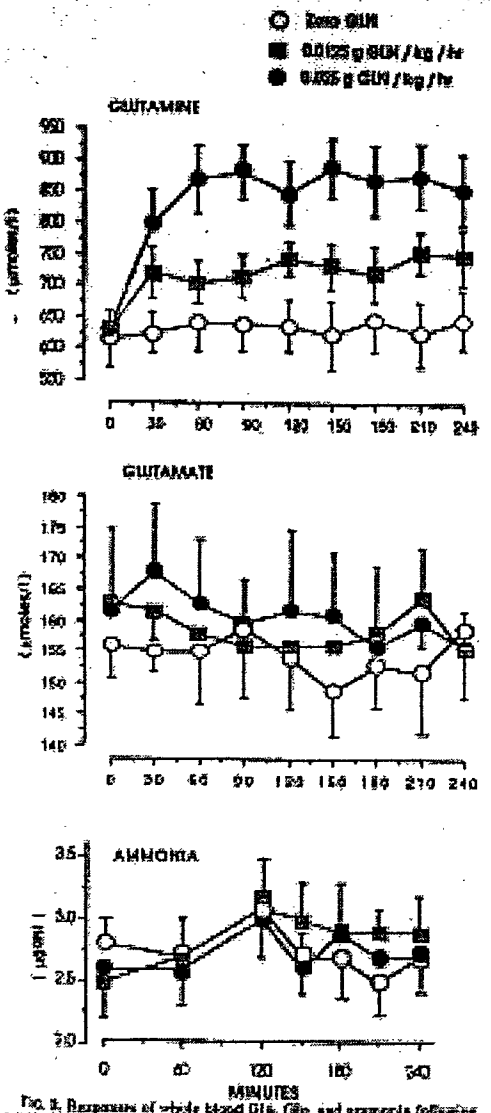

FIG. 1. Responses of whole blood Gln, Glu, and ammonia following a 4-hr infusion of L-Gln at 0.25 g/kg/hr in healthy humans. Gln levels rose in proportion to the Gln concentration of the infused solution. No appreciable changes in the Gln metabolites Glu or ammonia occurred with short-term Gln infusion.

TABLE V
Gln pharmacokinetic variables

k_e (min^{-1})	0.010 ± 0.002
$t_{1/2}$ (initial slope, min)	18 ± 2
$t_{1/2}$ (terminal slope, min)	67 ± 11
C_l (mg/kg/min)	2.9 ± 0.5
V_d (liters)	14.7 ± 3.1
V_d (ml/kg)	210 ± 30

Gln, 0.05 g/kg as iv bolus, was administered to three healthy volunteers. Systematic update for 0.2 g/kg and Gln dose: theoretical C_0 = measured C_0/theoretical C_0 = update. Gln dose/V_d = theoretical C_0 15,581 μM Gln/74.5 kg (liters) × 0.2 = 16,234 μM – 1670 μM (measured C_0)/16,234 μM = 0.896 or 89.6% splanchnic capture of oral Gln.

TABLE VI
Mean weekly blood values during TPN BMT study

	n	Baseline	Study week 1	2	3	4
Gln (μM)						
Control-TPN	9	705	718	660	704	673
Low Gln-TPN*	4	788	740	655	753	822
High Gln-TPN	2	872	659	701	838	662
Glu (μM)						
Control-TPN	2	37	74	40	36	33
Low Gln-TPN*	4	43	78	40	38	35
High Gln-TPN	2	41	53	46	40	39
NH_3 (μg/dl)						
Control-TPN	2	1.35	1.66	1.84	2.11	1.63
Low Gln-TPN*	4	0.85	0.76	1.23	1.16	1.01
High Gln-TPN	2	0.48	0.53	0.60	0.72	0.50
Alkaline phosphatase						
Control-TPN	2	197	180	162	178	180
Low Gln-TPN*	4	87	90	165	224	187
High Gln-TPN	2	96	99	202	227	144
Total bilirubin (mg/dl)						
Control-TPN	2	0.4	0.6	0.5	0.6	0.6
Low Gln-TPN*	4	0.4	0.6	0.7	0.7	0.4
High Gln-TPN	2	0.2	0.2	0.6	0.8	0.5
Blood urea nitrogen (mg/dl)						
Control-TPN	9	12	14	17	25	23
Low Gln-TPN*	4	15	16	24	29	31
High Gln-TPN	2	14	14	28	28	25
Glucose (mg/dl)						
Control-TPN	2	124	126	138	125	120
Low Gln-TPN*	4	122	126	129	140	114
High Gln-TPN	2	116	120	148	127	126

* n = 2 TPN week 1.

control and low-dose Gln-TPN groups during the first 2 weeks post-BMT; plasma Gln subsequently rose, especially in the low-dose group (Table VI). Plasma Gln levels were maintained above the basal value in two patients receiving the high-dose Gln-TPN and, in general, were greater than the concentrations found in the control TPN group. When plasma Gln was measured 3–5 days after discontinuation of Gln-enriched TPN, levels fell to 458 μM in the low-dose Gln patients and 559 μM in the high-dose Gln group. Plasma ammonia and Glu concentrations exhibited a similar pattern over time in the three TPN groups (Table VI). Plasma Gln rose during the first week of TPN in all groups and then stabilized; ammonia levels tended to rise in all groups during weeks 1 to 3 and then fall toward basal levels. Serum alkaline phosphatase rose slightly in the patients receiving Gln during weeks 2–4 of TPN and total bilirubin rose slightly in all three groups over time. Serum glucose levels remained stable in all patients.

Blood urea nitrogen rose during TPN in all groups to approximately double the basal value by the second to third week of parenteral feeding, but it then stabilized (Table VI). Nitrogen excretion appeared to be attenuated with Gln-enriched TPN. With the low-dose solution, daily and cumulative nitrogen balance was negative, but

slightly less so than that observed with the standard solution; with the high-dose Gln solution, nitrogen balance values approached equilibrium.

DISCUSSION

These studies in healthy humans demonstrate that the administration of L-Gln by either the enteral or parenteral route is well tolerated. The patient studies demonstrated the initial safety of Gln-enriched parenteral nutrition administered to severely catabolic patients for an average of 4 weeks.

Gln and its major metabolic end products, Glu and ammonia, can cross the blood-brain barrier[28] and potentially lead to altered central nervous system function. Therefore, we evaluated serial mental status function via objective standardized testing, close clinical monitoring, and subjective observations of the normal subjects and patients, in addition to monitoring of blood ammonia and Gln. These evaluations demonstrated no deleterious clinical effects due to administered Gln in any of the studies. In addition, blood concentrations of ammonia and Glu were not altered with Gln administration. Our clinical safety data support other anecdotal studies in which beneficial effects of oral L-Gln loads in disorders such as depression[29] and alcoholism[30] were described without evidence of clinical toxicity. In addition, these studies are similar to the reports of others who administered Gln-alanine dipeptides[25] or free L-Gln[31] by the intravenous route to postoperative patients. No clinical toxicity was reported in these studies; circulating ammonia levels, however, have not been previously reported.

Oral Gln appeared to be readily metabolized by splanchnic tissues to AA end products. The increased release of arginine, for example, may enhance body protein synthesis following Gln administration.[31] The fall in BCAA following oral Gln has not been previously described. This effect was most prominent for leucine and possibly occurs secondary to increased net skeletal muscle BCAA uptake due to enhanced insulin secretion.[32] Metabolic interaction between Gln and leucine is known to occur, as demonstrated by the stimulation of Gln release from the human forearm following oral leucine loads.[33] The integrated Gln levels were positively correlated with integrated plasma glucagon levels after the oral load, suggesting that Gln stimulated release of this hormone. Gln has previously been shown to stimulate glucagon release in Gln-enriched islet cell culture systems[34]; conversely, intravenous glucagon infusion decreased blood Gln concentrations.[35] In addition, glucagon infused into the portal vein stimulates gut-liver Gln uptake and decreases hepatic Gln production.[36] Thus, glucagon appears to serve as a key hormonal regulator of Gln metabolism. The route of Gln administration appeared to influence insulin, glucagon, and GH release in this study, for intravenous Gln infusion in the normal subjects did not alter hormonal responses.

The V_d of Gln derived from the oral and intravenous studies demonstrated that these estimated distribution spaces were different depending on the route of administration. The larger V_d with oral administration is related to either (1) incomplete absorption of the oral Gln or (2) splanchnic capture of the AA. There is little evidence that AA absorption in normal humans is impaired[37]; therefore, the most likely explanation for these differences is the extraction of Gln by gut-associated tissues (eg, intestinal mucosa, liver).

Blood Gln peaked at 30–45 min after an oral Gln load, similar to the peak of Gln after an oral mixed AA load.[3] The rise in Gln integrated area after 0.1 g/kg and 0.3 g/kg of oral Gln was approximately 5- and 15-fold greater than basal. The nonlinear rise in Gln within the extracellular compartment represents AA that has escaped the splanchnic bed. These data are consistent with the calculation of splanchnic capture that occurred following the low and high doses, which was 57% and 84% of the oral dose, respectively. These differences may reflect characteristics of splanchnic transport or glutaminase enzyme kinetics within the viscera or may be due to other aspects of Gln metabolism.[38] Our calculations are similar to the estimates of Dechelotte and colleagues,[39] who perfused unlabeled L-Gln into the jejunum of normal subjects over 2½ hr and observed that approximately 65% of a 7–17 g dose of Gln was taken up by splanchnic tissues. Previous studies in normal humans also demonstrated that whole blood Gln is extracted by the splanchnic bed[37] and to a much greater extent than other AA after 12–60 hr fasts[38] or after ingestion of mixed AA loads.[31]

The estimated Gln elimination half-time was longer after oral ingestion than that found for bolus Gln elimination kinetics. Because smaller doses were given in the bolus studies, it appears that either the dose or the route of administration may have affected elimination of this nutrient. Because the $t_{1/2}$ values of the two oral doses were similar, these data suggest that the route of administration is largely responsible for the differences observed.

The $t_{1/2}$ of the initial elimination slope after bolus injection (13 ± 2 min) is very similar to the $t_{1/2}$ calculated for free Gln after injection of alanine-Gln dipeptides (12 ± 8 min).[39] The apparent two-compartment elimination of Gln after bolus injection probably reflects initial distribution into the plasma compartment, followed by redistribution into other extracellular fluid compartments. Thus, Gln given at loads that greatly exceed usual dietary intakes was readily cleared from the bloodstream.

In the 4-hr infusion study, blood Gln rose as a function of Gln dose, plateauing between 30 and 90 min, depending on the dose administered. During the plateau phase, Gln clearance from the blood equaled its entry into this compartment. These findings are similar to those of a study in which an AA admixture incorporating 0.024 g/kg/hr of alanine-Gln dipeptide was infused intravenously into normal subjects; peak free Gln levels (33% above basal) were achieved by 60 min, followed by stabilization of the blood concentration.[40] Thus, short-term infusion of L-Gln appear to exhibit pharmacokinetics similar to those seen with infusion of dipeptide Gln. In this study, urinary Gln excretion was negligible (<0.3% of the dose administered), suggesting that the vast majority of the infused Gln was being utilized or assimilated by these subjects.

Excretion of urea and total nitrogen increased in a nonlinear fashion as the oral Gln load increased. This likely reflects the hepatic metabolism to urea of portal vein ammonia and other Gln end products, such as alanine and citrulline. Although animal studies show that parenteral and enteral Gln are equally metabolized by the small intestine,[27] excretion of nitrogen and urea did not change, as compared with saline infusion, when smaller Gln doses (≈3.5 or 7.0 g) were infused over 4 hr. This alteration may be a function of the doses used in the oral and intravenous studies and may reflect differing production rates of metabolic end products.

Nitrogen balance did not change with Gln loading in the 5-day infusion studies in normal subjects. This finding suggests that, in the unstressed, normal state, Gln is utilized as are other nonessential AA and that these AA may be substituted for one another without untoward effects. In contrast, when Gln was provided to catabolic patients, a nitrogen-sparing effect appeared to occur, especially with the highest Gln dose. These data are compatible with the hypothesis that Gln is a conditionally essential AA in stressed patients. These preliminary data also suggest that a critical level of Gln must be provided in the diet to improve nitrogen retention. Gln-enriched TPN maintains or increases plasma Gln levels; Gln concentrations (which peaked at levels 20–45% above baseline) fell rapidly after Gln-enriched TPN was discontinued. The fate of the administered Gln nitrogen, its impact on various tissues during catabolic stress, and the clinical conditions under which Gln may become conditionally essential in humans are largely unknown. One explanation is that an obligatory Gln requirement by certain tissues (such as the enterocytes of the small bowel and stimulated macrophages) must first be satisfied before improvement in whole body nitrogen economy occurs.

In summary, the results of these studies that L-Gln is well tolerated in healthy humans when administered as an oral or intravenous bolus, as a short-term infusion, or as a major component of intravenous nutrition formulas. Similarly, TPN solutions enriched with Gln also appeared to be safe when given for up to 4 weeks to patients undergoing BMT. Gln was readily metabolized and cleared from the bloodstream without significant generation of toxic end products. Catabolic patients appear to utilize this AA efficiently for protein anabolism. Additional studies are needed to define further the optimal dose of Gln in various nutrient mixtures and to determine the appropriate clinical situations in which Gln-enriched feeding should be initiated.

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4.2 Cover Sheet and OCPB Filing/Review Form

Office of Clinical Pharmacology and Biopharmaceutics

NEW DRUG APPLICATION FILING AND REVIEW FORM

General Information About the Submission

	Information		Information
NDA Number	21-667	Brand Name	Nutrestore• •
OCPB Division (I, II, III)	II	Generic Name	L-Glutamine
Medical Division	Division of Gastrointestinal and coagulation Drug Products (HFD-180)	Drug Class	Conditionally essential amino acid
OCPB Reviewer	Sue-Chih Lee	Indication(s)	As a cotherapy with rhGH for patients with short bowel syndrome
OCPB Team Leader	Suresh Doddapaneni	Dosage Form	Powder
Date of Submission	8/8/2003	Proposed Dosing Regimen	5 grams, 6 times daily
Estimated Due Date of OCPB Review	May 7, 2004	Route of Administration	Oral
Medical Division Due Date	May 10, 2004	Sponsor	Nutritional Restart Pharmaceutical, L.P.
PDUFA Due Date	June 11, 2004	Priority Classification	1S

Clin. Pharm. and Biopharm. Information

	"X" if included at filing	Number of studies submitted	Number of studies reviewed	Critical Comments If any
STUDY TYPE				
Table of Contents present and sufficient to locate reports, tables, data, etc.	x			
Tabular Listing of All Human Studies	x			
HPK Summary	x			
Labeling	x			
Reference Bioanalytical and Analytical Methods	x			
I. Clinical Pharmacology				
Mass balance:				
Isozyme characterization:				
Blood/plasma ratio:				
Plasma protein binding:				
Pharmacokinetics (e.g., Phase I) -				
Healthy Volunteers-				
single dose:				
multiple dose:				
Patients-				
single dose:				
multiple dose:				
Dose proportionality -				
fasting / non-fasting single dose:				
fasting / non-fasting multiple dose:				
Drug-drug interaction studies -				
In-vivo effects on primary drug:				
In-vivo effects of primary drug:				

In-vitro:				
Subpopulation studies -				
ethnicity:				
gender:				
pediatrics:				
geriatrics:				
renal impairment:				
hepatic impairment:				
PD:				
Phase 2:				
Phase 3:				
PK/PD:				
Phase 1 and/or 2, proof of concept:				
Phase 3 clinical trial:				
Population Analyses -				
Data rich:				
Data sparse:				
II. Biopharmaceutics				
Absolute bioavailability:				
Relative bioavailability -				
solution as reference:				
alternate formulation as reference:				
Bioequivalence studies -				
traditional design; single / multi dose:				
replicate design; single / multi dose:				
Food-drug interaction studies:				
Dissolution:				
(IVIVC):				
Bio-wavier request based on BCS				
BCS class				
III. Other CPB Studies				
Genotype/phenotype studies:				
Chronopharmacokinetics				
Pediatric development plan				
Literature References	**x**	**20**	**16**	
Total Number of Studies				

Filability and QBR comments

	"X" if yes	***Comments***
Application filable ?	x	Reasons if the application is not filable (or an attachment if applicable) For example, is clinical formulation the same as the to-be-marketed one?
Comments sent to firm	x	Comments have been sent to firm (or attachment included). FDA letter date if applicable.
QBR questions (key issues to be considered)	• **What PK information can be included in the label?** • **Is there a need for additional clinical pharmacology and biopharmaceutics studies?**	
Other comments or information not included above	**Reference books and other published articles were used to gather additional information.**	
Primary reviewer Signature and Date		
Secondary reviewer Signature and Date		

CC: NDA 21-667, HFD-870 (Electronic Entry or Lee), HFD-180 (Clayton), HFD-870 (Doddapaneni, Hunt, Malinowski)

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