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Texaphyrins: Tumor Localizing Redox Active Expanded Porphyrins

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Abstract

Texaphyrins, a class of tumor selective expanded porphyrins capable of coordinating large metals, have been found to act as redox mediators within biological systems. This review summarizes studies involving their experimental use in cancer chemotherapy. Mechanistic insights involving their presumed mode of action are also described, as well as certain structure activity relationships. Finally, newer texaphyrin-based applications associated with targeted drug delivery are presented.

Keywords

Texaphyrin; reactive oxygen species; anticancer activity; mechanism of action; zinc; platinum delivery; alpha and beta core emitters

INTRODUCTION

The regulation of reactive oxygen species (ROS) within biological systems plays a vital role in the health and longevity of the organism. For example, the serendipitous discovery by McCord and Fridovich [1] of superoxide dismutase (SOD), a major enzyme responsible for the regulation of ROS, helped pave the way for developing the discipline of free radical biology [2]. Herein, we discuss our approach to cancer chemotherapy through alteration of the redox environment within cancer cells. Our research has focused on a class of expanded porphyrins called texaphyrins. The foremost of these, motexafin gadolinium (MGd) and motexafin lutetium (MLu) (Fig. 1), were selected for clinical development in the context of radiation therapy and photodynamic therapy, respectively, by Pharmacyclics, Inc [3, 4]. This work has provided us with insights into the cellular antioxidant system and inspired the discovery and investigation of new molecules capable of ROS generation within cancer [5, 6].

REDOX ACTIVE DRUGS

The regulation and control of ROS is crucially important in many biological processes. While such ROS generation can be beneficial when effected in a cancer-selective manner, it is interesting to note that the over-production of ROS is implicated in a variety of inflammatory responses and diseases, including amyotrophic lateral sclerosis (ALS), Parkinson's Disease (PD), and Alzheimer's Disease (AD) [7–9]. The majority of redox active drug candidates are designed to decompose ROS and related species, including superoxide, hydrogen peroxide, and peroxynitrite. These systems generally employ a redox active transition metal catalyst and are often biologically inspired by metalloenzymes such as SOD and catalase.

Groves and co-workers have studied the use of both manganese and iron metalloporphyrins for the decomposition of peroxynitrite under physiological conditions [10–13]. These systems rely on oxidized (Fe^{III} or Mn^{III}) species that react with ONOO^- to form initially high-valent (Fe^{IV} or Mn^{IV}) oxo-intermediates. As shown in a collaboration between Sessler and Groves, Mn(II)-texaphyrins also proved to be effective peroxynitrite decomposition catalysts in the presence of ascorbate [14]. More recently, Crow and co-workers have demonstrated that a Mn(II)-texaphyrin complex that is analogous to MGd, except for the central metal and the overall complex charge, extends the lifespan of transgenic mice which over-express the G93A human Cu, Zn superoxide dismutase (SOD1) protein (a murine model for ALS) [15]. Gratifyingly, this texaphyrin complex proved to be as efficacious as the very best of the many porphyrin systems tested to date by Crow [15–18].

There is great interest in using manganese complexes for decomposition of ROS. For instance, Doctrow and colleagues have developed Mn-salen complexes that act as mimics of SOD, cata-lase, and peroxidase; these chemical entities show promising activity *in vivo* as supplemental antioxidants [8, 19–25] and are currently being developed by Eukarion, Inc. as potential treatments of neu-rodegenerative events such as stroke, AD, and PD. Many other small molecule SOD mimics have been developed [26]. These include Mn(III) and Fe(III) metalloporphyrins, [27–29] Cu(II) complexes, [30–32] nitroxides, [33–35] and other Fe (II/III) and Mn(II) macrocycles [36]. Riley and co-workers have developed this latter class of Fe/Mn macrocyclic complexes to the point of ongoing clinical trials [7, 9, 36]. Fridovich, Batinic-Haberle and co-workers have recently developed Mn-porphyrins as SOD-mimics [37–44] and decomposition catalysts for hydrogen peroxide, [45, 46] per-oxynitrite, [47] and carbonate radical anion [47].

Several classes of anticancer drugs, including quinone-based agents, [48] are believed to promote the generation of ROS at tumor sites *via* a redox cycling mechanism. Bioreductive drugs may also rely on *in vivo* reduction, by biological reductants such as NADPH, to produce reactive species that damage DNA. For instance, bioreductive activation of mitomycin C by one-electron reduction, can lead to the generation of ROS as well as alkylation of DNA (through opening of the aziridine ring) [49]. Other quinone antibiotics are known to form semiquinones or hydroquinones as the result of bioreduction [50]. Depending on the position and degree of cationic functionality, these bind to, and are hence capable of reacting directly with, DNA.

Natural products have been used in combination chemotherapy to treat cancers as diverse as lymphomas, Kaposi's sarcoma, cervical cancer, and squamous cell carcinoma of the head and neck. Among these, bleomycins represent a class of anticancer agents whose mode of action is believed to involve redox chemistry. This class of natural products is thought to derive its cytotoxicity from hydroxyl radical mediated cleavage of nucleic acids. However,

the clinical usage of bleomycins has been limited, most notably due to pulmonary toxicity [51].

Biological systems have a host of natural defenses to ROS, including enzymes such as SOD, glutathione peroxidase, and catalase. Additional cofactors, such as glutathione (GSH), thioredoxin (TRX)/thioredoxin reductase (TRXR), ascorbate (vitamin C), and α -tocopherol (vitamin E), are able to serve as ROS scavengers. Many strategies to enhance the efficacy of radiation therapy involve “shutting off these defense mechanisms. For instance, L-buthionine-(S,R)-sulfoximine (BSO) acts as a selective inhibitor of GSH synthesis [52, 53] and has been found to potentiate the effects of MGd, [54] thus providing important support for its proposed mode of action. TRX is further believed to be responsible for mediating cellular response to environmental stress, including radiation therapy [55]. Inhibition of TRX/TRXR is thus an attractive strategy for patients undergoing radiation therapy. Early work to target TRX involved the use of alkyl 2-imidazolyl disulfides, which thioalkylate critical cysteine residues on TRX, thereby rendering it biologically inactive [56]. More recently, rationally designed compounds, such as the cyclodextrin-derived diorganyl tellurides, have been developed as inhibitors of thioredoxin reductase and have shown inhibition of cancer cell growth [57].

STRUCTURE ACTIVITY RELATIONSHIPS OF TEXAPHYRINS: EFFECT OF REDOX POTENTIAL

One of the most important “lessons” learned is that MGd mediates its observed radiation sensitizing function, at least in part, by acting as a redox mediator [54]. This mechanistic rationale, summarized in Fig. (2), relies on the fact that MGd readily accepts an electron from an endogenous electron rich species (also referred to as reducing metabolites), such as ascorbate, NAD(P)H, or glutathione (GSH). This produces a reduced texaphyrin radical that reacts with oxygen to produce superoxide in a rapid equilibrium process which in turn regenerates MGd. *In vitro*, and presumably *in vivo*, this superoxide is converted quickly into hydrogen peroxide, a species that is known to be a potent apoptosis trigger. (The rate constant for non-enzymatic disproportionation of superoxide is estimated to be $1 \times 10^5 \text{ M}^{-1} \text{ s}^{-1}$ at pH 7.4 [58]).

Texaphyrins have also been shown to localize specifically to tumors [6]. *In vitro*, MGd uptake was found to be dependent on medium supplementation with serum protein and temperature [59, 60]. Tumor uptake and enhancement of MGd has been well documented with MRI signal enhancement, [61] fluorescence bioimaging, [62] and radiolabeling [63] *in vivo* with persistent uptake in the tumor relative to blood and neighboring tissue.

Because MGd, in contrast to other redox active agents, localizes in tumors, the net effect of this catalytic (in MGd) process is (1) the site selective production of ROS and enhancement of cancer cell death and (2) a corresponding decrease in the concentration of reducing metabolites, such as ascorbate and glutathione, that under normal circumstances serve to mitigate the effects of oxidative stress and ionizing radiation. Consistent with this rationale was the observation that the addition of BSO, antimycins A, or diamide, compounds involved in inhibiting glutathione metabolism or forming reactive oxygen species, enhanced the effects of MGd.

We see the mechanism presented in Fig. (2) as the beginning, rather than the end, of our structure-function based understanding of MGd. Indeed, as described below much of the work carried out over the last few years has been designed to extend and refine this working hypothesis. For instance, it provided a rationale for why MGd could prove active under certain *in vitro* conditions but not others (a realistic concentration of reducing metabolites is

required for activity, as independently established by Rockwell [64]) [65]. This mechanism also led to the inference that MGd should (1) show activity in the absence of ionizing radiation and (2) prove particularly useful in treating cancers characterized by a high level of oxidative stress. Such considerations, supported by follow-up preclinical work, led to further clinical testing of MGd, both in conjunction with a range of other cancer drugs or, in the case of chronic lymphocytic leukemia (CLL) [66] (a disease particularly susceptible to oxidative stress) and non-small cell lung cancer (NSCLC), as a single agent.

As implied above, much of our effort has been devoted to extending and refining our mechanistic understanding of MGd. For instance, we now know that replacing the central Gd(III) by a heavier (and smaller) lanthanide(III) cation facilitates reduction of the texaphyrin (ligand, not metal) and makes the electron transfer process more favorable in a thermodynamic sense (for instance, $E_{1/2} = -239$ vs. -294 mV for the first one-electron reduction of MLu and MGd, respectively, vs. Ag/AgCl in DMSO) [67]. However, depending on the nature of the biological reductant in question, the kinetics of the reaction can be slowed. In the case of ascorbate, where this process was studied in some detail, this disparity between kinetics and thermodynamics was rationalized in structural terms [68]. In particular, it was proposed that the larger lanthanide(III) cations, including Gd(III), which resides further above of the texaphyrin plane than, e.g., Lu(III) as in **2** (MLu), allow for better binding interactions between the anionic reductant and the positively charged metal center, thus facilitating inner sphere electron transfer. Unfortunately, MLu, in contrast to MGd, is aggregated in buffer and likely bound with greater affinity to serum proteins *in vitro* and *in vivo*, thus complicating interpretations based solely on structural characteristics.

A second effect of cation-anion binding interactions was also noted, namely that the products of the reaction between MGd, ascorbate, and air form an oxalate bridged coordination polymer in saline buffer [69]. This polymer, characterized by Gd-oxalate-Gd bridging interactions, was found to be taken up into A549 human lung cancer cells more rapidly than MGd and led to greater oxidative stress. This raises the intriguing possibility, as yet not pursued, that these or related polymers could provide the basis for new kinds of formulations that might improve further the efficacy of the compound.

The potential utility of the reactions with ascorbate as a possible indicator of biological function provided an incentive for us to study whether other metallotexaphyrins besides those containing lanthanide(III) cations could function as oxidation catalysts for this key reducing metabolite. Accordingly, several transition metal complexes of texaphyrin were prepared (cf. Scheme 1) and characterized including the water-soluble Mn(II) (**3**), Co(II) (**4**), and μ -oxo Fe(III) dimer (**5**) analogues of MGd (referred to as Mn-TeX, Co-TeX, and Fe-TeX, respectively) [70]. Again, the effect of the centrally coordinated metal center was found to be substantial. While the Mn(II) complex (**3**) displayed an initial rate that was ca. 3 times slower than MGd under identical experimental conditions ($V_o = 3.0$ (μ M/min vs. 8.7 (μ M/min, respectively), the Co(II) (**4**) and μ -oxo Fe(III) dimer (**5**) complexes gave initial rate values ($V_o = 23.8$ and 30.6 μ M, respectively) that were substantially larger [70]. This proved true in spite of the fact that these species are *harder* to reduce than MGd ($E_{1/2} = -571$ for Co-TeX vs. -294 for MGd; vs. Ag/AgCl in DMSO) [67]. In this instance, it is thought that the redox active metal centers participate in ascorbate decomposition. Unfortunately, the Co(II) complex (**4**) and the Fe(III) complex (**5**) are too lipophilic to be attractive in terms of further drug development, at least for the indications for which MGd is being tested. (Note: Fe-TeX (**5**) exists as a monomeric species and in equilibrium with a μ -oxo dimeric species at neutral pH.) On the other hand, the Mn(II) texaphyrin complex (**3**) has taken on a “life of its own” as a potential ALS treatment and has been studied carefully in this regard by Crow, as noted above.

The zinc complex of texaphyrin was prepared and found to contain a highly unusual η^3 -texaphyrin-metal center coordination mode [70]. Unfortunately, preliminary biological studies revealed that this latter complex is unstable in cell culture medium. On the other hand, the lability of this metal center led us to suggest that this complex could serve as a precursor for the generation of other more stable metal systems, an idea that was subsequently developed by researchers at Pharmacyclics using the corresponding calcium texaphyrin derivative [71]. Meanwhile, in an effort to achieve this same goal, and because it represented an important “missing link”, we worked to prepare the oxidized, metal-free form of texaphyrin (**6**); we succeeded in doing so by subjecting the starting “sp³ form” of an organic soluble texaphyrin to oxidation using ferrocenium as the oxidant, as shown in Scheme 1 [72]. While relatively unstable in its unprotonated form, this product did allow for the formation of certain metal complexes, including a Mn(II) derivative.

Another way of adjusting the redox potential of the texaphyrin ligand involves modifying the π -system. While there are a number of ways this could be done, one that looked particularly promising involves replacing the *o*-phenylenediamine moiety by the corresponding diamionaphthalene (cf. Fig. 3). Doing so leads to a substantial enhancement of *in vitro* activity relative to MGd [73]. This result is rationalized in terms of a change in the redox properties of the system. Specifically, the “exchange” of naphthyl for phenyl leads to a ca. 70 mV shift in the reduction potential in favor of initial electron transfer to the texaphyrin (−122 mV, −130, and −329 mV vs. SCE in DMF in the case of **7**, **8**, and MGd, respectively). Assuming this latter rationale is correct, it serves to justify the synthesis of other electronically modified texaphyrins. In a more general sense, these findings are important because they show that by modifying the texaphyrin skeleton appropriately it is indeed possible to prepare new systems that are more active than MGd, at least as judged from initial tests involving well-validated *in vitro* model systems (A549 human lung cancer; Fig. 3).

In the case of MGd, substantial effort has been devoted to exploring its reactions with reducing metabolites other than ascorbate. Although such analyses are far from complete, we have found that dithiothreitol (DTT), a model substrate containing two thiols in close proximity (a so-called “vicinal thiol”), reacts particularly quickly with MGd (cf. Fig. 4) [68, 74]. Presumably, this reflects the fact that the ultimate product of thiol oxidation is (generally) a disulfide and that eliminating the need for a bimolecular process is beneficial. In accord with such thinking, it was found that thioredoxin reductase provides a particularly favorable substrate for MGd [68, 72]. This enzyme is characterized by a thiol-selenothiol “active site” and serves as a general purpose cellular reductant [75]. Its deactivation *in vivo* is thus expected to play a key role in mediating MGd-induced oxidative stress [76]. We have also found that other enzymes, including cytochrome c reductase and cytochrome P450 reductase, may be effectively substituted for thioredoxin reductase, again leading to the concurrent formation of reactive oxygen species and oxidation of the enzyme cofactor, NADPH. These and similar studies led us to propose a probable role for MGd in inhibiting the thioredoxin reductase-derived antioxidant system, as distinguished from the glutathione-dependent system (see Fig. 5 for a schematic overview).

Another class of thiol rich proteins whose oxidation may play a critical role in mediating the effects of MGd, are the metallothioneins [77]. Metallothioneins (strictly speaking thioneins) possess multiple cysteine-rich sites that can bind metal cations. They are believed to be a major site of intracellular zinc storage and transport [78, 79] and, as such, play a key role in maintaining zinc homeostasis. Metallothionein oxidation, in the context of MGd reduction and catalytic ROS production, would thus liberate free (or, more precisely, chelatable) zinc in a similar fashion to the effect of MGd on thioredoxin reductase described above. The presence of this free zinc, in turn, would trigger biochemical responses designed to mitigate

the effects of excess zinc, including the expression of thionein and an increased production of zinc transporter 1 (*ZnT1*), a plasma membrane-bound protein that transports zinc to the outside of the cell [80–86]. However, the catalytic nature of the MGd-mediated oxidation process is expected to overwhelm these natural “defenses” leading to toxicity effects associated with excess free zinc (cf. Fig. 5). Moreover, it was appreciated that the disulfide bond in metal-free thionein must be reduced by thioredoxin reductase, a species that is also deactivated by MGd (*vide supra*), in order to (re)coordinate zinc. Thus, it was expected that MGd would act as a potent agent for the disruption of zinc homeostasis.

Considerable evidence has already been obtained in support of the above hypothesis [87]. For instance, MGd was found to lead to the release of free zinc in A549 human lung cancer, PC3 prostate cancer, and Ramos B-cell lymphoma cells [87]. This was inferred from Northern blotting and lipoate reduction inhibition measurements, as well as from direct measurements made using a zinc-specific fluorescent probe (FluoZin-3™) in combination with flow cytometry [88, 89]. Lipoate reduction and FluoZin-3 measurements also show that increased intracellular zinc levels can be observed in the absence of extracellular zinc (i.e., in RPMI 1640 medium without serum or exogenous zinc), and that inhibition of cellular transcription or translation by addition of actinomycin D or cycloheximide increases zinc levels and potentiates the effect of MGd treatment. Conversely, the addition of a zinc ionophore, zinc 1-hydroxypyridine-2-thione (ZnHPT), increases the inhibition of lipoate reduction following treatment of cells with MGd. It also decreases cell viability. Finally, gene expression profiling experiments, carried out in collaboration with Hacia and coworkers (Univ. of Southern California), revealed increased levels of metallothionein isoforms and *ZnT1* transcripts in plateau phase A549 cell cultures treated with MGd [87]. More recent studies demonstrated that MGd or zinc each elicited transcriptional responses in Ramos B-cell lymphoma cultures that were remarkably similar and consistent with those seen in the plateau phase A549 cultures. Interestingly, treatment with either MGd or zinc also led to the induction of hypoxia-inducible transcription factor 1 (HIF-1) regulated genes. Stabilization of HIF-1 after treatment with MGd or zinc was confirmed on the protein level using an ELISA for HIF-1. Cultures co-treated with both MGd and zinc displayed further increases in the levels of MTF-1 and HIF-1 regulated transcripts as well as additional transcripts regulated by NF-E2-related transcription factor 2 (NRF-2), a sign of oxidative stress [90]. Taken together, these findings lead us to suggest that an important component of the anticancer activity of MGd derives from its ability to alter the cellular availability of zinc.

The upregulation of zinc by MGd is of interest because the role intracellular free (non-protein bound) zinc plays in regulating cellular functions is of considerable relevance to cancer. For example, increased free zinc has been proposed to stabilize hypoxia-inducible factor-1 (HIF-1) and thus influence processes such as glycolysis, apoptosis, and angiogenesis [90–93]. Moreover, free zinc inhibits thioredoxin reductase, [79] a key mediator in the cellular response to oxidative stress that is frequently overexpressed in cancer [76, 94, 95]. The importance of these targets in tumor development led to the hypothesis that small molecules capable of modulating free zinc levels can serve as anticancer agents. As previously mentioned, MGd increases intracellular free zinc levels, enhances the cellular toxicity of zinc, and inhibits cellular bioreductive activity in several human cancer cell lines [79, 89]. In fact, the ability of MGd to disrupt cellular zinc homeostasis provides a possible mechanistic explanation for its anticancer activity noted in clinical trials [96]. Such considerations led to the proposal that synergistic interactions between MGd and exogenous zinc acetate are important for activity and that these interactions would occur regardless of the means of zinc delivery.

Indeed, MGd displayed synergy with the zinc ionophore ZnHPT in an *in vitro* assay measuring bioreductive activity as noted above [87]. ZnHPT is commonly used to increase intracellular zinc *in vitro* and has been given p.o. to laboratory animals for toxicity testing and to probe zinc homeostasis *in vivo* [97–100]. ZnHPT is extensively used as a bactericide and fungicide [101]. However, the complex is insoluble in aqueous media and is poorly bioavailable [100]. Functionalizing the ZnHPT cores with short, water solubilizing polyethylene glycol (PEG) chains (i.e. complexes **9**, **10**, and **11**, Fig. 6) increased solubility and biological utility [102]. No benefit was seen above a certain length PEG group, seemingly due to an unfavorable decrease in logP. It was also observed that co-incubation with additional Zn (Zn(OAc)₂ supplemented media) potentiated increases in intracellular free Zn and this trend directly correlated to rates of cell death (cf. Fig. 7).

Even more promising, these modified Zn ionophores also proved to suppress tumor growth in nude mice bearing either A549 or PC3 tumors. **10** and **11** were sufficiently soluble in aqueous media to permit i.v. administration and testing in these A549 and PC3 xenograft models. A549 tumor growth was inhibited transiently by both agents. Since we had shown that MGd would potentiate the effects of zinc *in vitro*, [87] the effect of i.p. treatment with MGd was also examined, followed after 1 hour by **11**. MGd treatment alone does not significantly inhibit A549 tumor growth using this protocol. However, in combination with **11**, significant tumor growth inhibition was observed. In the PC3 model, tumor growth was inhibited significantly by treatment using **11** after four doses, but not after two doses. This leads us to suggest that more effective tumor control might be observed after more prolonged treatment or in combination with MGd.

CURRENT INVESTIGATIONS GENERATED FROM BIOMECHANISTIC STUDIES OF TEXAPHYRIN

Selective Platinum Delivery

Building on the characterization of texaphyrins as tumor selective redox active agents, our group has been inspired to develop the texaphyrin core as an active delivery vehicle for other known cancer therapeutics. We rationalized this approach versus other methods (i.e. pegylation, liposomal formulation) in that the carrier (i.e. texaphyrin) is itself an anticancer agent thus delivering two different agents in one.

A major thrust involves the use of texaphyrin as a “carrier” with the goal of overcoming Pt-drug resistance, particularly as applied to ovarian cancer. The FDA-approved Pt drugs, cisplatin, carboplatin, and oxaliplatin (Fig. 8) are widely used cancer therapeutic agents [103–106]. Cisplatin and carboplatin, however, are the main agents used in ovarian cancer [107]. The mode of action in platinum based agents is the formation of Pt-DNA adducts, which in turn initiate several transduction pathways eventually leading to apoptosis. In several cell lines, Pt resistance has become a major factor, recapitulating a key limitation in terms of the clinical use of Pt-based drugs. This observed resistance has been attributed to a reduction in intra-cellular drug accumulation, drug inactivation by thiol-containing molecules, increased Pt-DNA adduct repair, and inactivation of apoptotic pathways (i.e., p53), to just name a few. In the clinic, resistance serves to compound the inherent limitations of the Pt drugs, including systemic (and often dose limiting) toxicity that reflects, at least in part, a lack of tumor-specific tissue distribution. Many strategies have been put forth in an effort to increase the tumor specificity and accumulation of platinum drugs, including conjugation to potentially site-directing molecules, such as folate, poly-(ethyleneglycol) (PEG), porphyrins, peptides, and nanoparticles among others [108–115]. While increased delivery of a Pt drug can augment tumor cell killing effects, we believe that the Pt species

delivered to resistant tumor cells should also have intrinsic properties that overcome diverse mechanisms of resistance.

Over the past two years, much of our effort has been focused on the development of texaphyrin-platinum conjugates with the ultimate goal of overcoming Pt resistance in ovarian cancer. Working in collaboration with Zahid Siddik at the M. D. Anderson Cancer Center, we began exploring the hypothesis that conjugation of platinum to a tumor localizing texaphyrin would serve to overcome some platinum resistance pathways such as reduced accumulation, reduced Pt-DNA lesions, and inactivation of p53 regulated apoptosis. Towards this end, we designed and synthesized a novel texaphyrin Pt conjugate (complex **12**; Fig. 8). An ovarian cancer model, consisting of a platinum sensitive A2780 cell line and its isogenic platinum resistant 2780CP cell line, was chosen to determine whether this conjugate was effective in overcoming resistance [116].

Cell proliferation assays were used initially to detect cytotoxicity and probe anti-resistance benefits (Table 1). Conjugate **12** provided cytotoxicity profiles similar to that of carboplatin and other controls in ovarian A2780. In addition, complex **12** provided higher cytotoxicity than MGd. Conjugate **12** provided greater cytotoxicity (i.e. lower IC₅₀) than carboplatin in platinum sensitive 2780CP. In terms of resistance factor, conjugate **12** provided the lowest resistance factor and about 32–55% lower relative to other platinum complexes. This is indicative of partial circumvention of cisplatin resistance. It was later discovered that the decrease in resistance factor of conjugate **12** is due to increased intracellular platinum provided by conjugation to texaphyrin (cf. Fig. 9) [117]. In fact, a 12-fold increase in intracellular platinum from conjugate **12** was detected relative to carboplatin. Additionally, no reduction was seen in the uptake of platinum between A2780 and 2780CP with conjugate **12** whereas a 50% reduction was observed in platinum based controls including cisplatin. This significant increase in intracellular platinum resulted in increased production of Pt-DNA adducts in both A2780 and 2780CP, presumably accounting for the reduced resistance as compared to control complexes.

Bismuth and Lead Coordinated Texaphyrins

In addition to exploring biotechnological studies of texaphyrins, much of our ongoing research effort is focused on exploiting their highly versatile complexation ability. In porphyrin chemistry, complexes with post-transition elements such as Ga, In, Tl, Pb, and Bi are rare as compared to that of the transition elements [118]. Yet the chemistry of bismuth has become of increasing interest as its Bi and ²¹³Bi isotopes have some potential as α -emitters in radiotherapy [119] due to effective cancer cell killing even under hypoxic conditions owing to high ionization density radiation of 100 keV/um. This ultimately leads to double-strand DNA breaks with little chance of cell repair and survival. However, the short half-life of the isotopes (60.55 min and 45.65 min for ²¹²Bi and ²¹³Bi, respectively) gave birth to the concept of an *in situ* generator based on the initial complexation of lead [120]. Lead-212 has a half-life of 10.64 hours and produces ²¹²Bi as its primary decay product along with a β -particle.

Finding suitable ligands for bismuth or lead has proved challenging. An ideal ligand would be one that is able to form rapidly stable complexes with both bismuth and lead and do so under mild conditions. Complexes of bismuth and lead that possess inherent tumor selectivity would be further advantageous since they would allow the radioactive species in question, namely ²¹²Bi, ²¹³Bi, or ²¹²Pb, to be delivered selectively to cancerous tissues. This led us to suggest that texaphyrin would be an ideal ligand for these metals. Texaphyrins have been shown to localize to, or be retained selectively in, rapidly growing tissues, including cancerous lesions and could therefore act as carriers for these radioisotopes in question (cf. Fig. 10) [121]. Moreover, we demonstrated recently that texaphyrin is indeed

able to complex these two cations rapidly. Specifically, spectroscopic and mass spectrometric evidence was put forward to support the formation of the first lead(II) texaphyrin complex. Similar methods were used to confirm the formation of the first discrete binuclear μ -oxo bismuth(III) macrocyclic complex (**14**) to be described in the literature, a system that was then characterized in a definitive manner *via* a single crystal X-ray diffraction analysis [122]. These newly prepared Pb(II) and Bi(III) texaphyrin complexes proved chemically stable despite the μ -oxo bond being present in the latter complex. This allowed the water soluble derivatives to be studied *in vitro* using the A2780 ovarian cancer cell line. On this basis, it was concluded that the Pb(II) texaphyrin **15** and the Bi(III) texaphyrin **16** gave IC₅₀ values of 2.9 and 2.2 μ M, respectively. This represents a 2–3 fold increase in cytotoxicity relative to MGd (6.3 μ M) [116]. Based on these findings and considering the tumor selectivity properties of texaphyrins, we suggest that the texaphyrins could emerge as useful complexants for ²¹²Pb, ²¹³Pb, or ²¹²Pb and, as such, warrant further study as candidates for radiotherapy.

CONCLUSIONS

The results obtained to date provide support for the notion that texaphyrins could have a role to play in a variety of biomedical application areas, including as anticancer agents, site-localizing carriers, isotope delivery vehicles, and treatments for neurodegenerative diseases. Their unique mode of action, involving in some instances the manipulation of reactive oxygen species, and their inherent versatility in terms of sites for chemical modification and functionalization certainly make them attractive scaffolds for further study. It is hoped that this overview will inspire additional efforts to develop fully the potential of this unique class of compounds, as well as investigations of findings, such as the potential therapeutic utility of zinc ionophores, that have emerged from the work carried out to date.

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Fig. 1. Expanded pentaaza porphyrins, MGd (**1**) and MLu (**2**). The two acetate counter anions present in the neutral complex are not shown.

Fig. 2.

Metabolic processes affected by MGd. The essence of this mechanistic proposal is that MGd deactivates the cellular antioxidant system while producing ROS in a catalytic manner. This figure is a modification of one that first appeared in ref. 54.

Fig. 3. Results of a cell proliferation assay showing the enhanced activity of the naphthalene texaphyrin (**8**) under conditions (RPMI media; A549 cells) where MGd is not active (ref. 73).

Fig. 4. Combined data for MGd-catalyzed, air-based oxidation of reducing metabolites in pH 7.5 NaCl/HEPES buffered aqueous media. The concentration of dissolved O_2 in 1 mm sealed cuvettes was monitored as a function of time for each sample. The initial reductant concentration was 1.25 mM and the substrate:MGd ratio was 20:1 in all cases. Taken from ref. 68.

Fig. 5.

Generalized effect of MGd and zinc on metabolism. Both agents have an effect on the thioredoxin reductase based antioxidant pathway and are thus expected to complement the activity predicted on the basis of Fig. (2).

Fig. 6.
Water-soluble zinc ionophores.

Fig. 7.

A) FluoZin-3 fluorescence assay of A549 cells incubated with various ionophores and supplemental zinc. Results indicate increased levels of intracellular zinc in the presence of the representative zinc ionophore. **B)** Annexin A5 affinity assay of A549 cells with various zinc ionophores and supplemental zinc indicating that the presence of the representative zinc ionophore induces apoptosis and is correlated to increased intracellular zinc from data in A).

Fig. 8.
FDA approved Pt agents and texaphyrin-Pt conjugate **12**.

Fig. 9. Intracellular platinum concentration of cells after a 4-hour incubation with 200 μ M of each complex.

Fig. 10.

Lead and bismuth texaphyrins **13–16**, and views of the single crystal X-ray diffraction-derived structure of complex **14**.

Scheme 1.

Synthesis of transition metal texaphyrin complexes.

Table 1

IC₅₀ Values of Platinum Complexes with Cisplatin Sensitive A2780 Ovarian and Its Isogenic Cisplatin Resistant Cell Line (2780CP)

Complex	IC ₅₀ (μM)	IC ₅₀ (μM)	Resistance
	A2780	2780CP	Factor
12(MGd-Pt)	1.4	14.4	10.3
Carboplatin	1.6	26.3	16.4
Cisplatin	0.31	7.1	22.9
MGd	6.3	13.7	2.2