Biometals (2007) 20:639–654
DOI 10.1007/s10534-006-9033-z

Metals ions and neurodegeneration

Francisco Molina-Holgado · Robert C. Hider · Alessandra Gaeta · Robert Williams · Paul Francis

Received: 3 July 2006 / Accepted: 20 July 2006 / Published online: 9 February 2007

Abstract Neurodegenerative disorders include a variety of pathological conditions, which share similar critical metabolic processes such as protein aggregation and oxidative stress, both of which are associated with the involvement of metal ions. In this review Alzheimer's disease and Parkinson's disease are mainly discussed, with the aim of identifying common trends underlying these neurological conditions. Chelation therapy could be a valuable therapeutic approach, since metals are considered to be a pharmacological target for the rationale design of new therapeutic agents directed towards the treatment of neurodegeneration.

Keywords Neurodegeneration · Protein aggregation · Oxidative stress · Aβ-amyloid · α-Synuclein · Chelation therapy · Iron

Abbreviations

AD	Alzheimer's disease
Aβ	β-Amyloid
APP	Amyloid precursor protein
BBB	Blood brain barrier
CNS	Central nervous system
CQ	Clioquinol
DFO	Desferrioxamine
EDTA	Ethylenediaminetetraacetic acid
IL	Interleukine
IRE	Iron-responsive element
PD	Parkinson's disease
PrP	Prion protein
PrP^{C}	Normal isoform of the prion protein
PrP^{Sc}	Scrapie isoform of the prion protein
ROS	Reactive oxygen species
SN	Substantia nigra
SNc	Substantia nigra pars compacta
SOD	Superoxide dismutase
TNF	Tumour necrosis factor

F. Molina-Holgado · R. Williams · P. Francis
Wolfson Centre for Age Related Diseases, King's College, London, UK

R. C. Hider (✉) · A. Gaeta
Division of Pharmaceutical Science, King's College, London, UK
e-mail: robert.hider@kcl.ac.uk

A clear classification of neurodegeneration can be achieved on the basis of the principal neuropathological changes, characterised by the presence of abnormal protein components (Butterfield and Kanski 2001; Shastry 2003), which accumulate in the brain leading to a selective loss of neurons in an age-dependent manner such as Alzheimer's disease (AD) (Aβ-amyloid neuritic plaques and neurofibrillary tangles), Parkinson's disease (PD) (α-synuclein, Lewy bodies), prion disease (amyloid plaque core surrounded by ''petals'' of sponge-like tissue,

spongiosis) (Prusiner 2001), Huntington's disease (huntingtin protein aggregates) (Bonilla 2000) and Pick's disease (Pick bodies) (Wisniewsky et al. 1972; Brion et al. 1973). AD, PD and prion disease are discussed in some detail in this review.

Protein aggregation in neurodegeneration

There is extensive evidence for the association of protein aggregation and neurodegeneration in many disorders. Interestingly, metals such as iron and copper appear to play an important role in protein aggregation and therefore are likely to provide a link between the two pathological processes of protein aggregation and oxidative damage.

The cause of abnormal protein folding (misfolding) and consequent protein accumulation in the brain is still unclear. Nevertheless, genetic and environmental factors as well as age are all involved. Thus, pathogenic mutations in genes encoding aggregating proteins, such as Aβ, α-synuclein and prion protein, are responsible for inherited forms of AD, PD and prion disease, respectively. The mutant proteins, in these examples, show an increased tendency to form so-called amyloid-like fibrils (amyloidogenic activity), the formation of which is pathogenic.

Amyloidogenic proteins can be different in terms of amino acid sequence and/or native fold, but their corresponding fibrils are structurally similar. Electron microscopy, solid-state NMR and X-ray diffraction techniques indicate the following common features (Dobson 2003a; Antzutkin et al. 2002; Torok et al. 2002; Sikorski et al. 2003):

- Unbranched and twisted structures rich in β-sheet;
- Fibril diameter between 5 and 13 nm;
- Rigid structures;
- So-called ''cross-β'' structure, with β strands perpendicular to the fibril axis and backbone hydrogen bonds parallel to fibril axis;
- The presence of a fibril core, which is stabilised by hydrogen bond interactions associated with the polypeptide chain.

It is still a controversial issue whether amyloid fibril formation occurs via self-assembly of parallel or antiparallel β-sheets or indeed β-helices. Both parallel and antiparallel β-sheets have been observed in amyloid fibrils using solid state NMR (Tycko 2004).

The fibril construction is a cascade process, which involves the formation of intermediate structures (e.g. Aβ1–42, Fig. 1). The proteins first coalesce to form small soluble oligomers that aggregate to produce high molecular weight assemblies, so called ''protofibrils''. The protofibrils eventually exceed solubility limits and are deposited as fibrils (Rochet and Lansbury 2000; Dobson 2003b). The current dominant opinion is that the plaques, which deposit surrounding the neurons, are intrinsically toxic (Bence et al. 2001; Bucciantini et al. 2002). However, it is still unclear how their formation leads to cell death. Indeed, it is difficult to propose a common mechanism of toxicity consistent with each different disease. Studies with Aβ and α-synuclein have demonstrated that small oligomeric aggregates are more likely to be toxic than the multi layer fibrils (Lambert et al. 1998; Walsh et al. 1999; Hardy and Selkoe 2002). This concept is

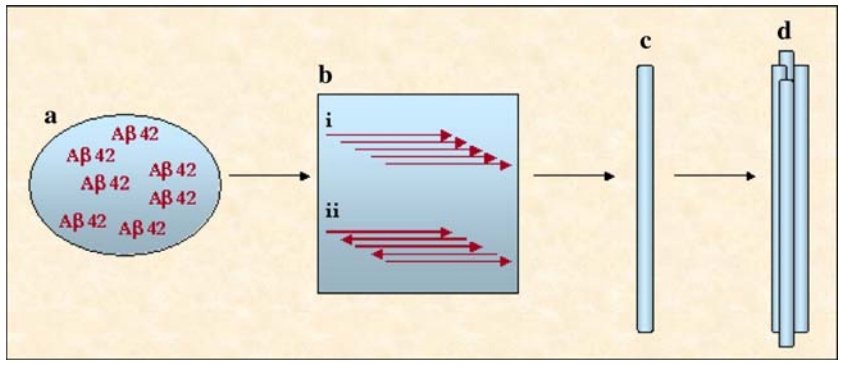

Fig. 1 Amyloidogenesis: fibril formation. (**a**) Aβ accumulation; (**b**) Aβ aggregation (oligomerisation), through (i) parallel or (ii) antiparallel alignment; (**c**) protofibrils formation; (**d**) mature fibril formation

consistent with the fact that fibril formation represents the final step of a cascade process, thereby providing a biomarker of neurodegeneration. The following sections focus on three proteins and their relative implications in neurodegenerative diseases: *Aβ*, *α-synuclein, prion protein*.

Aβ

The essential feature of the AD brain is the presence of extracellular plaques constituted of Aβ1–42 peptide deposits. The Aβ isoforms are 39–42 residues peptides, which are formed proteolytically in the cell from a large transmembrane glycoprotein called amyloid precursor protein (APP). Interestingly, these peptides have been found also in healthy individuals, suggesting a physiological role of Aβ (Vigo-Pelfrey et al. 1993) and recently a role for Aβ as an endogenous regulator of synaptic excitability has been proposed (Kamenetz et al. 2003). Aβ toxicity seems to be mainly associated with Aβ1–42 (DAEFRHDSGYEVHHQKLVFFAEDVGSNK GAIIGLMVGGVVIA). The observation of plaques as end-stage lesions in AD post-mortem brain tissue has led to the assumption that the accumulation of fibrils is responsible for the progression of the disease. Nevertheless, whether the oligomeric form or the fibrillated form (plaques) of Aβ is toxic still remains the subject of debate (Lue et al. 1999; Mc Lean et al. 1999). In fact, the soluble oligomeric forms seem to play a fundamental role in the preclinical and early progression of AD (Klein et al. 2001; Wang et al. 2002). These species are formed soon after the generation of the peptide within specific intracellular vesicles and are subsequently secreted from the cell. After their formation in the cell, Aβ monomers form dimers, trimers, and maybe higher oligomers. The monomeric class is the predominant water-soluble fraction and it is typically present in AD brains at six times higher concentration than that detected in control brains (Lue et al. 1999; Mc Lean et al. 1999). The secreted oligomers can interact with neurons in vivo, affecting their normal function. Correlation between soluble Aβ levels and the extent of synaptic loss and cognitive impairment is strong; even small doses of monomeric or dimeric Aβ1–42 can induce a dramatic loss of viability in neurones (Terry et al. 1991; Dickson et al. 1995; Roher et al. 1996). It has been demonstrated that naturally secreted human Aβ alters hippocampal synaptic efficacy at physiological levels (Walsh et al. 2002). The Aβ-protofibrillar metastable forms are also strongly involved in neurotoxicity, especially acting through rapid electrophysiological changes (membrane depolarisation and increase in action potentials), which eventually cause neuronal death (Hartley et al. 1999). The neuronal dysfunction is then initiated by the formation of oligomeric and protofibrillar species.

α-Synuclein

α-Synuclein is the main component of the abnormal protein depositions constituting the Lewy bodies, intracytoplasmic inclusions (5–25 μm diameter) recognised as an hallmark of PD (Duffy and Tennyson 1965). α-Synuclein is a relatively unfolded protein, possessing a random coil secondary structure. The strong electrostatic repulsion associated with the structure at neutral pH is responsible of the lack of the protein folding (Uversky et al. 2001). The non-amyloidogenic core (NAC), the central hydrophobic/amyloidogenic part of the protein, is responsible for the conformational change from random coil to *β*-sheet (protofibril and fibril formation) (Serpell et al. 2000). Both genetic mutations and exposure to metals accelerate the rate of α-synuclein fibril formation. Significantly high levels of Fe^{3+} have been found in Lewy bodies. Disruption of α-synuclein membrane binding ability is related to aggregation process (Paik et al. 1999; Miranda et al. 2000). In physiological conditions, α-synuclein is present in random coil conformation in cytoplasm and, after translation, becomes associated with the plasma membrane and the vesicular membrane, which both represent its functional sites. At these sites the protein is in α-helix conformation. The misfolded isoform of the protein may lose the ability to bind to membranes after the translation and thus accumulates as free α-synuclein in the cell. These events are believed to lead to oligomerisation and aggregation in vivo (Hedge and Jagannatha Rao 2003).

It is reported that soluble α-synuclein complexes are more likely to be mediators of neurotoxicity and the accumulation of α-synuclein in cultured human dopaminergic neurons results in reactive oxygen species-mediated apoptosis (Xu et al. 2002). In contrast, α-synuclein is not toxic in non-dopaminergic human cortical neurons, where it is reported to exhibit a neuroprotective activity (Xu et al. 2002). Apparently, soluble α-synuclein neurotoxicity is dopamine-dependent, which might offer an explanation for the selective neuronal loss, observed in PD (Xu et al. 2002).

Prion protein

Prion diseases provide a fascinating example of the relation between protein folding and neurodegenerative disease. The neuroanatomical distribution of the lesions varies with the specific type of prion disease. Irregular rods of protein aggregates and amyloid plaques, which are resistant to proteolytic degradation, accumulate in plasmalemma, Golgi and intracytoplasmic organelles of neurons. These species, which are formed of two or four subfilaments helically wound around each other, are mainly composed of PrP 27–30 (prion protein protease resistant core, with an apparent molecular mass of 27–30 kDa) (Cohen et al. 1982; Merz et al. 1981; Prusiner et al. 1983; Jeffrey et al. 1994; Laine et al. 2001).

While studying the molecular basis of the disease, it has become clear that protein conformation plays a critical role in the pathogenic process. The conversion of the cellular, primarily α-helical prion protein isoform (PrP^{C}), to a β-sheet-rich conformation results in the accumulation of a protease resistant disease-associated oligomeric isoform called scrapie (PrP^{Sc}). Interactions between ''normal'' and ''abnormal'' isoforms of the same proteins are well-known facilitators of aggregation (Pan et al. 1993; Prusiner 1991). PrP^{C} is characterised by a flexible and unstructured region of 100 residues at the N-terminal tail, a globular domain of nearly identical size (120–231) containing two short antiparallel β-strands and three α-helices. The protein is present at the pre- and post-synaptic level, heterogeneously distributed in healthy adult brain; it is attached to the cell surface (synaptic plasma membrane) via a glycosyl phosphatidylinositol anchor (Stahl et al. 1987). Since the first structural information was obtained, it has become evident that copper has a key role in the biological function of PrP^{C}. The protein is reported to bind copper in a specific manner and apparently this binding induces a conformation transition, which in turn modulates protein aggregation (Brown 2001). In particular, the formation of the disease causing isoform PrP^{Sc} involves the refolding of a specific amino acidic region (90–140) into a β-sheet, and the metal binding might be crucial in this conformational switching (Zecca et al. 2002). According to the model proposed in an interesting general theory on diseases characterised by protein deposition, PrP^{Sc} provides a template to assist the conversion of nascent PrP^{C} molecules, by lowering the activation energy barrier for the conformational change (Cohen 1999; Hijazi et al. 2003). If true, the disease-associated isoform would also be disease causing, because its presence would dramatically enhance the conversion of the normal cellular isoform.

Alzheimer's disease, Parkinson's disease and oxidative stress

One of the principal risk factors in most neurodegenerative disorders is age and this may be directly linked to oxidative stress (lipid peroxidation, protein oxidation, DNA and RNA oxidation), which increases in the brain with age and plays a central role in the pathogenic mechanisms of neurodegeneration.

Oxidative stress may be defined as an imbalance between the production of free radicals and the ability of the cell to defend against them through a set of antioxidants and detoxifying enzymes that include superoxide dismutase, catalase and glutathione. When this imbalance occurs, oxidatively modified molecules (lipids, proteins, nucleotides) accumulate in the cellular compartment causing dysfunction (Floyd and Hensley 2002). In the case of very sensitive cells such as neurons, the failure of limited defense systems may eventually lead to cell death. Under physiological conditions free radicals are

by-products of cellular oxygen metabolism, with superoxide ($O_2^{\cdot-}$), hydroxyl ($OH^{\cdot}$) and nitric oxide ($NO^{\cdot}$) species being prevalent. Hydrogen peroxide (H_2O_2) and peroxynitrite ($ONOO^-$) are not radicals themselves, but nevertheless contribute to the cellular redox state and eventually produce radicals through various chemical reactions. These species are referred to as reactive oxygen species (ROS). Mitochondrial oxidative metabolism (the major portion of the total ROS produced during aerobic metabolism comes from by-products of the electron transport chain of mitochondria), nitric oxide synthases, phospholipid metabolism, proteolytic pathways and metal ions are all potential sources of intracellular free radicals. The reactions leading to ROS production occur at all times within the cell and low levels activate neuronal survival signalling pathways (Crossthwaite et al. 2002). However, under certain conditions, such as stroke, very high levels are produced (Floyd 1999; Sherki et al. 2001). The brain is at particular risk from oxidative damage because of the following specific characteristics:

- High oxygen consumption (20% of the total body basal O_2 consumption);
- High levels of both iron and ascorbate (crucial in causing membrane lipid peroxidation);
- Relatively low levels of antioxidant protective agents are present;
- Tendency to accumulate metals.

In principle a target protein, which is able to interact with free radicals and metal ions, can generate redox activity and, as a consequence, oxidative damage. This latter has been found as a typical hallmark in the majority of neurodegenerative disorders, such as AD, PD and prion disease, and could in principle be either the primary cause or the consequence of disease progression.

Oxidative stress and Alzheimer's disease

Oxidative stress is believed to play a major role in the dysfunction and degeneration occurring in AD.

One of the hallmarks of AD is the presence of senile plaques constituted by a highly dense core formed of a mixture of 39–43 residue polypeptides derived from APP that accumulate in the cortical interstitium and cerebrovasculature in a characteristic manner. One such peptide, Aβ1–42 is the minor soluble species but possesses a fibrillogenic activity that renders it central to the pathogenesis and particularly toxic to cells in the early stage of the peptide aggregation process (Klein et al. 2001; Wang et al. 2002).

There is strong evidence of a relationship between oxidative stress and cortical Aβ deposits. Interestingly, this correlation seems to depend on the Aβ physicochemical properties, which are consistent with both anti-oxidant and pro-oxidant activities. This characteristic of Aβ is likely to derive mostly from its ability to bind metals and, as a consequence, to mediate redox reactions (Butterfield and Kanski 2001). In fact, Aβ1–42 is a metallo-binding peptide with binding sites for Zn(II), Cu(II) and Fe(III) (Huang et al. 1999). Metal homeostasis is altered during AD, and as a consequence, metals are reported to accumulate in the neuropil with concentrations which are 3–5 fold increased compared to age matched controls (Lovell et al. 1998). Three histidine residues (His6, His13 and His14) located in the hydrophilic N-terminal part of the peptide and a methionine (Met35) residue in the lipophilic C-terminal region have been identified as the crucial section involved with metal ion binding. Particularly, the histidine residues identify the site, which binds redox active Cu or Fe, while the methionine residue identifies a second site suggested to be involved in the reduction of Cu(II) and the generation of H_2O_2. Based on these findings, an Aβ-induced oxidative stress model has been developed (Atwood et al. 2003). However, it is still unclear whether Aβ generation is a cause or an effect of the oxidative damage observed in the process of neurodegeneration in the AD brain.

Interestingly, metal ion accumulation and oxidative stress are also associated with changes of both soluble Aβ and deposited Aβ concentrations. Nevertheless, when Aβ reaches a concentration sufficient to produce oxidative stress, it induces its own production, so generating a vicious cycle (Bush 2002). Therefore, if Aβ

synthesis is modulated by stress conditions, Aβ production can be considered a response to an increased oxidative stress in the brain, apart from being itself a potential source of additional oxidation processes. Oxidative modifications have been observed in the cell (neuronal cytoplasm) and in the extracellular lesions (amyloid plaques) of AD brains. In particular, reversible and rapidly degraded products have been detected in the cell, while stable glycation, carbonyl and lipid peroxidation products have been found in the lesions. This oxidative process is the result of an increased production of membrane permeable H_2O_2, at both intracellular level (mitochondria) and at extracellular level (activated microglia and Aβ-amyloid deposits) (Bush et al. 1999).

H_2O_2 is a reactive species, in the presence of redox-active metal ions producing hydroxyl radicals ($OH^{\cdot}$) via Fenton chemistry (Eq. 1). Since these radicals are only able to diffuse short nanometre distances, the changes observed at nucleic acid and protein sites may identify the sites of both $OH^{\cdot}$ generation and metal ion interaction. Redox active iron(II) is critical in the Fenton reaction (Eq. 1):

$$Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + OH^{\cdot} + OH^{-} \quad (1)$$

Iron is localised in the endoplasmic reticulum, but also in granulis formed of lipofuscin, or age pigment, as well as in their associated vacuoles (Brunk et al. 1992). Lipofuscin is an auto fluorescent pigment that accumulates progressively with age within secondary lysosomes. As a result it has often been used as a marker and index of ageing. It is formed during a process of production of partially reduced oxygen species by mitochondria (via Fenton reaction between iron and H_2O_2) and following degradation via autophagocytosis within secondary lysosomes. In AD, lipofuscin may play a role in the modulation of the release of iron from damaged mitochondria, which becomes an important generator of H_2O_2, thus an important site of oxidative damage (Brunk and Terman 2002).

The accumulation of metals in AD brains as well as the presence of a metal binding site on Aβ represent promising pharmacological targets. Therefore, compounds with chelation properties, but also with the ability to block the site, so preventing the adverse generation of H_2O_2 catalysed by the site and the metal-induced protein aggregation, could be useful in the treatment of AD.

Oxidative stress and Parkinson's disease: the role of iron

Post-mortem studies in PD brains indicate that a wide range of molecules undergo oxidative damage, including lipids, proteins and DNA (Dexter et al. 1989; Sanchez-Ramos et al. 1994; Alam et al. 1997). In fact, significant neurochemical, physical, histochemical and biochemical evidence confirm the hypothesis that oxidative stress generates the cascade of events, which are responsible of the preferential degeneration of melanised dopaminergic neurons in the substantia nigra pars compacta (SNc) in PD (Maguire-Zeiss et al. 2005). The following phenomena have all been observed in Parkinsonian brains:

(a) Decline in the mitochondrial activity (which might result from generation of ROS);
(b) Generation of H_2O_2 following the deamination of dopamine by monoaminooxidase and by autoxidation (dopamine oxidative metabolism);
(c) Increased activity of superoxide dismutase (which catalyses the conversion of superoxide anions to H_2O_2);
(d) Reduced concentration of glutathione (responsible of H_2O_2 clearance);
(e) Elevated level of iron in microglia, astrocytes, oligodendrocytes and dopaminergic neurons of SNc (Mizuno et al. 1989; Riederer et al. 1989; Saggu et al. 1989; Halliwell 1992; Sofic et al. 1992; Gotz et al. 1994; Olanow and Youdim 1996; Ye et al. 1996; Lan and Jiang 1997; Jelliger 1999).

Furthermore, the examination of brain material and the use of a variety of analytical techniques have demonstrated changes in the normal iron and antioxidant concentrations in SNc of PD patients (Sofic et al. 1988; Dexter et al. 1989; Hirsh et al. 1991; Olanow 1992;

Sanchez-Ramos et al. 1994; Alam et al. 1997). Interestingly, a study, in which antibodies against ferritin were used, indicated no increase in neuronal ferritin, suggesting that the elevated iron is unbound and therefore potentially reactive (Connor et al. 1995). Such iron would be able to initiate ROS-dependent oxidative stress in nigrostriatal dopamine neurons.

Neuroimmune interactions of iron chelators

Neuroimmune interactions also form an important point for consideration, as iron has conflicting roles in homeostasis and in neurodegeneration. Iron is essential to maintain homeostatic function during brain development, neurometabolism, myelination, and neurotransmitter function (Beard et al. 2003; Lozoff et al. 2006), but increasing concentrations of iron are associated with neuroinflammation, neurodegeneration and cell death (Gaeta and Hider 2005). Inflammatory processes play a key role in the pathogenesis of a number of neurodegenerative disorders such as PD and AD (Allan and Rothwell 2003). In the CNS, excessive iron accumulation is detrimental to neurones, astrocytes and microglia but not to oligodendrocytes. It has been suggested that the high iron concentration observed within the oligodendrocytes is due to an elevated expression of enzymes involved with myelin production (Levine and Chakrabarty 2004). The study of pro-inflammatory cytokines and pathological iron has been the subject of exhaustive investigation, but the mechanisms by which iron might affect neuronal survival through this mechanism remain elusive. Exposure of different brain cell types, including oligodendrocytes, to iron has been reported to produce a number of pro-inflammatory mediators, including interleukin-1β (IL-1β) and nitric oxide (Mandel et al. 2005 for review; Zhang et al. 2005). One of the suggested mechanisms for iron-induced inflammation in models of PD is by a direct activation of the transcription factor NFkβ with a subsequent release of pro-inflammatory cytokines such as TNF-α, IL-1β and IL-6 (Grunblatt et al. 2000; Youdim et al. 1999). In addition, it has been demonstrated that iron chelation abrogates endotoxin-induced inflammation by suppressing IL-1β transcription (O'Brien-Ladner et al. 2000) and that IL-1β up-regulates iron efflux through modulation of ceruloplasmin and ferroportin-1 synthesis as part of a local defence pathway set up by the organism (di Patti et al. 2004).

Furthermore, there is evidence for the presence of an iron-responsive element (IRE) in the 5′-untranslated region (5′-UTR) of the Alzheimer's amyloid precursor protein (APP) which is located immediately upstream of an interleukin-1 responsive acute box domain (Rogers et al. 2002). Iron-regulatory protein (IRP) binding to the APP 5′-UTR is reduced after treatment of cells with DFO and is increased after interleukin-1 (IL-1) stimulation (Rogers and Lahiri 2004). Rogers et al. (1999) showed that IL-1 stimulates a 12-fold increase in the rate of astrocytic APP protein synthesis through the APP 5′-UTR sequences and that iron levels regulate APP mRNA translation in astrocytes, which are responsible for providing both mechanical and metabolic support for neurons (Allan et al. 2005).

Thus, with any pharmacological manipulation of intracellular iron levels, it is likely that APP and IL-1 synthesis will be influenced.

Therapeutic strategies

Oxidative stress, protein aggregation and redox active metal ions can all considered to be promising pharmacological targets. The apparently critical involvement of metals, particularly iron and copper, in both oxidative stress and protein aggregation processes therefore renders chelation therapy a sensible strategy. The important features of a suitable chelating agent would be the ability firstly to scavenge the free redox active metal present in excess in the brain to form a non-toxic metal complex, which is then excreted, and secondly to cap the metal at its labile binding site. (Aβ-amyloid, α-synuclein, prion protein), preventing any mediated toxic action (Fenton activity and/or aggregation). In this second case, the newly formed stable metal complex would favour the state in which the metal is not redox active and therefore not toxic. The latter mechanism implies additional interactions between the

drug and the target protein, which have to be considered in the design.

Design features of clinically useful metal chelators

One of the dominant properties of any therapeutic chelator is metal selectivity, typically a high selectivity being required, for instance in the treatment of iron overload associated with β-thalassaemia. In this latter situation ligands with a high selectivity for iron over copper and zinc are essential, as chelation therapy is maintained for life. Unfortunately, with the proposed treatment of neurodegenerative diseases by chelation therapy, the identity of the putative toxic metal is not always firmly established. With AD, for instance, iron, copper and zinc have all been associated with the progression of the disease. In contrast, with PD, iron is clearly the major target.

Although there are clear guidelines for the design of iron-selective chelating agents (Liu and Hider 2002a), this is not the situation with copper and zinc. Furthermore, with the necessity of ready permeation of the blood brain barrier (BBB), the size of useful chelators should probably be limited to less than 300 Daltons, thereby excluding hexadentate ligands and seriously limiting the potential for the design of selective copper(II) and zinc(II) chelators (vide infra). Most agents that bind copper(II) tightly will also bind iron(II), zinc(II), nickel(II), cobalt(II) and manganese(II), thereby causing a potential toxic insult to all cell types (Liu and Hider 2002b). This limitation is a major issue for the design of chelators with potential for treating neurodegeneration.

In principle therefore, there are two major classes of ligands required:

- Iron selective chelators for the treatment of PD;
- Iron/copper/zinc chelators for the investigation and eventual treatment of AD; inevitably, this latter group of chelators are likely to be more toxic, at a given dose, than iron-selective chelators.

Iron-selective ligands

Chelating agents can be designed for efficient binding of either the iron(II) or the iron(III) oxidation state. Chelators that prefer iron(II) use nitrogen and sulphur atoms as ligands, for instance 2,2′-bipyridyl (**2**, Fig. 2). Although these compounds are selective for iron(II) over iron(III), they retain an appreciable affinity for other biologically important bivalent metals such as copper(II) and zinc(II) (Table 1). Thus, the design of a low molecular weight non-toxic iron(II)-selective ligand is extremely difficult and indeed may not be possible. In contrast, iron(III)-selective chelators favour oxygen atoms as ligands, notably hydroxypyridinones (**3**). Most tribasic cations, for instance aluminium(III) and gallium(III), are not essential for living cells and thus iron(III) is a practical target for ''clinical chelator'' design. An additional advantage of high-affinity iron(III) chelators is that, under aerobic conditions, they will

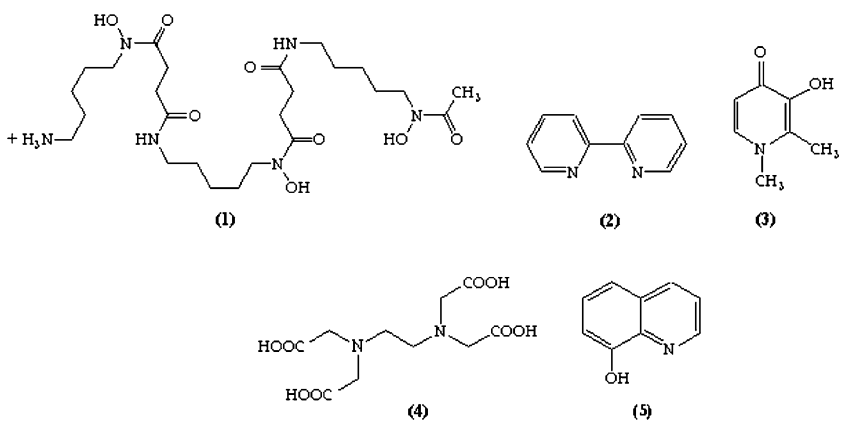

Fig. 2 General structure of iron(III) chelators. *Hexadentate*: desferrioxamine (DFO, **1**), ethylenedi aminetetraacetic acid (EDTA, **4**); *bidentate*: 2,2′-bipyridyl (**2**), deferiprone (**3**), 8-hydroxyquinoline (**5**)

Table 1 Metal affinity constants for selected ligands (Martell and Smith 1974–1989)

Ligand	Log cumulative stability constant						
	Fe(III)	Al(III)	Ga(III)	Cu(II)	Zn(II)	Fe(II)	pFe^{3+}
DFO (**1**)	30.6	25.0	27.6	14.1	11.1	7.2	26
2,2′-Bipyridyl (**2**)	16.3	–	7.7	16.9	13.2	17.2	–
3-Hydroxypyridin-4-one, (deferiprone) (**3**)	37.2	35.8	32.6	21.7	13.5	12.1	19
EDTA (**4**)	25.1	16.5	21.0	18.8	16.5	14.3	23.4
8-Hydroxyquinoline (**5**)	37.7	–	40.5	22.9	15.8	–	20.6

Hexadentate: DFO (**1**), EDTA (**4**); bidentate: 2,2′-bipyridyl (**2**), deferiprone (**3**), 8-hydroxyquinoline (**5**)

[a] $pFe^{3+} = -\log[Fe^{3+}]$ when $[Fe^{3+}]_{total} = 10^{-6}$ M and $[ligand]_{total} = 10^{-5}$ M at pH 7.4

chelate iron(II) and facilitate autoxidation to iron(III) (Harris and Aisen 1973). Therefore, high-affinity iron(III)-selective ligands bind both iron(III) and iron(II) under most physiological conditions.

General requirements of iron(III) complexes with therapeutic potential

Ligands can be structurally classified according to the number of donor atoms that each molecule possesses. When a ligand contains two, three, six or more donor atoms, it is termed bidentate, tridentate, hexadentate or generally multidentate respectively (Fig. 3). Under biological conditions the pM[1] value is a more useful parameter than the conventional stability constant to assess the ligand affinity for the metal (Raymond et al. 1984; Liu and Hider 2002a); for clinically useful iron scavengers a pFe^{3+} value ≥ 20 (Table 1) is considered to be essential. Molecular size is also a critical factor, as it influences the penetration of both the wall of the gastrointestinal tract (Holander et al. 1988) and the BBB (Oldendorf 1974). In order to achieve greater than 70% oral absorption, the chelator molecular weight should be < 500 (Maxton et al. 1986). This molecular-weight limit provides a considerable restriction on the choice of chelator and may effectively exclude hexadentate ligands from consideration. Bidentate and tridentate ligands, by virtue of their much lower molecular weights, are predicted to possess higher absorption efficiencies. The fraction of the absorbed dose for a range of bidentate hydroxypyridin-4-ones (e.g. **3**, Fig. 2) has, for instance, been found to fall between 50% and 70%, as assessed in the rabbit (Yokel et al. 1995). This type of molecule has also been demonstrated to penetrate the BBB (Habgood et al. 1999). Hydroxypyridin-4-ones are monoprotic acids at pH 7.0 and thus form the neutral *tris*-iron(III) complexes.

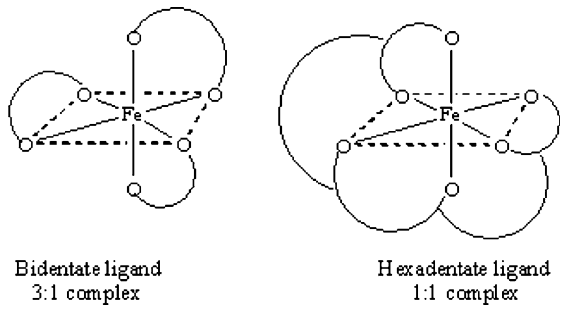

Fig. 3 Schematic representation of chelate ring formation in metal-ligand complexes

The toxicity associated with iron chelators originates from a number of factors, such as inhibition of iron-containing metalloenzymes, lack of metal selectivity, redox cycling of iron-complexes between iron(II) and iron(III) and the kinetic liability of the iron complex leading to iron redistribution.

In general, iron chelators do not directly inhibit haem iron-containing enzymes due to the inaccessibility of porphyrin-bound iron to chelating agents. In contrast, many non-haem iron-containing enzymes such as lipoxygenase, the aromatic hydroxylase families and ribonucleotide reductase are susceptible to chelator-induced inhibition

[1] pM is defined as the negative logarithm of the metal ion concentration under the following conditions: $[\text{Metal ion}]_{total} = 10^{-6}$ M, $[\text{Ligand}]_{total} = 10^{-5}$ M at pH 7.4.

(Hider 1995). Generally, hydrophobic chelators inhibit lipoxygenases, therefore the introduction of hydrophilic characteristics into a chelator tend to minimise such inhibitory potential (Abeysinghe et al. 1996), particularly if their introduction also induces steric interference of the chelation process at the enzyme active site (Liu et al. 2002). By careful modification of physicochemical properties, iron chelators can therefore be designed which exert minimal inhibitory influence on many metalloenzymes (Cooper et al. 1996; Liu et al. 2001).

In summary, a chelator suitable for the treatment of PD should possess a molecular weight in the region 200–400, should bind iron(III) much more tightly than iron(II), should not redox cycle or interfere with metalloenzyme activity. Hydroxypyridin-4-ones are suitable candidates, by virtue of their high selectivity for iron and their generally favourable physical-chemical properties. Deferiprone (**3**, Fig. 2), a hydroxypyridinone, has been used clinically for over 10 years in the treatment of transfusion-induced iron overload. It forms a stable 3:1 ligand iron complex, which is water soluble and readily excreted by the kidneys (Olivieri et al. 1990). Furthermore, deferiprone readily crosses the BBB (Habgood et al. 1999).

Iron/copper/zinc binding ligands

Chelators with a broad selectivity for transition metals generally use nitrogen atoms as ligands, for instance 2,2′-bipyridyl (**2**, Fig. 2) However, the metal complexes of these ligands are positively charged, tend to bind to membranes and, by virtue of their net charge, do not penetrate membranes readily. Thus, they are inefficient at excreting intracellularly localised transition metals. In contrast, 8-hydroxyquinoline (**5**, Fig. 2) contains both oxygen and nitrogen atoms and hence possesses intermediate properties between those of the di-nitrogen ligands and the di-oxygen ligands (Table 1) (Hider and Hall 1991; Liu and Hider 2002b). More importantly, because 8-hydroxyquinoline is monobasic, it forms neutral 3:1 complexes with iron(III) and neutral 2:1 complexes with both copper(II) and zinc(II). Thus, in principle, it can remove these metals from cells. Although hydroxyquinoline derivatives are used in many parts of the world for the treatment of diarrhoea (Claesen and Clements 1989), their use has been criticised (Chetley and Gilbert 1986) due to associated toxicity (Palm 1932; Rose and Gawel 1984).

At the present time, there is no obvious solution to the design of a non-toxic ligand with high affinities for iron, copper and zinc and the ability to mobilise such metals from intracellular sites.

Chelators investigated for their potential in the treatment of neurodegenerative diseases

Hexadentate chelators

Two hexadentate ligands have been investigated for the treatment of neurodegenerative disease, desferrioxamine (DFO) (**1**, Fig. 2) and a synthetic amino-carboxylate ligand, DP-109 (**6**, Fig. 4).

A 2-year, single blind study was undertaken to investigate whether the progression of AD dementia could be slowed by DFO (Crapper Mclachlan et al. 1991). With this purpose, 48 patients were assigned to three different groups: DFO treated (125 mg DFO given intramuscularly twice daily, 5 days per week, for 24 months), oral placebo (lecithin) and no treatment. Activities of daily living were monitored and recorded over 24-month period at regular intervals. No differences were observed in the rate of deterioration of patients receiving either placebo or no treatment. In contrast, it was reported that DFO treatment led to a significant reduction in the rate of decline of daily living activities, leading to the conclusion that sustained administration of DFO might slow the clinical progression of dementia associated with AD. Although interesting, these findings are surprising because, by virtue of this molecule's hydrophilic nature and size, it does not penetrate the BBB and hence, access to the brain could only be achieved in the presence of a damaged BBB. In contrast, DP-109 has been designed as a pro-drug, and optimal metal chelation will only occur subsequent to the cleavage of the two long chain ester functions (Lee et al. 2004). Indeed, this molecule has been demonstrated to possess a strong inhibition activity on plaque formation and deposition in female hAβPP-transgenic Tg 2576

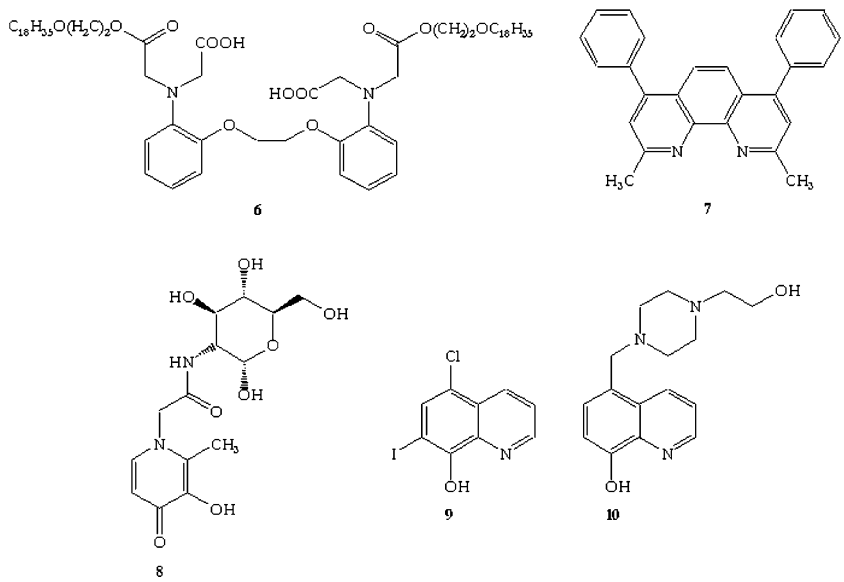

Fig. 4 Chelators investigated for their potential in the treatment of neurodegenerative diseases. *Hexadentate*: DP-109 (**6**); *bidentate*: bathocuproine (**7**), feralex (**8**), clioquinol (**9**), VK-28 (**10**)

mice (Hsiao et al. 1996; Lee et al. 2004). Nevertheless, under in vivo conditions a large trans BBB flux is unlikely to be achieved due to the relatively high molecular weight of DP-109 (>1,000) and the surface activity of this strongly amphiphilic molecule.

Bidentate chelators

Several ranges of bidentate ligands have been investigated for their potential to cross the BBB and to treat neurodegeneration. The hydrophobic phenanthroline analogue, bathocuproine (**7**, Fig. 4), has been demonstrated to facilitate the solubilisation of Aβ from AD brain samples (Cherney et al. 2000). However, all metal complexes of this ligand are positively charged and are, therefore, unlikely to penetrate the BBB. In contrast, a number of 3-hydroxypyridin-4-ones (e.g. **3**, Fig. 2) have been demonstrated to penetrate the BBB (Habgood et al. 1999). This ability is due to the formation of neutral complexes with both iron(III) and copper(II) and, therefore, they have the potential of facilitating metal efflux from the brain. A related molecule, feralex (**8**, Fig. 4), a closely related ligand to 3-hydroxypyridin-4-ones, has been reported to disaggregate in vitro hyperphosphorylated τ-protein, which is responsible for the formation of neurofibrillary tangle in AD (Shin et al. 2003).

To date, a range of 8-hydroxyquinoline analogues have demonstrated the greatest potential for the treatment of neurodegeneration and one compound, clioquinol (CQ) (**9**, Fig. 4), has entered clinical trial. Clioquinol is a small, lipophilic bio-available metal chelator, which leads to beneficial effects in both AD and PD animal models (Cherney et al. 2001; Kaur et al. 2003). Following oral treatment with CQ (30 mg kg^{-1} day^{-1}), Aβ accumulation was markedly inhibited (49% decrease), as shown in a blinded study of APP2576 transgenic mice treated for 9 weeks. There was no evidence of neurotoxicity or increased non-amyloid pathology. General health and body weight parameters were significantly stable in the treated animals, with a conspicuous improvement after only 16 days of treatment (Cherney et al. 2001).

In the PD studies, mice previously treated with the neurotoxin MPTP were administered orally with CQ (30 mg kg^{-1} day^{-1}) for 8 weeks to assess the ability of the compound to protect against MPTP-induced toxicity. Total substantia nigra (SN) iron levels were found to be reduced approximately 30% in the CQ-fed versus control animals, accordingly with the reported non-toxic range. Following CQ pre-treatment, oxidative stress markers and glutathione depletion were found significantly attenuated in SN (Kaur et al. 2003). Unfortunately, many halogenated

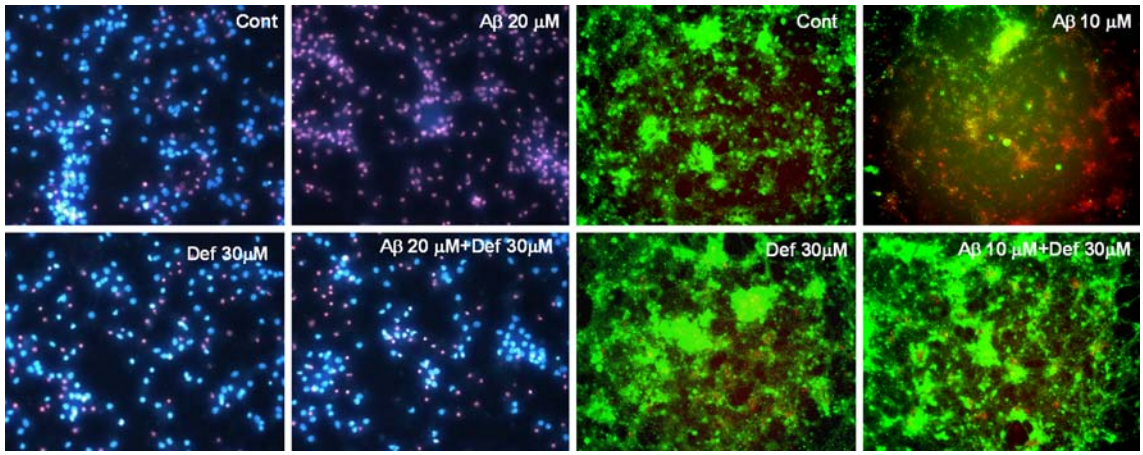

Fig. 5 Deferiprone protects against amyloid β-induced cell death (necrosis + apoptosis). On the left, Aβ induced neuronal cell death assessed by propidium iodide (PI) (red) and Hoescht 33342 (blue). Necrotic cells stain red and viable cells blue. PI: membrane impermeable DNA intercalator that stains necrotic cells. Hoescht 33342: cell permeable adenosine-thymidine-specific fluorescence stain, useful for staining DNA, chromosomes or nuclei. On the right, Aβ treated neuronal cultures. Annexin V-CY3 (AnnCy3) (red) versus 6-carboxyfluorescein diacetate (6-CFDA) (green) labelling. Apoptotic cells stain yellow, necrotic cells red and viable cells green. Annexin V-Cy3 binds to phosphatidylserine that is present in the outer leaflet of the plasma membrane of cells entering apoptosis. The binding is observed as red fluorescence. 6-CFDA is used to measure viability. When 6-CFDA (non-fluorescent) enters the cell, it is hydrolysed by esterases producing the fluorescent 6-carboxyfluorescein (6-CF) (green fluorescence). Viable cells: 6-CF (green), dead cells: AnnCy3 (red), apoptotic cells (staining both AnnCy3 and 6-CF) (yellow)

hydroxyquinolines possess neurotoxic side effects (Tsubaki et al. 1971; Oakley 1973). These side effects may be avoided by the use of non-halogenated analogues, for instance the brain permeable VK-28 (Warshawsky et al. 2000) (**10**, Fig. 4). A study centred on rats, with 6-OHDA-induced striatal dopaminergic lesions with (Ben-Shacar et al. 2004), has shown that, when injected either intraventricularly (1 μg in 5 ml) or intraperitoneally (1 or 5 mg kg^{-1} day^{-1} for 10 and 7 days respectively), VK-28 is able to provide neuroprotection against 6-OHDA at very low doses. This has been confirmed by the prevention of the reduction in striatal dopamine levels and by the decrease of dopamine turn-over, which are normally observed after 6-OHDA lesioning. Moreover, this study has shown that the mechanism of action of VK-28 is more likely to be related to iron chelation properties than to any direct interference with 6-OHDA, since intranigral or intraventricular 6-OHDA initiates an increase in total iron in the substantia nigra and striatum at the sites of neurodegeneration, in monkeys, rats and mice (Ben-Shachar et al. 2004).

Pharmacological studies undertaken recently on Deferiprone (**3**, Fig. 2) have shown an interesting profile, which suggests a neuroprotective effect in vitro against ferric nitrilotriacetate (FeNTA) and Aβ1–40. FeNTA and Aβ1–40 induced significant death of primary cortical neurons as determined by morphometric analysis of cell viability, using Hoescht 33324 and propidium iodide or 6-CFA and annexin V (Fig. 5). Deferiprone, when given up to 6 h after the insult, protected neurons in a concentration-dependent manner (Molina-Holgado et al. 2006). It is clear that deferiprone is able to protect neurones and their processes against a range of insults relevant to AD, when given after the insult. Compounds based upon this mechanism of action may have therapeutic potential in AD.

Conclusion

In summary, protein aggregation and oxidative stress have been demonstrated to be the major factors involved in the neurodegenerative process in AD, PD and prion disease. Metal ions play a

crucial role, acting as mediators of neurotoxicity either by favouring plaque formation or redox cycling. Thus, they provide a suitable pharmacological target for the treatment of neurodegenerative diseases. In particular, bidentate chelators such as hydroxypyridinones and hydroxyquinolines would appear to possess the greatest potential for this goal. The development of an effective non-toxic therapeutic agent for such complex and comprehensive brain disorders represents an extremely challenging task. Much needs to be understood in terms of ethiopathogenesis for AD, PD and prion disease. However, chelation therapy may be considered as a valuable strategy both for the treatment and for the investigation of neurodegeneration.

References

Abeysinghe RD, Roberts PJ, Cooper, CE, Maclean KH, Hider RC, Porter JB (1996) The environment of the lipoxygenase iron binding site explored with novel hydroxypyridinone iron chelators. J Biol Chem 271:7965–7972

Alam ZI, Daniel SE, Lees AJ, Marsden DC, Jenner P, Halliwell B (1997) A generalised increase in protein carbonyls in the brain of Parkinson's but not incidental Lewy body disease. J Neurochem 69:1326–1329

Allan SM, Rothwell NJ (2003) Inflammation in central nervous system injury. Phil Trans R Soc Lond B Biol Sci 358:1669–1677

Allan SM, Tyrrell PJ, Rothwell NJ (2005) Interleukin-1 and neuronal injury. Nat Rev Immunol 5:629–640

Antzutkin ON, Leapman RD, Balbach JJ, Tycko R (2002) Sopramolecular structural constraints on Alzheimer's β-amyloid fibrils from electron microscopy and solid-state nuclear magnetic resonance. Biochemistry 41:15436–15450

Atwood CS, Obrenovich ME, Liu T, Chan H, Perry G, Smith MA, Martins RN (2003) Amyloid-β: a chameleon walking in two worlds: a review of the trophic and toxic properties of amyloid-β. Brain Res Rev 43:1–16

Beard JL, Wiesinger JA, Connor JR (2003) Pre- and postweaning iron deficiency alters myelination in Sprague–Dawley rats. Dev Neurosci 25:308–315

Bence NF, Sampat RM, Kopito RR (2001) Impairment of the ubiquitin-proteosome system by protein aggregation. Science 292:1552–1555

Ben-Shacar D, Kahana N, Kampel V, Warshawsky A, Youdim MBH (2004) Neuroprotection by a novel brain permeable iron chelator, VK-28, against 6-hydroxydopamine lesion in rats. Neuropharmacology 46:254–263

Bonilla E (2000) Huntington disease. A review. J Clin Invest 41:117–141

Brion S, Mirol J, Psimaras A (1973) Recent findings in Pick's disease. In: Zimmerman HM (eds) Progress in neuropathology, vol 2. Grune and Stratton, New York, pp 421–452

Brown DR (2001) Copper and prion disease. Br Res Bull 55:165–173

Brunk UT, Jones CB, Sohal RS (1992) A novel hypothesis of lipofuscinogenesis and cellular aging based on interactions between oxidative stress and autophagocytosis. Mut Res 275:395–403

Brunk UT, Terman A (2002) Lipofuscin: mechanisms of age-related accumulation and influence on cell function. Free Rad Biol Med 33:611–619

Bucciantini M, Giannoni F, Chiti F et al (2002) Inherent toxicity of aggregates implies a common mechanism for protein misfolding diseases. Nature 416:501–511

Bush AI, Huang X, Fairlie DP (1999) The possible origin of free radicals from amyloid β-peptides in Alzheimer's disease. Neurobiol Ageing 20:335–337

Bush AI (2002) Metal complexing agents as therapies for Alzheimer's disease. Neurobiol Ageing 23:1031–1038

Butterfield DA, Kanski J (2001). Brain oxidation in age-related neurodegenerative disorders that are associated with aggregated proteins. Mech Ageing Dev 122:945–962

Cherny RA, Barnham KJ, Lynch T et al (2000). Chelation and intercalation: complementary properties in a compound for the treatment of Alzheimer's disease. J Struct Biol 130:209–216

Cherny RA, Atwood CS, Xilinas ME et al (2001) Treatment with a copper–zinc chelator markedly and rapidly inhibits b-amyloid accumulation in Alzheimer's disease transgenic mice. Neuron 30:665–676

Chetley A, Gilbert D (1986) Health action international. International Organisation of Consumers Unions, The Hangue

Claesen ME, Clements ML (1989) Ridding the world of hydroxyquinolines. Br Med J 299:527–528

Cohen AS, Shirahama T, Skinner M (1982) Electron microscopy of amyloid. In: Harris JR (eds) Electron microscopy of proteins, vol 3. Academic Press, London UK, pp 165–205

Cohen FE (1999) Protein misfolding and prion diseases. J Mol Biol 293:313–320

Connor JR, Snyder BS, Arosio P, Loeffler DA, Lewitt P (1995) A quantitative analysis of isoferritins in select regions of aged, parkinsonian and Alzheimer's diseased brains. J Neurochem 65:717–724

Cooper CE, Lynagh GR, Hoyes KP, Hider RC, Cammack R, Porter JB (1996) The relationship of intracellular iron chelation to the inhibition and regeneration of human ribonucleotide reductase. J Biol Chem 271:20291–20299

Crapper Mclachlan DR, Dalton AJ, Kruck TPA et al (1991) Effect of desferrioxamine on the clinical progress of Alzheimer's disease. Lancet 337:1304–1308

Crossthwaite AJ, Williams RJ (2002) Hydrogen peroxide-mediated phosphorilation of ERK1/2, Akt/PKB and JNK in cortical neurons: dependence on Ca^{2+} and PI 3-kinase. J Neurochem 80:24–36

Dexter DT, Wells FR, Lees AJ (1989) Increased nigral iron content and alterations in other metal ions occurring in brain in Parkinson's disease. J Neurochem 52:381–389

Dickson DW, Crystal HA, Bevona C, Honer W, Vincent I, Davies P (1995) Correlations of synaptic and pathological markers with cognition of the elderly. Neurobiol Aging 16:285–298

Di Patti MC, Persichini T, Mazzone V, Polticelli F, Colasanti M, Musci G (2004) Interleukin-1 beta upregulates iron efflux in rat C6 glioma cells through modulation of ceruloplasmin and ferroportin-1 synthesis. Neurosci Lett 363:182–186

Dobson MC (2003a) Protein folding and disease: a view from the first horizon symposium. Nat Drug Disc 2:154–160

Dobson MC (2003b) Protein folding and misfolding. Nature 426:884–890

Duffy PE, Tennyson VM (1965) Phase and electron microscopic observations of Lewy bodies and melanin granules in the substantia nigra and Locus caeruleus in Parkinson's disease. J Neuropathol Exp Neurol 24:398–414

Floyd RA (1999) Antioxidants, oxidative stress, and degenerative neurological disorders. Proc Soc Exp Biol Med 222:236–245

Floyd RA, Hensley K (2002) Oxidative stress in brain aging. Implications for therapeutics of neurodegenerative diseases. Neurobiol Aging 23:795–807

Gaeta A, Hider RC (2005) The crucial role of metal ions in neurodegeneration: the basis for a promising therapeutic strategy. Br J Pharmacol 146:1041–159

Gotz ME, Künig G, Riederer P, Youdim MBH (1994) Oxidative stress: Free radical production in neural degeneration. Pharmacol Ther 63:37–122

Grunblatt E, Mandel S, Youdim MB (2000) MPTP and 6-hydroxydopamine-induced neurodegeneration as models for Parkinson's disease: neuroprotective strategies. J Neurol 247(Suppl 2):95–102

Habgood MD, Liu ZD, Dehkordi LS, Khodr HH, Abbott J, Hider RC (1999) Investigation into the correlation between the structure of hydroxypyridinones and blood–brain barrier permeability. Biochem Pharmacol 57:1305–1310

Halliwell B (1992) Reactive oxygen species the central nervous system. J Neurochem 59:1609–1623

Hardy J, Selkoe DJ (2002) The amyloid hypothesis of Alzheimer's disease: progress and problems on the road to therapeutics. Science 297:353–356

Harris DC, Aisen P (1973) Facilitation of Fe(II) autoxidation by Fe(III) complexing agents. Biochim Biophys Acta 329:156–158

Hartley DM, Walsh DM, Ye CP et al (1999) Protofibrillar intermediates of amyloid b-protein induce acute electrophysiological changes and progressive neurotoxicity in cortical neurones. J Neurosci 19(20):8876–8884

Hedge ML, Jagannatha Rao KS (2003) Challenges and complexities of α-synuclein toxicity: new postulates in unfolding the mystery associated with Parkinson's disease. Arch Biochem Biophys 418:169–178

Hider RC, Hall AD (1991) Clinically useful chelators of tripositive elements. Prog Med Chem 28:41–173

Hider RC (1995) Potential protection from toxicity by oral iron chelators. Toxicol Lett 82–83:961–967

Hijazi N, Shaked Y, Rosenmann H, Ben-Hur T, Gabizon R (2003) Copper binding to PrP^C may inhibit prion disease propagation. Br Res 993:192–200

Hirsh EC, Brandel J-P, Galle P (1991) Iron and aluminium increase in the substantia nigra of patients with Parkinson's disease: an X-ray microanalysis. J Neurochem 56:446–451

Holander D, Ricketts D, Boyd CAR (1988) Importance of probe molecular geometry in determining intestinal permeability. Can J Gastroenterol 2:35A–38A

Hsiao K, Chapman P, Nilsen S et al (1996) Correlative memory deficits, Aβ elevation, and amyloid plaques in transgenic mice. Science 274:99–102

Huang X, Atwood CS, Hartshorn MA et al (1999) The Aβ peptide of Alzheimer's disease directly produces hydrogen peroxide through metal ion reduction. Biochemistry 38:7609–7616

Jeffrey M, Goodsir CM, Bruce ME, Mcbride PA, Scott JR (1994) Infection-specific prion protein (PrP) accumulates on neuronal plasmalemma in scrapie-infected mice. Ann NY Acad Sci 724:327–330

Jelliger KA (1999) The role of iron in neurodegeneration: prospects for pharmacology of Parkinson's disease. Drugs Aging 14:115–140

Kaur D, Yantiri F, Rajagopalan S et al (2003) Genetic or pharmacological iron chelation prevents MPTP-induced neurotoxicity in vivo: a novel therapy for Parkinson's disease. Neuron 37:899–909

Kamenetz F, Tomita T, Hsieh H et al (2003) APP processing and synaptic function. Neuron 37:925–937

Klein WL, Krafft GA, Finch CE (2001) Targeting small Aβ oligomers: the solution to an Alzheimer's disease conundrum? Trends Neurosci 24:219–224

Laine J, Marc M-E, Sy M-S, Axelrad H (2001) Cellular and subcellular morphological localization of normal prion protein in rodent cerebellum. Eur J Neurosci 14:47–56

Lambert MP, Barlow AK, Chromy BA et al (1998) Diffusible, nonfibrillar ligands derived from $Aβ_{1-42}$ are potent central nervous system neurotoxins. Proc Natl Acad Sci USA 95:6448–6453

Lan J, Jiang DH (1997) Desferrioxamine and vitamin E protect against iron and MPTP-induced neurodegeneration in mice. J Neural Transm 104:469–481

Lee J-Y, Friedman JE, Angel I, Kozak A, Kohj JY (2004) The lipophilic metal chelator DP-109 reduces amyloid pathology in brains of human β-amyloid precursor protein transgenic mice. Neurobiol aging 25:1315–1321

Levine SM, Chakrabarty A (2004) The role of iron in the pathogenesis of experimental allergic encephalomyelitis and multiple sclerosis. Ann NY Acad Sci 1012:252–266

Liu ZD, Lockwood M, Rose S, Theobald AE, Hider RC (2001) Structure-activity investigation of the inhibition of 3-hydroxypyridin-4-ones on mammalian tyrosine hydroxylase. Biochem Pharmacol 61:285–290

Liu ZD, Hider RC (2002a) Design of clinically useful iron(III)-selective chelators. Med Res Rev 22:26–64

Liu ZD, Hider RC (2002b). Design of iron chelators with therapeutic application. Coord Chem Rev 232:151–171

Liu ZD, Kayyali R, Hider RC, Porter JB, Theobald AE (2002) Design, synthesis, and evaluation of novel 2-substitued 3-hydroxypyridin-4-ones: structure-activity investigation of metalloenzyme inhibition by iron chelators. J Med Chem 45:631–639

Lovell MA, Robertson JD, Teesdal WJ, Campbell JL, Markesbery WR (1998). Copper, iron and zinc in Alzheimer's disease senile plaques. J Neurol Sci 158:47–52

Lozoff B, Beard J, Connor J, Barbara F, Georgieff M, Schallert T (2006) Long-lasting neural and behavioural effects of iron deficiency in infancy. Nutr Rev 64:S34–43

Lue LF, Kuo Y-M, Roher AE et al (1999) Soluble amyloid β-peptide concentration as a predictor of synaptic change in Alzheimer's disease. Am J Pathol 155:853–862

Maguire-Zeiss KA, Short DW, Federoff HJ (2005) Synuclein, dopamine and oxidative stress: co-conspirators in Parkinson's disease? Brain Res Mol Brain 134(1):18–23

Mandel S, Weinreb O, Amit T, Youdim MB (2005) Mechanism of neuroprotective action of the anti-Parkinson drug rasagiline and its derivatives. Brain Res Brain Res Rev 48:379–387

Martell AE, Smith RM (1974–1989) Critical stability constant, vol 1–6. Plenum Press, London

Maxton DG, Bjarnason I, Reynolds AP, Catt SD, Peters TJ, Menzies IS (1986). Lactulose, 51Cr-labelled ethylenediaminetetra-acetate, L-rhamnose and polyethyleneglycol 400 [corrected] as probe markers for assessment in vivo of human intestinal permeability. Clin Sci 71:71–80

Mc Lean CA, Cherny RA, Fraser FW et al (1999) Soluble pool of Aβ amyloid as a determinant of severity of neurodegeneration in Alzheimer's disease. Ann Neurol 46:860–866

Merz PA, Sommerville RA, Wisniewsky HM, Ikbal K (1981). Abnormal fibrils from scrapie-infected brain. Acta Neuropathol 54:63–74 (Berlin)

Miranda S, Opazo C, Larrondo LF et al (2000) The role of oxidative stress in the toxicity induced by amyloid β-peptide in Alzheimer's disease. Prog. Neurobiol. 62:633–648

Mizuno Y, Ohta S, Tanaka M et al (1989) Deficiencies in complex I subunits of the respiratory chain in Parkinson's disease. Biochem Biophys Res Commun 163:1450–1455

Molina-Holgado F, Williams RJ, Gaeta A et al (2006) Neuroprotective actions of an iron chelator against Alzheimer's disease-relevant insults. Poster session, 10th international conference on Alzheimer's disease and related disorders's, Madrid, Spain

Oakley GP (1973) The neurotoxicity of the halogenated hydroxyquinolines. JAMA 225(4):395–397

O'Brien-Ladner AR, Nelson SR, Murphy WJ, Blumer BM, Wesselius LJ (2000) Iron is a regulatory component of human IL-1beta production. Support for regional variability in the lung. Am J Respir Cell Mol Biol 23:112–119

Olanow CW (1992) Magnetic resonance imaging in parkinsonism. Neurol Clin North Am 405–420

Olanow CW, Youdim MB (1996) Neurodegeneration and neuroprotection in Parkinson's disease. Academic Press, pp 55–69

Oldendorf WH (1974) Lipid solubility and drug penetration of the blood–brain barrier. Proc Soc Exp Biol Med 147:813–816

Olivieri N, Koren G, Hermann C et al (1990) Comparison of oral iron chelator L1 and desferrioxamine in iron-loaded patients. Lancet 336:1275–1279

Paik SR, Shin H-J, Lee J-H, Chang C-S, Kim J (1999) Copper(II)-induced self-oligomerisation of α-synuclein. Biochem J 340:821–828

Palm A (1932) Untersuchung in des chinolin reihe. Arch Exp Pathol Pharmacol 199:176–185

Pan K, Baldwin M, Nguyen J et al (1993) Conversion of α-helices β-sheets features in the formation of the scrapie prion proteins. Proc Natl Acad Sci USA 90:10962–10966

Prusiner SB, Mckinley MP, Bowman KA et al (1983) Scrapie prions aggregate to form amyloid-like birefringent rods. Cell 35:349–358

Prusiner SB (1991) Molecular biology of prion disease. Science 252:1515–1522

Prusiner SB (2001) Neurodegenerative diseases and prions. N Engl J Med 344:1516–1526

Raymond KN, Müller G, Matzanke BF (1984) Complexation of iron by siderophores: a review of their solution and structural chemistry and biological function. Top Curr Chem 58:49–102

Riederer P, Sofic E, Rausch WD, Jellinger K, Youdim MBH (1989) Transition metals, ferritin, glutathione and ascorbic acid in Parkinsonian brains. J Neurochem 52:515–520

Rochet JC, Lansbury PT (2000) Amyloid fibrillogenesis: themes and variations. Curr Opin Struct Biol 10:60–80

Roher AE, Chaney MO, Kuo Y-M et al (1996) Morphology and toxicity of Aβ-(1–42) dimmer derived from neuritic and vascular amyloid deposits of Alzheimer's disease. J Biol Chem 271:20631–20635

Rogers JT, Lahiri DK (2004) Metal and inflammatory targets for Alzheimer's disease. Curr Drug Targets 5:535–551

Rogers JT, Leiter LM, Mcphee J et al (1999) Translation of the Alzheimer amyloid precursor protein mRNA is up-regulated by Interleukin-1 through 5′-untranslated region sequences. J Biol Chem 274:6421–6431

Rogers JT, Randall JD, Cahill CM et al (2002) An iron-responsive element type II in the 5′-untranslated region of the Alzheimer's amyloid precursor protein transcript. J Biol Chem 277:45518–45528

Rose FC, Gawel M (1984) Clioquinol neurotoxicity: an overview. Acta Neurol Scand 80(Suppl 100):137–145

Saggu H, Cooksey J, Dexter D (1989) A selective increase in a particular superoxide dismutase activity in Parkinsonian substantia nigra. J Neurochem 53:692–697

Sanchez-Ramos J, Overvick E, Ames BN (1994) A marker of oxyradical-mediated DNA damage (8-hydroxy-2′-deoxy-guanoxine) is increased in nigro-striatum of Parkinson's disease brain. Neurodegeneration 3:197–204

Serpell LC, Berriman J, Jakes R, Goedert M, Crowther RA (2000) Fiber diffraction of synthetic α-synuclein filaments shows amyloid-like cross β-conformation. Proc Natl Acad Sci USA 97:4897–4902

Shastry BS (2003) Neurodegenerative disorders of protein aggregation. Neurochem Int 43:1–7

Sherki YG, Melamed E, Offen D (2001) Oxidative stress induced-neurodegenerative diseases: the need for antioxidants that penetrate the blood brain barrier. Neuropharmacology 40:959–975

Shin R-W, Kruck TPA, Murayama H, Kitamoto T (2003) A novel trivalent cation chelator Feralex dissociates binding of aluminum and iron associated with hyperphosphorylated τ of Alzheimer's disease. Br Res 961:139–146

Sikorski P, Atkins EDT, Serpell LC (2003) Structure and texture of fibrous crystals formed by Alzheimer's Aβ (11–25) peptide fragment. Structure 11:915–926

Sofic E, Riederer P, Heinsen H (1988) Increased Iron(Iii) and total iron content in post mortem, substantia nigra of parkinsonian brain. J Neural Trans 74:199–205

Sofic E, Lange KW, Jellinger K, Riederer P (1992) Reduced and oxidized glutathione in the substantia nigra of patients with Parkinson's disease. Neurosci Lett 142:128–130

Stahl N, Borchelt DR, Hsiao K, Prusiner SB (1987) Scrapie prion protein contains a phosphatidylinositol glycolipid. Cell 51:229–240

Terry RD, Masliah E, Salmon DP et al (1991) Physical basis of cognitive alterations in Alzheimer's disease: Synapse loss is the major correlate of cognitive impairment. Ann Neurol 30:572–580

Torok M, Milton S, Kayed R et al (2002) Structural and dynamic features of Alzheimer's Aβ peptide in amyloid fibrils studied by site-directed spin labelling. J Biol Chem 277:40810–40815

Tsubaki T, Honma Y, Hosh M (1971) Neurological syndrome associated with clioquinol. Lancet 1:696–697

Tycko R (2004) Progress towards a molecular-level structural understanding of amyloid fibrils. Curr Opin Struct Biol 14:1–8

Uversky VN, Li J, Fink AL (2001) Metal-triggered structural transformations, aggregation and fibrillation of human α-synuclein. J Biol Chem 276:10737–10744

Vigo-Pelfrey C, Lee D, Keim P, Lieberburg I, Schenk DB (1993) Characterisation of amyloid peptide from human cerebrospinal fluid. J Neurochem 61:1965–1968

Walsh DM, Hartley DM, Kusumoto Y et al (1999) Amyloid β protein fibrillogenesis: structure and biological activity of protofibrillar intermediates. J Biol Chem 274:25945–25952

Walsh DM, Klyubin I, Fadeeva JV et al (2002) Naturally secreted oligomers of amyloid β protein potently inhibit hippocampal long-term potentiation in vivo. Nature 416:535–539

Wang SS, Becerra-Arteaga A, Good TA (2002) Development of a novel diffusion-based method to estimate the size of the aggregated Aβ species responsible for neurotoxicity. Biotechnol Bioeng 80:50–59

Warshawsky B., Youdim MBH, Ben-Shacar D (2000) Pharmaceutical compositions compromising iron chelators for the treatment of neurodegenerative disorders and some novel iron chelators. International Publication number WO 00/74664A2

Wisniewsky HM, Coblentz JM, Terry RD (1972) Pick's disease. A clinical and ultrastructural study. Arch Neurol 26:97–108

Xu J, Kao S-Y, Lee FJS, Song W, Jin L-W, Yankner BA (2002) Dopamine-dependent neurotoxicity of α-synuclein: a mechanism for selective neurodegeneration in Parkinson disease. Nat Med 8:600–606

Ye FQ, Allen PS, Martin WRW (1996) Basal ganglia iron content in Parkinson's disease measured with magnetic resonance. Mov Disord 11:243–249

Yokel RA, Fredenburg AM, Meurer KA, Skinner TL (1995) Influence of lipophilicity on the bioavailability and disposition of orally active 3-hydroxypyridin-4-one metal chelators. Drug Metab Dispos 23:1178–1180

Youdim MB, Grunblatt E, Mandel S (1999) The pivotal role of iron in NF-kappa B activation and nigrostriatal dopaminergic neurodegeneration. Prospects for neuroprotection in Parkinson's disease with iron chelators. Ann NY Acad Sci 890:7–25

Zecca L, Fariello R, Riederer P, Sulzer D, Gatti A, Tampellini D (2002) The absolute concentration of nigral neuromelanin, assayed by a new sensitive method, increases throughout the life and is dramatically decreased in Parkinson's disease. FEBS Lett 510:216–220

Zhang X, Haaf M, Todorich B et al (2005) Cytokine toxicity to oligodendrocyte precursors is mediated by iron. Glia 52(3):199–208