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REVIEW

Twenty years of metallo-neurobiology: where to now?

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Abstract The redox active transition metals Cu^{2+} and Fe^{3+} have been proposed as important factors in the neuropathology of Alzheimer's disease (AD) and other neurodegenerative diseases. The field that has been called metallo-neurobiology has expanded greatly in the last 20 years. Although there is much experimental evidence on various aspects of the interaction between these metals and the molecular and supramolecular components of the neuropil and the structural biology of metal binding, we are far from fully understanding the part this interaction plays in the normal CNS and in neurodegeneration. This understanding is needed if we are to move beyond the promising, but semi-empirical, approaches to therapies of these diseases based on metal attenuation.

Abbreviations

AD	Alzheimer's disease
Aβ	Amyloid β peptide
APP	Amyloid precursor protein
CNS	Central nervous system
CQ	Clioquinol (5-chloro-7-iodo-8-hydroxyquinoline)
EPR	Electron paramagnetic resonance spectroscopy
MMPs	Matrix metalloproteinases
NMR	Nuclear magnetic resonance spectroscopy
PrP^c	Prion protein, cellular isoform
SOD	Superoxide dismutase
XAFS	X-ray absorption fine structure

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Introduction

The Metals, Membranes and Neuroscience meeting organized by the Australian Society for Biophysics as a satellite of the 7th IBRO World Congress of Neuroscience in 2007 was held at the University of Melbourne's Bio21 Molecular Biology and Biotechnology Institute on July 11th 2007. The event was planned to bring together researchers from a variety of biophysical disciplines working in fields relevant to transition elements and membranes in neurology, in the expectation that the gathering would lead to a synthesis of ideas that could help clear the way to the development of therapies for neurodegenerative diseases based on the accumulating body of knowledge in these fields. The papers presented included data on X-ray absorption and diffraction studies on the Cu^{2+} coordination site of the amyloid β-peptide (Aβ) of Alzheimer's disease (AD), X-ray crystallographic, XAFS and EPR studies on the copper binding domain (CuBD) of the amyloid β precursor protein (APP), neuronal copper transport, brain metalloproteinase activity, models of PX2 allosteric receptor, a critical review of EPR approaches to the determination of metal coordination sites and, in the membrane area, NMR studies on the interaction between Cu binding proteins, membranes as templates for protein folding and prion-membrane interaction. Expanded versions of these papers are printed in this issue.

Background to metallo-neurobiology

It is over two decades since dysregulation of the redox active transition metal ions, as shown by high concentrations Cu^{2+} and Fe^{3+} in β-amyloid plaques in the brain, was first proposed to play an important part in the pathology of AD. Over this time, a new field of metallo-neurobiology has arisen with a steady growth in the proportion of papers about Zn or Cu in the AD literature (Fig. 1). There is also increasing evidence of the importance of metals in other neurodegenerative diseases.

Some landmark papers published over the last 20 years are those on stimulation-induced uptake and release of zinc in hippocampal slices (Howell et al. 1984; Kang and Kim 2003), on the resemblance between APP and a cell surface receptor (Kang et al. 1987), on the association between familial amyotrophic lateral sclerosis and mutations in the Cu/Zn superoxide dismutase gene (Rosen et al. 1993), on the rapid induction of Aβ amyloid formation by zinc (Bush et al. 1994), on Cu^{2+} binding by PrP^c (Brown et al. 1997), on increased iron-dependent aggregation and toxicity in the A53T alpha-synuclein mutant (Ostrerova-Golts et al. 2000) and on how Cu^{2+} mediates the progression of Huntington's disease (Fox et al. 2007).

Nevertheless, we are far from fully understanding the part played by these metals in the normal CNS and in neurodegeneration. Although there is much experimental evidence on various aspects of the interaction between the transition elements and the molecular and supramolecular components of the brain, much more needs to be done to understand the structure of the metal binding sites and their involvement in the physiology and biochemistry of the normal and pathological brain.

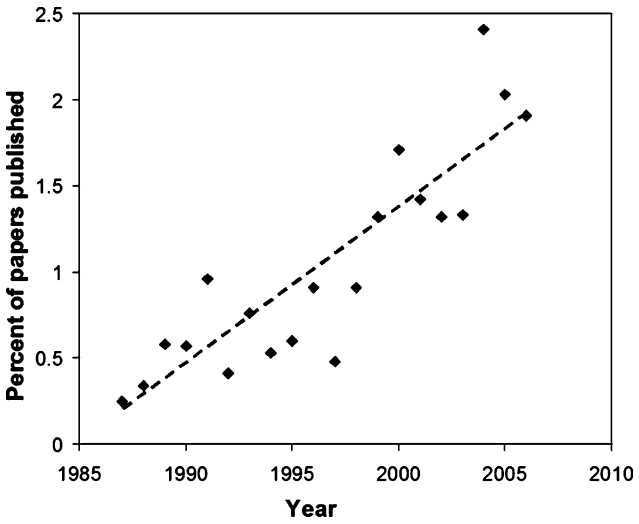

Fig. 1 Year by year plot of the number of papers referring to metals in AD (aggregate 76) as a proportion of the total number referring to AD (aggregate 3,143) published in the last two decades $R^2 = 0.7775$

Since the neurodegenerative diseases, aside from being individual human tragedies, will cause an increasing health burden in all societies where longevity is increasing, the development of effective therapies is urgent. There have been some encouraging results from animal studies and a small clinical trial based on a semi-empirical approach to correcting transition metal imbalances in AD. However the interactions involved in the high energy central nervous system are of daunting complexity; in all of them a bewildering array of metalloproteins and their interactions are deployed. For example, copper-binding proteins in the central nervous system may possess oxidant or anti-oxidant properties, possibly affecting neuronal function or triggering neurodegeneration.

Progress so far

It is well-established that oxidative damage to many classes of biological molecule produces lipid peroxidation adducts, protein carbonyl modifications, and nucleic acid adducts such as 8-OH guanosine that are typical of AD neuropathology (Baker et al. 1996; Sayre et al. 1997a, b; Smith et al. 1996, 1997) and precede Aβ deposition (Nunomura et al. 2000, 2001, 2006). Cu^{2+} and Fe^{3+} interact with Aβ (to make it toxic in cell culture. In vitro Aβ catalyses H_2O_2 generation through the reduction of Cu^{2+} and Fe^{3+}, using O_2 and biological reducing agents, such as cholesterol, vitamin C and catecholamines, as substrates (Huang et al. 1999a, b; Opazo et al. 2000; Opazo et al. 2002). Aβ is not toxic in the absence of Cu^{2+} or Fe^{3+} (Opazo et al. 2002), contradicting the once prevalent view that Aβ by itself was toxic. However, the question is open as to whether the so-called toxic soluble oligomeric species owe their toxicity to the presence of metal ions in their supramolecular structure making them redox active (Curtain et al. 2001; Smith et al. 2006) or whether their oligomerization is an outcome of metal catalysed redox reactions leading to dityrosine cross-linking (Barnham et al. 2004b).

Studies in vitro had shown that sequestering the metal ion with a suitable chelator inhibited the production of ROS by copper complexed Aβ (Huang et al. 1999a, b, 2000, 2004). Subsequently, it was shown using a range of metal chelators that the dissolution of Aβ in the amyloid plaques of post-mortem AD-affected brain tissue was correlated with the release of Cu and Zn, but not Fe (Cherny et al. 1999) and infrared Raman spectroscopic microscopy showed that treatment of amyloid plaques with the chelator tetraethyldiamine tetraacetate loosened their characteristic structure due to a reversal of Cu^{2+} binding by the histidine residues of the plaques' constituent Aβ peptides (Dong et al. 2003).

Consideration of the bioavailability of the chelators led to in vitro trials of CQ, a discontinued halogen-substituted

hydroxyquinoline antibiotic, which was found to be as effective as high affinity chelators in blocking the production of H_2O_2 by Aβ, in preventing precipitation of synthetic Aβ by Zn^{2+} and Cu^{2+}, and in extracting Aβ from post-mortem AD brain specimens (Cherny et al. 1999). In a blinded study, 9 weeks of oral treatment of human APP-expressing Tg2576 transgenic mice with CQ lowered brain Aβ deposition by 49% (~375 μg/g wet weight, $P = 0.0001$) (Cherny et al. 1999). CQ was not neurotoxic, and general health and body weights were significantly more stable in the treated animals, which were conspicuously improved after only 16 days of treatment. CQ treatment decreased the levels of extracellular Aβ not by preventing an Aβ–metal interaction outside the cell, but by facilitating the delivery of Cu and Zn across the plasma membrane into the cell (White et al. 2006). Once inside the cell, Cu and Zn, but not Fe, activated phosphoinositol 3-kinase mediated protein kinase pathways, leading to an increase in the secretion of MMPs whose ability to degrade Aβ has been reported by several groups (Backstrom et al. 1996; Stix et al. 2001).

Treatment with CQ may therefore work in two ways; by binding extracellular metals, it prevents metal mediated Aβ aggregation and toxicity and, by then delivering the bound metals into the cell, it activates specific protein kinases increasing the production of Aβ-degrading MMPs. Therefore, we need to recognise that neurotoxicity generated by the interaction between Aβ and metals may be more complex than merely the catalysis of Aβ aggregation. Numerous studies have now shown that several potential mechanisms of neurotoxicity for soluble Aβ are exacerbated by, if not dependent on, the presence of metals. This indicates that Aβ–metal interactions, possibly occurring within the cell, may induce mechanisms of neurotoxicity that involve soluble Aβ oligomers, and that the mechanisms of toxicity precede Aβ aggregation and accumulation (Lue et al. 1999; McLean et al. 1999). CQ showed promise as a "proof of principle" compound in a small phase 2 clinical trial (Ritchie et al. 2003), encouraging the development of a series of heterocyclic compounds (Barnham et al. 2004a) one of which is in clinical trial in AD.

The future

If we are to follow the metallo-biological path in the therapy of neurodegenerative diseases, we shall need a wide range of biophysical, biochemical and in vivo studies to move beyond a semi-empirical approach. Judging from the results with CQ, any metal attenuating compound must be hydrophobic, unlike most chelators, and readily cross the blood brain barrier. Hydrophobicity means a low aqueous solubility, which will reduce the chances of systemic metal depletion. Aside from its low solubility CQ is a relatively weak chelator (Ka is nanomolar for Zn^{2+} and Cu^{2+}), with the metal ions being redistributed rather than excreted. It is possible, also, that the effects of the drug may arise from its selective binding to the Aβ–metal complex, as well as/or from metal depletion of brain tissue and/or facilitating metal ion transport across cell membranes. Here, the hydrophobicity of CQ and its stereochemical properties would be the keys to its access to the metal binding site on Aβ. In this case, determination of the 3D structure of the Aβ metal ion coordination site is an important consideration in designing drugs based on CQ.

We must recognise, however, that Aβ (can bind up to 3.5 moles of Cu^{2+} and Zn^{2+} (Atwood et al. 2000) per mole peptide suggesting other, low affinity, metal binding sites. These are structurally ill defined. They are not obvious in either NMR or EPR spectroscopy (Curtain et al. 2001), the latter even when a sensitive instrument is used at high gain (Smith et al. 2006). These low-affinity metal binding sites on Aβ may also be important for the pathophysiology of AD (Curtain et al. 2001; Kaur et al. 2003). The affinity of CQ for Cu^{2+} is sufficient (nM) to dissociate Zn^{2+} and low affinity bound Cu^{2+} from Aβ, and CQ induced Cu^{2+} dissociation from Aβ by has been observed by NMR spectroscopy, while showing no direct interaction of CQ with Aβ (Cherny et al. 2001). It may not be too far-fetched to imagine CQ reacting with relatively low-affinity metals bound to Aβ, as well as binding selectively to the high-affinity binding site. Once in the brain, any metal attenuating compound might have to perform many actions, as well as interacting with $Cu2^{+}$Aβ and facilitating Cu^{2+} passage across the neural cell membrane to activate MMPs mentioned above. Other actions could be dissolving zinc-mediated Aβ plaques and inhibiting Cu^{2+}Aβ-catalysed H_2O_2 production by reducing the availability of Cu^{2+}, inhibiting formation of toxic Aβ oligomers. Zn^{2+} plays a complex role in the pathophysiology of Aβ. By precipitating Aβ to form amyloid plaques (Bush et al. 1994) it may be a defence where the ion acts to divert the formation of pathological Aβ oligomers (Lovell et al. 1998), explaining why H_2O_2-mediated oxidative damage in the neuropil is inversely correlated to plaque load. For these reasons, blocking Zn^{2+} as well as Cu^{2+} interactions with Aβ could be therapeutically important and dual Zn^{2+}/Cu^{2+}-binding properties could be an important property in any future drug's effectiveness in inhibiting Aβ accumulation in vivo.

It is interesting to reflect on the recent suggestion that altered Cu^{2+} coordination converts Zn-deficient SOD (normally binding equimolar Cu and Zn) from an antioxidant to a neurotoxic pro-oxidant (Estevez et al. 1999). Earlier, we had found that the coordination of Cu^{2+} by Aβ resembles the SOD1 active site, with Cu binding generating an allosterically ordered membrane-penetrating Aβ oligomer linked by SOD-like bridging histidine residues (Curtain et al.

2001). While further electron spin resonance studies combined with other biophysical and cell toxicity data (Smith et al. 2006) have furnished a more sophisticated analysis (Drew et al. 2007 this issue), it appears that membrane interaction of redox competent oligomers appears to be a necessary .condition for cytotoxicity. A full understanding of the nature of this interaction is an important task for the future.

From the list given above it can be seen that, while the determination of the structures of the key actors in the metallobiology of the brain is necessary for the rational development of therapies of disorders of transition ion regulation it is not sufficient until we can gain better understanding of the interactions between these actors.

Declaration of interest Ashley I. Bush is a shareholder and scientific consultant to Prana Biotechnology Ltd.

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