Investigation of anticancer mechanism of clavulone II, a coral cyclopentenone prostaglandin analog, in human acute promyelocytic leukemia

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Summary
The marine prostanoid clavulones were shown to exert cytotoxicity against several cancer cells. In the present study, we illustrate the pathways utilized by clavulone II to trigger apoptotic signaling in human acute promyelocytic leukemia HL-60 cells. Exposure of cells to clavulone II resulted in early induction of phosphatidylserine externalization, mitochondrial dysfunction, and alteration of the cell cycle. Down-regulated expression of cyclin D1 explained the effect of clavulone II on G1 phase arrest of the cell cycle. Clavulone II induced the disruption of mitochondrial membrane potential and activation of caspase-8, -9 and -3 in a time- and concentration-dependent manner. Furthermore, the effect of 3 μM clavulone II was accompanied by the up-regulation of Bax, down-regulation of Mcl-1, and cleavage of Bid. Taken together, it is suggested that low concentrations of clavulone II induce the antiproliferative effect through the down-regulation of cyclin D1 expression and G1 arrest of the cell cycle, while that of high concentration induce the apoptotic cell death via the modulation of members of caspases and Bcl-2 family proteins in HL-60 cells.

Introduction
Diverse action mechanisms of chemotherapeutic drugs are comprehensively used in malignant tumors. Defective ability to undergo programmed cell death (apoptosis) or deregulated proliferation is characteristic of cancer cells. Chemotherapeutic drugs that attempt to overcome defects in the apoptotic pathways may complement the capacity of treatment. Apoptosis is defined as distinct morphology and composed of the extrinsic and intrinsic pathways [1]. The extrinsic pathway is triggered by members of the death receptor superfamily to form a death-inducing signaling complex and result in activation of caspase-8. The intrinsic pathway is associated with the mitochondrial damage and releasing its proteins, such as cytochrome c, into the cytosol. Members of the Bcl-2 family proteins regulate mitochondrial membrane permeability, either pro-apoptotic or anti-apoptotic function. Crosstalk between the two pathways is mediated by Bid, a member of pro-apoptotic Bcl-2 family proteins. The intrinsic and extrinsic pathways converge at the activation of caspase-3, an executioner of caspase cascade, and lead to cell death.

Prostaglandins (PGs) are a family of structurally related molecules that are formed by cells in response to a variety of extrinsic stimuli and involved in various homeostatic functions. Within
this family, PGs of the A and J series constitute a cyclopentenone structure, which is characterized by the presence of α,β-unsaturated carbonyl group. In contrast to conventional PGs, most of the actions of cyclopentenone PGs do not appear to be mediated by G-protein coupled prostanoid receptors. Cyclopentenone PGs are actively transported into cells and exert its biological activities including antiviral effects, growth inhibition, and anti-neoplastic actions [2]. The growth-inhibitory ability of cyclopentenone PGs is thought to regulate the expression of cell cycle related genes such as N-myc, c-myc, cyclin D1, or cyclin-dependent kinase, and then induce growth arrest [2]. In addition, one prominent action of cyclopentenone PGs is to promote a stress response resulted in induction of various stress-related protein genes, including heat shock proteins, and heme oxygenase [3].

Marine coral prostanoids isolated from Clavularia viridis, such as clavulones, has been reported to possess antitumor [4], positive chronotropic effect [5], and antiviral activity [6]. Although the antileukemic effect of clavulones has been demonstrated in the late 1980s, the exact mechanism is unclear. In the present study, we have elucidated the antiproliferative and apoptotic mechanisms of clavulone II in human acute promyelocytic leukemia HL-60 cells.

Methods

Cell culture and treatment

Human acute promyelocytic leukemia HL-60 cells were obtained from the American Type Culture Collection (Rockville, MO, USA). Cells were maintained in RPMI-1640 medium containing 10% heat-inactive fetal bovine serum, 100 units/ml penicillin and 100 μg/ml streptomycin. Cells were cultured in a humidified incubator at 37 °C in 5% CO2/air. Clavulone II (Figure 1) was provided by Dr. Y.C. Shen (Department of Marine Resources, National Sun Yat-sen University, Kaohsiung, Taiwan). The powder was dissolved in dimethylsulfoxide (DMSO) and further diluted in culture medium. The final DMSO concentration in medium was 0.1% and did not affect cellular function and assay systems in this study.

Cell proliferation assay

Cell viability was determined by the enzymatic reduction of the yellow dye, 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide (MTT), to a purple formazan product in intact cells [7]. Cells at a density of 4 × 10⁵/ml were treated with DMSO (control) or different concentrations of clavulone II for 24 h. MTT dye (final concentration of 0.5 mg/ml) was added later and then the cells were incubated for 1 h at 37 °C. Finally, the cells were lysed and absorbance was detected at 550 nm using a microplate photometer (Packard, Meriden, CT, USA). For cell number determination, a standard correlation between the known cell numbers and the absorbance density values was constructed for the measurement of cell number from various detected absorbance density values.

FACScan flow cytometric analysis of apoptosis and cell cycle progression

The apoptotic status of the cells was evaluated by measuring the exposure of phosphatidylserine (PS) on cell membranes using Apoptosis Detection Kits (R&D systems, Minneapolis, MN, USA). After drug treatment, cells (2 × 10⁵) were harvested, and resuspended in a staining solution containing propidium iodide (PI, 50 μg/ml) and annexin V-FITC (25 μg/ml) for 15 min at room temperature in the dark. The cells were quantified using a FACSCalibur flow cytometer equipped with the Cell Quest software (Becton Dickinson, Heidelberg, Germany). Data analysis was performed using WinMDI program (J. Trotter, Scripps Research Institute, San Diego, CA, USA).

The DNA content of the cells was analyzed by measuring the distribution of the cells in the different phases of the cell cycle. After treatment with DMSO or clavulone II, cells were harvested and fixed with cold ethanol for at least 30 min at −20 °C. Fixed cells were washed twice with ice-cold phosphate-buffered saline (PBS), resuspended...