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Vitamin C exhibits pro-oxidant properties

Vitamin C is marketed as a dietary supplement, partly because of its 'antioxidant' properties. However, we report here that vitamin C administered as a dietary supplement to healthy humans exhibits a pro-oxidant, as well as an antioxidant, effect *in vivo*.

We conducted a study[1] involving 30 healthy volunteers (16 females and 14 males aged between 17 and 49) whose diets were supplemented with 500 milligrams per day of vitamin C (ascorbic acid) for 6 weeks. We assessed the levels of oxidative damage to peripheral blood lymphocytes in terms of modified DNA bases. The level of 8-oxoguanine was found to decrease on supplementation relative to both placebo (calcium carbonate; 500 mg per day for 6 weeks) and baseline measurements, whereas the level of 8-oxoadenine increased.

For each volunteer, blood was collected at 3-weekly intervals for up to 12 weeks (6 weeks on placebo, 6 weeks on vitamin C) and then another sample was taken 7 weeks after completion of the vitamin C course (washout period). In each case, levels of plasma ascorbate were determined, lymphocytes were isolated and their DNA extracted before analysing oxidative damage.

Supplementation of diets with 500 mg per day of vitamin C resulted in a significant increase in ascorbate levels in the plasma (about 60%) compared with both pre-supplementation and placebo (data not shown). Following the washout period, vitamin C levels returned to the concentrations observed at baseline and in the placebo. In contrast, there was no change in ascorbate concentrations during oral placebo treatment in comparison with the baseline value.

We used gas chromatography–mass spectrometry (GC–MS)[2–4] to assess the levels of 8-oxoguanine and 8-oxoadenine, which are markers for DNA damage mediated by oxygen radicals. Supplementation of diets with 500 mg per day of vitamin C resulted in a significant decrease ($P<0.01$ by ANOVA compared with both baseline and placebo) in 8-oxoguanine levels (Fig. 1). No significant differences from baseline damage to DNA bases was observed during the placebo treatment. Furthermore, following the 7-week 'washout' period, levels of 8-oxoguanine returned to those observed at baseline and during placebo treatment.

In contrast, supplementation with vitamin C resulted in a significant increase ($P<0.01$ by ANOVA compared with baseline and with placebo values) in 8-oxoadenine levels in DNA isolated from lymphocytes. Again, there were no significant differences during the placebo treatment. During the washout, the mean level of 8-oxoadenine returned to that observed at baseline or during placebo, such that there was a statistically significant decrease in 8-oxoadenine compared with the vitamin C supplementation period ($P<0.01$). There were no changes in either lymphocytes or neutrophil counts during either placebo or vitamin C treatments (data not shown), confirming that the alterations noted in 8-oxopurine levels in lymphocyte DNA were not due to gross changes in cell type.

We established baseline values for the 8-oxoguanine and 8-oxoadenine lesions in human lymphocyte DNA. Mean values obtained for 8-oxoguanine (30 lesions per 10^5 guanine bases) and 8-oxoadenine (8 lesions per 10^5 adenine bases) were remarkably similar to those found in other *in vivo* systems[5–7]. These results re-emphasize that GC–MS is a powerful technique for measuring oxidative DNA damage as it can simultaneously quantify more than one modified DNA base.

Endogenous oxygen radicals are capable of damaging cellular biomolecules such as DNA[8]. Most of these species produced *in vivo* are quenched by antioxidant defences. However, a fine balance exists that may be disrupted in favour of oxidants (oxidative stress), giving rise to an accumulation of biomolecular damage, which in turn may play a role in major diseases such as cancer, rheumatoid arthritis and atherosclerosis[9]. It has been postulated that dietary intervention with vitamin C may reduce oxidative stress and thereby prevent such diseases.

Although the antioxidant nature *in vivo* of vitamin C has been questioned[10], it is nonetheless marketed as supplements in doses of 500 mg or more per day as an 'antioxidant'. Our discovery of an increase in a potentially mutagenic lesion, 8-oxoadenine[11,12], following a typical vitamin C supplementation should therefore be of some concern, although at doses of less than 500 mg per day the antioxidant effect may predominate.

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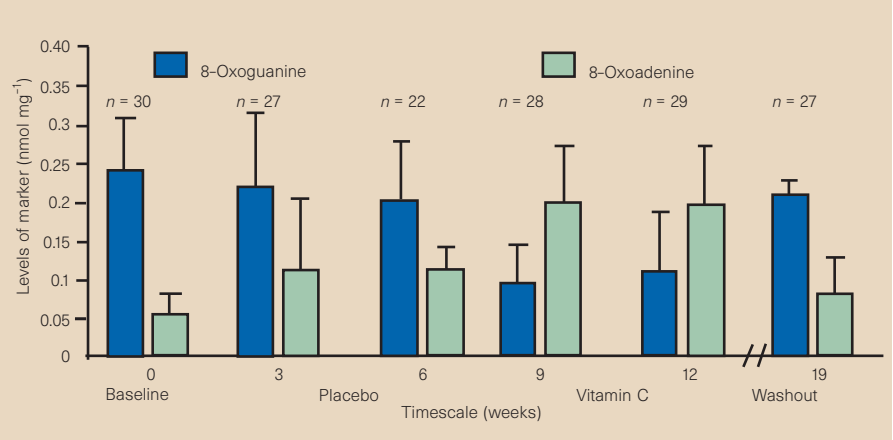

Figure 1 Levels of 8-oxoguanine and 8-oxoadenine in lymphocyte DNA from healthy subjects during vitamin C supplementation as measured by gas chromatography–mass spectrometry. Values represent the mean plus 1 s.d. Data were analysed by general linear model analysis of variance (ANOVA), with subsequent comparison between means using either Tukey one-way ANOVA, or Fisher's least significant difference test if there was a significant subject effect. One nanomol of 8-oxoguanine (8-oxoadenine) per mg DNA is equivalent to about 124 8-oxoguanines (8-oxoadenines) per 10^5 guanines (adenines).

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