Reviewer’s report

Title: Comparative Effects of Alpha- and Gamma-Tocopherol on Proliferation and Apoptosis in Human Colon Cancer Cell Lines

Version: 1 Date: 12 September 2005

Reviewer: Kimberly Kline

Reviewer’s report:

General
The role of vitamin E in health and disease is unclear and much critical information is missing. Studies addressing the differences among vitamin E forms, especially in regard to anticancer properties, are both timely and important.

Major Compulsory Revisions (that the author must respond to before a decision on publication can be reached)
None

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

Minor Essential Revisions

1. Tables giving statistics on numerous comparisons are confusing. Authors need to decide what is the central (primary) outcome of a particular study and provide statistics on this comparison. Also, biological relevance needs to be considered. Just because something is significant from a purely mathematical standpoint, this does not make it important from a biological standpoint. For example, what is the biological relevance of Fig 2 Panel A statistically significant data at 25 and 50 microMolar concentrations of the various pro-death agents? In this same set of experimental data, some type of evaluation of whether or not the pro-death agents produce a concentration-dependent response needs to be made.

2. More caution needs to be exercised in conclusions drawn. For example, the statement: “The amount [of] growth reduction was dependent upon the molecular signatures of the cell lines suggesting more than one pathway can be modulated by gamma-tocopherol to inhibit cell proliferation.” An equally likely speculation is some of the cells express elevated levels/enhanced functions of survival factors that block apoptosis by gamma-tocopherol. Also it is not justified to draw “conclusions” about the involvement of regulatory pathways [for example, Cox-2, lipoxygenase and sphingolipid metabolism), PPAR and wnt], based on descriptive data from non-isogenic cell lines.

3. There are two completely different types of alpha-tocopherol: RRR-alpha-tocopherol, a naturally occurring form and all-rac-alpha-tocopherol, a combination of eight stereoisomers only 1/8th of which is RRR-alpha-tocopherol. Thus, all references to alpha-tocopherol in the manuscript need to be clarified. For example, in the Background section the following statement is made: “The isoform found in highest concentration in the serum and dietary supplements is alpha-tocopherol.” RRR-alpha-tocopherol is the form found in highest concentration in the serum and synthetic all-rac-alpha-tocopherol is the form frequently used in dietary supplements and clinical studies.
4. What data from animal models of colon cancer support the hypothesis that gamma-tocopherol can act as either a chemopreventive or chemotherapeutic agent? If there is none, this needs to be clearly stated.

5. Figure 1 needs to be omitted since this information has been published previously.

6. Clarification of RRR versus all-rac form of alpha-tocopherol from Eastman Chemical is needed in the Methods section.

7. Clarification of whether or not bovine serum albumin was included in the vehicle is needed in the Methods section.

8. Clarification is needed whether or not CCD-112CoN cells are truly normal or if they are really an immortalized cell line. If the latter it is not accurate to refer to them as normal.

9. Vertical axis for Figures 2 and 3 need to be the same for all panels to permit easier comparisons among cell types.

10. Horizontal axis for Figure 3 and Figure 5 need to be labeled in days for easier analysis.

11. Figure 4 data is a repeat of data in Figure 3 except for normal cell data.

12. What was the rationale for using different positive controls for induction of cell death for the different cells types? Is there no single pro-apoptotic agent to which all these cell types respond?

13. Vertical axis of Figure 5 needs to be labeled in some easily understandable measure.

14. Figure 3 legend refers to 5 day data that is not in Figure.

15. Discussion / second paragraph. Since colonic epithelium is exposed to the contents of the gastrointestinal tract lumen it would seem that this exposure to dietary vitamin E forms would be as important as exposure to plasma levels. It would be helpful if this exposure was addressed. Also according to Maret Traber and colleagues there is no bioselective processes of vitamin E forms by the GI tract just by the liver alpha-tocopherol transfer protein.

What next?: Accept after minor essential revisions

Level of interest: An article whose findings are important to those with closely related research interests

Quality of written English: Acceptable

Statistical review: No

Declaration of competing interests:

We have a patent on analogs of vitamin E that exhibit anticancer properties.