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Potential Applications of Vanadium-Based Anticancer Drugs for Intratumoral Injections †

John Monganaro[1], Aviva Levina[2] and Peter A. Lay [2]*, Debbie C. Crans[1,3]*

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[1] Department of Chemistry, Colorado State University, Fort Collins, CO 80523, USA; John.Manganaro@colostate.edu (J.M.)

[2] School of Chemistry, The University of Sydney, Sydney, NSW 2006, Australia; aviva.levina@sydney.edu.au (A.L.)

[3] Cellular and Molecular Biology, Colorado State University, Fort Collins, CO 80523, USA

* Correspondence: peter.lay@sydney.edu.au (P.A.L.); Debbie.Crans@ColoState.EDU (D.C.C.);

† Presented at the title, place, and date.

Abstract: Administration of highly cytotoxic or immunomodulating drugs directly into a tumor is a method used in the clinic for late stages of cancers and in clinical trials for platinum-based drugs. A hydrophobic non-innocent Schiff base V(V) complex with a sterically hindered catecholate ligand was taken up rapidly into cancer cells and caused cell death. The synthesis was non-trivial at large scales and high purities. This class of complexes is sufficiently stable to survive briefly under physiological conditions before hydrolysis and/or redox reactions. Degradation reactions occur very rapidly for complexes with less sterically hindered catacholates.

Keywords: vanadium complexes; non-innocent; Schiff base; synthesis; properties; anticancer compounds.

1. Introduction

The first chemotherapeutic metal-complex, cisplatin, was approved by the FDA in 1978 and since that time, other cytotoxic metal-based chemotherapeutics have been approved, are in clinical trials, or under development [1-3], Figure 1. A major objective in the development of effective chemotherapeutics is increasing efficacy while reducing toxicity to healthy tissue to acceptable levels. After the approval of cisplatin, early investigations turned to the development of other metal-based drugs, including ruthenium and titanium based drugs. However, these drugs were cancelled during clinical trials due to nephrotoxicity, which limited dosage to ineffective levels [3]. One strategy to reduce toxicity relies on designing metal-complexes that degrade after intratumoral injection and before encountering healthy tissue.

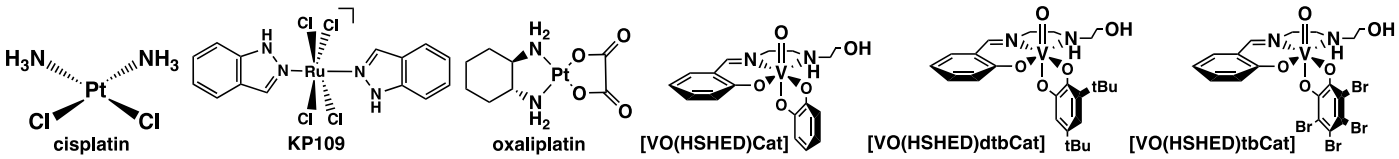

Figure 1. Anticancer metal-complexes used in the clinic, or under investigation [1-4].

A major obstacle facing chemotherapeutic metal-complexes is effective movement across cell membranes. It is well established that the cell membrane is distinctly hydrophobic and interacts favorably with hydrophobic drugs. Hydrophobic non-innocent vanadium(V) complexes are one example of such a class of potential drugs that may be suitable for intratumoral injection due to their rapid uptake [5,6]. These vanadium(V) complexes are designed to break down before diffusion or transport outside the tumor and into contact with healthy tissue. The breakdown products of these potential drugs are inoffensive to the human body and may have beneficial properties. In this way such a compound acts to support healthy tissue during an otherwise toxic event. Currently,

one intratumoral pharmaceutical, an oncolytic virus (T-VEC), is in clinical use for melanoma which cannot be surgically removed. Furthermore, seven platinum-based anticancer preparations are in clinical trials.

2. Synthesis and properties of hydrophobic non-innocent vanadium(V) complexes

Metal-complexes containing redox active ligands are named non-innocent metal complexes and are widely used in catalytic reactions, sensors in biological systems, drug delivery, and water remediation [7]. The term "non-innocent metal complex" refers to a complex where, when a redox reaction has occurred, it is not certain if the resulting charge is localized on the metal ion, or on the coordinated ligand. Our groups first became interested in these compounds after observing their unusual electronic, and later cytotoxic, properties. With that in mind, we continued our investigations into the biological activities of these compounds, and have since synthesized, and tested, a number of these derivatives, Figure 1 and Figure 2.

Among the vanadium(V) complexes we have synthesized are [VO(HSHED)cat], and the more sterically hindered derivative [VO(HSHED)dtb]. Regardless of the exact steric, electronic, or structural nature of a [VO(HSHED)cat] derivative, the synthetic scheme of these vanadium(V) complexes begins with the condensation product of the Schiff base ligand from 2-((aminomethyl)amino)ethan-1-ol and salicaldehyde under an inert atmosphere, Figure 2. Then, an aqueous solution of vanadyl sulfate was added to the Schiff base scaffold, followed by aqueous sodium hydroxide to give solid [VO_2(HSHED)]. To an acetone solution of this solid, catechol was added to give a purple crystalline powder, Figure 2 [8].

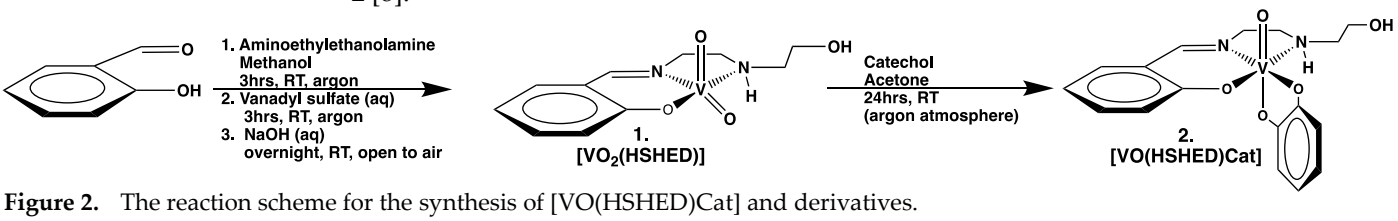

Figure 2. The reaction scheme for the synthesis of [VO(HSHED)Cat] and derivatives.

Due to the profound effect of the catechol ligands upon the electronic properties of these compounds, these reactions and their corresponding products are quite distinctive and can be monitored by both ^{51}V NMR spectroscopy and UV-vis spectrophotometry. Both methods are significantly influenced by the electronic properties of the vanadium(V) as they reflect the size of the HOMO-LUMO gap, Figure 3. For instance, when the catechol substituent is decorated with electron-donating functional groups, the HOMO-LUMO gap is lowered and the ^{51}V NMR is pushed downfield.

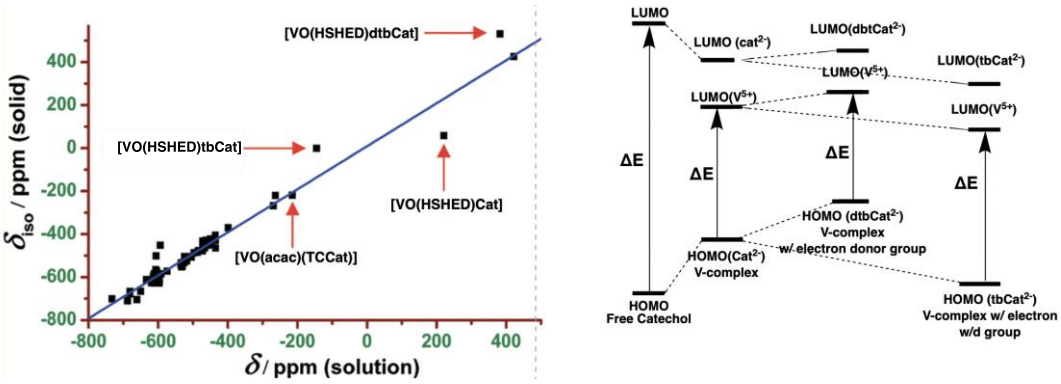

Figure 3. A qualitative molecular orbital diagram demonstrating the impact of different ligands in free and vanadium(V) coordinated catechol. Adopted with permission from Ref. [9].

When functionalized with electron-withdrawing functional groups, the HOMO-LUMO gap is widened and the ^{51}V NMR signal moves upfield. Similarly, the color of this class of compounds varies from the bright yellow vanadium scaffold to a non-innocent vanadium-complex that ranges from dark purple to green. Furthermore, the HOMO-

LUMO gap impacts redox reactions of these complexes [9]. While [VO(HSHED)cat] is a cytotoxic complex, it also rapidly hydrolyzes. With the substitution of two *t*-butyl (dtb) functional groups onto the catechol ligand, [VO(HSHED)(dtbCat)] demonstrated significantly prolonged lifetime in both water and cell culture medium as compared to [VO(HSHED)Cat]. Functionalization of catechol with electron donating di-*tert*-butyl groups raises both HOMO and LUMO, with a net decrease in HOMO-LUMO gap. In contrast, the tetra-brominated analogue, [VO(HSHED)tbCat], decreases both HOMO and LUMO, with a net increase in HOMO-LUMO gap, Figure 3.

3. Biological studies

To investigate if there is a relationship between hydrophobicity and V(V) complex cytotoxicity, we conducted experiments with [VO(HSHED)Cat] and [VO(HSHED)dtbCat] in a simple monolayer model membrane system. Using microemulsions (reverse micelles), we generated a self-assembled system containing H_2O/dioctyl sulfosuccinate sodium salt/organic solvent, where the organic solvent was either *iso*-octane or cyclohexane [10]. Using ^{51}V NMR and UV-vis spectroscopies to monitor the complex stability, we demonstrated that the more hydrophobic and sterically hindered [VO(HSHED)dtbCat] was stabilized by this monolayer and was slower to hydrolyze than the parent [VO(HSHED)Cat] complex. The greater cellular penetration of [VO(HSHED)dtbCat] would favor more potent anticancer properties and this was observed for human bone cancer cells (SW1353) [10].

Cellular experiments were designed to cross-test several cancer cell lines against intact and hydrolyzed complex. Fresh [VO(HSHED)dtbCat] was tested alongside its breakdown products (aged [VO(HSHED)dtbCat]). Also tested were fresh and aged cisplatin, as reference compounds. These substances were all tested against cancerous brain (T98g cells), breast (MDA-MB-231), pancreatic (PANC-1), lung (A549), and normal connective (HFF-1) tissues. In all cell lines, fresh [VO(HSHED)dtbCat] had the lowest IC_{50} (highest activity) of the substances tested. Furthermore, in T98g cells [VO(HSHED)dtbCat] was an order of magnitude more cytotoxic than its breakdown products or cisplatin, Figure 4 [6].

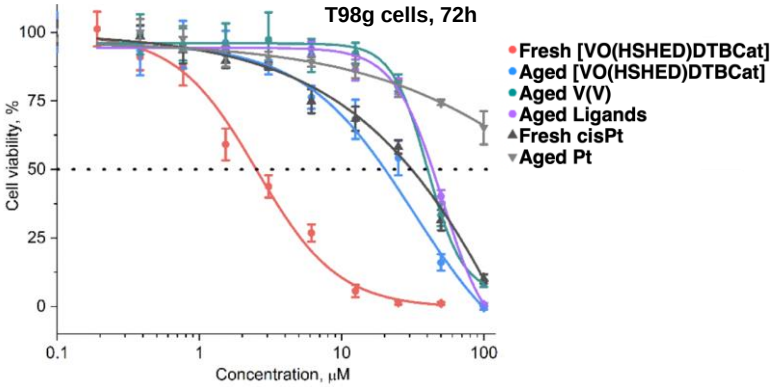

Figure 4. Dose-response curves of fresh [VO(HSHED)dtbCat], aged [VO(HSHED)dtbCat], fresh cisplatin, and aged cisplatin in T98g cells (72 h treatments). "Fresh" solutions were added to cell culture medium less than 60s prior to cell treatment. "Aged" solutions were incubated in cell culture medium under a 5% CO_2 atmosphere for 24 hrs at 310K before addition to cells. Adopted with permission from Ref. [6].

The IC_{50} values of [VO(HSHED)dtbCat], fresh (intact) and aged (decomposed), showed promise as a candidate for intratumoral injection into brain cancers. Fresh [VO(HSHED)dtbCat] may be more effective at killing cancerous cells than cisplatin, however, the decomposition products were comparatively safe and harmless. In fact, some of these products have demonstrated neuroprotective and neurostimulating properties and may serve to support healthy tissue through both cancer and chemotherapy, however further studies are needed to understand how complex reactivity, degradation, and speciation within a biological system affects activity [11-13].

4. Conclusions and future directions

We describe [VO(HSHED)(dtbCat)] as a potential intratumoral drug, which has the required rapid cellular uptake, enhanced reactivity, and non-toxic decomposition products. While some hydrophobic, sterically hindered vanadium(V) complexes perform quite well in *in vitro* experiments against cancer cell lines, it remains unclear if complexes with even greater still degrees of hydrophobicity and steric-hindrance will perform proportionally better. We are currently testing other complexes which we have designed with these properties. We have suggested that not only these non-innocent vanadium complexes, but also other complexes that have been investigated in clinical trials but abandoned because they did not exhibit sufficient stability to be administered by conventional methods could be excellent candidates for intratumoral administration [2,10].

Author Contributions: Conceptualization, D.C.C. and P.A.L.; writing—original draft preparation, J.M. and D.C.C.; writing—review and editing, J.M.; A.L.; P.A.L.; D.C.C. All authors have read and agreed to the published version of the manuscript.

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