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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 [¹⁴C] AA was studied in HCEC and D407 cells. Functional aspects of [¹⁴C] 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 RT-PCR.

Results: Uptake of [¹⁴C] AA was observed to be sodium, chloride, temperature, pH and energy dependent in both cell lines. [¹⁴C] AA uptake was found to be saturable, with K_m values of 46.14 ± 6.03 and 47.26 ± 3.24 μ M and V_{max} 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 626bp band corresponding to a vitamin C transporter (SVCT2) has been identified by RT-PCR analysis in both the cell lines.

Biography

Abhirup Mandal has completed his Bachelors in Pharmacy from Manipal University, India and is pursuing Ph.D. from University of Missouri-Kansas City, MO, USA. He has presented and co-authored a poster on a study entitled "Evaluation of Drug Advertisements in Medical Journals" at International Pharmaceutical Federation Centennial Congress held at Amsterdam, The Netherlands in October 2012. He has also co-authored an article entitled "India uniquely placed to offer cost-effective Biosimilars" published in The Chronicle Pharmabiz (South Asian Circulation), July 2012.

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