Nanoparticle and other metal chelation therapeutics in Alzheimer disease

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Abstract

Current therapies for Alzheimer disease (AD) such as the anticholinesterase inhibitors and the latest NMDA receptor inhibitor, Namenda, provide moderate symptomatic delay at various stages of disease, but do not arrest disease progression or supply meaningful remission. As such, new approaches to disease management are urgently needed. Although the etiology of AD is largely unknown, oxidative damage mediated by metals is likely a significant contributor since metals such as iron, aluminum, zinc, and copper are dysregulated and/or increased in AD brain tissue and create a pro-oxidative environment. This role of metal ion-induced free radical formation in AD makes chelation therapy an attractive means of dampening the oxidative stress burden in neurons. The chelator desferioxamine, FDA approved for iron overload, has shown some benefit in AD, but like many chelators, it has a host of adverse effects and substantial obstacles for tissue-specific targeting. Other chelators are under development and have shown various strengths and weaknesses. In this review, we propose a novel system of chelation therapy through the use of nanoparticles. Nanoparticles conjugated to chelators show a unique ability to cross the blood–brain barrier (BBB), chelate metals, and exit through the BBB with their corresponding complexed metal ions. This method may prove to be a safe and effective means of reducing the metal load in neural tissue thus staving off the harmful effects of oxidative damage and its sequelae.

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1. Introduction

Alzheimer disease (AD) is a devastating neurodegenerative disease with progressive and irreversible damage to thought, memory, and language. AD is the most common form of dementia among people age 65 and older, progressing slowly from mild forgetfulness to the need for total care (reviewed in [1]). Unfortunately, an explicative etiology or a viable cure is not available. An estimated 360,000 new cases will occur this year and this number will continue to increase as the population ages [2]. As such, the disease poses a heavy economic and societal burden, with associated annual cost of care over $100 billion (reviewed in [1]). In addition, AD places an incalculable emotional and physical drain on families and caretakers. The ravaging effects of the disease call for efforts to prevent, forestall, and reverse the disease. Unfortunately, despite much interest, currently available FDA-approved drugs, targeted towards managing cognitive symptoms such as changes in memory and perception, provide only partial benefit to select patients and fall tremendously short as adequate means of therapeutic management.

In addition to donepezil (Aricept®), Tacrine (Cognex®), rivastigmine (Exelon®), and galantamine (Reminyl®), Memantine (Namenda®) has recently won the Food and Drug Administration approval for the treatment of AD. Unlike the acetylcholinesterase inhibitors Aricept®, Exelon®, and Reminyl®, and the cholinesterase inhibitor Cognex®, this medicine blocks excess amounts of gluta-
mate that can damage or kill nerve cells and is becoming the first line choice for advanced stages of AD [3]. In contrast, earlier approved drugs mentioned above work in early stages of AD by delaying the breakdown of acetylcholine, vital for nerve cell communication [4, 5]. Although treatment with these drugs provides symptomatic improvements or delays in the progression of cognitive, behavioral, and functional deficits, it does not stop or reverse the progression of AD.

Other promising methods of treatment for AD include anti-inflammatory drugs [6–8], antioxidants [9], amyloid-β-targeted drugs, and even a drug (rosiglitazone) to treat insulin resistance [10]. Many medications and substances are being investigated at the present time including physostigmine and nerve growth factor [11]. Currently, new studies on regulating amyloid β protein precursor translation by phenserine [12], on inhibiting the activity of γ-secretase [13, 14], and on vaccinating against amyloid-β [15] as well as on CD45 [16] show promising progress toward new therapeutic agents. Also, other studies including targeting cholesterol and glycosaminoglycans, and metalloprotein attenuating compounds, clioquinol, and other chelation agents are under investigation [11, 17–25]. Unfortunately, none of the treatments or drugs, which are currently available can prevent or cure AD. It is likely that more than one type of drug will be required to control AD. Indeed, a recent study shows that Aricept® and Namenda® work in tandem to ameliorate symptoms of AD [26]. Obviously, there is a great need for a better understanding of AD development and more effective therapeutic agents.

2. The rationale of chelating therapy: a new strategy for the treatment of Alzheimer disease

The etiology of AD is not well understood. Accumulating evidence supports the hypothesis that oxidative stress generated by various mechanisms may be among the major risk factors that initiate and promote neurodegeneration [6, 27–34]. Compared with other tissues, the central nervous system may be particularly susceptible to oxidative damage [35, 36]. Oxidation reactions are catalyzed by transition metals such as iron and copper [37] and, as such, the likelihood that an oxidation reaction will take place is probably increased by the regional concentrations of transition metals [38]. Substantial studies show that the metabolism of iron is involved in AD and that the concentration of iron in the brain of AD patients is elevated [30, 39]. Aluminum has also received attention in AD, although a role has never been convincingly demonstrated. Nonetheless, aluminum has been found in high concentrations in both senile plaques and intraneuronal neurofibrillary tangles in the brains of subjects with AD, which suggests that this metal may be involved in the etiopathology of AD [30, 39, 43]. Aluminum, unlike transition metal ions, is unable to redox cycle in electron transfer reactions due to a fixed oxidation state of 3+ in biological systems, but growing evidence suggests that it can act synergistically with iron to increase free radical damage [35, 44]. Recent study shows that accumulated aluminum in the central nervous system modulates amyloid-β formation and deposition [45]. Strong evidence also shows that copper [31, 32, 46–50] and zinc [30, 46, 51–53] are also implicated in the development of AD. In the AD brain, the concentration of zinc is significantly elevated in senile plaques and the concentration of copper is elevated in the rim of senile plaques. Overall, these studies indicate that the environmental conditions in AD, exacerbated by imbalances in several metals, has the potential for catalyzing and stimulating free radical formation and enhancing neuron degeneration.

Simultaneously elevated concentrations of various metals promoting oxidative damage, and hence promoting neurodegeneration present a complex system of pathophysiology not yet fully understood. Despite this complexity, metal dysregulation may in fact be the Achilles’ heel of AD, opening a door for chelation therapy. A chelator, regardless of synthetic or natural origin, may have high affinity for one metal ion, but it can also undesirably chelate other metals in various tissues leading to serious side effects. Affinity for multiple metals such as aluminum, copper, and zinc may pose useful rather than detrimental effects since various metals are implicated as oxidative instigators. Perhaps, this is why desferrioxamine (DFO), a specific iron chelator with high affinities for aluminum, copper, and zinc, has demonstrated some therapeutic benefits for patients with AD.

3. Metal chelators in the treatment of Alzheimer disease

DFO was found to significantly slow the progression of AD in one clinical trial [54]. In this study, the chelation of aluminum was examined, but it is possible that the therapeutic effect may also have been due to removal of iron since DFO preferentially chelates iron [55, 56]. DFO also has an appreciable affinity for copper and zinc [55, 57]. The affinity constants of DFO for Fe(III), Al(III), Cu(II), and Zn(II) are 30.6, 22.0, 14.1, and 11.1 (logK), respectively [58]. In this particular clinical study, copper and zinc, unfortunately, were not monitored. Therefore, it is not clear if the removal of copper and zinc by DFO treatment also played a part in slowing the clinical progression of AD. This may have been important since some studies have shown that copper- and zinc-specific chelators have the ability to dissolve amyloid-β plaques, a hallmark of AD pathology [20, 55, 59, 60].

DFO is the only chelation drug approved by the FDA for iron overload. DFO therapy promotes iron excretion and has led to great improvements in the quality and duration of life of patients who suffer from β-thalassemia and other refractory anemias. In addition, DFO also inhibits nigros-
trial degeneration induced by 6-hydroxydopamine [61].

Unfortunately, DFO has serious side effects including neurotoxicity and neurological changes [37,54,62–67]. Furthermore, DFO is poorly absorbed by the gastrointestinal tract and rapidly degrades after administration [68]. Therefore, it requires long subcutaneous administration to yield significant iron excretion [56,69]. Moreover, some studies show DFO does not easily penetrate the blood–brain barrier (BBB) due to its hydrophilic nature [70], although this point remains open to debate [71]. Indeed, some penetration may occur due to a compromised BBB via lesion sites [70]. Nonetheless, the neurotoxicity and difficulty of administration and delivery present serious hindrances to the use of DFO for AD treatment.

Deferiprone or L1 (1,2-dimethyl-3-hydroxyl-4-pyridinone) is an iron and aluminum chelator approved in Europe, but not in the United States [72]. Although L1 has high oral activity and BBB penetration ability due to its lipophilicity, its use is limited because of serious side effects [73,74]. In addition, studies have shown that L1 lacks the ability to remove iron from the brain [71] probably due to strong hydrophilicity of the iron–L1 complex. Additionally, there is no carrier-mediated transport system available to remove the complex from the brain. Other L1 derivatives with higher lipophilicity also have the ability to cross the BBB and complex brain iron, but they also possess considerable neurotoxicity [62,65,73].

Thus, the use of the currently available iron chelators is limited by their toxicity and/or poor transference across the BBB. Most bi- or tridentate iron chelators with small molecular weight and high lipophilicity have the ability to penetrate the BBB, but show toxicity [75]. On the other hand, hexadentate iron chelators are considered better candidates for chelation therapy than bi- and tridentate ones because of their lower toxicity before and after chelation [75], but they have difficulty penetrating the BBB [71,75,76] due to their hydrophilicity and relative high molecular weight. One strategy to increase the BBB penetration is by enhancing the lipophilicity and lowering the molecular weight of the iron chelators, but this is believed to increase toxicity [77]. In addition, it is possible that many lipophilic drugs which normally should cross the brain endothelial cells are rapidly pumped back into the blood stream by extremely effective efflux pumps [78], which include multiple organic anion transporter and P-glycoprotein (multidrug resistance protein). Many promising attempts have been made to develop iron chelators with oral activity (membrane penetration) and low toxicity for the treatment of iron overload disease [79–85]. This leads to the hope, that if successful, some iron chelators may also be suitable for AD treatment.

Some existing USP drugs such as Iodochlorhydroxyquin (clioquinol) that possess chelation properties and BBB penetration ability have shown therapeutic benefits in AD [86]. Clioquinol is an antibiotic agent and chelator with higher affinity for zinc and copper than for calcium and manganese [20]. With clioquinol therapy, the clinical rate of cognitive decline is slowed in a subset of AD patients compared with that in controls [86]. Interestingly, the zinc concentration in plasma of AD patients treated with clioquinol was significantly higher that of controls although no parallel change in plasma copper concentration has been indicated [86]. This new approach, indeed, merits further investigation in larger clinical trials. It should be mentioned that these drugs generally may have a low affinity for iron and would be toxic at doses needed for sufficient iron removal [58]. Currently, iron chelators with a hydroxyquinoline backbone or with high lipophilicity show BBB permeability and neuroprotective potential [21,25]. However, the toxicity of these chelators related to their lipophilicity has not been studied. Other therapeutic approaches are being explored to overcome the impediment of the BBB. For example, a prochelator has been designed for the purpose of easily entering the BBB. The functional groups of the prochelator are then activated by enzymatic or non-enzymatic reactions only after they have entered the target organ [87]. Another example is to use simple inorganic silicate that can form very stable complexes with many metals and probably has the ability to enter the BBB [88]. Iron chelators designed with near-optimal lipophilic/hydrophilic balance of the free chelator and iron complex for the purpose of passage into and out of the cell have been synthesized and studied [74]. However, no clear-cut clinical evidence for a beneficial effect of these chelators in AD has been demonstrated, and new approaches are necessary.

We believe the use of nanoparticles presents an exciting therapeutic option that will prove safe and effective for chelation delivery. The potential use of nanoparticles conjugated to chelators, which mimic lipoprotein particles and transfer iron chelators in and out brain by selected apolipoprotein absorption, provides not only a useful means of treatment, but insight into the mechanism of AD.

4. Nanoparticle systems with chelation agents: increased BBB permeability and lower toxicity

Nanoparticles made of natural or artificial polymers ranging in size from about 10–1000 nm [78,89] present a possible tool to transport drugs across the BBB [78] and nanoparticles of a size around or less than 300 nm coated with surfactants such as polysorbate 80 have been demonstrated to possess this ability [90–92]. The advantages of nanoparticles include reduced drug toxicity, improved biodistribution and therapeutic efficacy [93]. The mechanism by which the nanoparticles deliver drugs into the brain may be involved in the preferential absorption of ApoE and/or B. The particles also appear to mimic LDL and interact with the LDL receptor, resulting in their uptake by brain endothelial cells [78,90,94–96]. The transferrin transcytosis systems may be also employed by the particulated drug delivery systems to deliver drugs into the brain [78,94,97].
If an iron chelator can be covalently bonded to a nanoparticle, the particle may serve as a targeting vehicle to deliver the chelator to the brain and cross the BBB. There are three advantages to this approach. First, the chelators need not be lipophilic to cross the BBB. Second, the lipophilic character of the chelator no longer contributes to potential toxicity. And third, hydrophilic hexadentate iron chelators with large molecular weights may be used, as previously demonstrated with nanoparticle technology [91,92].

For iron chelation to be effective, the chelators must be capable of leaving the brain with the corresponding complexed metal ions. If the nanoparticles are not biodegradable and can mimic lipoprotein particles by preferentially absorbing ApoAI, known to facilitate the removal of particles from the brain [78,98], the same carrier-mediated transport systems will be able to carry the iron complex nanoparticles out of the brain. This is in contrast to lipophilic chelators which can enter the brain, but when complexed, they are unable to cross the BBB due to a change in their lipophilicity. For example, the distribution coefficient (DC) of free L1 determined in n-octanol/Tris–HCl buffer system is 0.24, but when complexed is down to 0.0009 [99]. Therefore, although L1 can reportedly penetrate the BBB, it fails to remove iron from the brain [71]. Apparently, there is no carrier-mediated transport system to eliminate the synthetic complex from the brain. In addition, the increase in lipophilicity will decrease the solubility in aqueous solution that probably decreases the bioavailability [74].

Our preliminary studies show that nanoparticles potentially transfer chelators in and out brain. We have developed synthetic methods and synthesized a series of iron chelators with functional side chains that can be used to conjugate with nanoparticles [100–102]. We have also tested the metal binding properties of the chelators and some biological properties such as the in vitro ability of iron removal from tissue sections of Alzheimer’s disease brain, and removal from ferritin, an important protein for iron storage [100,101]. The synthesized chelators removed iron from ferritin more effectively than DFO and were capable of removing iron from tissue sections of AD brain. Next, we developed the methods for conjugation of various iron chelators to nanoparticles. The particles can be made of biocompatible synthetic or natural macromolecules [78,89] with functional groups such as amino and carboxyl groups on their surface for covalently bonding with chelators [103]. Then, we quantitatively measured the amount of chelators that conjugated to the particles and determined the ability of the chelator–particle systems to bind iron. Interestingly, we found that some bi-dentate iron chelators after conjugation to particles converted to hexadentate chelators because the particles provided backbone linkages. This phenomenon greatly improved the metal binding stability and lowered the toxicity caused by metal–chelator complexes. We have also determined the human-plasma-protein-absorption patterns on iron chelator particle systems by using of two-dimensional polyacrylamide gel electrophoresis (2-D PAGE) technology [78]. Our results show that the protein pattern on iron chelator particle systems is totally different from that of the human plasma proteins. Through changing the system-surface properties, such as chelators and surfactants, the chelator–particle systems can preferentially absorb ApoE [92]. With the same kind of changes, we also find that the chelator–particles systems after binding metals can preferentially absorb ApoAI. Such preferential absorptions allow the systems to mimic the ApoE or ApoAI nanoparticles and cross the BBB through LDL transport mechanism [92,98]. Uniform coating of the systems with ApoE, B, or AI can also be achieved by overcoating of the apolipoproteins, which may enable the systems to cross the BBB with higher efficiency [90]. These studies indicate that iron chelator–nanoparticle systems have the potential to enter brain and bring excess metals out of the brain thereby prevent metal-associated oxidative damage. Our results also show the potential to optimize the surface property of chelator–nanoparticle systems by changing chelators, linkages, coating materials, and nanoparticles with different surfaces, which suggest that a chelator–nanoparticle system with optimal apolipoprotein absorption patterns can be developed. Our findings are promising and we are optimistic that this novel method of chelation will prove useful in the treatment of AD. More studies are warranted to demonstrate the protective efficacy of the chelator–nanoparticle systems to evaluate their toxicity and to optimize their capability to cross the BBB.

In addition to designing useful means of treatment, future studies with nanoparticle-linked chelators will provide insights into the mechanisms of AD pathophysiology, and may also show utility in other iron-mediated neurodegenerative diseases such as Friedreich’s ataxia, Parkinson’s disease and Hallervorden–Spatz Syndrome.

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References


