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# LETTERS TO THE EDITOR

## Vitamin C, vitamin E and immune response

Dear Editor:

In a recent original article published in the *Journal of Nutritional Biochemistry* [*J Nutr Biochem* 15 (2004) 45-50], Bergman et al. reported on some in vitro effects of incubating human peripheral blood cells derived from healthy subjects with vitamins C and E. The results showed that vitamin C increased phagocyte activity after 60 min of incubation in polymorphonuclear cells without affecting the number of active phagocytes. This effect was not observed in monocytes (M∅). After 24 h of incubation, vitamin C produced a 39% increase in propidium iodide incorporation (reported by the authors as apoptotic index) but did not influence caspase 3 activity. Vitamin C also produced a concentration-dependent decrease in the IL-10, but not TNFα, release in the supernatant of cell cultures.

Vitamin E had no effect on any of these parameters. Both vitamins decreased the 24-h $^{3}$H-thymidine incorporation as an index of DNA synthesis of mononuclear leukocytes. Again, this effect was particularly marked and concentration dependent in the case of vitamin C.

Apart from the several limits in the experimental design and methods (such as different times of incubation and number of cells used in the different assays, the absence of information on vitamin uptake by the cells, the use of propidium iodide incorporation as a marker of apoptosis assessed by cytofluorimetry without other and more specific tests that can distinguish dying cells as necrotic or apoptotic such as the Annexin-V test, the absence of specific information on the extent and rate of apoptosis in leukocyte subsets), to the eyes of this reader, the definition of "doses extrapolated from clinical practice" that the authors used appears questionable to describe the doses of vitamins C and E used in the different in vitro incubation protocols. In point of fact, they added to the incubation media the two vitamins at the final concentrations of 0.2 and 0.125 mg/ml (1.16 and 0.29 mmol/L), respectively. These concentrations of the two vitamins largely surpass physiological plasma concentrations of well-nourished nonsupplemented healthy subjects, which are around 0.060 and 0.030 mmol/L, respectively. These represent approximately 20 and 10 times less than the concentrations used in this study. Even after prolonged oral supplementation with daily doses of 1 g or more of both vitamins, plasma levels at saturation cannot increase above approximately two times the baseline concentrations. Specific metabolic and excretion routes keep under narrow control circulating and tissue levels of the nutrients investigated in the study. For this reason, in vitro studies on cells challenged with these food-derived antioxidants should point out to biological effects produced by concentrations close to or lower than physiological ones (i.e., concentrations obtained in vivo in the biological context of such cells in the presence of a standard or fortified nutritional regimen). The concentrations used by the authors in this study might have pharmacological or toxicological relevance only in in vitro or ex vivo experimental settings.

Other possible biases in this study should be considered. With vitamin E, for instance, the evaluation of some nonantioxidant effects is strongly influenced by the length and method of incubation in different types of cells. In my experience using several cancer cell lines, short (less than 12 h) and long (more than 48 h) incubation times may produce similar steady-state levels of α-tocopherol in cell membranes but produce completely different results with regard to cell growth, apoptosis and signaling element expression. Therefore, time-course experiments accompanied by the evaluation of cell uptake and transformation of the test compound(s) are of key relevance in these kinds of studies. Moreover, important points to verify the specificity of the results would be the practice of reconditioning the cells in the absence of the test compounds and the comparison of complete and serum-free culture media (to exclude the presence of growth factors, cytokines and other interfering elements).

In light of these considerations, I believe that the authors may comment about their experiments and provide some more information on the criteria adopted to build this research in a way that it might be better interpreted and reproduced in other laboratories.

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