Author's response to reviews

Title: Decreased erythrocyte nucleoside transport and hENT1 transporter expression in glucose 6-phosphate dehydrogenase deficiency

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Author's response to reviews: see over
Dear Sir;

In spite of the negative appraisal given at the end of the ‘Editors Comments’ I was encouraged by the BioMed Central Editorial Office to submit an amended manuscript. I submit point-by-point responses to the Editors Comments below.

Editor’s comment

‘The revised manuscript is certainly enhanced in response to the reviewer comments. The findings described in the manuscript are certainly interesting and are hypothesis generating. However, additional experiments are needed to explore the underlying mechanisms that explain the decreased hENT1 expression on RBCs and to explain the relationship between decreased hENT1 and decreased nucleoside uptake in G6PD-deficient RBCs. The findings are not sufficient to publish as a full paper.’

The Authors thank the Editor for thorough consideration of the manuscript. We present some point-by-point responses below.

Editor’s comment:
1. “… additional experiments are needed to … explain the relationship between decreased hENT1 and decreased nucleoside uptake in G6PD-deficient RBCs.”

Authors’ response:
I believe that this point has been fully addressed in the studies described in this manuscript; the relationship between decreased hENT1 and decreased nucleoside uptake in G6PD-deficient RBCs is intrinsic to much of the discussion section; in support of this assertion I submit some further remarks focused on this point (Author’s remarks) below.

I regret that a statement in the Conclusions section that makes explicit reference to this finding was not included in the original manuscript.

Rectification of this deficiency has been undertaken through inclusion of a revision to the Conclusions section to include sentence given in lines 271-275 in the revised manuscript to clarify and emphasize this point.
Authors' remarks concerning Editors Comments (1):

Kinetic studies undertaken by a number of research groups, and dating from the 1970’s and 1980’s, have established that in human erythrocytes physiological nucleosides traverse the membrane by way of a saturable equilibrative carrier system showing reversible inhibition by nanomolar concentrations of nitrobenzylthioinosine (NBTI, NBMPR) and related compounds (for example see Cass and co-workers; 1972, 1973, 1974, below). Kinetic evidence suggested that only a single transport pathway was responsible for this ‘facilitated diffusion’, and demonstrated that transmembrane passive diffusion is very slow for physiological nucleosides. Use of high affinity inhibitors subsequently allowed identification and cloning of the transporter polypeptide, hENT1 (Griffiths et al, 1997), that mediates nucleoside fluxes in human red cells.

Thus, an extensive and well established body of evidence predicts that a decrease in expression of hENT1 in the red cell plasma membrane would result in decrease in nucleoside transport capacity. The results presented in this manuscript strongly support this prediction in that they demonstrate decrease in hENT1 expression with concomitant decrease in uridine transport, in G6PD deficiency; consequently, the results also indicate that no major changes to the catalytic activity of the transporter (for example; change in turnover number, sequestration from the plasma membrane, oxidative inactivation, .... etc.) have occurred in the G6PD deficient samples studied.


Editor’s comment:
2. “However, additional experiments are needed to explore the underlying mechanisms that explain the decreased hENT1 expression on RBCs”.

The Authors share the Editor’s interest in understanding the biochemical mechanism(s) that have produced the selective decrease in hENT1 discovered in this study. However, a significant new set of investigations with a revised scientific objective, “explore the underlying mechanisms”, will require new resources (reagents etc.).
Briefly, to address the Editor’s comment we would propose to take blood samples from G6PD deficient and control individuals and use a conventional density step gradient separation to produce reticulocyte-enriched and reticulocyte-depleted red cell fractions. Immunoblot quantitation of hENT1 and Glut1 expression in membrane preparations from both fractions to be undertaken as described in the manuscript, and a ratio for hENT1 expression between reticulocyte enriched and depleted fractions determined for both G6PD deficient and control individuals. Statistical difference in the hENT1 reticulocyte:erythrocyte ratio between G6PD deficient subjects and controls would argue that the cause of the decrease in membrane hENT1 expression is associated with changes in cell membrane remodeling in G6PD deficiency, rather than differences at transcriptional or translational levels for this polypeptide. Since Glut1 expression was not different in G6PD deficient and control individuals, this may be used to normalize results for experimental variation in separation, transfer and detection efficiencies.

We share the interest of the Reviewers in the potential for variation of membrane properties with differences in genotype and would very much like to employ a commercial multiplex PCR kit to confirm that the G6PD deficient subjects are all of Mediterranean genotype.

Given the positive comments from Reviewers we have investigated the logistics of undertaking this investigation, but it is not possible within a short timescale;

I. We require institutional approval of an amendment to our Ethics approval to encompass use of substantially larger blood samples (minimum about 20-25 ml) from G6PD deficient and control subjects than the small hematological samples that were approved by the Ethics Committee for this initial study. As noted in Methods Section, none of the subjects in this study exhibited overt reticulocytosis. 

II. We need time to obtain some funding support for PCR genotyping; quotations for a suitable kit have so far been subject to minimum order value since Kuwait is not a regular market and administrative and processing costs for import are significant.

We find ourselves with an interesting discovery – a decrease in nucleoside transporter expression that decreases red cell nucleoside permeation; unfortunately experimental exploration of the mechanism(s) responsible requires further time and resources and thus cannot be completed in a short time for rapid resubmission of the manuscript establishing the phenomenon.

Editor’s comment:
3. The findings are not sufficient to publish as a full paper

The Authors thank the Editor, Editorial Office, and Reviewers for their time and constructive suggestions.

We appeal to the Editor in the hope that further contemplation of the revised manuscript might allow reconsideration of this initial assessment.
Publication of our findings would inform the scientific community that in G6PD deficiency erythrocytes show changes in nucleoside permeability, even in the absence of exogenous stress, and thus stimulate further research that may elucidate processes that cause membrane changes in G6PD deficiency.