OPEN PEER REVIEW REPORT 1

Name of journal: Neural Regeneration Research
Manuscript NO: NRR-D-17-00739
Title: Hydrogen peroxide mediates pro-inflammatory cell-to-cell signaling: a new therapeutic target for inflammation?
Reviewer: Paulina Carriba, Cardiff University, UK.

COMMENTS TO AUTHORS

The most interesting point of view is to consider H_2O_2 as an intercellular messenger that can boost the inflammatory response. The weaknesse is that this is not totally new. It has been previously described that activation of immunity cells produce H_2O_2, that H_2O_2 can cross the membrane and that H_2O_2 is able to initiate the production of NO and TNFa. They put these premises together.

In the present paper the author joining some evidence previously established to generate a new hypothesis that they try to demonstrate. Using two validate facts: a) H_2O_2 can cross the plasmatic membrane (by simple passive diffusion or mediated by channels) and b) H_2O_2 is involved in the regulation of NO and TNFa; the authors try to evidence that H_2O_2 produced in one cell can act as an intercellular messenger able to activate in neighboring cells the production of the aforementioned inflammatory molecules. They arrive to this conclusion because the addition of extracellular catalase, which cannot enter inside the cells, is able to regulate the production of these inflammatory molecules, in a dose-depended fashion.

Despite the results present are considerable, in my opinion a convincing experiment is necessary. Without too much details about how they conduct the experiments, I have understood that in the same dish/well in which the macrophages were treated they determined the production of NO and TNFa. This could generate doubts about if H_2O_2 is really an intercellular messenger. Do the inductors used produce NO and TNFa by themself? Could catalase interfere in the proper activation of the macrophages and for that to reduce the levels of these cytokines? To avoid these questions, an elegant experiment could be to try to do these experiments in insert culture wells in which cells are separated by a pore-determined membrane.

Several papers show the involvement of H_2O_2 in the regulation of production of NO, especially through the induction of Nitric-Oxide Synthase expression. The production of TNFa induced by H_2O_2 has as well been described. Curiously in the same macrophage cell line, RAW 264.7, it has been described that H_2O_2 is involved in the production of TNFa but not NO (Innate Imm. 2008 Jun:14(3):190-6). Could you explain what could be the discrepancy with your results?

Introduction: - There are two confusing sentences related to superoxide (O_2-). Line 33 "O_2- is then release into the extracellular space", but as it is depicted in Figure 1, O_2- is in the cytosol. And in line 35 " … extracellular O2- does not easily travel through the cytoplasmic membrane". Then if O_2- is release into the extracellular space and then it does not cross the membrane into the cytosol, how does is it converted in intracellular H_2O_2?

Materials and Methods: - It is necessary to include a brief description of the protocols used. For example, in materials, the name of the chemicals, how were prepared, etc. Please give more information in this section.

Results: - Please, readjust the size of the typo in the graphs, or increase the resolution because it is really difficult to read what is determined in each graph, letters and symbols are blurred.
- Line 92-93: "NO and TNFα production (as readouts for activation) as well as cell viability, and were determined.
- Please check Table 1. It does not match with the text (cell viability - % inhibition TNF at maximum catalase concentration).
- As it is written in the figure legend 1, cells were activated only with one concentration.
- Please, include statistical significances in the results, also indicating which test you have used. When you mention n=6, what does it means? 6 independent experiments or one experiment in sixplicate?