Glutamine’s protection against cellular injury is dependent on heat shock factor-1

Angela L. Morrison, Martin Dinges, Kristen D. Singleton, Kelli Odoms, Hector R. Wong, and Paul E. Wischmeyer. Glutamine’s protection against cellular injury is dependent on heat shock factor-1. Am J Physiol Cell Physiol 290: C1625–C1632, 2006. First published January 25, 2005; doi:10.1152/ajpcell.00635.2005.—Glutamine (GLN) has been shown to protect cells, tissues, and whole organisms from stress and injury. Enhanced expression of heat shock protein (HSP) has been hypothesized to be responsible for this protection. To date, there are no clear mechanistic data confirming this relationship. This study tested the hypothesis that GLN-mediated activation of the HSP pathway via heat shock factor-1 (HSF-1) is responsible for cellular protection. Wild-type HSF-1 (HSF-1+/+) and knockout (HSF-1−/−) mouse fibroblasts were used in all experiments. Cells were treated with GLN concentrations ranging from 0 to 16 mM and exposed to heat stress injury in a concurrent treatment model. Cell viability was assayed with phenazine methosulfate plus tetrazolium. GLN led to a dose-dependent increase in HSF-1+/+ cells in a dose-dependent manner; however, the survival benefit of GLN was lost in HSF-1−/− cells. GLN led to a dose-dependent increase in HSF-70 and HSP-25 expression after heat stress. No inducible HSP expression was observed in HSF-1−/− cells. GLN increased unphosphorylated HSF-1 in the nucleus before heat stress. This was accompanied by a GLN-mediated increase in HSF-1/HSE binding and nuclear content of phosphorylated HSF-1 after heat stress. This is the first demonstration that GLN-mediated protection against a lethal heat stress injury is related to HSF-1 expression and cellular capacity to activate an HSP response. Furthermore, the mechanism of GLN-mediated protection against injury appears to involve an increase in nuclear HSF-1 content before stress and increased HSF-1 promoter binding and phosphorylation. We believe that GLN may act on one or all of the steps leading to activation of an HSP response.

On the basis of this preliminary data, we hypothesized that GLN-mediated protection against a lethal heat stress injury is due to expression of HSF-1 and subsequent activation of an HSP response. We compared the effects of a range of GLN doses and iso-nitrogenous amino acid controls on survival in HSF-1−/− mouse embryonic fibroblasts. HSF-1−/− murine fibroblasts were cultured in high-glucose (4.5 mg/ml) Dulbecco’s modified Eagle’s medium (DMEM), supplemented with 2 mM GLN, 1× iso-nitrogenous nonessential amino acid solution (NEAA), 1× β-mercaptoethanol, 10% fetal calf serum (FCS), 100 IU/ml penicillin, and 100 μg/ml streptomycin. The selection of HSF-1−/− cells was maintained with 500 μg/ml G418 sulfate. Cultured cells were maintained in a humidified 37°C incubator with 5% CO₂.

MATERIALS AND METHODS

Cell culture. All experiments were performed in mouse embryonic fibroblasts. HSF-1−/− and HSF-1+/+ mouse embryonic fibroblasts were a gift from Dr. Ivor Benjamin, University of Utah. HSF-1−/− or HSF-1+/+ murine fibroblasts were cultured in high-glucose (4.5 mg/ml) Dulbecco’s modified Eagle’s medium (DMEM), supplemented with 2 mM GLN, 1× iso-nitrogenous nonessential amino acid solution (NEAA), 1× β-mercaptoethanol, 10% fetal calf serum (FCS), 100 IU/ml penicillin, and 100 μg/ml streptomycin. The selection of HSF-1−/− cells was maintained with 500 μg/ml G418 sulfate. Cultured cells were maintained in a humidified 37°C incubator with 5% CO₂.

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Address for reprint requests and other correspondence: P. E. Wischmeyer, Univ. of Colorado Health Sciences Center, Dept. of Anesthesiology, 4200 E. 9th Ave., Campus Box B113, Denver, CO 80262 (e-mail: Paul.Wischmeyer@UCHSC.edu).
Heat injury for cell viability. HSF-1+/+ and HSF-1−/− cells were seeded in 96-well plates at 1.25 × 10^4 cells/well in minimal media (DMEM plus 10% FCS only). Twenty-four hours later, 0–16 mM GLN or 1 × [NEAA] were added immediately before submersion in a 43°C water bath for 50 min. A second injury condition at 43°C for 45 min was carried out as well, utilizing GLN concentrations of 0–8 mM, because 16 mM GLN did not increase cell survival >8 mM GLN. The plates were sealed with parafilm to prevent leakage. In a separate set of experiments, the cells were treated with glycine, taurine, or GLN, and injured at 43°C for 45 min. Cells recovered at 37°C until a single well reached 50% confluence, the point when all cells were assayed for viability. This occurred at ~48 h in all plates. In a final experiment, uninjured control cells were assayed. Cells were treated as above with 0–8 mM GLN, serum, or NEAA, and no injury occurred. Cells were then assayed for cell viability at 48 h as were the cells after heat stress injury.

**MTS/MIT cell proliferation assay.** Cell viability was measured using the MTS (Promega) and 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT; Sigma, St. Louis, MO) assay according to the manufacturer’s recommendations. For the MTS assay, 1 part phename methane-sulfate was added to 20 parts tetrazolium salt immediately before the solution was diluted 1:5 in phenol red-free DMEM, and was then added to PBS-washed cells. MTS was bioreduced by cells into a colored, soluble formazan product. Absorbance values were read every 60 min for 4 h at 490 nm; references included readings at 650 nm and no-cell blank wells. Higher absorbance values reflect greater cell proliferation/viability.

**Western blot analysis.** HSF-1+/+ and HSF-1−/− cells were seeded at 2 × 10^5 cells/10 cm dish in minimal media. Twenty-four hours later, 0–16 mM GLN were added immediately before the cells were submerged in a 43°C water bath for forty-five minutes. Control plates remained at 37°C. Cells recovered at 37°C for 6 h before being harvested. Western blot analysis was performed as previously described (25). For HSF-70 detection, the membranes were incubated with a specific mouse monoclonal antibody, C92 (catalog no. SPA-810; Stressgen, Victoria, BC, Canada). The membranes were washed and incubated with horseradish peroxidase-conjugated secondary goat anti-mouse antibody (catalog no. 610094; Santa Cruz Biotechnology, Santa Cruz, CA). For HSF-25, membranes were incubated overnight with rabbit polyclonal antibodies specific to HSF-25 (catalog no. SPA-801, Stressgen). The membranes were washed and incubated with secondary donkey anti-rabbit antibody HRP (catalog no. SC-2305, Santa Cruz Biotechnology). Nuclear extracts were then performed for HSF-1 phosphorylation detection. All nuclear protein extractions were performed with ice-cold reagents. Protease inhibitors were added to reagents before use, and the NE-PE Nuclear and Cytoplastic Extraction Kit (Pierce, Rockford, IL) was utilized to obtain nuclear fractions. The fractions were then stored at −80°C and used for Western blot analysis. For nuclear HSF-1 detection, an additional set of Western blot analysis was performed. The membranes were incubated with an anti-HSF-1 antibody (catalog no. SC-9144, Santa Cruz Biotechnology) overnight. Blots were then washed and incubated with a secondary donkey anti-rabbit HRP (Santa Cruz Biotechnology). All Western blots were normalized against β-actin to control for protein loading. For β-actin measurements, the aforementioned Western blot technique was applied utilizing a specific mouse monoclonal antibody to β-actin (catalog no. A5441; Sigma).

**Electrophoretic mobility shift assay.** HSF-1+/+ and HSF-1−/− cells were prepared as for Western blot analysis. Cells were harvested 5 and 15 min postheat stress and then fractionated using the NE-PER kit (Pierce). EMSA for HSF-1 was performed using an oligonucleotide probe corresponding to a heat shock element (HSE) consensus sequence (5′-GGCTCGATGTGCGGCAGTTCG-3′) synthesized at the University of Cincinnati DNA Core Facility. The HSE oligonucleotide was labeled with [γ-32P]ATP using T4 polynucleotide kinase (GIBCO Technologies) and purified in Bio-Spin chromatography columns (Bio-Rad, Hercules, CA). For each sample, 10 μg of nuclear proteins were preincubated with EMSA buffer composed of 12 mM HEPES, pH 7.9, 4 mM Tris·HCl, pH 7.9, 25 mM KCl, 5 mM MgCl2, 1 mM EDTA, 1 mM DTT, 50 ng/ml poly [d(I-C)], 12% glycerol vol/vol, and 0.2 mM PMSF, on ice for 10 min before the addition of the radiolabeled oligonucleotide probe for an additional 10 min. Supershift assays were performed using an anti-HSF-1 antibody (Stressgen) and controlled using an irrelevant antibody to AP-1 (Santa Cruz). Cold competitor assays were performed using 100-fold molar excess of unlabeled HSF-1 oligonucleotide and controlled using an irrelevant oligonucleotide to AP-1.

**Protein–nucleic acid complexes were resolved using a nondenaturing polyacrylamide gel consisting of 5% acrylamide (29:1 ratio of acrylamide:biacylamide) and run in 0.5 × 45 mM Tris·HCl, 45 mM boronic acid, and 1 mM EDTA, for 1 h at a constant current (30 mA). Gels were transferred to Whatman 3M paper, dried under a vacuum at 80°C for 1 h, and exposed to photographic film at −70°C with an intensifying screen.

**Statistical analysis.** All data are expressed as means ± SE. Experimental groups were compared using Student’s t-test or ANOVA, followed by Student-Newman-Keuls test where applicable. Results were considered significant at P < 0.05. Statistical analysis software (version 10.07, SPSS, Chicago, IL) was used for all analyses.

**RESULTS**

**Effect of GLN on cell survival.** In these experiments, we exposed HSF-1+/+ cells and HSF-1−/− cells to a range of GLN concentrations or NEAA control treatments (n = 16/per treatment/per cell type (HSF-1−/− or HSF-1+/+)). Cell survival was measured via MTS assay. GLN led to a dose-dependent improvement in survival in HSF-1+/+ cells at both temperatures tested (Fig. 1, A and B). No benefit was observed from 10% fetal calf serum alone or NEAA. No additional survival benefit was obtained when NEAA was added to 2 mM GLN. All of GLN’s protective effects were lost in the HSF-1−/− cells (Fig. 1, A and B). Furthermore, there was no benefit on cell survival after treatment with increasing concentrations of taurine or glycine (Fig. 2). These control amino acids were chosen because GLN is thought to be an important osmoregulator in the cell (6); taurine has also been found to have this effect (15). Glycine was chosen because preexisting data have indicated that glycine may have cell protective properties (18, 32). Finally, the effect of GLN, NEAA, and serum in uninjured controls was assayed (Fig. 3). This experiment revealed that HSF-1−/− cells consistently had increased proliferation vs. HSF-1+/+ cells. This proliferation was not effected by the dose of GLN administered. These data indicate GLN protects HSF-1+/+ cells after lethal heat stress injury and that this effect is independent of nitrogen content of the media. Furthermore, this protective effect is dependent on HSF-1 expression.

**Effects of glutamine on HSP-70 and HSP-25 expression.** Having demonstrated that GLN attenuated heat-stress injury only in HSF-1 wild-type cells, we hypothesized that it was related to the HSP expression. The lower temperature (43°C for 45 min) was utilized to evaluate HSP expression as the higher temperature (44°C for 50 min) utilized in the survival studies led to inadequate protein yield for analysis via Western blot analysis in HSF-1−/− cells. No increase in HSP expression was observed in cells treated with 1 × NEAA (data not shown). We first studied the dose response of GLN on HSP-70 expression in HSF-1+/+ cells only. We found GLN induced HSP-70 expression after heat stress in a dose-dependent fashion (Fig.
Our data indicated that maximal HSP-70 response occurred at 8 mM GLN. Thus we examined only 8, 2, and 0 mM GLN for the remainder of our experiments. We then examined the effect of GLN on unstressed HSF-1+/+ and HSF-1−/− cells. We found that HSF-1+/+ cells had a baseline HSP-70 expression that was significantly greater than the HSF-1−/− cells. However, the HSF-1−/− cells did express a basal level of HSP-70, as has been previously described (31) (Fig. 4B). We next examined HSP-70 expression in HSF-1+/+ cells and HSF-1−/− cells after heat stress. We found that GLN increased HSP-70 expression in a dose-dependent fashion in HSF-1+/+ cells only, no increase in HSP-70 expression was observed in the HSF-1−/− cells (Fig. 4C). We then examined HSP-25 expression in HSF-1+/+ cells and HSF-1−/− cells after heat stress. We found that GLN increased HSP-25 expression in a dose-dependent fashion in HSF-1+/+ cells only, no increase in HSP-25 expression was observed in the HSF-1−/− cells (Fig. 5). The results demonstrate that GLN leads to a dose-dependent increase in HSP-70 and HSP-25 after heat stress in HSF-1+/+ cells only.

GLN’s effect on translocation, HSE binding, and phosphorylation of HSF-1 in the nucleus. Our next goal was to examine the mechanisms by which GLN may enhance HSP expression.
after heat stress. We focused on the nuclear translocation, HSE binding, and phosphorylation of HSF-1 after GLN treatment and heat stress. Initially, we examined the HSE binding of HSF-1 in nuclear extracts via EMSA. HSF-1+/+ cells were heat stressed and cells harvested at 5 and 15 min. We found that GLN led to a dose-dependent increase in HSF-1 to HSE binding after heat stress injury. A small, but significant increase in HSF-1/HSE binding was found in non-heat-stressed cells treated with GLN for 50 min in the 8 mM GLN-treated cells vs. the cells that received 0 mM GLN. Figure 6A depicts increased HSF-1/HSE binding at 5-min postheat stress. Similar results were obtained at 15 min (data not shown). To determine the specificity of the bands identified in Fig. 6A, we performed cold competitor and supershift assays. Cold competitor assays were performed by coincubation of nuclear proteins from cells subjected to heat shock and 4 nM GLN, with a radiolabeled HSE oligonucleotide plus 100-fold molar excess of a nonradiolabeled HSE oligonucleotide, which eliminated the band (Fig. 6B; lane 2 vs. lane 3). In contrast, when this cold competitor assay was performed with 100-fold molar excess of a nonradiolabeled oligonucleotide corresponding to AP-1, there was no effect on the band (Fig. 6B; lane 2 vs. lane 4). Supershift assays were performed by coincubation of nuclear proteins from cells subjected to heat shock and 4 nM GLN, with a radiolabeled HSE oligonucleotide plus an anti-HSF-1 IgG, which led to a slower migrating band (Fig. 6B; lane 2 vs. lane 5). In contrast, when this supershift assay was performed with an anti-AP-1 IgG, there was no effect on the band (Fig. 6B; lane 2 vs. lane 6). Collectively, these data indicate that the bands seen in Fig. 6A correspond to a HSF-1:DNA complex.

Our next goal was to examine the effect GLN had on phosphorylated and unphosphorylated HSF-1 in the nucleus in non-heat-stressed cells and after heat stress. We examined this in the HSF-1+/+ cells only. We found that before heat stress, GLN treatment at 8 mM led to a significant increase in unphosphorylated nuclear HSF-1 vs. cells not receiving GLN (Fig. 7A). After heat stress, GLN treatment led to a significant increase in phosphorylated nuclear HSF-1 (Fig. 7B). This data indicates that GLN alone appears to lead to a small increase in nuclear HSF-1/HSE binding and a marked increase in unphosphorylated HSF-1 in the nucleus before or without the presence of a heat stress. After heat stress, GLN leads to a significant increase in the binding of HSF-1 to the HSE. GLN also leads to a significant increase in phosphorylated nuclear HSF-1.

DISCUSSION

This data represents the first demonstration that GLN-mediated protection of heat-injured cells appears to be related to the expression of HSF-1 and the ability of the cell to mount an HSP response. These data indicate that GLN exerts a dose-dependent protective effect in HSF-1+/+ cells and this protection is lost when HSF-1 is not present. The mechanism of this effect seems to be related to GLN, causing enhanced transcription of unphosphorylated HSF-1 to the nucleus before stress, increased binding of HSF-1 to the HSE, and enhanced phosphorylation of nuclear HSF-1, which is a vital step in its transcriptional activation.

We have significant previous data implicating activation of the HSP pathway in GLN’s protective effect at the cellular, tissue, and organismal level (19, 20, 25, 28–30). We initially showed that GLN could protect intestinal epithelial cells in a dose-dependent fashion against heat stress and oxidant injury (28). This protection was lost when quercetin, a nonspecific chemical inhibitor of HSP expression, was given. Subsequently, we showed that GLN could enhance HSP expression after endotoxin shock and this was correlated with improved survival (25). We have also shown that GLN can enhance lung
HSP-70 and HSP-25 expression and improve survival after cecal ligation and puncture-induced sepsis (20). This was correlated with improved survival and decreased lung injury. Administration of quercetin blocked GLN-mediated increases in HSP-70 and HSP-25 and attenuated GLN’s survival benefit. Finally, we (33) have recently shown that GLN administration can enhance HSP-70 expression in a pilot study of critically ill patients and this enhanced HSP-70 expression was correlated with decreased length of intensive care unit stay. However, none of these studies could provide a solid mechanistic link between GLN-mediated increases in HSP expression and cellular or organismal protection. This study provides the first data confirming this association. Previous data by Ropeleski et al. (17) implied an association of GLN-mediated protection against apoptosis with enhanced HSP-70 expression (17); however, a significant loss of protection was not observed when small interfering RNA to HSP-70 was utilized in GLN and non-GLN-treated cells. The authors of this study concluded that GLN-mediated protection must work through additional pathways independent of HSP-70 alone. The results of our study indicate that perhaps other HSPs, such as HSP-25, which is known to be a vital protective protein via interaction with the cytoskeleton (4), may play an important role in GLN’s cellular protection. Our data demonstrates that when all inducible HSP expression is inhibited a marked loss of GLN’s protective effects are observed. Other differences between our present data and the aforementioned study by Ropeleski et al. include a different cell line (fibroblasts vs. epithelial cells), different heat stress injury conditions, and higher doses of GLN in our study.

To attempt to determine the specificity of GLN’s protective effects vs. other amino acids, and iso-nitrogenous NEAA control, and serum, we utilized multiple controls. We chose taurine because GLN has been shown to be an important cellular osmoregulator (6); it is possible an osmotic effect of GLN could be implicated in enhanced HSP expression. Taurine is also known as a cellular protective amino acid in various settings (18, 32). However, neither of these amino acids led to significant cellular protection in our model. We chose a NEAA

Fig. 4. GLN enhances heat shock protein (HSP)-70 expression after heat-stress injury (43°C for 45 min). A: HSP-70 expression after an increasing dose of GLN (*P < 0.01 vs. 0 mM GLN). AU, arbitrary units. B: HSP-70 expression in HSF-1+/− and HSF-1−/− cells following varying concentrations of GLN and no heat-stress injury (*P < 0.01 vs. HSF-1−/− cells). KO, knockout; WT, wild type. C: HSP-70 expression in HSF-1+/− and HSF-1−/− cells following varying concentrations of GLN and heat-stress injury (43°C for 45 min; *P < 0.01 vs. 0 mM GLN). Western blot is representative of results from 4 separate experiments.

HSP-70 and HSP-25 expression and improve survival after cecal ligation and puncture-induced sepsis (20). This was correlated with improved survival and decreased lung injury. Administration of quercetin blocked GLN-mediated increases in HSP-70 and HSP-25 and attenuated GLN’s survival benefit. Finally, we (33) have recently shown that GLN administration can enhance HSP-70 expression in a pilot study of critically ill patients and this enhanced HSP-70 expression was correlated with decreased length of intensive care unit stay. However, none of these studies could provide a solid mechanistic link between GLN-mediated increases in HSP expression and cellular or organismal protection. This study provides the first data confirming this association. Previous data by Ropeleski et al. (17) implied an association of GLN-mediated protection against apoptosis with enhanced HSP-70 expression (17); however, a significant loss of protection was not observed when small interfering RNA to HSP-70 was utilized in GLN and non-GLN-treated cells. The authors of this study concluded that GLN-mediated protection must work through additional pathways independent of HSP-70 alone. The results of our study indicate that perhaps other HSPs, such as HSP-25, which is known to be a vital protective protein via interaction with the cytoskeleton (4), may play an important role in GLN’s cellular protection. Our data demonstrates that when all inducible HSP expression is inhibited a marked loss of GLN’s protective effects are observed. Other differences between our present data and the aforementioned study by Ropeleski et al. include a different cell line (fibroblasts vs. epithelial cells), different heat stress injury conditions, and higher doses of GLN in our study.

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Fig. 5. GLN enhances HSP-25 expression after heat-stress injury (43°C for 45 min). *P < 0.01 vs. 0 mM GLN; **P < 0.01 vs. 2 mM GLN. Western blot is representative of results from 4 separate experiments.
amino acid control as this is the standard control utilized in most clinical trials of GLN supplementation in illness and injury (7, 9, 27). No benefit of an isonitrogenous control was observed following heat injury. Interestingly, in uninjured controls, HSF-1 cells had increased cellular proliferation vs. HSF-1 cells. The explanation for this is unclear. This was not effected by the dose of GLN administered either cell type. One limitation of this study is that the HSF-1 cells are more susceptible to heat injury than the HSF-1 cells; however, given that GLN showed no benefit at the lower injury temperature where measurable survival was observed in the HSF-1 cells. This indicates that the survival benefit after GLN treatment in the HSF-1 cells is not due to increased cellular proliferation in the wild-type cells.

An additional question that has remained unanswered is the mechanistic steps by which GLN enhances HSP expression. Our previous data in cecal-ligation and puncture (CLP)-induced sepsis indicated an increase in phosphorylated and

Fig. 6. GLN potentiates HSF-1 DNA binding detected by EMSA in heat-shocked HSF-1 cells. A: GLN affects protein/DNA complex abundance in electromobility shift assays. *P < 0.05 vs. 0 mM GLN in non-heat-stressed cells; **P < 0.01 vs. 0 mM GLN in heat-stressed cells. Data are representative of 3 experiments. B: supershift and cold-competitor EMSA for HSF-1/HSE binding. Data are representative of 3 experiments.

Fig. 7. Effect of GLN on nuclear HSF-1. A: GLN enhances nonphosphorylated HSF-1 expression in nucleus of non-heat-stressed cells. Cells in non-heat-stressed group were treated with GLN for 45 min and then harvested 1 and 2 h later for analysis. *P < 0.01 vs. 0 mM GLN in nonheated cells. B: GLN enhances phosphorylated HSF-1 expression in heat-stressed cells. *P < 0.01 vs. all 0 mM GLN conditions and 8 mM GLN without heat. Data are representative of 3 separate experiments.
unphosphorylated HSF-1 in the lung tissue after GLN administration. Thus we examined the HSF-1 activation pathway in more detail. Activation of HSF-1 is a multistep process, involving trimerization, acquisition of HSE binding activity, and inducible phosphorylation, which results in the transcription of HSP genes (2). Although heat-inducible nuclear localization and DNA binding are necessary steps in HSF-1 activation, these events are not sufficient for full transcriptional competence; HSF-1 can be activated to an intermediate HSE binding state, in which it does not stimulate transcription (1, 16). The second step in the activation of HSF-1 involves protein phosphorylation (8, 16). Thus, we examined the translocation of HSF-1 to the nucleus, nuclear binding of HSF-1 to the HSE, and phosphorylation of nuclear HSF-1 because all of these steps are required to transactivate HSF-1-mediated HSP expression. We found that GLN treatment could lead to increased unphosphorylated HSF-1 in the nucleus in the absence of stress. This is the first description of this phenomenon. This indicates that GLN may be able to “prime” the cell to mount a more robust HSP response by activating HSF-1 and translocating an increased amount of HSF-1 to the nucleus. We next demonstrated that GLN could enhance HSF-1 binding to the HSE. This effect was most pronounced after heat stress. However, a small but significant increase in HSF-1/HSE binding was observed in cells treated with 8 mM GLN in the absence of heat stress. Finally, we showed that GLN could enhance the phosphorylation of intranuclear HSF-1, which is the final step in the HSF-1 transactivation pathway. This data is supported by a recent study by Ropeleski et al. (17), which showed that GLN can enhance HSF-1/HSE element binding in intestinal epithelial cells. This study did not detect an increase in cellular phosphorylated HSF-1 immediately after heat stress. However, they did not examine phosphorylated HSF-1 in nuclear fractions in their study. Furthermore, aforementioned differences in cell line, heat-stress conditions, and GLN doses also exist. A key unanswered question is how GLN treatment leads to manipulation of HSF-1 transcriptional activity. GLN may act on the following emerging drug targets for the activation and regulation of HSP expression: 1) cytoplastic complex of HSF-1 and its repressor HSP-90; 2) activation of HSF-1 and translocation to the nucleus; 3) intranuclear distribution of HSF-1; 4) binding of HSF-1 to the HSE; 5) phosphorylation of HSF-1; 6) nuclear complex of HSF-1 and HSP-90; and 7) retrotranslocation of HSF-1 to the cytoplasm (21). Our data would indicate that perhaps the increase in unphosphorylated HSF-1 in the nucleus might be a key step. On the basis of their results, Ropeleski et al. (17) postulated mechanisms by which GLN may exert its effect on enhanced HSP expression include interactions between HSF-1 and the core transcriptional machinery, the chromatin organization of the 5′-flanking regions of heat shock genes, and/or other trans-factors active upstream in the HSP-70 promoter. However, significant research is necessary to understand this pathway better.

Our current data indicate that GLN-mediated cellular protection after heat stress appears to be dependent on HSF-1 expression and the ability of the cell to activate the HSP pathway. These data may be clinically important because many previous studies (i.e., 4) have demonstrated the benefit of enhanced HSP-70 expression after experimental illness and injury. The concentrations of GLN found to be beneficial in our model (2–8 mM) have been found to be easily attainable in an in vivo model without adverse consequences to the organism (25). These plasma levels of GLN led to significant increases in tissue HSP-70 and survival benefit after injury (25). Furthermore, data from an update of a recent meta-analysis of all clinical trials utilizing GLN as a sole agent in critical and surgical illness indicate GLN treatment shows a strong trend toward the reduction of infectious complication rates in postsurgical patients and a reduction in complication and mortality rates in critically ill patients (Ref. 14; see also www.criticalcarenutrition.org for the latest updated data). Finally, we (33) recently demonstrated that GLN can enhance HSP-70 in critically ill patients and this enhancement of HSP-70 expression was correlated with decreased intensive care unit length of stay. Although it is difficult to extrapolate from an in vitro study to the clinical setting, when the data from this study are examined in light of the aforementioned clinical studies, it is possible that an important mechanism of GLN’s improvement in outcome after illness and injury maybe due to modulation of the HSP pathway.

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