Reviewer's report

Title: Boron neutron capture therapy induces apoptosis of glioma cells

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Reviewer: Romina Flavia RFA Aromando

Reviewer's report:

The study is rather interesting. However several points need to be clarified.

The paper addresses a topic under study in the field of BNCT. Animal models have shown very little or no apoptosis after BNCT mediated by BPA and other boron compounds. This paper studies three tumor cell lines and compares the effect of BPA-BNCT, neutron irradiation only and gamma radiation. It is interesting to study the effect of BNCT in cell cultures although it is very difficult to correlate these findings with the very complex interactions of tumor cells in tumoral tissue. We hope these studies will help us understand the mechanisms underlying BNCT effect.

Major points

For the sake of clarity and easier comprehension of the paper I suggest a clearer name for experimental groups D and E

Group D: treated with BPA-BNCT 4 Gy
Group E: treated with BPA-BNCT 8 Gy

Results:
BPA-BNCT induces apoptosis at 12 hours. How do you calculate the apoptosis rate?

Survival fractions for the three cell cultures were inhibited by BPA-BNCT. At what time was the inhibition of colony formation calculated? 12, 24, 48 hours?

In the statistical analysis you state that data was compared using student`s t test, but in table 2 you compare several groups. It does not say how this statistical analysis was performed. The correct analysis would have been ANOVA, please state the statistical analysis or correct it if you performed t test.

Figure 4: how was the apoptotic rate calculated? Please perform ANOVA for apoptotic rate for groups A, B, D, F and H and for the groups A, C, E, G and I in order to clarify if apoptosis was elicited by BPA-BNCT, neutron beam alone or gamma rays alone.

Discussion:
Bcl-2 down-regulation and Bax up-regulation were detected at 12 and 24 hs. You could compare these data with the data published for BPA-BNCT apoptosis induction in different animal models.
In the discussion you do not discuss the very important issue of BPA-BNCT diminishing cell proliferation, which has been proved to be a mechanism of tumor remission in animal models. You should include this.

In the last paragraph of the discussion you state that BPA-BNCT may damage mitochondrial membrane integrity. However, the activation of the mitochondrial pathway is mediated by noxious stimuli that induce DNA DAMAGE rather than mitochondrial damage. The down-regulation of Bcl-2 and the upregulation of Bax after BPA-BNCT could demonstrate DNA damage induced by the therapy that triggers the activation of the mitochondrial pathway.

**Level of interest:** An article of importance in its field

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

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

**Declaration of competing interests:**

I declare I have no competing interests