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Review

Metal based neurodegenerative diseases—From molecular mechanisms to therapeutic strategies☆

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Contents

Abstract

The hypothesis is presented that changes in metal ion homeostatic control, particularly of redox-active metals such as iron and copper, in specific brain regions, leads to the generation of reactive oxygen species, which either directly damage key proteins, or lead to the formation of reactive aldehydes. These, in turn, generate protein carbonyls, leading to protein denaturation, aggregation, and a failure of the ubiquitin/proteasome system to eliminate these defective proteins. We present the evidence for metal based neurodegeneration in Parkinson's and Alzheimer's disease. Possible therapeutic strategies are presented which could remove such excesses of these specific metals and lead to the diminishment of the neurodegenerative processes.

Keywords: Neurodegeneration; Iron; Copper; Zinc; Parkinson's disease; Alzheimer's disease; Therapeutics

1. Metals in brain, metal transport, storage and homeostasis

Metal ions are absolutely essential to fulfil a series of important biological functions in the brain, such as nerve transmission, synthesis/metabolism of neurotransmitters and oxygen transport. The alkali metal ions Na^+ and K^+ play a crucial role in the transmission of nervous impulses. Calcium ions also play an important role in signal transduction; most eukaryotic cells either export or deposit calcium within membrane-enclosed storage sites to maintain free cytosolic Ca^{2+} levels at 100–200 nM, roughly 10,000 times less than in the extracellular space. This allows calcium to function as a second messenger and a carrier of biological signals that guide cells from their origins to their ultimate death. More recently, considerable attention has been directed to the role of transition metal ions, e.g. copper, zinc and iron, in the brain and their involvement in neurological diseases. Relatively high concentrations of these metals are present within the different cellular compartments, at concentrations ranging from 100 to 1000 μM, such that if misregulation of their homeostatic controls occurs, toxicity could

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ensue, mediated by oxidative stress in the case of copper and iron.

Oxidative stress has been identified in many neurodegenerative diseases, and is commonly associated with increased levels of at least one of these transition metal ions in specific brain regions. Transporters for copper, zinc and iron play an important part in the distribution of these metals intracellularly, such that defects in their regulation, which could possibly occur with ageing, may create an environment which could result in protein aggregation, thereby accelerating degenerative conditions. In addition, several of the metal ion transporter systems are energy dependent such that changes in the production/consumption might perturb metal content.

Zinc plays an important role in both catalysis and stabilization of protein structures, including zinc-finger proteins, many of which bind to DNA promoter sites. Zn homeostasis in humans requires the coordinated activity of several Zn transporter families, as well as metallothioneins for its storage. Zinc uptake across the blood brain barrier into the blood-cerebrospinal fluid is facilitated by L-and D-histidine, but a specific transporter, if involved, has not been identified. The putative roles of zinc transporters, ZnT and Zip, in regulating brain zinc content between different brain regions require further studies. Zinc metalloproteins are present in neurons and glial cells, as well as being concentrated in the limbic system, i.e. the hippocampus and amygdala. Approximately 10–15% of the total zinc in the brain, probably as ionic zinc, exists in the synaptic vesicles, and may serve as an endogenous neuromodulator in synaptic neurotransmission. Excessive excitation of zinc-containing glutamatinergic neurons causes a decrease in vesicular zinc, with a transient increase in the synaptic cleft, from a basal level of <0.5 μM to around 300 μM [1] which may influence neurotransmission [2]. Zn^{2+} reuptake after synaptic release is a rapid, energy dependent process, such that any energy depletion could cause an increase in this pool of extracellular Zn^{2+}. The synaptic zinc pool, is regulated by the zinc transporter ZnT3, which is influenced by oestrogen, thus explaining why females are more prone to Alzheimer's disease (AD) than males [3].

Copper (Cu) is an essential element in many metalloproteins which are involved in a multitude of cellular functions which range from cellular respiration, ferroxidase activity, pigment formation, neurotransmitter biosynthesis to antioxidant defences. Copper is transported from the plasma into the brain via Ctr1, a plasma membrane protein. Intracellular copper metabolism is dependent on copper transporters, Atpases, Atp7a and Atp7b. In the brain Atp7a is expressed in endothelial cells of the blood brain barrier and facilitates copper movement across the basolateral membrane into the extra-vascular space of the brain. Atp7a is expressed within specific populations of neurons in several brain regions including the cerebellum and hippocampus. Intracellular trafficking of copper requires metallo-chaperones that direct copper to specific cellular pathways [4] which include Atox1 (delivers copper to copper transporting ATPases in the late Golgi), CCS (required for copper incorporation into Cu/Zn superoxide dismutase), and Cox17, Sco1 and Sco2 (delivers copper to mitochondrial cytochrome *c* oxidase). Copper is most abundant within the basal ganglia as well as in the cerebellar

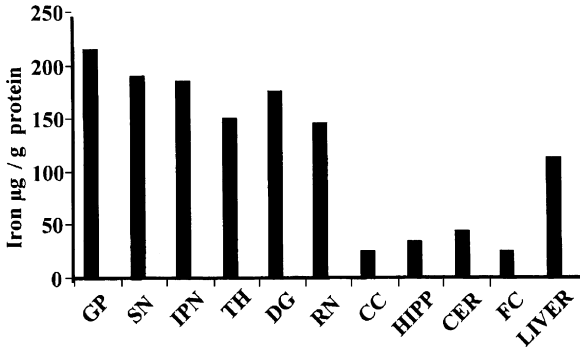

Fig. 1. Distribution of iron in human brain. GP globus pallidus; SN, substantia nigra; IPN, interpeduncular nucleus; TH, thalamus; DG, dentate gyrus. RN, red nucleus; CC, cerebral cortex; HIPP, hippocampus; CER, cerebellum; FC, frontal cortex [7].

granular neurons, the neuropil of the cerebral cortex and the hippocampus. Copper can also be released at the synapse in some neurons, reaching micromolar concentrations, which may abrogate long-term potentiation in the hippocampus.

Iron (Fe) is required as a co-factor in the central nervous system metabolic processes which include oxidative phosphorylation, neurotransmitter production, nitric oxide metabolism and oxygen transport. Enormous advances have been made in the last 10 years in understanding iron homeostasis [5,6], from the identification of genes and proteins involved in its uptake and transport, e.g. DcytB, DMT1, ferroportin, modulation of their translation by IRP-1 and IRP-2, and proteins involved in its storage, ferritin and haemosiderin. Specific point mutations in these proteins, e.g. ferroportin, lead to excessive iron loading in some tissue although, as yet, no excessive accumulation of iron in brain has been reported in such affected subjects. This could indicate other unique mechanisms for the regulation of brain iron. Exactly how the brain regulates fluxes and storage of iron into neurons, oligodendrocytes, astrocytes and glial cells remains an enigma. Although iron content within the brain is less than 2% of total body iron content, there is extensive regional variations with some brain regions, such as the substantia nigra and the globus palidus, containing higher iron concentrations than liver when expressed as μg/g protein [7] (Fig. 1).

2. Oxidative stress and redox-active metal ions

The most potentially dangerous reactive species are the reactive oxygen species (ROS), for example the short lived hydroxyl ion, and reactive nitrogen species (RNS), for example nitric oxide (NO) and peroxynitrite ($ONOO^-$), which can cause damage to most biological molecules. Under normal conditions, such free radicals will be rapidly detoxified by the body's defence systems, but when greater amounts of ROS and RNS are produced, these overwhelm the cellular defence mechanisms leading to oxidative stress. This is the so-called oxygen paradox—oxygen is an absolute necessity for our energy-economical aerobic life style, yet it is a potential toxin.

There is considerable evidence that both ROS and RNS are involved in a number of neurodegenerative pathologies. ROS can initiate lipid peroxidation, attacking polyunsaturated fatty acids

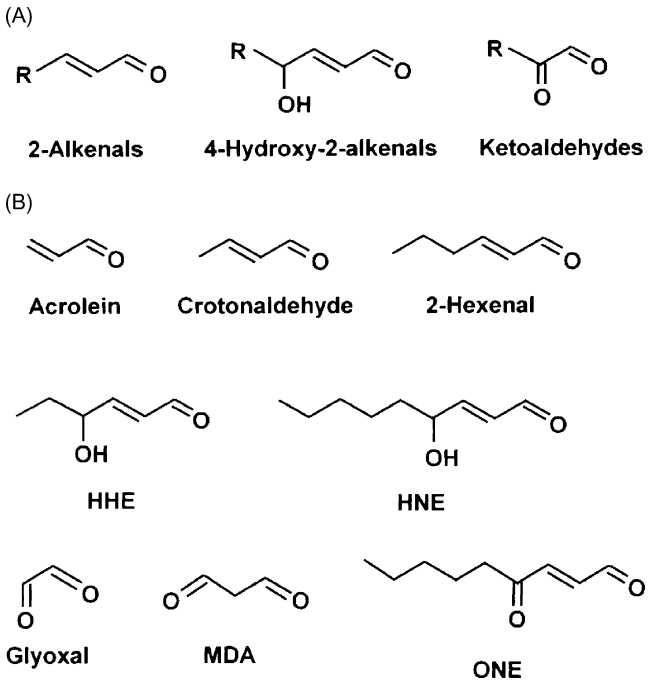

Fig. 2. Structures of reactive aldehydes a (A) and lipid-peroxidation-specific aldehydes (B) and (right) Michel type addition of 4-hydroxynonenol (HNE) to proteins [61].

in membrane phospholipids with production of a series of reactive aldehydes (Fig. 2, left). These can then lead to formation of protein carbonyls by Michael type addition to protein thiols, imidazoles and amines (Fig. 2, right). These will have particularly deleterious effects causing cellular damage, frequently associated with cell death either by necrosis or by apoptosis. In many of the neurodegenerative diseases increased levels of lipid peroxidation [8], protein carbonyl [9] and DNA damage [10] have been identified indicating enhanced oxidative stress. An important point to emphasise here is the very obvious reduced capacity of brain cells to protect themselves from ROS, the activity and levels of many of the cytoprotective enzymes and antioxidants are markedly reduced by comparison to other tissues, apart from anaerobic muscle [11,12]. Although the brain constitutes only 2% of adult body mass, it is responsible for 20% of resting oxygen consumption (even when sleeping). This is on account of its high demand for ATP production, around 50% of which is used to power the plasma membrane (Na^+–K^+)-ATPase, which maintains the membrane potential required for transmission of nerve impulses.

There is a growing body of evidence that changes in the homeostatic control of iron, copper, zinc and manganese will lead to mis-regulation of numerous proteins and metabolic systems (see [6]). Deficiency of certain metal ions during ageing or perhaps in brain development in utero, during early development or maturation in adolescent, could lead to decreases of such metals in specific brain regions could also contribute ultimately to neurodegenerative disorders. Increases in such metal ions may lead to inflammation, orchestrated by activated microglia which may also play a prominent role in the degenerative process. Recent studies have also indicated that an endogenous 'cholinergic anti-inflammatory pathway' is also involved in the regulation of inflammatory events, via α7 nicotinic acetylcholine receptors, present on phagocytic cells, i.e. macrophages and microglia [13,14].

3. Degradation of cellular proteins via proteasomal system

An important feature of many neurodegenerative diseases is the apparent inability of the cell to rid itself of unwanted proteins, which have been designated for degradation via the proteasomal system. In normal circumstances cellular proteins are degraded via the ATP-dependent ubiquitin system, which also involves the 26S proteasome (Fig. 3). Proteins, that have been marked out for degradation, are identified in the cell by covalent coupling to ubiquitin. Three steps are involved; firstly, ubiquitin is bound by its carboxyl terminus to ubiquitin-activating enzyme (E_1) in an ATP-dependent process. Secondly, ubiquitin is transferred to the thiol group of one of a number of ubiquitin-conjugating enzymes (E_2). Then, ubiquitin-protein ligase (E_3) transfers the ubiquitin from E_2 to the amino group of a Lys residue of its target protein, forming an isopeptide bond. Ubiquinated proteins are subsequently proteolytically degraded by the large (2000 kD) multi-subunit protein complex, the 26S proteasome. In this process, the ubiquitin molecules themselves are not degraded, but are returned to the cell for reutilisation. In addition, the activity of the 26S proteasome declines with age, which is a predisposing factor for many neurodegenerative conditions.

One of the fundamental molecular mechanisms underlying the pathogenesis of cell death in Parkinson's disease (PD), Alzheimer's disease and, possibly other 'protein conformational diseases', could be the production of ROS. These primarily occur as a result of increasing concentrations of redox-active metal ions, such as iron which catalyse the formation of the aggregated proteins. Electron spin resonance (ESR) spin-trapping method

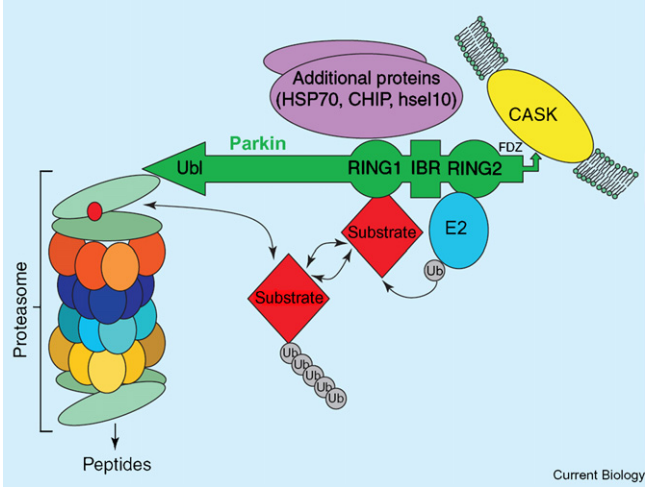

Fig. 3. The reaction involved in the attachment of ubiquitin to a protein. In the first part the carboxyl group of ubiquitin in coupled to E_1 by a thioether linkage in a reaction driven by ATP hydrolysis. The activated ubiquitin is subsequently transferred to a sulphydryl group of E_2 and then in a reaction catalysed by E_3 to the amino group of a Lys residue on a condemned protein, thereby flagging the protein for proteolytic degradation by the 26S proteasome [62].

Table 1
Characteristic protein aggregates in various neurodegenerative diseases and their location within the brain

Disease	Regions most affected	Characteristic pathology	Disease proteins deposited
Parkinson's	Substantia nigra Cortex, locus ceruleus	Lewy bodies Lewy neuritis	α-synuclein
Alzheimer's	Cortex, hippocampus Basal forebrain Brain stem	Neuritic plaques Neurofibrillary tangles	Aβ peptide (from APP) Hyperphosporylated tau
Huntington's	Striatum, basal ganglia Cortex	Intranuclear inclusions Cytoplasmic aggregates	Huntingtin with polyglutamine expansion
Amyotrophic lateral Sclerosis	Spinal motor neurons	Bonina bodies axonal spheroids	Unknown (neurofiliaments)
Prion	Cortex, thalamus Brain stem, cerebellum	Spongiform degeneration amyloid, other aggregates	Prion proteins

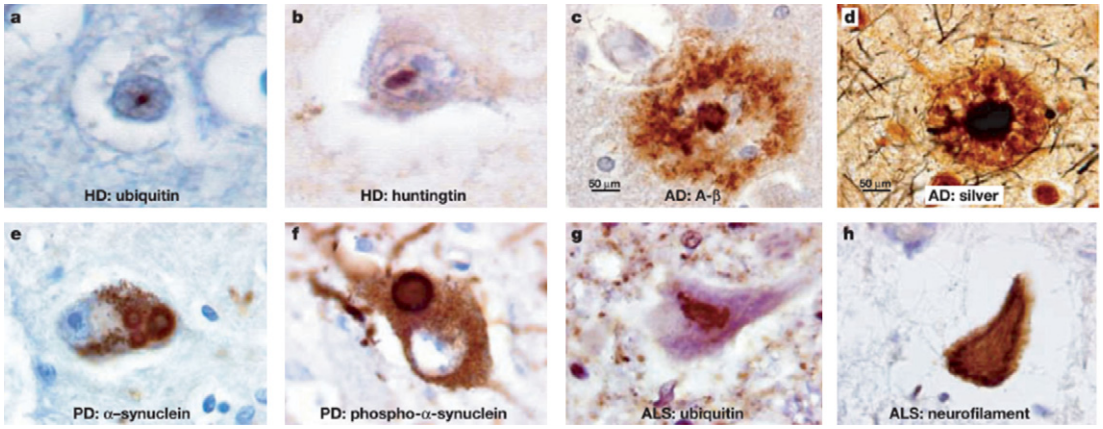

Fig. 4. Characteristic neurodegenerative disease neuropathological lesions involve deposition of abnormal proteins, which can be intranuclear, cytoplasmic or extracelular. All are labelled with antibodies (except d) as indicated. (a) and (b) HD, intranuclear inclusion labelled for ubiquitin and huntingtin (cerebral cortex). (c) and (d) AD, neuritic plaque labelled with Aβ (cerebral cortex) and silver stained. (e) and (f) PD, Lewy bodies labelled for α-synuclein (fine granular brown label in this and next panel represent neuromelanin) and phosphorylated α-synuclein (substantia nigra). (g) and (h) ALS, cytoplasmic skein of neurofilaments labelled with ubiquitin and with neurofilament (medulla oblongata) [63].

has shown that solutions of either Aβ or α-synuclein, incubated with small amounts of Fe^{2+} will rapidly release hydroxyl radicals [15]. In addition, various biophysical techniques have shown that both Aβ-40 and Aβ-42 deposits are formed, in vitro, after incubation of immobilised β-amyloid oligomers, with Cu^{2+}, Zn^{2+} or Fe^{3+} [16]. Each redox-active ion catalysed the formation of such amyloid deposits although only Fe^{3+} induced the deposition of fibrillar amyloid plaques, while Cu^{2+} and Zn^{2+} only induced the formation of amorphous aggregates [16]. Furthermore when Aβ is pre-treated with the iron chelator, deferoxamine, neuronal toxicity was significantly attenuated [17]. Such aggregates are a prominent pathological feature of many different 'protein conformational disease' including PD, AD, motor neurone disease, and 'prion' dementias etc., and are deposited in specific brain regions (Table 1, Fig. 4).

4. Parkinson's disease

Parkinson's disease was initially described as Shaking Palsy in 1817 by the English surgeon James Parkinson. PD is the second most common form of motor system degeneration, characterised by a progressive loss of dopaminergic neurons in the substantia nigra pars compacta in the ventral midbrain. Cumulative evidence supports an 'oxidative stress hypothesis' for initiation of nigral dopamine neuron loss (Fig. 5). The pres-

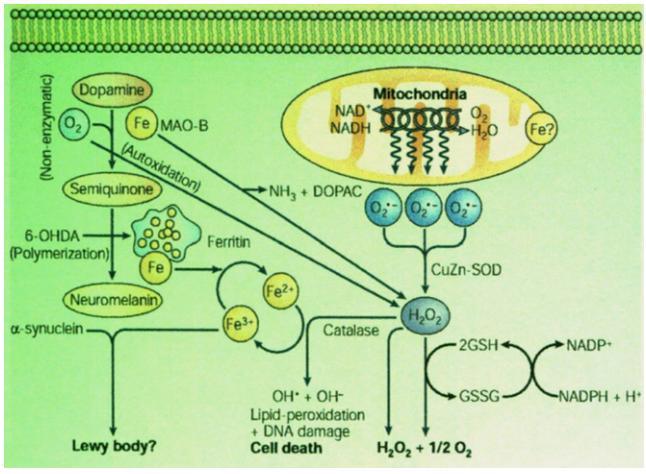

Fig. 5. Iron induced oxidative stress of Parkinson's disease [64].

ence of an increased burden of iron, approximately 2-fold, in specific brain regions, the substantia nigra and lateral globus pallidus will enhance oxidative stress [7]. H-ferritin rather than L-ferritin is present in the iron loaded SN and lateral pallidus of PD brain [18] with large amounts of iron being sequested into neuromelanin in dopaminergic neurons [19]. Furthermore, since the substantia nigra has a relatively high metabolic rate, with a high content of dopamine, neuromelanin, polyunsaturated fatty acids and iron, but low antioxidant protection, e.g. glutathione [20,21], oxidative stress will be enhanced. Both reactive oxygen and nitrogen intermediates will contribute to the demise of the dopaminergic neurons, leading to the formation of lipid peroxidation products, as well as protein carbonyls and DNA damage [22]. In addition, ROS, generated as a result of mitochondrial malfunction, will contribute to this toxicity [23].

The etiology of this enhanced brain iron content may be attributable to a variety of factors which include changes in iron release mechanisms across the blood brain barrier (BBB), or perhaps more likely, a mis-regulation of iron homeostatic control in the substantia nigra. In our recent studies [24] mRNA was isolated from two regions of Parkinson's brain, the substania nigra and the cortex, and the expression of a number of iron genes quantitated and compared with those from control post mortem material. A significant number of genes were specifically upregulated in the substantia nigra in comparison to the cortex in the PD brains as well as controls (Table 2). Such upregulation of both transferrin and transferrin receptor2 in other cell types is associated with iron deficiency [25] and inflammation [26]. The high iron content of the substantia nigra might have been expected to diminish IRP-1 and IRP-activity. However IRP-1 expression did not alter significantly whilst IRP-2, which dominates post-transcriptional regulation of brain iron metabolism [27], was upregulated. A previous study [28] similarly reported no alteration of IRP-1 in SN of Parkinsonian brain. The increased mRNA expression of ferroportin in SN might indicate an elevated flux of iron from certain cell types within the SN. In other cell types, alveolar macrophages, the imposition of iron deficiency increased mRNA expression of ferroportin [29].

Table 2
Changes in mRNA expression of iron genes involved in iron homeostasis in the substantia nigra and cortex of PD patients compared with controls in post mortem tissue

Descriptor	Gene	SnM *p*-value	Sni	Cortex
Upregulated				
IRP-binding protein 1	IRP-1	ns	ns	ns
IRP-binding protein 2	IRP-2	0.025	ns	ns
Transferrin	Tf	0.0001	0.0030	ns
Transferrin receptor 2	TfR2	ns	0.0017	ns
Transferrin receptor 2	TfR2	ns	0.0184	ns
Ferritin H	FTH1	0.0019	ns	ns
Ferritin H pseudogene 1	FTHP1	0.0010	0.0348	ns
Ferritin L	FTL	0.0291	ns	ns
Ferritin L	FTL	0.0335	ns	ns
Ferritin L	FTL	ns	0.006	ns
Caeruloplasmin	Cp	0.0276	0.0276	ns
Caeruloplasmin	Cp	ns	0.0343	ns
Caeruloplasmin	Cp	ns	0.0336	ns
Hephastin	HEPH	ns	0.009	ns
Haemochromatosis	HFE	0.0416	0.0005	ns
Haemochromatosis	HFE	ns	0.0111	ns
Haemochromatosis	HFE	ns	0.0295	ns
Haemochromatosis	HFE	ns	0.0039	ns
Ferroportin	FPN1	0.0192	ns	ns
Ferroportin	FPN1	0.0353	ns	ns
Solutecarrier family11	SLC11A2	ns	ns	0.0291
Downregulated				
Ferrochetalase	0.006	0.0223	ns	
Sideroflexin 1	0.006	0.0314	ns	
Friedreich ataxia		0.031	ns	ns

Iron will enhance intracellular aggregation of α-synuclein leading to the formation of advanced glycation end products while α-synuclein liberated hydroxy radicals when incubated with Fe^{2+}. Pre-treatment of cells with cell-permeable iron chelators, transferrin receptor antibodies or transfection with glutathione peroxidase inhibited intracellular oxidant generation, α-synuclein expression/aggregation as well as apoptosis [6]. Interestingly magnesium inhibited aggregation, by preventing conformational changes.

5. Alzheimer's disease

Alzheimer's disease is one of the most common neurodegenerative maladies in Western societies. Clinical symptoms occur between the ages of 60–70 year. This disease presents with symptoms of memory loss, followed by a progressive decline of both cognitive and motor function occurs. Both genetic and environmental factors are implicated in its development. Females are more susceptible than males, which may be attributable to the higher constitutive activity of the synaptic zinc transporter ZnT3. The characteristic histology of AD is the deposition of both the amyloid peptide (Aβ) as neurotic plaques (Fig. 6a) and of the protein tau, as neurofribrillary tangles (Fig. 6b) predominantly in the cerebral cortex and hippocampus.

There is considerable evidence that mis-regulation in the metal homeostasis of iron, zinc and copper, which occurs in the hippocampus, amygdala, neocortex and olfactory bulb, contributes to the neuropathology of AD. Increased concentrations of these metals have been assayed in the cortical and accessory basal nuclei of the amygdala and in the neuropil of AD patients, compared to age-matched controls [30] as well as within Aβ plaques; (copper (390 μM), zinc (1055 μM) and iron (940 μM)), neurons and NFTs [31,32], when compared to the neuropil of normal age-matched copper (70 μM), zinc (350 μM) and iron (340 μM)) [30]. Such redox-active iron (Fe^{2+} and Fe^{3+}) associated with the amyloid plaques as well as the neurofibrillary tangles, could contribute towards oxidative damage [33]. Such changes in the metal content of specific brain regions might indicate alterations in brain iron homeostasis in AD patients. In one study where both iron and its transport protein transferrin were assayed in various brain regions of AD and PD patients and compared to elderly controls, diminished transferrin/iron ratios were identified in specific brain regions in both neurodegenerative diseases possibly indicating dysregulation of iron homeostasis [34]. Higher transferrin C2 allele frequencies have been reported in Alzheimer's disease compared with normal controls, as well

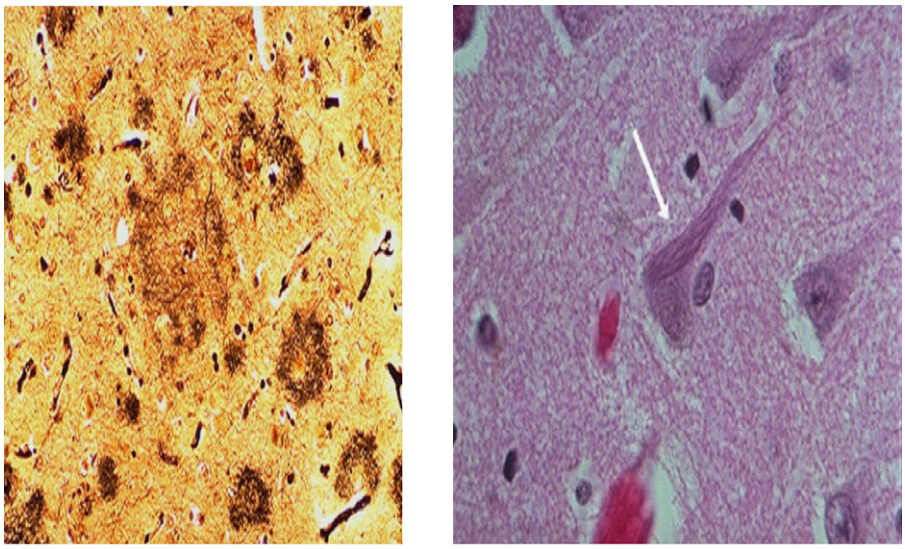

Fig. 6. (a) Characteristic histo-pathological findings of Alzheimer's disease are senile plaques-a collection of degenerative presynaptic endings with astrocytes and microglia. Plaques are stained with silver stains and are of varying size. (b) Neurofibrillary tangles of Alzheimer's disease. The tangles are present as long pink filaments in the cytoplasm. Each is composed of cytoskeletal intermediate filaments [6].

as a potential interactions between such C2 alleles and APOE (epsilon 4) [35]. A recent study [36] which investigated memory and ageing showed that bicarriers of the HFE C282Y and the transferrin C2 gene variants were at a 5-fold higher risk of developing AD. Furthermore the combination of either HFE C282Y and HFE H63D or of HFE C282Y and transferrin C2 markedly raised transferrin saturation in subjects without dementia which may predispose such people to higher iron loads and oxidative stress in the preclinical stages of AD. In addition, alterations in different metalloenzymes and metalloproteins, such as caeruloplasmin [37], the zinc transporter protein-1 (ZnT-1) [38] as well as metallothioneins [39] may also play an important role in the development of AD.

Amyloid precursor protein (APP), a type I membrane protein, resembles a cell surface receptor and is physiologically processed by site specific proteolysis. APP is cleaved by α-secretase (within the Aβ domain between Lys^{687} and Leu^{688}), to yield APPsα and the C-terminal fragment containing p3 (Fig. 7). The production of the Aβ is thus precluded. The membrane anchored α-carboxy terminal fragment, α-CTF, is then cleaved by γ-secretase within the membrane, releasing p3 peptide and the APP intracellular domain (AICD) (Fig. 7) [40]. The presence of increasing amounts of iron may alter α-secretase activity; one hypothesis suggested that iron might be required as a co-factor or be an allosteric modifier of α-secretase activity. Iron may also decrease α-secretase cleavage rates. In amyloidogenesis, the APP is cleaved sequentially by the proteolytic enzymes β-secretase (aspartyl protease, BACE or Asp-20) and then by γ-secretase. β-secretase has a C-terminal transmembrane domain and two active site motifs located in the luminal domain. Beta secretase cleaves APP between Met^{671} and Asp^{672} and yields APPβs and C99 fragments. The enzyme γ-secretase, a multi-subunit complex (containing presenilins 1 and 2 (PS1 and PS2)), will then cleave APPβs to produce beta-amyloid peptide Aβ ($Aβ_{42}$ and $Aβ_{40}$) and AICD.

Aβ accumulation and aggregation is considered to be the initiating factor in AD pathogenesis although it is known that such deposition occurs over many years, if not decades, prior to the clinical cognitive impairment. Aβ may be oxidised within the membrane, perhaps as a result of the increased Cu and Fe levels in the brain, from where it is ultimately liberated in a soluble form, to precipitate in the amyloid plaques. Aβ has a very effective binding domain for copper in its N-terminal domain and can bind copper in nmol amounts (Fig. 8) [41]. Aβ peptides will increase calcium influx through voltage gated calcium channels (N and L types) by reducing magnesium blockade of NMDA receptors, as well as forming cation-selective ion channels after Aβ peptide incorporation into the cellular membrane, thereby increasing excitotoxicity. Aβ peptides may interfere with long-term hippocampal potentiation and cause synaptic mis-function in Alzheimer's disease. It is unknown whether Aβ, which is continuously secreted under normal physiological conditions, may have a physiological role, possibly functioning as an antioxidant. There is an inverse relationship between Aβ content and in vivo oxidative damage [42] suggesting that Aβ might be a modulator of ROS generation. The precipitation of Aβ into plaques, associated with increased levels of metal ions, may be an efficient means of presentation to phagocytic cells for its removal from the cell. Other physiological functions assigned to Aβ are a superoxide scavenger (SOD activity), a cholesterol binding molecule, and an acute phase reactant [43].

Iron may modulate APP processing, by virtue of the presence of a putative iron response element in APP mRNA (based on sequence homology). The IRE was mapped within the 5′-untranslated regions (5′-UTR) of the APP transcript (+51 to +94) from the 5′-cap site (Fig. 9). The APP mRNA IRE is located immediately upstream of an interleukin-1 responsive acute box domain (+101 to +146). In response to intracellular iron chelation, translation of APP was selectively down regulated, thereby causing a striking decrease in the production of

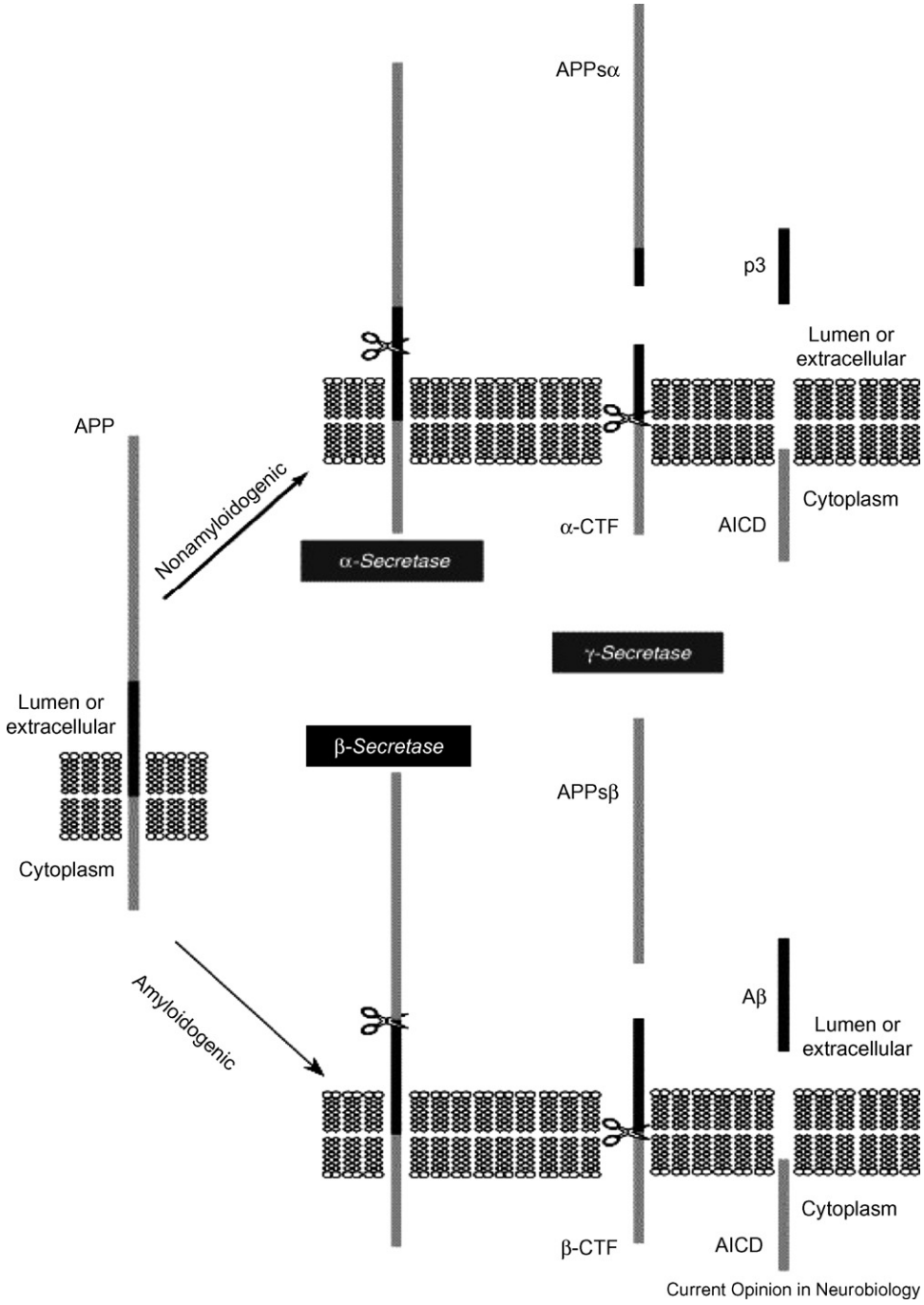

Fig. 7. Proteolytic processing of the amyloid precursor protein (APP) by secretases. APP is a type 1 transmembrane glycoprotein. The majority of APP is processed by the non-amyloidogenic pathway (thick arrow). APP is first cleaved by α-secretase leading to APPsα secretion and precluded Aβ generation. Membrane anchored α-carboxy terminal fragment (CTF) is then cleaved by γ-secretase within the membrane releasing the p3 peptide and the APP intracellular domain (AICD). Alternatively amyloidgenesis takes place when APP is first cleaved by β-secretase, producing APPsβ. Aβ and AICD are generated upon cleavage by γ-secretase of the β-CTF fragment retained in the membrane [40].

APP_{sol}. Iron influx reversed this inhibition, by a pathway similar to iron control of the translation of the ferritin-L mRNAs by iron responsive elements in its 5′-UTRs. It has been suggested that the abnormal distribution of iron in the brains of AD patients might result from alterations in iron homeostasis mechanisms regulated, in part, by the iron-regulatory proteins, IRP-1 and IRP-2. In an early study, an alteration in the localization of IRP-2, but not IRP-1, was reported in AD [44]. However in a later study [45] no significant differences in IRP-2 expression between controls, mild-moderate AD, severe AD, and cases of dementia with Lewy bodies were discernible. In addition, IRP-2 did not colocalize with senile plaques, NFT, or Lewy bodies in this study. It should also be noted that an increase in cytokine production, namely IL-1, which might occur due to inflammatory processes in AD, would increase IRP binding to the APP 5′-UTR, thereby decreasing APP production. Further studies are

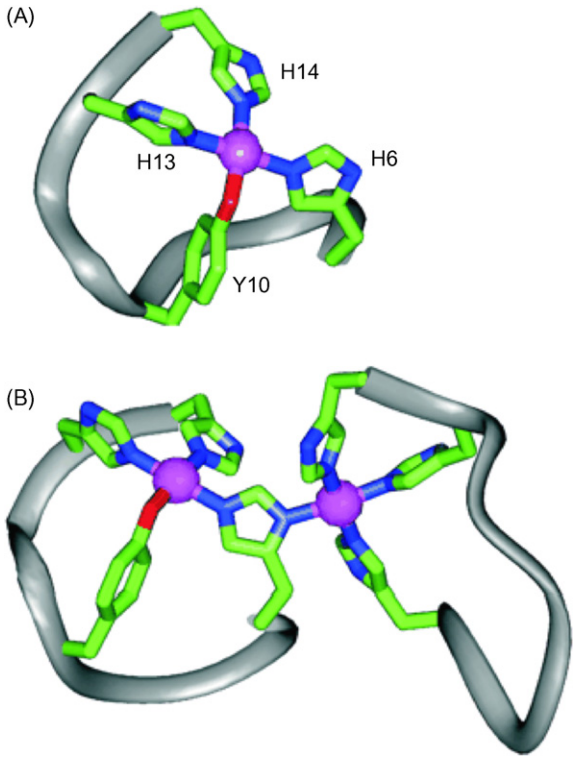

Fig. 8. Binding domain for copper in APP [65].

currently needed in this area.There is a considerable amount of data that demonstrates that tau can bind metals, such as copper and iron [46], which play a role in the aggregation of tau. It is also recognised that many of the kinases that phosphorylate tau (ultimately resulting in hyperphosphorylation and dissociation from the microtubules), can be induced by metal ions such as zinc [47,48]. Accumulation of tau in neurofibrillary tangles is associated with the induction and increased synthesis of haem oxygenase-1 (HO-1) [49,50], a potent antioxidant, which plays an important role in metabolising haem released from damaged mitochondria. The products of the haem oxygenase reaction, free ferrous iron, carbon monoxide and biliverdin/bilirubin are all biologically active molecules that may profoundly influence tissue redox homeostasis in a wide range of pathophysiological conditions, HO-1 will reduce oxidative damage but Fe^{2+} will be released which may participate in Fenton chemistry to produce hydroxyl radicals. Tau exhibits oxidative modification which include nitrotyrosine [51], glycoxidation [52] and lipid peroxidation (Takeda et al. [53]).

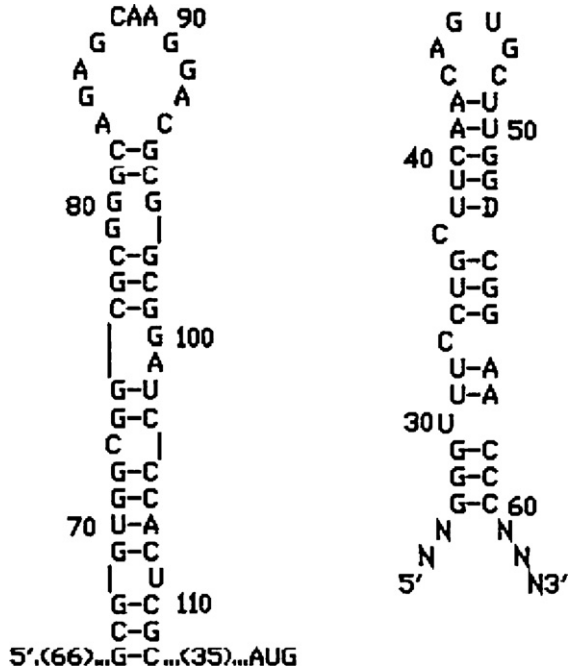

Fig. 9. The iron-responsive element in the 5′-UTR of APP mRNA [66].

6. Other metal based neurodegeneration diseases

Space precludes further presentation of other neurodegenerative diseases where metal ions may be involved in the aetiology and pathogenesis. Further information on each of these diseases, including prion disease, Huntington's disease, amyotrophic lateral sclerosis, together with the role played by metals in their etiology and pathology, can be found in our recently published book [6].

7. Therapeutic strategies

At the present time, the treatment of each of these neurological diseases relies almost exclusively on therapeutic agents that merely treat the pathology of each of the diseases rather than their etiology. Apart from drugs which increase (e.g. dopamine) or diminish (e.g. acetyl choline) levels of neurotransmitters, there is an overwhelming requirements for better therapeutic approaches.

One of the main features of PD is the increase of iron in the substantia nigra and corpus pallidium. Its removal may therefore be of importance in preventing toxicity, reviewed in [54]. One of the major causes for concern in the use of iron chelators in PD patients, is that their iron metabolism is essentially normal such that chelators, currently in clinical use to remove large excesses of iron from tissues, primarily the liver, e.g. desferrioxamine, deferipone or desferasiox (Fig. 10), may not be appropriate unless administered at relatively low doses. Extensive studies will be needed to ensure that iron is removed from specific brain areas and that the iron-chelated complex is not redistributed to other brain regions. Molecules which have iron chelating properties as well as other beneficial effects, e.g. an inhibitor of monoamine oxidase B, VK-28 (5-[4-hydroxyethyl) piperazine-1-ylmethyl]-quiniline-8-ol), e.g. antioxidant properties, epigallactocatechin-3-gallate, are worthy of further investigation (Fig. 10). Recent clinical studies have yielded promising results for the compound V-28 [55].

Oxidative stress has been put forward as one of the major causes of the nigral degeneration, such that its amelioration may retard the progression of the disease. An active inflammatory process occurs, with the induction of the transcription factor $NF_{kappa}B$ and of iNOS in brain glial cells. Drugs which

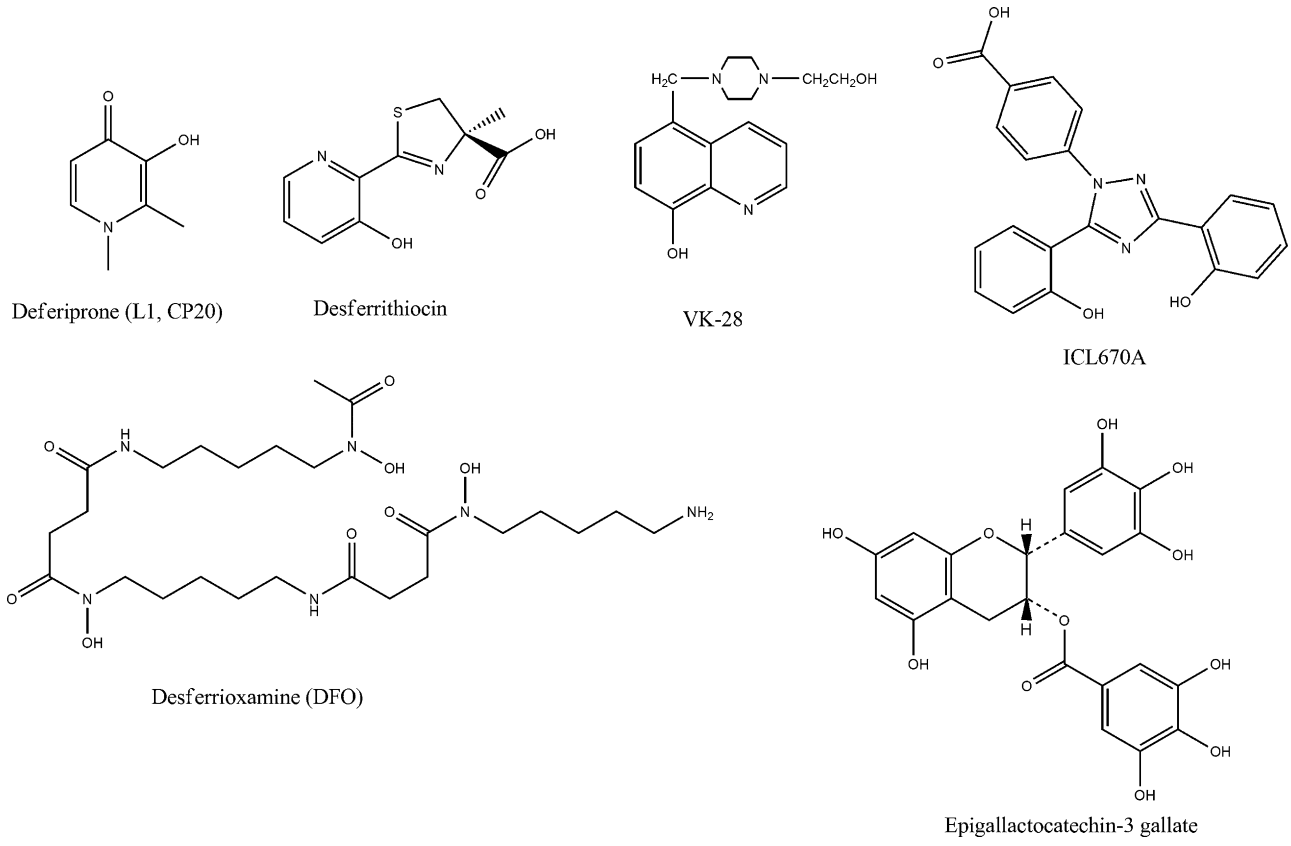

Fig. 10. Iron chelators, past and future therapeutic agents. (a) Chelators in current use for the removal of excess iron, bidentates, tridentates, and hexadentates and (b) for possible use in removing excess brain iron, epigallactocatechin and VK28.

inhibit nNOS (7-nitroindazole) and iNOS (ginsenoside), one of the biological active ingredients of ginseng) may help to prevent the destruction of dopaminergic neurons.

Non-steroidal drugs, such as ibuprofen and aspirin, will primarily inhibit COX-1 (which is present in activated microglia) and COX-2, the rate limiting enzymes in prostaglandin synthesis and thereby inflammation. Whereas COX-1 is constitutively expressed in most mammalian tissues, COX-2 is only expressed in certain tissues in response to inflammatory stimuli and is hence responsible for the elevated levels of prostaglandins found in inflammation. It therefore plays a role in the pathogenesis and selectivity of the PD neurodegenerative process. COX-2 inhibitors may be a useful adjunct therapy since they rapidly traverse the BBB. However, recent concern about their toxicity, via raised blood pressure and cardiovascular risks as well as changes in fatty acid synthesis, may preclude their use.

Currently the treatment for Alzheimers disease patients is to enhance neurotransmitter systems, e.g. acetylcholine, which does not address the underlying etiology. Since Aβ is implicated in the pathogenesis of the disease, inhibition of its production by protease (secretase) inhibitors or enhancement of its clearance may be of therapeutic advantage [56]. Recently there has been some concern about a vaccination approach, as to the exact target, either Aβ plaque deposit (fibrillar) or the oligomeric form, for irradication [57]. As already stated, we consider that the Aβ is a protective entity such that its removal might be detrimental. Further studies are clearly warranted. Metal ions also play a major role in its aggregation, such that decreases in their concentration may also prevent this process. In early studies [58] a single blind study of 48 AD patients who received 125 mg DFO i.m. 2×/day for 5 days/week for 2 years, induced a significant reduction in the rate of decline of daily living skills. Unfortunately no haematological or biochemical markers of iron status were assessed. Recently a novel system has been described which conjugates chelators to nanoparticles to facilitate their passage across the blood brain barrier, as well as to aid their exit with their corresponding complexed metal ion [59]. This latter therapeutic approach may prove to be safe and effective in reducing the metal load in neural tissue. The development of new orally active metal chelators e.g. deferasirox, may also be of therapeutic use in AD. Clioquinol, a Cu/Zn chelator may have potential therapeutic effects, in pre- and clinical trials, it induced a marked reduction of Aβ deposition in APP transgenic mice. However they have been concerns about its use in humans as there have been some reports of myelo-optic neuropathy.

Non-steroidal anti-inflammatory drugs (NSAIDs), such as indomethacin, attenuate inflammatory reactions and protect against nerve cell death that results from the generation of free radicals. Many different NSAIDs have Alzheimer's-protective effects and will reduce the generation of free radicals from activated microglial cells. Some NSAIDs, ibuprofen, indomethacin, and sulindac sulphide, can lower toxic Aβ levels by as much as 80%, independently of the inhibition of cyclooxygenase (COX)

activity, with a preferential increase of Aβ38. The latter effect is possibly due to alterations in the activity of α and β secretase.

Chloroquine, the anti-malarial drug reduces the inflammatory stimuli by decreasing both tissue iron content and down-regulating $NF_{kappa}B$ activation in rat macrophages [60]. However, a double blind parallel group multicentre trial of hydroxychloroquine for 18 months, did not slow the rate of decline in minimal or mild AD and showed no advantage, by comparison to placebo, with respect to quality of life and cognitive assessment.

8. Conclusions

We hope that in this brief review we have substantiated the hypothesis that metal mis-regulation play an important role in a number of neurodegenerative diseases (a) by increasing the concentration of redox sensitive metals, which (b) catalyse the formation of reactive oxygen species which (c) damage specific proteins causing them to (d) aggregate within cells. It is of vital importance that progress can be made in our understanding of the molecular basis of these neurodegenerative diseases such that treatments may become available to both treat and ultimately prevent their occurrence. The increasing longevity of the population, particularly in the Western World, in part due to the major advances that have been made in preventing and treating many maladies, including cardiovascular disease and cancer, means that we are all at risk of this advancing, unseen and undetectable changes in our brain physiology and function.

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