

Commentary

Commentary on “Faster plasma vitamin E disappearance in smokers is normalized by vitamin C supplementation”

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Commentary

The paper in this issue of *Free Radical Biology and Medicine* titled “Faster plasma vitamin E disappearance in smokers is normalized by vitamin C supplementation” by Richard Bruno and colleagues [1] provides the first direct evidence that vitamins C and E work together as coantioxidants *in vivo*.

In the 1920s and 1930s the research of Dr. Albert Szent-Györgyi had vitamin C as a central focus. In his seminal work on vitamin C he provided the first evidence that ascorbic acid (then called hexauronic acid) could function as an antioxidant [2]; he discovered that it was the preferred reducing substrate for the activated form of hemeperoxidases (now known as compound-I) thereby sparing the oxidation of other potential substrates. At this same time Dr. Henry A Mattill was doing research on vitamin E [3]. Although vitamin C is water soluble and vitamin E oil soluble, they were encountering somewhat similar problems when trying to determine the exact chemical nature of these two substances; because both substances are excellent reducing agents, they were easily destroyed in the presence of oxidants. In 1936 Olcott and Mattill [4] demonstrated that vitamin E functions as a classic antioxidant. In 1941 Mattill's group [5] clearly demonstrated that these two substances could work as coantioxidants to protect fats from oxidation; he observed that tocopherol was necessary for this protection and that it was preserved as long as ascorbate was present. After all the ascorbate was oxidized, tocopherol was subject to loss and the oxidation of the fats/oils ensued. However the chemical mechanism of this protection was not understood.

In 1979 J.E. Packer *et al.* [6] demonstrated a likely mechanism by which these coantioxidants functioned as

partners to protect against lipid oxidation. Using pulse radiolysis, a fast kinetic technique, in a solvent system that allowed a homogenous solution of both ascorbic acid and tocopherol, they generated the tocopherol free radical (TO[•]) and observed that it reacted rapidly with ascorbate (AscH⁻) with the resulting formation of the ascorbate free radical (Asc^{•-}), thus, the now well-known figure of lipid peroxy radicals (LOO[•]) being reduced to lipid hydroperoxide (LOOH) by tocopherol (TOH), with formation of the chromanoxyl radical of tocopherol. TO[•] is in turn reduced by AscH⁻; i.e., vitamin E is recycled. Enzyme systems and thiols reduce the oxidation products of ascorbate back to AscH⁻ (Fig. 1). Their results show that at the heart of the process are one-electron reactions; i.e., ascorbate reacts rapidly with the one-electron oxidation product of tocopherol.

Lipid peroxy radicals are quite oxidizing ($E^{\circ} \approx 1000$ mV [7]); the tocopherol radical is less oxidizing ($E^{\circ} \approx 500$ mV) but not innocuous. If not removed it will initiate free-radical-mediated lipid oxidation as demonstrated in human low-density lipoprotein, albeit slowly [8]. The ascorbate free radical is even less oxidizing ($E^{\circ} \approx 280$ mV) but rapidly dismutates or is reduced by enzymes and thus the possibility of the initiation of additional oxidations is removed.

In the complex environment of blood plasma, ascorbate had been found to preserve tocopherol and extend the lag phase preceding detectable lipid oxidation when the plasma is subjected to a continuing flux of peroxy radicals [9,10]. Because of the rapid reaction of ascorbate with the tocopherol radical ($k = 3 \times 10^5 \text{ M}^{-1} \text{ s}^{-1}$ [11]), the ascorbate radical can be observed, but the tocopherol radical remains below the limit of detection as long as there is sufficient ascorbate present in plasma [12].

The protection against the loss of vitamin E by C has also been demonstrated in cell culture [13,14]. Using freshly isolated hepatocytes, it was observed that ascorbate could

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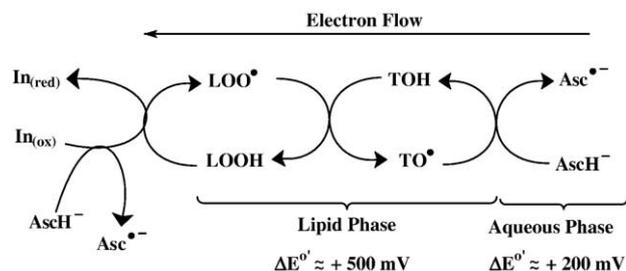


Fig. 1. Ascorbate is an excellent one-electron reducing agent that can spare tocopherol. AscH[•] can intercept the primary oxidants (In_(ox)) that can initiate free-radical-mediated lipid oxidation. This would spare TOH as the need for its reducing capacity would be lessened. AscH[•] can also recycle tocopherol by reducing TO• back to TOH, not allowing TO• to be oxidized further and then in turn go on to form irreversible products and be lost.

preserve tocopherol, but it also appeared that each acted independently as an antioxidant. The observations are consistent with the recycling of E by C through one-electron processes but are not direct evidence supporting this mechanism. Both groups concluded that ascorbate could preserve tocopherol by intercepting the oxidants that would initiate lipid oxidation.

The concept of C and E working as coantioxidants prompted K.F. Gey to reexamine epidemiological data to see how plasma levels of C and E might correlate with human health [15]. He found that plasma values for C and E for prevention of cardiovascular disease and cancer were (a) $\geq 50 \mu\text{M}$ vitamin C, (b) $\geq 30 \mu\text{M}$ lipid-standardized vitamin E, and (c) a vitamin C/vitamin E ratio >1.3 – 1.5 in plasma for primary prevention. He found that all data published were consistent with the assumption that the functional couple of vitamins C and E is a crucial part of the overall antioxidant network.

The work of Bruno et al. [1] in this issue of *FRBM* provides the first direct evidence in humans that a chronic oxidative stress, smoking, increases the rate of loss of vitamin E (both α -TOH and γ -TOH) in plasma and that ascorbate slows this loss in smokers; increasing plasma ascorbate produced no change in the rate of loss of E in nonsmokers. Importantly, increasing the plasma level of ascorbate produced no changes in the rate of metabolism of vitamin E via the cytochrome P-450 system in both smokers and nonsmokers. This observation provides strong support for the coantioxidant function of these two antioxidants *in vivo*. There are two possibilities that cannot be deconvoluted for these data for the sparing of E: (1) AscH[•] reduces TO• back to TOH, not allowing TO• to lose another electron to form the tocopherol quinone, an oxidation product in which the nonaromatic ring undergoes rupture and is thought to be irreversible; thus, loss of TOH; or (2) AscH[•] could reduce the primary oxidants that initiate free-radical-mediated lipid peroxidation, removing the burden on TOH and thereby sparing it.

No matter which mechanism is operating, it is clear that AscH[•] spares tocopherol in smokers. This is not due to changes in the rate of metabolism of TOH by the P-450 system, which

points directly to these two reductants acting as coantioxidants. In the blood this sparing is probably due to the reaction of AscH[•] with TO•; however, in tissue AscH[•] may be acting as a preventative antioxidant, Fig. 1 far left, and as a recycling agent, Fig. 1 far right. That an increase in plasma AscH[•] did not change the rate of the loss of E in nonsmokers is probably due to the sufficiency of the very robust levels of C ($\approx 60 \mu\text{M}$) and the absence of stress. The levels of C in smokers was somewhat less ($\approx 50 \mu\text{M}$), but the long-term, chronic oxidative stress of smokers appears to be key to the increased rate of loss of E. That this loss of TOH is slowed with increased levels of plasma ascorbate suggests that an organism subjected to oxidative stress should have increased intake of TOH and AscH[•]. This study does not address this directly but is clearly suggestive.

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