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PKC 412 sensitizes U1810 non-small cell lung cancer cells to DNA damage

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Abstract

Non-small cell lung carcinoma (NSCLC) is characterized by resistance to drug-induced apoptosis, which might explain the survival of lung cancer cells following treatment. Recently we have shown that the broad-range kinase inhibitor staurosporine (STS) reactivates the apoptotic machinery in U1810 NSCLC cells [Joseph et al., Oncogene 21 (2002) 65]. Lately, several STS analogs that are more specific in kinase inhibition have been suggested for tumor treatment. In this study the apoptosis-inducing ability of the STS analogs PKC 412 and Ro 31-8220 used alone or in combination with DNA-damaging agents in U1810 cells was investigated. In these cells Ro 31-8220 neither induced apoptosis when used alone, nor sensitized cells to etoposide treatment. PKC 412 as a single agent induced death of a small number of U1810 cells, whereas it efficiently triggered a dose- and time-dependent apoptosis in U1285 small cell lung carcinoma cells. In both cell types PKC 412 triggered release of mitochondrial proteins followed by caspase activation. However, concomitant activation of a caspase-independent pathway was essential to kill NSCLC cells. Importantly, PKC 412 was able to sensitize etoposide- and radiation-induced death of U1810 cells. The best sensitization was achieved when PKC 412 was administered 24 h after treatments. In U1810 cells, Ro 31-8220 decreased PMA-induced ERK phosphorylation as efficiently as PKC 412, indicating that the failure of Ro 31-8220 to induce apoptosis was not due to weaker inhibition of conventional and novel PKC isoforms. However, Ro 31-8220 increased the basal level of ERK and Akt phosphorylation in both cell lines, whereas Akt phosphorylation was suppressed in the U1810 cells, which might influence apoptosis. These results suggest that PKC 412 could be a useful tool in increasing the efficiency of therapy of NSCLC.

Keywords: Apoptosis; Chemotherapy; Radiation; PKC; Lung cancer

Introduction

Worldwide, tumors of the lung are the most common forms of cancer. With more than 1 million new cases and 900,000 deaths per year, they are believed to remain a major cause of tumor-related death during the next decades [2]. The most frequent type of lung cancer, the non-small cell lung carcinoma (NSCLC), generally exhibits a low response to conventional anti-cancer agents [3]. The other type of lung cancer, the small cell lung carcinoma (SCLC), typically shows an initial response to treatment at an early stage of disease but often relapses. Secondary primary tumors in

Abbreviations: AIF, apoptosis-inducing factor; AMC, 7-amino-4-methylcoumarin; CHAPS, 3-[(3-cholamidopropyl)dimethylammonio]-1-propanesulfonate; DEVD, Asp-Glu-Val-Asp; DTT, dithiothreitol; ERK1/2, extracellular signal-regulated kinase 1/2; FIGE, field inversion gel electrophoreses; fmk, fluoromethyl ketone; NSCLC, non-small cell lung carcinoma; PARP, poly(ADP-ribose)polymerase; PBS, phosphate buffered saline; PKC, protein kinase C; PMA, phorbol 12-myristate 13-acetate; SCLC, small cell lung carcinoma; STS, staurosporine; TMRE, tetramethyl rhodamine ethyl ester; VAD, Val-Ala-Asp.

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long-term survivors with SCLC are usually of the NSCLC type [4]. Conventional radio- and chemotherapy of lung cancer has reached a plateau, and new and more effective methods are required [4,5].

Commonly used regimens in cancer therapy rely on the induction of apoptotic cell death. At present two main apoptotic pathways, receptor- and mitochondria-mediated, are well characterized. Death receptor stimulation leads to a fast activation of a family of proteases called caspases, followed by cleavage of many proteins, eventually leading to apoptosis-specific changes, such as chromatin condensation and cleavage of DNA, cell shrinkage, and alterations in the plasma membrane that function as an engulfment signal to macrophages or neighboring cells. Following DNA damage caspases are activated as a result of mitochondria-mediated events [6]. Mitochondria play an important role in apoptosis by the release of certain proteins, normally located in their intermembrane space, to the cytosol. Upon release some of these proteins, such as cytochrome *c* and apoptosis-inducing factor (AIF), are involved in the regulation and execution of caspase-dependent and/or caspase-independent apoptosis [7]. Cytochrome *c* is released either as a consequence or independently of a decrease in mitochondrial membrane potential (MPT) and drives apoptosis by activation of caspases [8]. AIF, which is released upon a drop in MPT, can independently of caspases induce chromatin condensation and formation of high molecular weight DNA fragments [8].

Dysregulation of apoptosis contributes to the development of cancer, as well as to resistance to anti-cancer therapy [9]. It has been shown that failure of death can occur even though all components of the apoptotic machinery are present in the cell. One possible reason for this inhibition is the aberrant signaling of kinases, favoring cell survival [10]. Resistance to treatment of NSCLC is often associated with an aberrantly activated protein kinase B/Akt [11–14]. There is also evidence for involvement of protein kinase C (PKC) [15–17] and its downstream target extracellular signal-regulated kinase 1/2 (ERK1/2) [18,19] in sensitivity of NSCLC to treatment. One of the promising approaches in tumor therapy is to use protein kinase inhibitors alone or in combination with well-known anti-cancer agents [20,21]. Indeed, strong apoptotic death in response to the broad-spectrum protein kinase inhibitor STS was observed in U1810 NSCLC cells, which are usually very resistant to treatment [1]. In these cells, even though caspases were activated upon treatment with αFas, VP-16, and irradiation, there was no subsequent translocation of caspase-3 to the nucleus and no apoptosis-related nuclear changes could be seen [22]. STS-mediated death was associated with induction of mitochondrial dysfunction and subsequent nuclear translocation of AIF and caspase-3 [1]. The underlying mechanism of STS-induced apoptosis in NSCLC cells is not known, but most likely it is dependent upon kinase inhibition. STS is a non-selective kinase inhibitor [23,24], which might explain the high toxicity of this compound. Because of high toxicity, STS cannot be used for the treatment of patients. However, by modifying the structure of STS more selective PKC inhibitors have been obtained [23].

One such derivative, *N*-benzoyl staurosporine (PKC 412/CGP 41251/midostaurin), exerts anti-proliferative activity in different tumor-derived cell types [24], including both SCLC and NSCLC [25–28], and has been shown to enhance apoptosis and/or increase growth inhibition when combined with conventional anti-cancer agents in different murine and human cells [29–33]. Although there are several reports of PKC 412-induced death with apoptotic features in certain tumor-derived cells [29,34–37], to our knowledge, there has been no detailed investigation concerning the involvement of apoptotic mechanisms in response to the treatment of lung cancer-derived cells with this drug. Following treatment with this kinase inhibitor an increase in central necrosis and appearance of condensed nuclei was observed in human NSCLC inoculated in mice, suggesting the involvement of apoptotic cell death in the reduction of tumor growth [34]. However, in SCLC the apoptotic index was not changed following PKC 412 exposure [25]. The bisindolylmaleimide Ro 31-8220 is an STS analog that reduces growth of A549 cells [27,28] and induces apoptosis in non-lung-derived cell lines [38,39]. However, the apoptosis-inducing ability of Ro 31-8220 has not been investigated in lung cancer cells.

The main goal of this study was to investigate the possible use of the STS analogs PKC 412 and Ro 31-8220 alone, or in combination with conventional anti-cancer treatments, in the killing of lung cancer cells. Our primary aim was to make an attempt to kill therapy-resistant U1810 NSCLC cells. We found that PKC 412 was a more potent inducer of apoptosis compared to Ro 31-8220. The most efficient apoptosis induction was achieved when PKC 412 was combined with conventional anti-cancer treatments. There was no difference between PKC 412 and Ro 31-8220 in their abilities to inhibit PMA-induced signaling to extracellular signal-regulated kinase1/2 (ERK1/2), whereas they had opposite effects on PKB/Akt phosphorylation; Ro 31-8220 increased while PKC 412 decreased PKB/AKT phosphorylation. The differences in the pharmacological profiles of the two PKC inhibitors might explain the ability of PKC 412 to reverse the resistance of NSCLC cells to radiation- and etoposide-mediated apoptosis.

Material and methods

Cell lines, culture conditions, and treatment

The SCLC cell line U1285 and the NSCLC cell line U1810 used in this study have been previously investigated with regard to apoptosis resistance [1,22]. Cells were maintained at 37°C, 5% CO_2 in RPMI 1640 medium supplemented with 10% heat-inactivated fetal calf serum, 2 mM L-glutamine, penicillin (100 U/ml), and streptomycin