Reviewer’s report

Title: Pirfenidone prevents Acute Kidney Injury in the Rat

Version: 1 Date: 06 Oct 2018

Reviewer: Rajesh Mohandas

Reviewer's report:

This is a study of the efficacy of pirfenidone to prevent acute kidney injury in rats. The authors find that pirfenidone administered 24 hours prior to ischemia reperfusion injury decreases tubular injury and attenuated/prevented oxidative stress as well as decreases in urine output, renal blood flow and creatinine clearance.

I have some concerns.

Major Concerns

Some key experimental details have not been included in the manuscript. What strain of rats were included? How old were they? These are important factors that determine the extent of renal injury beyond the ischemia time and I could not find where it was mentioned in the manuscript.

While the initial discussion focuses on decreased blood flow as the cause of ATN, the authors clamped the renal pedicle instead of just the renal artery. This would cause venous obstruction, induce renal venous congestion and increase the severity of renal injury. Was there a reason to favor clamping the pedicle versus the renal artery?

The authors report creatinine clearance. In rats there is significant secretion of creatinine. Moreover in acute kidney injury creatinine levels or excretion is not in steady state. Use of Dextran-FITC or inulin-FITC would have been ideal to determine GFR. The authors should report serum BUN and Creatinine in the animals and note the limitation in the discussion.

"Pirfenidone was administered 24 hours before surgery at 700 mg/kg/day". How was this administered? In water? In food? How was the dose determined? Was a dose response assessed? The half life of pirfenidone is short < 2 hours. How was it ensured that rats had pirfenidone when they underwent ischemia reperfusion.

What does the authors mean by the statement "mRNA levels were significant by t-test but not by ANOVA". An ANOVA and t-test should produce the same results if there are only two groups. If there are more than two groups and you do multiple t-tests then you increase the chances of Type-I error. Such tests have to be adjusted for multiple post-hoc testing, which will
compromise their power. So if the tests was not significant in ANOVA then the authors cannot claim any difference between groups.

Figure-1 legend: The authors say both I/R group and the IR+PFN group as shown by black bars. I suspect the authors meant to say the former as black and the latter as grey. Details need to be specified in the figure legends as to how many mice were used and how the p-value was derived. Is this ANOVA? Multiple t-tests? According to the figure there is a significant increase in body weight with IR. Is that true? If so why would that be?

Figure-2C. There are no loading controls shown for the western blot. How were they normalized? AKI is associated with changes in proteinuria. So I would think a loading control/normalization is essential.

Figure-3. The authors show 3 figures representative of sham, IR and IR+PFN. However, figures A and C show cortex with plenty of glomeruli. The cortex will not contain the S2/S3 segments were damage is expected to be worse. Only Fig B with IR injury shows the medulla with casts containing tubules. We are not comparing apples to apples here.

Figure-4. The number of replicates is not mentioned. Also what statistical tests were done.

Minor concerns

Page 3: Line 4: "AKI is caused by a reduction in blood flow". This statement is not entirely correct. Not all causes of AKI are characterized by a reduction in blood flow. There is ample evidence that sepsis is associated with increased renal blood flow. There is no decreased blood flow in AKI due to nephrotoxins.

There is no control group of Pirfenidone in sham operated rats. The effects of Pirfenidone could be independent of renal injury.

I would examine the mRNA levels of SOD1 (in addition to Catalase and GPX) as well as hsp72.

The authors speculate the protein in the urine is tubular in origin. This can be easily assessed by measuring urine micro-albumin creatinine ratio in addition to total protein.

Are the methods appropriate and well described?
If not, please specify what is required in your comments to the authors.

No

Does the work include the necessary controls?
If not, please specify which controls are required in your comments to the authors.

No
Are the conclusions drawn adequately supported by the data shown?
If not, please explain in your comments to the authors.

No

Are you able to assess any statistics in the manuscript or would you recommend an additional statistical review?
If an additional statistical review is recommended, please specify what aspects require further assessment in your comments to the editors.

I am able to assess the statistics

Quality of written English
Please indicate the quality of language in the manuscript:

Acceptable

Declaration of competing interests
Please complete a declaration of competing interests, considering the following questions:
1. Have you in the past five years received reimbursements, fees, funding, or salary from an organisation that may in any way gain or lose financially from the publication of this manuscript, either now or in the future?
2. Do you hold any stocks or shares in an organisation that may in any way gain or lose financially from the publication of this manuscript, either now or in the future?
3. Do you hold or are you currently applying for any patents relating to the content of the manuscript?
4. Have you received reimbursements, fees, funding, or salary from an organization that holds or has applied for patents relating to the content of the manuscript?
5. Do you have any other financial competing interests?
6. Do you have any non-financial competing interests in relation to this paper?

If you can answer no to all of the above, write 'I declare that I have no competing interests' below. If your reply is yes to any, please give details below.

I declare that I have no competing interests' below. If your reply is yes to any, please give details below

I agree to the open peer review policy of the journal. I understand that my name will be included on my report to the authors and, if the manuscript is accepted for publication, my named report including any attachments I upload will be posted on the website along with the authors' responses. I agree for my report to be made available under an Open Access Creative Commons CC-BY license (http://creativecommons.org/licenses/by/4.0/). I understand that any comments
which I do not wish to be included in my named report can be included as confidential comments to the editors, which will not be published.

I agree to the open peer review policy of the journal