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DN604: A platinum(II) drug candidate with classic SAR can induce apoptosis *via* suppressing CK2-mediated p-cdc25C subcellular localization in cancer cells

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ABSTRACT

DN604, a carboplatin analogue with a functional dicarboxylato ligand, was deeply investigated to explore its ability to induce apoptosis as well as its antitumor mechanism of action. Both *in vitro* and *in vivo* assays indicated that DN604 could effectively inhibit cell viability of SGC-7901 gastric cancer cells and exhibited stronger antitumor activity than carboplatin and comparable activity to cisplatin. Significantly in contrast to cisplatin, DN604 resulted in negligible toxic effects *in vivo* with the same tumor growth inhibition effect as cisplatin. The mechanism study indicated that DN604 inhibited CK2-phosphorylated cdc25C activation to decrease p-cdc25C subcellular localization, leading to the inactivation of cdc2/Cyclin B and G2/M cell cycle arrest and apoptosis in SGC-7901 cancer cells. Our research revealed for the first time that the dicarboxylato ligand containing a suitable functional moiety as the leaving group in the platinum(II) complex can effectively induce cell cycle arrest and apoptosis *via* inhibiting key checkpoint proteins.

1. Introduction

Gastric cancer (GC) is one of the most frequently diagnosed cancers with high incidence and mortality rates over the past several decades [1]. Since most patients are either diagnosed at an advanced stage or a relapse following apparently curative operation, the platinum-based drug cisplatin is regularly applied as the most effective chemotherapeutics in the first-line treatment of an advanced stage of gastric cancer [2]. However, the severe side effects and cisplatin resistance are inevitable, which could be attributed to its high reactivity, low aqueous solubility, reduced drug accumulation and increased the repair of DNA damage. Thus, carboplatin, designed with a dicarboxylato ligand, was used to diminish the side effects of cisplatin especially the toxicity with the treatment of solid tumors. Application of 1,1-cyclobutyldicaboxylate as the leaving ligand, which is coordinated to the platinum atom through a six-membered ring, enhances the stability and aqueous solubility of carboplatin. Despite some misfeatures of cisplatin like reactivity, aqueous solubility and toxicity were remarkably improved by carboplatin, the drug was found to possess weak anticancer activity. So it is of much significance to hunt for novel platinum drugs to enhance platinum-based drugs anticancer effects and conquer its side effects for successful treatment.

Increasing evidences indicated that the reactivity of platinum(II) complexes is crucial to their properties including biological profiles, hence, many efforts have been devoted to investigating their reactivity [3–5]. Previous studies have been mainly focused on the impact of the carrier ligands of the platinum(II) complexes with their steric and electronic effects, but few of them were concerned with the influence and underlying mechanism of the leaving group [6]. So far, numerous mono- and dicarboxylates have been applied as leaving groups to modulate the lipophilicity and biodistribution of the resulting platinum (II) complexes [7], however, the detailed knowledge of the factors that carboxylato ligands affecting the biological activity of the platinum(II) complexes are not fully understood. For example, many reports described the improved pharmaceutical properties of the platinum(II) complexes by introducing a substituent to the skeleton of malonate or 1,1-cyclobutanedicarboxylate (CBDC) [8,9]. In contrast to their parent compounds, the activity of the derivatives can be greatly changed due to minor modifications in the ligands [10]. Upon our previous report [11], DN604, a carboplatin analogue containing 3-oxocyclobutane-1,1-dicarboxylate (OCBDC) as a leaving group, exhibited potent and comparable cytotoxicity to cisplatin against a number of human cancer cell

Abbreviations: SAR, structure activity relationship; CBDC, 1,1-cyclobutanedicarboxylate; OCBDC, 3-oxocyclobutane-1,1-dicarboxylate; CDKs, cyclin-dependent kinases; MPF, M-phase-promoting factor; Pt, Potassium tetrachloroplatinate; ICP-MS, inductively coupled plasma mass spectrometry; DAPI, diamidino-phenyl-indole; ROS, reactive oxygen species

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lines. Its structural difference from carboplatin is the existence of a carbonyl group at the 3-position of CBDC in DN604.

It has been known that cisplatin interacts with DNA to form inter- and intrastrand DNA-adducts after entering cancer cells [12], which could induce DNA damage and trigger cell cycle arrest, initiating a signaling cascade that leads to cell death [13]. Previous studies showed that cell cycle arrest could be alleviated by the activation of checkpoint proteins such as cyclin-dependent kinases (cdks) to promote cell proliferation in cancer cell lines [14]. It is well known that the activity of cdks is mediated by the regulatory cyclin subunits assembly with the catalytic cdks, its subcellular localization and specific phosphatases [15]. Cdc25c, one of the cell cycle regulating phosphatases, primarily controls the progression through the G2-phase and entry into mitosis [16]. Among the crucial cyclin-dependent kinases in cell cycle, a cdc2/cyclin B complex called M-phase-promoting factor (MPF) plays a vital role in the transition from G2 to M phase. Dephosphorylation in MPF by cdc25C is used to activate the cdks mediated cell cycle progression. Additionally, cdc25C is also phosphorylated by protein kinase CK2 to retard the nuclear localization of cdc25C, which is a prerequisite for activation of the MPF complex [17]. However, cdc2 activity was supported by CK2-induced p-cdc25C in cancer cells following the treatment of platinum-based drugs, which resulted in G2/M progression and a lower chemo-sensitivity to them. These findings hypothesized that obstacle of CK2-induced p-cdc25C could enhance the effect of cell cycle arrest and death in cancer cells treated by platinum-based drugs.

Based on the above, the *in vitro* biological activity of DN604 toward SGC-7901 gastric cancer cell line was herein evaluated to determine whether DN604 has ability to mediate anticancer effects. Furthermore, its capability of inhibiting tumor growth with a gastric cancer xenograft mice model was assayed. For extensive *in vivo* test on DN604, a HCT-116 colon cancer xenograft mice model was also used to evaluate its anticancer ability. And finally, the role of CK2-induced p-cdc25C activation in DN604-mediated cancer cell cycle arrest and apoptosis was studied.

2. Materials and methods

2.1. Instruments and materials

All reagents and solvents were used as received from commercial vendors. Potassium tetrachloroplatinate(II) was purchased from a local chemical company (Shandong Boyuan Chemical Co., Ltd., China). DN604 was prepared and characterized as reported previously [11]. Platinum contents were typically determined *via* using ICP-MS (Optima 2100DV, PE, USA). Fluorescence spectra were recorded *via* using a Hitachi F-4600 fluorescence spectrometer with a 1 cm path length cuvette.

2.2. Cell cultures and reagents

Human cancer cell lines SGC-7901, A549 and HCT-116, normal cell lines HUVEC were originally purchased from the Cell Bank of Shanghai Institute of Cell Biology. SGC-7901, A549 and HCT-116 cancer cells were cultured at 37 °C in 5% CO_2 with RPMI-1640 supplemented with 10% FBS (Hyclone, Lifescience, MI), 100 U/mL benzyl penicillin and 100 mg/mL streptomycin (Beyotime, Nantong, China), while HUVEC cells were cultured at 37 °C in 5% CO_2 with DMEM supplemented with the same FBS and penicillin/streptomycin. The cells were restarted from frozen stocks and passed every one day upon reaching pass number 20.

2.3. In vitro cell viability assay

Cytotoxicity profiles of cisplatin, carboplatin and DN604 against different cell lines were evaluated *via* the MTT assay. Cancer cells were plated at density of 10^5/mL per well in 96-well plates. After overnight growth, cells were exposed to medium containing cisplatin, carboplatin and DN604 separately at varying concentrations and incubated for 72 h at 37 °C. Then the cell viability was determined by the MTT method according to previous description [18].

2.4. Cell morphological assessment

SGC-7901 cancer cells were plated in six-well plates at a density of 2×10^5 cells/well and incubated overnight. 15 μM cisplatin and carboplatin, 7.5 μM, 15 μM and 30 μM DN604 were added. After 24 h, the treated six-well plates were detected and photographed using the inverted light microscope.

2.5. Antitumor effects in mice

Six weeks old BALB/c nude mice were bought from SLAC Laboratory Animals (Shanghai, China) and raised in the air-conditioned room with standard laboratory food and water ad libitum. Experimental protocols were in accordance with National Institutes of Health regulations and approved by the Institutional Animal Care and Use Committee. Forty-eight nude mice (BALB/c) with body weight ranged from 18 to 22 g were randomly divided into 7 groups. The SGC-7901 single-cell suspension in PBS (1×10^7 cells/mouse) was injected subcutaneously into the right oxter of nude mice. When tumor grew to a size of 80–150 mm^3 at 12 days, the mice were administrated *via* injecting intravenously of cisplatin (dosed intravenously at 4 mg/kg twice a week), carboplatin (dosed intravenously at 20 mg/kg and 40 mg/kg once every three days), DN604 (dosed intravenously at 5 mg/kg, 10 mg/kg and 20 mg/kg once every three days). The control group was administered glucose. Meanwhile, other Forty-eight nude mice (BALB/c) with body weight ranged from 18 to 22 g were randomly divided into 7 groups. The HCT-116 single-cell suspension in PBS (1×10^7 cells/mouse) was injected subcutaneously into the right oxter of nude mice. When tumor grew to a size of 80–150 mm^3 at 12 days, the mice were administrated *via* injecting intravenously of cisplatin (dosed intravenously at 4 mg/kg twice a week), carboplatin (dosed intravenously at 20 mg/kg and 40 mg/kg once every three days), DN604 (dosed intravenously at 5 mg/kg, 10 mg/kg and 20 mg/kg once every three days). The control group was also administered glucose.

The tumor growth was monitored *via* measuring the perpendicular diameter of the tumor by calipers every two days and calculated according to the formula:

$$\text{Tumor volume } (mm^3) = 0.5 \times \text{length} \times \text{width}^2$$

The growth curves were plotted using tumor volume in average within each group at the corresponding time points. The mice were sacrificed after the last treatments, then tumor weight was evaluated as the antitumor activity of the corresponding groups. The sera were collected for biochemical studies. The kidneys and livers were excised, embedded in paraffin for immunohistochemistry analysis. The physical state and body weight of the mice were determined as an indicator of systemic toxicity. The CK2 kinase activity was detected with purified CK2 extracted from the groups treated with cisplatin, carboplatin and DN604, respectively, according to manufacturer's instruction [27].

2.6. Immunohistochemistry

The expression of p-cdc25C in tumor tissues of *in vivo* nude mice model was measured as previously described [19]. All reagents used in the experiment were provided by Maixin-Bio Co. (Fuzhou, China).

2.7. Reactions of DN604 with DNA

Herring sperm DNA was dissolved in 10 mM phosphate buffer (pH 7.4) containing 10 mM $NaClO_4$. The DNA concentration was determined by UV–vis spectra at 260 nm with an extinction coefficient of