Reviewer’s report

Title: DanQi Pill protects against heart failure through the arachidonic acid metabolism pathway by balancing different cyclooxygenase patterns

Version: 2 Date: 20 November 2013

Reviewer: Gary Asher

Reviewer’s report:

The manuscript describes a set of studies that used a predictive model to estimate biological pathways that would be affected by DanQi pill and then used a rodent model of heart failure to estimate the putative effects of the prediction modeling. The use of such a prediction/validation scheme is interesting, especially when considering the multi-targeted effects of complex botanical formulations. A well described study of this methodology is welcome. However, there are many important missing elements of the studies contained in this manuscript, which makes it very difficult for any reader to fully understand the studies that were conducted or to evaluate the meaning of the reported results.

Background

The leading cause of mortality worldwide is ischemic heart disease, not heart failure (http://who.int/mediacentre/factsheets/fs310/en/). I’m sorry, but you have not done your homework with this background section.

Roifman, et al., (citation 2), is a systematic review of the association between chronic inflammatory diseases and coronary artery disease; the sentence that cites this study has nothing to do with either chronic inflammatory disease or CAD. Perhaps this citation has been misplaced, although other citations in the background section also appear to be misapplied (eg. citation 1, Panza, et al. is about inducible MI and LV dysfunction, not the leading causes of mortality worldwide).

The term effective (paragraph 2) implies both efficacy (preferably demonstrated in large, well done, randomized controlled trials), and the ability to implement the efficacious treatment in real world environments beyond the RCT. Citation 5 appears to be review article on pattern differentiation in Chinese medicine, which seems a bit far-fetched to support the authors claim that ‘some herbal formulas have been proven to be effective’.

I suggest that this background section be rewritten with reference to appropriate, primary studies where appropriate.

Methods

There are many acronyms used throughout the manuscript. Please spell them out the first time they are introduced to the reader (eg. PNS, KEGG, SMILES,
Information on providing names of mouse strains can be found here (http://www.informatics.jax.org/nomen/)

Please more clearly describe the sham, model, and DQP group. Also state in methods how many rats were in each group and the method used to allocate them to groups.

What does the phrase ‘...within a short period of time.’ at the end of 2.2 refer to?

Please avoid non-specific indicators such as ‘... other indicators.’ (section 2.3, paragraph 1). If the indicators are important, then please list them.

What was the PST 65A sector scanner used to do?

Section 2.4 includes ‘dose consideration’ in the section title, however there is no information on the dose used, nor how the choice was made and justified. Importantly, how was product integrity independently tested/verified? Which plant parts were used? Was the hot water extraction an infusion or decoction? How was the water extract concentrated?

Section 2.5.2 begins ‘The tissue....’ This is the first time the reader learns that tissue, other than whole blood, has been collected for analysis (ie. section 2.2 only mentions blood). What are the tissue samples, how and when were they collected? What method was used for protein extraction?

Section 2.6, Statistical analysis. This section provides very little detail about the very important statistical procedures performed during these studies. Although I am unfamiliar with DrugCIPHER-CS, other genome-wide studies typically need to adjust for false discovery (FDR). How was this done for the current methods? How was the value of 0.1% targets determined? How did you choose the final 7 pathways to be analyzed? What was the prespecified plan for the KEGG analysis? An ANOVA is appropriate for analysis of the 3 rat groups, but what was the prespecified plan for between-group analyses when the ANOVA was significant (it should be adjusted for multiple testing – if all pairs were tested, a bonferroni corrected p-value would be 0.015 for each individual t-test, for example)? Additionally, a t-test is often used after ANOVA, but there is no mention of how pair-wise groups were tested. There appear to be many ANOVAs – how was multiple testing controlled?

Results
The search for DQP compounds appears to have been done through the Chinese Materia Medica only, which is far from comprehensive. But, what was the search strategy? Furthermore, how did you verify that the compounds frequently found in Danshen and Sanqi are found in the processed DanQi pill? This is an essential piece of information.

It is unnecessary (and misleading) to be overly precise reporting your results (eg. EF values … dropped by 49.03%). Also, please report exact p-values rather than
NS, p<0.05, p<0.01.

What are the actual data values for figure 1?

3.2.2. how was ‘significantly improved’ defined for cardiomyocyte hypertrophy?

3.2.3 Please report absolute values rather than relative values – this should be done throughout the manuscript. Were baseline levels of COX, etc. measured? The appropriate comparisons here should be the amount of change from baseline among groups, which is an analysis that was not described in the section on analysis plan. At the end of paragraph 5, cardioprotective effects are demonstrated clinically, not by estimating metabolite levels.

Tables and Figures
In general, a table or figure should be able to be read and understood entirely on its own (without having to refer back to the text).

Table 1 – the method for the KEGG analysis should be fully explained in the Methods section (foot note a). The concept of coverage is not entirely clear.

Table 2 – method for GO analysis needs to be explained in methods.

Table 3 – this table would read better with the rat models across the top and the outcome measures vertically. P-values for ANOVA can be clearly reported in a 4th column and t-tests (or whatever was actually used) can also be reported for pair-wise comparisons.

Table 4 – what is ratio of P/T. Please report actual p-values (for both ANOVA and other testing).

Figure 1 – although the pictographs are nice, actual measurement values would be far more informative.

Figure 3 – why are there 2 asterixes over the ‘model’ group?

Manuscript title – ‘balancing’ and ‘patterns’ are vague descriptions. Furthermore, these studies did not look at the protective effects of DQP against HF development. In a rodent HF model, you demonstrated that DQP could reverse protein expression that is induced in the HF model.

**Level of interest:** An article of limited interest

**Quality of written English:** Needs some language corrections before being published

**Statistical review:** Yes, but I do not feel adequately qualified to assess the statistics.

**Declaration of competing interests:**
I declare that I have no competing interests