Reviewer's report

Title: Berberine enhances inhibition of glioma tumor cell migration and invasiveness mediated by arsenic trioxide via the inactivation of protein kinase C

Version: 2 Date: 12 July 2007

Reviewer: Yok Lam Kwong

Reviewer's report:

General

In this revised manuscript, Lin et al attempted to address some of the concerns raised. However, serious conceptual and technical problems remain.

Major Compulsory Revisions (that the author must respond to before a decision on publication can be reached)

1. Lin et al stated clearly in the introduction that they aimed to study “the molecular mechanisms of As2O3-mediated suppression of glioma cell invasion which involves PKC signaling, ERK phosphorylation, and MMP-2 activation.” However, most of the data in the manuscript have been focused on the action of berberine. The author also stated, in the letter accompanying the revision, that berberine was the active compound evaluated in this study. Therefore, the actual conceptual design of this study remains unclear. In fact, reading through it once again, one finds it difficult to really understand which compound is being evaluated here, As2O3 or berberine, or both.

2. It is unsatisfactory for the authors to believe that there is no need to separately investigate the molecular mechanisms of As2O3 and berberine on their cellular model. This makes their experimental approach empirical and not analytic.

3. It is unknown whether the concentration of berberine used (10 μM) can be achieved safely in a therapeutic context.

4. The explanation of the statistical analyses is still inadequate. Were the data derived from triplicates in the same experiment, or was each experiment repeated three times, each experiment generating one datum? This remains unclear in the legends. What did the authors mean by “means were considered to be significantly different from control”? Please specify the statistical package used to analyze the data. Also, please specify the types of post-hoc tests used to calculate the p values for the various groups.

5. In figure 1C, there was only minimal decrease (although statistically significant as claimed) in cell survival in the presence of 10μM of berberine. This is unlikely to be biologically significant. Why did Lin et al not use berberine 20 μM in subsequent experiments?

6. In figure 6, Lin et al presumed that the ratio of membrane to cytosol PKCs was
one. This is clearly not the case, as shown in N10 and N1 of figure 6A.
7. Please explain the reason accounting for the difference of N10 and N1 in figure 6A, with respect to the membrane and cytosolic distribution of PKCs.
8. The biochemical data shown are without biological significance. Lin et al should at least attempt to inhibit some of the pathways, and examine the effect of inhibition of these pathways on the cytotoxicity of As2O3 or berberine on the glioma cells. Otherwise, no significance can be attributed to these pathways, as the alterations of these pathways may simply be incidental.

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Minor Essential Revisions (such as missing labels on figures, or the wrong use of a term, which the author can be trusted to correct)
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Discretionary Revisions (which the author can choose to ignore)

What next?: Unable to decide on acceptance or rejection until the authors have responded to the major compulsory revisions

Level of interest: An article of limited interest

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

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

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