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Cytotoxicity of a non-cyclooxygenase-2 inhibitory derivative of celecoxib in non-small-cell lung cancer A549 cells

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Summary Lung cancer is one of the most common causes of cancer death worldwide. Although recent advances in chemotherapy and radiation therapy have yielded modest improvements in patient outcomes, overall survival remains poor. Therefore, new therapeutic targets are needed. Phosphoinositide-dependent kinase-1 (PDK1) is one potential target. The aim of the present studies was to investigate the potential of a celecoxib-derived PDK1 inhibitor (OSU03013), that does not inhibit cyclooxygenase-2, to kill lung cancer cells in vitro. Using human non-small-cell lung cancer A549 cells, OSU03013 dose-dependently induced apoptosis. After 6 h of treatment with 7.5 μM OSU03013, 26% of the cells were apoptotic, compared to 4% of the control cells as determined by measuring the sub-G1 peak of propidium iodide stained cells with flow cytometry. A similar increase in apoptosis was evident using the Cell Death ELISA assay. OSU03013-induced apoptosis was accompanied by a reduction in the mitochondrial membrane potential, the release of cytochrome *c* and the cleavage of caspase-3. Surprisingly, the phosphorylation of Akt at serine 473 was increased in A549 cells treated with 7.5 μM OSU03013. However, the toxicity of OSU03013 was reduced in A549 cells expressing a constitutively active form of Akt. These data demonstrate that OSU03013 induces apoptosis in A549 cells via the mitochondrial pathway. Inhibition of the Akt pathway appears uninvolved in this toxicity, although Akt can provide protection. These results also suggest the potential of celecoxib-derived agents to treat some forms of lung cancer.

Abbreviations: COX, cyclooxygenase; PDK1, phosphoinositide-dependent kinase-1; NSCLC, non-small-cell lung cancer

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1. Introduction

Lung cancer is one of the leading causes of cancer death in the USA [1]. Although surgical resection together with chemotherapy offers the best hope for a cure, the 5-year survival rate remains poor, even in patients with earlier stages of the disease [2]. Non-small-cell lung cancer (NSCLC) accounts for over 80% of newly diagnosed lung cancer and the majority of patients are diagnosed with advanced and un-resectable disease [2]. The standard treatment for advanced NSCLC is chemotherapy. However, NSCLC is extremely resistant to chemotherapeutic agents [3] and such therapy only modestly increases the survival rate, although the symptoms and the quality of life in patients with advanced NSCLC are improved [4,5]. Thus, new chemotherapeutic agents are needed for the treatment of NSCLC.

Recent advances in tumor cell biology have identified a number of molecular pathways that may be responsible for tumor growth and resistance of NSCLC to chemotherapy. One such pathway involves the cyclooxygenase (COX) enzymes [6] that convert arachidonic acid to prostaglandins. There are two isoforms of COX. COX-1 is constitutively expressed in all tissues, whereas COX-2 is induced by growth factors, tumor promoters and cytokines [7—9]. There is considerable evidence that COX-2 represents a potential pharmacologic target for inhibiting tumor growth. For example, increased COX-2 expression has been observed in a variety of human cancers, including lung cancer [10]. Furthermore, the formation and growth of tumors in COX-2 null mice are reduced [11,12]. Celecoxib, the best studied inhibitor of COX-2, has been approved by the United States Food and Drug Administration as an adjunct therapy for patients with familial adenomatous polyposis, a disease that left untreated almost always leads to colorectal cancer. Importantly, celecoxib is able to inhibit human NSCLC A549 cell proliferation in vitro and tumor growth in vivo [13] and enhances the response of NSCLC to preoperative paclitaxel and cardoplatin [14].

The antitumor activity of celecoxib is thought to be associated with its ability to induce apoptosis in a variety of cancer cells including those from colon, stomach, prostate, breast and lungs [15]. The biochemical mechanism underlying celecoxib-induced apoptosis remains elusive. Although celecoxib is an inhibitor of COX-2, substantial data indicate that COX-2 inhibition is not the primary mechanism underlying celecoxib-induced apoptotic cell death [16,15]. Based on this premise, a series of celecoxib derivatives that lack COX-2 inhibitory activity but are more effective at inducing apoptosis than celecoxib have been developed [17]. These compounds, like celecoxib [18], appear to induce apoptosis in prostate cancer cells by the inhibition of PDK1/Akt pathways [17,19].

In the present study, we investigated the pro-apoptotic effects and underlying mechanisms of the celecoxib derivative OSU03013 in the NSCLC A549 cell line. The data demonstrated that OSU03013 is extremely toxic to A549 cells. Mechanistic studies showed that the apoptosis induced by OSU03013 in A549 cells is though the mitochondrial pathway. Surprisingly, effects on Akt appear not to be involved. The potency of OSU03013 against A549 cells, that are highly resistant to many toxics, suggests these celecoxib derivatives may be effective for the treatment of NSCLC.

2. Materials and methods

2.1. Cell culture

Human lung adenocarcinoma A549 cells were obtained from the American Type Culture Collection (Manassas, VA) and were cultured in Dulbecco's Modified Eagles medium supplemented with 10% fetal bovine serum (Atlanta Biologicals, Norcross, GA) and 50 μg/ml gentamicin. Cells were passaged at 70% confluency using 1 mM EDTA—0.025% trypsin for 3—5 min.

2.2. Reagents and treatments

OSU03013 was synthesized as described in [17]. Rabbit polyclonal cytochrome *c* antibodies and caspase-3 antibodies were obtained from Santa Cruz Biotechnology (Santa Cruz, CA). Antibodies against total Akt, or Ser473 and Thr308 phosphorylated Akt were purchased from Cell Signaling Technology Inc. (Beverly, MA). 5,5′,6,6′-Tetrachloro-1,1′,3,3′-tretraethylbenzimidazolcarbocyanine (JC-1) was purchased from Sigma (St. Louis, MO). All chemicals were dissolved in DMSO. Before treatment, A549 cells were plated and allowed to adhere overnight. Exponentially growing cells were used for all experiments. All treatments, including appropriate vehicle controls, were added directly to the culture medium.

2.3. Construction of plasmid expressing constitutively active, myristoylated Akt

To construct the plasmid containing constitutively active, myristoylated Akt (mAkt), the full-length human mAkt cDNA was obtained, by restriction enzyme cleavage, from a vector containing this sequence (a gift from Dr. David Plas, University of Cincinnati, Cincinnati, OH). The full-length of human mAkt cDNA was then subcloned into the mammalian expression vector pcDNA3.1/His© (Invitrogen, Carlsbad, CA) in sense orientation. The identity and orientation of this construct were confirmed by DNA sequencing.

A549 cells were transiently transfected with 2 μg DNA of pcDNA3.1/His©/mAkt using lipofectamine reagent (Invitrogen) according to the manufacturer's protocol. pcDNA3.1/His© DNA was used as a control. The transfected A549 cells were treated with xenobiotic after 24 h. The expression of mAkt was determined by western blotting using antibodies against phosphorylated Akt.

2.4. Measurements of apoptosis

Apoptosis of A549 cells was detected using either the Cell Death Detection ELISA kit following the manufacturer's instructions (Roche Applied Science, Indianapolis, IN) or by flow cytometric analysis of the sub-G1 peak of propidium iodide (PI) stained cells. For the ELISA assay, lysates from treated and untreated cells were added into a 96-