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COMMENTARY

Vitamin C: new role of the old vitamin in the cardiovascular system?

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In 1928, Albert Szent-Györgyi, professor of Chemistry and Biochemistry at the University of Szeged, Hungary, isolated ascorbic acid (vitamin C) from green pepper. Mainly due to this discovery, he was awarded the Nobel Prize for Medicine or Physiology in 1937 (Raju, 1999). Almost a century after the discovery of vitamin C, the old vitamin is still able to show us something new.

In this issue of *British Journal of Pharmacology*, Davis *et al*. (2006) describe that ATP induces a purinoceptor-mediated, Ca^{+}-dependent release of ascorbate from pig coronary endothelial cells. However, this phenomenon has not been observed in vascular smooth muscle cells. This observation is of particular interest as it suggests that the antioxidant capacity of coronary endothelial cells can be actively regulated by receptor-mediated mechanisms leading to release of ascorbate. It may further suggest that, in man, ascorbate is not a 'passive' antioxidant depending on the dietary intake. Endothelial cells are able to accumulate (May & Qu, 2005) and release (Davis *et al*., 2006) ascorbate for yet unidentified reasons, possibly for regulation of the antioxidant capacity of the plasma and the extracellular space neighboring the endothelial cells (Figure 1). Elucidation of vitamin C transport and function in health and disease may lead to development of new drugs acting on vitamin C transporters to increase the antioxidant capacity of the vasculature in cardiovascular diseases. Here, we briefly summarize the physiology and pharmacology of vitamin C in the light of the new observation of Davis *et al*. (2006).

As an electron donor, vitamin C acts as a cofactor for eight enzymes involved in collagen hydroxylation, biosynthesis of carnitine and norepinephrine, tyrosine metabolism and amidation of peptide hormones (see for a review Padayatty & Levine, 2001). Vitamin C has many nonenzymatic actions as well. It is a powerful water-soluble antioxidant, it protects low density lipoproteins from oxidation, reduces harmful oxidants in the stomach and promotes iron absorption (see for reviews Carr & Frei, 1999a; May, 1999; Padayatty *et al*., 2003).

Most mammalian species synthesize ascorbic acid *de novo* from glucose in the liver, through a biosynthetic pathway involving gulono-gamma-lactone oxidase for the terminal step. However, primates and guinea pigs are absolutely dependent on exogenously supplied dietary vitamin C due to inactivity of the gulono-gammalactone oxidase gene. Nonhepatic cells (and even hepatic cells in primates) need to take up ascorbate actively. The cellular uptake of ascorbate is regulated by both glucose and insulin (see for reviews Cunningham, 1998; Wilson, 2005). Ascorbic acid is transported into the cell by sodium-dependent vitamin C transporters SVCT1 and SVCT2, one or both of which are found in most tissues. Ascorbic acid can be easily oxidized to the unstable dehydroascorbic acid, which is transported into the cells by glucose transporters GLUT1 and GLUT3, and, in insulin-sensitive tissues, also by GLUT4, where it is rapidly reduced to ascorbic acid by glutaredoxin and thioredoxin reductase (May *et al*., 1998). As a result of this recycling of extracellular ascorbic acid in some cells, the concentration of intracellular ascorbic acid may increase up to 30-fold as seen, for example in neutrophil granulocytes. Brain, adrenal cortex, liver, spleen, pancreas and kidney tissues concentrate vitamin C for yet unknown reasons. Recent results have shown that endothelial cells also recycle and accumulate ascorbate by glutathion-dependent mechanisms (May *et al*., 2001, 2003; May & Qu, 2005) (Figure 1).

Although release of ascorbate has been shown in some cell types, there has been very little research on how ascorbate is transported out of the cells. Incubation with extracellular vitamin C stimulates efflux of preloaded [^{14}C]-vitamin C as observed in some epithelial and nonepithelial cells (see for a review Wilson, 2005). Intracellular reduction of dehydroascorbic acid to ascorbic acid can lead to ascorbate efflux as reported for erythrocytes (Mendiratta *et al*., 1998) and hepatocyte-like HepG cells (Upston *et al*., 1999). The mechanism of cellular ascorbate efflux is not known, although the involvement of volume sensitive anion channels and connexins was suspected (see for a review Wilson, 2005). The novel observation of Davis *et al*. (2006) shows that ascorbate efflux from endothelial cells can be regulated by a P2Y2-receptor-mediated mechanism. Whatever the underlying mechanism, the recycling of ascorbate allows the reducing equivalents derived from cell metabolism to be transferred to dehydroascorbic acid and carried into the extracellular fluid as ascorbate, thus becoming available to neighbouring cells.

Plasma ascorbate level is tightly controlled. Efflux of ascorbate from enterocytes and renal tubular cells, to the blood, is essential for intestinal absorption and renal conservation of vitamin C. Furthermore, hepatic ascorbate release is essential for the maintenance of plasma ascrobate level (Upston *et al*., 1999). When given orally, ascorbic acid is

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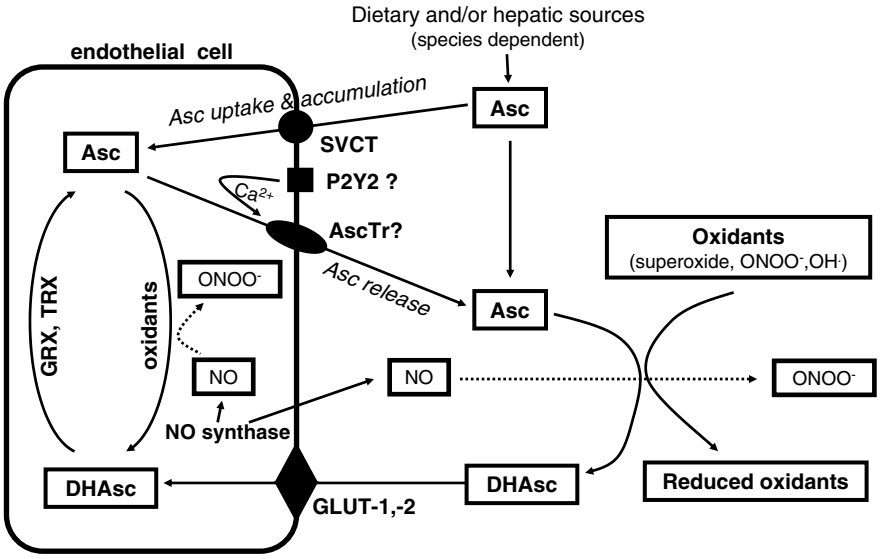

Figure 1 Recycling of ascorbate (Asc) by endothelial cells to maintain local nitric oxide (NO) bioavailability. Asc is taken up *via* sodium-dependent Asc transporters (SVCT) and accumulated by endothelial cells. Asc is oxidized by oxidants to dehydroascorbate (DHAsc). Extracellular DHAsc is taken up by glucose transporters (GLUT-1, -2). DHAsc is reduced to Asc by glutaredoxin (GRX) and thioredoxin reductase (TRX). Asc is released by a calcium-dependent, purinoceptor-mediated (suspected receptor is PYP2) mechanism *via* an unidentified Asc transporter (AscTr?). The role for receptor-mediated Asc release is not known. It is hypothesized that the regulation of Asc uptake, accumulation, and release by the endothelial cells maintains the optimal antioxidant capacity of the plasma and the neighbouring extracellular space to increase local NO bioavailability and prevent formation of the cytotoxic peroxynitrite ($ONOO^-$). How the mechanism of Asc recycling is affected in disease states is not known.

well absorbed at lower doses, but absorption decreases as the dose increases. Ascorbic acid is not protein bound, so it is filtered and reabsorbed by the kidneys in healthy subjects. Ascorbic acid begins to appear in urine when plasma concentration rises above about 60 $\mu mol\,l^{-1}$. Regulation of absorption and renal excretion keeps plasma vitamin C at less than 100 $\mu mol\,l^{-1}$, even with an oral dose of 1000 mg. In men at steady state, a 30-mg daily intake results in a mean plasma concentration of 9 $\mu mol\,l^{-1}$, 60 mg results in 25 $\mu mol\,l^{-1}$, 100 mg in 56 $\mu mol\,l^{-1}$ and 200 mg in 75 $\mu mol\,l^{-1}$. Oral doses greater than 500 mg daily contribute little to further increase in plasma concentration or tissue stores. The optimum intake of vitamin C, its function in various tissues and its antioxidant actions *in vivo* remain to be elucidated. In the late nineties, on the advice of the Food and Nutrition Board of the US National Academy of Sciences, US and Canadian recommended dietary allowances (RDA) were increased from 60 mg per day to 75 mg per day for women and 90 mg per day for men. However, for prevention of cardiovascular and cancer diseases in healty subjects, even 120 mg has been proposed as a new RDA for vitamin C (Carr & Frei, 1999b). Nevertheless, some of the large scale, chronic intervention studies with vitamin C have shown no clear clinical benefit possibly due to inappropriate dosing and selection of patient groups (Padayatty *et al*., 2003).

The role of recycling of ascorbate by endothelial cells and erythrocytes (Mendiratta *et al*., 1998; May *et al*., 2004) which involves uptake of ascorbate and dehydroascorbate, reduction of dehydroascorbate, and release of ascorbate, may be of great importance for regulation of local antioxidant capacity of the vasculature to maintain physiological levels of nitric oxide (NO). When NO combines with superoxide, the potential cytotoxic species, peroxynitrite is formed with a concomitant decrease in NO bioavailability as seen in various cardiovascular pathologies including ischemic heart disease and hyperlipidemia (see for reviews Ferdinandy & Schulz, 2001; Ferdinandy & Schulz, 2003), nitrate tolerance (see for a review Csont & Ferdinandy, 2005), and atherosclerosis (see for a review Carr *et al*., 2000). Ascorbate has been suggested to exert a tonic protective effect of NO in rat aorta (Dudgeon *et al*., 1998), to protect nitrergic neurotransmission in the vasculature (Lilley & Gibson, 1997), and to prevent endothelial dysfunction (see for a review May, 2000). Ascorbate has been proved to protect against peroxynitrite-induced tissue injury in the myocardium (Gao *et al*., 2002) and in other tissues as well (see for a review Arteel *et al*., 1999). However, little is known about ascorbate recycling in disease. The proinflammatory cytokines tumor necrosis factor-alpha and interleukin-1beta have been shown to inhibit ascorbate uptake in human endothelial cells (Seno *et al*., 2004). Further studies on the mechanism of ascorbate recycling in endothelial cells in health and disease may lead to development of new drugs that increase the ascorbate-mediated local vasculoprotective mechanisms to treat various cardiovasascular diseases.

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