Construction and Application of a Rh–Pt DNA Metalloinsertor Conjugate

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Supporting Information

ABSTRACT: We report the synthesis and characterization of a bimetallic conjugate (RhPt) in which an oxaliplatin derivative is tethered to a rhodium metalloinsertor through an aminomalonate leaving group ligand. The complex interacts with DNA through metalloinsertion at a base pair mismatch followed by formation of a covalent Pt–DNA adduct. Characterization of RhPt in mismatch repair-deficient HCT116O cells reveals increased cytotoxicity compared to cisplatin and oxaliplatin as well as relative to the unconjugated rhodium and platinum counterparts. Caspase and poly-ADP ribose polymerase inhibition assays indicate that RhPt induces apoptotic cell death. Inductively coupled plasma mass spectrometry (ICP-MS) experiments reveal that RhPt exhibits enhanced cellular uptake properties that contribute to its increased efficacy.

Platinum anticancer compounds are among the most successful and most widely used chemotherapeutics to date.1 However, cancers that exhibit deficiencies in the DNA mismatch repair (MMR) pathway, including 15% of sporadic colorectal cancer cases and 18% of all solid tumors, have encountered limited success in treatment with classical platinum therapeutics.2,3 Such cancers are largely resistant to cisplatin and platinum counterparts. Caspase and poly-ADP ribose polymerase inhibition assays indicate that RhPt induces apoptotic cell death. Inductively coupled plasma mass spectrometry (ICP-MS) experiments reveal that RhPt exhibits enhanced cellular uptake properties that contribute to its increased efficacy.

Rhodium metalloinsertors may offer a promising strategy in the development of new therapies for such cancers. These bulky, octahedral complexes bind specifically to DNA base pair mismatches,6 which are amplified in cells with defective MMR machinery.2,3 Metalloinsertors exhibit cytotoxicity preferentially in MMR-deficient cells, and the extent of this selectivity correlates with mismatch binding affinity and localization to the nucleus, where they target mismatches in genomic DNA.7,8

The design of bifunctional drug conjugates is a burgeoning field in chemotherapy, especially as a strategy to circumvent resistance.9 Here, we present a bimetallic oxaliplatin–metalloinsertor conjugate (RhPt) that displays dual DNA binding behavior. Additionally, RhPt exhibits enhanced cytotoxicity and cellular uptake in MMR-deficient HCT116O cancer cells compared to first-line platinum therapeutics as well as its unconjugated subunits. The cytotoxicity of RhPt appears to be triggered by an apoptotic cell death pathway, and its potency is attributed to the improved cellular uptake of the complex.

The RhPt conjugate, shown in Figure 1, consists of a trisheteroleptic Rh(III) scaffold tethered to an oxaliplatin derivative by an aminomalonate leaving group ligand. RhPt was constructed via a linear synthesis in which the rhodium scaffold was first functionalized with the aminomalonate, followed by complexation to platinum (see the Supporting Information).10 The platinum unit employs the same (1R,2R)-1,2-diaminocyclohexane nonleaving group ligand as oxaliplatin and, therefore, is expected to form the same DNA adducts.11 The rhodium unit contains a sterically expansive 5,6-chrysene diimine ligand (chrysi), which is responsible for the recognition of DNA mismatches.12 Too wide to intercalate into well-matched DNA, the chrysi complexes instead target thermodynamically destabilized mismatches from the minor groove, ejecting the bases from the duplex in a binding mode known as metalloinsertion.13 The aminomalonate linker is tethered to one of the noninserting ancillary ligands, which allows the conjugate to remain intact temporarily but ultimately enables the release of platinum, via hydrolysis, for DNA binding. The biological activity of RhPt was compared to several complexes, including cisplatin and oxaliplatin (Figure 1). The rhodium hydrolysis product, Rh(Amal), was included as a control to test whether the biological activity of RhPt may be attributed to the intact conjugate and not premature hydrolysis of the subunits. The unconjugated platinum complex, Pt(Amal) was included to explore the effects of the aminomalonate ligand on activity.

DNA binding studies were performed with RhPt and radiolabeled duplex DNA containing a CC mismatch (Figure 2). As RhPt does not cleave DNA upon irradiation, a competition titration was carried out using rac-[Rh(bpy)2chrysi]3+, which does photocleave DNA at the site of a mismatch.6 RhPt inhibits photocleavage by rac-[Rh(bpy)2chrysi]3+ at the mismatched site;
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RhPt inhibits photocleavage by [Rh(bpy)$_2$chrysi]$^{3+}$ on 1 with a CC mismatch. Controls without irradiation (ØRh), and without Rh (ØRh) were included. RhPt inhibits photocleavage by [Rh(bpy)$_2$chrysi]$^{3+}$ at the mismatched site. Right: DMS footprinting of duplex DNA containing a CC mismatch. Lanes (left to right): Maxam-Gilbert sequencing (C+T; A+G); 1, DMS alone; 4, oxaliplatin (1 μM); 5, RhPt (1 μM); 6, RhPt (50 μM). Bands of high electrophoretic mobility indicate cleavage at guanine residues; covalent binding of platinum to guanine inhibits cleavage.

Figure 2. DNA binding of RhPt. Left: Competition titration of increasing concentrations of RhPt (0–50 μM) with 1 μM rac-[Rh(bpy)$_2$chrysi]$^{3+}$ on 1 μM $^{32}$P-labeled 17mer duplex DNA with a CC mismatch. Controls without irradiation (ØRh), and without Rh (ØRh) were included. RhPt inhibits photocleavage by [Rh(bpy)$_2$chrysi]$^{3+}$ at the mismatched site. Right: DMS footprinting of duplex DNA containing a CC mismatch. Lanes (left to right): Maxam-Gilbert sequencing (C+T; A+G); 3, DMS alone; 4, oxaliplatin (1 μM); 5, RhPt (1 μM); 6, RhPt (50 μM). Bands of high electrophoretic mobility indicate cleavage at guanine residues; covalent binding of platinum to guanine inhibits cleavage.

Figure 3. Cellular accumulation of metal complexes in HCT116O cells. Rh and Pt counts were normalized to cellular protein: (left) whole-cell uptake of Pt complexes as a function of time; (right) whole-cell uptake of Rh for RhPt and hydrolysis product Rh(Amal).

Figure 4. Cell viability in HCT116O cells after 72 h with PARP and caspase inhibitors. Viability is normalized to untreated controls. Left (gray): PARP inhibition assay. Cells were treated with 0 or 20 μM RhPt and 0, 25, or 50 μM DPQ, DPQ does not increase the viability of cells treated with RhPt. Right (blue): Caspase inhibition assay. Cells were treated with 0, 10, or 20 μM RhPt and 0 or 20 μM Z-VAD-FMK. Z-VAD-FMK increases viability in RhPt-treated cells. Addition of either inhibitor alone does not affect viability. *p < 0.0001 (unpaired two-tailed t test).
inhibitor Z-VAD-FMK. The viability of RhPt-treated cells increases in the presence of Z-VAD-FMK, signifying that RhPt triggers caspase-dependent death (Figure 4). This is consistent with studies of platinum cytotoxicity generally; it is well established that cisplatin and oxaliplatin typically trigger apoptosis. This result may, in part, explain the lack of cell-selectivity observed for RhPt. By initiating apoptosis, rather than necrosis, it is possible that the highly selective biological response to mismatch recognition by rhodium is overridden by the effects of high concentrations of platinum in the cell.

In this work, we examined the biological effects of conjugation of a DNA metalloinsertor with a platinum drug. In vitro, the complex successfully exhibits bifunctionality via dual DNA binding. In MMR-deficient cells, this strategy affords enhanced cellular uptake and potency over the individual subunits as well as versus traditional chemotherapeutics. However, RhPt is not without its limitations. The platinum subunit appears to dominate the cellular response, resulting in a loss of cell selectivity. Nevertheless, the biological analysis of RhPt provides insight into the behavior of bifunctional DNA targeting agents as well as a foundation for the design of future conjugates that are both potent and selective in their cellular targeting.

ASSOCIATED CONTENT

Supporting Information

Experimental procedures and supporting figures. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

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REFERENCES

(10) RhPt and Rh(Anam) each showed only one peak by HPLC; thus, all studies were carried out on the racemic complexes.

(14) No difference in platination levels was observed in DNA binding experiments with well-matched DNA.
(16) Adenine methylation is also observed with DMS treatment, but there is no evidence of platinum binding at these sites.
(17) At 50 μM RhPt, only one guanine is protected, possibly due to distortions to the DNA that impede the formation of 1,2-d(GpG) adducts.
(21) In fact, a significant decrease in viability is observed during combination treatment of RhPt and DPQ, although the reasons for this cooperative effect are unclear at present.
(22) By irreversibly binding to the active site of caspases, Z-VAD-FMK inhibits apoptosis. See: Vandenabeele, P.; Vanden Berge, T.; Festjens, N. Sci. STKE 2006, 358, pe44.