Peer Review File
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Reviewer A

Dr. Joshua H and colleagues conducted a systematic review and meta-analysis of preclinical studies examining the effects of cyclosporine on myocardial ischemia-reperfusion injury. They discussed what strategies should be used in clinical trials for cyclosporine, which has no proven efficacy. The authors focused on cyclosporine, a promising drug for ischemia-reperfusion injury, but their speculations were based on animal studies, and the following limitations remain.

Comment 1: A sub-analysis conducted by the authors stated that experiments with porcine, females, and elderly animals were less effective or failed to show efficacy (Page 15). This suggests a reason for the lack of efficacy in clinical trials. Since the actual cases include older people and women, how can these findings be used to plan clinical trials that can demonstrate cyclosporine's efficacy? Is it possible that subgroup analyses of past clinical trials show the efficacy of cyclosporine in patients from which older people and women are excluded?

Reply 1: Thank you for taking the time to review our manuscript. While we would like to emphasize that our analysis was intended to generate hypotheses from the existing data rather than arrive at definitive conclusions, it is possible that age and/or sex did impact the outcome of the clinical trials. Perhaps clinical trials of cyclosporine considering these factors (e.g., by recruiting younger patients) would show a positive result, but it still remains an area of opportunity to clearly establish these parameters in well-designed preclinical studies before planning any subsequent clinical trials.

It is possible that subgroup analyses of past clinical trials could show efficacy if they excluded older people and women. Interestingly, one of the larger clinical trials (the CIRCUS trial reported by Cung *et al.*) included a subgroup analysis looking at men and women separately, as well as older and younger patients. They did not find a difference in any of these subgroups; however, the age cutoff used was 75, so patients in the ‘younger’ group were still quite a bit older, relative to the animals used in most preclinical studies. We have added comments on these points to the discussion (see Page 15, lines 305-308).

Comment 2: Reports showing that nanoparticle-mediated administration of cyclosporine demonstrated its efficacy suggests that it may not be pharmacokinetically effective in humans. Most preclinical studies that showed efficacy used doses of 10 mg/kg or higher, which are higher than those used in human clinical studies. This may be an important reason for the lack of efficacy.

Reply 2: That is a good point. It seems that higher doses of cyclosporine are required to achieve a protective effect in the face of ischemia compared to its use as an immunosuppressant. It may be that doses of 10mg/kg or greater are prohibitive in humans because of side-effects, such as

nephrotoxicity. Thus, human-specific pharmacokinetic studies are definitely an important consideration for any subsequent clinical trial.

Reviewer B

Comment: This is a very well written review which highlights the discrepancy of preclinical data and the transition into clinical reality. The authors describe their methods and the limitations of this subgroup meta analysis in great detail. They focus on the reduction of ischemia reperfusion injury with cyclosporine A. They highlight the differences in the preclinical studies and compare it to clinical studies. I do not have any concerns.

Reply: Thank you for your generous comments!

Reviewer C

This preclinical meta-analysis by Hefler and colleagues aims to assess the effect of cyclosporin on myocardial infarct size in preclinical models of myocardial ischemia-reperfusion injury. They found that cyclosporin significantly reduced myocardial infarct size in these models, however, important subgroup analyses show that this effect might not be relatable to clinically relevant conditions. These might reinforce why clinical trials failed to show any significant effect of cyclosporin on infarct size in patients with acute coronary syndrome. Some further points could be clarified.
Major comments:
Comment 1: The authors should acknowledge that a previous preclinical meta-analysis already assessed the effect of cyclosporin on myocardial infarct size (doi: 10.1111/j.1476-5381.2011.01691.x), however, novel subgroup analyses are provided here as novelty. The authors might elaborate on the added value of their present study, and highlight similarities and differences between the two analyses.

Reply 1: Thank you for bringing this to our attention. We have made reference to this review in our discussion (see Page 18, lines 374-379). The added value of our study is several-fold. First, we include subgroup meta-analyses, as you mentioned. Our systematic review also updates the findings of Lim *et al.* (which was published in 2011) and found a further 23 studies suitable for a meta-analysis of infarct size. In addition, looking at the preclinical studies in light of the results from large randomized controlled trials (which were not available in 2011) greatly informs our discussion.

Comment 2: The authors should better describe the statistical methods used. For example, it is unclear from the statistical methods which effect measure was used? Based on the data, it is weighted mean differences. Why was it used instead of standardized mean differences? Which random-effects model was used?

Reply 2: We have clarified this in our methods section (Pages 8-9, lines 158-9). Weighted mean

differences were used rather than standardized mean differences because the outcome of interest (infarct size) was reported in the same units and measured by the same method (Evans-Blue staining) in the studies in which it was reported. We used a random-effects model, as opposed to a fixed-effects model as we could not assume that the underlying effect of cyclosporine was the same between trials, owing the differences in species, duration of ischemia, timing of administration, etc.

Comment 3: The authors should also assess within-study bias using a validated tool specifically designed for studies of myocardial ischemia-reperfusion injury as published previously (doi: 10.1007/s00125-020-05359-2). Accordingly, it would be interesting to see whether cyclosporin also reduces infarct size in ex vivo heart models, so that important mechanistic data could be derived.

Reply 3: That is a great suggestion. We have added this to our assessment of bias (Page 8, lines 149-150; Page 10, lines 183-184, 191-193, 196-198).

We agree that it would be interesting and potentially relevant to review *ex vivo* models. However, we had pre-specified the inclusion of only *in vivo* models, as we felt that these would be more clinically relevant.

Comment 4: Several grammatical errors throughout the manuscript should be corrected (e.g. ‘randomized control trials’ etc..)

Reply 4: Thank you for bringing this to our attention. We have reviewed the manuscript thoroughly for spelling and grammatical errors (Page 3, line 44; Page 5, lines 70, 78; Page 6, lines 91, 103, 110; Page 7, line 114; Page 11, line 208; Page 12, line 244; Page 14, line 281; Page 15, line 30; Page 17, line 349).

Comment 5: The authors should provide some numeric data in the abstract as well, at least the result of the main analysis.

Reply 5: We have included the result of the main meta-analysis in the abstract (Page 3, line 55).