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Original Article

A CK2-targeted Pt(IV) prodrug to disrupt DNA damage response

Feihong Chen [1], Xiaochao Huang [1], Mian Wu, Shaohua Gou*, Weiwei Hu

Jiangsu Province Hi-Tech Key Laboratory for Biomedical Research, Pharmaceutical Research Center and School of Chemistry and Chemical Engineering, Southeast University, Nanjing 211189, China

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ABSTRACT

A Pt(IV) prodrug, Cx-platin, containing CX-4945 (a CK2 inhibitor) as an axial ligand was designed and prepared by targeting CK2 to disrupt DNA damage response. *In vitro* study indicated that Cx-platin had superior cytotoxicity to cisplatin against a number of cancer cell lines with distinct CK2-expressed levels, caused CK2-overexpressed cancer cells death via suppressing CK2-mediated DNA damage repair and reversed cisplatin resistance. Mechanistic investigation suggested that the potent antitumor activity of Cx-platin resulted from its major suppression of CK2-phosphorylated MDC1 to combine FHA domain of aprataxin to DNA double strand breaks (DSBs) caused by improved cellular uptakes of Pt and ATM deactivation. Further *in vivo* tests exhibited that Cx-platin displayed high tumor inhibition rates, increased weight gain, and hardly toxicity effects in contrast to cisplatin.

Introduction

DNA-targeted anti-cancer chemotherapeutics are fundamental components of the most effective chemotherapeutics in clinic. The effect of such DNA-targeted agent like cisplatin has been demonstrated to function via initiating DNA damage to induce cell death [1,2], which can be survived by repairing DNA lesions, potentially leading to innate or acquired drug resistance [3–5]. Thus, a practical approach to reduce the resistance caused by DNA-targeted agents is to combine them with inhibitors [6], particularly those to inhibit multiple DNA repair pathways to increase the sensitivity of cancer cells to platinum-based chemotherapies [7].

Protein kinase CK2, consisted of two catalytic subunits (α and α′) and two regulatory β subunits, is widely overexpressed in human tumors [8,9]. As a key regulator of various cellular events, CK2 could generally regulate diverse pathways in various chemotherapeutics-induced resistance [10–13]. More importantly, CK2 could be dimed as a key director in DNA repair of both single-strand break (SSB) and double-strand break (DSB) [14–16]. Among the basic components of DNA damage repair pathways, the key proteins XRCC1 and MDC1 are two of the best characterized substrates of the CK2-dependent SSB and DSB repair, respectively. As required for the rapid and slight SSB repair, XRCC1 could be phosphorylated by CK2 at S/T residues in amino acid sequence 403–538 [17–19]. It is well known that SSB-mediated ataxia telangiectasia mutated kinase (ATM) activation is followed by a G1 cell cycle delay that allows more time for repair and thus prevents DNA replication and DSB accrual [20]. Moreover, ATM activation is critical to distinguish SSBs from DSBs. Whereas, the strengthened resistance was associated with a slow and heavy repair of DSB, which was the most damaging form of DNA lesions [21]. As a key mediator of DSB repair, MDC1, activated by CK2 at N-terminal S-D-T-D motifs, can co-localize with aprataxin FHA domain and then bind to γ-H2AX [15,22–24]. Thus, DNA damage repair pathways could be destructed by the inhibitors of CK2. Despite several ATP-competitive CK2 inhibitors have been discovered so far, only one of them, CX-4945, as an orally bioavailable selective inhibitor, entered phase II clinical trials to act as a potential anti-cancer drug [25]. Based on that, the approach of combing cisplatin with CX-4945 in a molecule may have the potential superiority to reverse cisplatin-induced resistance.

Recently, several Pt(IV) complexes as prodrugs have been designed and studied to enhance anti-tumor efficacy of traditional Pt(II) drugs, improving their cellular uptake and blood-circulation time [26–29]. Compared with their Pt(II) counterparts, six-coordinated Pt(IV) complexes with octahedral geometry have higher coordination numbers with two extra axial ligands, which facilitate their kinetic inertness and low reduction potential till entering tumor cells [30,31]. As the first bifunctional Pt(IV) complex, Mitaplatin with two dichloroacetate (DCA) moieties in the axial positions was found to attack both nuclear DNA and mitochondria, and expected to reduce drug resistance and heighten

* Corresponding author.
E-mail address: sgou@seu.edu.cn (S. Gou).
[1] These authors contributed equally to this work.

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tumor cell apoptosis [32]. Inspired by that, vitamin E has also been coupled to a Pt(IV) complex that can cause simultaneous DNA and mitochondria damage [33]. Before long, a hybrid of Pt(IV)-aspirin, Platin-A, was designed to release aspirin, which exhibited anticancer and anti-inflammatory features better than the corresponding combination therapy [34]. Although the above-mentioned Pt(IV) complexes shown desirable ability to reduce platinum resistance via mitochondrial and other possible mechanisms [35,36], specific DNA repair pathways that play a crucial role in the resistance response have not been explored. In this paper, CX-4945 has been designed in a Pt(IV) prodrug (Cx-platin) to occupy one of two axial positions of the Pt(IV) octahedral coordination sphere derived from cisplatin. It is anticipated that Cx-platin could reinforce cellular uptake of Pt and particularly suppress DNA repair response essential for aberrant CK2 activity to overcome cisplatin-induced resistance together with potent anticancer efficacy.

Results

Synthesis and characterization of Cx-platin

The preparation of Cx-platin is simply shown in Scheme 1. Treatment of *cis*,*cis*,*trans*-[$Pt(NH_3)_2Cl_2(OH)_2$] with equivalent molar amount of CX-4945 in the presence of TBTU and TEA in DMF produced Cx-platin, which was characterized by ^1H NMR, ^{13}C NMR and ESI mass spectroscopy (Fig. S1) together with microanalysis. It is noted that only one hydroxyl group was substituted by CX-4945 under the reaction condition, and Cx-platin was a little dissolved in water like cisplatin.

The stability of Cx-platin and its released ability under reduction

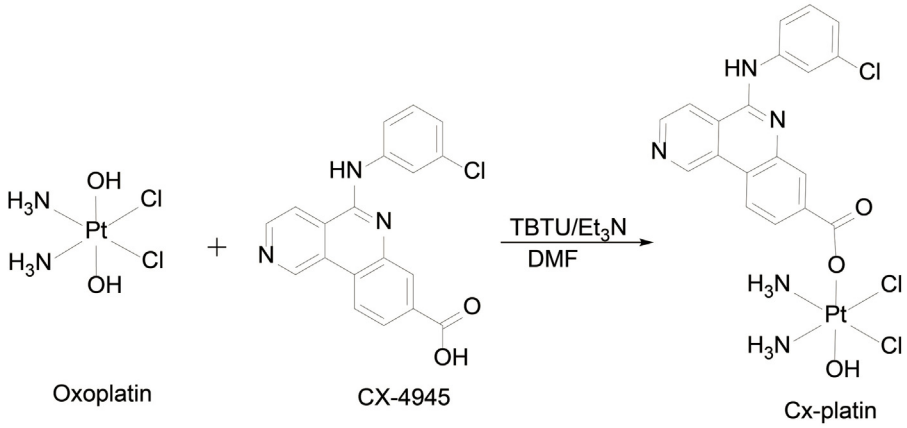
The stability of Cx-platin dissolved in acetonitrile and water (2:1) was first examined by HPLC technique at different time, and the corresponding chromatograms are shown in Fig. S2A. It was observed that Cx-platin kept unchangeable in a period of 24 h, indicating that the designed compound is stable under the normal condition. The capability of Cx-platin in a solution of acetonitrile and water (2:1) to release CX-4945 and cisplatin under reduction with ascorbic acid (AsA) was studied by HPLC as well. The HPLC chromatograms (Fig. S2B) from the reduction reaction were recorded every three hours in a period of 24 h. It was noticed that Cx-platin was gradually reduced to release CX-4945 as the time passed, accompanied by the peak of Cx-platin falling down and that of CX-4945 rising up. At the end of 24 h, Cx-platin disappeared, leaving the presence of CX-4945 alone. Although cisplatin in the related chromatograms, monitored under the ultraviolet detecting condition, was not observed due to its weak chromophore, the above experiment proved that Cx-platin is able to release both CX-4945 and cisplatin under reduction condition as expected.

DNA platination induced by reduction

The cytotoxicity of Cx-platin owing to the DNA platination could be detected in the presence of EtBr as a probe, because the DNA platination of Cx-platin could prevent the formation of EtBr-DNA complex, resulting in its stoichiometric loss of fluorescence. It was observed in Fig. 1A that Cx-platin did not cause much fluorescent intensity change until it was reduced to a Pt(II) moiety in the presence of ascorbic acid, while cisplatin containing divalent Pt(II) species could definitely lead to the loss of fluorescent intensity under the same condition. The above results indicated that under the activation of reductant AsA, Cx-platin can be regarded as the prodrug of cisplatin to exert its cytotoxic effect.

Cellular uptake

In order to investigate the mechanism of the enhanced cytotoxicity of Cx-platin compared with cisplatin, the amounts of cellular uptake of Cx-platin and cisplatin in all tested cancer cells were measured. After the treatment for 4 h, the platinum content in these cancer cells was analyzed via ICP technique. As Fig. S3 shown, the increased cellular uptake of Cx-platin in cancer cells was dramatically higher than that of cisplatin, especially in cancer cells that CK2 are expressed in high and medium levels. Among them, the maximum difference of the amount of Pt between Cx-platin and cisplatin nearly reached 3–4 folds in SGC-7901 and SGC-7901/cDDP cells, while the minimum difference is about 1.5 fold in HepG2 cell. As for CK2 low-expressed cancer cell PANC-1, there is only a small difference of the amount of cellular uptake between Cx-platin and cisplatin, in which Cx-platin exhibited a little high value. These could account for the higher cytotoxicity of Cx-platin than cisplatin in CK2-expressed cancer cells. Comparing the Pt levels in SGC-7901 with SGC-7901/cDDP cancer cells (Fig. 1B), Cx-platin and cisplatin as well as the equimolar mixture of cisplatin and CX-4945 had higher amount of Pt in SGC-7901/cDDP than in SGC-7901 cells, despite only Cx-platin presented the ability to overcome the cisplatin resistant cancer cell. To further illustrate the unique ability of Cx-platin to overcome drug resistance, we studied the Pt levels of Cx-platin, cisplatin and the equimolar mixture of cisplatin and CX-4945 in genomic DNA of gastric cancer cells SGC-



Scheme 1. Preparation of Cx-platin.