

Reviewer's report

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

Authors:

Mark JS Miller (millermj@mail.amc.edu)
Fausto M Angeles (fmangelesf@usa.com)
Brian K Reuter (ok_go@hotmail.com)
Paul Bobrowski (bobrowski@amazonmedicines.com)
Manuel Sandoval (sandovm@mail.amc.edu)

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Reviewer: Dr C Payne

Level of interest: A paper whose findings are important to those with closely related research interests

Advice on publication: Other (see below)

Summary:- This interesting manuscript addresses a very important issue related to the use of antioxidants in the prevention of G.I. cancer. The authors conclude that dietary antioxidants can reduce oxidant-induced apoptosis using cultured cell lines derived from the G.I. tract. In addition, the data suggest that green tea and cat's claw extracts may exert its cytoprotective effects by affecting intracellular signaling pathways.

Critique

- 1) Apoptosis experiments that utilize biochemical assays need to be confirmed using morphology. At least one of the experiments needs to be repeated using brightfield microscopy and scoring apoptotic cells. At least 200 cells should be scored per time point. For the necrosis experiments, at least one experiment needs to be repeated using annexin V and propidium iodide (P.I.) in conjunction with flow cytometry. Annexin V(+)/P.I.(-) cells represent early apoptosis, annexin V(+)/P.I.(+) cells represent late apoptosis and annexin V(-)/P.I. (+) cells are necrotic.
- 2) The details regarding the ELISA cell death assay needs to be provided. Specifically, it is stated on page 5 that the harvested cells are allowed to grow to confluence over a 24 hour period before use. However, on page 6 it is stated that cells need to be incubated with BrdU to label the DNA for the assay. Since confluent cells are not dividing and cannot incorporate BrdU into DNA, how is this labeling accomplished?
- 3) The use of the MTT assay to assess viability can be misleading since the MTT assay measures dehydrogenase activity from various sources within the cell, including mitochondrial dehydrogenase activity. Another assessment of cell death should be included, such as P.I., ethidium bromide or trypan blue uptake to confirm the MTT assay results.
- 4) The authors state that the antioxidants failed to affect cell proliferation over a 72 hour period. This is a very important point to make since a pro-oxidant state usually promotes entry into the cell cycle. Growth curves need to be provided to substantiate the statement that the antioxidants used do not reduce cell proliferation.
- 5) The source of the peroxyxynitrite is not stated in the Materials and Methods section.

6) The Discussion section is too long and not focused enough. Specifically, the role of apoptosis in cancer progression needs to be discussed with respect to G.I. epithelial cells and the types of survival and apoptotic signaling pathways that are likely to be modulated by antioxidants. Since the experiments were performed on cultured cells and not primary tissue, the protective effect on apoptosis may actually enhance tumor progression, especially since proliferation is not reduced by antioxidant treatment. The authors, therefore, also need to discuss how these antioxidants will be tested in various tumor progression models before they could be potentially applied to human G.I. cancer prevention.

7) Typos need to be corrected: page 4: cucumerin to curcumin; page 13 references 7 (releassing) ,8 (NG-kB),22 (endotheleial) ,29 (supplemnts).

Competing interests:

None declared.