Pramanicin induces apoptosis in Jurkat leukemia cells: A role for JNK, p38 and caspase activation

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Pramanicin is a novel anti-fungal drug with a wide range of potential application against human diseases. It has been previously shown that pramanicin induces cell death in fungal organisms is seen with minimal inhibitory concentrations of 20–100 µM. Pharmacological applications of pramanicin on mammalian systems have not been studied extensively. It has been previously shown that pramanicin increases cytosolic calcium concentrations and induces cell death in endothelial cells, but the effects of pramanicin on cancer cell lines have not been investigated.

The principal aim of all anti-cancer therapies and cancer prevention approaches is to eliminate all tumor cells from the human body. Disequilibrium between cell proliferation and death has been proposed to be a fundamental step in carcinogenesis. Additionally, induction of apoptosis (programmed cell death) is an effective mechanism used to eradicate transformed, deleterious cells; as well, many chemotherapeutic or chemopreventive agents act through triggering of apoptotic pathways in tumor cells. The cellular apoptotic machinery is formed by protein signalling networks, which are finely tuned by protein-protein interactions and protein modifications. The intrinsic and extrinsic apoptotic pathways have been defined previously and protein kinases as well as various cysteinyl-specific aspartate proteases (caspases) have been proposed to mediate apoptosis induced by cytokines, chemotherapeutics and cellular stress through a highly organized network at different signalling levels. Briefly, cleavage of initiator caspases (caspase 8 and caspase 9) and the effector caspases (caspase-3/7) and typical cellular features of apoptosis (nuclear condensation and formation of apoptotic bodies) have been observed following the release of cytochrome c from mitochondria in response to death receptor stimulation or a direct intracellular insult.

In mammals three distinct groups of mitogen-activated protein kinases (MAPKs) have been identified. c-Jun N-terminal kinases (JNK) and p38 MAPKs have been shown to be activated by cellular stress, UV radiation, growth factor withdrawal and pro-inflammatory cytokines (mainly TNFα and IL-1). Upon activation through a dual tyrosine/threonine phosphorylation mechanism by their corresponding upstream kinases, JNK and p38 phosphorylate various transcription factors such as...
c-jun, ATF-2 and p53 with different substrate specificities and control their transcriptional activity. Both JNK and p38 kinases have been shown to be involved in pro-apoptotic or anti-apoptotic signaling pathways in many different studies. However, many stimuli have been shown to activate these kinases without inducing apoptosis. The third group of MAPKs, extracellular signal regulated kinases (ERK 1/2, p42/p44 kinases) were demonstrated to be mainly activated by growth factors and other mitogenic stimuli. In general, intensive research on MAPKs has suggested that JNKs and p38 are mainly involved in apoptosis and growth arrest, but ERK 1/2 are involved in cellular transformation, differentiation and proliferation. Indeed, the cell type, origin of the stimuli, co-activation of other signalling cascades as well as the initial magnitude, duration and further amplification of the activated signal transduction pathway determine the pro-apoptotic or anti-apoptotic characteristic of the cellular target response. Thus activation patterns and pro-/anti-apoptotic properties of each MAPK should be evaluated carefully in the light of above parameters.

Here we report on the apoptotic effect of pramanicin on Jurkat T lymphoblastic leukemia cells in a dose- and time-dependent manner, as shown by MTT assay and DNA fragmentation. In order to gain insight into the mechanisms of this apoptotic response we followed the activation of MAPKs and caspases in response to pramanicin treatment. Our results have clearly demonstrated the involvement of JNKs and p38 as well as caspase-9 and caspase-3 activation with respect to pramanicin-induced apoptosis in Jurkat T lymphoblastic leukemia cells. Mitochondrial cytochrome c is released by pramanicin with similar time-kinetics to caspase-9 activation. Pramanicin also induces an early and transient ERK activation, which contributes to a partial protective effect against apoptosis. To our knowledge, this is the first study that reports pramanicin as a potential novel therapeutic approach against cancer, which acts through an intelligibly JNK- and p38-dependent mechanism.

Materials and methods

Reagents and antibodies

Caspase-3 inhibitor, Z-DEVD-FMK (benzyloxy carbonyl-Asp-Glu-Val-Asp-fluoromethylketone), caspase-9 inhibitor, Z-LEHD-FMK (benzyloxy carbonyl-Leu-Asp-fluoromethylketone) and general caspase inhibitor, Z-VAD-FMK (benzyloxy carbonyl-Val-Ala-Asp-fluoromethylketone) were obtained from BD Biosciences Pharmingen, (San Diego, CA, USA). RPMI 1640 Medium was purchased from Biological Industries, Rehovot, Israel. JNK inhibitor SP600125 (anthra[1,9-cd]pyrazol-6(2H)-one), p38 inhibitor SB203580 (4-(4-fluorophenyl)-2-(4-methylsulfinylphenyl)-5-(4-pyridyl)-1H-imidazole) and MEK1/ERK inhibitor PD98059 (2′-amino-3′-methoxy flavone) were from Calbiochem (San Diego, CA, USA). JNK, phospho-JNK (Thr 183/Tyr 185), p38, phospho-p38, ERK 1/2, phospho-ERK 1/2 and cytochrome c antibodies were from Santa Cruz Biotechnology Inc. (Santa Cruz, CA, USA). Caspase-9, caspase-3 and β-actin antibodies were from Cell Signaling Technology Inc. (Beverly, MA, USA). CoxIV (cytochrome c oxidase subunit IV) antibody was purchased from Abcam (Cambridge, UK). Milk Diluent Concentrate Kit was obtained from KPL (Maryland, USA). Phosphatase Inhibitor Cocktail 1, Phosphatase Inhibitor Cocktail 2, Digitonin, fetal bovine serum and other chemicals were purchased from Sigma (Darmstadt, Germany) otherwise indicated.

Growth of Stagonospora and purification of pramanicin

Stagonospora Sp. ATCC 74253 (American Type Culture Collection, Rockville, MD, USA) was cultured in liquid medium LCM, with the glucose content reduced to 40 g/L (100 mL in each of twelve 500 mL Erlenmeyer flasks). After seven days, the cultures were centrifuged and the supernatant extracted with methyl ethyl ketone. After concentration, the organic extracts were purified by column chromatography (SiO2, 10% MeOH/EtOAc). Final purification was by MPLC on a Merck LOBAR RP-8 column in MeOH–H2O (70:30), giving approx. 75 mg of pramanicin, as previously described.

Cell cultures and treatments

Jurkat T lymphoblastic leukemia cells were a kind gift of Dr. Jean-François Peyron, Faculté de Médecine Pasteur, Nice, France and have been previously described. The cells were grown in RPMI-1640 supplemented with 10% fetal bovine serum and penicillin/streptomycin (100 U/mL, respectively) in a humidified incubator at 37°C and 5% CO2. Cells were seeded in 6-well culture plates (1 × 105 cells/well), 60 mm culture flasks (1 × 10⁶ cells/well) or 96-well plates (10⁴ cells/well) and treated as indicated in the experimental protocols. Ethanol (≤0.05%, v/v) was added to all control wells in each experiment.