L-Ascorbic acid induces apoptosis in acute myeloid leukemia cells via hydrogen peroxide-mediated mechanisms

Seyeon Park\textsuperscript{a,b,*}, Seong-Su Han\textsuperscript{a}, Chan H. Park\textsuperscript{a,c}, Eun-Ryeong Hahn\textsuperscript{a}, Sook J. Lee\textsuperscript{a}, Hye K. Park\textsuperscript{a}, Se-Hoon Lee\textsuperscript{a}, Won S. Kim\textsuperscript{a}, Chul Won Jung\textsuperscript{a}, Keunchil Park\textsuperscript{a}, Hugh D. Riordan\textsuperscript{c}, Bruce F. Kimler\textsuperscript{d}, Kihyun Kim\textsuperscript{a,1}, Je-Ho Lee\textsuperscript{a,1}

\textsuperscript{a} Samsung Medical Center, Sungkyunkwan University School of Medicine, Seoul 135-710, Republic of Korea
\textsuperscript{b} Anesthesia and Pain Research Institute, Yonsei University College of Medicine, 134 Shinchon-dong, Seodaemun-gu, Seoul 120-752, Republic of Korea
\textsuperscript{c} The Center for the Improvement of Human Functioning International, Wichita, KS 67219, USA
\textsuperscript{d} University of Kansas Medical Center, Kansas City, KS 66160, USA

Received 20 March 2004; received in revised form 15 April 2004; accepted 15 April 2004

Abstract

L-Ascorbic acid (LAA) is being investigated clinically for the treatment of patients with acute myeloid leukemia (AML) based on the observed effects of LAA on AML progenitor cells in vitro. However, the mechanism for LAA-induced cytoreduction remains to be elucidated. LAA at concentrations of 0.25–1.0 mM induced a dose- and time-dependent inhibition of proliferation in three AML cell lines and also in leukemic cells from peripheral blood specimens obtained from three patients with AML. In contrast, ovarian cancer cell lines were only minimally affected. Flow cytometric analysis showed that LAA at concentrations of 0.25–1.0 mM could significantly induce apoptosis in the AML cell lines. LAA induced oxidation of glutathione to oxidized form (GSSG) and subsequent \( \text{H}_2\text{O}_2 \) accumulation in a concentration-dependent manner, in parallel to induction of apoptosis. The direct role of \( \text{H}_2\text{O}_2 \) in the induction of apoptosis in AML cells was clearly demonstrated by the finding that catalase could completely abrogate LAA-induced apoptosis. Induction of apoptosis in LAA-treated AML cells involved a dose-dependent increase of Bax protein, release of cytochrome C from mitochondria to cytosol, activation of caspase 9 and caspase 3, and cleavage of poly(ADP-ribose)polymerase. In conclusion, LAA can induce apoptosis in AML cells, and this is clearly due to \( \text{H}_2\text{O}_2 \) which accumulates intracellularly as a result of oxidation of reduced glutathione by LAA.

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Keywords: L-ascorbic acid; Apoptosis; Hydrogen peroxide; Glutathione; Acute myeloid leukemia

Abbreviations: AML, acute myeloid leukemia; APL, acute promyelocytic leukemia; BSO, buthionine sulfoximine; DCF, dichlorodihydrofluorescein; DHA, dehydroascorbic acid; FITC, fluorescein-isothiocyanate; GSH, reduced glutathione; \( \text{H}_2\text{DCFDA} \), 2',7'-dichlorodihydrofluorescein diacetate; HPLC, high performance liquid chromatography; LAA, l-ascorbic acid (vitamin C); MDS, myelodysplastic syndromes; MPA, meta-phosphoric acid; \( \text{O}_2^- \), superoxide; PARP, poly(ADP-ribose)polymerase; PI, propidium iodide; RA, retinoic acid; SBA, sodium 5,6-benzylidene-l-ascorbate; SOD, superoxide dismutase

* Corresponding author. Tel.: +82-2-3410-6810; fax: +82-2-3410-6808. E-mail address: seypark21@hotmail.com (S. Park).
1 Co-corresponding authors.

1357-2725/ - see front matter 2004 Elsevier Ltd. All rights reserved. doi:10.1016/j.biocel.2004.04.005