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Panchromatic Osmium Complexes for Photodynamic Therapy: Solutions to Existing Problems and New Questions

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Abstract

This article is a highlight of the paper by Lazic *et al.* in this issue of *Photochemistry and Photobiology*. It describes the validation of osmium coordination complexes as photosensitizers for photodynamic therapy, with very promising *in vivo* results that demonstrate radical improvements in survival following irradiation with visible (635 nm) or near-IR (NIR; 808 nm) light. An unusual feature in the study is that the different complexes exhibit disparate photophysical and photobiological characteristics, despite sharing common structural motifs. These findings raise hopes for the development of novel photosensitizers that overcome the limitations of current commercially available systems for PDT, but also raise questions regarding the most efficacious biological mechanisms of action for this treatment modality.

COMMENTARY

Photodynamic Therapy (PDT) is a treatment approach for localized cancer, and offers advantages that include low systemic side effects and reduced pain and scarring compared to surgical excision. While it is often considered primarily an option for superficial lesions, the use of fiber optics have allowed for treatment of esophageal, endobronchial, and lung cancer, and recent clinical trials demonstrated safety and promise for intratumoral irradiation in pancreatic cancer.(1) There is increasing evidence that PDT can also induce an immunological response,(2, 3) which would greatly enhance the potential for treatment of metastatic disease. There is a persistent impression, however, that PDT has not lived up to its promise, which is due to a combination of factors that include the complexity of multivariable optimization for the treatment plan (photosensitizer choice, photosensitizer dose, time delay before light treatment, and light treatment features, including wavelength, light dose, intensity, pulsed vs. continuous light source etc.).(4) Basic research efforts have generated incremental improvements in photosensitizers and treatment plans, but the field has experienced potentially more significant gains in the last few years with the introduction of inorganic coordination compounds that provide alternative mechanisms of action and modular features that can be adjusted systematically to optimize photophysical, photochemical, and photobiological responses. This highlight article of the paper by Lazic *et al.* in this issue of *Photochemistry and Photobiology*(5) describes new osmium-based coordination complexes (Figure 1) that overcome several key challenges in photosensitizer

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development: they can be synthesized via a modular strategy in two steps, absorb light over the range of the ultra-violet to the near-IR region, do not require oxygen for cytotoxic activity, and exhibit promising effects in an *in vivo* model.

SYNTHESIS

McFarland and Lilge have previously collaborated with Theralase Inc. to develop ruthenium-based photosensitizers, and the remarkable success of those efforts have culminated in an on-going clinical trial for bladder cancer.(6) The paper in this issue represents a continuation of their conceptual approach to develop enhanced photosensitizers for PDT, but these next-generation compounds present several key advantages. Similar to other coordination complexes, the synthesis is modular, and compounds can be constructed in only two steps. The molecules are three dimensional octahedral structures and chiral at the metal center (the compounds were synthesized and studied as a mixture of the Δ and Λ enantiomers). A key difference, however, is the use of osmium. In contrast to ruthenium complexes, which have been investigated for photobiological applications for decades, osmium has been comparatively ignored.(7, 8) This is due to long-standing concerns about the toxicity of the metal center, and the suboptimal photophysical properties of Os(II) coordination complexes: their short excited state lifetimes have been presumed to reduce their potential utility as photosensitizers. It turns out that both concerns do not always hold true.

In this work, McFarland and co-workers used a rational design approach to capitalize on the osmium's advantageous characteristics in order to obtain panchromatic absorbers. The π -extended biq ligand was chosen as it is known to facilitate red-shifted absorption when combined with metal centers (due to a lower-energy metal to ligand charge transfer (MLCT) absorption), while Os is known to exhibit formally forbidden singlet-triplet transitions to the MLCT, which also occur at lower energy. As anticipated, the complexes were black absorbers, with extinction coefficients of $2,000\text{--}3,000\text{ M}^{-1}\text{ cm}^{-1}$ in the 700–900 nm PDT “therapeutic window”, and all exhibited good photostability. Both features are in contrast to the properties of analogous Ru complexes such as TDL1433(9) and Ru(biq)₂phen, shown in Figure 1; systems without biq ligands generally require activation in the blue-green region, and compounds containing biq ligands exhibit red and NIR absorption, but are not photostable.(10, 11) This instability is not always unfavorable, as this property may be exploited to develop compounds that are cytotoxic in the therapeutic window (using red or NIR light with wavelengths longer than 650 nm) through formation of covalent ruthenium-DNA adducts.(12)) Most remarkably, the three Os compounds were synthesized in > 95% yield and did not require purification, so construction of large libraries of similar systems should be synthetically accessible and low in cost, in terms of the number of synthetic steps and processing required.

BIOLOGICAL AND PHOTOBIOLOGICAL PROPERTIES

Biological evaluation included *in vitro* studies in various cancer cell lines and *in vivo* experiments in a xenograft model using Balb/C immunocompetent mice implanted with CT26.WT colon cancer cells. While the *in vitro* results were promising, they were

overshadowed by the *in vivo* studies, where significant extension in survival was found with TLD1829. The compound was dosed via intratumoral injection at half the MTD; only a single treatment was performed. More than 50 and 75% survival was observed past 40 days for treatment with NIR (808 nm; 600 J cm⁻²) and red light (635 nm, 266 J cm⁻²); by comparison, all animals had succumbed by day 20 for the light-only control.

In addition to presenting a new class of compounds for PDT, the biological results in this paper both dispel misconceptions and raise a number of important questions for those in the field of medicinal inorganic chemistry and phototherapy. First, as the authors note, this work directly contradicts the long-standing belief that osmium complexes are intrinsically toxic and thus not of interest in medicinal chemistry. It is essential to move beyond these biases, as classes of compounds (and heavy metals) have not been pursued for years due to concerns about possible toxicity. Second, while TLD1824 had a respectable MTD of 47 mg kg⁻¹, TLD1822 was 37-fold more toxic, demonstrating that seemingly small modifications in compound structures can have very significant ramifications for systemic toxicity. Third, modification of one of the three ligands in the complex can alter the location of the excited state (from the biq ligand in TLD1822 and TLD1829 to the dppn ligand in TLD1824) and change the possible mechanism of action (as discussed below) without altering the absorption profile. Thus, this modular approach shows that significant modulation of biological and photobiological characteristics can be achieved in closely related molecules.

MECHANISM OF ACTION

Organic photosensitizers for PDT generally rely on the photocatalytic generation of singlet oxygen (¹O₂) through so-called type II (energy transfer) photoreactions, but metal complexes are also capable of electron transfer (type I) photoreactions. In the case of TLD1433,(9) extreme sensitivity to quenching by molecular oxygen and type I/II switching have been identified as likely causes for its large phototherapeutic indices; this molecule is highly photo-cytotoxic even under very low O₂ tensions, where typical PDT photosensitizers are ineffective. McFarland has coined the term “metal-organic dyads” to describe photosensitizers for PDT that combine a metal center that facilitates intersystem crossing (leading to long-lived excited states) with π -expansive ligands that provide low lying ³ $\pi\pi^*$ states that can equilibrate with metal to ligand charge transfer (³MLCT) states. This has proven to be a very fruitful approach for the generation of effective photosensitizers.

In order to elucidate the mechanism of action of the osmium dyads, photophysical analysis was undertaken. These studies revealed that TLD1822 and TLD1829 are poor ¹O₂ sensitizers ($\Phi = 0.04$), and no singlet oxygen was detected at all with TLD1824. This is interesting in light of the fact that all compounds possess excited states with more than sufficient energy to sensitize ¹O₂ (94 kJ/mol, or 7,886 cm⁻¹). Nonetheless, the photobiological activity of the compounds must be due to some oxygen-independent pathway, whether through other reactive oxygen species (ROS) or electron transfer reactions remains to be determined. Indeed, it appears that O₂ can have a deleterious effect, depending on compound structure. TLD1822 exhibited significant toxicity in the dark under normoxic conditions, and was *less* cytotoxic upon light activation; under hypoxic conditions the compound became non-toxic in the dark and potent when irradiated. The mechanistic basis

for this switch in biological behavior as a function of O₂ levels was not detailed and may not be known, but it is tantalizing to consider that it might be related to the compound's significant toxicity *in vivo*.

The mechanistic studies provided additional important ramifications. They demonstrate that modification of one of the three ligands in the complex can change the nature of the excited state, and thus, the likely mechanism of action. Moreover, it appears that environmental features and the excitation wavelength can alter coupling between the initial excited state and the excited state responsible for the photobiological effects. It is also clear from this work that photoreactions that are *not* type II can be highly efficacious *in vivo*. While the active species responsible for the photobiological effects are not defined, it is highly unlikely that ¹O₂ plays a significant role. Thus, there are other reactive species or electron transfer processes that can be exploited for oxygen-independent PDT. While the immunological component to the *in vivo* response was not reported as a part of this study, Theralase has reported antitumor immunity, which they refer to as an “anticancer memory response”, as being a characteristic of the photosensitizers developed by McFarland.(13) It remains to be seen if the excellent *in vivo* results are due to activation of an immune response, but if this is the case, the treatment approach would likely work on disseminated tumors.

CONCLUSIONS

Lazic et al. demonstrate that focusing on efficacy, rather than using a biological target focused approach, can result in identification of highly promising compounds; mechanistic studies will of course follow. One of the greatest implications of this work is that it serves as an example of the remarkable progress that can be made when academic and industrial groups collaborate in an underexplored area. The combination of the chemical and photophysical expertise, provided by McFarland, the medical biophysics expertise of Lilge, along with the clinical light-based medical devices developed by the scientists at Theralase Inc. has resulted in one compound in the clinic and more in the pipeline. It also serves as reminder that conventional wisdom can be misleading, and that there is no replacement for chemical intuition and experimental evaluation.

Acknowledgments

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Figure 1. Structures of the compounds discussed in this highlight and the full spectrum that can be used to activate the new osmium-based “panchromatic” compounds.