Dietary chelators as antioxidant enzyme mimetics: implications for dietary intervention in neurodegenerative diseases
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Following recent reviews on the role of metal ions in oxidative stress and neurodegenerative diseases, this article reports advances in the study of dietary components for the control of these conditions. Poor metal ion homeostasis is credited with pathological roles in the progression of a number of disorders including Alzheimer’s disease, Parkinson’s disease and multiple sclerosis. Synthetic metal ion chelators continue to show promise as a new therapeutic approach for neurodegenerative disorders. Dietary chelators, unlike most vitamins, are, however, capable of negating or even reversing the roles of metal ions by: (i) decorporation of metal ions, (ii) redox silencing, (iii) dissolution of deposits, and (iv) generation of an antioxidant enzyme mimic. This review gives a critical evaluation of recent progress in, and potential for, dietary control of neurodegeneration on the basis of the formation of antioxidant enzyme mimetics. Behavioural Pharmacology 17:425–430 © 2006 Lippincott Williams & Wilkins.

Introduction
Detailed studies of many intricate pathological events occurring during neurodegeneration have widened our understanding of these disease processes (e.g. Esposito et al., 2006; Nichols et al., 2006). We are, however, far from comprehensively understanding, let alone combating, many neurological disorders. Persistent inflammation and metal ion deposition are characteristic features of many neurological disorders including Alzheimer’s disease (AD), Parkinson’s disease (PD) and multiple sclerosis (Bossy-Wetzel et al., 2004; Doraiswamy and Finferock, 2004; Gaeta and Hider, 2005; Moreira et al., 2005; Tabner et al., 2005). Chronic inflammation is a complex multifaceted process, which is driven by a matrix of pathways with complexities that do not lead to an optimistic prognosis or to the expectation of an imminent cure. In the half century, since the Nobel Prize was awarded for the treatment of rheumatoid arthritis with corticosteroids, mankind has endeavoured to treat chronic inflammatory diseases by combating inflammatory pathways, but without a glimpse of a cure. Even the latest anti-inflammatory agents exhibit serious side effects upon long-term use, as do corticosteroids, for which the Prize was awarded.

From a drug discovery perspective, the presence of metal ion deposits in inflamed tissues is intriguing. Uncontrolled metal ion homeostasis is considered to play an important role in inflammatory pathogenesis via the mediation of oxidative damage (Halliwell and Gutteridge, 1999). Oxidatively damaged cellular components may in turn elicit an immune response leading to the perpetuation of autoimmune disease. In vitro, the redox-active transition metal ions Fe(III) and Cu(II) enhance oxidative damage by well-established reactions such as the Fenton and Haber–Weiss reactions for the generation of the potent hydroxyl radical (–OH) (Halliwell and Gutteridge, 1999; Fisher and Naughton, 2004, 2005a). The presence of redox-active ions in neurological disorders is a clear target for therapeutic intervention. Recent reviews have highlighted the potential for metal-ion-based therapeutic intervention in neurological disorders (Fisher and Naughton, 2005a; Valko et al., 2005) and put the case for the ineffectiveness of vitamins (Fisher and Naughton, 2005b). This approach aims to prevent or slow down formation of deposits, and where formed to: (i) remove deleterious metal ions and their contribution to oxidative stress, (ii) dissolve the ferric ion deposits, (iii) prevent metal ion based biomolecule aggregation and (iv) generate catalytic antioxidants (enzyme mimetics) that unlike vitamins are capable of independently destroying large quantities of reactive oxygen and nitrogen species (RONS) (Fisher and Naughton, 2005a).

The focus of this review is to give a critical overview of the potential for dietary intervention involving natural products that chelate metal ions to form antioxidant enzyme mimetics. Thus, natural chelators have the...
potential to: (i) remove deleterious redox active metal ions and their contributions to biomolecule aggregation, (ii) convert metal ions into potent antioxidant mimetics to combat oxidative stress and (iii) ‘dissolve’ biomolecule and metal oxide aggregates.

**Reactive oxygen and nitrogen species and metal ions**

Oxidative damage results from an imbalance between the body’s defences and the levels of RONS generation in a specific tissue (Halliwell and Gutteridge, 1999; McLaren et al., 2006; Rahman et al., 2006). The defence mechanisms include compartmentalization, antioxidant enzymes [eg, superoxide dismutase (SOD), catalase and peroxidase] and careful homeostasis of oxidizing and reducing agents and redox-active metal ions that could aggravate oxidative damage. A further consideration is the capacity for damage repair that may vary with organelle, cell and tissue type, and health status or type (and stage of progression) of disease (Mahmoudi et al., 2006). RONS are generated by inflammatory cells, normal metabolic (enzymatic) processes, signalling mechanisms, xenobiotics and ionizing radiation, among other sources (Blomgren and Hagberg, 2006; Roncone et al., 2006; Tompkins et al., 2006; Zweier and Talukder, 2006).

Commonly studied RONS include hydrogen peroxide (H$_2$O$_2$), superoxide (O$_2^-$), hydroxyl radicals (OH), nitric oxide (NO), peroxy nitrite (ONOO$^-$) and hypochlorous acid (HOCl). It should be, however, noted that under physiological conditions, many of these species can rapidly interconvert and readily convert to a much larger number of reactive intermediates that are outside the scope of this article. In broad terms, RONS can be grouped into those whose formation or removal normally falls under enzymatic control in vivo (H$_2$O$_2$, O$_2^-$, NO) and those that are relatively uncontrollable (OH, ONOO$^-$, HOCl). The latter uncontrollable RONS are key targets for therapeutic intervention, as they exceed the scope for antioxidant protection in mammals. The complexities of RONS investigations, however, parallel those of mediators of inflammation, except an added dimension arises from the dynamics of RONS interconversion. This interchange coupled to the paucity of real-time measurement capacity for multiple RONS leads to caution in assessing the pathological roles of individual RONS. An alternative approach involves preventing the formation of specific RONS by applying ‘chemical knockouts’. Rapid progress has been made in the discovery of numerous small molecule antioxidant enzyme mimetics that destroy copious quantities of their target RONS (Fisher and Naughton, 2005a).

**Neurological disorders and redox-active metal ions**

Over 20 neurodegenerative disorders have been associated with metal ion imbalances. In PD, increased iron levels found in the substantia nigra coupled to caeruleoplasmin mutations imply a state of poor metal homeostasis (Hochstrasser et al., 2004; Morawski et al., 2005). Other studies have revealed an almost two-fold increase in the incidence of PD in daily iron supplement users, demonstrating that diet can play a key role in disease progression (Powers et al., 2003). The iron chelator desferrioxamine was effective in models of PD in mice, indicating that reducing iron levels prevents dopaminergic neuron degeneration and reduces abnormal protein aggregation (Zhang et al., 2005).

In AD, plaques are formed from the amyloid-$\beta$-protein (A$\beta$), which is proteolytically derived from the amyloid precursor protein (APP). APP binds to Cu(II) reducing it to Cu(I), leading to the formation of a disulfide bond in the APP (Multhaup et al., 1997). Aggregation of amyloid-$\beta$ plaques is initiated by submicromolar levels of metal ions in vitro (Huang et al., 2004). Previous studies have implicated leading roles for metal ions in the progression of AD. Plaques are associated with accumulations of redox-active metal ions (Cu $\approx$ 0.4 mmol/l, Fe $\approx$ 1 mmol/l) (Strausak et al., 2001). Increased ferritin and decreased transferrin levels are observed in brains of AD patients (Fischer et al., 1997). RONS are produced during metal ion binding by A$\beta$ (Huang et al., 1999) and plaques can be dispersed with metal ion chelators (Cherny et al., 2001; Rottkamp et al., 2001). More recently A$\beta$ has been shown to redox cycle iron leading to RONS generation (Khan et al., 2006).

In a preliminary study, magnetite and maghemite deposits have been found in brains of patients suffering from AD (Dobson, 2001; Hautot et al., 2003). These highly magnetic (ferromagnetic) crystals are found in regular patterns that suggest they are deposited in a carefully controlled biological process, analogous to their biochemical control in birds where they assist navigation. Deposition of these iron oxides suggests that a defect has occurred in normal control mechanisms for this process. Dissolving these crystals with chelators is one of a number of therapeutic approaches that may also include controlling the biochemical regulation of deposition and dietary intervention.

In several other neurodegenerative disorders characterized by inflammation, poor metal ion homeostasis has been observed. Brain iron localization in reactive microglia and macrophages is likely to contribute to oxidative damage in multiple sclerosis (LeVine, 1997). Iron misregulation is credited with a pathological role in a variety of other neurological disorders including Huntington’s disease, Friedreich’s ataxia and prion diseases (Mattson, 2004; Cerpa et al., 2005). Furthermore, for a number of conditions, neurodegeneration is just one component in which poor metal ion homeostasis affects
several organs. In Wilson’s disease, poor copper regulation results in copper accumulation in the liver followed by deposition in the brain (Kitzberger et al., 2005).

Neurological disorders, behaviour and antioxidants

The approach of treating neurological disorders with chelators with the capability to form antioxidant mimetics, while preventing or reversing metal ion-mediated plaque formation, is still in its infancy. A number of recent studies have, however, demonstrated the potential for antioxidant intervention strategies to rescue and/or protect against neurodegenerative disorders. Further work is warranted to explore the precise active constituent(s) and/or the mechanism(s) of action in each case given below.

Potential experimental antioxidant therapies for PD, or models of PD, include pretreatment of caffeine against intrastriatal 6-hydroxydopamine-lesioned rats (Joghataie et al., 2004). In this study, the observed attenuation of the apomorphine-induced rotational behaviour, coupled to protection of neurons of the substantia nigra pars compacta by caffeine, may reflect its antioxidant activity (Weiss and Landauer, 2003). The antioxidant selenium was also protective in the 6-hydroxydopamine-induced rat model of PD (Zafar et al., 2003). In this study, selenium treatment resulted in an enhancement of the antioxidant status along with near baseline functional recovery. In the same model of PD, pretreatment with the iron chelator desferrioxamine ameliorated locomotor activity, rearing and exploratory behaviour (Youdim et al., 2004). In a more recent study, the protective effects of black tea extracts (BTE) have been investigated (Chaturvedi et al., 2006). This study also revealed recovery (both pretreatment and post-treatment with BTE relative to 6-hydroxydopamine) in spontaneous locomotor activity and in D-amphetamine-induced circling behaviour, as well as in striatal antioxidant status (lipid peroxidation and SOD levels). The authors report the pretreatment schedule as the more effective, and further exploration of these results are warranted in light of the potential for BTE constituents to act as dietary chelators, with the potential to form complexes with SOD-mimetic activities as outlined below.

Potential antioxidant treatments for AD are under investigation in models of disease. Following intrahippocampal injections with aggregated Aβ in rats, daily oral administration of α-tocopherol improved temporal discrimination under a recycling conjunctive schedule of food reinforcement (McDaid et al., 2005). In a previous report, intracerebroventricular infusion of Aβ in rats was employed to show that daily oral administration of α-tocopherol prevented impairment of maze performance (Yamada et al., 1999). Apple juice concentrate was selected as a rich source of antioxidants, and has been shown to prevent oxidative damage and deterioration of maze performance in aged mice (Tchantchou et al., 2005).

In a study reported by Frautschy et al. (2001), dietary curcumin reduced Aβ deposits and prevented spatial memory deficits induced by infusions of Aβ. The recent identification of curcumin as a dietary antioxidant chelator, which can exhibit SOD-like activity in the complexed form (Table 1), further enhances the significance of these key observations.

Antioxidant enzyme mimetics

A wide range of metal complexes has been prepared which exhibit antioxidant enzyme activities. These range from small peptide mimetics of the antioxidant enzyme active sites (metal binding centres) to numerous complexes bearing little or no similarity to the enzymes (Table 1). A large number of these diverse agents have been tested in numerous model systems and these have been reviewed elsewhere (Fishier and Naughton, 2005a). Numerous key parameters such as the specific antioxidant enzyme activities of various mimetics are not known however, thus, the field lacks standardization. Current efforts are underway to enhance our understanding of these agents which includes: (i) the full characterization of all the antioxidant enzyme activities (i.e. catalase, SOD, peroxidase) for each agent, (ii) the tissue and cellular distribution profiles (e.g. permeability into cells, organelles, membranes etc.), (iii) the pharmacokinetics and (iv) pharmacodynamics.

Several approaches to categorize therapeutic chelators exists and these may be based on the source, chelator structure, metal ion(s) complexes formed or pharmacodynamic properties of the end complex. It is becoming clear that a large number of redox-active metal ion complexes can partake in redox reactions attributable to antioxidant enzyme mimetics. These chelators arise from numerous sources including synthetic chemistry projects,

<table>
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<tr>
<th>References</th>
<th>SOD mimic</th>
<th>IC_{50}(μmol/l)</th>
</tr>
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<tbody>
<tr>
<td>Jiang et al. (2003)</td>
<td>M40403</td>
<td>31.6</td>
</tr>
<tr>
<td>Kostyuk et al. (2004)</td>
<td>Cu(I)-luteolin</td>
<td>0.80</td>
</tr>
<tr>
<td>Fisher et al. (2004)</td>
<td>2Cu(II)-EGTA</td>
<td>0.60</td>
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<tr>
<td>Kostyuk et al. (2004)</td>
<td>Cu(I)-ruizin</td>
<td>0.50</td>
</tr>
<tr>
<td>Baudry et al. (1993)</td>
<td>Manganes salen C12</td>
<td>0.32</td>
</tr>
<tr>
<td>Kostyuk et al. (2004)</td>
<td>Cu(II)-(–)-epicatechin</td>
<td>0.32</td>
</tr>
<tr>
<td>Fisher et al. (2005)</td>
<td>Mn(II)-monensin</td>
<td>0.31</td>
</tr>
<tr>
<td>Fisher et al. (2004)</td>
<td>Mn(II)-EGTA</td>
<td>0.19</td>
</tr>
<tr>
<td>Bark et al. (2003)</td>
<td>Cu(I)-curcumin</td>
<td>0.16</td>
</tr>
<tr>
<td>Boka et al. (2004)</td>
<td>Cu(I)-Ac-HisValHis-H_2</td>
<td>0.16</td>
</tr>
<tr>
<td>Fisher et al. (2005)</td>
<td>2Cu(II)-monensin</td>
<td>0.09</td>
</tr>
<tr>
<td>Spasojevic et al. (2001)</td>
<td>1/2[Mn(III)BVDME]_2</td>
<td>0.047</td>
</tr>
<tr>
<td>Baticini-Haberle et al. (1999)</td>
<td>Mn(III)TE-2-PyP^{2+}</td>
<td>0.045</td>
</tr>
<tr>
<td>Jitsukawa et al. (2001)</td>
<td>CuZn-SOD</td>
<td>0.0028</td>
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Dietary antioxidant chelators

As a wide variety of molecules have been shown to complex redox-active metal ions and impart antioxidant enzyme activities, a large number of dietary components may be expected to exhibit these properties (Table 1). As for the general field of antioxidant chelators, clearly a great deal of further study is required to ascertain which dietary components are active and under which conditions. These studies should include the usual considerations of relative stabilities of the chelators and/or their complexes towards stomach acids and enzymes, ingestion potential, and profiles of RONS that are inactivated. In addition, consideration needs to be given to the preference for administration as chelator or complex, the optimal ratios of metal ions to chelators, the availability of redox-active metal in vivo to enact complex formation from the chelator and the stability constants for the complex. The stability constant is critical to ensure competing metal ions do not destabilize the complex and that key metal centres in biomolecules remain unaffected, while removing deleterious metal ions that initiate and/or perpetuate pathological biomolecule aggregation. Further key issues for treating NDs are the permeability of therapeutic agents and their complexed forms to the blood–brain barrier and this key concept is addressed below.

Drug discovery programmes, microbial isolates and more recently from plant sources. Recently, chelators have been categorized by their pharmacodynamic activities that are imparted upon formation of complexes with redox-active metal ions (Fisher and Naughton, 2005a). Thus, the categories of (i) antioxidant enzyme mimetic, (ii) a RONS scavenger and (iii) a redox silencer have been employed.

To date, these detailed studies have not been conducted on any dietary foodstuff. One of the most studied common sources of dietary chelators is green tea, which has well-established antioxidant effects. A large number of publications detail the beneficial effects of green tea and of polyphenolic and other extracts of green tea on in-vitro model systems, cellular systems and animal models (Sutherland et al., 2006). These effects range from epidemiological studies on humans relating to cancer, cardiovascular and neurological diseases to animal models of PD and AD (Cabrera et al., 2006). Polyphenolics constitute up to one-third of the dry weight of green tea with the catechins being the principal remedial components (Zaveri, 2006). The major catechin epigallocatechin gallate (EGCG) accounting for some two-thirds of the catechin content is protected by the steaming and drying process used for green teas (Zaveri, 2006). The pharmacological effects of this and related catechins have been widely studied. EGCG inhibits cardiac hypertrophy by blocking the RONS-dependent nuclear factor-kB and RONS-independent activator protein-1 pathways (Li et al., 2006a). It ablates RONS-induced apoptosis in motor neurones transfected with G93A CuZnSOD (Koh et al., 2004). It upregulates the cytoprotective enzyme heme oxygenase via the phosphatidylinositol 3-kinase and extracellular signal-regulated protein kinase pathways (Wu et al., 2005). More recently, EGCG has been shown to ameliorate disease progression in an amyotrophic lateral sclerosis mouse model initiated by transfection with G93A CuZnSOD (Koh et al., 2006). In oral carcinoma cells, EGCG inhibited secretion of APP in a dose-dependent manner (Ko et al., 2006). Similar results were found in human neuroblastoma cells in which the inhibitory effect of EGCG on levels of membrane-bound APP secretion was reversed with the administration of ferrous sulphate (Reznichenko et al., 2006). The authors suggest that this brain-permeable potent chelator may be a therapeutic agent for AD and other iron-associated disorders (Reznichenko et al., 2006). EGCG when administered both intraperitoneally and orally reduced the amyloid depositions in brains of APP transgenic mice (Li et al., 2006b).

In contrast, it is notable that a number of studies report detrimental effects of green tea and its extracts (Navarro-Peran et al., 2005; Galati et al., 2006; Yu et al., 2006). Toxic effects were found in isolated hepatocytes with EGCG being most toxic with the mechanism of toxicity being disruption of the mitochondrial membrane potential and formation of RONS (Galati et al., 2006). Furthermore, a prostate cancer cell line treated with Cu(II) ions and EGCG exhibited rapid cell death via disruption of the cytoplasmic membrane, which may result from generation of hydroxyl radicals (Yu et al., 2006). Intake of EGCG during pregnancy may cause birth defects owing to its inhibitory effect on dihydrofolate reductase at common levels found in the sera of green tea drinkers (Navarro-Peran et al., 2005).

In addition to the ferric ion chelation study on EGCG (Reznichenko et al., 2006), a number of other catechins have been subjected to metal ion binding studies. These include ferric and cupric ion interactions with catechin (Fernandez et al., 2002), and (−)-epicatechin, which exhibited moderately enhanced SOD activity in vitro in the complexed form relative to the free chelator (Kostyuk et al., 2004). A conversion of redox active metal ions from mediating disease initiation and/or progression to preventing metal or peptide deposition, along with the concomitant generation of antioxidant enzyme mimetics, is very attractive from a therapy perspective.

A wide variety of dietary products or isolated natural products have been examined in vitro and in model systems for their antioxidant and anti-inflammatory potentials, with emphasis on their efficacy in treating...
neurodegenerative diseases (e.g. Ono et al., 2006). Many have not addressed the issues pertaining to the pathological roles of metal ions, either in discussion or by appropriate experimental design (Fisher and Naughton, 2005b). More recently, several isolated compounds have been shown to exhibit levels of antioxidant enzyme activity that are commensurate with some of the best synthetic mimetics available (Barik et al., 2005). This subject area requires considerable work to establish the potential of dietary-based chelators to treat or protect against neurological disorders in which metal ions are credited with pathological roles.

**Concluding remarks**

Although a comprehensive understanding of the parameters raised above remains elusive, a great deal of effort is bringing the prospect of diet-based therapeutic agents for neurological diseases somewhat closer. A number of uncertainties, however, remain to be clarified. For many synthetic and natural products, the antioxidant mimic activities have only been reported for the SOD reaction. Hence, a highly active SOD mimic generating large amounts of hydrogen peroxide may be deleterious in the absence of catalase activity. It is imperative that we gain an in-depth understanding of the profile of antioxidant enzyme activities exhibited by each complex. Where, for example, do these chelators decarboxylate the metal ions? (are the complexes permeable to the blood–brain barrier for excretion?). What becomes of the hydrogen peroxide generated by the SOD reaction? (do the mimetics also exhibit catalase activities?). Do these chelators impair metalloprotein function by chelating the metal ions in the active site or by extracting them? What other non-oxidant-related enzyme activities will these chelators exhibit?

**References**


