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Metal-amyloid-β peptide interactions: a preliminary investigation of molecular mechanisms for Alzheimer's disease

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Although humans have spent exactly 100 years combating Alzheimer's disease (AD), the molecular mechanisms of AD remain unclear. Owing to the rapid growth of the oldest age groups of the population and the continuous increase of the incidence of AD, it has become one of the crucial problems to modern sciences. It would be impossible to prevent or reverse AD at the root without elucidating its molecular mechanisms. From the point of view of metal-amyloid-β peptide (Aβ) interactions, we review the molecular mechanisms of AD, mainly including Cu^{2+} and Zn^{2+} inducing the aggregation of Aβ, catalysing the production of active oxygen species from Aβ, as well as interacting with the ion-channel-like structures of Aβ. Moreover, the development of therapeutic drugs on the basis of metal-Aβ interactions is also briefly introduced. With the increasingly rapid progress of the molecular mechanisms of AD, we are now entering a new dawn that promises the delivery of revolutionary developments for the control of dementias.

Alzheimer's disease (AD), amyloid-β peptide, Cu^{2+}, Zn^{2+}, molecular mechanisms, aggregation, active oxygen species, ion channel

On November 3, 1906, Alois Alzheimer, a Bavarian psychiatrist, first described the pathological characteristics of the neurodegenerative disease that bears his name at a meeting in Tübingen, Germany[1]. Alzheimer's disease (AD), the most common form of senile dementia, is a progressive neurodegenerative disorder and has an incidence rising almost logarithmically with age. Until the 1960s, however, the advent of electron microscopy and the utilization of it to examine the two classical pathological hallmarks, namely, extracellular senile plaques and intracellular neurofibrillary tangles, broke the lastingly stagnant complexion of the study on the molecular mechanisms of AD[2]. In the mid 1970s, the first clear neurochemical clue as to what might underlie the dementing symptoms came from the observation that neurons synthesizing and releasing acetylcholine underwent variable but usually severe degeneration. According to the cholinergic theory, substantial pharmacological research focused on attempting to enhance acetylcholine levels in the synaptic cleft, primarily by inhibiting the degradative enzyme, acetylcholinesterase (AChE)[3]. These efforts ultimately led to the success of a serial of AChE-inhibitor type drugs for the treatment of AD, such as tetrahydroaminoacridine, donezepil, galantamine, huperzine A, and so forth[4,5]. However, the clinic effect indicates that the therapy with these types of drugs is just symptomatic palliative interventions[6]. Since the mid 1980s, with the discovery of the major components of the senile plaques, that is, amyloid-β peptide (Aβ), the study of the molecular mechanisms of AD reached a new rapidly developmental period[7,8].

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Both of the two defining hallmarks of AD, i.e., the aggregation of Aβ and the generation of reactive oxygen species within the neocortex, may be closely related to metal-Aβ interactions[9]. In the most recently decade, the study of the mechanisms of metal-Aβ interactions has made increasingly rapid progress, and has become one of the mainstream research directions of the molecular mechanisms of AD[10].

1 Aβ, metal ions and amyloid plaques

Aβ is one of the normal products of the β-amyloid precursor protein(APP) metabolism[11−13]. APP is a 695—770-residue ubiquitously expressed glycosylated transmembrane protein and has two proteolytic pathways *in vivo* (Figure 1)[2]. One is the non-amyloidogenic pathway in which APP is first cleaved by α-secretase after the 687-residue yielding α-amyloid precursor proteins (α-APPs) and the C-terminal C83 fragment; the latter is then further cleaved by γ-secretase after the 711/713-residue into P3 peptide. The other is the amyloidogenic pathway in which APP is first cleaved by β-secretase after the 671-residue yielding β-amyloid precursor proteins (β-APPs) and the C-terminal C99 fragment; the latter is then further cleaved by γ-secretase after the 711/713-residue into $Aβ_{1-40}$/$Aβ_{1-42}$ peptides. $Aβ_{1-40}$ is the major soluble Aβ species, which accounts for 90-95% of the secreted Aβ in cultured neuronal cells[14]. $Aβ_{1-42}$ is a minor Aβ species, but more fibrillogenic than $Aβ_{1-40}$[15]; and is the predominant form of Aβ in senile plaques[16]. The 'nucleation' or 'seeding' hypothesis was proposed to elucidate the formation of senile plaques, whereby $Aβ_{1-42}$ forms the nucleus of a plaque initially, enabling the subsequent deposition of otherwise soluble $Aβ_{1-40}$ in some instances[17].

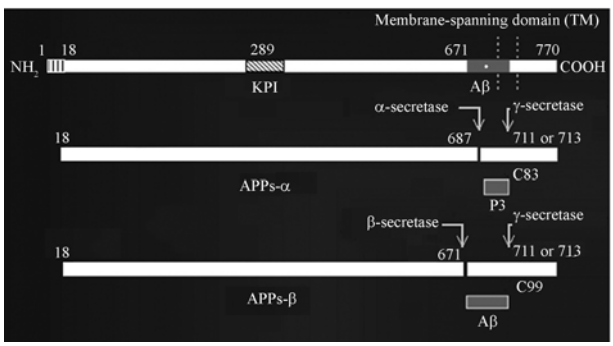

Figure 1 The metabolism of APP and production of Aβ[2].

At present the physiological functions of APP and Aβ remain unclear. The mainstream 'amyloid hypothesis' states that the abnormal processing of APP results in the increased production of Aβ, especially of its more amyloidogenic form $Aβ_{1-42}$, as well as the aggregation and deposition of Aβ. This accumulation of toxic fibrillar Aβ injures neurites and disrupts neuronal function and homeostasis, and eventually causes neuronal death[18,19]. As increasing numbers of amyloid plaques are formed, there is a cascade of neuronal loss that leads to dementia. In 2002, Robinson et al.[20] proposed the 'bioflocculant hypothesis' which is distinct from the above 'amyloid hypothesis'. It posits that Aβ is neuroprotective and serves to bind neurotoxic solutes (pathogens, proteins, and metal ions) so that they can be phagocytosed and prevented from causing further damages. Several lines of recent data support a role for APP and Aβ in modulating tissue metal-ion homeostasis and in controlling metal-ion-mediated oxidation. The biochemical behaviour of Aβ is therefore pleiotropic: at a high peptide to metal-ion stoichiometry, Aβ removes the metal ion and is protective; however, at high metal-ion-to-peptide stoichiometry, Aβ becomes aggregated and catalytically pro-oxidant, and therefore is neurotoxic[10].

Metal ions, such as Zn^{2+}, Cu^{2+}, and Fe^{3+}, are maintained at abnormal high concentrations with Aβ in amyloid plaques, which suggests that metal ions are an important neurochemical factor closely related to the aggregation of Aβ. While these metals play important roles in normal physiology, they are found in relatively high concentrations in the neocortical regions of the brain most susceptible to AD neurodegeneration, such as the amygdala and hippocampus. Using the modern simultaneous analytical techniques for multielements, such as the micro Particle-Induced X-ray Emission (μ-PIXE)[21] and X-ray fluorescence microscopy (μ-XRF)[22], high concentrations of Zn^{2+}, Cu^{2+} and Fe^{3+}(1055, 390, 940 μmol/L, respectively; 3—4-fold background brain tissue concentrations)[21,23,24] have been detected in amyloid deposits in AD affected brains, so amyloid plaques are described as 'metallic sinks'. The typical detection limit of μ-PIXE and μ-XRF are 1—10 μg/g. Moreover, μ-XRF technique has a higher sample penetration depth (1000 μm) and spatial esolution(0.1 μm) than μ-PIXE (100 and 0.3 μm, respectively)[25]. Liu et al.[22] reported that combining with the

laser capture microdissection(LCM), a lesion-specific tissue procurement technique, the elemental profiles (S, Fe, Cu, and Zn) in typical amyloid plaques of submicron size were obtained by μ-XRF. Conventional tissue procurement techniques usually acquire larger pieces of brain tissue, in which the actual amyloid-bearing plaques are 'diluted' such that the elemental signals from the plaques may be obfuscated, or even lost entirely, in the background noise from the neutrophils and other cellular components residing in close proximity. By using the LCM to excise individual amyloid plaques along with a portion of the immediately proximal area, μ-XRF analysis can image both areas with high resolution and high signal-noise ratio. As shown in Figure 2, a typical amyloid plaque from an AD brain gives strong signals for Fe, Cu, and Zn. The significance of sulfur (S) element may reflect its high abundance in proteinaceous elemental composition. Further, its marked presence may also be an indicator for amyloid plaque-associated oxidative stress since protein S-glutathionylation is a salient feature for oxidative stress[22,28].

2 The inducing effect of metal ions on the aggregation of Aβ

There is now compelling evidence that Aβ does not spontaneously aggregate, but that there is an age-dependent reaction with excess brain metal (copper, iron, and zinc), which induces Aβ to precipitate into metal-enriched masses(plaques). In the recent decade, a series of intensive studies of the metal-ions-Aβ interactions, especially Zn^{2+}[29,30], Cu^{2+}[31—37] with Aβ, has been made by a range of complementary spectroscopies, such as EPR spectroscopy, NMR, Raman spectroscopy, CD, fluorescence and UV-vis, as well as potentiometric curves, aggregation assays, and competitive metal capture analysis techniques. *In vitro* studies have shown that metal ions are able to promote Aβ aggregation, fibril, and amyloid formation[29,38—40]. Compounds/Cu-Zn chelators that interdict metal-ion binding to Aβ dissolve brain deposits *in vitro* and inhibit Aβ deposition in brain of Tg2576 transgenic mouse model for AD[41]. A more recent study indicates that coprecipitant(s), such as Zn^{2+}, may be needed for *in vivo* Aβ aggregation since $Aβ_{1-40}$ peptide is thermodynamically soluble at physiological concentrations[42]. Data also imply that metal ions may lower the kinetic barrier for Aβ precipitation, although the peptide's thermodynamic solubility remains little changed. Aβ is a metalloprotein with multi-binding sites of metal ions, among which histidine residues at positions 6, 13, and 14 near its N-terminus are the crucial sites for metal binding[43—47]. Using NMR and electron paramagnetic resonance (EPR) spectroscopy, the coordination of Aβ with Cu^{2+} has been probed both in aqueous solution and membrane-mimetic environments, which indicates that the three histidine residues are all involved in the coordination[48]. The ability of metal ions to aggregate human Aβ, is diminished by modifying all three histidine residues at positions 6, 13 and 14 with diethyl pyrocarbonate[39]. Rat Aβ, which contains three amino acid substitutions, R5G, Y10F and H13R, binds Zn^{2+} and Cu^{2+} much less avidly than human Aβ[29,39,49]. Especially, the reduced affinity of rat Aβ for Zn^{2+} is reproduced by the single H13R mutation of human Aβ[50].

The affinity of variant $Aβ_{1-40}$ and $Aβ_{1-42}$ species for Zn^{2+} is equal[10], but the affinity for Cu^{2+} and Fe^{3+} differentiates variant Aβ species in a manner that echoes their participation in AD pathology [$hAβ_{1-42}$>$hAβ_{1-40}$>$mAβ_{1-42}$>$mAβ_{1-40}$][39,27]. Aβ forms 3.5 metal-ion-binding sites (per subunit, as oligomers) of various affinities[48]. *In vitro* experiments indicate that there are two binding sites for Zn^{2+} with $Aβ_{1-40}$: one is the high affinity site (K_D=107 nmol·L^{-1}), and the other is the low affinity site (K_D=5.2 μmol·L^{-1})[29]. The affinity of $Aβ_{1-40}$

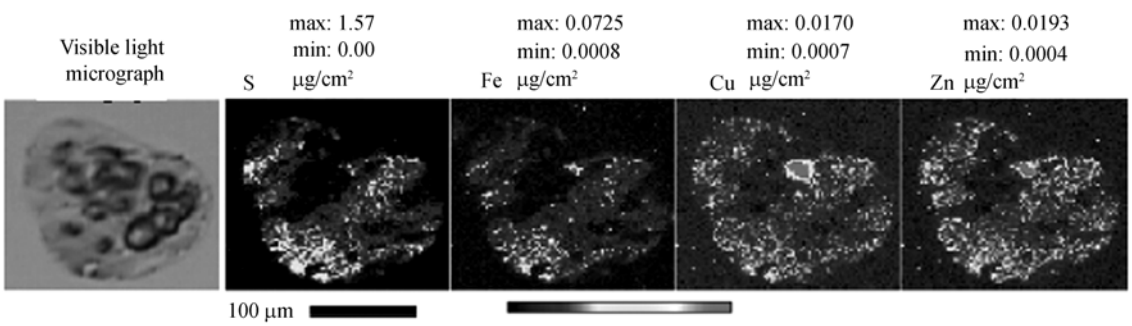

Figure 2 Elemental profiles in a typical Aβ amyloid plaque[22].

and $A\beta_{1-42}$ for Cu^{2+} are 10^{-10} and 10^{-18} $mol \cdot L^{-1}$, respectively[48,51]. The affinity of Aβ for Zn^{2+} and Cu^{2+} are so high that it is likely that Aβ is bound to them physiologically. Seeing the high affinity of Aβ for Cu^{2+} and the redox activity of Cu^{2+}, intensive studies of the interaction of Cu^{2+} with Aβ have been made, and the main results obtained so far are summarized in Table 1.

Experimental data suggest that the coordination of metal ions makes the conformation of Aβ transit markedly, and further promote or inhibit the aggregation of

Table 1 The main results of Cu-Aβ interactions[31]

Peptide	Solvent	Method	Observations	K_D ($\mu mol \cdot L^{-1}$)	Binding site	Ref.
Haβ6	H_2O	potentiometric curves, EPR, UV-vis, CD	$CuH_{-2}L$ dominant at neutral pH	lgβ provided	3N His, 2 amide NH	[32]
Haβ9	H_2O	potentiometric curves, EPR, UV-vis, CD	$CuH_{-1}L$ dominant at neutral pH	lgβ provided	3N His, 1 amide NH, NH_2	[32]
Maβ9	H_2O	potentiometric curves, EPR, UV-vis, CD	$CuH_{-1}L$ dominant at neutral pH	lgβ provided	3N His, 1 amide NH, NH_2	[32]
Haβ16	H_2O	Raman spectroscopy aggregation assays	induction of aggregates at pH < 6.6		His-Cu-His bridges (insoluble) His, 3 amide NH (soluble)	[52]
	H_2O	potentiometric curves, EPR, UV-vis, CD	CuH_2L dominant at neutral pH	logβ provided	3N His, His, His	[33]
	H_2O	fluorescence		47		[43]
Maβ16	H_2O	potentiometric curves, EPR, UV-vis, CD	$CuH_{-1}L$ and CuHL dominant at neutral pH	lgβ provided	2N (CuHL) His, His 3N ($CuH_{-1}L$) His, 2 amide NH	[33]
Haβ28	H_2O/D_2O	NMR, EPR, CD	formation of precipitate no reduction to Cu(I)		3N1O His, His, His, Tyr	[46]
	H_2O	potentiometric curves, EPR, UV-vis, CD	CuHL dominant at neutral pH	lgβ provided	3N His, His, NH_2	[33]
	H_2O	EPR (20 K), CD, NMR, fluorescence	EPR spectra are pH Dependent	0.1－0.01	3N1O (pH 5) 4N (pH 10) His, His, His, NH_2	[45]
	H_2O	EPR (20 K)				[43]
	H_2O	EPR (110 K)	Ph dependence and Cu(II) conc dependence	28	type 2 square-planar Cu(II) change of coordination at pH 5.5－6 and 7－7.5	[34]
Maβ28	H_2O	potentiometric curves, EPR, UV-vis, CD	CuHL dominant at neutral pH	lgβ provided	3N His, amide NH, NH_2	[33]
Raβ28	H_2O/D_2O	NMR, EPR, CD	formation of precipitate no reduction to Cu(I)		$2N_2O$	[46]
Haβ40	H_2O	competitive metal capture analysis		5.0×10^{-5} (pH 7.4) 2.5×10^{-4} (pH 6.6)		[48]
	SDS/D_2O	NMR, EPR	no reduction to Cu(I)		3N1O His, His, His, Tyr	[46]
	H_2O	Raman spectroscopy aggregation assays	induction of aggregates at pH < 6.6		His-Cu-His bridges (insoluble) His + 3 amide NH (soluble)	[52]
	H_2O	fluorescence		1.6 (pH 7.4)		[35]
	H_2O	EPR, cyclic voltammetry	76% reduction to Cu(I)		3N1O	[47]
	H_2O+glycerol 50%	EPR (10 K)	protofilaments 6 nm wide 1:1 ratio in fibrils		3N1O in both soluble complex and fibrils	[44]
	H_2O+glycerol 50%	EPR (20 K), fluorescence	filaments 5.5 nm wide 1:1 ratio in fibrils	11	3N1O in both soluble complex and fibrils His, His, NH_2, O donor not Tyr	[43]
Haβ42	H_2O	competitive metal capture analysis		6.3×10^{-12}(pH 7.4) 5.0×10^{-11} (pH 6.6)		[48]
	H_2O	fluorescence		2.0 (pH 7.4)		[35]
	H_2O	EPR (77 K)		role of Tyr-10 in reduction to Cu(I)	3N1O	[36]
	H_2O	Raman spectroscopy	senile plaque cores were also investigated; extensive Met oxidation observed		His residues	[37]

Aβ[38]. A rigorous study of the mechanism of the confomational transition of metal-Aβ complexes is critical to understanding the effects of metal ions on the conformational conversions of Aβ and on its aggregation. Because of the fibril's extreme insolubility and the monomer's high propensity to aggregate, investigation of the conformational conversions of Aβ in the atomic detail by means of experimental methods is still intractable[53]. Computational simulation with its extremely high time resolution and atomic level representation has been increasingly used in understanding the conformational conversions and the aggregation mechanism of Aβ[53—59].

Evidence obtained so far indicates that Zn^{2+} appears to be the major neurochemical factor responsible for aggregating Aβ. Bush et al.[29,30] originally found that Zn^{2+}, at low micromolar concentrations, rapidly precipitated soluble Aβ into proteaseresistant amyloid aggregates *in vitro*. Although Zn^{2+} is the only physiologically available metal ion to precipitate Aβ at pH 7.4[29,39], Cu^{2+}(and Fe^{3+} to a lesser extent) induces limited Aβ aggregation[39], which is exaggerated by slightly acidic conditions. Recently, for elucidating the mechanism of Zn^{2+}-induced aggregation, we have explored the possible binding modes of Zn^{2+} with $Aβ_{10-21}$ in different environments, that is, in soluble complexes and in insoluble aggregates, by molecular modeling[60]. The computational results show that the basic mode of Zn^{2+}-induced aggregation is the His13(Nτ)-Zn^{2+}-His14(Nτ) bridges through which different Aβ strands are crosslinked. It is consistent with Miura's deduction from the Raman spectra[52]. This Zn^{2+}-bridge formation results in the rapid decrease of the system potential from −152.15 to −1692.98 kJ/mol and accordingly stabilizes the structure of the amyloid core, which clearly elucidates that the binding of Zn^{2+} with Nτ atoms is an important structural factor for stabilizing the aggregates. The study of the zinc transporter 3 (ZnT3) indicates that the amyloid deposits in the neocortex may be closely associated with the cerebral homeostasis of Zn^{2+}. ZnT3 is situated in the vesicular membrane, but the mechanism of its participation in transport of Zn^{2+} into the synaptic vesicle remains to be elucidated[61]. ZnT3 loads Zn^{2+} into synaptic vesicles within glutamatergic corticofugal fibers, which represent about 30% of brain Zn. During neurotransmission, Zn^{2+} is released in a low-affinity bound or exchangeable chemical form, and extracellular Zn concentrations reach <300 μmol·L^{-1} in the neocortex. Zn^{2+} reuptake after synaptic release is rapid and energy dependent, maintaining minimal basal levels in the interstitial spaces that are estimated to be <500 nmol·L^{-1}[62]. This efficient, energy-dependent Zn^{2+} flux would be a vulnerable site for energy depletion, which could cause pooling of extracellular Zn and the initiation of Aβ deposition. High concentration of Zn^{2+} (300 μmol·L^{-1}) during neurotransmission may explain why the precipitation of Aβ commences in the synapse. Genetic ablation of ZnT3 markedly inhibits cortical amyloid plaque deposition and congophilic angiopathy in the brains of the Tg2576 transgenic mouse model for AD[63].

At pH 7.4, Aβ precipitation by Cu^{2+} and Fe^{3+} is far less than that induced by Zn^{2+}[39], yet Cu^{2+} and Fe^{3+}[21,22] are also found at high levels in amyloid plaques. This suggests that when Aβ is precipitated by synaptic Zn^{2+}, it coprecipitates with Cu^{2+} and Fe^{3+}, a possibility supported by the observation of selective Cu^{2+}- and Zn^{2+}-binding sites on Aβ[48]. The Fe^{3+} in plaques is found predominantly in neuritic processes, probably complexed with ferritin[64], and might not directly interact with plaque Aβ because, unlike Cu^{2+} and Zn^{2+}, Fe^{3+} does not co-purify with Aβ from postmortem AD brain tissue[65]. In addition, studies of various metal-ion chelators in solubilizing Aβ from postmortem AD-affected brain tissue have correlated the dissolution of precipitated Aβ with the release of Cu^{2+} and Zn^{2+}, but not Fe^{3+}[66].

Different from Zn^{2+} in a wide pH range (>6.0), Cu^{2+} induce Aβ aggregation only at mildly acidic pH which represents physiological acidosis, and yet strongly inhibit Aβ from aggregation at neutral and basic pH[27,39]. This interesting di-direction(up/down) modulation property of Cu^{2+} on the aggregation of Aβ is also found in the interaction of Cu^{2+} with the Prion protein[67] and the silk fibroin[68]. Cu^{2+} not only strongly inhibit Aβ aggregation under physiological pH[69—71], but also compete with Zn^{2+} and inhibit the Zn^{2+}-induced aggregation of Aβ at a Cu^{2+}/Aβ molar ratio of around 4[70]. We have examined the inhibitory mechanism of Cu^{2+} on the aggregation of Aβ by molecular modeling. The interaction of Cu^{2+} with Aβ is characterized in the soluble binding mode which differs significantly in the insoluble binding mode of intermolecular cross-linking by Zn^{2+}-bridge-bond. In the mono-ring mode, the Y10 residue promotes

quasi-helix conformation of Aβ. Particularly, [Cu-H13 (Nπ)-Y10(OH)] complex forms a local 3.010 helix conformation (Figure 3). In the multi-ring mode, the side chains of Q15 and E11 residues collaborate harmoniously with other chelating ligands and result in the lower potential and quasi-helix conformations[72]. [Cu-3N-Q15(O)-E11 (O1)] and [Cu-H13(Nπ)-Y10(OH)] complex with quasi-helix conformations may prefer soluble forms in solution. The above results are in good agreement with that of the relative experiments[73]. In brief, metal-ions- induced aggregation of Aβ is a key pathological event in the molecular mechanism of AD, but its detailed mechanisms remain to be further elucidated. In addition, the abnormal combination of Aβ with Cu or Fe induces the production of hydrogen peroxide, which may mediate the conspicuous oxidative damage to the brain in AD.

3 Metal-Aβ interactions and the oxidative stress mechanism of AD

Increasing evidence has implicated oxidative stress in the pathogenesis of AD. Metabolic signs of oxidative stress in AD-affected neocortex include oxidative species of almost all essential biomacromolecules, such as proteins, DNA, and lipids[74]. Oxidative damage to neurons is one of the earliest pathological events in AD[75], and the link between the oxidative stress and the pathology of AD has drawn people's attention[9,10,76]. Aβ, both in soluble state and in aggregation state, catalyses H_2O_2 generation through the reduction of Cu^{2+} and Fe^{3+}, using O_2 and biological reducing agents (e.g., cholesterol and vitamin C) as substrates[47,65,77]. The metal-reducing activity and H_2O_2 production of Aβ species follows the order $hA\beta_{1-42}$>$hA\beta_{1-40}$>>$mA\beta_{1-40}$[47,78], corresponding to the neurotoxicity of the respective peptides in neuronal culture and their involvement in AD neuropathology. Aβ is not toxic in the absence of Cu^{2+}[65]. Aβ is markedly vulnerable to Cu^{2+}-mediated auto-oxidation, leading to carbonyl adduct formation, histidine loss, and dityrosine cross-linking, modifications which have been found on human Aβ extracted from AD amyloid[9]. The rat/mouse Aβ lacks tyrosine and does not form the crosslink after incubation with Cu^{2+}, perhaps also explaining why these animals do not develop AD pathology. The aggregate of Aβ may be the form with the enhanced redox activity, especially when it contains high concentrations of Cu^{2+} and Fe^{3+}[77]. Moreover, the enhanced redox activity may be the cause of higher neurotoxicity for the aggregate of Aβ. The high affinity chelators of Cu^{2+} and Fe^{3+} inhibit Cu^{2+} and Fe^{3+} from being reduced by Aβ, which indicates that the selective coordination of metal ions with some certain active sites of Aβ is essential to the eletronic tranfer between Aβ and Cu^{2+}/Fe^{3+}[47]. The single methionine residue at Position 35 in the lipophilic C-terminal part of Aβ, namely Met35, can reduce transition metals to their high-active low-valency forms[79], which further trigger the Fenton reaction and generate the highly reactive $OH^{\bullet}$, as well as is one of the key residues of metal-induced oxidative stress[80,81].

In vitro experiments indicate that the triad system, consisting of Aβ, redox metal ions and molecular oxygen, may be one of the important ways by which Aβ achieves its redox activity[77]. The substance basis for constructing the triad system exists in the brain, especially in the AD brain. First, the concentrations ofAβ in the brain are high enough to generate significant H_2O_2 levels that have the potential to harm the brain tissues. The concentration of Aβ in the brain is on the order of 100 μg/g of tissue, which corresponds to ap

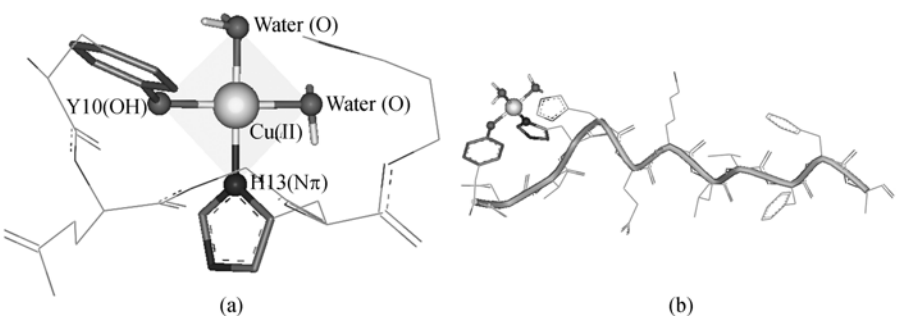

Figure 3 Coordination microenvironment (a) and local quasi-3.0_{10} helix conformation (b) of [Cu-H_{13}(Nπ)-Y_{10}(OH)] complex[72].

proximately 20 μmol·L^{-1}[82], assuming a tissue density of 1 g/mL. The experimental data indicate that 10 μmol·L^{-1} Aβ generates up to 25 μmol·L^{-1} H_2O_2 in one hour[77], depending on the O_2 tension. Second, the brain is a specialized organ that concentrates metal ions, the catalyzers of ROS. Cu and Fe are concentrated in the neocortex, but are highly enriched in cerebral amyloid deposits in AD, up to about 0.4 and 1.0 mmol·L^{-1}, respectively[21]. In addition, the brain is the right organ with the highest activity of metabolism since the brain accounts for 20%—25% of the total body oxygen consumption but for less than 2% of the total body weight[83]. About 5% of the cell oxygen consumption is reduced as ROS. One of the sideproducts of the highly active metabolism is the increase of superoxide anion (O_2^-). Compared with the other tissues with the lower oxygen consumption, the ROS concentrations of the brain are generally higher, so the brain is the vulnerable region of oxidative damage.

Based on *in vitro* experimental results, Huang et al.[77] proposed the molecular mechanism of Aβ reduces Cu^{2+}, to a lesser extent, Fe^{3+}, to Cu^+ and Fe^{2+} with concurrent generation of ROS-H_2O_2 and $OH^{\bullet}$. This mechanistic scheme is outlined in the following reactions[22]: (1) Aβ reduces Cu^{2+}/Fe^{3+} to Cu^+/Fe^{2+}; (2) reduced Cu^+/Fe^{2+} reacts with molecular oxygen (O_2) to generate the superoxide anion (O_2^-); (3) the O_2^- generated undergoes dismutation to H_2O_2 and O_2 either catalyzed by SOD or spontaneously; (4) the reaction of reduced metals with H_2O_2 generates the highly reactive $OH^{\bullet}$ by the Fenton reaction (Cu^+ catalyzes this reaction at a rate constant magnitude higher than that for Fe^{2+}); (5) the Haber-Weiss reaction can form $OH^{\bullet}$ in a reaction catalyzed by $M^{(n+1)+}/M^{n+}$.

$$(A\beta)_2+M^{(n+1)+}=A\beta{:}A\beta^{+\bullet}+M^{n+} \quad (1)$$

$$M^{n+}+O_2=M^{(n+1)+}+O_2^- \quad (2)$$

$$O_2^-+O_2^-+2H^+=H_2O_2+O_2 \quad (3)$$

$$M^{n+}+H_2O_2=M^{(n+1)+}+OH^{\bullet}+OH^- \text{ (Fenton reaction)} \quad (4)$$

$$O_2^-+H_2O_2=OH^{\bullet}+OH^-+O_2 \text{ (Haber-Weiss reaction)} \quad (5)$$

Unless scavenged by catalase and/or glutathione peroxidase, H_2O_2 that is freely permeable across all tissue boundaries, will react with reduced metal ions (Fe^{2+}, Cu^+) to generate $OH^{\bullet}$, which generates lipid peroxidation adducts, protein carbonyl modifications and nucleic acid adducts (such as 8-OH guanosine) in various cellular compartments[84]. H_2O_2 production by Aβ can be inhibited by chelators[47]. Zn^{2+} partially quenches H_2O_2 production from Aβ-Cu complexes, rescuing its toxicity in cell culture while simultaneously precipitating the peptide[85,86]. This effect is consistent with the observation that the quantity of H_2O_2-mediated 8-OHG adducts in neocortical tissue affected by AD is inversely proportional to plaque surface area[86]. These findings suggest that plaque β-amyloid, although conspicuous, is not as toxic as soluble or diffuse forms of Aβ, which are probably not bound to Zn^{2+}[10]. In sum, metal ions, Aβ and ROS, all together compose a complex redox system, and the investigation of the redox mechanism of metal ions with Aβ is significant for understanding the pathology of oxidative stress to AD.

4 Effects of metal-Aβ interactions on ion-channels

A growing number of reports suggest that Aβ can directly incorporate into lipid bilayer membranes to form ion-channel-like structure[87—92]. Kawahara et al.[88] exposed the inside-out membrane patches from immortalized hypothalamic neurons (GT1-7 cells), which are derived from murine hypothalamic neurons, to a solution of $A\beta_{1-40}$. Within 3—30 min of the addition of $A\beta_{1-40}$ to the bath solution, a current appeared across the excised membrane patches with spontaneous conductance changes over a wide range of 50—500 pS. The amyloid channels were cation-selective, and the channel activity was inhibited by the addition of 250 mmol·L^{-1} of zinc in the bath solution. Thus, it is concluded that Aβ is directly incorporated into neuronal membranes to form channels. Aβ induces non-selective current in neurons of rat[89] and frog[90] and transports Ca^{2+} into lipids. This process can be interdicted by Aβ antibody[91,92]. In addition, other amyloid proteins also form channels which are interdicted by Zn^{2+} and disrupt Ca^{2+} homeostasis[93].

Using the whole-cell voltage-clamp technique, we showed that Zn^{2+}-induced aggregation of $A\beta_{10-21}$ potentiates its action on outward potassium currents in hippocampal CA1 pyramidal neurons[94]. $A\beta_{10-21}$ blocked the fast-inactivating outward potassium current (I_A) in a concentration- and aggregation-dependent manner, but with no effect on the delayed rectifier potassium current (I_K). Both the unaggregated and aggregated forms of

$A\beta_{10-21}$ significantly shifted the activation curve and the inactivation curve of IA to more negative potentials. However, the aggregated form has more effects than the unaggregated form. In addition, we found that the action of AChE inhibitors may be related to potassium channels, which might be another new target for AChE inhibitors besides AChE itself[95].

Curtain et al.[34,46] have used a combination of EPR(utilizing spin-labelled lipids) and CD spectroscopy to study the interaction of Aβ with bilayer membranes and the effect of metal ions. The EPR data indicate that $A\beta_{1-40}$ and $A\beta_{1-42}$ bound to Cu^{2+} or Zn^{2+} penetrated the lipid membrane. The lipid∶peptide ratio is approximately 4∶1. This stoichiometry can be satisfied by 6 helices arranged in a pore surrounded by 24 boundary lipids, and this structure is consistent with atomic force microscopy studies of $A\beta_{1-42}$ reconstituted in a planar lipid bilayer (Figure 4(a)—(c)) that exhibited multimeric channel-like structures[96]. In the presence of Zn^{2+}, $A\beta_{1-40}$ and $A\beta_{1-42}$ both were inserted into the bilayer over the pH range 5.5—7.5, as did $A\beta_{1-42}$ in the presence of Cu^{2+}. However, $A\beta_{1-40}$ only penetrated the lipid bilayer in the presence of Cu^{2+} at pH 5.5—6.5; at higher pH, there was a change in the Cu^{2+} coordination sphere that inhibited membrane insertion. CD spectroscopy revealed that the Aβ peptides had a high α-helix content when membrame penetrated, but were predominantly β-strand.

Employing molecular dynamics simulations, Kim et al.[97] showed an amphipathic α-helical conformation of $A\beta_{1-28}$ that allows for favourable electrostatic interactions between monomers and consequently makes possible the formation of tetrameric (Figure 4(d)) and pentameric (Figure 4(e)) aggregates. The mechanism of an α-helix channel formation may occur through voltage-induced insertion of the monomer (aided by the helix dipole 'driving force') followed by aggregation within the bilayer. The length of both pores are ~38 Å with an average pore radius of ~4 Å for the tetramer and ~8 Å for the pentamer. The tetrameric and the pentameric pores have a conductance of ~52 and ~311 pS, respectively. The minimal radii for both pores were due to the His6 imidazol and the Tyr10 phenol moieties pointing to-wards the pore axis. These rings form the bottleneck of the aggregate pores reducing the radius of

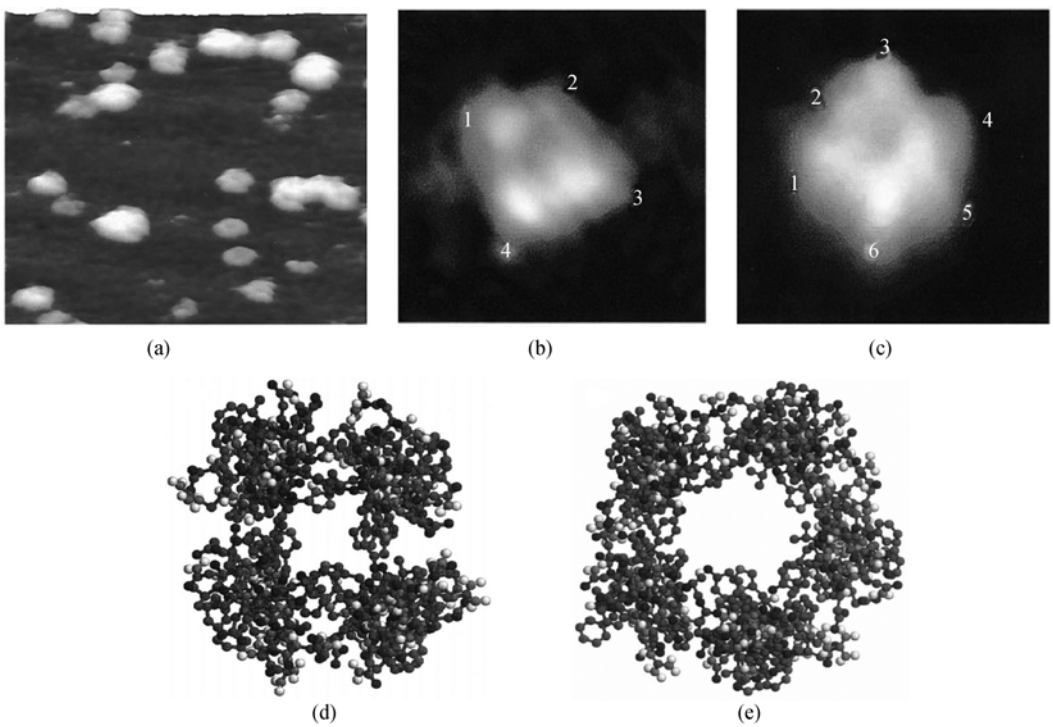

Figure 4 High-resolution images of $A\beta_{1-42}$ channels[96]. (a) Surface plots. Off-line zoomed images of $A\beta_{1-42}$ channels: four apparent subunits (b), six apparent subunits (c). Channels of tetrameric (d), pentameric aggregates of $A\beta_{1-28}$ (e)[97].

the channels by several angstroms. The protrusion of the His6 side chains not only acts as a size filter for the channel, but also may transiently bind Ca^{2+} ions. It is predicted that the transient binding of Ca^{2+} ions to histidine may aid cationic selectivity.

The cellular toxicity of Aβ channels includes the following points. First, Aβs or Aβ aggregates insert into the membranes. They not only disturb the normal structure of membranes and reduce the fluidity, but also influence the structure and function of ion channels located in membranes originally, even destroy the integrality of membranes, and result in the death of neurons[98]. Second, Aβ channels reduce the membrane potential, allow excessive calcium influx and disrupt the normal cellular calcium homeostasis[99,100]. Aβ antibody markedly inhibits the increase of $[Ca^{2+}]_i$ by Aβ, and yet the interdiction agent of calcium channels does not, which indicates that the path via which $[Ca^{2+}]_i$ increases is Aβ channels rather than calcium channels[101]. The overburden of $[Ca^{2+}]_i$ not only damages oxidative phosphorylation, but also results in the aberrant action of calcium-dependent ATP enzyme, leading to lack of energy for cells, even being exhausted, and the dysfunction and structure destruction of cells. Especially, the increase in $[Ca^{2+}]_i$ itself enhances the secretion of Aβ from APP, by which unregulated calcium influx is amplified and a vicious circle is initiated[102]. Third, Aβ oligomers in the channels interact with redox metal ions (Fe^{3+}/Cu^{2+}) and generate H_2O_2, which further generate $OH^{\bullet}$ by Fenton reaction and other ROS resulting in the oxidative lesion of essential macromolecules in cells (DNA, proteins, and lipids)[47,77], and the dysfunction, even death of cells[74].

5 Bush's model for metal-Aβ interactions

Based on a large body of the recent research results, Bush[10] proposed the model for the metallobiology of Aβ in AD in 2003, which clearly draws the outline of the molecular mechanism of AD from the viewpoint of the metal-Aβ interactions (Figure 5). The proposed sequence of biochemical events leading to AD is the following. (1) The concentrations of Fe and Cu rise with increasing age in the brain cortex. This leads to an overproduction of amyloid precursor protein and Aβ in an attempt to suppress cellular metal-ion levels. If metal-ion levels continue to rise, hypermetallation of Aβ occurs, facilitated by mild acidosis. (2) Some forms of hypermetallated Aβ catalytically produce H_2O_2 from O_2 and biological reducing agents. (3) Aβ-Cu reacts with H_2O_2 to generate oxidized and crosslinked forms that are liberated from the membrane. Oxidation of Aβ makes it protease resistant. (4) These oxidized forms of Aβ are the major components of plaque deposits. The release of soluble Aβ presents the peptide for precipitation by the high concentrations of Zn released in the synaptic vicinity. Plaques are, therefore, an admixture of Aβ with high concentrations of Zn, Cu, and Fe. (5) The oxidized Aβ initiates microglial activation. The microglia characteristically react by producing high concentrations of H_2O_2 and myeloperoxidase, which fosters further crosslinking of Aβ and H_2O_2 build-up outside the cortical cells. (6) H_2O_2 is freely permeable across lipid boundaries and it crosses from the outside of the cell into cellular compartments, where it reacts with Cu and Fe (the levels of these having been elevated by age), causing the production of the highly reactive hydroxyl radical, and the oxidation of nucleic acids, proteins, and lipids that characterize AD-affected brain tissue.

6 Pharmacological interdiction of metal-Aβ interactions as the basis for novel AD therapeutics

Despite approval of several drugs for AD, the disease continues to rob millions of their memories and their lives. As the most recently reviewed by Mucke et al.[3], basic research is identifying many of the pathways that contribute to this devastating disease, providing unprecedented opportunities for the development of new treatments aimed at the root causes of AD. Among the investigative AD therapies in clinical trials are several strategies to block pathogenic Aβ and to rescue vulnerable neurons from degeneration. In this paper, the greatest emphasis is on the abolition of Aβ accumulation in the brain, which is based on the metal-Aβ interactions.

The molecular mechanism of AD metioned above indicates that metal-Aβ interactions play a crucial role in the neurotoxicity of Aβ. It is the interdiction of metal-Aβ interactions that is the key step to depress the neurotoxicity of Aβ[10]. Cu and Zn are enriched in Aβ deposits in AD, which are solubilized by Cu/Zn-selective chelators *in vitro*[41]. Cherny et al. reported two high efficient chelators for Cu^{2+}/Zn^{2+}, *N*, *N*, *N*′,*N*′-tetrakis (2-pyridylmethyl)-ethylene diamine (TPEN) and bathocuproine

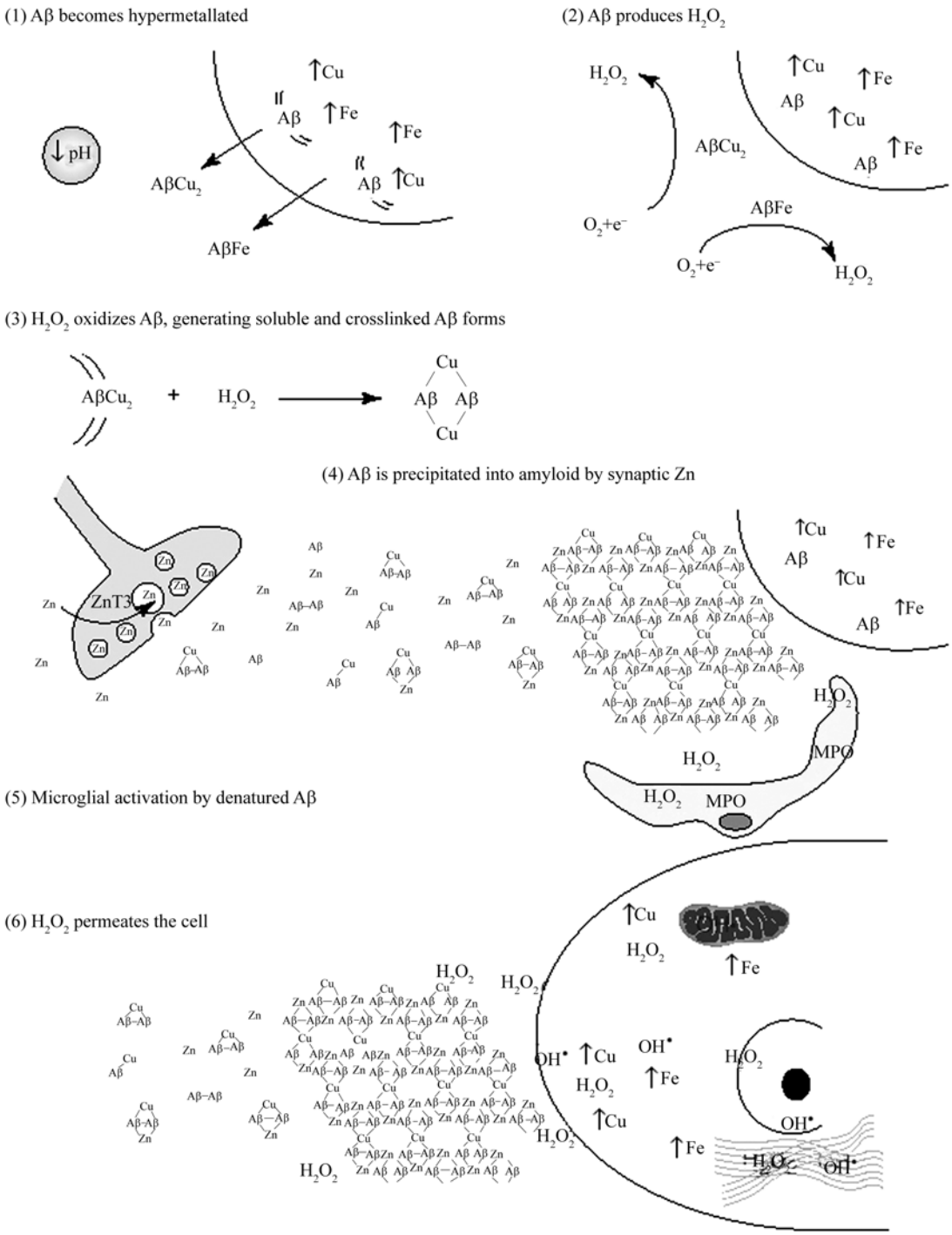

Figure 5 The model for the metallobiology of Aβ[10].

(BC), which redissolve the Aβ deposits[66]. The pK_a values of TPEN with Cu^{2+} and Zn^{2+} are 20.2 and 15.4, respectively. These data indicate that metal ions are the important compositions of amyloid deposits, and chelators that redissolve the deposits have the possibility of being a kind of therapeutic agent. However, the animal model and preclinical trials suggest that systemic metal-ion depletion, e.g., 'chelation therapy', is not likely to be a useful therapeutic strategy for AD[10,103]. DFO (desferrioxamine), a metal chelator with high-affinity for zinc, copper, iron, and aluminum, was reported to induce a significant slowing in the rate of progression

of dementia. Further clinical research into the effects of DFO may have been met with diminished enthusiasm since the administration of DFO is associated with discouraging difficulties including the non-specific problems of systemic metal ion depletion (e.g., anemia). Also, DFO is a charged molecule that does not easily penetrate the blood-brain barrier and is easily degraded after it is administered[103]. TETA (triene), another high-affinity copper/zinc chelator that does not penetrate the BBB, failed to inhibit amyloid deposition in the transgenic mice[41]. As a further therapeutic strategy, a new type of compounds based on inhibiting metal-Aβ interactions, known as metal-protein attenuating compounds(MPACs), has been designed and developed prosperously[104]. Unlike common chelators which systematically remove metal ions, MPACs target at interdicting aberrant metal-Aβ interactions through the following: (1) competition with the target protein for the metal ions; (2) leading to a normalisation of metal homeostasis. Clioquinol (CQ), 5-chloro-7-iodo-8-hydroxyquinoline, is a typical potential MPACs-type compound, originally being a hydrophobic Zn and Cu chelator that freely crosses the blood-brain barrier[105]. Its efficacy tested in transgenic Tg2576 mice showed a dramatic 49% decrease in brain Aβ deposition after 9 weeks of oral treatment[41]. Oral clioquinol treatment was statistically significant in preventing cognitive deterioration in the moderately severe Alzheimer's disease patient group, with no evidence of toxicity[106]. CQ may exert its efficacy on AD by the following two approaches: one is to reduce Aβ amyloid burden by reversing the precipitation of Aβ; the other is to depress the neurotoxic from oxidative stress by inhibiting the generation of ROS species, such as hydrogen peroxide[107].

The most prominent characteristic of CQ treatment may be its capability of restoring homeostatic defects of normal brain metal metabolism which may occur in AD[106]. Experiments find that brain Cu and Zn levels are relatively decreased by APP transgene expression in APP2576 mice, despite Aβ levels accumulating several hundred fold. This relative decrease must either be due to secreted APP and/or Aβ promoting the efflux of the metal ions, or APP/Aβ preventing their uptake. Treatment of 21-month-old Tg2576 mice with CQ for 9 weeks paradoxically elevated brain Cu by 19% and Zn by 13% while markedly inhibiting Aβ deposition[106]. A marked increase (about 50%) in Cu and Fe levels occurred after 6 months of age. The paradoxical increase in Cu and Zn in CQ-treated APP2576 mice may be explained by CQ preventing Cu^{2+} and Zn^{2+} from complexing with extracellular Aβ, securing metal for uptake into metal-deficient brain tissue instead of being sequestered into amyloid. The consequent lowering of extracellular metal concentrations inhibited the formation, or facilitated the dissolution, of amyloid deposits.

Compared with traditional chelators, e.g., DFO and TETA, CQ treatments in the Tg2576 mice model exhibit excellent performance, namely, high efficacy and low toxicity. Summarization of the underlying cause is significant for guiding the development of this type of drug candidate. It might be the following characteristics which ensure that CQ not only interdicts metal-Aβ interactions, but also does not result in the metal depletion for tissues[106]: (1) the ability to bind selectively to the metal-Aβ complex, not to deplete metal from brain tissue without selectivity; (2) the moderate affinity of CQ for metal ions. CQ is as effective as high-affinity chelators in blocking the production of H_2O_2 by Aβ *in vitro*, in preventing precipitation of synthetic Aβ by Zn^{2+} and Cu^{2+}, and in extracting Aβ from post-mortem AD brain specimens[41]; (3) the penetration of the blood-brain barrier. Clioquinol hopefully becomes the first therapeutic medication that interdicts the metal-Aβ interactions, and has been in a randomised, double blind, placebo-controlled pilot Phase II clinical trial[106,108]. Other similarities or novel compounds are also in active development, such as PBT2, a new chemical entity based around the 8-OH quinoline structure that has passed phase I clinical trial[107].

7 Prospects

Along with the rapid growth of the oldest age groups of the population, AD has become the leading neurodegenerative disorder which seriously threatens the health of the aged worldwide. Prevalence estimates indicate that there were 4.5 million persons with AD in the US population in 2000, and at least $100 billion is spent a year on direct care alone. By 2050, the projected number of AD patients could range from 11.3 million to 16 million in the United States of America if no cure or preventive measure for AD is found[109]. According to a conservative estimate, there are above 6 million persons affected by AD in China, the morbidity of the group who are 65

years and older is 5%. By 2050, it is predicted that the aged who are 65 and 80 years and older will account for 35% and 22% of the total population, respectively; and China will meet her first peak of the incidence of AD. The predicted increase in AD cases over the next few decades makes the development of better treatments a matter of utmost importance and urgency.

As a disorder of the most complex of physiological systems, the human cerebral cortex, the molecular mechanism of AD is doubtless a real challenge[110]. As shown in this paper, metal-Aβ interactions may be one of the hinges of the whole molecular mechanism of AD[10]. Although 'the tip of the iceberg' has emerged, further systematic research still needs to be accelerated: (1) completely understanding the normal metabolism mechanism of metal ions and Aβ in the brain; (2) elucidating the mechanism and key factors resulting in the abnormal metabolism of metal ions and Aβ in the brain; (3) deeply understanding the biochemistry of the abnormal metal-Aβ interactions and its relationship with the pathological events of AD. With the increasingly rapid progress of the molecular mechanisms of AD and many new therapies directly targeting the mechanisms underlying AD are now in the pipeline[3,107], we are entering a new dawn that promises the delivery of revolutionary developments for the control of dementias. We hope that by ceaseless exertions humans could overwhelm AD eventually.

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