

Critical Review

Metal Ionophores – An Emerging Class of Anticancer Drugs

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Summary

Compounds that bind metals such as copper and zinc have many biological activities, including the ability to induce apoptosis in cancer cells. Although some of these compounds have been considered to act as chelators of metals, decreasing their bioavailability, others increase intracellular metal concentrations. We review recent work regarding the recognition of the biological effects of metal ionophores with different structures, particularly with regard to their actions upon cancer cells focusing on dithiocarbamates, pyrithione, and the 8-hydroxyquinoline derivative, clioquinol. We provide a biologically based classification of metal-binding compounds that allows an experimental distinction between chelators and ionophores that can be readily used by biologists, which may lead to further study and classification of metal-binding drugs. Metal ionophores may kill cancer cells by a number of mechanisms, including lysosomal disruption and proteasome inhibition, and likely others. Because some of these compounds have been safely administered to animals and humans, they have the potential to become clinically useful anticancer agents. © 2009 IUBMB

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INTRODUCTION

Metals play a critical role in cellular biology affecting many key cellular processes. Zinc, for example, can alter cellular signal transduction (1), DNA synthesis (2, 3), gene transcription (4, 5), post transcriptional modifications (6), protein-protein interactions (7), and protein function (8, 9). Increases or decreases in intracellular zinc have been associated with apoptotic cell death (10–13). As the essential role of metals in cell biology is well recognized, there have been relatively few

attempts to alter the biology of cancer cells by altering their metal content. The majority of work performed has been directed toward decreasing the availability of metals, using metal-binding compounds. The use of the term “metal-binding” has generally implied that the drug “sequesters” (or “chelates”) the metal ion, rendering it biologically unavailable.

Physiologists and cell biologists have long used drugs to raise or lower ion concentrations in cells. Although much of their efforts were directed toward studying the effects of sodium and calcium upon biological processes, they have also used metal-binding compounds to probe cellular processes. “Ionophores” are a subset of metal-binding drugs, which are capable of moving multiple members of a given ion across cell membranes. Although most of the work performed has utilized sodium and calcium ionophores, a zinc ionophore (pyrithione) has been utilized by a diverse group of investigators. In this article, we will review a series of reports that collectively indicate that metal ionophores have anticancer properties. We will provide a framework for differentiating ionophores from other types of metal-binding compounds, consider why a given compound may function as an ionophore, and discuss how they act as anticancer agents.

Metal-binding Compounds May Act as “Chelators” or “Ionophores”

Until recently, the effects of metal-binding drugs upon cancer cells have instinctively been attributed to their ability to sequester the metal in question. Drugs such as desferrioxamine, an iron chelator (14), and tetrathiomolybdate, a copper chelator (15, 16) have been shown to suppress tumor growth, tumor metastases, and angiogenesis and are currently being investigated in clinical trials (14–16). We initiated our studies of the metal-binding drug clioquinol with the thought that it would serve as a chelating agent, able to inactivate superoxide dismutase-1 (SOD1) and kill cancer cells. In the course of those studies we recognized that its activity was not due to its acting as a chelator, but as an ionophore, which caused it to raise intracellular zinc concentrations.

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Table 1
A biologically based classification of metal-binding compounds

Class	Functional properties	Biological response when metal is increased	Example (metal)	References
Metal chelator	Removal of metal from biologically active role	Decreased drug effect	TPEN (Zn) TTM (Cu) EDTA (multiple) Desferrioxamine (Fe)	(17, 18) (15) (14)
Metal shuttle	Transport of a single metal ion across biological membranes	No change	Metalloporphyrins and derivatives	(19, 20)
Metal ionophore	Transfer of multiple metal ions across biological membranes	Increased drug effect	Dithiocarbamates (Zn, Cu) Pyrithione (Zn, Cu, Pb) 8-hydroquinolines (Zn, Cu, Pb) Monensin (Pb) Zincophorin (Zn)	(21) (22, 23, 24) (25, 26, 24) (27) (28, 29)

TPEN - tetrakis(2-pyridylmethyl) ethylenediamine

TTB - tetrathiomolybdate

EDTA - ethylenediaminetetraacetic acid

In the course of those studies, in which we compared clioquinol with other metal-binding drugs (17), we found no focused discussion of metal-binding compounds to guide us, and derived operational definitions of different metal-binding compounds that may be of use to others (Table 1). A compound may be classified as a “metal chelator”, “metal shuttle”, or “metal ionophore” based upon how the compound’s biological effects are altered by an increase in metal concentration. If increasing metal concentrations reverse the drug’s effect it is classified as a “metal chelator”. If the metal-binding drug’s effect is not altered by increasing the metal concentration, and the drug-metal conjugate enters the cell, we consider it to be a “metal shuttle”. The metal-shuttle conjugates may exhibit biological activity because the metal dissociates from the complex, or because the conjugate itself is biochemically active. If the compound’s effect is amplified by increasing the metal concentration, it is classified as a “metal ionophore”. Ionophores may theoretically diffuse from the extracellular space to the intracellular space, and back to the extracellular space or may remain in the plasma membrane as it transports metal ions between intracellular and extracellular spaces.

Recognition that Some Metal-Binding Compounds Act as Ionophores and Have Anticancer Activity

In the mid 1990s, many investigators studied the ability of dithiocarbamates to inhibit NF-kappa B activity in malignant and non-malignant cells. Some reports showed inhibition of apoptosis in malignant cells (30), whereas others showed promotion of apoptosis in both nonmalignant (31) and malignant cells (32). The actions of these drugs were often attributed to their antioxidant (32) or metal chelating properties (33). In 1999, Kim et al recognized that zinc was required for a dithiocarbamate to exhibit inhibition of NF-kappa B and labeled it as a

zinc ionophore (21, 34). The same group then reported that a known zinc ionophore, pyrithione, was a 10-fold more potent inhibitor of NF-kappa B (22).

Pyrithione (1-hydroxypyridine-2-thionine, omadine) had long been recognized to have antimicrobial properties, and remains in common use as a topical agent in over-the-counter health products and an industrial biocide. Its anticancer activity was first reported in the 1980s, though it was thought to be functioning as an iron chelator (35, 36). When added to cells along with zinc it induced apoptosis (37–39). Although the sodium salt of pyrithione is water soluble, the zinc salt is not, prompting the synthesis and description of several water soluble derivatives designed to be used as anticancer drugs (23).

Clioquinol Has Anticancer Activity

Clioquinol (5-chloro-7-iodo-8-hydroxyquinoline) is a chelator of copper, zinc, and iron (Fig. 1). It was first produced in Switzerland in the early part of the last century, and was widely used as an antibiotic for the treatment of diarrhea and skin infection (40). More recently, clioquinol has been used in clinical trials for the treatment of Alzheimer’s disease (41,42), and explored as a potential anticancer agent (25). We were initially interested in finding a copper and zinc chelator that would inactivate SOD1, which we thought might play a role in determining cancer cells’ sensitivity to docosahexaenoic acid (43). Clioquinol did indeed interact synergistically with docosahexaenoic acid (44), but our studies suggested that its activity was not simply due to SOD1 inhibition. We found that clioquinol induces apoptosis in cancer cells through a caspase-dependent apoptotic pathway, and its IC₅₀ for eight different human cancer cell lines was in the low micromolar range. Importantly, these

IC₅₀s level are close to the blood clioquinol concentrations reported in a modern clinical trial of clioquinol in Alzheimer's disease patients (42). Using xenograft mouse models, we found that clioquinol slows down the growth of mouse tumor xenografts without noticeable toxicity (25). Further studies demonstrated that the synergistic interaction of docosahexaenoic acid and clioquinol is mediated in part by the PPAR α signaling pathway (45). Daniel et al subsequently confirmed the anticancer activity of clioquinol against breast (33) and prostate cancer cells (46), and showed that it inhibits angiogenesis. Schimmer and coworkers have recently demonstrated that clioquinol inhibits growth of leukemia and myeloma cells *in vitro* and *in vivo* (47).

Clioquinol Acts as a Zinc Ionophore

Understanding the mechanisms of clioquinol's anticancer action is critical for further development of this, and related, compounds. Although clioquinol had a moderate inhibitory effect on SOD1 activity, adding copper and zinc did not reverse its cytotoxicity, as one would expect with a metal chelator. In fact, adding metals potentiated its cytotoxicity, suggesting that

it was transporting metals into cells. Its ionophoric properties were confirmed using both a zinc-sensitive reporter cell and confocal microscopy. The latter, used in conjunction with a fluorescent zinc tracer, showed increased free zinc in the cytoplasm of cancer cells upon clioquinol treatment (Fig. 2). Others have subsequently reported that clioquinol can transport zinc and copper into cells (48–50).

Properties of Metal-Binding Compounds That Allow Them to Act as Ionophores

An ionophore binds a metal, transports it across a lipid bilayer, releases the metal, and then re-engages with metal ions not yet transported across the membrane, repeating the process. (Ionophores can transfer metals either into or out of cells, but for clarity's sake we will discuss the case of those that carry metals into cells). Some high-affinity metal-binding compounds (such as EDTA) are not able to cross cell membranes, whereas others (such as metalloporphyrins) are able to enter cells, but remain within the cell and do not re-cycle to the extracellular space. Compounds with "low" to "moderate" metal affinity may serve as ionophores because they bind the metal in regions of high concentration, and release it in regions where the metal concentration is lower.

Other molecules may act as ionophores because they are protonated upon entering a given compartment, which induces the release of the bound metal. Thus, if the pH of the extracellular space is higher than the pK_a of the ionophore, the ionophore will complex with the metal. If the complex then enters an intracellular compartment with a pH lower than the ionophore's pK_a, the complex will dissociate and free metal will be released. Differences in pH between intracellular compartments may cause some compounds to load the cytosol (pH close to 7),

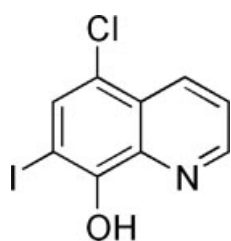


Figure 1. Structure of clioquinol (5-chloro-7-iodo-8-hydroxyquinoline).

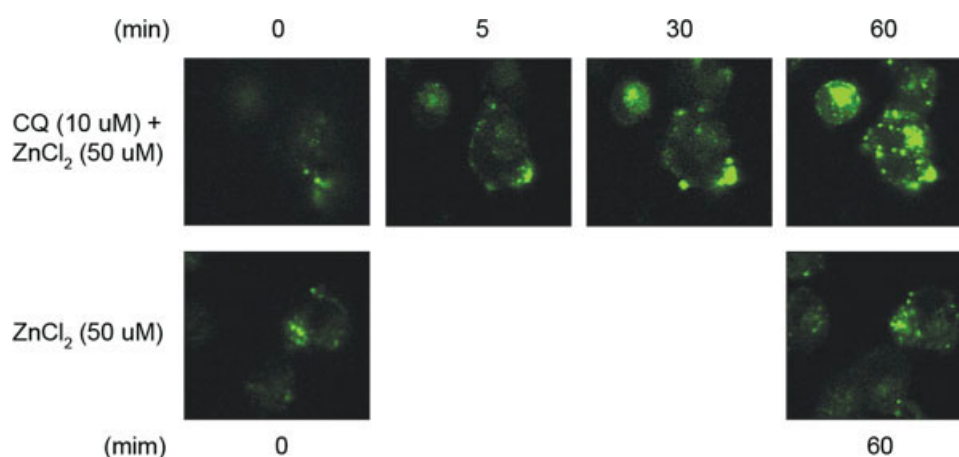


Figure 2. Clioquinol is a zinc ionophore. Confocal images of MCF-7 cells stained with FluoZin-3 probe (Molecular Probes, Eugene, OR). Cells were loaded with 3 μ M FluoZin-3 and treated with 10 μ M clioquinol plus 50 μ M ZnCl₂ for 5–60 min. The images were captured with the Leica TCS NT Confocal Microscopy using 63 $\times$ Plan APO 1.2 NA on living cells. A: Time-course of clioquinol-induced increase in zinc staining. B: ZnCl₂ alone did not enhance the staining.

whereas others may release metals only in more acidic spaces, such as endolysosomes.

We recently used a human prostate cancer cell line to study the intracellular location of free intracellular zinc, following zinc-clioquinol treatment. Using a fluorescent probe, we found a pattern consistent with a membrane bound intracellular localization, which co-localized with lysosomes. The lysosomes were subsequently disrupted, with release of cathepsins into the cytosol and the induction of apoptosis (26). Clioquinol is thus an example of an ionophore whose properties depend upon a combination of moderate affinity and a suitable pK_a . Other control mechanisms may be found that regulate the dissociation of metal-ionophore complexes.

Cell Killing by Metal-Ionophore Complexes

In addition to causing lysosomal disruption, some metal-ionophore complexes may kill cells by serving as proteasome inhibitors. Both the dithiocarbamate ionophore PDTC and clioquinol have been shown to inhibit proteasome activity in the presence of metals (46), and treatment of cells with zinc pyridithione causes results consistent with proteasome inhibition (51). Similarly, we have found that clioquinol inhibits NF-kappa B activity in ovarian cancer cells (17, 25), a well-recognized consequence of proteasome inhibition (52).

Although these experiments show that metal-ionophore complexes with ionophore activity may serve as proteasome inhibitors, they do not establish that the ionophore activity *per se* is essential. Thus, some compounds capable of serving as ionophores may bring about a biological response simply by acting as "metal shuttles", a distinction that may require specialized testing for clarification.

Other Mechanisms of Action

In a co-culture model system, Du et al reported that clioquinol stimulates TNF α secretion from macrophages, which in turn induces apoptosis of HeLa cells (53). We have reported that clioquinol targets both Akt and MAP kinase pathways when inducing apoptosis of cancer cells (17, 25). Magda described water soluble zinc ionophores that activate stress response pathways involving the metal-responsive transcription factor 1, the hypoxia-inducible transcription factor 1, and heat shock transcription factors (23). It is likely that additional mechanisms will be discovered, and the ability of a given drug to affect multiple pathways may determine its ultimate clinical usefulness.

Will Metal Ionophores Be Clinically Useful?

Whether these compounds can be developed into a novel group of anticancer agents is uncertain. Not all 8-hydroxyquinoline derivatives act as ionophores (Ding and Lind, unpublished observations) or have anticancer activity. Some derivatives with ionophore activity have been developed for treatment of neurological diseases (54), but it is not known which, if any,

have anticancer activity. Why metal ionophores should selectively kill cancer cells *in vivo* is unclear, but the fact that they are tolerated by animals and humans is encouraging. Disulfiram, for example, a drug that is hydrolyzed into dithiocarbamates *in vivo*, has a long track record of safety in humans and has demonstrated anticancer and antiangiogenic activity (55, 56). The cancer-specificity of these compounds may lie in their ability to alter metabolic signatures, such as NF-kappa B overexpression, that are unique to cancer cells. Regardless of their ultimate fate, examination of metal ionophores seems likely to advance our knowledge of cancer cell biology.

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