Author’s response to reviews

Title: Curcumin activates the p38MPAK-HSP25 pathway in vitro but fails to attenuate diabetic nephropathy in DBA2J mice despite urinary clearance documented by HPLC

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Author’s response to reviews:

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Dear Biomed Central Editorial Team,

Thank you for the opportunity to once again send a revision of our manuscript. Please see the responses to the Reviewers (below).

One of the contentious issues regarding this paper, particularly for Reviewer 1, was why this experiment failed to show a benefit of curcumin in diabetic nephropathy when other published works showed apparent benefit. Of interest, since the last submission of this paper, a paper came out in FASEB J showing that a mutation in the protein gpnmb in the DBA2J mouse, (which was the strain used in our experiment), impairs kidney healing by interfering with the reparative functions of macrophages. In the original submission, we suggested in the paper that mouse strain may have impacted on the negative result. This interesting piece of data underscores that argument. I have now included this reference. Thus, therapies that may work in certain individuals for diabetic nephropathy may not work in others due to underlying genetic influences. This is an interesting additional novelty of the work. I have addressed this issue at some length in my response to Reviewer 1 below.

I have tried very hard to meet virtually all of the concerns of the Reviewers, whenever I had the data to respond. I hope that with these changes, the Editorial Team will find this manuscript now suitable for publication.

Best,
Sharon Adler, MD

Reviewer 1

1-1/2 of paper are recycled from Tikoo et al (2008), paper entitled (Change in post-translational modifications of histone H3, heat-shock protein-27 and MAP kinase p38 expression by curcumin in streptozotocin-induced type I diabetic nephropathy), but on unsuitable animal model.

Reply: The authors fundamentally disagree with this Reviewer that a negative study in a particular animal model means that the animal model was “unsuitable”. The role of research is not to find an animal in which a specific therapy may work. The role of research is to understand the spectrum of responses and resistances that exist in nature, to better understand mechanisms of action. Indeed, a lack of response may be a window to reasons for resistance to therapy. In the prior version of this submission, we suggested that strain factors may have been one explanation for a failure to respond. Indeed, a paper by Li et al in the FASEB J available online (Aug 13, 2010), but not yet published, addresses this issue. The DBA2J mouse which was used in our paper has a naturally occurring mutation (stop codon) in the gene glycoprotein non-metastatic melanoma protein b (gpnmb). Li et al showed that this mutation is associated with a defect in renal repair processes. The “failure” of curcumin in the DBA2J mouse reported herein is likely due to a genetic mutation which interferes with reparative processes in injured kidney, which the provision of curcumin did not overcome. It is well-known that both susceptibility to disease and response to therapy is influenced by genetic inheritance. We believe that this “failure” elucidates this phenomenon. We believe further that it is not only justified, but important to publish papers on disease resistance that can be well documented. There is much to learn from these reports.

We have added the following to the manuscript in the Discussion section “Indeed, in a recently published paper, Li et al (Reference) showed that in the DBA2J mouse used herein, which has a naturally occurring mutation in the gene glycoprotein non-metastatic melanoma protein b (gpnmb), there is a defect in renal reparative processes. It is possible that the negative results observed for curcumin in this mouse are due to this inherited reparative defect. It is well-known that both susceptibility to disease and responsiveness to therapy are influenced by genetic predisposition.”

Furthermore, we disagree with Reviewer 1 regarding the statement that this is simply a repeat of Tikoo et al. Indeed, Tikoo (Reviewer 3) doesn’t think so either. Among the following are findings that distinguish the paper from Tikoo et al: 1) Our mice didn’t respond to curcumin; and 2) We demonstrated, for the first time, that a good measure of renal curcumin bioavailability is measurement of
Curcumin and its metabolites in the urine. In order to underscore these differences, we have changed the title of the paper. The new title is: “Curcumin activates the p38MAPK-HSP25 pathway in vitro but fails to attenuate diabetic nephropathy in DBA2J mice despite urinary clearance documented by HPLC”. Although the title is a bit long, we believe this new title makes the novel contributions of this paper more clear to the reader and to the Reviewer.

2- A significant amount of paper focus on the monitor of urinary curcuminoid and its renal pharmacodynamics effect. The new material in the paper describes how the authors confirmed the presence of urinary curcuminoid.

Reply: Agreed. We changed the title to reflect this (above).

3- The paper is long. What I have in front of me is a long text in a very bland format. The document needs considerable additions to make it readable. In results and discussion I wondered about the need for 11 papers in this 37-page report.

Reply: We have made an effort to shorten where we could, but in the aggregate, it is only shortened by ~10 lines. The paper is double-spaced, so it also appears longer than it would in print. I did not see either a page limit or a word limit in the Instructions to Authors. I have done the best I can regarding the length.

4- Dose selection is doubtful.

Reply: Through our HPLC measurements, we have shown that the kidney was exposed to curcumin and its metabolites. We have also shown that other biologic effects of curcumin were achieved at the doses used (eg lowering of renal HSP25 and increasing urine 12-HETE). We used a dose that worked in a published paper in experimental Alzheimer’s disease, and we purchased the food from the same compositor. We believe the failure to respond was related to the mouse strain and not the dose. The Reviewer is certainly entitled to his/her opinion!

5- The document needs careful proofreading and copy-editing.

Level of interest: An article of importance in its field

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

Reply: I leave that to the capable journal editorial staff. I was a Biology major and an English minor at an Ivy League college in New York City, where I was born. I think I know how to write English. If you don’t think so, please feel free to correct the text.
Statistical review: No, the manuscript does not need to be seen by a statistician.

Reviewer 2

The authors thank the Reviewer for helpful comments on the first round and appreciate that the Reviewer is now satisfied.

Reviewer 3

1) If the authors could not perform the western blotting for p38 and hsp25 in normal control animals for figure 6 due to funding issues, the authors should give some earlier references comparing the in vivo changes in these protein levels between control and diabetic mice in results for easily evaluating the effect of curcumin administration in Stz-DM mice.


In DBA2J mice we also have published values in pancreas, but once again, that would also not be of any use (Dai T, Natarajan R, Todorov I, Ma J, LaPage J, Nast CC, Becerra D, Chuang P, Tong L, de Bellerocche J, Wells DJ, and Adler SG: Overexpression of heat shock protein 27 (HSP27) confers resistance to cytokine-induced beta cell apoptosis and attenuates the development of streptozotocin-induced diabetes mellitus. Endocrinology 150(7):3031-9, 2009).

Although the data requested would be of some interest, our goal in this experiment was not to compare the control and the diabetic mice. Instead, it was to determine whether or not, in diabetic mice, curcumin feeding had a biological effect. The data do show a biological effect. We agree that it would also have been interesting to have shown how this compares to healthy control mice, but we don’t have the data.

2) The authors should mention the effect of curcumin in increasing urinary 12-HETE/cr excretion in vivo at higher doses in discussion.
Reply: The following has been changed in the Discussion, as follows: “Urine 12-HETE is a reliable measure of activation of the 12/15-LO pathway in vivo (96), and in these curcumin-treated mice, the urine 12-HETE/cr ratio was increased”.

3) Even though the numbers of animals in each group are different, the authors should write the number of animals in each group in brackets at respective positions.

Reply: My apologies to the Reviewer, who did request this in the last version. I did include this information in the revision, but forgot to mention it in the reply and failed to highlight it in the manuscript. The following is now highlighted in purple font in the manuscript:

“In Experiment 1, non-diabetic (noDM) or DM mice were assigned to one of the following diets at the time the DM was confirmed in the Stz-injected group (Day 0): 1) control chow with 0 ppm Cur (n=8 for noDMCur0; n=11 for DMCur0); 2) test chow with Cur5,000ppm (n=10 for noDMCur5000; n=6 for DMCur5000)”.

“In Experiment 2, mice were randomly assigned to receive a control or Cur diet one week prior to Stz injections. Mice were then injected with Stz daily for 5 days as described above. DM was ascertained one week after the last Stz injection (Day 0), and then again in steady state from weeks 5-7, and in some mice specially maintained for glycemic monitoring, up to 11 weeks. The following experimental conditions were compared: 1) control chow with 0 ppm Cur (n=5 for noDMCur0; n=5 for DMCur0); 2) test chow with Cur5,000ppm (n=6 for noDMCur5000; n=7 for DMCur5000); or 3) test chow Cur7,500ppm (n=6 for noDM Cur7500; n=5 for DMCur7500)”. 