

Author's response to reviews

Title: Dietary antioxidants protect epithelial cells from oxidant-induced apoptosis

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Reply to Reviewers' Comments

Dr. Andre Buret

1. Details of the statistical analysis has been included
2. Agreed with comments as to initial cell numbers but that was accounted for. The comment on proliferation/ cell numbers in the presence of antioxidants alone was noted. It was also raised by Dr. Payne. In order to address this issue we have included the data previously noted as "not shown" in a double figure. It does clearly describe that there was no effect on cell proliferation for either AGS or IEC-18 cells, in contrast to the protective effects on oxidant-induced cell death.
3. Corrected
4. Corrected
5. Corrected
6. An additional sentence as to dosing ahs been included in the discussion. We had previously hinted at this but now it is discussed with greater clarity.
7. Corrected and clarified.
8. Minor corrections made.

Dr. Matthew Grisham

1. Peroxynitrite was used in this study as a tool to study oxidant damage. It was not used to address issues of whether peroxynitrite was a participant in any human disease. Oxidants may well be pollutants and other exogenous chemicals, and as such it is worthwhile addressing the cellular response to a potent oxidant. Thus we have respectfully retained the data.

Dr. C. Payne

1. We have confirmed the apoptosis response that was quantified by ELISA of DNA fragmentation by acridine orange but we have not quantified this method. The DNA fragmentation ELISA is a standard commercial method that is widely used and we have considerable experience with this technique. In addition we performed studies addressing necrotic cell death by LDH release and these results add weight to the observations that cell death was via apoptosis.
2. We have clarified the confusion as to whether the cells were at confluence when BrdU was added. They were spiked with BrdU when they were seeded (and not confluent) and so the tag was incorporated.
3. As noted above, we have added data from a LDH assay for cell death by necrosis.

4. Cell proliferation in the presence of the antioxidants for both AGS and IEC-18 cells over a 72 hour period was not affected as we stated previously. We have included this result in a new figure. It was not included before as it was negative data and we were attempting to limit the burden of figure numbers. Considering this is a journal in cyberspace we should have been less concerned and we apologise for the appearance that data was not forthcoming.
5. Details on how to make peroxyxynitrite have been included.
6. The comments on the discussion are quizzical. The items that the reviewer wanted to be discussed have been discussed. Considering that this paper has 6 figures and 5 tables a discussion of 3 pages double-spaced is not too long. The other reviewers' comments were not directed at the discussion or interpretation, so this section has had only minor modifications.
7. Typos have been corrected.