Glutamine in acute lung injury: the experimental model matters

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GLUTAMINE (Gln) has undergone extensive study as a therapeutic agent in the treatment of acute lung injury, sepsis, and shock in the past 15 years. Models ranging from acute cellular injury to animal models of sepsis to human clinical trials have been published (9, 10). The majority of these studies demonstrate a protective effect of Gln against cellular injury, decreased whole organism mortality, and improved clinical outcome (9, 10). The key mechanism underlying the majority of these findings may be Gln’s ability to induce expression of heat shock protein (HSP), which is known to enhance cell survival in the face of injury and attenuate the systemic inflammatory response (9). Cells and animals that have undergone genetic knockout of key HSP pathway mediators are not protected by Gln administration (4, 7). The possible importance of HSP expression in Gln’s clinical benefit in critically ill humans has been shown as well (11). Other hypothesized key pathways of Gln’s benefit in acute lung injury and critical illness include attenuation of peroxisome proliferator-activated receptor (PPAR) activation, buffering of oxidant stress via glutathione generation, protection of the gut barrier, and providing substrate for the appropriate division of immune cells (1, 5, 9).

In their recent article in the American Journal of Physiology-Lung Cellular and Molecular Physiology, Hou et al. (3) examine a unique model of acute lung injury via pulmonary lipopolysaccharide (LPS) injection in the rodent. The authors hypothesize that Gln may increase neutrophil infiltration, oxidant stress, inflammation, and lung injury. As the authors point out, this is a model that is quite different from the more clinically relevant models of lung injury in which a systemic insult, such as peritonitis, leads to lung injury. This model is unique because the injury is not caused by bacteria that are cleared by a competent immune system or via the gut-lymph hypothesis that is felt to be responsible for lung injury following hemorrhage, traumatic injury, or systemic endotoxin injection. The authors’ model of LPS injection into the trachea leads to a direct, chemical-induced, inflammatory injury of the lung in a very short time frame.

The authors base their hypothesis of increased lung inflammation and injury on previous data showing that appropriate physiological Gln levels are vital to adequate immune function, immune cell proliferation, and cytokine expression (5). It is clear that Gln is required for immune cell division, bacterial killing by neutrophils, and prevention of immune cell apoptosis (5). These are all required for bacterial clearance and immune cell response to bacterial invasion. The other key method of note in this trial is that the administration of Gln is part of the chow before injury. The authors do not report chow intake, Gln intake, or Gln plasma levels for the animals in their data. This is a vital omission, because no inferences can be made about the actual Gln intake or Gln levels of these animals at time of lung injury. Furthermore, it is not clear how much chow or Gln was fed after injury. It is likely that the sick animals had minimal intake orally after the injury, but this is not described. This would have been an appropriate point to supplement the animals with intravenous or gavage (enteral) Gln after injury (as has been done in all clinical trials of Gln performed thus far). Thus it is very possible that the animals became Gln deficient in both groups after injury.

The authors’ findings confirm previously known data showing that Gln prevents the fall in white blood cell count after injury and allows for a more rapid neutrophil response after injury. However, this increased neutrophil response did not persist, and by 12 h the control animals had significantly more neutrophils infiltrating the lung. By 24 h the neutrophil counts appear to be similar in both groups. The NF-κB data are largely uninterpretable because the authors only measured whole cell p65 subunit and it is unclear why there would be more whole cell p65 subunit protein at 0 h in the Gln group (before injury) and much less 6 h after injury. The key data that are needed here are those regarding nuclear fraction p65 subunit (to show nuclear translocation of NF-κB) or actual NF-κB binding by EMSA.

The cytokine data show increased cytokine expression in the Gln group at 6 h, which then is below control levels at 12 h. It would have been useful to know whether the anti-inflammatory cytokine expression (such as IL-10) was also elevated in the Gln group given the rapid resolution of inflammation in the Gln group (which the authors’ data showed was reduced to zero expression of the measured cytokines at 12 h). There is slightly more septal thickening in the Gln group, but the difference, which appears to be about 0.1% at 24 h after LPS and 0.2% at 2 wk, is probably not clinically relevant. Finally, the thiobarbituric acid-reactive substances (TBARS) data are interesting because they show that there is a large increase in oxidant stress at 6 h in the control animals and a reduction in oxidant stress in the Gln animals from preinjury levels. This is curious, because the largest increase in cytokine expression and neutrophil infiltration appears to occur before 6 h in the Gln-treated animals, which would lead one to hypothesize that the Gln animals would have much more oxidant stress at 6 h than the control animals. However, based on the authors’ data, the control animals have very few neutrophils and essentially no lung cytokine expression at 6 h. The increased TBARS levels at 12 and 24 h likely represent an exhaustion of the Gln supply that buffered the oxidant stress at earlier time points. The lack of oxidant stress at 12 and 24 h in the control group contradicts the authors’ conclusion that the oxidant stress is likely coming from the neutrophils infiltrating the lung. The authors’ data show that at 12 h there are more neutrophils infiltrating the lung in the control group and at 24 h the numbers are similar. It could be possible that more Gln led to a greater respiratory burst of the neutrophils, but then, as the authors correctly state, there should more Gln to generate glutathione and buffer the stress. This reader is unclear as to how to explain these contradictory data. In the end, despite 110 animals being

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studied, no statistical difference in mortality was observed between the two groups. Thus the inflammatory, lung injury, and oxidant marker changes did not impact clinical outcome.

Finally, it is disappointing that no HSP levels were measured in the lung, because this might have indicated the adequacy of Gln tissue levels at time of injury. This is due to the fact that adequate Gln levels are required for appropriate HSP generation (5, 9).

In conclusion, the key to these data comes back to the model of injury. Gln is, as the authors correctly point out, key to the body mounting an immune response to invading bacteria and prevention of the end-organ injury that systemic or tissue-level infection can cause. This model does not account for this key function of Gln because the injury is “chemical” in nature and does not allow for the appropriate prevention of bacterial invasion and/or appropriate bacterial clearance, toward which early neutrophil activation is targeted. Furthermore, in systemic injury Gln likely prevents the bacterial invasion of the gut wall and thus prevents the activation of the gut immune system. This is vital, because much of the lung injury that follows systemic injury (particularly in the rodent) has been shown to be due to prevention of lymph toxicity, which subsequently leads to acute lung injury (2). Again, this model of direct lung tissue injury by LPS does not allow for the normal protective physiological processes, which Gln mediates, to function appropriately. It is possible that HSP expression could be induced by this injury process, but it is unclear whether enough Gln was given to provide for this expression. Furthermore, it is possible there was not sufficient time for transcription and translation of protective HSPs before the rapid lung injury this model induces. The difference in the authors’ method of Gln administration is also key, because our laboratory (6–8) and others (1) studying Gln have historically given Gln as a specified dose intravenously or by gavage to ensure accurate dose delivery of the amino acid. The authors’ model of Gln feeding with no reporting of chow intake or Gln levels does not allow for any interpretation of their results relative to previous published data. Overall, this is an important set of data as it confirms that Gln is vital to a rapid immune response to prevent infection following injury. However, the lack of key data with regard to Gln intake and NF-κB activation by nuclear fractionation or EMSA, the contradictory data regarding oxidant stress versus tissue neutrophil infiltration, and a non-clinically relevant lung injury model lead to difficulty in interpreting how these data fit with previously published findings regarding Gln and acute lung injury. I applaud the authors on this work and hope that they continue their research in the field of Gln therapy for acute lung injury. In the future, I encourage the authors to employ a more clinically relevant model of acute lung injury and to utilize precise and measurable Gln doses so their results may be readily compared with the existing data in the field.

REFERENCES