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REVIEW

The SLC23 family of ascorbate transporters: ensuring that you get and keep your daily dose of vitamin C

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The ascorbate transporters SVCT1 and SVCT2 are crucial for maintaining intracellular ascorbate concentrations in most cell types. Although the two transporter isoforms are highly homologous, they have different physiologic functions. The SVCT1 is located primarily in epithelial cells and has its greatest effect in reabsorbing ascorbate in the renal tubules. The SVCT2 is located in most non-epithelial tissues, with the highest expression in brain and neuroendocrine tissues. These transporters are hydrophobic membrane proteins that have a high affinity and are highly selective for ascorbate. Their ability to concentrate ascorbate inside cells is driven by the sodium gradient across the plasma membrane as generated by Na+/K+ ATPase. They can concentrate ascorbate 20 to 60-fold over plasma ascorbate concentrations. Ascorbate transport on these proteins is regulated at the transcriptional, translational and post-translational levels. Available studies show that transporter function is acutely regulated by protein kinases A and C, whereas transporter expression is increased by low intracellular ascorbate and associated oxidative stress. The knockout of the SVCT2 in mice is lethal on day 1 of life, and almost half of SVCT1 knockout mice do not survive to weaning. These findings confirm the importance both of cellular ascorbate and of the two transport proteins as key to maintaining intracellular ascorbate.

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Abbreviations
AFR, ascorbate free radical; DHA, dehydroascorbate; DIDS, 4,4′-diisothiocyanodihydrostilbene-2,2′-disulfonic acid; DTNB, 5,5′-dithiobis(2-nitrobenzoic acid); SVCT, sodium-dependent vitamin C transporter

Introduction/Overview

Humans and higher primates are unable to synthesize vitamin C (ascorbic acid) and will develop scurvy if long deprived of the vitamin in their diets. Most other species can convert glucose to ascorbic acid in the liver and thus are minimally dependent upon dietary vitamin C. To preserve ascorbate, several mechanisms have evolved to efficiently recycle it from its two oxidized forms, the ascorbate free radical and dehydroascorbate (DHA), which are the one and two electron-oxidized forms respectively (May and Asard, 2004). Almost all of this recycling occurs inside cells, where ascorbate has its major functions to scavenge radical species, to recycle vitamin E in cell membranes, to serve as a co-factor for various dioxygenase enzymes, and to help generate and preserve nitric oxide that is needed for vascular regulation (May, 2000). Several of these functions require relatively high ascorbate concentrations; indeed, ascorbate concentrations in most cells and organs (0.5–4 mM) are much higher than in plasma (40–60 μM) (Evans *et al.*, 1982). High intracellular ascorbate concentrations can be generated by transport of either ascorbate or DHA into cells, although as noted next, the latter is not usually the major route.

Ascorbate has long been known to enter cells on a sodium and energy-dependent transporter that is of high affinity and specificity, capable of generating a steep concentration gradient, but which is relatively slow compared with uptake of DHA (Rose, 1988; Wilson, 2005). Cells rapidly take up DHA by facilitated diffusion on the ubiquitous glucose transporters of the GLUT family (Wilson and Dragan, 2005). Although ascorbate itself has little or no affinity for GLUTs, DHA assumes a bicyclic hemiketal form in solution (DiLabio and Wright, 2000; Pastore *et al*. 2001) that is able to use the GLUT transporters even in the presence of glucose (Vera *et al*., 1993). Once inside cells, DHA is rapidly reduced to ascorbate by NADH and NADPH-dependent reductases, as well as by reduced glutathione (GSH) (May and Asard, 2004). GSH-dependent reduction of DHA occurs either directly or is facilitated by thiol transferase enzymes (Wells *et al*., 1990). The relative importance of DHA transport and reduction versus ascorbate transport to the intracellular ascorbate concentration depends on the extracellular concentration of DHA. If this is high enough to compete with glucose, such as in areas of inflammation near inflammatory cells that are releasing reactive oxygen species (Nualart *et al*., 2003), DHA uptake and reduction can be significant, albeit transient. On the other hand, DHA levels in plasma and probably the interstitium are very low and thus likely to contribute very little to overall uptake (Okamura, 1979; Dhariwal *et al*., 1991). Perhaps the strongest evidence that ascorbate rather than DHA transport is the primary means by which most cells accumulate ascorbate is that embryonic mice lacking one isoform of the ascorbate transporter (the absence of which is lethal past the embryonic stage) have very low levels of ascorbate in brain and numerous other tissues (Sotiriou *et al*. 2002; Harrison and May, 2009). Moreover, inflammatory macrophages from adult mice that selectively lack this ascorbate transporter in hematopoietic cells also have undetectable levels of ascorbate when placed in culture (Babaev *et al*., 2010). Even though ascorbate in the diet or synthesized in the liver can provide sources of ascorbate for cells and tissues, it is clear that the ascorbate transporter is necessary to prevent cellular 'scurvy.'

Nomenclature

The transporter for ascorbate was cloned in 1999 and given the trivial name of sodium-dependent vitamin C transporter, abbreviated SVCT (Tsukaguchi *et al*., 1999). Two functional isoforms were identified in the rat (Tsukaguchi *et al*., 1999) and subsequently in the human (Daruwala *et al*., 1999; Rajan *et al*. 1999; Wang *et al*., 1999; 2000). The human isoforms have 65% identity at the amino acid level, but their functions differ due to their divergent tissue distributions (Tsukaguchi *et al*., 1999). Initial gene names given the two isoforms differed from those ultimately assigned in 2003 in the HUGO nomenclature (Takanaga *et al*., 2004), such that the human SVCT1 now corresponds to the *SLC23A1* gene and maps to chromosome 5q31.2–31.3 (Stratakis *et al*., 2000; Wang *et al*. 2000), and the human SVCT2 now corresponds to the *SLC23A2* and maps to chromosome 20p12.2–12.3 (Hogue and Ling, 1999; Stratakis *et al*. 2000). There is little homology between the SVCTs and other mammalian membrane transporters (Tsukaguchi *et al*., 1999), although both human transporters are weakly homologous in sequence to an orphan yolk-sac permease-like protein (YSPL1, *SLC23A3*) and a related variant (*SLC23A4*), as well as to a family of nucleobase transporters in lower organisms (Tsukaguchi *et al*. 1999; Takanaga *et al*., 2004). However, no isoforms beyond the SVCT1 and SVCT2 with clear function as ascorbate transporters have been identified.

Pharmacology

Quantification

SVCT1 and SVCT2 are low abundance transporters and have not thus far been quantified by binding or coupling with radioligands or other labels. Ascorbate derivatives involving carbons 2 and 3 are poor inhibitors of ascorbate transport and also lose the reducing capacity of the molecule. Although numerous carbon-6-modified ascorbate derivatives have been prepared (Cousins *et al*., 1977; Raic-Malic *et al*., 1999; Manfredini *et al*. 2002; Dalpiaz *et al*. 2004; 2005), affinity ligands for the SVCT transporters have not been reported.

Endogenous substrates

The SVCT's are very selective for *L*-ascorbic acid. As reviewed by Savini and colleagues, (Savini *et al*., 2008 14324/id), the apparent affinities of the SVCTs for ascorbate vary with species, cell type and assay conditions (e.g. pH). Apparent K_m values for ascorbate typically range over concentrations found in plasma, or 25–100 μM. In general, the SVCT2 tends to be of higher affinity but lower capacity than the SVCT1, albeit with considerable overlap (Savini *et al*., 2008 14324/id). *Xenopus* oocyte expression studies showed that neither isoform has appreciable affinity for isoascorbate or DHA (Rumsey *et al*., 1999; Tsukaguchi *et al*. 1999).

Synthetic substrates and their selectivities

Ascorbate carbon-6 iodo, bromo and fluoro derivatives have been shown to be transported into cells with affinities similar to that of ascorbate (Osmak *et al*. 1990; Rumsey *et al*., 1999; Nishikawa *et al*., 2003; Corpe *et al*., 2005; Kim *et al*. 2009), which implicate influx on the SVCTs. Significantly, oocyte expression studies showed that 6-bromo-6-deoxy-*L*-ascorbate is transported on the SVCT2 (Corpe *et al*., 2005), whereas its oxidized iodo derivative is transported only on glucose transporters (Rumsey *et al*. 1999; Corpe *et al*., 2005). Although other carbon-6-modified ascorbate derivatives inhibit ascorbate transport into cells, they have not been shown to be transported (Manfredini *et al*., 2002; Dalpiaz *et al*., 2004; 2005). A caveat in this regard is the slow uptake of ascorbate-2-phosphate on several different cell types that likely occurs on SVCTs. Although ascorbate 2-phosphate is not a substrate or inhibitor of the SVCTs (Tsukaguchi *et al*., 1999), in the presence of cells the phosphate group is removed by cell-surface phosphatases and the resulting ascorbate is then taken up on the SVCT (Hitomi *et al*. 1992; Fujiwara *et al*., 1997). Ascorbate 2-phosphate has been used to prolong ascorbate effects in culture, as dephosphorylation appears to be rate limiting for uptake (Nowak and Schnellmann, 1996; Fujiwara *et al*., 1997; Kashino *et al*. 2003). Fatty acid carbon-6

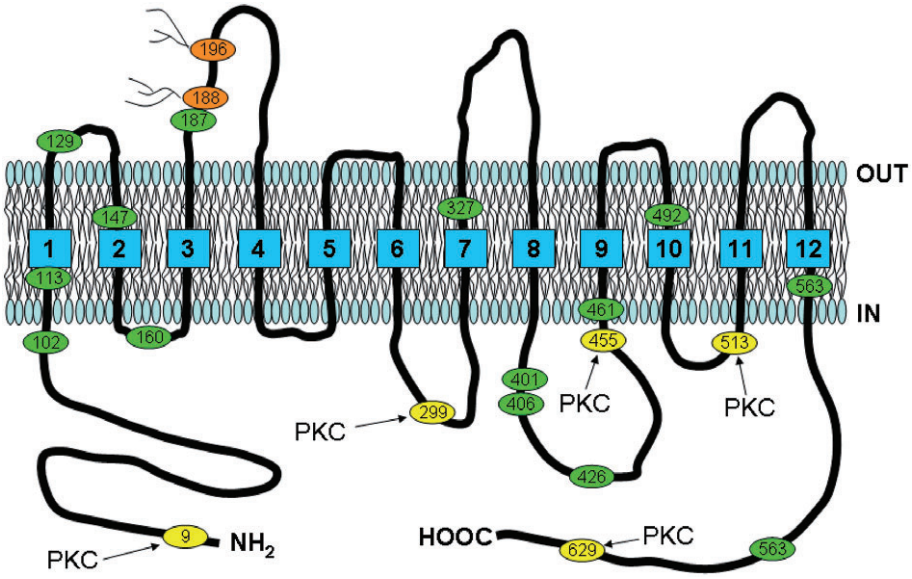

Figure 1

Schematic diagram based on the proposed sequence and hydropathy analysis of the human SVCT2 (Rajan *et al.*, 1999). The human SVCT2 is predicted to have 12 membrane-spanning regions with intracellular amino (NH_2-) and carboxy (HOOC-) termini. Locations of potential extracellular glycosylation sites are noted in brown, cysteine residues are noted in green and sites for protein kinase C phosphorylation are noted in yellow.

ascorbate derivatives such as ascorbate 6-palmitate have antioxidant activities (Nagao and Terao, 1990; Ross *et al.*, 1999; Wang *et al.* 2000; Pinnell, 2002). This likely relates to intercalation into and protection of the plasma membrane due to the lipophilic fatty acid group, although ascorbate 6-palmitate did have weak inhibitory activity of the SVCT1 in oocyte expression studies (Wang *et al.*, 2000).

Transport inhibitors of the SVCTs

For sodium- and energy-dependent transporters such as the SVCTs, transport inhibitors could inhibit the transporter, inhibit the Na^+/K^+ ATPase that maintains the sodium gradient or affect the driving sodium gradient itself. Indeed, Na^+/K^+ ATPase inhibition with agents such as ouabain has long been used to provide evidence that specific ascorbate transporters (and not the GLUT-type glucose transporters) are involved (Diliberto *et al.*, 1983; Castro *et al.*, 2001). Removal of the sodium gradient by replacement of extracellular sodium with lithium or choline also effectively blocks ascorbate uptake (Rajan *et al.* 1999; Godoy *et al.*, 2006).

Several non-covalent inhibitors of high-affinity ascorbate transport have been described in a variety of cell types. Sulfinpyrazone and 4,4′-diisothiocyanodihydrostilbene-2,2′-disulfonic acid at low millimolar concentrations are well established inhibitors of sodium-dependent high-affinity ascorbate transport (Franceschi *et al.*, 1995; Holmes *et al.* 2000; Daskalopoulos *et al.* 2002; Best *et al.*, 2005). However, these inhibitors are not selective for the SVCTs and will inhibit anion transporters as well. For the most part, inhibitors of glucose or DHA transport (e.g. glucose derivatives, cytochalasin B) do not affect ascorbate transport. An exception to this is flavonoid compounds such as phloretin and quercetin. Phloretin concentrations of 20–100 μM substantially inhibit both glucose (LeFevre, 1961; Kotyk *et al.* 1965; Craik and Elliott, 1979) and ascorbate transport (Wang *et al.* 2000; Caprile *et al.*, 2009). Quercetin has a similar relatively high affinity for the SVCTs as for the glucose transporters, with K_i values of 18 and 23 μM respectively (Song *et al.*, 2002). Other agents with low micromolar K_i values for the SVCT2 include steroid hormones and non-steroidal anti-inflammatory agents (Biondi *et al.*, 2007). Regarding the latter, diclofenamic acid, which has a K_i of 2.7 μM for inhibition of ascorbate transport in human retinal pigmented epithelial cells, when coupled to carbon-6 of ascorbate, had increased affinity for the SVCT2, with a K_i of 0.16 μM (Manfredini *et al.*, 2002). Perhaps the highest affinity ascorbate transport inhibitor known is palytoxin, which inhibited ascorbate transport in adrenal chromaffin cells half maximally at 0.1 nM, a concentration two orders of magnitude less than its inhibition of rubidium uptake or the Na^+/K^+ ATPase (Morita *et al.*, 1996).

Both cell permeant and impermeant thiol reagents have been shown to inhibit ascorbate transport, suggesting the presence of free cysteines on the protein (May and Qu, 2004). There are 16 cysteines on the rat SVCT2, although only 14 on the human SVCT2 (Figure 1). That the reactive cysteines are actually on the transporter and not on the Na^+/K^+ ATPase is indicated by the ability of ascorbate to protect ascorbate transport against inhibition by 5,5prime;-dithiobis(2-nitrobenzoic acid) (J.M. May, unpubl. data).

Transport stoichiometry and kinetic mechanism

The SVCTs couple the inward movement of ascorbate against its concentration gradient to the simultaneous movement of sodium down its electrochemical gradient. Studies in *Xenopus*

oocytes (Tsukaguchi *et al.*, 1999) and SVCT2-transfected human melanoma cells (Godoy *et al.*, 2006) showed that ascorbate transport is driven by the sodium gradient established by the Na^+/K^+ ATPase. Sodium acts to initiate ascorbate transport by increasing the affinity of the transporter for ascorbate (Godoy *et al.*, 2006). Once ascorbate has bound, it is proposed that a second sodium ion binds and drives the transport cycle (Godoy *et al.*, 2006). This process is also regulated by the required presence of both Ca^{2+} and Mg^{2+} ions for efficient function (Godoy *et al.*, 2006). Whereas ascorbate transport for the SVCT1 is electrogenic and associated with an inward sodium current during ascorbate transport, the SVCT2 appears to lack this property (Tsukaguchi *et al.* 1999; Godoy *et al.*, 2006).

Distribution

A major feature that differentiates between the SVCTs is their distribution. The SVCT1 is primarily expressed in epithelial cells of the intestine and renal proximal tubules (Tsukaguchi *et al.*, 1999), where it absorbs and reabsorbs ascorbate respectively. The sub-cellular distribution of the SVCT1 is primarily apical in columnar epithelial cells derived from the intestine (Caco-2) (Boyer *et al.*, 2005) and lung (Jin *et al.*, 2005), where it likely functions to increase intracellular ascorbate. On the other hand, the SVCT2 is found on the basolateral membrane of Caco-2 cells (Boyer *et al.*, 2005). In the lungs, both transporters are found on the apical surfaces of columnar epithelium (Jin *et al.*, 2005). In either case, dual expression of the transporters with their expected orientation would simply bring ascorbate into the cells. How ascorbate effluxes from intestinal cells after uptake or from hepatocytes after synthesis in most mammals has not been determined, although ascorbate efflux from hepatocytes is appreciable (Upston *et al.*, 1999).

The highest expression of the SVCT2 is in adrenal, brain, lung and bone (Tsukaguchi *et al.*, 1999). The SVCT2 is also functionally expressed in muscle (Savini *et al.*, 2005), lymphoid organs (Tsukaguchi *et al.*, 1999) and reticuloendothelial cells (Babaev *et al.*, 2010) including platelets (Savini *et al.*, 2007). Brain astrocytes lack the SVCT2 *in vivo* (Berger and Hediger, 2000), but develop it when placed in culture (Siushansian *et al.*, 1997). Maturing erythrocytes lose the SVCT2 during maturation and extrusion of the nucleus, such that they lack it entirely when they enter the circulation (May *et al.*, 2007). This accounts for the fact that they contain the same ascorbate concentration as does the blood plasma in which they circulate (Evans *et al.*, 1982).

Most organs take up ascorbate directly across the endothelium from internal capillaries. Recent studies have shown that although endothelial cells express high levels of the SVCT2, this does not facilitate ascorbate entry into the tissues (May *et al.*, 2009). Rather, ascorbate moves around endothelial cells in a paracellular manner through gaps between the cells and very likely across adherins and tight junctions. An exception to this is the brain, where the blood-brain barrier endothelium is impermeable to ascorbate (Agus *et al.*, 1997) and also lacks the SVCT2 (García *et al.*, 2005; Qiao & May, 2008). Rather, ascorbate enters the brain through the choroid plexus (Spector and Lorenzo, 1973), which contains the SVCT2 (Angelow *et al.*, 2003; García *et al.* 2005). Through this mechanism, the ascorbate concentration is stepped up from that in plasma to 200–300 μM in the cerebrospinal fluid, and then further into neurons, where the concentration can be as high as 10 mM in rodents (Rice and Russo-Menna, 1998). Although DHA has been shown to rapidly cross the blood-brain barrier on the GLUT1 by facilitated diffusion (Agus *et al.*, 1997), this mechanism is not likely to be of physiologic significance, given the very low circulating DHA concentrations noted previously.

The SVCT1 has been shown to translocate from the cytoplasm to the plasma membrane upon activation of protein kinase C (Liang *et al.*, 2002) and following UVB irradiation in skin keratinocytes (Kang *et al.*, 2007). Treatment of murine MC3T3-E1 cells with prostaglandin E_2 caused translocation of the SVCT2 from the cytoplasm to the plasma membrane and activation of transport on the SVCT2 (Wu *et al.*, 2007). The SVCT2 has also been detected in a perinuclear distribution in neurons (Mun *et al.*, 2006), and it may be functional intracellularly. High-affinity ascorbate transport has long been known to occur in chromaffin granules and likely accounts for their high intra-vesicular ascorbate concentrations (Ingebretsen *et al.*, 1980). The SVCT2 has also been localized to punctate structures in the axons of primary culture hippocampal neurons (Qiu *et al.*, 2007), where it could serve to take up ascorbate from the cytoplasm into neurosecretory vesicles. The presence of ascorbate in neurosecretory vesicles is well established, as glutamate activation releases significant amounts of ascorbate into the cerebrospinal fluid (O'Neill *et al.* 1984; Cammack *et al.*, 1991). This presumably derives from release of these vesicles at synaptic terminals following stimulation (Rebec and Pierce, 1994).

Regulation

Transcriptional and translational regulation of ascorbate transport

Regulation of ascorbate transport at the transcriptional level has been described for both the SVCT isoforms and generally occurs as changes in total cell and plasma membrane transport protein expression. For the SVCT1, expression increases with ascorbate deprivation in human Caco-2 intestinal cells (Maulen *et al.*, 2003) and decreases with aging in rat hepatocytes (Michels *et al.*, 2003). SVCT2 expression is increased during the establishment of primary culture of microcapillary brain endothelial cells (Qiao and May, 2008), following stimulation by various growth factors in human trophoblastic cells (Biondi *et al.*, 2007), and by treatment of cultured osteoblasts with glucocorticoids (Fujita *et al.*, 2001), zinc (Wu *et al.*, 2003b), or calcium and phosphate ions (Wu *et al.*, 2004). *In vivo* up-regulation of the SVCT2 has been demonstrated in the peri-infarct area of rodent brain following ischaemic injury (Berger *et al.*, 2003), as well as following dietary ascorbate deprivation in the liver of mice unable to synthesize ascorbate (Amano *et al.*, 2010).

Regulation of SVCT2 function by protein–protein interaction has also been proposed. This is based on the generation of an alternatively spliced variant of the protein that acts as a dominant-negative inhibitor of ascorbate transporter function (Lutsenko *et al.*, 2004).

Translational regulation of the SVCT2 has been demonstrated in human platelets, where activation by thrombin and phorbol ester increases both SVCT2 expression and function (Savini *et al.*, 2007).

Acute changes in transporter function

Post-translational regulation of the SVCTs has been proposed, based on the presence of consensus phosphorylation sites for both protein kinase A and protein kinase C on the endofacial transporter (Tsukaguchi *et al.*, 1999). The SVCT2 is known to be stimulated by agents that increase intracellular cyclic AMP (Wilson, 1989; Korcok *et al.*, 2000). Further, the previously noted translocation of the SVCT2 and resulting increases in ascorbate transport in murine MC3T3-E1 cells by prostaglandin E_2 were blocked by inhibition of protein kinase A and by mutation of serine residues in either or both of two protein kinase A consensus sequences (Wu *et al.*, 2007). Phorbol ester-dependent activation of protein kinase C halved ascorbate transport mediated by both SVCT1 and SVCT2 in COS-1 cells expressing the proteins (Liang *et al.*, 2001; 2002). For the SVCT1, this decrease was due to a decreased translocation of the protein to the plasma membrane, while for the SVCT2 it was due to decreased catalytic efficiency. On the other hand, SVCT2 message and protein were increased by phorbol ester-induced differentiation of THP-1 macrophages, an effect also blocked by inhibition of protein kinase C activity during differentiation (Qiao and May, 2009).

Biochemistry and genetics of the SVCTs

The human SVCT1 gene encodes 15 exons, while the much larger (10-fold) SVCT2 gene encodes 17 exons (Erichsen *et al.*, 2006). Despite differences in gene size and existence on different chromosomes, the genomic organization of the two genes is quite similar (Eck *et al.*, 2004), except for the promoter and transcription start site sequences. The genomic structure of the human SVCT1 shows that the region 100 base pairs upstream of the transcription start site to contain both CAAT and TATA boxes, as well as two AP-1 binding sites and a GATA binding site in the expected promoter region. The promoter of the SVCT2 is more complex (Rubin *et al.*, 2005) in that it lacks classical TATA box and uses two different promoters immediately upstream of the first two exons (termed 1a and 1b), with the translation start site in exon 3. Reporter constructs showed increased activity for the exon 1b promoter compared with the 1a promoter. Key features of the two promoter variants are USF, NFY and HIF-1 sites for the exon1a promoter, and multiple SP1 and EGR1 sites for the exon 1b promoter.

The SVCTs code for proteins of different lengths. At least for the SVCT2, these vary with the species. For example, immunoblots of the mouse SVCT2 show bands typically of 65–75 kDa (corresponding to 647 amino acids) (Wu *et al.*, 2003a; Jin *et al.*, 2005; May *et al.* 2005), whereas most studies show human SVCT2 as a band about 50 kDa (corresponding to 650 amino acids) (Li *et al.* 2003; Godoy *et al.*, 2006; Savini *et al.*, 2007). Hydropathy analysis suggests that both isoforms cross the plasma membrane 12 times, with both N- and C-terminal regions intracellular (Tsukaguchi *et al.*, 1999) (Figure 1). There are N-glycosylation sites between membrane-spanning regions 3 and 4 of the SVCTs, which may account in part for variation in band locations on immunoblot studies.

Genomic and functional analyses of the SVCT1 initially showed 22 single nucleotide polymorphisms in Caucasians and African-Americans, but no significant differences in transporter function when expressed in *Xenopus* oocytes (Eck *et al.*, 2007). Subsequent studies revealed that several human polymorphic variants expressed in oocytes did in fact have substantial decreases in ascorbate transport (Corpe *et al.*, 2010). An analysis of the larger SVCT2 gene also revealed numerous polymorphisms, but a functional analysis was not performed (Eck *et al.*, 2004). However, an evaluation of polymorphisms in a large study of pregnancy showed that intron variants of the SVCT2 had 1.7 to 2.4-fold increased risk of preterm delivery, whereas polymorphisms of the SVCT1 had no such association (Erichsen *et al.*, 2006). Variants of neither transporter were associated with changes in the incidence of colorectal adenoma (Erichsen *et al.*, 2008).

Clinical significance

Changes in expression or function of the SVCTs have not yet been associated with human disease, and no drugs have been shown to affect either of the two transporters in the clinical setting. Nonetheless, the importance of the transporters for maintaining cellular ascorbate concentrations and of cellular ascorbate for maintaining the health of both cells and the organism is clear from studies in knockout mice. Targeted deletion of the SVCT1 (Corpe *et al.*, 2010) resulted in 45% perinatal mortality (a fivefold increase) of the offspring of $SVCT1^{-/-}$ dams. This occurred in both the heterozygous and knockout pups and was prevented by ascorbate supplementation of the dam during pregnancy. This highlights the importance of ascorbate provided by the dam during pregnancy, even though mice can make their own ascorbate starting about day 15 of gestation (Kratzing and Kelly, 1982). Loss of the SVCT1 decreased plasma ascorbate concentrations by 50–70%, tripled ascorbate lost in the urine, but only marginally affected intestinal ascorbate absorption. As ascorbate absorption was similar in knockout mice and controls, there must be an alternative mechanism for ascorbate absorption beyond the SVCT1. Loss of up to 70% of body stores of ascorbate was ameliorated by increased hepatic synthesis of the vitamin. These results show the importance of the renal SVCT1 in maintaining ascorbate stores. Further, in a human unable to synthesize ascorbate with a dysfunctional SVCT1 polymorphism, this could lead to a significant drain of ascorbate and clinical consequences, especially during pregnancy (Corpe *et al.*, 2010).

Deficiency of the SVCT2 causes mice to die shortly after birth, with respiratory failure and cortical brain haemorrhage in the absence of classical or biochemical signs of scurvy (Sotiriou *et al.*, 2002). A subsequent study showed that there was also haemorrhage in lower brainstem areas and increased oxidative stress in several organs (Harrison *et al.*, 2010). Ascorbate levels in these mice are very low in tissues served by the SVCT2, including brain, adrenal, pituitary, skeletal muscle and pancreas (Sotiriou *et al.* 2002; Harrison *et al.*,

2010). Decreased placental ascorbate levels (Harrison *et al.*, 2010) and inability to prevent death by supplementing the dam with ascorbate during pregnancy suggest that the SVCT2 is also crucial for placental ascorbate transport. Mice heterozygous for SVCT2 deficiency appear completely normal and are fertile. Nonetheless, these results show clearly the requirement for the SVCT2 during gestation.

Conclusions

Both of the two ascorbate transporter isoforms play crucial roles in maintaining plasma and tissue ascorbate levels. Key to this function is their selectivity and high affinity for ascorbate as well as their ability to move the vitamin into cells against its concentration gradient. Their tissue distributions complement their ability to retain ascorbate systemically (e.g. renal reabsorption of ascorbate by the SVCT1) and to move it into cells that require it for crucial functions (ascorbate transport into most organs and especially brain, lung and neuroendocrine tissues). These features become especially important in humans and higher primates, who cannot synthesize their own ascorbate. The clear dependence on both transporters for prenatal development in mouse models could explain why significant defects in either transporter have not been discovered in humans.

Conflict of Interest

The author reports no conflict of interest for the content of this review.

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