Cytotrienin A, a Novel Apoptosis Inducer in Human Leukemia HL-60 Cells

Sir:

Cell death occurs via two distinct mechanisms: necrosis and apoptosis\(^1\). While necrosis is a degenerative phenomenon produced by severe environmental disturbances, apoptosis is regarded as an active suicidal response to various physiological or pathological stimuli. Apoptosis is involved in embryonic development, tissue remodeling, and tumor regression. Autoreactive T cells are selectively depleted by apoptosis during clonal selection in the immune system.\(^2\)\(^-\)\(^4\) Recently, it has been shown that the efficacy of various anticancer agents is dependent on the intrinsic propensity of the target cells whether if the cells respond to the anticancer agents by apoptosis or not. In fact, apoptosis is induced by various anticancer agents such as cisplatin\(^5\), camptothecin\(^6\), etoposide\(^7\), and taxol\(^8\). The compounds which induced apoptosis in tumor cells might be a good candidate for anticancer drugs. From this point of view, we started to explore new apoptosis inducers derived from microorganisms by utilizing a human promyelocytic leukaemia cell line, HL-60 as an indicator of apoptosis. In the course of our screening, we found that an actinomycete strain RK95-74 isolated from a soil sample collected in Shiki City, Saitama Prefecture, Japan, produced a new potent apoptosis inducer, cytotrienin A (1) containing a triene-ansamycin, a 1-aminocyclopropane carboxylic acid and a 1-cyclohexene-1-carboxylic acid moieties. (Fig. 1).

The strain RK95-74 was identified to be \textit{Streptomyces} sp. and deposited in the National Institute of Bioscience and Human-Technology, Agency of Industrial Science and Technology, Japan, with the accession No. FERM P-15904.

Cytotrienin A was obtained by the general purification procedures as a yellowish powder with mp 132~135°C and optical rotation \([\alpha]_D^{\mathrm{25}} +271^\circ\) (c 1.0, MeOH). It is soluble in MeOH, CHCl\(_3\) and DMSO, and substantially insoluble in H\(_2\)O and n-hexane. The molecular formula of 1 was determined to be C\(_{37}\)H\(_{48}\)N\(_2\)O\(_8\) from the result of positive HR-EI-MS spectrometry (Found: m/z 648.3355, Caled: m/z 648.3407), which was supported by \(^{13}\)C and \(^1\)H NMR data. \(^1\)H NMR and \(^{13}\)C NMR are shown in Figs. 2 and 3. The UV spectrum showed absorption maxima at 262 nm (c 45500), 273 nm (c 46600) 283 nm (c 45400) in MeOH, which suggested the presence of triene moiety. Its IR spectrum (KBr) revealed the absorption at 3400, 1720, 1660, 1000 cm\(^{-1}\). Cytotrienin A has a novel structure consisting of a triene-ansamycin and a unique 1-aminocyclopropane carboxylic acid moieties. The structure determination will be a subject.

Fig. 1. Structure of cytotrienin A (1).

Fig. 2. \(^1\)H NMR spectrum of cytotrienin A (400 MHz, in DMSO-\(d_6\)).
Fig. 3. $^{13}$C NMR spectrum of cytotrienin A (100 MHz, in DMSO-$d_6$).

Fig. 4. Effect of cytotrienin A on the cell growth in HL-60 and WI-38 cells.

Fig. 5. Phase contrast and fluorescence micrographs of HL-60 cells treated with cytotrienin A.

HL-60 (- - - - ) and WI-38 (- - - - ) cells were cultured in RPMI-1640 containing 10% fetal bovine serum and DMEM containing 10% fetal bovine serum, respectively, for 1 day with various concentrations of cytotrienin A at 37°C in 5% CO$_2$ humidified atmosphere. The growth was measured by MTT method.

The antibacterial activity in vitro was determined by the standard agar dilution techniques. Cytotrienin A showed antimicrobial activity against Pyricularia orizae with a MIC value of 12.5 µg/ml. However, cytotrienin A showed no activity against Staphylococcus aureus FDA 209P, Escherichia coli BE1186, Pseudomonas aeruginosa N-10 L-form, Candida albicans, and Chlorella vulgaris at the dose of 100 µg/ml.

The cytotoxic effect of cytotrienin A on mammalian cell lines, HL-60 and human lung fibroblast WI-38 cells were shown in Fig. 4. The growth inhibitory effects of cytotrienin A in HL-60 and WI-38 cells are illustrated in Fig. 4. The growth inhibitory activity was determined by the MTT method.

Cytotrienin A is closely related to mycotrienin II$^{10,11}$ in its structure, however, different from the ansamycin group compounds reported in the respect of the 1-amino-cyclopropane carboxylic acid moiety which was found in plant metabolites but not in this group.
cytotrienin A on these cells with the IC$_{50}$'s values of 0.005 and >10 μg/ml in HL-60 and WI-38 cells, respectively. The HL-60 cells treated with 0.01 μg/ml of cytotrienin A for 16 hours contained condensed chromatin and fragmented nuclei as visualized by staining with Hoechst Dye 33258 (Fig. 5). When HL-60 cells were treated with 0.01 μg/ml of cytotrienin A, agarose gel electrophoresis of DNA revealed a "ladder" pattern as shown in Fig. 6, indicating preferential DNA degradation at the internucleosomal cleavage of genomic DNA. These data suggest that cell death induced by cytotrienin A resulted a programmed cellular response, apoptosis$^3$. Further studies on the biological studies of cytotrienin A are in progress.

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Fig. 6. DNA fragmentation caused by cytotrienin A in HL-60 cells.

HL-60 cells were incubated for 16 hours with 0 (lane 2), 0.1 μg/ml (lane 3), 0.001 (lane 4), and 0.01 (lane 5) of cytotrienin A, respectively. Cellular DNA was extracted and electrophoresed on agarose gel as described in the previous report$^8$. Lane 1: HaeIII-digested φX174 DNA.

References