Author's response to reviews

Title: Development of an invasively monitored porcine model of acetaminophen-induced acute liver failure

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Author's response to reviews: see over
Dear Sir/Madam,

This letter accompanies the revised submission of a manuscript entitled, “Development of an invasively monitored porcine model of acetaminophen-induced acute liver failure.” We would like to thanks both reviewers for their positive and constructive comments on the submission, and have incorporated changes as detailed below.

**Editorial request:** Please ensure you document a statement of ethical approval (including the name of the body which gave approval) in the Methods section of the manuscript.

We have added this into the text. Approval was given by the Home Office under the Animal (Scientific Procedures) Act 1986 as per Project licence PPL 60/2389. This has been added to the methods section. Page 5.

**Reviewer 1 (Professor H Hodgson)**

Point 1: Anaesthetic strategy and fluid requirements
Anaesthesia was induced with Ketamine and Midazolam, and subsequently maintained with isoflurane and nitrous oxide according to tidal volume. Background hydration was maintained at a rate of 2mls/kg/hr using a combination of 0.9% Normal Saline and 5% Dextrose according to electrolyte results from arterial blood gas sampling and urine output. Boluses of colloid (Gelofusine) were administered for episodes of hypotension. Page 5.

Point 2: Figure 4
On checking the data we are certain that the pulmonary artery occlusion pressure is not mis-labelled. It is the pulmonary vascular resistance which is different between the two groups, being higher in the injury group.

Point 3: Lack of lung histology
Lung histology is not available as the samples were not adequately inflated before fixation, thus we were unable to assess them satisfactorily. We have addressed this shortcoming in the text. Page 9.

Point 4: Fisher’s ratio
We agree with the caution regarding over-interpretation of Fisher’s ration and have added a comment to that effect. Page 10.
Point 5: Reason for lack of intra-cranial pressure changes
We have incorporated this suggestion into the discussion section. It may reflect timing issues, as well as the lack of sepsis, or indeed the fact that toxicity models such as this are quite different to those models involving hepatic ischaemia. Page 10.

Point 6: Variation in survival
This study highlights many of the issues that confront researchers working in the field of acetaminophen induced liver injury, namely the inherent variation in response. Whilst the time-frames of death in this survival are reasonably homogeneous there is still an element of variation which we have reiterated in the revised manuscript. Page 9.

Point 7: Criteria for death under anaesthesia
As this work was governed by Home Office regulations animals which were deemed to be critically unwell were euthanased despite being under general anaesthesia. Refractory hypotension and/or difficulties in ensuring successful ventilation led to a joint decision to euthanase pigs. With hindsight use of inotropes or more sophisticated ventilation equipment may have prevented or delayed cardiovascular collapse although the costs of this may be prohibitive. Page 7.

Reviewer 2 (Professor A Carraro)

Point 1: Features of syndrome of ALF and acetaminophen levels
The purpose of measuring acetaminophen levels was to prevent methaemoglobinaemia which occurs in animals such as pigs and cats, but not in humans. The association between acetaminophen levels and liver injury has previously been examined and was not within the scope of this study. The numbers of pigs involved would not allow us to draw robust conclusions about any association.

The syndrome of ALF incorporates a range of features and whilst cerebral oedema is one of those it is not mandatory. We observed biochemical changes which were compatible with encephalopathy but no changes in ICP or histological evidence of cerebral oedema. As detailed in Point 5 for Reviewer 1 there are many possible reasons for this. Extending the period of monitoring from 28hours onwards was not possible due to clinical deterioration of the pigs as detailed.

Point 2: Meaning of critically unwell.
This point has been addressed in Point 7 for Reviewer 1.

Point 3: cytochrome p450 levels
This has been re-written to make it clearer. All animals in the in vivo study received phenobarbital. Page 7.

Point 4: Animal survival
Microbiological analysis was performed routinely for all pigs. This has been included in the methods section. Page 5.

Point 5: Cardio-respiratory evaluation
The comparison is against uninjured pigs and a sentence to that effect has been added to the results section. Page 7.
Point 6: Fig1 is unclear
We considered this was the best way of presenting the data on acetaminophen and methaemoglobin data. It highlights the variations that can arise in levels despite accurate monitoring. We note that Reviewer 1 passed no comment on the figure and feel that it is fine as it is.

Point 7: Figure 2 severity score
We agree with the comments and have qualified the figure legend to provide extra clarity on how we derived this score. The histology was scored by a pathologist who felt that +++ covered severe hepatic coagulative necrosis, ++ covered moderate hepatic coagulative necrosis and + covered mild hepatic coagulative necrosis. Similarly the scoring scale for renal injury covered the range from mild (+) to moderate (++) to severe (+++) tubular necrosis. Page 16.

Point 8: Figure 4 difference between PAOP.
This difference was present before acetaminophen was administered and reflects random variation between animals.

Point 9: The word albumin is missing from legend
We thank the reviewer and have added the missing word. Page 19.

Point 10: Description of anaesthesia.
We agree and have added this in as per Point 1 for Reviewer 1.

We hope this addresses the changes required.

With kind regards.

Yours sincerely,

Phil Newsome