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Minding Metals: Tailoring Multifunctional Chelating Agents for Neurodegenerative Disease

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Abstract

Neurodegenerative diseases like Alzheimer's and Parkinson's disease are associated with elevated levels of iron, copper, and zinc and consequentially high levels of oxidative stress. Given the multifactorial nature of these diseases, it is becoming evident that the next generation of therapies must have multiple functions to combat multiple mechanisms of disease progression. Metal-chelating agents provide one such function as an intervention for ameliorating metal-associated damage in degenerative diseases. Targeting chelators to adjust localized metal imbalances in the brain, however, presents significant challenges. In this perspective, we focus on some noteworthy advances in the area of multifunctional metal chelators as potential therapeutic agents for neurodegenerative diseases. In addition to metal chelating ability, these agents also contain features designed to improve their uptake across the blood-brain barrier, increase their selectivity for metals in damage-prone environments, increase antioxidant capabilities, lower A β peptide aggregation, or inhibit disease-associated enzymes such as monoamine oxidase and acetylcholinesterase.

Introduction

Iron, copper, and zinc play complicated roles in human health and disease. While all three are essential nutrients utilized as various protein cofactors, their misappropriation within cells and tissues can lead to significant damage. Biological systems tightly regulate these metals at both the systemic level of absorption and distribution as well as the cellular level of storage, recycling, and utilization so that the organism meets metabolic demand without over accumulation (for recent reviews of iron, copper, and zinc homeostasis see references¹⁻³). Excess metal that is not appropriately contained by the proteins or vesicles that utilize, store, or transport it becomes available for unintentional and potentially toxic reactivity. One of the dangers of redox-active metals like iron and copper is their ability to promote the formation of highly toxic hydroxyl radicals that oxidize biomolecules and subsequently lead to cell death.^{4, 5} Even in the the absence of redox activity, metal cations like Zn²⁺ and Cu²⁺ may also cause damage by inducing aberrant protein aggregation.^{6, 7}

Both kinds of metal-promoted damage, oxidative stress and protein misfolding, have been linked to Parkinson's, Alzheimer's, and other neurodegenerative diseases.⁸⁻¹³ Furthermore, there is increasing recognition that these diseases are associated with localized accumulation of metal ions in disease-affected regions.¹⁴ Iron-induced oxidative stress in particular is speculated to play a role in a wide variety of progressive inflammatory and degenerative diseases, ranging from atherosclerosis and aging to diabetes and macular degeneration.¹⁵ The emphasis here is on *localized* misregulation, as these diseases are not characterized by

systemic metal imbalances, as is the case with traditional iron overload diseases like hemochromatosis and thalassemia.

Inhibiting metal-promoted damage by using small molecule chelating agents is a promising strategy for treating diseases associated with localized metal accumulation.^{10, 16-18} The target pool of metal ions in these diseases, however, is significantly different from diseases of systemic metal overload where the target metal is plentiful and more readily available for sequestration. Furthermore, our current understanding of metal homeostasis in the central nervous system is limited, which further constrains the interpretation of the effects of manipulating metals in the brain.¹⁹ Designing chelators to target localized metal imbalance therefore presents unique challenges. Generalized metal chelation can indeed protect against acute oxidative stress or inhibit protein aggregation, but long-term treatment with metal chelators poses serious risks associated with depletion or redistribution of healthy metal ions, inhibition of metalloenzymes, or other unintended consequences.

Research on medicinal iron chelators over the last 40 years has been dominated by the search for alternatives to desferrioxamine B (DFO, see Fig. 1), the naturally occurring siderophore that has been used clinically since the 1970s for treating transfusional iron overload diseases.²⁰ DFO is a hydrophilic, hexadentate chelator that is not absorbed by the gut and has a short plasma half-life, requiring it to be administered daily in large doses via subcutaneous transfusion. Extensive research to identify alternatives has led to the commercial development of two orally available agents, the bidentate hydroxypyridinone known as L1 or deferiprone, and the tridentate chelator deferasirox, or Exjade (Fig 1).²¹⁻²³ The targets of all these drugs are primarily non-transferrin bound iron in the plasma, followed by intracellular iron stores in the liver, heart and endocrine tissues.

While chelating agents developed for systemic iron overload diseases are feasible starting points for developing agents for localized metal imbalance, the variation in the target metal pools requires chelators with different chemical properties. For example, avoiding chelator access to the brain is critical for transfusional iron overload, whereas crossing the blood–brain barrier (BBB) is obligatory for treating neurodegeneration. DFO is not a reasonable candidate due to its inability to cross membranes. While increasing the lipophilicity of a chelator may improve BBB penetration, this same property will also improve general intracellular access where it can inhibit essential non-heme iron enzymes like ribonucleotide reductase, lipoxygenase and tyrosine hydroxylase.^{24, 25} For example, deferiprone crosses the BBB, but it also inhibits catechol-*O*-methyltransferase and tyrosine and tryptophan hydroxylases by forming ternary complexes with iron in the enzyme active site.²⁶ Tyrosine hydroxylase is a critical enzyme for synthesis of the key neurotransmitter dopamine, therefore its inhibition is undesirable in Parkinson's patients.

For localized metal overload, an optimal chelator needs to readily pass cellular membranes, specifically sequester only that fraction of metal that is causing damage without depleting healthy metal stores, form complexes that do not redox cycle under physiological conditions and that also pass cellular membranes for elimination, avoid redistribution of iron or other metals within a cell or between cells, and be non-toxic.

It is now becoming apparent that the next generation of therapeutically relevant metal chelators must have multiple functions to be most effective. Some noteworthy advances in these multifunctional metal chelating agents involve functions that increase cell and BBB permeability, increase antioxidant capabilities, increase selectivity for metals in damage-prone environments, lower Ab peptide aggregation, and inhibit key enzymes associated with specific disease pathways. This article will highlight recent advances and clever strategies in the design of multifaceted metal chelating agents targeted against neurodegenerative disease.

Increasing uptake into cells and across the blood brain barrier

If metal chelating agents are to be used to treat neurodegenerative disease, a prerequisite is that they must bypass the blood–brain barrier, a continuous layer of endothelial cells connected by tight junctions where the bloodstream meets the neural tissue.¹³ Its function is to restrict high molecular weight compounds and most ions from entering the brain while allowing essential nutrients to pass through, thereby blocking entry of neurotoxic materials while minimizing fluctuations in levels of vital substances in the central nervous system. As mentioned previously, abnormally elevated levels of metals have been found localized in the brains of patients with neurodegenerative diseases.¹⁴ In order to sequester potentially damaging sources of aberrant metal ions, chelating agents must be able to pass through the BBB; however, creating a compound capable of permeating the BBB is not trivial. Conventional wisdom posits that the molecule must be small (<500 Da), hydrophobic enough to diffuse passively through the membrane, yet hydrophilic enough to stay soluble in physiological environments.¹³ Several strategies have been employed to overcome these obstacles, including linking carbohydrates, peptides, and nanoparticles to chelators to facilitate entry into cells.

Multifunctional agents that target specific transporters or receptors

Carbohydrate conjugates to facilitate BBB uptake—The brain consumes a significant amount of glucose and needs a mechanism that quickly passes it through the BBB to meet this need.²⁷ The high concentration of glucose transporters (GLUTs) at the BBB therefore provides an appealing pathway for drug delivery if the drug can be tagged with glucose moieties to facilitate GLUT-mediated transport. Orvig and coworkers have reported several glucose–chelator conjugates with this premise in mind. The multifunctional conjugates are based on tetrahydrosalen or hydroxypyridinone families of ligands that are decorated with glucose molecules, as shown in Fig. 2.^{27–30}

Using carbohydrates as the actual metal binding moiety poses a challenge since typical carbohydrates have problematic stereochemistry and bind weakly to metals.³¹ The carbohydrate tag is instead installed as a pendant moiety onto a metal-binding ligand. In the first-generation tetrahydrosalen glucoconjugates (as in **1**, Fig. 2), the sugar tags are on the outer rim of the chelator where they do not interfere with the metal-binding core. In later-generation hydroxypyridinone and tetrahydrosalen glucoconjugates (**2**, **3** in Fig. 2), the glucose appendages mask the metal binding site, which has the advantage of preventing systemic metal binding prior to enzymatic cleavage of the sugar. The glucose modification has been found to increase water solubility, minimize toxicity, and shows promise for specific targeting of these compounds.^{27, 30}

In vivo, these glucoconjugate pro-ligands are anticipated to gain entry into the brain via glucose transporters on the surface of endothelial cells on the BBB.²⁷ Once inside cells, the glucose masking group would be enzymatically cleaved to release the active chelating agent, as shown at the bottom of Fig. 2. As proof of principle, it was shown that both a broadspectrum glucosidase from *Agrobacterium faecalis* and a rat brain homogenate as a model of glucosidase activity convert the glucoconjugate prochelators into their metal-binding hydroxypyridinone or tetrahydrosalen versions.^{28, 30} A rat brain perfusion experiment with a radiolabeled hydroxypyridinone glucoconjugate demonstrated adequate cerebral uptake, showing that these compounds are indeed taken across the BBB.²⁸

Once the carbohydrate groups are cleaved by β -glucosidases, the released tetrahydrosalen or hydroxypyridinone chelators are available to bind metals. In addition, both classes of compounds are effective radical-scavenging antioxidants.^{28, 29} *In vitro* studies show that both chelators compete with amyloid-beta (A β) peptides for binding Cu²⁺ and Zn²⁺, and

decrease A β ₁₋₄₀ peptide aggregation induced by these metals.²⁷⁻³⁰ The ability of **1** to prevent A β peptide aggregation was comparable to that of EDTA despite its lower affinity for Cu²⁺. This observation could be attributed to the increased lipophilicity and/or intercalating ability of the tetrahydrosalen compound into A β peptide aggregates.³⁰

Iron binding peptides that target neuronal receptors—Functionalizing neuropeptides with metal chelating groups is another strategy to target agents to specific brain locations.³² Vasoactive intestinal peptide, VIP, is widely distributed in areas of the brain associated with learning and memory and is associated with specific neuronal receptors in the brain.³³ An eight amino acid peptide abbreviated as NAP with amino acid sequence Asn-Ala-Pro-Val-Ser-Ile-Pro-Gln is the smallest active portion of activity-dependent neuroprotective protein, ADNP, which is a glial cell mediator of VIP-induced neuroprotection.³² NAP has demonstrated potent neuroprotection in both cell culture and animal models of neurodegeneration and is capable of crossing the BBB.³⁴ In order to create a multifunctional agent that would take advantage of the targeting ability of the NAP neuropeptide to sites of neurodegeneration and expand its antioxidant potential, Fridkin and coworkers modified the native peptide by adding hydroxamate (**4**) or 8-hydroxyquinoline (**5**) moieties to derivatives of NAP, as shown in Fig. 3.^{32, 34} The modified peptides form stable metal ion complexes with Fe^{2+/3+}, Cu²⁺ and Zn²⁺ in water, pH 5–7, whereas the parent NAP peptide shows no binding affinity to these metal ions. The peptides containing two hydroxamate units inhibit lipid peroxidation and iron-catalyzed hydroxyl radical formation *in vitro* and protect neuroblastoma cells against oxidative stress induced by exogenous hydrogen peroxide.³⁴ Although not yet tested, these peptides could allow for transport and localization of therapeutic chelators into the brain.

An alternative strategy for targeting chelators into the brain is to modify them with a neutral amino acid carrier group that would facilitate uptake by system L, a known brain uptake pathway for hydrophilic amino acids including L-DOPA. Fridkin and coworkers therefore synthesized and studied a bifunctional iron chelator, M10 (**6**), that fuses an 8-hydroxyquinoline unit with an alanine amino acid, as shown in Fig. 3.³⁵ They have shown that **6** is a metal chelator that exhibits free radical scavenging properties and is water soluble. The hydrophilicity of **6** may prevent its passive diffusion into cells, which would avert its interference with normal, systemic metal metabolism. It is hoped that the carrier group would thus allow selective targeting of the agent into the brain while minimizing effects on systemic metal balance.³⁵

Chelators with moieties that target integrin receptors—Overexpression of the cell surface $\alpha_v\beta_3$ integrin receptor (ABIR) has been found on activated endothelial cells in the neovasculature of tumors and has been associated with tumor growth, invasion, and metastasis.³⁶ Fluorescent probes and anticancer agents have previously been shown to be taken up when coupled to ABIR-binding peptides.^{37, 38} In order to use this approach to improve the uptake of metal chelators, Achilefu and coworkers synthesized a tri-functional agent consisting of a DFO chelating unit, a near-infrared cypate fluorescent probe, and a cyclic RGD peptide that is known to bind the integrin receptor. The multifunctional molecule (**7**) is shown in Fig. 4.

They found that the ABIR-avid RGD peptide improved the cellular internalization of the DFO analogue without permeating the cell nucleus, which can be damaging to the cell.³⁶ While this bioconjugate is not directed at a brain-specific receptor, the strategy shows that receptor-specific peptides can improve cellular uptake of metal chelators that otherwise have limited cellular access.

Nanoparticles as chelator carriers—Nanoparticles have been found to cross the BBB by receptor-mediated transport systems through brain endothelial cells and have shown promise in drug delivery.³⁹ This strategy is being pursued as a means to transport metal chelators into the brain. Mumper and coworkers coupled the Cu(I) chelator D-penicillamine, an FDA approved drug used to treat Wilson's disease patients, to nanoparticles by a disulfide bond, as shown in **8**, Fig. 5.⁴⁰ The nanoparticles used for the chelator conjugates, which were composed of emulsifying wax and surfactants, had been previously found to cross the BBB with no effect on cerebral perfusion flow or BBB integrity and permeability in an *in situ* rat brain perfusion model.⁴⁰ The D-penicillamine, found to be highly hydrophobic on its own, was released from the nanoparticles when exposed to reducing conditions and was able to solubilize copper-A β peptide aggregates *in vitro*.⁴⁰

With a similar concept in mind, Smith and coworkers devised polystyrene nanoparticles decorated with pyridinone chelators, as shown for Nano-N2PY, **9**, in Fig. 5.^{41, 42} In this case, conjugation of the chelators to the nanoparticle does not alter its metal chelating ability. The Nano-N2PY conjugates were shown to inhibit A β aggregation *in vitro* and to protect neuronal cells from A β -associated neurotoxicity.⁴²

Strategies for increasing passive diffusion

Metal chelators with increased lipophilicity—Synthetic derivatives of the well-known siderophore, ferrichrome, have been shown to bind Fe³⁺ with a tris hydroxamate binding site and display no toxic properties in cell studies *in vitro*. Cabantchik and Shanzer presented a new class of lipophilic ferrichrome analogues containing acetoxymethyl ester moieties, (**10**) Fig. 6.⁴³ The acetoxymethyl moieties provide the ferrichrome analogue with a hydrophobic region that facilitates entry of the chelator into cells. Once inside the cell, these molecules become hydrophilic by esterase-mediated hydrolysis of the acetoxymethyl groups. The R group on the tripodal anchor can be modified, allowing fluorescent labeling of the compound. Once the carboxylic acid groups are exposed, the diffusion of the chelator out of the cell is delayed when compared to a control containing stable ethyl ester groups instead of labile ester groups.⁴³

In another strategy aimed at improving the lipophilicity of iron chelators, Shanzer and colleagues prepared a tris-hydroxamate chelator, **11** in Fig. 7, wherein an isopentyl group that has been shown to assist in membrane crossing is used to anchor two ligand arms that contain hydroxamate chelating units.⁴⁴ In order to attain the proper octahedral geometry required for iron binding, one of the two arms contains an additional hydroxamate that is properly oriented for metal chelation by judicious incorporation of a proline residue. One of the analogs protects oligodendritic cells exposed to excess iron and H₂O₂ with a 25-fold increase in protection compared to DFO.⁴⁴ Future studies will consist of improving the lipophilicity of the analogues to improve the chelators activity.

Prochelators for triggered activation against oxidative stress

A leading hypothesis for the cause of cell death in degenerative disease is an increase in oxidative stress that results when reactive oxygen species (ROS) overwhelm the cell's inherent antioxidant mechanisms and damage cellular components.^{13, 14} Oxidative stress results primarily from the highly reactive hydroxyl radical, OH $\cdot$, formed by the oxidation of Fe²⁺ to Fe³⁺ by H₂O₂, the Fenton reaction, shown in Scheme 1.^{4, 5} The iron can be catalytic if its coordination environment favors redox cycling and cellular reductants can reduce Fe³⁺ to Fe²⁺. Iron-promoted oxidative stress may therefore be a critical component in diseases where normal iron homeostasis is impaired or where aberrant iron accumulation occurs.⁴⁵⁻⁴⁸ The premise for using chelators to thwart oxidative stress is to sequester any "free" iron capable of undergoing this redox cycle into unreactive complexes to prevent further free

radical production. The challenge for using chelating agents for these diseases is developing agents that are selective only for the harmful metal without disturbing healthy metal balance. The following examples show how selectivity can be achieved by reactivity of the chelating agent itself.

Selectivity based on chelator reactivity

Masked iron chelators—In 2006 our lab introduced a prochelator strategy that takes advantage of the reactivity associated with oxidative stress to generate metal chelators *in situ* to inhibit further oxidative damage.⁴⁹⁻⁵¹ The concept is shown in Fig. 8.

A boronic ester conceals a phenolic oxygen on SIH (salicylaldehyde isonicotinyl hydrazone), which is the active iron-binding agent. In its masked form, BSIH (**12**) has little to no affinity for metal ions. SIH is a well known aroylhydrazone chelator that has favorable attributes of membrane permeability and high affinity iron binding.²¹ Fig. 8 shows that the boronic ester on BSIH reacts selectively with hydrogen peroxide to convert BSIH to SIH, thus allowing the chelator to bind iron and prevent hydroxyl radical formation.⁴⁹ Several analogs of BSIH have been studied to tune properties including lipophilicity, iron binding affinity, and the rate of hydrogen peroxide dependent unmasking of the prochelator.⁵⁰ BSIH itself shows protection against cell death induced by hydrogen peroxide in cultured retinal pigment epithelial cells.⁵¹ Importantly, prolonged and repetitive exposure of healthy cells to BSIH was not toxic, whereas similar treatment with SIH or DFO caused cell death, presumably due to metal depletion.⁵¹ This finding suggests that the prochelator strategy may be effective for turning on metal chelation only under oxidative stress conditions.

Photo-caged iron chelators—An alternative strategy for masking a chelating unit is to block metal binding with a photoactive protecting group to create a photo-caged chelator.⁵² Exposure of UVA radiation to skin cells has been found to cause an immediate release of labile iron that exacerbates oxidative damage.⁵³ Pourzand and coworkers therefore prepared caged iron chelators that are activated when irradiated with UVA light.⁵² Aroyl hydrazone chelators SIH, PIH and derivatives of the two were masked with ortho-nitrobenzyl protecting groups that prevent metal binding, as shown in **13** in Fig. 9. Exposure to physiologically relevant levels of UVA light cause release of the active chelator. The caged compounds were found to be lipophilic enough to enter cells and exhibited no cytotoxic effects. Of the prochelators analyzed, 2-NPE-SIH (**13**) showed the most promise as a sunscreen component exhibiting protection at 500 kJ/m², the highest UVA dosage administered.⁵²

Iron chelators activated by hydroxyl radicals—In another example where the reactivity of a chelating unit can be used as a switch to prevent further Fenton reactivity, Galey and coworkers showed that aminocarboxylate ligands with relatively weak iron affinity can be converted to high-affinity chelators under oxidative stress conditions. An example is shown in Fig. 10. The compounds contain aromatic rings that are readily attacked by hydroxyl radicals generated at the iron center to produce phenolates that are well positioned to coordinate iron.⁵⁴⁻⁵⁷ Thus under pro-oxidant conditions, *i.e.* H₂O₂ and a reductant, metal complexes like **14** are hydroxylated and their iron affinity increases. In addition to an increase in affinity, another consequence of the added phenolate donor is a decrease in Fe³⁺/Fe²⁺ redox potential that prevents further redox cycling of iron.⁵⁵ The early compounds were not efficient at protecting cells from oxidative stress, probably because they were unable to cross cell membranes. Altering functional groups on the aromatic rings and esterifying the carboxylic acid groups alleviated this problem.⁵⁶ Non-specific esterases presumably cleave the esters intracellularly to give the active prochelator, which was found to protect skin fibroblasts against H₂O₂ toxicity with an IC₅₀ of 3 μM.⁵⁶

Similarly, Naughton and coworkers studied the potential radical scavenging of EDTA bis-(ethyl phenylalaninate), EBEP, **15** in Fig. 10.⁵⁸ In the presence of H₂O₂ and low concentrations of ascorbate, the oxidation products, *p*-, *o*-, and *m*- tyrosine are obtained. However, the phenolate in the *o*-position is then the only one that can participate in intramolecular iron binding.

Chelator–antioxidant hybrid molecules

L-DOPA/antioxidant hybrid molecules

Although many advances have been made in the area of Parkinson's Disease therapy, currently available drugs only treat the symptoms of the disease and can neither reverse nor slow its progression. The most clinically useful drug is still L-DOPA (3-hydroxyphenylalanine), a brain-accessible precursor to the neurotransmitter dopamine.⁵⁹ However, there are significant disadvantages to L-DOPA, beginning with its poor bioavailability and susceptibility to chemical and enzymatic degradation. Moreover, L-DOPA has pro-oxidant properties that generate free radicals as a result of its autoxidation, which is accelerated in the presence of iron or copper. In order to improve its bioavailability and provide a slow release mechanism for L-DOPA administration, several prodrug strategies have been evaluated.⁶⁰ As one example, Di Stefano and coworkers fused L-DOPA or dopamine with (*R*)- α -lipoic acid, a radical scavenging antioxidant that is taken up in all neuronal cell types.^{61, 62} α -Lipoic acid is rapidly reduced intracellularly to dihydrolipoic acid (DHLA), which has been found to lower the redox activity of free iron and copper without extracting the metals from protein sites.⁶³ The hybrid molecules (one example shown as **16** in Fig. 11) are enzymatically hydrolyzed in human plasma to release L-DOPA and DHLA as codrugs, with the idea being that sustained release of therapeutic L-DOPA would coincide with release of an antioxidant that can attenuate iron- or copper-mediated oxidative damage.⁶¹

Radical scavaging/ iron binding hybrid molecules

In another hybrid approach, Bebbington *et al.* combined the radical scavaging properties of *tert*-butylphenolic antioxidants with the metal-chelating hydroxypyridinone unit of deferiprone; an example of one of these hybrid molecules is shown as **17** in Fig. 12.^{64, 65} The dual-action agents were shown to inhibit lipid peroxidation in rat brain homogenates and protect cells against toxicity induced by ROS-generating iodoacetate.⁶⁴ Some of the derivatives showed superior neuroprotection compared to dual administration of antioxidants like BHT (butylhydroxytoluene) or Trolox and deferiprone.⁶⁴

Chelators that target A β

Amyloid-binding metal chelators

A diagnostic feature of Alzheimer's disease includes the formation of extracellular plaques composed primarily of aggregated amyloid beta peptide (A β).⁶⁶ Metal ions, particularly Cu^{+/2+} and Zn²⁺ but also Fe^{2+/3+}, accelerate A β peptide aggregation *in vitro* and have been found at elevated levels in neurotoxic oligomers.⁶⁷ The notion that metal chelation could be a promising treatment option for Alzheimer's was encouraged by promising phase IIa clinical trials of clioquinol (Fig. 13), a derivative of 8-hydroxyquinoline.^{68, 69} However, as expressed in the recurrent theme of this review, it is challenging to use general chelating agents to mitigate damaging effects of some metal ions without disturbing the beneficial properties of others. In an attempt to target metal-binding agents directly to amyloid fibrils, several groups have developed multifunctional agents that combine a metal-chelating unit with an amyloid-binding unit.

Thioflavin-T is a traditional dye used as a marker to detect amyloid deposits in tissue sections because of its strong affinity for amyloid fibrils. Amyloid-targeted chelators have therefore been designed that borrow the core structure of thioflavin-T and modify it with metal-binding functionality. Two examples are shown in Fig. 13. The bifunctional metal chelator XH1 (**18**), wherein two amyloid-binding units flank an aminocarboxylate chelating unit, was shown to minimize Zn²⁺-induced A β ₁₋₄₀ aggregation *in vitro* and attenuate A β amyloid pathology in a transgenic mouse model without toxicity.⁷⁰

In another example, virtual screening methods were employed to find compounds with combined features of thioflavin-T and clioquinol, along with common druglike properties and a lipophilic balance that would enable passage through the BBB.⁷¹ One of the compounds identified is shown in Fig. 13 in its iodinated form (HBTI, **19**), which could permit its use as a non-invasive imaging agent.⁷¹ HBTI is comparable to clioquinol in its ability to reduce both Zn²⁺ and Cu²⁺-induced A β aggregation, as confirmed by the A β aggregation turbidity assay.⁷¹ In addition, fluorescent measurements of HBTI demonstrate significant changes in the presence of amyloid fibrils, indicating that the compound is intercalating within the peptide aggregates as envisioned in the design of the multifunctional agent.⁷¹

Prochelators activated by Cu–A β

In addition to copper's role in accelerating A β aggregation, it is also implicated in pro-oxidant mechanisms associated with neuronal damage.⁸ In the presence of reductants, A β –Cu²⁺ complexes generate H₂O₂ *in vitro*.^{72, 73} Excessive H₂O₂ and a surplus of copper ions may therefore create a local environment conducive to oxidative stress. In order to target a chelator for conditions that mimic early Alzheimer's pathology, we adapted our boronate prochelator strategy to create the hydroxyquinoline-based prochelator QBP (**20**).⁷⁴ In the absence of H₂O₂, QBP does not prevent or disaggregate metal-promoted A β aggregates, a feature that may be beneficial as it may not be desirable to disaggregate already formed plaques. In the presence of ascorbic acid and O₂, however, Cu²⁺–A β assemblies generate enough H₂O₂ to convert QBP to the hydroxyquinoline metal chelator that diminishes copper's ROS-forming reactivity and inhibits further A β aggregation (Fig. 14).⁷⁴

Artificial peptidases to break up A β peptides

In the aforementioned examples, the overall strategy is to use a chelating agent to extract metals from A β in order to prevent aggregation and ROS formation. Suh and coworkers recently introduced a completely different paradigm where a metal complex is used to catalyze the hydrolytic degradation of neurotoxic A β oligomers.⁷⁵ To develop these artificial A β -specific proteases, they screened a library of compounds that combine a Co^{III} cyclen complex as the catalytic center with aromatic moieties having known affinity for A β plaques.⁷⁵ One of the lead compounds (**21**) that was capable of cleaving A β oligomers is shown in Fig. 15. By altering binding sites, linkers, and catalytic units, protein-cleaving catalysts specific for other proteins have also been developed.⁷⁶

In an extension of this protein cleaving tactic, Wu *et al.* reasoned that cyclen would be able to extract copper from A β to generate a hydrolytically active complex that could then degrade A β .⁷⁷ Their constructs contain cyclen covalently fused to an A β recognition motif such as the peptide sequence Lys-Leu-Val-Phe-Phe in **22** in Fig. 15, or curcumin. *In vitro* the cyclen can be metallated by competing with A β for Cu(II) to generate the active [Cyc(Cu)-KLVFF] complex that inhibits A β oligomerization, suppresses H₂O₂ formation, and produces A β peptide cleavage fragments.⁷⁷ Furthermore, the apo Cyc-KLVFF hybrid complex was shown to rescue cultured neurons from A β /Cu-induced toxicity, suggesting that it is able to acquire Cu(II) *in situ* to generate the active compound.⁷⁷

Metal chelators with enzyme inhibitory activity

Monoamine oxidase (MAO) dual action agents

The substantia nigra is a small part of the basal ganglia which is important for movement, reward, and addiction and is the region most affected in Parkinson's disease. In addition to elevated levels of iron in the substantia nigra, the brains of Parkinson's and Alzheimer's patients also show increased activity of monoamine oxidases (MAO), enzymes that oxidatively degrade neurotransmitters like dopamine and generate H_2O_2 as a byproduct.⁷⁸ These factors, combined with diminished antioxidant stores of glutathione, lead to a localized environment primed for oxidative stress. In order to increase the effectiveness of iron chelation as a strategy against these diseases, multifunctional agents have been developed that combine iron chelation ability with MAO inhibition.^{78, 79}

Both of the lead compounds M-30 (**23**) and HLA-20 (**24**) shown in Fig. 16 contain a brain-permeable 8-hydroxyquinoline chelating unit together with an *N*-propargylamine moiety. The latter has been found to be responsible for the neuroprotective effects of rasagiline and selegiline, two MAO inhibitors used clinically for treating Parkinson's patients.⁸⁰ *In vitro* the bifunctional agents coordinate Fe^{3+} to give 3:1 ligand:metal complexes that inhibit lipid peroxidation at levels comparable to desferrioxamine, while also showing modest MAO inhibitory activity and reasonable cell permeability.^{79, 81} The compounds show significant protective effects in cell culture models used for studying neuronal oxidative stress and show a wide range of pharmacological activities that include regulation of the amyloid precursor protein (APP) and reduction of $A\beta$ peptide levels.⁸¹⁻⁸³ The propargyl substituent on the chelator plays a crucial role in the multifaceted activity of M-30 and HLA-20 that make these compounds promising for future development.⁸⁴

Acetylcholinesterase (AChE) triple action agents

Acetylcholinesterase (AChE) is the enzyme responsible for breaking down acetylcholine, a neurotransmitter that is in short supply in Alzheimer's disease. Classical inhibitors of AChE, like tacrine, the first drug approved for Alzheimer's, block the active site in order to normalize cholinergic levels and improve cognitive function.⁸⁵ They do not, however, address the underlying pathology. In addition to its hydrolysis active site, AChE also contains a peripheral anionic site that has been identified as being responsible for promoting $A\beta$ fibrillization. Dual binding site inhibitors that block both the active site and the peripheral anionic site are being sought as they potentially could alleviate cognitive symptoms associated with acetylcholine deficiency while simultaneously reducing $A\beta$ fibrillization, which is believed to be central to the disease mechanism.⁸⁵

In order to take this concept one step further, Bolognesi *et al.* modified a dual-action AChE inhibitor with a metal chelating function to create the triple action agent **25** shown in Fig. 17.⁸⁶ The parent inhibitor, in which two tacrine units were linked together via a polymethylene spacer, had been validated for its ability to interact with both the catalytic and peripheral sites.⁸⁷ By incorporating either carbonyl or oxalamide functional groups into the spacer, the resulting inhibitors also maintain some ability to interact with divalent metal ions. The new bis-tacrine derivatives showed potent AChE inhibition, were able to reverse AChE-induced $A\beta$ fibrillization, and showed spectral changes in the presence of Cu^{2+} or Fe^{2+} indicating metal binding ability.⁸⁶ Although the modest μM affinities observed for these divalent metals might not be strong enough to extract metals from $A\beta$, the potential of generating triple-action AChE inhibitors that incorporate metal chelation was demonstrated.

Youdim and Fridkin have also recently incorporated AChE inhibition into a multifunctional chelator. In this case, they masked their existing multifunctional chelator HLA-20 (**24**) with an AChE inhibiting moiety to generate a prochelator that requires AChE enzyme activation

to release the metal-binding functionality, Fig. 18.⁸⁸ The prochelator **26** is an amalgamation of structural features found in HLA-20 in addition to rivastigmine and donepezil, two AChE inhibitors currently in clinical use. The *N*-benzylpiperidine unit of donepezil targets the peripheral site of AChE, while the carbamyl and ethylmethylamino moieties of rivastigmine bind at the active site. The prochelator **26** inhibited AChE activity with an IC₅₀ value of 0.5±0.06 μM that was slightly more potent than rivastigmine.⁸⁸ Furthermore, it was found that AChE efficiently cleaves the carbamyl protecting group to release the quinolinol which forms 2:1 and 3:1 complexes with Cu²⁺ and Fe³⁺, respectively.⁸⁸ Compared with HLA-20, **26** exhibits lower cytotoxicity in SH-SY5Y neuroblastoma cells, further substantiating the prochelator strategy for minimizing toxicity associated with generalized metal chelation.

Conclusions

Metal chelation research aimed at developing potential treatments for neurodegenerative diseases such as Alzheimer's and Parkinson's is moving in a new direction that blends metal chelation with additional functionality. In this Perspective, we have highlighted some of the creative approaches synthetic and inorganic chemists are taking to generate novel compounds that not only bind metals, but do so in ways that mitigate their potential cellular damage while also providing some element of specificity for disease conditions. These various elements include moieties that target the compounds across the blood-brain barrier or provide a trigger to release the metal-binding agent when the local environment is under conditions of oxidative stress. Other design strategies are used to create hybrid molecules that combine metal chelation with either antioxidant or enzyme inhibitory moieties, or domains that target the chelator to amyloid fibrils. Many of these compounds are at a very early stage of development, and significant work is still required to test these strategies in cell and animal models of disease. The concepts, however, may have broad applicability in a range of conditions, including cancer and bacterial and viral infection, where interference in cellular metal regulation via targeted metal chelation may provide a therapeutic angle.⁸⁹

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Fig. 1.
Structures of select medicinal iron chelators.

Fig. 2.
Top: structures of metal ion chelators containing pendant or masking carbohydrate groups.
Bottom: schematic example of cellular uptake by glucose transporters of prochelator **3**, followed by intracellular enzymatic activation and metal binding.

Fig. 3. Neuroprotective NAP peptides modified with bis-hydroxamic acid (**4**) or hydroxyquinoline (**5**) groups; and amino acid derivative of 8-hydroxyquinoline (**6**).

Fig. 4. A trifunctional chelator composed of: (A) an ABIR-binding peptide to facilitate entry through cell membranes, (B) cypate as a near-IR probe for detection, and (C) DFO as the iron chelator.³⁶

Fig. 5.
Nanoparticle carriers for penicillamine (8) and deferiprone (9) analogues.

Fig. 6. Acetoxymethyl protecting groups (highlighted in red) provide a lipophilic ferrichrome analogue (**10**) that is converted to a hydrophilic version (black) by intracellular esterases.⁴³

Fig. 7. Tris-hydroxamate iron chelator (**11**) with a dipodal anchor to assist in crossing the cell membranes.⁴⁴

Scheme. 1.
The catalytic cycle of Fenton Chemistry.

Fig 8.

The masked chelator, BSIH (**12**), binds Fe^{3+} only following deprotection by H_2O_2 .⁴⁹

Fig. 9.

Exposure to UVA light induces release of the photoactive protecting group (colored in red) on **13** to generate the active chelator SIH available for metal binding.

Fig. 10.

The tetradentate aminocarboxylate **14** binds iron weakly, but increases affinity following hydroxylation (highlighted in red) in the presence of H_2O_2 and a reductant. [X= coordinating solvent]. In a similar fashion, the aromatic rings in **15** can also be hydroxylated. In this case, only modifications at the ortho positions (highlighted in red) result in compounds with improved iron affinity.

Fig. 11. Hybrid molecule **16** fuses L-DOPA with lipoic acid. Enzymatic processing releases DHLA as a metal-attenuating antioxidant and dopamine.⁶¹

Fig. 12.
Structure of BHT antioxidant (red) fused to hydroxypyridinone chelator (blue).

Fig. 13.

Examples of chelators that incorporate features of thioflavin-T as an amyloid-directing group. XH1 (**18**) contains an aminocarboxylate binding site, whereas HBTI (**19**) uses an O/ N bidentate unit reminiscent of clioquinol.

Fig. 14.

The H_2O_2 generated from Cu-A β species in the presence of O_2 and ascorbic acid unmasks prochelator QBP (20) to release 8-hydroxyquinoline that extracts Cu^{2+} from A β and prevents further redox-cycling and A β aggregation.

Fig. 15. Catalytic Co(III) (**21**) and Cu(II)-cyclen (**22**) chelators found to break up A β peptides.

Selegiline

Rasagiline

M-30 (**23**) HLA-20 (**24**)

Fig. 16.
Structures of bifunctional metal-ion chelators, M-30 (**23**) and HLA-20 (**24**) that contain an *N*-propargylamine moiety found in MAO inhibitors like Selegiline and Rasagiline.

Fig. 17.

A bis-tacrine molecule (**25**) where the linker can interact with metal ions. The tacrine units are colored in red. **25** has been found to inhibit AChE activity, reverse AChE-induced amyloid fibrillogenesis, and bind metal ions.

Fig. 18. The multifunctional prochelator **26** combines structural features of HLA-20 (**24**, see Fig. 16) and AChE inhibitors rivastigmine and donepezil. The carbamyl group is cleaved by AChE to release the 8-hydroxyquinoline metal binding group (highlighted in bold).