Photoactivated Silkworm Metabolite (CpD) induces Apoptosis mediated by Mitochondrial Cytochrome-c and Caspase-3 in a Human Tumor Cell Line.

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Abstract

Back ground : Photodynamic therapy (PDT) has been established to be a beneficial therapeutic measure for cancers. The success of PDT mainly depends upon the photosensitizer. We found that silkworm metabolite (CpD) has yielded an advantageous photosensitizing effects in experimental PDT (1).In this, a mechanism of cytotoxicity by PDT in a CpD-sensitized human tumor cell line, Jurkat, was studied.

Methods : CpD was prepared by extracting soluble fractions of silkworm metabolites dissolved in acetone. The final concentration of CpD was adjusted to be 10μg per ml of DMF for the tests. A human T cell leukemia cell line, Jurkat was used throughout the experiments since human healthy peripheral blood mononucleated cells (PBMC) were available for normal control. For photodynamic analysis, target cells ($1x10^6$ in 35 mm flask) were incubated with 1.5 ml of RPMI containing CpD (33 -66 mg/ml of DMF) for 30 min. to 1 hr at 37℃. The total light out put for the irradiation of the cells were adjusted to be was 120 mJ per cm. The cells treated were then examined for viability assays by employing PI and annexin V staining by use of fluorescent activated cell sorter (FACS) analysis. The other parts of the cells were examined for the signs of apoptosis.

Results : A rapid cell death was demonstrated in 30 min. following CpD-PDT. Membrane blebbing, DNA fragmentation was prominent in the cultures treated with CpD-PDT. Presence of cytochrome-c, activation of caspase 3 and cleavage of PARP were evidenced in cytosolic fractions of the cells. However, activation of caspase 8 had not been detected. Direct disruption of mitochondria by CpD was thought to be responsible for the caspase 8 independent apoptosis. Detecting CpD specific emission spectra in mitochondrial fraction in the spectrophotometry supported the possibility of direct disruption of mitochondria. Taken together, these results suggest that direct mitochondria disruption followed by immediate release of cytochrome c and activation of caspase 3 are the major events in apoptosis of the cells by CpD-PDT. In particular, caspase 8 activation was not involved at any stages during CpD-PDT-induced Jurkat cell apoptosis. Target cell specificity of these phenomena is under study.

Keywords: Silkworm metabolite;; Chlorophyll derivatives; Photodynamic therapy; Apoptosis; Cytochrome c; Caspase-3; Mitochondrion