Functional Characterization and Molecular Identification of Vitamin C Transporter (SVCT2) in Human Corneal Epithelial (HCEC) and Retinal Pigment Epithelial (D407) Cells

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Purpose
The main goal of this study is to investigate the existence of sodium dependent vitamin C transport system (SVCT2) and to define time dependent uptake mechanism and intracellular regulation of ascorbic acid (AA) in human corneal epithelial (HCEC) and human retinal pigment epithelial (D407) cells.

Methods
Uptake of \[^{14}\text{C}]\text{AA}\) was studied in HCEC and D407 cells. Functional aspects of \[^{14}\text{C}]\text{AA}\) uptake were studied in the presence of different concentrations of unlabeled AA, pH, temperature, metabolic inhibitors, substrates and structural analogs. Molecular identification of SVCT2 was examined with reverse transcription–polymerase chain reaction (RT-PCR).

Results
Uptake of \[^{14}\text{C}]\text{AA}\) was observed to be sodium, chloride, temperature, pH and energy dependent in both cell lines. \[^{14}\text{C}]\text{AA}\) uptake was found to be saturable, with Km values of 46.14±6.03 and 47.26±3.24 μM and Vmax values of 17.34±0.58 and 31.86±0.56 pmol/min/mg protein, across HCEC and D407 cells, respectively. The process is inhibited by structural analogs (L-AA and D-Iso AA) but not by structurally unrelated substrates (glucose and PAHA). Ca"/calmodulin and protein kinase C (PKC) pathways play an important role in modulating uptake of AA. A 626 bp band corresponding to a vitamin C transporter (SVCT2) has been identified by RT-PCR analysis in both the cell lines.

Conclusion
This study reports regarding the ascorbic acid uptake mechanism, kinetics, and regulation by sodium dependent vitamin C transporter (SVCT2) in HCEC and D407 cells. Also, SVCT2 can be utilized for targeted delivery in enhancing ocular permeation and bioavailability of highly potent ophthalmic drugs.