Reviewer’s report

Title: Dietary Diallyl Disulfide supplementation attenuates ethanol-mediated pulmonary vitamin D speciate depletion in C57Bl/6 mice.

Version: 4 Date: 29 April 2015

Reviewer: Adrian Gombart

Reviewer’s report:

Major Compulsory revisions
1. The authors were unresponsive to several of the major compulsory critiques from the two reviewers. Those that required only written changes to the manuscript were accepted with the exception of adding gene expression data in Figure 5. This reviewer’s major compulsory critique 1 asked the authors’ to cite Shankar et al., 2008 and discuss it in relation to the findings of this work. Also, the Kent et al. paper should be discussed as both paper show that alcohol affects the metabolism of vitamin D. In their response the authors wrote nearly a page about why they are not going to cite the Shankar paper or discuss it in relation to their findings. This could be considered a form of “fraud” as the authors appear to be excluding or avoiding publications that are relevant to their study for the purpose of making their own findings appear more unique or novel. The organ systems may be different, the doses may be different, and the animal models may be different, but the fact that vitamin D metabolism is affected by ethanol in both the previously published studies and in this manuscript demands a fair and balanced discussion of the relevant published studies. Why not incorporate the lengthy rebuttal into the paper’s discussion? The readers of this manuscript should be informed of these other studies so that they can refer to them if they wish. The inclusion of the citation was listed as compulsory not discretionary. Also, the authors were asked to examine kidney expression of CYP27B1, CYP24A1 and CYP2E1 in the liver to determine if mice have a similar renal response as rats. This was not done and no reason was given why.

2. In Critique 3, this reviewer asked for data that authors acknowledge they have and would “assist in elucidating these observations presented in this manuscript”, but refuse to incorporate it into the current manuscript for the reason of splitting the studies into two papers. The authors should incorporate the data from the human cells and write a much more compelling single manuscript. Let the editors and reviewers decide if the manuscript is to large. Alternatively, the authors could measure CYP enzyme activities as requested by the other reviewer and put the human data into a second manuscript. This would address a major problem with this manuscript in that it is purely an observational study of a phenomenon in mice and offers little in the way of a mechanism. There is a lot of speculation with heavy reliance on prior studies and in some cases the citations don’t seem to support the argument being made (see Critique 4 in the original review and below). Measuring the activity levels of the CYP enzymes would provide at least
some explanation or showing that it occurs in humans would at least take it out of the realm of mice. An alternative set of experiments would be to identify the metabolites of CYP2E1 that are increasing catabolism of the two forms of vitamin D or performing studies similar to those presented in the Shankar, 2008 paper.

3. In Critique 4, the use of citations did not seem to support the statement’s being made. It does not appear that this has been rectified although the authors stated it was. Please see my original critique and address this.

4. Additional comments

The manuscript has many grammatical errors and uses language that is incorrect. For example “vitamin D speciates” is used. I have never seen this sort of language used in reference to metabolites of vitamin D. This must be corrected throughout the manuscript.

Added text highlighted in yellow should be reread and corrected for errors. In particular, the new section in the Material and Methods that describes the use of qPCR arrays. It seems that it was lifted from the manual supplied by the manufacturer. It describes in gruesome detail how qPCR works. This is completely unnecessary in a scientific journal. Also, it goes from past tense to present tense and back. It is poorly written.

A citation is missing in this section of the text.

225 related morbidity[42], and mortality[43] (Moss 1996). We believe that the deleterious
226 health endpoints associated with the alcoholic lung involve ethanol’s propensity to
227 disturb vitamin D presence in the lungs.

The authors should cite Kent et al. and Shankar et al. as their studies would support this statement because they showed that ethanol impacts metabolism of vitamin D.

In the following section after “1) Control” it should be “2) Ethanol”

258 Study C utilized the same methodology as Study A (N=7) with a total of 4 treatment
259 groups: 1) Control, 4) Ethanol (20%) 3)DADS 0.05µg/1g DADS (~0.15µg/mouse/day) (260 4) Ethanol (20%) and 0.05µg/1g DADS (~0.15µg/mouse/day). DADS

The following text reads as if it belongs in the results section:

A percentage of orally consumed ethanol diffuses from the bronchial circulation into the
309 lumen of the airways, where some of the ethanol is then expelled during expiration. The
310 remainder of the ethanol in the lumen condenses onto the mucosal/epithelial
layer of the large airways. This can lead to localized high levels of ethanol exposure [13]. Due to this localized exposure to ethanol, we assayed lung tissue and bronchial alveolar lavage fluid to determine the effect of chronic ethanol exposure on inactive and active vitamin D availability in the lungs of ethanol-fed mice.

This following section of text proposes that studies on VDR and RXR function should be performed, but instead metabolism is studied. Again, it would be more appropriate to cite the Kent and Shankar studies. Also, in the manuscript a period is required after the word “activity”.

coats the pulmonary epithelium [13]. As an inducible metabolic pathway for ethanol metabolism, CYP2E1 creates increased amounts of oxidative stress[14] and acetaldehyde, which inhibits Retinoic X receptor (RXR). The RXR is a co-activator with VDR to induce VDRE [15-17]. We believe that extended exposure of the pulmonary epithelium to ethanol can possibly affect VDR function antimicrobial peptide activity

**Level of interest:** An article of importance in its field

**Quality of written English:** Not suitable for publication unless extensively edited

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

**Declaration of competing interests:**

No competing interests.