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Toward a better understanding of the oxaliplatin mode of action upon the steric hindrance of 1,2-diaminocyclohexane and its analogue

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ABSTRACT

The present research is concerned with the mechanism investigation on the interaction between oxaliplatin and guanosine 5′-monophosphate (GMP) in the presence of glutathione (GSH). The binding modes of oxaliplatin with GMP and GSH were explored by HPLC and LC–MS techniques, respectively, in which four key intermediates were found and five adducts were determined in the reaction. The results indicated that GSH can interfere with the reaction between oxaliplatin and DNA in two ways. One is by competing with GMP to bind the active platinum unit, and the other is by substituting the guanine-N7 atom of DNA to form inactive platinum species. In contrast to oxaliplatin with trans 1,2-diaminocyclohexane as spatial framework, a known platinum(II) complex, characteristic of *trans*-bicyclo[2.2.2]octane-7,8-diamine possessing dicyclic steric hindrance, was also studied in the same way to explore its mode of action with DNA.

1. Introduction

Cisplatin, as a platinum based antitumor drug for clinical use, is usually limited for toxicity and intrinsic and/or acquired resistance, which is believed to be due to its interaction with sulfur-containing biological molecules [1]. It has been well documented that platinum drugs indeed interact with many biological molecules [2–4], especially sulfur-containing species like glutathione (GSH) before they reach the putative target DNA [5–7]. Since these interactions can cause the deactivation of the platinum-based drug, high dose of drug is needed to achieve the satisfactory therapeutic effect, but unfortunately leading to the generation of more side effects. It has been reported that high level of intracellular GSH in tumor cells, 7–10-fold higher than in normal cells [8], is clearly correlated with the resistance of platinum-based drugs [9,10], in which active platinum units are very reactive toward the cysteine residue of GSH instead of binding to the guanine-N7 atom of DNA [11,12]. Therefore, some studies on platinum anticancer drugs are focused on how platinum(II) complexes mainly bind to DNA rather than GSH [13]. Several analytical strategies (*e.g.* kinetic measurement) have been proposed to evaluate antitumor efficacy so far [14–16]. The kinetic measurement doesn't directly provide information on the reaction adducts. In comparison, the quantitative measurements performed by proton NMR and MS provide a very sensitive measurement of adducts' formation. Moreover, LC–MS has been proven to be a useful way to study the interaction of platinum complexes with biological molecules by providing direct information on the nature of adducts that are formed [17–19].

Oxaliplatin, a platinum based antitumor drug used especially in clinical treatment with metastatic colorectal cancer, has been reported to show activity against cisplatin and carboplatin resistant cancers [20]. Such a feature is attributed to its carrier ligand, 1*R*,2*R*-diaminocyclohexane (abbreviated as DACH), whose cyclohexyl skeleton can provide steric hindrance when oxaliplatin interacts with DNA. It is believed that the bulky ligand, DACH, contributes to high cytotoxicity against cisplatin-resistant cell lines, possibly due to the steric hindrance effect of the DACH–Pt–DNA adducts [21–24].

In view of the function of DACH in oxaliplatin stated above, we launched a project to explore platinum(II) complexes of DACH derivatives or its analogues with steric hindrance about their antitumor activity and potential to reduce/overcome cisplatin drug resistance. In our recent reports, several kinds of oxaliplatin derivatives with N-monoalkyl or N,N′-dialkyl *1R,2R*-diaminocyclohexane as carrier ligands were investigated, in which different sized linear alkyl or cycloalkyl groups were introduced to act as steric hindrance [25–28]. Although some typical compounds exhibited potent antitumor activity superior or comparable to cisplatin and oxaliplatin, the flexible N-substituted alkyl groups seemed not feasible to offer fixed spatial resistance in addition to bringing about extra stereogenic centers with complicity. Before long, an optical pair of oxaliplatin analogues (**2a**/**2b**) with chiral *trans*-bicyclo[2.2.2]octane-7,8-diamine (BCODA) as ligand possessing dicyclic steric hindrance was reported by our group, which has good lipophilicity, improved kinetic reactivity and potent antitumor activity especially to a cisplatin resistant cell line [28]. Because of the structural similarity to oxaliplatin, both complexes **2a** and **2b** displayed almost the similar

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antitumor behavior to oxaliplatin except to the cisplatin resistant cell line SGC-7901/CDDP. These findings motivated us to explore the possible mechanism of action about how sterically hindered oxaliplatin or complex **2a** interacts with DNA in the absence and presence of GSH, so as to shed light on the platinum drug design for reducing drug resistance caused by GSH. In this work, the mechanism about the interaction between oxaliplatin and guanosine 5′-monophosphate (GMP) with or without GSH was at first studied and illustrated by applying HPLC and LC–MS techniques, and then complex **2a** as an oxaliplatin analogue was investigated in the same way for the purpose of comparison.

2. Materials and methods

2.1. Materials and measurements

Complex **2a** was prepared according to the literature method [28]. Oxaliplatin was obtained from Jiangsu Hengrui Medicine Company Limited. GSH, GMP, and HPLC-grade methanol were all purchased from Sigma-Aldrich. For all preparations of aqueous solutions, ultrapure water was used.

High performance liquid chromatography (HPLC) was performed on an Agilent 1260 system equipped with a Phenomenex C18 column (250 × 4.6 mm, 5 μm). HPLC profiles were recorded on UV detection at 210 nm. Mobile phase A: water containing 15 mM ammonium acetate (NH_4HCO_3); mobile phase B: methanol. For analytical assays, the flow rate was 0.8 mL/min. The gradient (solvent B) for oxaliplatin was as follows: 95% from 0 to 5 min, 95% to 80% within 15 min, and reset to 95% from 20 to 25 min. The gradient (solvent B) for **2a** was as follows: 95% from 0 to 3 min, 95% to 75% within 17 min, and reset to 95% from 20 to 25 min.

High performance liquid chromatography electrospray ionization time of flight mass spectrometry (HPLC ESI TOF MS) was performed on an Agilent 1260-6224 system. The LC analysis method was the same as the HPLC-UV, and a splitting ratio to ESI-MS was 1/5. Positive-ion electrospray ionization mass spectra were obtained at a capillary temperature of 350 °C with spray voltage 4000 V. And the negative spectra were obtained at a capillary temperature of 350 °C with spray voltage 3500 V. The mass accuracy of all measurements was within 0.0001 m/z unit.

2.2. Reactions of oxaliplatin

Reaction mixtures of oxaliplatin with GMP and/or GSH at various molar ratios were prepared by mixing aliquots of 0.5 mM oxaliplatin, 0.5–5 mM GMP, and 0.5–5 mM GSH. No buffer was used and the pH value was 6. The final solution (oxaliplatin:GMP = 1:1, 1:2, 1:10; oxaliplatin:GSH = 1:2; oxaliplatin:GMP:GSH = 1:1:1, 1:5:5) was kept at 37 °C in the dark. The sample was taken for HPLC analysis or LC–MS analysis without further purification.

2.3. Reactions of complex *2a*

Reaction mixtures of complex **2a** with GMP and/or GSH at various molar ratios were prepared by mixing aliquots of 0.5 mM complex **2a**, 1 mM GMP, and 1 mM GSH. The final solution (complex **2a**:GMP = 1:2, complex **2a**:GMP:GSH = 1:1:1) was kept at 37 °C in the dark. The sample was taken for HPLC analysis or LC–MS analysis without further purification.

3. Results and discussion

3.1. Reaction of oxaliplatin with GMP

Reaction of oxaliplatin with GMP was firstly carried out in the absence of GSH in deionized water (unbuffered solution) [29]. The time-dependent decrease of the peak area of oxaliplatin and GMP, accompanied by the appearance of one major adduct ([Pt(DACH)(GMP)$_2$], **I**), is shown in Fig. 2. The mass spectrum of **I** showed a positive ion at m/z 517.5978 and a negative ion at m/z 1032.1679 in high abundance, which can be assigned to the product derived from oxaliplatin combining with two GMP by loss of oxalate (ox). The observed isotopic pattern was in agreement with the theoretical calculations (Fig. S2). Meanwhile, LC–MS detected other two intermediates, $[Pt(DACH)(GMP)(ox)]^-$ (**II**) and $[Pt(DACH)(GMP)]^+$ (**III**), whose concentrations were too low to be detected by HPLC (UV detector). Intermediate **II** had a pseudo-molecular ion peak of $[M + 2H]^+$ at m/z 761.1256, which corresponded to the molecular formula of $[C_{18}H_{29}N_7O_{12}PPt]^+$ by isotope modeling. This intermediate was defined as originated from the open oxalate chelating ring of oxaliplatin that was combined with one GMP afterward. Intermediate **III** had a pseudo-molecular ion peak of $[M]^+$ at m/z 671.1303, which was suggested by isotope modeling to be related to the molecular formula of $[C_{16}H_{27}N_7O_8PPt]^+$, corresponding to the peak derived from intermediate **II** by losing the oxalate group. These assignments were also confirmed by theoretic molecular weights and isotopic distribution patterns (Figs. S3–S4). (See Fig. 1.)

The influence of the concentrations of GMP has also been considered. In Fig. S1, the chromatograms clearly indicated a steady increase in the area of the peak for **I** with increasing concentrations of GMP, while the intensities of intermediates **II** and **III** were still too low to be detected. Several reports have indicated that oxaliplatin reacted with GMP to form the bi-functional adduct as major species [30,31]. So it can be concluded that the concentration of GMP has no obvious influence on products formed in the reaction, but remarkably affects the reaction rate. Upon the adducts of the reaction, a pathway of the reaction between oxaliplatin and GMP was proposed in Scheme S1.

3.2. Reaction of oxaliplatin with GMP in the presence of GSH

As mentioned before, GSH plays an important role in the platinum drug resistance, so the competitive binding of GMP and GSH to oxaliplatin should be considered in the reaction. The reaction of oxaliplatin with GMP in the presence of GSH was monitored by HPLC and LC–MS, and typical spectra are shown in Fig. 3. Besides the major product **I**, the reaction in the presence of GSH gave rise to four products as detected by HPLC. Positive ESI-MS analysis of **V** (Fig. S5A) showed a doubly-charged cationic peak at m/z 615.1556, corresponding to the molecular formula of $[C_{32}H_{60}N_{10}O_{12}S_2Pt_2]^{2+}$, and negative ESI-MS analysis of **V** (Fig. S5B) showed a single-charged anionic peak at m/z 1227.2953, corresponding to the molecular formula of $[C_{32}H_{57}N_{10}O_{12}S_2Pt_2]^-$. The peak **V** (corresponding to $[Pt_2(DACH)_2(GS)_2]^{2+}$) can be assigned to the $[Pt(DACH)]^{2+}$ fragment that reacts with glutathione forming a dimeric sulfur-bridged adduct containing two platinum units and two GSH moieties [32,33]. Positive ESI-MS analysis of **VII** (Fig. S6A) showed a doubly-charged cationic peak at m/z 461.6120, corresponding to the molecular formula of $[C_{26}H_{48}N_8O_{12}S_2Pt]^{2+}$, while negative ESI-MS analysis of **VII** (Fig. S6B) showed a single-charged anionic peak at m/z 920.2204, corresponding to the molecular formula of $[C_{26}H_{45}N_8O_{12}S_2Pt]^-$. The peak **VII** (corresponding to [Pt(DACH)(GS)$_2$]) can be ascribed to the $[Pt(DACH)]^{2+}$ fragment that binds two GSH. These assignments were also confirmed by comparison of experimental and theoretical isotopic pattern distributions.

To ensure the assignment of GSH's combination with oxaliplatin, the reaction of oxaliplatin with GSH (oxaliplatin:GSH = 1:2) was investigated as well. Typical spectra are shown in Fig. S7. The reaction led to products **V** and **VII** as detected by HPLC, whereas LC–MS detected another intermediate **VIII** (corresponding to $[Pt(DACH)(GS)]^+$) (Fig. S8) in the reaction. Intermediate **VIII** had a pseudo-molecular ion of $[M]^+$ at m/z 615.1567, corresponding to the molecular formula of $[C_{16}H_{30}N_5O_6SPt]^+$, which can be assigned to the $[Pt(DACH)]^{2+}$ fragment that is linked with one GSH [34]. The data of the reaction of oxaliplatin with GSH were consistent with the molecular arrangement above, and the mechanism of this reaction was proposed in agreement