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Ligand modulation of a dinuclear platinum compound leads to mechanistic differences in cell cycle progression and arrest

Vijay R. Menon [a,c], Erica J. Peterson [b,c], Kristoffer Valerie [c,d], Nicholas P. Farrell [a,b,c,*], Lawrence F. Povirk [a,c,*]

[a] Department of Pharmacology & Toxicology, Virginia Commonwealth University, Richmond, VA 23298-0613, United States
[b] Department of Chemistry, Virginia Commonwealth University, Richmond, VA 23298-0613, United States
[c] Massey Cancer Center, Virginia Commonwealth University, Richmond, VA 23298-0613, United States
[d] Department of Radiation Oncology/Department of Biochemistry and Molecular Biology, School of Medicine, Virginia Commonwealth University, Richmond, VA 23298-0613, United States

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ABSTRACT

Despite similar structures and DNA binding profiles, two recently synthesized dinuclear platinum compounds are shown to elicit highly divergent effects on cell cycle progression. In colorectal HCT116 cells, BBR3610 shows a classical G_2/M arrest with initial accumulation in S phase, but the derivative compound BBR3610-DACH, formed by introduction of the 1,2-diaminocyclohexane (DACH) as carrier ligand, results in severe G_1/S as well as G_2/M phase arrest, with nearly complete S phase depletion. The origin of this unique effect was studied. Cellular interstrand crosslinking as assayed by comet analysis was similar for both compounds, confirming previous *in vitro* results obtained on plasmid DNA. Immunoblotting revealed a stabilization of p53 and concomitant transient increases in p21 and p27 proteins after treatment with BBR3610-DACH. Cell viability assays and cytometric analysis of p53 and p21 null cells indicated that BBR3610-DACH-induced cell cycle arrest was p21-dependent and partially p53-dependent. However, an increase in the levels of cyclin E was observed with steady state levels of CDK2 and Cdc25A, suggesting that the G_1 block occurs downstream of CDK/cyclin complex formation. The G_2/M block was corroborated with decreased levels of cyclin A and cyclin B1. Surprisingly, BBR3610-DACH-induced G_1 block was independent of ATM and ATR. Finally, both compounds induced apoptosis, with BBR3610-DACH showing a robust PARP-1 cleavage that was not associated with caspase-3/7 cleavage. In summary, BBR3610-DACH is a DNA binding platinum agent with unique inhibitory effects on cell cycle progression that could be further developed as a chemotherapeutic agent complementary to cisplatin and oxaliplatin.

1. Introduction

Platinum drugs are a mainstay of cancer therapeutics especially for the treatment of head and neck, testicular, ovarian, lung, and colorectal cancers [1,2]. To date, all clinically useful platinum drugs contain a single Pt(II) center with two exchangeable ligands in *cis* geometry. Interaction of these drugs with cellular biomolecules such as sulfur-containing glutathione and metallothionein can render them inactive before reaching their pharmacological target, DNA [3,4]. Subsequently, polynuclear platinum complexes (PPCs) that are structurally different from cisplatin, and exhibit a different mode of DNA binding, were developed to circumvent the cellular resistance arising toward the mononuclear compounds, primarily decreased uptake, increased efflux, and increased DNA repair. Especially, structural conformational changes induced by long-range inter- and intrastrand crosslinks are distinctly different from those induced by the mononuclear cisplatin and oxaliplatin [5]. The prototype of this class, BBR3464 [{trans-$PtCl(NH_3)_2$}$_2${μ-trans-$Pt(NH_3)_2(H_2N(CH_2)_6NH_2)$}]$^{4+}$ (Fig. 1A), is the only platinum compound without two exchangeable ligands in *cis* to have reached human Phase II clinical trials [2]. It is cytotoxic in

Abbreviations: ATM, Ataxia Telangiectasia Mutated; ATR, Ataxia Telangiectasia Mutated and Rad3 related; BrdU, bromodeoxyuridine; CDK, cyclin dependent kinase; Chk1, checkpoint kinase 1; DACH, 1,2-diaminocyclohexane; Gy, Gray; MTT, (3,4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide; PAGE, polyacrylamide gel electrophoresis; PARP, poly (ADP-ribose) polymerase; PBS, phosphate buffered saline; PCNA, proliferating cell nuclear antigen; PPC, polynuclear platinum complex; PVDF, polyvinylidene difluoride; SDS, sodium dodecyl sulfate.

* Corresponding authors.
E-mail addresses: npfarrell@vcu.edu (N.P. Farrell), lpovirk@vcu.edu, lfp98@yahoo.com (L.F. Povirk).

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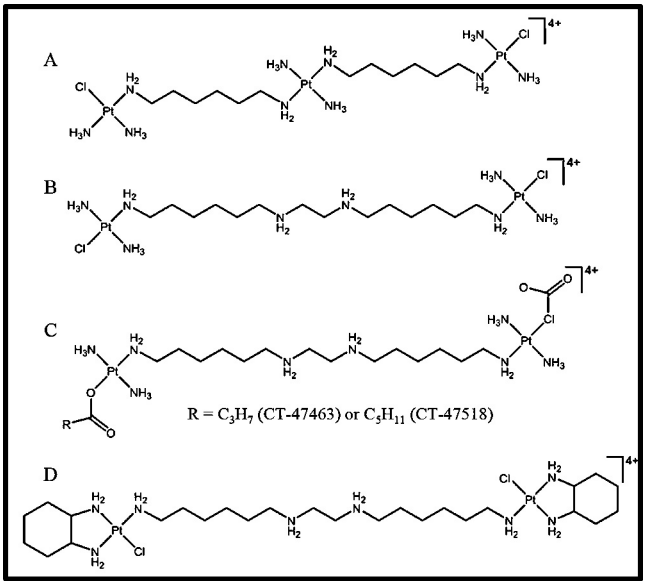

Fig. 1. Structures of the polynuclear platinum compounds (PPC) mentioned in the present study. (A) BBR3464; (B) BBR3610; (C) CT-47463 and CT-47518; (D) BBR3610-DACH.

cisplatin-resistant cell lines and shows high efficacy in p53 mutant tumor cells [6]. However, despite promising responses in Phase II clinical trials, BBR3464 had an unacceptably low therapeutic index, possibly due to increased metabolism in human plasma [7,8].

In analyzing the structure–activity relationship of the trinuclear complexes, it was seen that the charged central platinum atom provided H-bonding and an electrostatic pre-association with duplex DNA in the minor groove. Replacement of this central platinum with linear polyamines has yielded second-generation analogs of BBR3464 that retain its biological activity [9]. BBR3610 (Fig. 1B), which was developed by replacing the central platinum with a spermine-like linker, is one of the most cytotoxic platinum compounds, with nanomolar toxicity in gliomas and colon cancer cells [10,11]. However, like its predecessor, BBR3610 was also found to undergo serum biotransformation leading to its degradation [12,13]. This was attributed to the substitution of the Pt–Cl bond by thiol compounds, resulting in bridge cleavage.

To improve metabolic stability and circumvent irreversible plasma protein binding, different analogs of BBR3610 have been developed by modifying either the leaving groups or the carrier ligands. Replacement of the chloride leaving groups in BBR3610 with butyrate or capronate alkylcarboxylates (CT-47518 and CT-47463 respectively, Fig. 1C), improved pharmacokinetic and pharmacodynamic profiles of the parent drug [14]. These compounds were found to overcome resistance due to defects in DNA mismatch repair and were highly effective in cisplatin- and oxaliplatin-resistant cell lines. The use of the 1,2-diaminocyclohexane (DACH) as the carrier ligand gave BBR3610-DACH (Fig. 1D), where the chelating effect of the DACH ring contributed to the increased metabolic stability of BBR3610-DACH in the presence of sulfur-containing compounds at physiological pH [15]. *In vitro* studies showed this compound formed DNA adducts that persisted longer, escaped DNA repair, and inhibited transcription [16].

Deregulation of cell cycle progression is one of the key contributors toward cancer development [17]. Exposure to DNA damaging agents induces G_1/S or G_2/M cell cycle arrest by activating cell cycle checkpoint proteins that inhibit cyclin-dependent kinases (CDKs) [18]. The resulting cell cycle arrest can afford an opportunity for DNA repair and have negative effects on apoptosis [19]. Like cisplatin and most of its analogs, BBR3464 and BBR3610 disrupt DNA synthesis, resulting in transient S phase accumulation, followed by arrest in G_2. However, as shown below, BBR3610-DACH unexpectedly induced a dramatically different cellular response, consisting of a biphasic cell cycle arrest in G_1 and G_2, with a complete depletion of cells in S phase. These unusual cellular effects indicate a marked divergence from other PPCs and raise the possibility that BBR3610-DACH may have a unique spectrum and/or mechanism of antitumor activity.

2. Materials and methods

2.1. Chemicals

Cisplatin was obtained from Sigma–Aldrich (St. Louis, MO, USA; Cat# 479306) and dissolved in water. BBR3610 and BBR3610-DACH were synthesized as discussed earlier [12,15]. The stock solutions of platinum compounds were prepared at the concentration of 1 mM in water, stored at −20 °C and diluted in complete medium. The ATM inhibitor, KU-60019, was obtained from Selleck Chemicals (Houston, TX, USA). BrdU was obtained from Sigma-Aldrich (St. Louis, MO, USA; Cat# B9285).

2.2. Cell culture and PPC treatments

The human colorectal cancer cell line, HCT116, and its p53 and p21 knockout derivatives (denoted as p53−/− and p21−/−; kind gift from Dr. Bert Vogelstein) were cultured in RPMI 1640 (Invitrogen, Grand Island, NY, USA; Cat# 11875-093) supplemented with 10% fetal bovine serum (Quality Biologicals, Gaithersburg, MD, USA; Cat# 110-001-101US) and 100 U/mL penicillin and 100 μg/mL streptomycin (Invitrogen, Grand Island, NY, USA; Cat# 15140). The mismatch repair-proficient HCT116 + chr3 cell line (a kind gift from Dr. C. Richard Boland, Baylor Health Care System, Dallas, Texas) was cultured in IMDM (Invitrogen, Grand Island, NY, USA; Cat# 12440-053) supplemented with 10% fetal bovine serum and 100 U/mL penicillin and 100 μg/mL streptomycin. Cells were maintained in logarithmic growth as a monolayer in T75 tissue culture flask at 37 °C in a 5% CO_2 incubator. For the treatment, cells were seeded at a density of 5×10^5 cells/10 mL medium and treated with 20 μM compound, unless otherwise specified.

2.3. Growth inhibition assay

Five thousand cells/well in 100 μL were seeded in a 96-well plate and allowed 24 h to attach. Cells were then treated with the PPCs for 72 h. Following PPC removal, the cells were incubated in the presence of 0.5 mg/mL MTT reagent [(3,4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide; Sigma–Aldrich, St. Loius, MO, USA; Cat# M2128] in RPMI medium for 3 h at 37 °C. The MTT reagent was removed and 100 μL DMSO was added to each well. The plate was then incubated on a shaker at room temperature in the dark. The spectrophotometric reading was taken at 570 nm using a microplate reader.

2.4. Analysis of cell cycle distribution

HCT116, HCT116 p53−/−, HCT116 p21−/−, and HCT116 +chr3 cells were seeded in 100-mm tissue culture dishes at a density of 5×10^5 cells per dish. After 24 h, cells were treated with an equimolar dose of the PPCs (20 μM) for 6, 24, and 48 h. Both attached and floating cells were harvested at the different timepoints. One million cells were then suspended in 1 mL of propidium iodide solution (3.8 mM sodium citrate; 0.05 mg/mL propidium iodide; 0.1% Triton X-100) with added RNaseB at a final