Reviewer's report

Title: Triggering of suicidal erythrocyte death by uremic toxin indoxyl sulfate

Version: 1 Date: 11 June 2013

Reviewer: Christos Stournaras

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In this paper Ahmed et al. analysed the effects of the uremic toxin indoxyl sulfate on the suicidal erythrocyte death (eryptosis). The authors show that a 48 hours exposure to indoxyl sulfate significantly increases cytosolic Ca2+ activity ([Ca2+][i]), significantly increases ceramide formation, decreases cell volume and increases phosphatidyl.serine exposure at the erythrocyte surface. The effect of indoxyl sulfate on phosphatidylserine exposure was virtually abolished in the nominal absence of extracellular Ca2+. The authors conclude that indoxyl sulfate stimulates suicidal erythrocyte death or eryptosis thus contributing to the accelerated loss of circulating erythrocytes.

The present paper addresses an important topic. The experiments appear carefully done, the paper is well written and the conclusions of the paper are well supported by the results.

Minor Essential Revisions.

Several points need to be addressed prior to publication:

1. The concentrations of indoxyl sulfate required to stimulate annexin V binding are apparently lower than those required to increase Fluo3 fluorescence or forward scatter. The authors should comment on this seeming discrepancy.

2. Inspection of Fig. 2 suggests that even at the highest concentrations applied, indoxylsulfate does not elicit hemolysis. The authors should comment on the lack of hemolysis.

3. The authors should comment on the putative quantitative contribution of eryptosis to anemia of patients with CKD.

4. In the discussion the authors focus on cytosolic Ca2+ as the trigger of eryptosis by indoxyl sulfate. This may be an oversimplification. The authors should discuss additional mechanisms possibly involved, such as ceramide or p38 MAP kinase.

5. Several typos should be corrected, e.g.:
   ...previously been shown... instead of ...previously bveen shown...
   ... vascular wall at least in ... instead of ...vascular wall at leasdt in...
   ... adherence to the vascular wall ... instead of ...adherence ot the vascular wall...
Level of interest: An article of outstanding merit and interest in its field

Quality of written English: Acceptable

Statistical review: No, the manuscript does not need to be seen by a statistician.

Declaration of competing interests:
No competing interest to this paper