

# Glutamine: a precursor of glutathione and its effect on liver \*

YU Jian-Chun, JIANG Zhu-Ming and LI De-Min

**Subject headings** glutamine; glutathione/biosynthesis; liver/drug effects

## Abstract

**AIM** To investigate the relationship between alanyl-glutamine (ALA-GLN) and glutathione (GSH) biosynthesis in hepatic protection.

**METHODS** Twenty male Wistar rats were randomly divided into two groups: one receiving standard parenteral nutrition (STD) and the other supplemented with or without ALA-GLN for 7 days. The blood and liver tissue samples were examined after 5-fluorouracil (5-FU) was injected peritoneally.

**RESULTS** The concentration measurements were significantly higher in ALA-GLN group than in STD group in serum GLN ( $687 \mu\text{mol/L} \pm 50 \mu\text{mol/L}$  vs  $505 \mu\text{mol/L} \pm 39 \mu\text{mol/L}$ ,  $P < 0.05$ ), serum GSH ( $14 \mu\text{mol/L} \pm 5 \mu\text{mol/L}$  vs  $7 \mu\text{mol/L} \pm 3 \mu\text{mol/L}$ ,  $P < 0.01$ ) and in liver GSH content ( $6.9 \mu\text{mol/g} \pm 2.5 \mu\text{mol/g}$  vs  $4.4 \mu\text{mol/g} \pm 1.6 \mu\text{mol/g}$  liver tissue,  $P < 0.05$ ). Rats in ALA-GLN group had lesser elevations in hepatic enzymes after 5-FU administration.

**CONCLUSION** The supplemented nutrition ALA-GLN can protect the liver function through increasing the glutathione biosynthesis and preserving the glutathione stores in hepatic tissue.

## INTRODUCTION

Glutamine (GLN) was considered as a nonessential amino acid previously which is most abundant in the circulation system and in the amino acid pool of the body. The traditional standard amino acid solution does not contain glutamine since glutamine is unstable during sterilization at high temperature. In recent studies, GLN has also been demonstrated as a conditional essential amino acid, which plays a central role in the response to injury; and GLN also supports acid-base homeostasis<sup>[1]</sup>, maintains the function and morphology of the gastrointestinal epithelium<sup>[2,3]</sup>, preserves the stores of antioxidants in tissues<sup>[4,5]</sup>, enhances the immune response<sup>[6]</sup> and augments host defenses. The standard parenteral nutrition supplemented with GLN could significantly increase GSH stores and the survival rate of injured animal models. Therefore, GLN plays a major role in various anti-injury processes.

In this study, we used alanyl-glutamine dipeptide (ALA-GLN) solutions which are stable with the same biological effects as GLN and selected a model of 5-FU induced free radical-mediated hepatic injury, so as to establish a causal relationship between preservation of hepatic glutathione stores by glutamine administration and diminished hepatic injury caused by the toxicity of chemotherapy.

## MATERIAL AND METHODS

### Animals

Male Wistar rats ( $211 \text{ g} \pm 16 \text{ g}$ ) obtained from Animal Laboratories of Chinese Academy of Medical Sciences were allowed to acclimatize for 5-7 days at constant temperature with a 12 h light/dark cycle. Only those animals demonstrating normal food intake and weight gain were entered into the study. The rats were randomized to receive either the standard as control (STD,  $n = 10$ ) or alanyl-glutamine (ALA-GLN,  $n = 10$ ) parenteral nutrition. At the fifth day after catheterization 5-FU, a chemotherapeutic drug, was administered intraperitoneally ( $100 \text{ mg/kg}$ ) for GSH depletion as a model of stress injury and intestinal inflammation. Animals were killed on the seventh day after catheterization for harvest of tissue and blood. Mortality rate was assessed.

Department of Surgery, Peking Union Medical College Hospital, Chinese Academy of Medical Sciences, Beijing 100730, China

YU Jian-Chun, M.D., female, born on 1962-03-25 in Changchun City, Jilin Province, graduated from Postgraduate School of Peking Union Medical College, as a postgraduate in 1988, associate professor of general surgery, majoring gastrointestinal surgery, parenteral and enteral nutrition, having 15 papers published.

\*Supported by the Youth Scientific Foundation of Chinese Academy of Medical Sciences, No.31016.

**Correspondence to:** YU Jian-Chun, M.D., Department of Surgery, Peking Union Medical College Hospital, Chinese Academy of Medical Sciences, 1 Shuaifuyuan, East District, Beijing 100730, China  
Tel. +86 • 10 • 65296038, Fax. +86 • 10 • 65124875  
E-mail: yujch@csc.pumch.ac.cn

**Received** 1998-11-20

### **Catheterization for parenteral nutrition**

The animals were anesthetized with intraperitoneal sodium pentobarbital (45 mg/kg). The hair was clipped from the cervical and interscapular regions, and the skin prepared with alcohol and povidone-iodine. Under sterile conditions, a Silastic catheter with an internal diameter of 0.76 mm and external diameter of 1.52 mm (Dow Corning Corp, USA) was inserted into the right jugular vein, advanced into the superior vena cava, and secured in place. The catheter was tunneled subcutaneously to the interscapular region and anchored to the deep fascia using a stainless steel button and prolene (Ethicon, USA). After threading the catheter through a flexible wire sheath, the combined unit was attached to a swivel apparatus (Instech Laboratories, Inc. USA) that allowed free movement of the animal during sustained intravenous feeding. After recovery from anesthesia, the animals were housed in individual metabolic cages.

### **Diet preparation**

Parenteral feeding solutions were prepared in a laminar flow hood and were sterilized using membrane filtration (0.22  $\mu$ m filter). Stock solutions were prepared on the day of catheterization and stored at 4°C. Both the standard glutamine-free parenteral solution (STD) and the 3% alanyl-glutamine dipeptide supplemented solution (ALA-GLN) provided the necessary calories, nitrogen, vitamins, and minerals required for the growing rats<sup>[7]</sup>. The diets were isocaloric (260 Kcal  $\cdot$  kg<sup>-1</sup>  $\cdot$  d<sup>-1</sup>, in which 20% Intralipid provides 20% calories) and isonitrogenous (1.66 g  $\cdot$  kg<sup>-1</sup>  $\cdot$  d<sup>-1</sup>), and differed only in the composition of alaninyl-glutamine dipeptide (alaninyl-glutamine 6 g + standard amino acid solution 100 mL + non-protein caloric solution 100 mL, i.e. 3% ALA-GLN, equals to 2% GLN). After catheterization and recovery from anesthesia, animals were randomized to receive either the STD ( $n = 10$ ) or ALA-GLN ( $n = 10$ ) parenteral nutrition at an initial rate of 24 mL/day. On the second day after the operation, the infusion rate was increased to 48 mL/day and maintained at this level throughout the experiment. At 08:00 am of the fifth day after catheterization, 5-FU was administered intraperitoneally (100 mg/kg). Animals were killed on the seventh day after catheterization for harvest of tissue and blood. Mortality rate was assessed only in those animals killed at 24 hours.

### **Harvest procedures**

At the time of harvest, animals were weighed and

anesthetized with intraperitoneal sodium pentobarbital (45 mg/kg) and the skin prepared with povidone-iodine. Under sterile conditions, a midline incision was made from the pubis to the suprasternal notch, and whole blood was drawn from the right ventricle into a heparinized syringe. The whole blood was centrifuged immediately and plasma obtained for determination of glutathione, glutamine, alanine aminotransferase (ALT), alkalophospholase (ALP), total and direct bilirubin concentration. The plasma (200  $\mu$ L) added with 86.2 g/L-SSA (5-sulfosalicylic acid solution 200 mL) was centrifuged for 1 minute immediately. The supernatant was removed and stored at -80°C for determination of the reduced glutathione. A 400 mg liver without capsule was resected from the middle lobe and immediately placed in a 4.31% SSA solution with a weight to volume ratio of 1:10. These samples were then homogenized in a glass manual tissue grinder. The acid suspension was centrifuged and the supernatant sample was removed. For determination of disulfid glutathione (GSSG), an aliquot of previous supernatant was mixed with TRIS buffer and 2-vinylpyridine (Sigma Company) to protect the SH group directly during harvesting. Those samples were stored at -80°C until analysis of the GSH and the oxidized form of glutathione (GSSG).

### **Analytical procedures**

Biochemical assay of plasma and hepatocyte cellular glutathione was based on the "Glutathione, Glutathione Reductase-DTNB Recycling Method" originally described by Tietze<sup>[8]</sup>:  $2\text{GSH} + \text{DTNB} \rightarrow \text{GSSG} + \text{TNB} + 2\text{H}^+$ ;  $\text{GSSG} + \text{NADPH} + \text{H}^+ \rightarrow 2\text{GSH} + \text{NADP}^+$ .

The principle of the assay is illustrated as follows. GSH is oxidized by 5,5'-dithiobis-2-nitrobenzoic acid (DTNB) (Sigma Company), yielding 2-nitro-5-thiobenzoic acid (TNB) monitored at 412 nm spectrophotometrically (UVIKON 930, KONTRON INSTRUMENTS); and GSSG is reduced by nicotinamide-adenine dinucleotide phosphate (NADPH, reduced form, Sigma) in the presence of glutathione disulfide reductase. In the process, GSH is oxidized to GSSG, and the reaction is maintained by adding the enzyme glutathione reductase (Sigma Company) and the electron donor NADPH, so that the concentration of GSH is rate limiting. The GSSG was measured with the samples treated with 2-vinylpyridine masking GSH specifically. A standard curve with known concentration of glutathione was generated for each sample analy-

sis. Plasma glutamine was determined with amino acid autoanalyzer (Model 119 CL, Beckman, USA). ALT, ALP and total and direct bilirubin concentrations were assayed using an automated laboratory analyzer (RA 2000).

### Statistical analysis

All calculations were performed on an Apple Macintosh SE computer using STATVIEW standard statistical software. Data were expressed as mean  $\pm$  standard error. Chi square analysis was used for mortality data. Other data were analyzed with ANOVA.

## RESULTS

### Body weight

Although the body weight loss occurred during parenteral nutrition (PN) and 5-FU treatment, there were no significant differences in body weight between STD and ALA-GLN groups before starting PN ( $215.2 \text{ g} \pm 22.5 \text{ g}$  vs  $207.2 \text{ g} \pm 14.6 \text{ g}$ ,  $P > 0.05$ ) or after finishing PN ( $188.1 \text{ g} \pm 19.9 \text{ g}$  vs  $190.6 \text{ g} \pm 19.8 \text{ g}$ ,  $P > 0.05$ ).

### Plasma glutamine and glutathione

Forty-eight hours after 5-FU administration, animals in the experimental group had significantly higher plasma concentrations of GLN ( $687.3 \text{ } \mu\text{mol/L} \pm 49.8 \text{ } \mu\text{mol/L}$  vs  $504.9 \text{ } \mu\text{mol/L} \pm 39.6 \text{ } \mu\text{mol/L}$ ,  $P < 0.05$ ) and GSH ( $14.37 \text{ } \mu\text{mol/L} \pm 5.16 \text{ } \mu\text{mol/L}$  vs  $7.08 \text{ } \mu\text{mol/L} \pm 3.16 \text{ } \mu\text{mol/L}$ ,  $P < 0.01$ ) than in the control group (Figure 1).

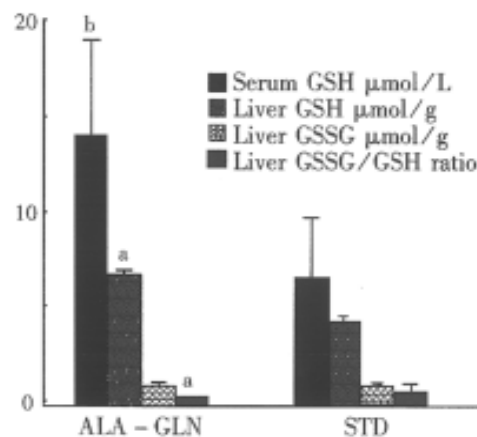
### Hepatic glutathione

Forty-eight hours after drug administration, hepatic glutathione concentrations were significantly lowered in the STD group. But the GSSG level was similar between the two groups. The GSH concentration was in the normal range in the ALA-GLN group, but significantly higher than that in the STD group ( $6.9 \text{ } \mu\text{mol/g} \pm 2.5 \text{ } \mu\text{mol/g}$  vs  $4.4 \text{ } \mu\text{mol/g} \pm 1.6 \text{ } \mu\text{mol/g}$  liver tissue,  $P < 0.05$ ). The redox ratio of GSSG/GSH in ALA-GLN group was significantly lower than that in the STD group ( $0.12 \pm 0.03$  vs  $0.47 \pm 0.33$ ,  $P < 0.05$ , Figure 1).

### Hepatic injury and mortality

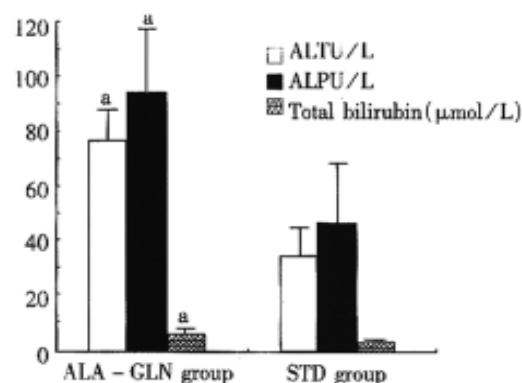
Forty-eight hours after drug administration, STD animals had greater elevations in plasma ALT, ALP and total and direct bilirubin levels as compared with the ALA-GLN animals ( $P < 0.05$ ) (Figure 2). One rat in ALA-GLN group and two rats in STD group died at 24 hours after drug administration,

and one rat in STD group died at 34 hours after drug administration. Although mortality rate was higher in STD (30%) than in ALA-GLN animals (10%), there was no significant difference in mortality rate between the two groups ( $P > 0.05$ ).



**Figure 1** Glutathione concentrations in serum and in liver tissue after 5-FU administration.

<sup>a</sup> $P < 0.05$ , <sup>b</sup> $P < 0.01$  as compared with STD.



**Figure 2** Liver function changes after 5-FU administration. <sup>a</sup> $P < 0.05$  compared with STD group.  $\times 10$

## DISCUSSION

Glutathione (GSH) is a major antioxidant and a vital component of host defenses. It protects tissues from free radical injury via detoxification of active species and/or repair of injury, and plays an important role in the metabolism of drugs and endogenous substances<sup>[9]</sup>. The depletion of tissue GSH concentration and protein alkylation results in the drug toxicity of target tissues. The drug toxicity to normal tissues limits the chemotherapy doses.

GSH is a tripeptide consisting of glutamate, cysteine and glycine. Although experiments showed that GSH biosynthesis is rate-limited by cysteine during glutathione depleted states<sup>[10]</sup>, the availability of glutamine appears to be important for the regeneration of glutathione stores during our experi-

ment of hepatic injury. The reason is that glutamate is poorly transported into cells. Under various experimental conditions, the glutamate portion of the molecule is derived from glutamine. Glutamine is efficiently transported across the cell membrane and deaminated in the mitochondria to produce glutamate and  $\text{NH}_3$ . Glutamate is then transported back to the cytosol, where it is readily available for glutathione synthesis. GLN is a vital precursor of glutamate for GSH synthesis.

After 5-FU was administered in our study, STD animals exhibited a significantly lower glutathione stores as compared with that in the ALA-GLN group animals. The concentration of GSH which was in the normal range in GLN group was significantly higher than that in the STD group. Ninety-six hours after chemotherapeutic drug administration, hepatic glutathione concentrations, GSH concentration and the GSH/GSSG-ratio in STD animals did not recover to the normal range. The possible reason may be the deficiency of GLN as a processor for GSH biosynthesis, limiting glutathione synthesis. This level of GSH depletion was associated with increased hepatic injury, as suggested by higher concentrations of hepatic enzymes. The normal concentration of GSH was associated with better hepatic function in the ALA-GLN animals.

These results suggest that 3% ALA-GLN has equivalent biological and metabolic effects with 2% GLN<sup>[11]</sup>. The study confirms the important function of glutamine in the causal relationship between preservation of hepatic glutathione stores by glutamine administration and decrease of hepatic injury and

mortality. This may be due to the mechanism of increased cellular reduced glutathione through the cell membrane<sup>[12]</sup>. GSH may also protect cells via detoxification by binding with the toxicant and repairing the injury of hepatocytes.

## CONCLUSION

We demonstrated that alanyl-glutamine could protect the liver function after chemotherapy through increasing the glutathione biosynthesis and preserving the glutathione stores of hepatic tissue.

## REFERENCES

- 1 Welbourne TC, Joshi S. Interorgan glutamine metabolism during acidosis. *JPEN*, 1990;14(Suppl):77s
- 2 Hwang TL, O'Dwyer ST, Smith RJ, Wilmore DW. Preservation of small bowel mucosa using GLN-enriched parenteral nutrition. *Surg Forum*, 1986;37:56
- 3 Rene RWJ, Bernard K, Maarten F. Glutamine and the preservation of gut integrity. *Lancet*, 1993;334:1364
- 4 Wilmore DW, Smith RJ, O'Dwyer ST. The gut: A central organ after surgical stress. *Surgery*, 1988;104:917
- 5 Roy W Hong, Jan D Rounds, William S Helton. Glutamine preserves liver glutathione after lethal hepatic injury. *Ann Surg*, 1992;215:114-119
- 6 Burke D, Alverdy JC, Aoye E, Moss GS. Glutamine supplemented total parenteral nutrition improves gut immune function. *Arch Surg*, 1989;124:1396
- 7 Popp MB, Brennan MF. Growth and body composition during long-term total parenteral nutrition in the rat. *Am J Clin Nutr*, 1982;36:1119-1128
- 8 Meister A, Anderson ME. Glutathione. *An Rev Biochem*, 1983; 52:711-760
- 9 Deneke SM, Fanburg BL. Regulation of cellular glutathione. *Am J Physiol*, 1989;257:L163-L173
- 10 Black M. Acetaminophen hepatotoxicity. *Gastroenterology*, 1980; 78:382-392
- 11 Jiang ZM, Wang LJ, Qi Y. Comparison of parenteral nutrition supplemented with L glutamine or glutamine dipeptides. *JPEN*, 1993;17:134
- 12 Le CT, Hollaar L, Van der Valk EJ. Protection of myocytes against free radical induced damage by accelerated turnover of the glutathione redox cycle. *Eur Heart J*, 1995 Apr;16:553-562

Edited by MA Jing-Yun