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Original article

Non-substituted N-heteroaromatic selenosemicarbazone metal complexes induce apoptosis in cancer cells via activation of mitochondrial pathway

Tatjana Srdić-Rajić [a], Manja Zec [a], Tamara Todorović [b], Katarina Anđelković [b], Siniša Radulović [a,*]

[a] Institute for Oncology and Radiology of Serbia, National Cancer Research Center, Department of Experimental Oncology, Laboratory for Experimental Pharmacology, Pasterova 14, 11 000 Belgrade, Serbia
[b] Faculty of Chemistry, University of Belgrade, Studentski trg 12-16, 11000 Belgrade, Serbia

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ABSTRACT

We previously published the synthesis, characterization and cytotoxic effect of the novel Zn(II), Ni(II), and Cd(II) complexes with 2-formylpyridine selenosemicarbazone. Here we further investigate the mechanism of their antiproliferative activity against several cancer and vascular endothelial cell lines and compared it to the activity of the ligand itself, corresponding salts and, as a referent compound, cisplatin. Investigated complexes induced apoptosis in a time- and dose-dependent manner as well as changes in a cell cycle distribution. Caspase-3 activation in HeLa cells, MDA-MB-361 and vascular endothelial cells EA.hy 926 cells by ligand alone, as well as Zn(II), Ni(II), and Cd(II) complexes was preceded by the activation of the p53 tumor-suppressor gene family protein p73. In addition to activation of p73, these compounds also trigger cytochrome C release by upregulation of Bax expression. The release of cytochrome C has been linked to loss of mitochondrial membrane potential. However, our data indicated that the increased phosphorylation of ERK could be also one of the mechanism involved in the Zn(II), and Cd(II) complexes- induction of apoptosis. Selenosemicarbazone complexes with Cd(II) and Ni(II), possess dual ability to induce apoptosis as well as necrosis, and might present an added advantage for inducing cell death in a diverse array of malignant cells. Taken together, our findings could indicate potential role of these complexes as activator of cross-talk between different signaling pathways that leads to cell death, and thus making the complex intriguing field for further scientific, and maybe clinical investigations.

1. Introduction

Primary or acquired resistance of many tumors to established treatment regimens still constitutes a major concern in oncology [1]. Thus, attempts to improve the survival of cancer patients largely depend on strategies to target tumor cell resistance. Induction of apoptosis in cancer cells is a key killing mechanism for most anti-tumor therapies including chemotherapy, γ-irradiation, immunotherapy, or cytokines [2,3]. Apoptosis pathways may be initiated through different entry sites, such as death receptors (receptor pathway) or mitochondria (mitochondrial pathway), resulting in activation of effector caspases [4]. Mitogen-activated protein kinase (MAPK) signaling pathways, including the extracellular signal-regulated kinase (ERK), p38 and c-Jun N-terminal kinase (JNK) pathways, play important roles in cellular responses to stress conditions, including various anti-cancer agents [2]. The ERK signaling pathway is involved in the regulation of cell growth, differentiation, proliferation and survival. By contrast, the p38 and JNK signaling pathways are stress dependent and have apoptotic regulatory functions. The mitochondrial pathway plays a crucial role in drug-induced apoptosis [5]. On induction of apoptosis, apoptogenic factors such as cytochrome C, apoptosis-inducing factor (AIF), or second mitochondria-derived activator of caspase (Smac)/DIABLO are released from mitochondria into the cytosol [6]. Cytochrome C triggers caspase-3 activation through formation of the cytochrome C/Apaf-1/caspase-9-containing apoptosome complex, whereas

Abbreviations: ERK, extracellular signalregulated kinase; JNK, c-Jun N-terminal kinase; MAPK, Mitogen-activated protein kinase; PT, permeability transition; PBS, phosphate-buffered saline; Rh 123, rhodamine 123; Δψm, Mitochondrial membrane potential; RPMI, Roswell Park Memorial Institute; DMEM, Dulbecco's modified Eagle's medium; HEPES, 4-(2-hydroxyethyl)piperazine-1- ethanesulfonic acid; FCS, fetal calf serum; CDDP, cisplatin; SDS, sodium dodecyl sulfate; PI, propidium iodide; FACS, fluorescence activated sorting cells; ANNEXIN V, Ca2+-dependent phospholipid-binding protein with high affinity for phosphatidylserine; FITC, fluoroscein isothiocyanate; TRIS, tris(hydroxymethyl)aminomethane; SRB, sulforhorodamine B.

* Corresponding author. Tel.:+381 11 2067434; fax: +381 11 2067292.
E-mail addresses: tsrdic@yahoo.com (T. Srdić-Rajić), manjazec@gmail.com (M. Zec), tamarat@chem.bg.ac.rs (T. Todorović), kka@chem.bg.ac.rs (K. Anđelković), sinisar@ncrc.ac.rs (S. Radulović).

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Smac/DIABLO promotes caspase activation by neutralizing the inhibitory effects to inhibitor of apoptosis protein (IAP) [6]. A p73-dependent apoptotic pathway requires activation of c-Abl tyrosine kinase. The c-Abl tyrosine kinase can activate p73 by phosphorylating the p73 protein and induce apoptosis in response to DNA damage [7]. Because most antitumor therapies including chemotherapy primarily act by inducing apoptosis in cancer cells, defects in apoptosis programs may cause resistance [2,7,8].

Many researchers are focused on search for novel strategies to enhance the efficacy of chemotherapy.

Investigations of biological activity of selenosemicarbazones have been focused mainly on antimicrobial [9–13] and antimalarial activity [14,15]. It was shown that in almost all cases studied, activity of selenosemicarbazones were more pronounced in comparison to sulfur and oxygen analogues. On the other hand, only few publications focus on antineoplastic effects of selenosemicarbazones [13,15–20]. It was shown that complexation of selenosemicarbazones with metal ions causes an increase in cytotoxic activity [19,20]. Moreover, our previous studies [21] showed that Zn(II), Ni(II), and Cd(II) complexes with condensation derivative of 2-formylpiridine and selenosemicarbazide (Hfpsesc = L) exhibited antiproliferative activity against a panel of seven human tumor cell lines and two endothelial cell lines.

The present work was designed to continue with research in the field of cytotoxic activity of N-heteroaromatic selenosemicarbazone metal complexes. In this study we investigated anticancer activity of complexes of Hfpsesc with Zn(II), Cd(II) and Ni(II) on two metastatic tumor cell lines (human cervical carcinoma-HeLa, human epithelial breast cancer-MDA-MB-361) and two endothelial cell lines (human endothelial-EA.hy 926 cells and murine endothelial-MS1 cells) as well as molecular signaling involved in the cytotoxic effect of selenosemicarbazone metal complexes.

Our results show that the anticancer activity of investigated agents against tested cell lines include perturbation of cell cycle phase distribution as well as apoptosis through different signaling pathways. Selenosemicarbazone metal complexes-induced apoptosis in tested cell lines was associated with cytochrome C release into the cytosol, activation of p73 and phosphorylation of ERK.

2. Chemistry

2.1. Synthesis of the ligand Hfpsesc and corresponding Zn(II), Cd(II) and Ni(II) complex

The ligand Hfpsesc and corresponding Zn(II), Cd(II) and Ni(II) complexes were prepared as described previously [21,22]. In short, the ligand was obtained by condensation reaction of ethanolic solution of 2-formylpyridine with selenosemicarbazide in the presence of acetic acid. The title complexes were obtained by reaction of corresponding metal salt with the etanolic solution of the ligand. Fig. 1 presents the structures of Zn(II), Cd(II) and Ni(II) complexes with selenosemicarbazone ligand Hfpsesc.

3. Pharmacology

The newly synthesized Zn(II), Ni(II), and Cd(II) complexes with 2-formylpyridine selenosemicarbazone were investigated for their *in vitro* anticancer activity against human cervix carcinoma cells (HeLa), human breast cancer cell line (MDA-MB-361), human endothelia (EA.hy 926) cells and murine endothelial (MS1). Some of the molecular and biochemical pathways involved in metal complexes with selenosemicarbazones-induced apoptosis were investigated in this study.

4. Results

4.1. Alterations of cell cycle

Our previously study shown cytotoxic activity of the complexes of Hfpsesc with Zn(II), Cd(II) and Ni(II) on HeLa and MDA-MB-361 cells with IC_{50} value in the range of 5–60 μM, among the others tested cell lines [22]. In order to obtain more information regarding events related to cytotoxic activity of investigated compounds changes in cell cycle progression were evaluated using FACS analysis. For that purpose, HeLa, and MDA-MB-361 cells as well as EA.hy 926, and MS1 cells were treated for 24 h with Zn(II) (compound 1), Cd(II) (compound 2) and Ni(II) (compound 3) complexes, as well as ligand Hfpsesc·1/2 EtOH, and cisplatin (CDDP). Each of these compounds were applied in tree concentrations: one in the range of IC_{50} value, one concentration below IC_{50} value and one concentration under IC_{50} value.

Investigated compounds 2 and 3, as well as ligand Hfpsesc, after 24 h of continual incubation with HeLa cells induced increase of the percent of apoptotic cells (sub-G1 peak), and a strong decrease of the percent of cells in G1 phase (Fig. 2A). The maximum accumulation of cell in sub-G1 peak was detected at concentration corresponding to IC_{50} of compounds 2, 3 and Hfpsesc (Fig. 2A). Also significant accumulation of cells in S phase of cell division was found after 24 h of continual treatment of HeLa cells with compounds 1, 3 and Hfpsesc (Fig. 2A). This accumulation is dose dependent for compound 1, while maximum accumulation of cell in S phase was detected at concentration corresponding to IC_{50} of compounds 3 and Hfpsesc. CDDP induces slight increase in the

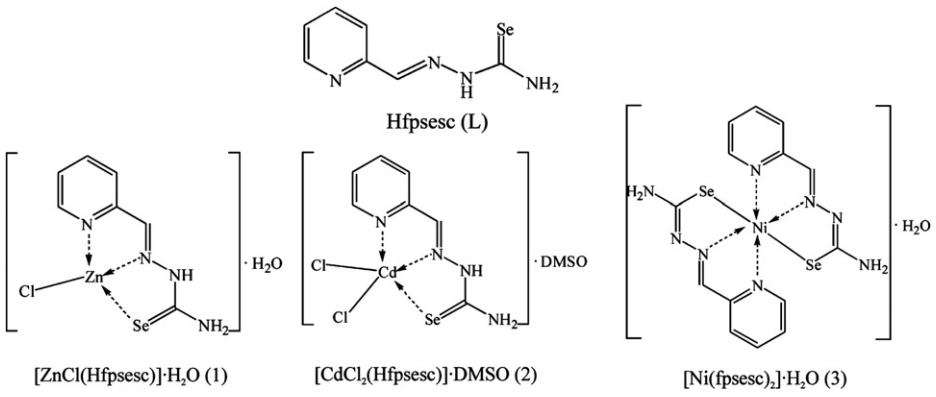

Fig. 1. Structure of the ligand Hfpsesc (L), Zn(II), Cd(II), and Ni(II) complexes used in this work.