Author's response to reviews

Title: Red wine and components flavonoids inhibit UGT2B17 in vitro

Authors:

Carl Jenkinson (k0713450@kingston.ac.uk)
Andrea Petroczi (A.Petroczi@kingston.ac.uk)
Declan P Naughton (D.Naughton@kingston.ac.uk)

Version: 2 Date: 24 July 2012

Author's response to reviews: see over
Dear Editor,

Thank you for the useful comments from the referees and the opportunity to resubmit the manuscript.

Please find responses below. We have addressed all of the points as shown.

Kind regards
Declan Naughton

Version: 1 Date: 21 May 2012
Reviewer: Lena Ekström
Reviewer's report:
The manuscript is very well written and the finding that red wine inhibits UGT2B17 is an interesting finding. The aims are well defined and the methods are clearly described.
Thank you.

However there are some major concerns that the author´s needs to address before it can be acceptable for publication.

Major complementary revisions:
1) The title is too speculative, considering this is an in vitro study. I strongly suggest the authors to remove the last part of the title “and potentially increase circulating testosterone levels”. They should instead clarify in the title that red wine flavonoids inhibit UGT2B17 in vitro.
This has been amended as suggested.

This reference has been changed.

3) Methods (page + relevant figures and table): Please use Mean ± Standard Deviation (SD) instead of Standard Error of Mean (SEM), since SEM only describes the standard deviation of the sample distribution, not the variability in your sample.
Thank you. This has been changed throughout.

4) For figure 1 and 2 the results presented are from a duplicate sample. If I understand correct the experiment was only performed at one time point? If so, the experiment should be repeated to include results from at least two independent experiments.
The data shown are from two independent experiments.

It would be more scientifically correct if the results are accompanied with statistical analyses which now are missing.
These have been added.
Moreover, why was 100 uM testosterone used? This level is in line with our previous published study (on green tea) to facilitate comparison. It also facilitates assessment over a range of inhibition levels below the concentration that is excluded owing to the competitive nature of inhibition.

Minor revisions:
5) Figure 3 – the inhibition of different phenolic compounds were studied for 60 min. In respect of their finding that a more pronounce inhibition was found after 2 hours it is surprising that they choose 60 min for the inhibition study – what was the reason for choosing 60 here?
In our previous report on green tea, we studied the inhibition between 30-90 minutes. For this paper, there was a major inhibitory effect observed at 60 minutes in all cases for red wine. However, for the addition of red wine at 8% this inhibitory effect was substantially greater at 2 hours. In addition, for the individual components we found reductions in glucuronidation in the range of ca. 2-70% at the 60 min time point. Thus, for comparative purposes (to the previous paper) and to capture a wide range of inhibition effects with individual components, we chose to use the 60 min duration.

6) Discussion: In addition to the animal studies referred to here, ethanol has been linked to increased testosterone excretion in humans (i.e. it could elevate your T/E ratio) (Falk, Palonek et al Clin Chem. 1988 Jul;34(7):1462-4. and Grosse, Anielski et al., Drug Test Anal. 2009 Nov;1(11-12):526-30). Ethanol of >1 g/kg body weight significantly increases the T/E ratio and it was speculated that this was due to phase I enzymes, which is consistent with your finding that ethanol did not affect UGT2B17 in amounts # 1%. It is worth thinking about if you are going to perform an in vivo study using red wine.
Thank you.

7) Discussion (paragraph 4) “Whilst it has been found… “: Reference 29 should be replaced with reference Ekström et al (Pharmacogentic and genomics 2011Jun 21(6):325-32 (as serum concentrations were not measured in reference 29).
Thank you. This reference has been substituted.

In addition a very large study has measured serum testosterone concentrations in 1000 young and 1000 elderly men and not found any significant differences between ins/ins+ ins/del and del/del (Swanson et. al., J Clin Endocrinol Metab. 2007 Dec;92(12):4878-82.) and this large study should be added.
Thank you. This reference has been added (as new ref 31).

8) Introduction: Is reference no 14 relevant?
This reference has been substituted with a more apposite recent reference.

Level of interest: An article of importance in its field
Thank you.

Version: 1 Date: 4 July 2012
Reviewer: Martin Fanzone
Reviewer's report:
Discretionary Revisions
Level of interest: An article of importance in its field
Thank you.

Quality of written English: Acceptable
Statistical review: No, the manuscript does not need to be seen by a statistician.
Declaration of competing interests:
I declare that I have no competing interests