Chapter

Metal Ions-Mediated Oxidative Stress in Alzheimer's Disease and Chelation Therapy

Dongjin Yeo, Tae Gyu Choi and Sung Soo Kim

Abstract

Alzheimer's disease (AD), ranked as the seventh leading cause of death worldwide, is one of the most incidental neurodegenerative disorders. AD patients experience irreparable damages to the brain, indicated as progressive, insidious, and degenerative. Past research has discovered that the *amyloid cascade hypothesis* best describes the pathophysiological etiology of AD, designating amyloid-β plaques and neurofibrillary tangles as the 'hallmarks' of AD pathology. Furthermore, accumulating evidence show that the oxidative stress state, the imbalance between reactive oxygen species (ROS) production and antioxidation, contributes to AD development. This chapter describes the oxidative stress process in AD. It mainly tackles the correlation of metal-catalyzed ROS production with amyloid-β and how it oxidatively damages both the amyloid-β itself and the surrounding molecules, potentially leading to AD. Additionally, both the role of metal chelation therapy as a treatment for AD and its challenges will be mentioned as well. This chapter specially focuses on how metal ions imbalance induces oxidative stress and how it affects AD pathology.

Keywords: Alzheimer's disease, Amyloid-β, Reactive oxygen species, Oxidative stress process, Metal ions, Metal chelation therapy

1. Introduction

Past research has tried to find the pathogenesis and etiologies regarding Alzheimer's disease (AD). Recent studies show that reactive oxygen species (ROS) are linked with the progression and development of AD, especially superoxide anion, hydrogen peroxide, and hydroxyl radical. Reactive oxygen species have been found as the by-products of metal-catalyzed oxidation associated with amyloid-$\boldsymbol{\beta}$. These findings are crucial for the treatment of AD, as they provide the underlying mechanism for metal chelation therapy, which involves the use of metal chelators for metal removal.

This chapter discusses both past and current research with regards to AD pathology and treatment in the following order: Alzheimer's disease, reactive oxygen species, oxidative stress and Alzheimer's disease, metal chelation therapy, and challenges of metal chelation therapy.

2. Oxidative stress and Alzheimer's disease

2.1 Alzheimer's disease

Alzheimer's disease (AD) is one of the most common neurodegenerative diseases characterized as insidious, progressive, and degenerative. It accounts for 70% of all dementia cases in people aged 65 years and older [1]. The World Health Organization revealed that AD ranked as the seventh leading cause of death worldwide from 2000 to 2019. Although it is assumed that AD is triggered from genetics, environment, and dietary factors, the exact causes of AD are still not fully understood [2].

Patients with AD experience irreversible damage to the brain which leads to cognitive and behavioral deterioration, shrinkage of brain tissue, and progressive memory loss [3]. $A\beta$ and NFTs are considered as the two key factors in the neurodegeneration of AD patients. The forebrain cholinergic neurons are damaged due to neurofibrillary tangles (NFTs) of P-tau and the accumulation of senile plaques composed of amyloid-$\beta(A\beta)$ in the hippocampus, neocortex, amygdala, and basal nucleus of Meynert [4].

Although still debated, the amyloid cascade hypothesis best explains the pathology of AD. The $A\beta$ protein, a 36 to 43 residue polypeptide (in several studies, 39 to 43 residues/38 to 43 residues), is generated in the process of amyloid precursor protein (APP) enzymatic proteolysis, a transmembrane protein responsible for neuron growth and repair. Among the two main pathways for disposal of APP, a non-amyloidogenicα -secretase-mediated pathway and an amyloidogenic β-and γ -secretase-mediated pathway, the neuropathology of AD derives from the latter, in which $A\beta$ peptide is produced [5].

APP consists of both a cytoplasmic C-terminus and an extracellular glycosylated N-terminus. In the amyloidogenic pathway, APP is initially cleaved by a β-secretase creating a membrane bound 99-amino-acid C-terminal fragment. The C-terminal fragment, now acting as a substrate, is serially cleaved by a γ-secretase, resulting in a full length $A\beta$, mainly the 40-amino-acid $A\beta$40 and the 42-amino-acid $A\beta$42 [6, 7].

Due to the insolubility in AD patients, the $A\beta$ monomers abnormally aggregate into higher order assemblies, oligomers, protofibrils, and fibrils, which ultimately deposit into senile plaques. Amyloid senile plaques spread throughout the brain, eventuating in the interference of intercellular communication and the activation of immune cells which provoke inflammation. Neurological brain damage induced from amyloid plaques are commonly detected in the neocortex of AD patients [7, 8].

NFTs, another factor regarded as a key contributor of AD, is linked with $A\beta$ as well. Microtubules (MTs) in neurons work as directional highways between the axon and dendrites for organelle transport such as nutrients, neurotransmitters, motor proteins. The MT arrays also act as architectural elements that stabilize the structure and shape of the neuron [9]. The firmness of MTs depends on tau, a microtubule-associated protein (MAP), which plays a vital role in regulating the dynamic network and assembly of MTs [10].

There is accumulating evidence that $A\beta$ peptide induces tau hyperphosphorylation, which reduces the MT-tau affinity. Tau, no longer able to bind to MTs, start to aggregate forming tau clumps. Consequently, due to the decreased stability, MTs start to disintegrate. Separated tau cluster into tau oligomers, which eventually develop into neurofibrillary tangles (NFTs) [11]. With the breakdown of the MT system, neurons are incompetent to transmit organelles, resulting in the neurodegeneration of nerve cells which explains the memory loss and cognitive and behavioral decline of AD patients.

2.2 Reactive oxygen species

Reactive oxygen species (ROS) are unstable, highly reactive molecules and radicals which are derived from molecular oxygen. ROS production takes place in aerobic organisms that utilize mitochondrial electron transport for respiration or undergo oxidation catalyzed by metals and intracellular enzymes [12]. In normal settings, ROS play a crucial role for cell signaling such as cell cycle regulation, enzyme activation and apoptosis. Yet, under oxidative stress conditions, the immoderate production of ROS has detrimental effects on cells causing protein, DNA, and lipids damage and eventually, cell death [1].

When a molecular oxygen goes through a monovalent reduction, superoxide anion radical ($O_2^{\cdot-}$), a precursor compound of ROS, is formed [13]. $O_2^{\cdot-}$, due to its unstable state, react with other radicals such as nitric oxide (NO), forming highly reactive peroxynitrite ($ONNO^-$). $0_2^{\cdot-}$ also propagates further oxidative chain reactions, producing hydrogen peroxide (H_2O_2) with the help of superoxide dismutase (SOD). H_2O_2 are sequentially reduced either to hydroxyl radical ($OH^{\cdot}$), one of the most reactive oxidants, or fully reduced to water [14, 15].

ROS generation, mainly in forms of $O_2^{\cdot-}$, H_2O_2, $OH^{\cdot}$, are induced by both endogenous and exogenous pathways. The endogenously produced ROS are mainly byproducts of mitochondrial respiratory chain and phagocytic nicotinamide adenine dinucleotide phosphate (NADPH) oxidase, circumstances in which the reduction of oxygen is enabled (**Figures 1** and **2**). Transition metals and numerous intracellular enzymes such as, Xanthine oxidase (XO), Lipoxygenases (LXO), and Cyclooxygenase (COX) are also principal endogenous ROS generators (**Figures 3–5**) [16].

ROS are produced in response to exogenous or environmental factors as well, such as radiation, air pollutants, diet, tobacco smoke, drugs and xenobiotics, chemotherapy, and pesticides [16, 17]. Exposure to UVR from solar radiation develops high concentrations of ROS, which causes an imbalance between ROS and cellular antioxidants, thus provoking oxidative stress [17]. Tobacco smoke, another notable factor of ROS production, consists of 10^{14}-10^{16} free radicals per puff which can potentially produce H_2O_2 and $OH^{\cdot}$ [16].

The right duration, quantity, and location of ROS production is required for normal physiological processes. In cases where the appropriate conditions are not met,

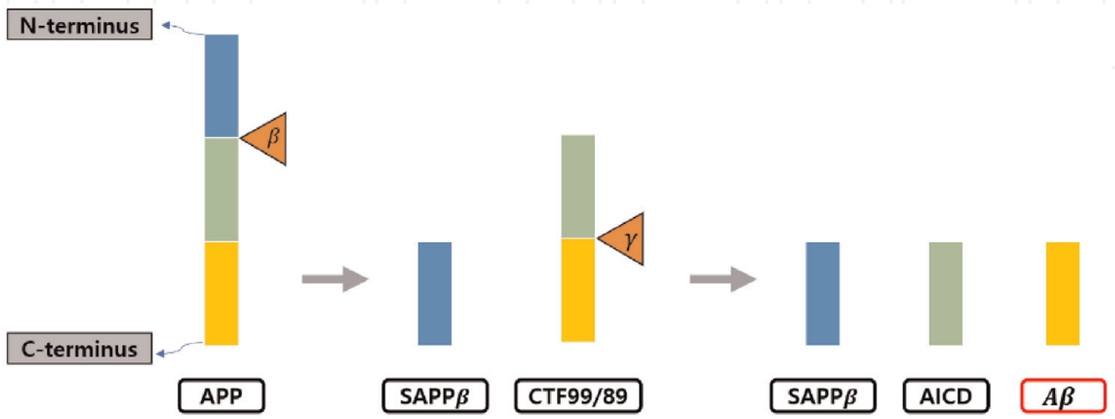

Figure 1.
Amyloidogenicβ -and γ-secretase-mediated pathway of APP disposal In the amyloidogenic pathway of APP disposal, APP is first cleaved by a β-secretase yielding SAPPβ and CTF99/89. Subsequently, CTF99/89 is cleaved by a γ-secretase creating AICD and Aβ.

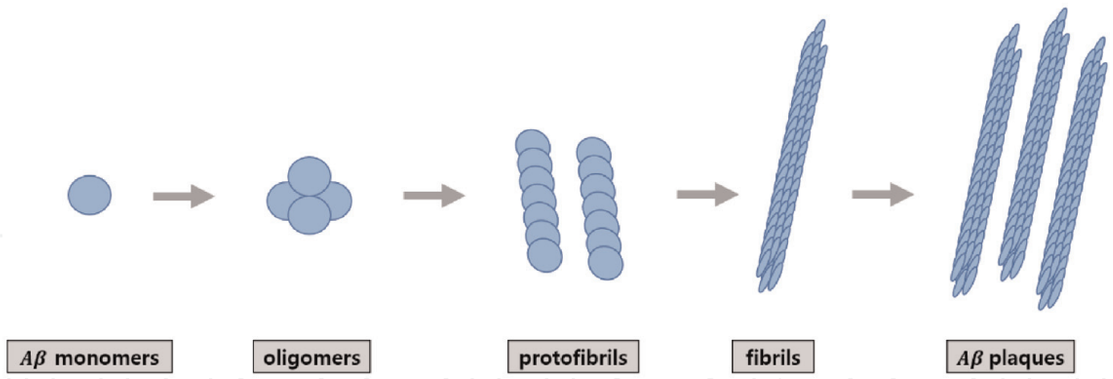

Figure 2.
Aβ plaques formation abnormal aggregation ofAβ monomers into oligomers, protofibrils, fibrils, and ultimately plaques can be seen in AD patients.

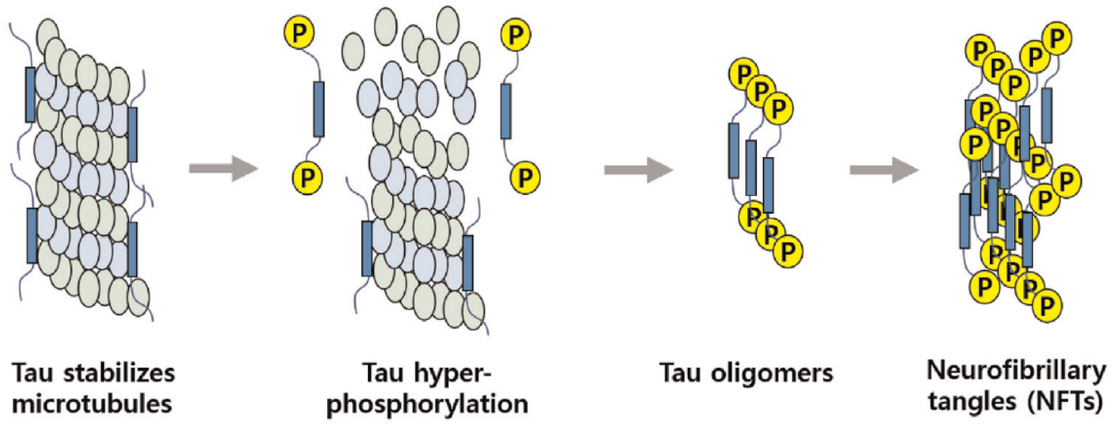

Figure 3.
NFTs formation Aβ peptide induces tau hyperphosphorylation, which reduces MT-tau affinity. Separated tau develop into oligomers and eventually NFTs.

both insufficient and excessive ROS production, ROS-related diseases can arise [15]. Such medical conditions include glucolipotoxicity, insulin resistance, diabetes mellitus, mitochondrial dysfunction, cancer, autoimmune disorders, cardiovascular, neurological, and psychiatric disease [15, 18].

Antioxidants work as the defense mechanism against ROS induced damage. Its role is to maintain the effective functions of ROS while at the same time, regulate its level. Oxidative stress is attenuated by both endogenous antioxidant system and the exogenous intake of antioxidants [19, 20]. The former includes enzymes such as SOD, glutathione (GSH), catalase and glutathione peroxidase (GPx) [19]. Meanwhile, the essential exogenous antioxidants are absorbed through vegetables, whole grains, fruits, and omega-3 fatty acid containing diet. Vitamin C, vitamin E, β-carotene, selenium, carotenoids, and polyphenols represent exogenous antioxidants [19, 20].

2.3 Oxidative stress and Alzheimer's disease

Majority of current research show that oxidative stress, the imbalanced state of ROS production level and antioxidative level, is related to the pathogenesis of neurodegenerative diseases, representatively AD [21]. This chapter approaches mainly the

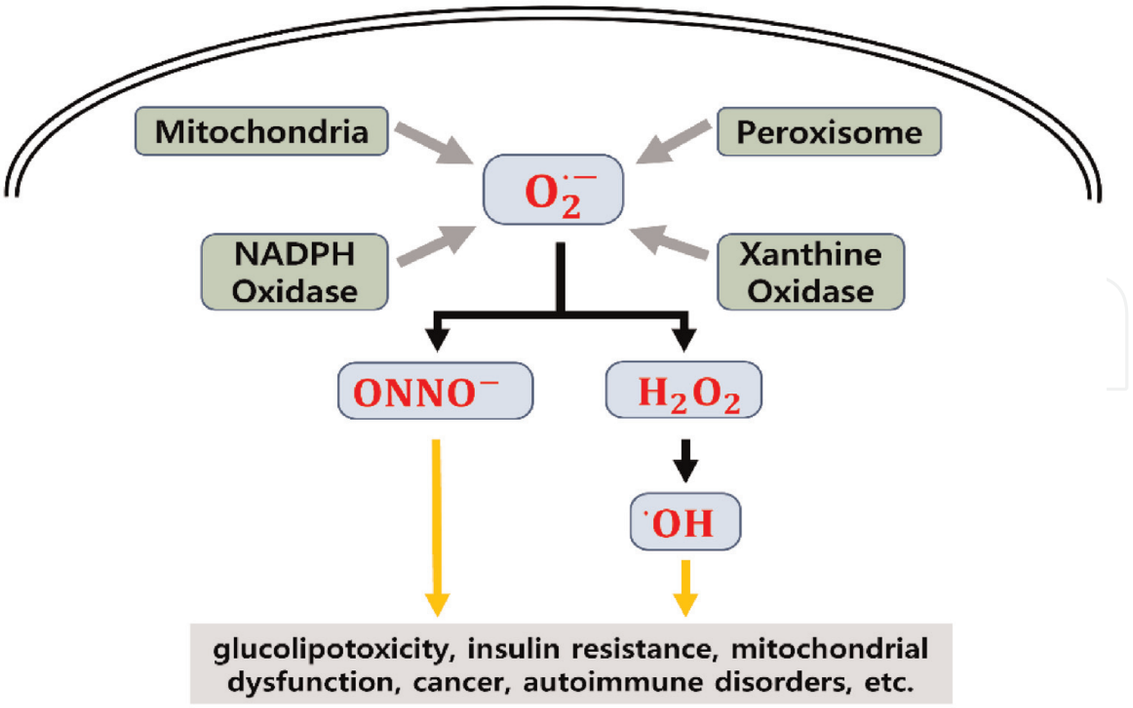

Figure 4.
ROS production pathways endogenous and exogenous pathways of ROS production include mitochondrial production, NADPH oxidase, peroxisome, and xanthine oxidase. Through such pathways, $O_2^{\cdot-}$, $ONNO^-$, H_2O_2, and $OH^{\cdot}$ are yielded.

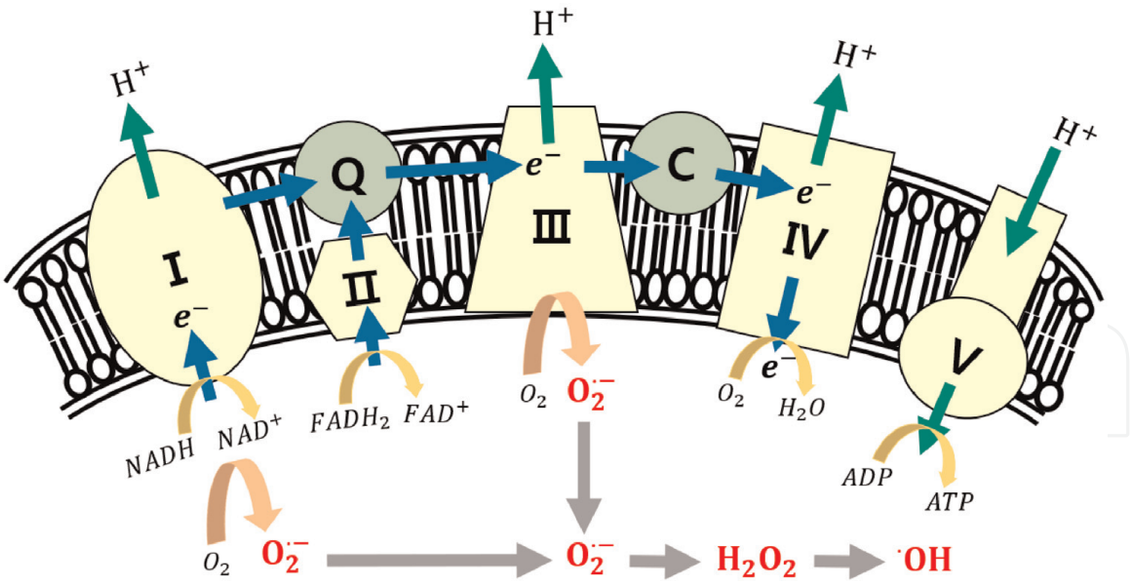

Figure 5.
Mitochondrial ROS production complex Iand complex III of the inner mitochondrial membrane create $O_2^{\cdot-}$ through oxidative phosphorylation. $O_2^{\cdot-}$, through further reactions, can also yield H_2O_2, and $OH^{\cdot}$.

association of oxidative stress with AD, mostly regarding the correlation between $A\beta$ and ROS production and how it affects the neighboring neural molecules.

As previously stated above in the *Alzheimer's Disease* section, amyloid plaques and NFTs are regarded as the 'hallmarks' of AD. Overwhelming evidence show that amyloid plaques are highly concentrated in metal ions, such as copper(Cu), iron(Fe), zinc(Zn)

and calcium(Ca), which are present in the synaptic areas. Such metal ions are interconnected with the amyloid cascade reaction and NFT formation [22].

Metal ions imbalance induces oxidative stress which triggers ROS production. Increased production of ROS leads to secretases imbalance and phosphatases imbalance, each interconnected with the formation of $A\beta$ and P-tau. Accordingly, $A\beta$ and P-tau production increases, which eventually leads to neurodegenerative diseases including AD [23]. Thus, the $A\beta$ toxicity, NFTs, oxidative stress, and ultimately neuronal cell death depend on the existence of redox metals [24]. This chapter mainly discusses the correlation of metal-catalyzed ROS production with Aβ (**Figure 6**).

2.3.1 Copper

Among the metal ions, copper is considered the most redox reactive. The association of copper ions with $A\beta$ can be described as a three-step process. First, endogenous reductants bind with the copper, followed by the reduction of Cu(II) to Cu(I). The reductive state of copper triggers the reduction of molecular oxygen as well, producing ROS [25]. Copper directly interacts with $A\beta$, promoting increased aggregation of $A\beta$ and the toxicity of amyloid oligomers and plaques [22, 26].

Histidine $(His6, His13, His14)$ and Tyrosine (Tyr10) amino acid residues modulate the binding of copper to $A\beta$.Cu(II) is reduced to Cu(I), after its chemically binding to $A\beta$(higher affinity to $A\beta$1- 42 compared to $A\beta$1- 40), generating hydrogen peroxide as a byproduct which has high potential to be reduced to hydroxyl radical. Accordingly, the complexation of copper in $A\beta$ elevates the neurotoxicity, now endowed with enlarged

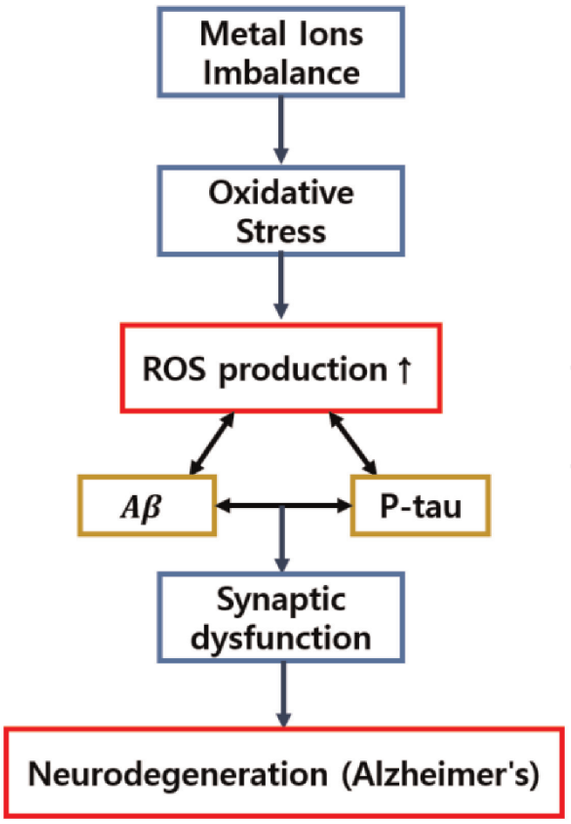

Figure 6.
Metal ions imbalance, increased ROS production, and neurodegeneration imbalance of metal ions, such as copper, iron, zinc, and calcium, creates oxidative stress condition. This is followed by increased production of ROS, and consequently Aβ and NFTs, which eventually provokes neurodegenerative diseases including AD.

reduction potential [24]. The *Cu- Aβ* couple correspondingly assists the further process of ROS production. The copper- *Aβ*-mediated oxidation of reductant species such as ascorbate, which are abundant in the brain, induces generation of ROS: hydrogen peroxide, hydroxyl radical and superoxide anion [25].

2.3.2 Iron

Iron, as a redox active metal, is also significantly linked with AD pathology. However, unlike copper, iron ions do not directly interact with and bind to *Aβ* [27]. Iron exists in both in redox-inactive forms (Fe^{3+}) and redox-active forms (Fe^{2+}) within the brain. They are also found in zero-oxidation-state (Fe^{0}) or as ionic compounds such as magnetite(Fe_3O_4) as well. All forms are possible inducers for *Aβ* aggregation, prompting the iron redox cycle and ROS production [28, 29]. Iron concentration and increased free radical production had been noticed in the cerebellum and glia cells of AD patients [23].

After iron's indirect interaction with *Aβ*, the redox cycle of Haber-Wiess and Fenton reaction is triggered, yielding ROS in forms of hydrogen peroxide, hydroxyl radical and superoxide anion, as in the process of copper-mediated oxidation. The resulting ROS effects *Aβ* aggregation and other oxidative damages in local organelles as well. Research results based on high-resolution transmission electron microscopy (HR-TEM) and synchrotron-based X-ray absorption studies support the storage of iron within *Aβ* and the iron-catalyzed ROS production [27, 29].

During the process of the metal-catalyzed ROS production in correlation with *Aβ*, both the *Aβ* peptide itself and the surrounding molecules undergo oxidative damages. The amino acid residues of *Aβ*, cysteine, methionine, arginine, histidine, lysine, phenylalanine, tryptophan, and tyrosine, are oxidated as well, chemically changed, and impaired. The ROS produced through metal-mediated oxidation also cause protein carbonylation and nitration, lipid peroxidation, and protein modification. The mitochondria of nearby cells also experience oxidation, leading to increased mitochondrial and nuclear DNA &RNA damages which all potentially lead to the etiology of AD [30].

2.3.3 Zinc

The impact of zinc(Zn^{2+}) in AD is rather controversial [23]. Some research suggests irregularly high concentration of Zn^{2+} have been investigated in AD patients' brains, inferring the linkage between imbalance of Zn^{2+} homeostasis with AD pathogenesis [31]. One study indicated that Zn^{2+} promotes both *Aβ*40 and *Aβ*42 aggregation, but only at the early stage [32]. In another study, high concentration of Zn^{2+} was shown to induce NADPH-oxidase reaction and ROS production (especially mitochondrial ROS production) in AD pathological state. Excessive zinc therefore prompted *Aβ* cascade reaction [23]. On the contrary, other research analysis show significant decrease of Zn^{2+} in AD patients [33].

2.3.4 Calcium

Calcium(Cu^{2+}) elevation also significantly contributes to *Aβ* production in AD patients. Sequentially, increased *Aβ* level in turn promotes an increase in Cu^{2+} level by triggering the opening of voltage-dependent Cu^{2+} channels. Moreover, high degree of Cu^{2+}provokes further influx of Cu^{2+} by enabling overexpression of L-type calcium

channel subtype (Cav1.2). Excessive Cu^{2+} consecutively stimulate $A\beta$ production and aggregation [23].

2.4 Metal chelation therapy, a potential treatment for Alzheimer's disease

Based on the thesis that AD pathology relates to the interplay between metal ions and $A\beta$, treatments for AD have been proposed established on this characteristic. Metal chelation therapy has been raised as a method to agitate metal- $A\beta$ interactions to treat AD in a lot of research [27]. Metal chelation therapy is initiated by injection of chelators (chelating agents) into the bloodstream which bind to the targeted metals and excrete them [34].

Studies show that metal chelating agents must satisfy the following conditions to manipulate as prospective treatments for AD.

1. Low molecular weight

2. Target certain: must be able to selectively attach to targeted metal ions bound to $A\beta$

3. Free or poor charge: must be able to cross blood brain barrier (BBB)

4. Low toxicity

5. Low possibility of side effects

Metal chelators content with above properties will successfully affix to aimed metal ions associated with $A\beta$, engendering their break-up and removal [27].

Among the various chelator drugs, only a few are suitable for AD; drugs that fulfill the properties stated above. The common chelator drugs adopted for AD treatments that have shown favorable results include desferrioxamine (DFO), bathophenanthroline, bathocuproine (BC), trientine, penincillamine, bis (thiosemicarbazone), tetrathiomolybdate (TTM) [35–37].

In one clinical trial in 48 patients with AD, DFO has shown its positive effects. Using trace-metal analysis, the research team confirmed that DFO decreased the aluminum level in neocortical brains of AD patients dosed with DFO;125 mg per injection, twice a day, five days a week [38]. Although it showed outcomes regarding aluminum, one research insisted that, considering the affinity DFO has for iron, the result might have also been due to the elimination of iron [39]. In addition to iron, DFO also shows binding affinity towards copper [40].

Penincillamine, bathophenanthroline, bathocuproine (BC), and trientine have also been proven to be effective copper chelators. In one research test, these agents showed interaction with Cu- $A\beta$ couple, deleting copper and improving $A\beta$ solubility. Furthermore, BC has been proved to be the most efficacious, showing constant results across the broad range of AD brain tissue samples [37].

It has been suggested that the bis(thiosemicarbazone) compounds can regulate the concentration of copper in $A\beta$ as well [41]. In one study, chemical compounds of the bis (thiosemicarbazone) metal complex family have shown successful treatment for animal models with AD [42]. Similar results have been noticed in another study using APP/PS1 transgenic AD mice model as well. Bis(thiosemicarbazone) enhanced the soluble $A\beta$ level by deleting copper and led to the restoration of cognitive activity [43].

The effect of tetrathiomolybdate (TTM) as a copper chelator has been demonstrated as well. In one experiment, TTM was applied to Tg2576 transgenic mice model for five months. Positive effects were derived, showing that TTM lowered both the level of *Aβ* and *Aβ* plaques present in the brain [44].

2.5 Challenges of metal chelation therapy

Although the above-stated metal chelating agents have shown positive effects in reducing *Aβ* levels in AD patients, there are still challenges surrounding the metal chelation therapy.

First, in addition to the originally aimed effects, metal chelating agents can induce undesirable outcomes as well. One study revealed that the application of divalent chelators, such as *Cu*, *Fe* and *Zn*, to severe AD patients lessened the requisite divalent metals that were already in their appropriate levels, as well as the targeted metal ions. Accordingly, the depletion of essential metals aggravated rather than treated AD pathology [45].

Furthermore, as stated in *Metal Chelation Therapy, a Potential Treatment for Alzheimer's Disease*, metal chelating agents have been proved to lower*Aβ* level through solubilization. However, it is still rather controversial whether metal chelators can not only *solubilize* but *reverse* the *Aβ* plaques to any forms of intermediates such as monomers, oligomers, protofibrils, short fibrils, or extended fibrils [27, 45].

Finally, there are remaining questions concerning the efficacy of certain metal chelating agents. For instance, clioquinol (CQ), a*Cu - Zn* chelator capable of agitating *Aβ* aggregation has been used in numerous clinical trials. However, the clinical and experimental results show that the effectiveness of CQ is yet contentious [45, 46]. In one experiment, the utilization of CQ perturbed *Cu* and *Zn* homeostasis which elevated metal ion concentrations, which is contradictory to the predicted results. CQ also showed side effects, arising astrogliosis, spongiosis, and brain edema to the mice model [47].

For further development of metal chelation therapy, such disadvantages should be improved.

3. Conclusion

Aβ and amyloid plaques are determining symbols of AD. Metal ions, especially copper and iron, interact with *Aβ* in AD patient's brain which generates ROS, such as superoxide anion, hydrogen peroxide, and hydroxyl radical. This process promotes the aggregation *Aβ* and increases the toxicity of *Aβ* plaques. ROS induces damages to both *Aβ* itself and the surrounding molecules leading to protein, lipid, DNA, and RNA impairment. Metal chelation therapy has been proposed as method to agitate metal-*Aβ* interactions for AD treatment. Metal chelators injected into the bloodstream will target metals associated with *Aβ* and eliminate them, cutting off the activity of causative substances. The metal chelating agents that have shown positive effects towards AD so far include desferrioxamine (DFO), bathophenanthroline, bathocuproine (BC), bis(thiosemicarbazone), tetrathiomolybdate (TTM), trientine, and penicillamine. However, there are ongoing challenges facing the metal chelation therapy. The remaining questions regarding the efficacy of chelating agents and the precise mechanism of chelation therapy should be solved.

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Conflict of interest

The authors declare no conflict of interest.

Appendices and nomenclature

Aβ	amyloid-*β*
AD	Alzheimer's disease
APP	amyloid Precursor Protein
BBB	blood brain barrier
Cav1.2	L-type calcium channel subtype
COX	cyclooxygenase
CQ	clioquinol
DOS	desferrioxamine
GPx	glutathione peroxidase
GSH	glutathione
HR-TEM	high-resolution transmission electron microscopy
LXO	lipoxygenases
MAP	microtubule-associated protein
MT	microtubules
NADPH	phagocytic nicotinamide adenine dinucleotide phosphate
NFT	neurofibrillary tangle
ROS	reactive oxygen species
SOD	superoxide dismutase
TTM	tetrathiomolybdate
XO	Xanthine oxidase

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