2-Chlorodeoxyadenosine induces apoptosis through the Fas/Fas ligand pathway in human leukemia cell line MOLT-4

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Halogenated nucleoside derivative 2-chrolodeoxyadenosine (2CdA) is now utilized as a chemotherapeutic agent for leukemia. Recently, using cell free system it was demonstrated that 2CdATP, which is formed by phosphorylation of 2CdA, activated an apoptosis-executive protease, caspase 3, by cooperation with Apaf-1, capase 9 (Apaf-3) and cytochrome C (Apaf-2) from mitochondria. As another pathway of apoptosis in T cells, the transmembrane receptor Fas and Fas ligand (Fas-L) are known to be key regulators of apoptosis. After ligation of Fas with Fas-L, procaspase 8 (FLICE) was transformed to its activated form (caspase 8) which further transform procaspase 3 to its activated form (caspase 3).

In this study, to get evidence for the presence of this Fas/Fas-L/caspase-8 mediated pathway in 2CdA-treated leukemia cell line MOLT-4 cells, the effects of a protein synthesis inhibitor cycloheximide (CHX), a caspase 8 inhibitor Ac-IETD-CHO and a caspase 3 inhibitor Ac-DEVD-CHO on 2CdA-induced apoptosis as well as the expression of Fas and Fas-L. 2CdA increased the formation of 3'-OH end in genomic DNA, the ladder-like fragmentation of DNA and the translocation of phosphatidylserine to the outer membrane, which were apoptotic characteristics. These apoptotic phenomena induced by 2CdA were inhibited by CHX, deoxycytidine (a substrate of deoxycytidine kinase), Ac-IETD-CHO and Ac-DEVD-CHO. The protein synthesis-dependent expression of Fas and Fas ligand was detected by treatment with 2CdA. The proteolytic processing of procaspases-8 and-3 to produce active fragments, caspases-8 (p18) and-3 (p17), respectively, was observed after treatment with 2CdA, and suppressed by CHX. Increase in the activities of caspases-8 and-3 was observed after 2CdA treatment and was attenuated by CHX. These results suggested that 2CdA-induced apoptosis was triggerd by phosphorylation of 2CdA followed by the protein synthesis-dependent expression of Fas and Fas-L and the activation of caspases-8 and -3.

From these experiments, in addition to the mechanism through 2CdA-induced activation of Apaf family, the activation of caspase 3 induced by Fas/Fas-L/caspase 8 was also considered to induce apoptosis in 2CdA-treated leukemia cell line. These data may contribute to its effective use to leukemia chemotherapy.