Reviewer's report

Title: Glutamine Therapy Reduces Endothelial Adhesion of Sickle Red Blood Cells to Human Umbilical Vein Endothelial Cells

Version: 1 Date: 21 January 2005

Reviewer: Gilda Barabino

Reviewer's report:

General

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Major Compulsory Revisions (that the author must respond to before a decision on publication can be reached)

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Minor Essential Revisions (such as missing labels on figures, or the wrong use of a term, which the author can be trusted to correct)

1. The study reported in this manuscript builds on previous work in which oral l-glutamine supplementation of sickle patients resulted in enhanced RBC redox capacity in vitro, and was accompanied by self-reported clinical improvement (Niihara, et al, Am J Hematol, 58:117, 1998). In the present study, RBC adhesion to LPS-activated HUVEC was examined in the presence of autologous plasma under static conditions. Two separate investigations were undertaken: a cross-sectional study that compared RBC adhesion between supplemented and unsupplemented populations, and a longitudinal study that measured RBC adhesion in the same individuals before and after supplementation. The impact of the results of this study would be higher if it was possible to directly compare the findings from the cross-sectional and longitudinal studies and if the sickle RBCs were tested for redox potential as was done in previous work by the primary author. Divergent methods for separating RBC and culturing endothelial cells for the two studies impede the ability to make direct comparisons and maximize the strengths of the study. The manuscript would be strengthened by inclusion of a rationale for the choice of assays and use of LPS and/or plasma to treat endothelial cells in the cross-sectional and longitudinal studies. With further discussion of the rationale of the experimental design as noted, this study could be published without additional experiments, however, it would be strengthened by a parallel study of the RBC redox capacity that could be tied directly to cell adhesion. It would also be stronger if the investigators were to employ the adhesion assay that was used in the cross-sectional study for the entire project.

2. Throughout the manuscript, the level of RBC adhesion is incorrectly referred to as an adhesion "rate". Rate implies a number of events per unit time, yet the measures of adhesion that are presented are defined as ratios of SS (+/-gln) over AA and therefore should be unitless.

3. The captions for Figures 1 and 2 that describe processing of the HUVEC and RBCs do not match that given in the text. It is unclear which description is more accurate.

4. The categories of RBC adhesion in the longitudinal study are quite detailed (“at the edges”, “in the gaps”, and “on EC”), however, this level of detail does not appear in the results or discussion. If the experiments took place within 24 hours of the cultures achieving confluence, gap formation would not be expected, therefore it would be difficult to distinguish RBC adhesion in gaps and at edges. This issue needs to be clarified. The authors report that 16 fields were counted in at least 4 separate experiments, however, Figure 3 lacks error bars. Figures 1 and 2 also lack error bars though
p-values are reported and the text states that statistical analysis was performed.

Discretionary Revisions (which the author can choose to ignore)

What next?: Accept after minor essential revisions

Level of interest: An article of importance in its field

Quality of written English: Needs some language corrections before being published

Statistical review: No

Declaration of competing interests:

I declare that I have no competing interests