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Journal of Nutritional Biochemistry 16 (2005) 258
Journal of Nutritional Biochemistry

Vitamin C, vitamin E and immune response: reply

In Response:

We would like to draw Dr. Galli's attention that most experiments in our study (i.e., apoptosis, DNA synthesis and cytokine production) were carried out at the same incubation times (24 h). As for phagocytosis, it was evaluated after 60 min, a period that, according to our experience, awards satisfactory results. We acknowledge that the number of cells in the various experiments was different. However, the procedures were carried on according to accepted protocols. In addition, the vitamin concentrations in each of the experimental setups remained constant. As for ways to examine apoptosis, we wish to clarify that it was done by both propidium iodide and caspase-3 activity as stated in the paper. We share Dr. Galli's concern as for distinguishing dying cells as necrotic or apoptotic. However, since cell viability using trypan blue dye exclusion test was over 90% and since there was no effect on cell viability in the presence of vitamins, it is conceivable that the results obtained with propidium iodide after incubation of cells with vitamin C indicate apoptosis and not necrosis. The lack of effect of vitamin E on apoptosis in our study is in accordance with the suggestion of Galli et al. [1] that vitamin E averts oxidative stress, one of the apoptosis inducers, both vivo and in vitro. DNA synthesis and cytokine release were carried out at vitamin concentrations in a range suggested by Dr. Galli himself. The vitamin C concentration in the remaining experiments was indeed higher than the suggested one. On the other hand, the dosage used in our study was even lower than that found in the plasma after oral administration of 1 g daily (1.2–2.48 mg/ml) [2] and similar to that described by Padayatty et al. [3]. However, one should keep in mind that, for in vitro studies, drug concentration usually exceeds clinical dosage [4]. Contrary to malignant cell lines, peripheral blood mononuclear cells are short lived in culture conditions — therefore, time-course experiments over 24 h are feasible.

Although evaluation whether the effect of the vitamins is reversible is a subject of interest, it should be a goal for a separate study. We did not compare the effect of the vitamins using complete versus serum-free culture media. However, since inactivated FCS does not contain detectable amounts of interleukins, it is conceivable that the cytokines found in our experiments were secreted by PBMC.

References

[1] Galli F, Canestrari F, Buoncristiani U. Biological effects of oxidant stress in hemodialysis: the possible roles of vitamin E. Blood Purif 1999;17:79–94.
[2] Zhao B, Tham SU, Lu J, Lai MH, Lee LKH, Moochhala S. Simultaneous determination of vitamins C, E and β-carotene in human plasma by high-performance liquid chromatography with photodiode-array detection. J Pharm Pharm Sci 2004;7:200–4.
[3] Padayatty SJ, Sun H, Wang Y, Riordan HD, Hewitt SM, Katz A, et al. Vitamin C pharmacokinetics: implications for oral and intravenous use. Ann Intern Med 2004;140:533–7.
[4] Heckelen A, Hermel M, Kondring B, Schrage NF. Ascorbic acid reversibly inhibits proliferation of retinal pigment epithelial cells. Acta Ophthalmol Scand 2004;82:564–8.

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DOI of the original article 10.1016/j.jnutbio.2005.01.014

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doi:10.1016/j.jnutbio.2005.01.015