Research Article

Effect of Previous High Glutamine Infusion on Inflammatory Mediators and Mortality in an Acute Pancreatitis Model

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Received 16 August 2016; Revised 10 November 2016; Accepted 16 November 2016

Academic Editor: Michael Conlon

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Parenteral glutamine supplementation in acute inflammatory conditions is controversial. We evaluated the inflammatory and survival responses after parenteral glutamine infusion in sodium taurocholate-induced acute pancreatitis (AP) model. Lewis rats received 1 g/kg parenteral glutamine (n = 42), saline (n = 44), or no treatment (n = 45) for 48 h before AP induction. Blood, lung, and liver samples were collected 2, 12, and 24 h after AP to measure serum cytokines levels and tissue heat shock protein (HSP) expression. From each group, 20 animals were not sacrificed after AP for a 7-day mortality study. Serum cytokine levels did not differ among groups at any timepoint, but the intragroup analysis over time showed higher interferon-γ only in the nontreatment and saline groups at 2 h (versus 12 and 24 h; both p ≤ 0.05). The glutamine group exhibited greater lung and liver HSP90 expression than did the nontreatment group at 2 and 12 h, respectively; greater liver HSP90 and HSP70 expression than did the saline group at 12 h; and smaller lung HSP70 and liver HSP90 expression than did the nontreatment group at 24 h (all p ≤ 0.019). The 7-day mortality rate did not differ among groups. In experimental AP, pretreatment with parenteral glutamine was safe and improved early inflammatory mediator profiles without affecting mortality.

1. Introduction

Glutamine can become essential during hypercatabolic stress and under critical conditions, such as severe trauma, sepsis, inflammatory diseases, and burns [1]. Glutamine is a fuel source for lymphocytes and enterocytes, a substrate for glutathione and heat shock protein (HSP) synthesis, and a potential inhibitory agent for inflammatory cytokine release [2, 3]. These biological properties could contribute to improving gut barrier and lymphocyte function and to attenuate inflammatory responses [4].

In critically ill patients, glutamine supplementation has been suggested to properly support increased cell proliferation rates, gut barrier protection, and inflammatory dysfunction attenuation [5, 6]. The intravenous administration of glutamine can result in its earlier availability for cell use and could be advantageous for the achievement of rapid inflammatory modulation and protection of cells against damage in clinical critical care conditions. However, unexpected harmful effects of parenteral glutamine supply, mainly in patients with multiple organ failure, have been reported recently [7–9].

These observations have challenged the development of new guidelines for safe glutamine supplementation and have made apparent the need for new experimental studies to better understand this nutrient’s mechanisms of action in critical illness. Experimental acute pancreatitis (AP) is an effective model for the study of systemic responses that can be applied to test immunomodulatory therapies [10]. The present study aimed to evaluate the impact of previous parenteral glutamine infusion on inflammatory mediator levels and mortality in acute critically ill conditions, using experimental AP as a systemic inflammation-reproducing model.
2. Methods

2.1. Animals. Adult male isogenic Lewis rats (n = 131, 300–350 g) were purchased from the Animal Laboratory of the Multidisciplinary Center for Research in Biological Science (Campinas, São Paulo, Brazil). Prior to the experiment, the animals were adapted for 5 days in metabolic cages at a controlled room temperature (22 ± 25°C) with a 12 h light/dark cycle and free access to water and standard rodent chow (Quimita®; Nutrilav, Jundiaí, Brazil). All experimental procedures were approved by the Research Ethics Committee of the School of Medicine, University of São Paulo, São Paulo, Brazil.

2.2. Intravenous Access. Animals were anesthetized with an intraperitoneal injection of ketamine (Ketamin-S(+), 100 mg/kg body weight; Cristália, Itapira, Brazil) and xylazine (Rompun®, 8 mg/kg body weight; Bayer, São Paulo, Brazil). Intravenous access was achieved by jugular central venous catheterization (CVC), according to a standard technique, followed by connection to a swivel apparatus that allowed the animals to have free mobility [11, 12]. After CVC, all animals received 0.9% saline solution infusion for 24 h. After this period, the animals were randomized to receive 48 h intravenous infusion of 6 mL/day 0.9% saline solution (saline group, n = 44) or 1 g/kg body weight dypeptide alanyl-glutamine (Dipeptiven®, 20%, Fresenius-Kabi, Bad Homburg, Germany; glutamine group, n = 42), or no infusion (nontreatment group, n = 45). All animals had access to a standard oral diet (AIN-93M) and water ad libitum during this period.

2.3. Experimental Acute Pancreatitis. After 72 h intravenous access, all animals were anesthetized with an intraperitoneal injection of 100 mg/kg body weight ketamine (Ketamin-S(+)), Cristália and 8 mg/kg body weight xylazine (Rompun®, Bayer). The pancreas was exteriorized through an abdominal incision and the pancreatic duct was catheterized using a 24-gauge angiocatheter. AP was then induced by retrograde injection of 0.5 mL 3% sodium taurocholate solution (Sigma Chemical, St Louis, MO, USA), according to a standard technique [13–15]. Following AP induction, 71 animals were sacrificed after proper anesthetization at 2 h (saline group, n = 8; glutamine group, n = 9; nontreatment group, n = 10), 12 h (saline group, n = 9; glutamine group, n = 6; nontreatment group, n = 9), and 24 h (saline group, n = 7; glutamine group, n = 7; nontreatment group, n = 6) by cardiac puncture for blood and tissue (lung and liver) collection, and 60 animals (n = 20/group) were kept alive for mortality analysis.

2.4. Serum Cytokine Measurement. Blood samples were centrifuged at 1,000× g at 4°C for 10 min to obtain serum. Concentrations of cytokines ( interleukin-1β [IL-1β], IL-2, IL-4, IL-6, IL-10, interferon-γ [IFN-γ], tumor necrosis factor-α [TNF-α] ) were assessed in 500 μL serum by multiplex microsphere immunoassays, using a commercial kit for rats (RECYTMAG® 07-65K; Genesis Ltd., MO, USA). Plates were read in a Luminex analyzer (MiraiBio, Alameda, CA, USA), according to the manufacturer’s instructions [16].

2.5. Heat Shock Protein Measurement. Approximately 100 mg lung tissue and 50 mg liver tissue were pulverized in liquid nitrogen. The material was homogenized in RIPA lysis buffer (100 mM Tris-HCl [pH 7.5], 1% sodium deoxycholate, 1% NP40, 150 mM NaCl, 0.1% sodium dodecyl sulfate [SDS]) plus protease inhibitors (1 mg/mL pepstatin A, 100 mM phenylmethysulfonyl fluoride). The samples were then centrifuged at 14,000× g for 10 min at 4°C. The supernatants were collected and protein concentrations were quantified using the Bradford method (Bio-Rad Laboratories, Hercules, CA, USA). Protein samples were added to sample buffer (2% SDS, 60 mM Tris [pH 6.8], 5% mercaptoethanol, 0.01% bromophenol blue) and subjected to electrophoresis in a sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) system (1.5 M Tris-HCl, 10% SDS, 30% bis-acrylamide, 10% ammonium persulfate, and 3,3,5,5-tetramethylmethylenediamine). Proteins were then transferred to nitrocellulose membranes using a semidy transfer apparatus (both from Bio-Rad Laboratories). The membranes were incubated in a blocking solution of 5% skim milk in TBST buffer (50 mM Tris buffer [pH 8.0], 100 mM NaCl, and 1% Tween 20) for 1 h at room temperature. Then, they were washed in TBST and incubated with the primary antibody against the protein of interest (HSP polyclonal goat anti-rat; Santa Cruz Biotechnology, Santa Cruz, CA, USA) overnight at 4°C. Subsequently, the membranes were incubated in a solution containing the peroxidase-conjugated secondary antibody (1:1000; Santa Cruz Biotechnology), and Super Signal detection (Pierce, Rockford, IL, USA) was performed. Protein expression was compared by gel densitometry using the ImageJ public domain software created by Wayne Rasband at the US National Institutes of Mental Health, which has been used previously for the determination of HSP70 and HSP90 [13].

2.6. Mortality Observation. After AP induction, 20 rats in each group remained under observation for a maximum of 7 days, with access to standard oral diet (AIN-93M) and water ad libitum. The animals were observed individually every 8 h for death registration. Animals that survived until 7 days after AP were sacrificed with an intraperitoneal injection of 80 mg/kg ketamine hydrochloride (Ketamin-S(+)); Cristália) and 8.0 mg/kg xylazine hydrochloride (Rompun® 2%; Bayer).

2.7. Statistical Analysis. All inflammatory variables were compared using the Kruskal–Wallis and Behrens–Fisher tests, as Kolmogorov–Smirnov tests showed that they were not distributed normally. These comparisons were performed between groups at each time point and within groups over time. Mortality and survival data were evaluated by the Fisher test and Kaplan–Meier analysis, respectively. All analyses were based on a 5% level of significance and were performed.
using SPSS software (ver. 18.0 for Windows; SPSS, Chicago, IL, USA).

3. Results

3.1. Serum Cytokine Concentrations. Serum cytokine levels did not differ among groups at any time point (Table 1). Serum IFN-γ levels in the nontreatment and saline groups were significantly higher at 2 h after AP than at 12 h ($p = 0.026$ and 0.001, resp.) and 24 h ($p = 0.002$ and 0.050, resp.) after AP (Figure 1(a)). In addition, animals in the nontreatment group exhibited higher serum IL-2 levels (Figure 1(b)) and lower serum IL-10 levels (Figure 1(c)) at 24 h after AP than at 2 h (both $p < 0.001$) and 12 h ($p = 0.005$ and $p < 0.001$, resp.) after AP. Animals in the saline and glutamine groups exhibited only lower IL-10 levels at 24 h after AP relative to 

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**Figure 1:** Median serum concentrations of interferon- (IFN-) gamma (a), interleukin- (IL-) 2 (b), IL-10 (c), and tumor necrosis factor- (TNF-) alpha (d) at 2, 12, and 24 h after acute pancreatitis induction with sodium taurocholate in Lewis rats previously treated or not treated with 48 h infusion of parenteral saline or glutamine. $^* p \leq 0.050$ versus 12 and 24 h; $¥ p \leq 0.050$ versus 2 and 12 h; $#$ $p \leq 0.050$ versus 24 h; $€ p = 0.051$ versus 2 and 12 h; $\phi p = 0.060$ versus 12 h.
Table 1: Serum cytokine levels in rats with acute pancreatitis, according to treatment and time point.

| Cytokine | Treatment       | 2 h Median | 1st quartile | 3rd quartile | p value | 12 h Median | 1st quartile | 3rd quartile | p value | 24 h Median | 1st quartile | 3rd quartile | p value |
|----------|----------------|------------|--------------|--------------|---------|------------|--------------|--------------|---------|------------|--------------|--------------|---------|
| IL-1α    | Saline         | 14.41      | 4.67         | 47.89        |         | 15.60      | 11.40        | 31.86        | 0.552   | 1.84       | 1.84         | 46.08        | 0.583   |
|          | Glutamine      | 4.67       | 4.67         | 12.27        | 0.552   | 13.57      | 9.48         | 33.94        | 0.574   | 12.49      | 1.84         | 25.98        | 0.249   |
|          | Nontreatment   | 8.47       | 4.67         | 47.89        |         | 8.57       | 6.13         | 17.86        |         | 72.61      | 1.84         | 128.00       |         |
| IL-4     | Saline         | 3.98       | 3.98         | 8.90         |         | 5.91       | 4.88         | 9.23         |         | 1.30       | 1.30         | 13.10        |         |
|          | Glutamine      | 3.98       | 3.98         | 3.98         | 0.970   | 5.91       | 3.91         | 15.38        | 0.464   | 1.30       | 1.30         | 4.88         | 0.398   |
|          | Nontreatment   | 3.98       | 3.98         | 3.98         |         | 2.17       | 1.35         | 6.98         |         | 19.72      | 1.30         | 42.82        |         |
| IL-2     | Saline         | 25.50      | 22.32        | 31.21        |         | 49.40      | 10.97        | 72.34        |         | 74.03      | 27.12        | 117.21       |         |
|          | Glutamine      | 23.89      | 22.31        | 30.38        | 0.833   | 46.75      | 18.16        | 90.19        | 0.814   | 70.94      | 17.49        | 167.75       | 0.249   |
|          | Nontreatment   | 23.10      | 17.69        | 33.71        |         | 37.89      | 24.44        | 48.50        |         | 135.50     | 83.31        | 166.00       |         |
| IL-6     | Saline         | 200.00     | 76.71        | 289.50       |         | 220.00     | 50.13        | 1270.00      |         | 369.00     | 184.21       | 766.50       |         |
|          | Glutamine      | 178.00     | 33.11        | 452.00       | 0.644   | 331.25     | 29.70        | 619.00       | 0.971   | 903.00     | 486.50       | 1089.00      | 0.458   |
|          | Nontreatment   | 336.00     | 33.11        | 3135.00      | 0.288   | 1789.00    | 113.00       | 5319.00      | 0.964   | 464.50     | 293.00       | 599.00       |         |
| IL-10    | Saline         | 400.00     | 294.50       | 560.00       |         | 342.00     | 99.08        | 691.00       |         | 65.83      | 34.62        | 183.18       |         |
|          | Glutamine      | 539.00     | 298.00       | 893.00       | 0.288   | 482.50     | 65.28        | 1521.00      | 0.964   | 77.82      | 54.16        | 125.41       | 0.553   |
|          | Nontreatment   | 336.50     | 168.00       | 482.00       |         | 273.00     | 96.09        | 617.00       |         | 51.42      | 39.50        | 57.66        |         |
| IFN-γ    | Saline         | 72.62      | 30.44        | 89.41        |         | 6.50       | 6.50         | 6.50         |         | 4.01       | 4.01         | 4.01         |         |
|          | Glutamine      | 55.67      | 55.67        | 64.15        | 0.142   | 6.50       | 6.50         | 6.50         | 0.908   | 4.01       | 4.01         | 4.01         | 0.776   |
|          | Nontreatment   | 85.25      | 64.15        | 93.61        | 0.142   | 6.50       | 6.50         | 6.50         | 0.908   | 4.01       | 4.01         | 4.01         | 63.08   |
| TNF-α    | Saline         | 8.44       | 1.82         | 15.97        |         | 4.84       | 2.17         | 5.86         |         | 13.02      | 9.19         | 23.27        |         |
|          | Glutamine      | 11.22      | 1.82         | 11.22        | 0.390   | 3.91       | 2.17         | 8.05         | 0.862   | 16.31      | 3.94         | 18.50        | 0.579   |
|          | Nontreatment   | 1.82       | 1.82         | 8.53         |         | 4.19       | 2.43         | 9.21         |         | 22.27      | 14.12        | 38.39        |         |

IL, interleukin; INF, interferon; TNF, tumor necrosis factor.
2 h after AP (both $p < 0.001$; Figure 1(c)). No significant change in the serum IL-1, IL-4, IL-6, or TNF-α level occurred over time, although marginally nonsignificant higher TNF-α levels were observed at 24 h after AP in the nontreatment ($p = 0.051$ versus 2 and 12 h after AP) and saline ($p = 0.060$ versus 12 h after AP) groups (Figure 1(d)).

3.2. Heat Shock Protein Expression. Data on HSP expression are presented in Table 2. Animals in the glutamine group exhibited greater lung and liver HSP90 expression than did those in the nontreatment group at 2 h ($p = 0.007$; Figure 2(a)) and 12 h ($p = 0.001$; Figure 2(b)) after AP, respectively, and greater liver HSP90 and HSP70 expression

Figure 2: Lung and liver expression of heat shock proteins (HSPs) at different time points after acute pancreatitis induction with sodium taurocholate in Lewis rats previously treated or not treated with 48 h infusion of parenteral saline or glutamine. (a) Expression of HSP90 in lung tissue at 2 h. (b) Expression of HSP90 in liver tissue at 12 h. (c) Expression of HSP70 in liver tissue at 12 h. (d) Expression of HSP70 in lung tissue at 12 h. (e) Expression of HSP70 in lung tissue at 24 h. (f) Expression of HSP90 in liver at 24 h. Data are expressed as medians. $^* p \leq 0.050$ versus glutamine group; $^\# p \leq 0.050$ versus saline group; $^\phi p = 0.066$ versus glutamine group; $^\Delta p \leq 0.050$ versus nontreatment group.
Table 2: Heat shock protein expression in rats with acute pancreatitis, according to treatment and time point.

| Variable | Treatment  | Median   | 1<sup>st</sup> quartile | 3<sup>rd</sup> quartile | p value | Median   | 1<sup>st</sup> quartile | 3<sup>rd</sup> quartile | p value | Median   | 1<sup>st</sup> quartile | 3<sup>rd</sup> quartile | p value |
|----------|------------|----------|--------------------------|--------------------------|---------|----------|--------------------------|--------------------------|---------|----------|--------------------------|--------------------------|---------|
|          |            | 2 h      |                          |                          |         | 12 h     |                          |                          |         | 24 h     |                          |                          |         |
| HSP70 lung| Saline    | 15615447 | 14285740                | 20267619                | 0.372   | 7848     | 6817                    | 9901                    | 0.066   | 24064   | 21086                    | 27654                    | 0.0194  |
|          | Glutamine | 13645497 | 814814                  | 15673669                |         | 11684    | 11021                   | 15036                   |         | 23499   | 22976                    | 26745                    |         |
|          | Nontreatment | 12956058 | 10096912                | 16820669                |         | 7219     | 5854                    | 10299                   |         | 33718   | 28039                    | 37863                    |         |
| HSP90 lung| Saline    | 16654811 | 15125912                | 24525217                | 0.009   | 8079     | 6392                    | 9511                    | 0.065   | 14031   | 11409                    | 28005                    | 0.1067  |
|          | Glutamine | 18875083 | 16923497                | 25849518                |         | 11742    | 9796                    | 13052                   |         | 8108    | 6184                     | 9750                     |         |
|          | Nontreatment | 8037912  | 7573083                 | 12943462                |         | 8745     | 5670                    | 10700                   |         | 9094    | 7174                     | 12214                    |         |
| HSP70 liver| Saline   | 27154796 | 2216953                 | 32188418                | 0.175   | 7497     | 6644                    | 9658                    | 0.027   | 15817   | 13442                    | 20652                    | 0.1495  |
|          | Glutamine | 28513246 | 26185806                | 35924403                |         | 23252    | 15236                   | 26715                   |         | 18071   | 13442                    | 20652                    |         |
|          | Nontreatment | 34420660 | 30578261                | 41040332                |         | 9875     | 7099                    | 11308                   |         | 20686   | 14079                    | 21387                    |         |
| HSP90 liver| Saline   | NS       | NS                      | NS                      | NS      | 10014    | 7396                    | 10263                   | 0.008   | 16841   | 16074                    | 21436                    | 0.0045  |
|          | Glutamine | NS       | NS                      | NS                      | 19218   | 15843    | 23452                   | 0.008                   |         | 337601  | 30857                    | 37946                    |         |
|          | Nontreatment | NS     | NS                      | NS                      | 8671    | 5527     | 11237                   | 0.008                   |         | 337601  | 30857                    | 37946                    |         |

NS, nonsignificant expression to be detected; HSP, heat shock protein.
than did those in the saline group at 12 h after AP ($p < 0.001$ and $p = 0.006$, respectively; Figures 2(b) and 2(c)). Marginally nonsignificant greater lung HSP70 expression was observed in animals in the glutamine group compared with the other groups at 12 h after AP ($p = 0.066$; Figure 2(d)). Lung HSP90 expression was greater in the saline group than in the nontreatment group at 2 h after AP ($p = 0.017$; Figure 2(a)). The nontreatment group presented increased lung HSP70 and liver HSP90 expression relative to the other groups at 24 h after AP ($p = 0.019$ and 0.004, respectively; Figures 2(e) and 2(f)).

### 3.3. Mortality

No significant difference in 7-day mortality was observed among groups (nontreatment, 28%; saline, 47%; glutamine, 27%) (Figure 3). In addition, the median interval of mortality occurrence did not differ among groups (nontreatment, 24–48 h; saline, 30–36 h; glutamine, 48 h).

### 4. Discussion

Our study aimed to contribute to the understanding of potential inflammatory mechanisms that may impact the risk-benefit balance of parenteral glutamine infusion in critical care. AP was chosen as a critical condition model due to the central roles of inflammatory mediators in its physiopathology and in multiple organ dysfunction syndrome, which is usually its primary cause of death [10]. In addition, the systemic effects of AP are similar to those observed in other critical conditions, such as sepsis, severe burns, and trauma [17]. Specifically, sodium taurocholate-induced AP has been reported to be a representative model of the disease, with severe and measurable systemic inflammatory response and multiple organ failure, as evidenced by lung, liver, and intestinal impairment in rats [18–21].

In human and experimental models, marked release of the proinflammatory mediators IL-1, IL-6, and TNF-α is the main detrimental finding associated with AP [15, 22]. This release is usually followed by increased release of anti-inflammatory mediators (e.g., IL-10), which may induce immunosuppression in the late stage of the disease [15]. In our study, high doses of parenteral glutamine infused for 48 h before the induction of experimental AP did not change serum levels of IL-1, IL-6, and TNF-α at any post-AP time point. Marginally nonsignificant increases in TNF-α levels were observed at 24 h after AP in the nontreatment and saline groups, but not in the glutamine group.

The glutamine group also did not show the significant decreases in IFN-γ level observed over time in the nontreatment and saline groups. In addition, the IL-2 levels were maintained overtime in the glutamine and saline groups and the decrease in IL-10 level, observed in all groups at 24 h after AP, occurred more slowly in the glutamine and saline groups than in the nontreatment group. Possible harmful effects and benefits associated with these cytokines must be interpreted in light of the timing of their release over the inflammatory stages of AP progression. For instance, IFN-γ and IL-2 can activate inflammation, but these cytokines also have benefits related to pathogen clearance that can be relevant in efforts to avoid infection in the later stages of critical aggression [23, 24].
Accordingly, immunotherapy with IFN-γ seems be detrimental in the early stage of AP (when inflammation has harmful effects) and beneficial in the later stage of the disease (when infectious complications and immunoparalysis are dominant causes of mortality) [25–28]. Similarly, decreased IL-2 release and 90% nonspecific mortality were observed after the intraperitoneal administration of lipopolysaccharide in mice with AP, and therapy with recombinant IL-2 reduced lipopolysaccharide-induced mortality in the later stages of the disease [24]. In addition, due to its potent contraregulatory effects, IL-10 has been found to be beneficial in a sodium taurocholate-induced AP model [29]. However, substantial release of this cytokine may hyperintensify its anti-inflammation effect and favor immunoparalysis [30]. Therefore, the dynamics of IFN-γ, IL-2, and IL-10 release over time observed in animals in the glutamine group in this study seem to be protective, enabling the maintenance of immunocompetence for pathogen clearance in the later stages of AP progression.

In our study, marked effects on HSP expression were also observed in the glutamine group in relation to the other groups. This effect included increased liver HSP70 expression and a tendency for increased lung HSP70 expression 12 h after AP, as well as an early significant increase in lung and liver HSP90 expression. Xue et al. [31] reported improvement in the expression of heat shock transcription factor-1 (a master regulator of HSP expression) after parenteral infusion of glutamine in rats. HSP expression may be vital to cellular and tissue protection in the context of stress or injury, as HSPs act as molecular chaperones that stabilize and refold damaged intercellular proteins and prevent intracellular protein aggregation [32]. Indeed, the main metabolic and stress-signaling effects of glutamine in illness and injury seem to occur due to its ability to induce HSP expression [33].

Increases in HSP70 expression induced by glutamine are associated with improvements in survival, tissue injury, and inflammatory response [32]. HSP90 also has cytoprotective properties, but most of its target proteins are kinases and transcription factors that can act as cellular regulators of gene expression, including the transcription of proinflammatory molecules via nuclear factor kappa B [34, 35]. However, we found no systemic detrimental increase in proinflammatory cytokines in parallel with increased HSP90 in the glutamine group. Moreover, compared with the nontreatment group, the increased liver HSP90 expression in the glutamine group occurred early after AP induction and was reduced significantly after 24 h. Because HSP expression increases in response to detrimental stimuli, this observation is highly suggestive of early liver homeostasis in response AP injury in the glutamine group [36].

Parenteral glutamine supplementation was recently associated with high mortality rates in critically ill patients with multiple organ failure [7, 8]. With consideration of systemic disturbances that could culminate in multiple organ failure, our AP model did not confirm this harmful effect, despite our parenteral infusion of high glutamine doses. The most recent multicentric trial showed that parenteral glutamine infusion did not change the mortality rate of patients in the surgical intensive care unit but also did not improve clinical outcomes [37]. In our study, the mortality rate was lower and death occurred later in animals in the glutamine group compared with the other groups, although these differences were not significant.

Our study has some limitations in addition to its experimental nature, which may limit the applicability of the findings to humans. First, glutamine was infused alone and before critical stress. As the release of inflammatory mediators is transient, this strategy was adopted to provide glutamine to cells and tissue in time to observe its modulatory effects on these mediators in our model. However, we do not know whether the same effect would be observed if glutamine were infused with other nutrients and in the presence of stress factors. In addition, parenteral supply of glutamine before critical stress cannot be applied fully in clinical practice. Second, saline was used as a parenteral control for glutamine. Saline hydration can attenuate AP by mitigating changes in pancreatic microcirculation and circulatory disorders of the intestinal wall, which facilitate bacterial translocation and perpetuate the inflammation mechanism [38]. These effects may explain the greater benefits of glutamine on systemic proinflammatory mediator profiles and tissue HSP expression in comparison with the nontreatment group than in comparison with the saline group.

Within these limitations, our data suggest that a high dose of parenteral glutamine protects against stress-induced organ damage by improving cytokine profiles and increasing HSP70 and HSP90 expression in our AP model. These protective effects are of particular interest for the treatment of critically ill patients. Further studies must seek to design a protocol for parenteral administration of glutamine that allows us to take clinical advantage of its potential benefits.

Competing Interests

Dan L. Waitzberg has received speaking honoraria from Fresenius-Kabi, the manufacturer of parenteral glutamine solution.

Acknowledgments

This study was supported by the Fundação de Amparo a Pesquisa do Estado de São Paulo (Process no. 2011/02071-7). The authors thank Fresenius-Kabi for kindly providing the glutamine solution (Dipeptiven), Baxter Laboratory for lending the peristaltic pumps, Farnometrállica for partnership in preparing the parenteral solution, and João Italo Dias França for statistical discussion.

References

[1] P. Déchelotte, M. Hasselmann, L. Cynober et al., “L-alanyl-L-glutamine dipeptide-supplemented total parenteral nutrition reduces infectious complications and glucose intolerance in critically ill patients: the French controlled, randomized, double-blind, multicenter study,” Critical Care Medicine, vol. 34, no. 3, pp. 598–604, 2006.

[2] P. Fürst and P. Stehle, “Glutamine and glutamine-containing dipeptides,” in Metabolic and Therapeutic Aspects of Amino
Mediators of Inflammation

Acids in Clinical Nutrition, L. Cynober, Ed., pp. 613–632, CRC Press, Boca Raton, Fl, USA, 2004.

[3] K. D. Singleton and P. E. Wischmeyer, “Glutamine’s protection against sepsis and lung injury is dependent on heat shock protein 70 expression,” American Journal of Physiology—Regulatory, Integrative and Comparative Physiology, vol. 292, no. 5, pp. R1839–R1845, 2007.

[4] A. L. Buchman, “Glutamine: is it a conditionally required nutrient for the human gastrointestinal system?” Journal of the American College of Nutrition, vol. 15, no. 3, pp. 199–205, 1996.

[5] P. E. Wischmeyer, R. Dhaliwal, M. McColl, T. R. Ziegler, and D. K. Heyland, “Parenteral glutamine supplementation in critically ill: a systematic review,” Critical Care, vol. 18, article R76, 2014.

[6] Q. H. Chen, Y. Yang, H. L. He et al., “The effect of glutamine therapy on outcomes in critically ill patients: a meta-analysis of randomized controlled trials,” Critical Care, vol. 18, article R8, 2014.

[7] D. Heyland, J. Muscedere, P. E. Wischmeyer et al., “A randomized trial of glutamine and antioxidants in critically ill patients,” New England Journal of Medicine, vol. 368, no. 16, pp. 1489–1497, 2013.

[8] D. K. Heyland, G. Elke, D. Cook et al., “Glutamine and antioxidants in the critically ill patient: a post hoc analysis of a large-scale randomized trial,” Journal of Parenteral and Enteral Nutrition, vol. 39, no. 4, pp. 401–409, 2015.

[9] A. R. H. van Zanten, F. Sztark, U. X. Kaisers et al., “High-protein enteral nutrition enriched with immune-modulating nutrients vs standard high-protein enteral nutrition and nosocomial infections in the ICU: a randomized clinical trial,” The Journal of the American Medical Association, vol. 312, no. 5, pp. 514–524, 2014.

[10] M. Bhatia, M. Brady, S. Shokuhi, S. Christmas, J. P. Neoptolemos, and J. Slavin, “Immunoalimentary mediators in acute pancreatitis,” Journal of Pathology, vol. 190, no. 2, pp. 117–125, 2000.

[11] E. Lima-Gonzálves, N. Yamaguchi, D. L. Witzberg, and G. B. Mello Filho, “Nutrition parental in rats: aspect technical,” Acta Cirúrgica Brasileira, vol. 5, pp. 17–22, 1990.

[12] M. S. Galizia, C. C. Alves, É. M. Tamanaha et al., “A new swivel model for parenteral and enteral infusion in rats,” Journal of Surgical Research, vol. 128, no. 1, pp. 3–8, 2006.

[13] A. I. S. Moretti, E. C. S. Rios, F. G. Soriano et al., “Acute pancreatitis: hypertonic saline increases heat shock proteins 70 and 90 and reduces neutrophil infiltration in lung injury,” Pancreas, vol. 38, no. 5, pp. 507–514, 2009.

[14] K. H. Su, C. Cuthbertson, and C. Christophi, “Review of experimental animal models of acute pancreatitis,” HPB, vol. 8, no. 4, pp. 264–286, 2006.

[15] J. Granger and D. Remick, “Acute pancreatitis: models, markers, and mediators,” Shock, vol. 24, supplement 1, pp. 45–51, 2005.

[16] W. de Jager, B. Prakken, and G. T. Rijken, “Cytokine multiple immunoassay: methodology and (clinical) applications,” Methods in Molecular Biology, vol. 514, pp. 119–133, 2009.

[17] G. A. Minkov, K. S. Halacheva, Y. P. Yovtchev, and M. V. Gulubova, “Pathophysiologic mechanisms of acute pancreatitis define inflammatory markers of clinical prognosis,” Pancreas, vol. 44, no. 5, pp. 713–717, 2015.

[18] T. De Campos, J. Derec, J. O. Martins et al., “Pentoxifylline attenuates pulmonary inflammation and neutrophil activation in experimental acute pancreatitis,” Pancreas, vol. 37, no. 1, pp. 42–49, 2008.

[19] X. Chen, S.-L. Li, T. Wu, and J.-D. Liu, “Proteasome inhibitor ameliorates severe acute pancreatitis and associated lung injury of rats,” World Journal of Gastroenterology, vol. 14, no. 20, pp. 3249–3253, 2008.

[20] A. Paszt, T. Takács, Z. Rakonczay et al., “The role of the glucocorticoid-dependent mechanism in the progression of sodium taurocholate-induced acute pancreatitis in the rat,” Pancreas, vol. 29, no. 1, pp. 75–82, 2004.

[21] P. Leveau, X. Wang, Z. Sun, A. Börjesson, E. Andersson, and R. Andersson, “Severity of pancreatitis-associated gut barrier dysfunction is reduced following treatment with the PAF inhibitor lepxipafant,” Biochemical Pharmacology, vol. 69, no. 9, pp. 1325–1331, 2005.

[22] A. Meyer, M. S. Kubrusly, V. M. Salemi et al., “Severe acute pancreatitis: a possible role of intramyocardial cytokine production,” Journal of the Pancreas, vol. 15, no. 3, pp. 237–242, 2014.

[23] J. Zhang, “Yin and yang interplay of IFN-γ in inflammation and autoimmune disease,” Journal of Clinical Investigation, vol. 117, no. 4, pp. 871–873, 2007.

[24] P. Curley, M. Nestor, K. Collins et al., “Decreased interleukin-2 production in murine acute pancreatitis: potential for immunomodulation,” Gastroenterology, vol. 110, no. 2, pp. 583–588, 1996.

[25] R. Amanvermez and G. S. Özbalı, “Clinics related to acute pancreatitis wonder whether IFN-γ can attenuate pancreatic injury or not,” Bosnian Journal of Basic Medical Sciences, vol. 13, no. 3, p. 203, 2013.

[26] H. Meng, J. Gong, L. Fang et al., “Effect of interferon-γ on NF-κB and cytokine IL-18 and IL-27 in acute pancreatitis,” Bosnian Journal of Basic Medical Sciences, vol. 13, no. 2, pp. 114–118, 2013.

[27] T. Hayashi, Y. Ishida, A. Kimura, Y. Iwakura, N. Mukaida, and T. Kondo, “IFN-γ protects cerulein-induced acute pancreatitis by repressing NF-κB activation,” Journal of Immunology, vol. 178, no. 11, pp. 7385–7394, 2007.

[28] B. M. Rau, C. M. Krüger, C. Hasel et al., “Effects of immunosuppressive and immunostimulative treatment on pancreatic injury and mortality in severe acute experimental pancreatitis,” Pancreas, vol. 33, no. 2, pp. 174–183, 2006.

[29] W.-G. Zou, D.-S. Wang, M.-F. Lang et al., “Human interleukin 10 gene therapy decreases the severity and mortality of lethal pancreatitis in rats,” Journal of Surgical Research, vol. 103, no. 1, pp. 121–126, 2002.

[30] M. L. Steinhauser, C. M. Hogaboam, S. L. Kunkel, N. W. Lukacs, R. M. Strieter, and T. J. Standiford, “IL-10 is a major mediator of sepsis-induced impairment in lung antibacterial host defense,” The Journal of Immunology, vol. 162, no. 1, pp. 392–399, 1999.

[31] H. Xue, D. Slavov, and P. E. Wischmeyer, “Glutamine-mediated dual regulation of heat shock transcription factor-1 activation and expression,” Journal of Biological Chemistry, vol. 287, no. 2, pp. 40400–40413, 2012.

[32] A. Saluja and V. Dudeja, “Heat shock proteins in pancreatic injury and inflammation,” Journal of Pathology and Experimental Pathology, vol. 162, no. 1, pp. 392–399, 1999.

[33] H. Xue, D. Slavov, and P. E. Wischmeyer, “Glutamine-mediated dual regulation of heat shock transcription factor-1 activation and expression,” Journal of Biological Chemistry, vol. 287, no. 2, pp. 40400–40413, 2012.

[34] A. Saluja and V. Dudeja, “Heat shock proteins in pancreatic injury and inflammation,” Journal of Pathology and Experimental Pathology, vol. 162, no. 1, pp. 392–399, 1999.
[35] J. E. Bohonowych, M. W. Hance, K. D. Nolan, M. Defee, C. H. Parsons, and J. S. Isaacs, "Extracellular Hsp90 mediates an NF-κB dependent inflammatory stromal program: implications for the prostatetumor microenvironment," *Prostate*, vol. 74, no. 4, pp. 395–407, 2014.

[36] M. R. Jacquier-Sarlin, L. Jornot, and B. S. Polla, "Differential expression and regulation of hsp70 and hsp90 by phorbol esters and heat shock," *Journal of Biological Chemistry*, vol. 270, no. 23, pp. 14094–14099, 1995.

[37] T. R. Ziegler, A. K. May, G. Hebbare et al., "Efficacy and safety of glutamine-supplemented parenteral nutrition in surgical ICU patients: an American multicenter randomized controlled trial," *Annals of Surgery*, vol. 263, no. 4, pp. 646–655, 2016.

[38] P. J. Kinnala, K. T. Kuttila, J. M. Grönroos, T. V. Havia, T. J. Nevalainen, and J. H. Niinikoski, "Pancreatic tissue perfusion in experimental acute pancreatitis," *European Journal of Surgery*, vol. 167, no. 9, pp. 689–694, 2001.