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A Potential Rhodium Cancer Therapy: Studies of a Cytotoxic Organorhodium (I) Complex that Binds DNA

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Abstract

Described is a novel organorhodium(I) complex that is cytotoxic to the colon cancer cell line HCT116 and alters cell migration, DNA replication, and DNA condensation. Most importantly, the mechanism observed is not seen for the parent organorhodium dimer complex $[(\text{RhCl}(\text{COD}))_2]$, RhCl_3 , or the free ligand/proligands (COD and 1-*n*-butyl-3-methylimidazolium chloride). Thus, the activity of this organorhodium complex is attributable to its unique structure.

Keywords

DNA binding; cisplatin; metastasis inhibition; anticancer; rhodium

The FDA approved metallodrug cisplatin¹ (Figure 1) is used to treat more than 50% of cancer patients, making it the most widely used oncological therapy.²⁻⁶ The side-effect profile and resistance development to cisplatin have driven the development of new metallodrug therapies. However, the development of other clinical metallo-drugs has been slow and only one is currently in clinical trials, the ruthenium based KP1019.⁷⁻¹² Other metals are in development as anticancer agents including promising reports of rhodium species.^{13,14,15}

Rhodium (III) complexes are the most highly researched organorhodium. The Barton group has led the way with Rh(III) metalloinsertors (Figure 1) that bind DNA mismatches, disrupt DNA synthesis, and have IC₅₀ values in the low micro-molar range. These rhodium complexes are more potent in cells unable to repair DNA mismatches, which makes them good candidates for cancer.¹⁶⁻¹⁸ Other investigations involving Rh(III) complexes include compounds that intercalate DNA via polypyridyl coligands. These Rh complexes have anti-topoisomerase II activity, and act as photo-inducible agents.^{14, 19-21,15}

Rhodium (II) complexes have demonstrated efficacy in Ehrlich ascites sarcoma and P 388 leukemia.^{22, 23} Very recently Rh(II) citrate was reported to prolong survival and reduce the

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Supporting information

Supporting information includes experimental details for biological assays and imaging experiments. This material is available free of charge at <http://pubs.bmcl.org>.

size of breast cancer carcinoma in mice when administered with maghemite nanoparticles.^{22, 23} There is little recent data on the biological activity of Rhodium (I) complexes. However, previous reports show that Rh(I) complexes have anticancer activity against Ehrlich ascites, leukemia L1210, sarcoma 180, and metastatic lung cancer.^{22, 24, 25}

Herein we report the mechanistic characterization of a potential lead structure, the organorhodium(I) complex; [RhCl(IBuMe)(COD)] (**1**) (IBuMe = 1-ⁿbutyl-3-methylimidazol 2-ylidene and COD = 1,8-cyclooctadiene) (Figure 1).^{26, 27} Preliminary data indicate that compound **1** is cytotoxic to HCT-116 colon cancer cell line, which led to profiling its mechanism of action. Mechanistic studies involved DNA binding, DNA synthesis, wound healing, and cell cycle assays. Compound **1** displayed a mechanism similar to other organometallic drugs, whereby **1** targets DNA, inhibits metastasis, prevents cell division, and reduces DNA condensation.

Cisplatin, **1** and several controls including the parent non-IBuMe coordinated dimer [{RhCl(COD)}₂], and RhCl₃ were run in cytotoxicity assays against HCT116 cells. The controls were used to evaluate if specific ligands were necessary for cytotoxicity. The IC₅₀ values (Table 1) for **1** and cisplatin after 72 hours are both in the low micro-molar range, whereas [{RhCl(COD)}₂] and RhCl₃ are too high to be measured. These data indicate that the cytotoxicity is unique to the complex as a whole. The IC₅₀ of compound **1** is much lower than previously reported values of >200 μM for Rh(I) complexes.²⁸

Given that all rhodium complexes previously reported effect cells by interacting with the DNA,¹³ we evaluated compound **1**'s ability to bind DNA. Cisplatin and **1** were incubated with pBR322 DNA, and the DNA binding patterns of **1** and controls were assessed (Figure 2A). The profile of compound **1**'s interaction with pBR322 DNA is different from that of cisplatin, and the non-toxic ligand RhCl₃. Treatment with increasing concentrations of cisplatin shows a slow decrease in supercoiled DNA, as well as a slight decrease in the separation of the bands. These data confirm that cisplatin induces conformational changes in the DNA. In contrast, treatment of DNA with increasing concentrations of **1** leads to a significant disappearance of supercoiled DNA and the appearance of nicked DNA. This result indicates that **1** uncoils and damages the DNA. In contrast, DNA is only minimally degraded when treated with RhCl₃. These data demonstrate that like cisplatin, and other organorhodium structures, compound **1** binds to DNA.

To fully assess compound **1**'s interaction with DNA, a BamHI digestion was completed. BamHI is a restriction endonuclease with a recognition site (G'GATCC) that hydrolyzes the phosphodiester bond between adjacent guanine sites.^{29, 30} The pBR322 DNA contains only one BamHI site. BamHI digestion of untreated pBR322 plasmid DNA converts the supercoiled and singly nicked circular DNA to a linear form. pBR322 DNA was treated as described above with cisplatin, **1**, and RhCl₃. (Figure 2B). The BamHI restriction digestion assays (Figure 2B) show that both cisplatin and **1** inhibit digestion by BamHI. These results indicate that cisplatin and **1** induce a DNA conformational change, inhibit the binding of BamHI to the DNA, and partially prevent DNA digestion. Cisplatin shows maximum inhibition at 10 μM compared to 40 μM for **1**.

Through this interaction with DNA, organorhodium complexes have been shown to disrupt DNA synthesis, and thereby halt cell division, acting as cytostatic drugs before causing cell death.¹⁷ We completed a BrdU incorporation assay in HCT116 cells to determine if **1** disrupts DNA synthesis. BrdU is a thymidine analogue that is incorporated into DNA of replicating cells.³¹ The relative amounts of BrdU uptake were then visualized by probing for BrdU with a Phycoerythrin (PE) conjugated antibody and the nucleus was counter-stained with Hoescht 33342.^{32, 33} Extensive BrdU uptake indicates cells are actively synthesizing

DNA, whereas minimal staining of BrdU shows that cells are not replicating their DNA. The decrease in BrdU fluorescence after treatment with **1** and cisplatin shows that both compounds halt DNA synthesis and have anti-proliferative effects on HCT116 cells (Figure 3A and B)¹⁷ These data are consistent with evidence reported by others using Rh(I) analogs.¹⁷

Rhodium(I) complexes have also demonstrated activity against metastatic cancers. Thus, we evaluated the ability of **1** and cisplatin to inhibit cell division and migration using wound-healing assays with HCT116 cells, a metastatic cancer cell line.^{13,34} Cells were seeded into wound creating culture-inserts, treated with **1** or cisplatin, and imaged every 30 minutes over 24 hours. Analysis of these time-lapse images showed that **1** inhibits wound closure (Figure 4, and Supplementary material S1). The wounds treated with **1** and cisplatin remained 59% and 70% open respectively, indicating that the cells were migrating and dividing at a significantly slower rate than that of the vehicle control (DMSO) after 24 hours. Nuclear staining of **1**-treated cells at this time point indicate that the reduced migration rate is not due to cell death. Along with the halt in DNA synthesis these data demonstrate that compound **1** is anti-proliferative and inhibits cell division and migration after 24 hours.

Since **1** halts DNA synthesis and causes a decrease in cell mobility, we used cell cycle phase analysis of synchronized cells to quantitate the number of cells in cell cycle phases. Employing propidium iodide (PI) as a stain to quantitate the number of cells located in the resting phase (i.e. G₀/G₁-phase) *versus* those preparing to divide (i.e. S-phase) we generated data on how compound **1** affects the cell cycle.³⁵ Organorhodium complexes have been reported to cause a cell cycle shift with a reduction in S-phase and an increase in G₁-phase.¹⁶ Synchronized HCT116 cells were left untreated, treated with 1% DMSO, 2 μ M of **1**, or 7.5 μ M cisplatin for 40 hours. Analysis using flow cytometry we observed treatment with either **1** or cisplatin results in a disproportionate shift of the cells into S-phase relative to the DMSO control (Supplementary material Figure S6).

Quantification of PI intensity indicates that **1** and cisplatin increase the number of PI-bright cells by 43% and 78% respectively, compared to 23% increase in DMSO treated cells (Figure 5A). These data suggest two possibilities: (i) that the cells are dividing, which is inconsistent with the wound healing and BrdU incorporation assays (Figures 2 and 4), or (ii) that cells treated with cisplatin or **1** incorporate more PI relative to the DMSO control. Since an increase in PI incorporation can serve as an estimate of the number of cells in S-phase or indicate a change in DNA condensation,³⁶ it is logical that treatment of DNA with binding agents such as cisplatin and **1**, which partially opens DNA, will result in higher PI fluorescence. This is supported by the observed total PI fluorescence for cisplatin (*ca.* 3 $\times$ DMSO) and **1** (*ca.* 2 $\times$ DMSO) in treated cells (Supplementary material Figure S6). Our data lead us to propose the uptake model illustrated in Figure 5B.

Taken together, our wound healing assays (Figure 4), DNA binding assays (Figure 2A), and cell division analysis (Figure 3) all support the hypothesis that treatment of HCT116 cells with **1** halt DNA replication by binding DNA and opening the double helix. In conclusion, we have identified a novel organorhodium(I) complex that: is cytotoxic to HCT116, has an IC₅₀ value much lower than other Rh(I) complexes, halts DNA replication, alters cell migration, and induces DNA condensation. Most importantly, the mechanism observed is unique to **1** and is not seen in studies with the parent organorhodium dimer complex [$\{\text{RhCl}(\text{COD})\}_2$], RhCl₃, or the free ligand/proligands (COD and 1-nbutyl-3-methylimidazolium chloride). The recent successes of Rh(II) and (III) organorhodiums as anticancer agents support exploration of Rh complexes. The data reported here are expected to provoke new interest in Rh(I) complexes as an potential oncogenic agents.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Abbreviations

DMSO	dimethyl sulfoxide
IC ₅₀	inhibitory concentration (50%)

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Figure 1. Structures of cisplatin, [Rh(HDPA)2-chrysi]3+ a lead Rh(III) anticancer molecule and compound 1.

Figure 2.

A) pBR322 DNA treated with compounds at following conditions: no treatment (control), 1.25 μ M, 2.5 μ M, 5 μ M, 10 μ M, 20 μ M, 40 μ M, 60 μ M, 80 μ M and 100 μ M. Intensity of the bands was measured by ImageJ software. **B)** pBR322 treated with compounds and digested with 10 units of BamHI at following conditions: untreated undigested (control), untreated BamHI digested (control) followed by 1.25 μ M, 2.5 μ M, 5 μ M, 10 μ M, 20 μ M, 40 μ M, 60 μ M, 80 μ M and 100 μ M.

Figure 3.

1 inhibits DNA replication. A) Fixed and permeabilized HCT116 cells were probed for BrdU with a PE conjugated antibody. The dramatic decrease in fluorescence demonstrates that **1** and cisplatin halt DNA replication. Hoescht 33342 acts to confirm the nuclear localization of BrdU. B) Cells were treated with 2 μ M cisplatin and 5 μ M **1**. The amount of BrdU incorporation was determined from 10 fields of view, counting all cells, and repeated in duplicate. Values are reported as DMSO representing 100% BrdU incorporation (*denotes $p < 0.0001$ when compared to DMSO using student t's test)

Figure 4.

Wound closure comparison of non-treated, cisplatin and **1** treated HCT 116 cells. Cells were seeded into wound creating culture-inserts (Ibidi). After 24 hours the inserts were removed. The cells were treated with the respective compounds (cisplatin 2 μ M, **1** 5 μ M) 1 hour before imaging, and then imaged every 30 minutes for 24 hours on a Nikon® Biostation IM. Composite images for specific time points were used to compare the impact of each compound on wound closure. (Supplementary material S3) The width of the wound was measured with ImageJ software. Thirty points along the wound were used to analyse wound closure, values reported here are mean $\pm$ SEM. (* denotes $p < 0.0001$ when compared to the corresponding time point for DMSO)

Figure 5.

A) Synchronized HCT-116 cells were treated for 40 hours under the following conditions: no treatment (control), 1% DMSO, 2 μ M **1**, or 7.5 μ M cisplatin. Cells were harvested and analyzed by flow cytometry, as described in supplementary material. The DNA content (PI fluorescence; y-axis) versus treatment (x-axis) are plotted. The number of PI bright cells in S phase are compared per treatment group. Results are shown for samples undergoing DMSO-treatment (black bars), cisplatin treatment (grey bars), and **1**-treatment (white bars). Error bars indicated standard deviation, and results are representative of four ($n = 4$) independent experiments. Statistical analysis was performed using the Student's t test. The “***” indicates $P < 0.0001$, “**” indicates $P < 0.001$, and “*” indicates $P < 0.04$. (B) Model system illustrating that the drug facilitates PI incorporation.

Table 1

Cytotoxicity of cisplatin, **1**, RhCl₃ and [{RhCl(COD)}₂] against the colon cancer cell line HCT116. Data represent results from four independent experiments, and the average IC₅₀ values are shown. Concentration curves are available in the supplementary material.

	Cisplatin	1	RhCl ₃	[(RhCl(COD)) ₂]
HCT116	1.5 μM ±0.5	3 μM ±1	>100 μM *	>100 μM *

* Highest concentration measured.