Iron chelation as a potential therapy for neurodegenerative disease

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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. Chelation therapy could provide a valuable therapeutic approach to such disease states, since metals, particularly iron, are realistic pharmacological targets for the rational design of new therapeutic agents.

Introduction

A classification of neurodegeneration can be achieved on the basis of the principal neuropathological changes, characterized by the presence of abnormal protein components [1,2], which accumulate in the brain leading to a selective loss of neurons in an age-dependent manner. A classic example is AD (Alzheimer’s disease).

There is extensive evidence for the association of protein aggregation with neurodegeneration in many neurodegenerative disorders. Interestingly, metals such as iron and copper appear to play an important role in protein aggregation and are therefore 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. Genetic factors are clearly involved, as pathogenic mutations in genes encoding aggregating proteins, such as Aβ (amyloid β-peptide) and α-synuclein are responsible for inherited forms of AD and Parkinson’s disease respectively. The mutant proteins, in these examples, show an increased tendency to form so-called amyloid-like fibrils, 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: unbranched and twisted structures rich in β-sheet of the ‘cross-β’ type, with β-strands perpendicular to the fibril axis and backbone hydrogen bonds parallel to the fibril axis [3–5]. Fibril construction is a cascade process which involves the formation of intermediate structures. The proteins first coalesce to form small soluble oligomers that aggregate to produce high-molecular-mass assemblies, so-called ‘protofibrils’. The protofibrils eventually exceed solubility limits and are deposited as fibrils [6,7]. The essential feature of the AD brain is the presence of extracellular plaques constituted of Aβ42 peptide deposits. The Aβ peptides are formed proteolytically in the cell from a large transmembrane glycoprotein called APP (amyloid precursor protein). Aβ42 is a 42-mer peptide which, in APP, is located at the outer surface of the plasma membrane. Studies with Aβ have demonstrated that small oligomeric aggregates are more likely to be toxic than the multilayer fibrils [8–10]. The soluble oligomeric forms seem to play a fundamental role in the pre-clinical and early progression of AD [11,12]. 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 6-fold higher concentration than that detected in control brains [13,14]. 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 Aβ42 can induce a dramatic loss of viability in neurons [15–17].

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

In principle, a protein or peptide, which is able to interact with metal ions, can generate redox activity and, as a consequence, oxidative damage. This latter property has been found to be a typical hallmark in the majority of neurodegenerative disorders, such as AD, and could, in principle, be either the primary cause or the consequence of disease progression. There is strong evidence of a relationship between oxidative stress and the presence of cortical Aβ deposits. In fact, Aβ42 is a metallo-binding peptide with binding sites for
Figure 1 | Secondary-structure prediction for $\text{A}_\beta^{42}$

The $P_\alpha$, $P_\beta$ and $P_\gamma$ values are defined in [26] and are modified by use of a larger dataset [27]. Structures of deferiprone (1), CQ (2) and VK-28 (3).

![Secondary-structure prediction for $\text{A}_\beta^{42}$](image)

Metal homoeostasis is altered during AD, and, as a consequence, metals are reported to accumulate in various brain sections with concentrations which are 3–5-fold increased compared with age-matched controls [19]. On the basis of these findings, an $\text{A}_\beta$-induced oxidative stress model has been developed [20]. 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.

The accumulation of metals in AD brains as well as the presence of a metal-binding site on $\text{A}_\beta$ represent promising pharmacological targets. Chelating compounds could, in principle, prevent the adverse generation of $\text{H}_2\text{O}_2$ catalysed by such metal-binding sites.

Structural analysis of $\text{A}_\beta^{42}$

There have been a number of solution structural studies [21] and molecular modelling investigations with the $\text{A}_\beta$ peptide [22], and, as is typical of a relatively small non-cross-linked peptide, a large degree of flexibility is demonstrated. In the past, we have successfully used a statistical-based analysis [23] for the correct structural analysis of melittin [24] and apamin [25], both relatively short peptides. Application of this method to $\text{A}_\beta^{42}$ is presented in Figure 1. Essentially, each amino acid is allocated a parameter for $\beta$-sheet, $\alpha$-helix and reverse-turn formation. Regions with an average parameter greater than 1 are predicted to adopt the related secondary structure. Thus $\alpha$-helix is predicted to form for regions 1–7 and 13–24 and $\beta$-sheet for regions 17–24, 30–36 and 39–42. The predicted $\beta$-sheet regions are complemented by two predicted reverse-turn regions, namely 25–28 and 36–39. The overlapping predictions of $\beta$- and $\alpha$-structures for region 17–23 are typical for a conformationally labile structure. However, from a range of physical measurements, it is known that $\beta$-sheet dominates the secondary structure of $\text{A}_\beta$ aggregates. Thus a reasonable assignment of structure for $\text{A}_\beta^{42}$, based on this analysis, is presented in Figure 2(a), where a large proportion of the peptide forms an antiparallel sheet and the N-terminal region of approx. 15 amino acids contains metal-binding residues, namely glutamate, aspartate, tyrosine and histidine. Parts of the long hairpin $\beta$-structure are relatively hydrophobic, but these regions are
close to the predicted hydrophilic reverse turn 25–28. A slightly modified structure (Figure 2b) demonstrates the possibility of the formation of highly predicted hydrophobic β-sheet core of 12 amino acids (ΔP2, 1.28). This core is connected to a longer hydrophilic hairpin loop and the metal-binding N-terminal region. Such a structure offers a ready explanation for the surface activity of Aβ42 (Figure 3). The calculated free energy of transfer of this core between water and lipid is 22.5 kcal/mol (1 kcal ≈ 4.184 kJ). Such a structure would possess the ability to form oligomers via strong hydrogen-bonding and hydrophobic interaction between the hydrophobic triple-stranded β-sheet units (Figure 4). Interestingly, this structure also offers an explanation for the dramatic difference in surface properties between Aβ42 and Aβ40. Aβ40 would not be expected to form oligomers as efficiently as does Aβ42, owing to disruption of the β-sheet strand region 39–42. The ligands in the N-terminal region are ideal for binding both iron(III) and iron(II), and therefore redox cycling of iron bound to such a structure is a reality.

**Therapeutic strategies**

The important features of a therapeutic iron-chelating agent are to scavenge the free redox-active iron present in excess in the brain and to form a non-toxic iron complex, which is then excreted. A second mechanism of beneficial chelation would be to cap the iron at its labile binding site, preventing any mediated toxic action (Fenton activity). In this second case, the newly formed stable iron complex would favour a complexed state in which iron is not redox active and therefore the toxicity would be blocked. The latter mechanism implies additional interactions between the drug and the target protein, which have to be considered in the design.

Another dominant property of any therapeutic chelator is metal selectivity, typically a high selectivity being required. There are clear guidelines for the design of iron-selective chelating agents [28]. In addition, for the treatment of neurodegeneration, the ready permeation of the BBB (blood–brain barrier) is critical. The size of useful chelators should therefore probably be limited to less than 300 Da. Such a molecular mass limit excludes hexadentate 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 sulfur atoms as ligands, for instance 2,2′-bipyridyl. 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). In contrast, iron(III)-selective chelators favour oxygen atoms as ligands, notably hydroxyypyridinones (Figure 1). 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 chelate iron(II) and facilitate autoxidation to iron(III) [29]. Therefore high-affinity iron(III)-selective ligands bind both iron(III) and iron(II) under most physiological conditions. Under biological conditions, the pM value (defined as the negative logarithm of the metal ion concentration under the following conditions: [metal ion]total = 1 μM, [ligand]total = 10 μM at pH 7.4) is a more useful parameter than the conventional stability constant to assess the ligand affinity for the metal [28,30]; for clinically useful iron scavengers, a pFe3+ value ≥ 20 is considered to be essential.

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 inhibit haem iron-containing
enzymes directly because of the inaccessibility of porphyrin-bonded iron to chelating agents. In contrast, many non-haem iron-containing enzymes such as lipooxygenase, the aromatic hydroxylase families and ribonucleotide reductase are susceptible to chelator-induced inhibition [31]. However, by careful modification of physicochemical properties, iron chelators can be designed which exert minimal inhibitory influence on many metalloenzymes [32,33].

In summary, a chelator suitable for the treatment of AD should possess a molecular mass in the region of 200–400 Da, should bind iron(III) much more tightly than iron(II) and should not redox cycle or interfere with metalloenzyme activity.

8-Hydroxyquinoline derivatives

To date, a range of 8-hydroxyquinoline analogues have demonstrated the greatest potential for the treatment of neurodegeneration and one compound, clioquinol (CQ) (Figure 1, 2), has entered clinical trials. CQ is a small lipophilic bioavailable metal chelator, which leads to beneficial effects in both AD and Parkinson’s disease animal models [34,35]. Following oral treatment with CQ (30 mg/kg of body weight per day), 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 stable in the treated animals, with a marked improvement being observed after only 16 days of treatment [34]. In model Parkinson’s disease studies, mice treated previously with the neurotoxin MPTP (1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine) were administered orally with CQ (30 mg/kg of body weight per day) for 8 weeks to assess the ability of the compound to protect against MPTP-induced toxicity. Total substantia nigra iron levels were found to be reduced by approx. 30% in the CQ-fed compared with control animals. Following CQ pre-treatment, oxidative stress markers and glutathione depletion were found to be significantly attenuated in substantia nigra [35]. Unfortunately, many halogenated hydroxyquinolines possess neurotoxic side effects [36,37]. These side effects may be avoided by the use of non-halogenated analogues, for instance the brain-permeable VK-28 (Figure 1, 3) [38]. A study centred on rats, with 6-OHDA (6-hydroxodopamine)-induced striatal dopaminergic lesions [39], has shown that, when injected either intraventricularly (1 μg in 5 ml) or intraperitoneally (1 or 5 mg/kg of body weight per day for 10 and 7 days respectively), VK-28 is able to provide neuroprotection against 6-OHDA.

Ideally for efficient removal of iron from the brain, the iron(III) complex should have a molecular mass less than 500 Da and be uncharged. With CQ, the 3:1 iron(III) complex is neutral, but its molecular mass is 922 Da; with VK-28, the iron complex will have a net charge of 3+ and a molecular mass of 878 Da. Thus neither of these complexes is likely to efflux from the brain in an efficient manner using a non-facilitated diffusion pathway.

Hydroxypyridin-4-one derivatives

Hydroxypyridinones possess a high affinity and selectivity for iron(III) and the dimethyl derivative (deferiprone; Figure 1, 1) has been used clinically to treat iron-overloaded thalassaemia patients for the last 15 years [40]. This chelator forms a stable 3:1 iron(III) complex (molecular mass, 470 Da), which is water-soluble and yet, by virtue of its neutral character, is able to transverse membranes by non-facilitated diffusion [28]. Furthermore, deferiprone readily crosses the BBB [41]. Pharmacological studies undertaken recently with deferiprone have shown an interesting profile, which suggests a neuroprotective effect in vitro against FeNTA (ferric nitrilotriacetate) and Aβ40 [42]. FeNTA and Aβ40 induce significant death of primary cortical neurons as determined by morphometric analysis of cell viability, using Hoechst 33342 and propidium iodide or 6-carboxyfluorescein and annexin V. Deferiprone, when given up to 6 h after the insult, protected neurons in a concentration-dependent manner [42]. It is clear that deferiprone is able to protect neurons and their processes against a range of insults relevant to AD, when given after the insult. On the basis of the knowledge that deferiprone is able to cross the BBB, the chelator has been successfully introduced into several clinical trials for the treatment of Friedrich’s ataxia [43]. Analogues of deferiprone are currently under investigation for their potential for the treatment of a range of neurodegenerative diseases.

Conclusion

In summary, oxidative stress has been demonstrated to be a major factor involved in the neurodegenerative process in AD. Metal ions and iron in particular play a crucial role, by catalysing redox cycling. Thus iron provides a suitable pharmacological target for the treatment of neurodegenerative diseases. Bidentate chelators such as hydroxyypyridinones and hydroxyquinolines would appear to possess the greatest potential for this goal. The development of an effective non-toxic therapeutic agent for such complex brain disorders represents a challenging task. Chelation therapy should be considered as a valuable strategy for the treatment of neurodegeneration.

References


