Reviewer's report

Title: Jacarehyperol A induced apoptosis in leukaemia cancer cell through inhibition the activity of BCL2 Proteins

Version: 3 Date: 20 May 2014

Reviewer: Karin Joehrer

Reviewer's report:

In this paper, Zhang et al state that Jacarehyperol A induces apoptosis in leukaemia cancer cells via the inhibition of BCL-2 proteins. They clearly show in MTT assays that the cell number of tumor cells of various origin is lowered by Jac-A. They further prove that Jac-A is binding to Bcl-2 family members by inhibition experiments and modelling. In vitro, Jac-A induces apoptosis in K562 cells. They show that K562 tumor cells exhibit lower growth in the mouse model under Jac-A treatment.

All together this data especially shows that Jac-A, a novel natural compound, is an efficient anti-tumor reagent in a mouse model, which is very interesting. However, the in vitro data on the mechanism is not convincing, since basic proofs are not done and in vivo mechanisms are neither completely delineated nor properly discussed.

• Major Compulsory Revisions

1) There is no experimental proof that binding of Jac-A to bcl-xL etc is also the causative reason for apoptosis induction neither in in vitro nor in vivo experiments – confirmative Western blots (pull-downs) showing the modulation and/or binding properties of the bcl-2 family members +/- Jac-A are missing. In addition, and to underline the possible therapeutic preference for this novel compound, control experiments with already utilized drugs (such as e.g. ABT-737) should be performed to compare the observed effects: ABT-737 has anyway already been used for the modelling.

2) Previous fundamental work (the isolation and characterization of the compound) is stated but not cited.

3) Methods: apoptosis and cell cycle: there is no cell cycle data in this paper although the experimental features were (wrongly) described in the Meth & Mat section;

4) Results: Fig.3: Ann/PI staining: it is not mentioned how many times this assay was performed and there is no graph showing the results including SD – I have to assume that this was a single experiment with an unusual buffer that might not work, since there is a specific Annexin-buffer normally used for the staining. The last plot says that Q1 and Q2 together should contain about 40% of cells but this is hard to assume from the shown plot.

5) Fig 4B: Bcl-2 family proteins should be shown and the effects of a known
inhibitor eg ABT 737 should be included as a control.

6) Fig 5C: according to the text the p value was <0.001; this should also apply for the 2mg/kg group? Fig.5D: p values?

7) High weight in the high concentration treated group: there could be also other reasons for that beside mere well-being, please discuss! To which concentrations has Jac-A be tested in animals? What would be the effective dose in humans? Could this be reached by any means? What are the expected side effects (extrapolated from ABT-737, e.g.?)? What are the side effects of a similar effective dose of ABT-737?

• Minor Essential Revisions

The author can be trusted to make these. For example, missing labels on figures, the wrong use of a term, spelling mistakes.

8) The modeling of the proposed binding is well done, however, fig description is incomplete and from the shown figure the reader cannot delineate the described mechanism.

9) first page results: BH3-binging pocket - spelling error

10) In the background section: the protein used for FP is mentioned as Flu-Bak-BH3, first line in methods mentions Bid-BH3 domain peptide # which one was used?

11) Fig 3: annotation of the lower right quadrant (Q4) is wrong (AnnV +/-Pi -)

12) Fig 4A gives no essential information and is a cartoon only which can be removed

13) The specific antibody clones utilized in western blots should be mentioned in the Materials section

14) Results: Fig 2a: residues are not numbered as described in the text, sub-pockets P4 and P5 are not shown?

15) Fig.5A: no p-values given.

• Discretionary Revisions

Level of interest: An article whose findings are important to those with closely related research interests

Quality of written English: Acceptable

Statistical review: Yes, and I have assessed the statistics in my report.

Declaration of competing interests:

I declare that I have no competing interests