Title: Intracellular dehydroascorbic acid inhibits SVCT2-dependent transport of ascorbic acid in mitochondria

Author: Mara Fiorani Catia Azzolini Andrea Guidarelli Liana Cerioni Maddalena Scotti Orazio Cantoni

PII: S1043-6618(15)00137-1
DOI: http://dx.doi.org/doi:10.1016/j.phrs.2015.07.004
Reference: YPHRS 2866

To appear in: *Pharmacological Research*

Received date: 11-2-2015
Revised date: 7-7-2015
Accepted date: 9-7-2015

Please cite this article as: Fiorani Mara, Azzolini Catia, Guidarelli Andrea, Cerioni Liana, Scotti Maddalena, Cantoni Orazio.Intracellular dehydroascorbic acid inhibits SVCT2-dependent transport of ascorbic acid in mitochondria.*Pharmacological Research* http://dx.doi.org/10.1016/j.phrs.2015.07.004

Intracellular dehydroascorbic acid inhibits SVCT2-dependent transport of ascorbic acid in mitochondria

Mara Fiorani, Catia Azzolini, Andrea Guidarelli, Liana Cerioni, Maddalena Scotti and Orazio Cantoni*

Dipartimento di Scienze Biomolecolari Università degli Studi di Urbino "Carlo Bo", 61029 Urbino, Italy

Corresponding author at: Prof. Orazio Cantoni, Dipartimento di Scienze Biomolecolari Università degli Studi di Urbino "Carlo Bo", 61029 Urbino, Italy. Tel: +39-0722-303523; Fax: +39-0722-305321.

E-mail address: orazio.cantoni@uniurb.it

Abbreviations: AA, L-ascorbic acid; Cyt B, cytochalasin B; DHA, dehydroascorbic acid; DTT, dithiothreitol; pCMB, 4-hydroxymercuribenzoic acid; S-Pyr, sulfinpyrazone; TBA, tetrabutylammoniumhydrogensulfate; EB, extracellular buffer; MB, mitochondrial buffer.

Abstract

Exposure of U937 cells to low concentrations of L-ascorbic acid (AA) is associated with a prompt cellular uptake and a further mitochondrial accumulation of the vitamin. Under the same conditions, dehydroascorbic acid (DHA) uptake was followed by rapid reduction and accumulation of identical intracellular levels of AA, however in the absence of significant mitochondrial uptake. This event was instead observed after exposure to remarkably greater concentrations of DHA. Furthermore, experiments performed in isolated mitochondria revealed that DHA transport through hexose transporters and Na^+-dependent transport of AA were very similar. These results suggest that the different subcellular compartmentalization of the vitamin is mediated by events promoting inhibition of mitochondrial AA transport, possibly triggered by low levels of DHA. We obtained results in line with this notion in intact cells, and more direct evidence in isolated mitochondria. This inhibitory effect was promptly reversible after DHA removal and comparable with that mediated by established inhibitors, as quercetin.

The results presented collectively indicate that low intracellular concentrations of DHA, because of its rapid reduction back to AA, are a poor substrate for direct mitochondrial uptake. DHA