Neurodegenerative Diseases and Therapeutic Strategies using Iron chelators

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Summary

This review will summarise the current state of our knowledge concerning the involvement of iron in various neurological diseases and the potential of therapy with iron chelators to retard the progression of the disease. We first discuss briefly the role of metal ions in brain function before outlining the way by which transition metal ions, such as iron and copper, can initiate neurodegeneration through the generation of reactive oxygen and nitrogen species. This results in protein misfolding, amyloid production and formation of insoluble protein aggregates which are contained within inclusion bodies. This will activate microglia leading to neuroinflammation. The evidence for metal involvement in Parkinson's and Alzheimer's Disease as well as Friedreich's Ataxia and Multiple Sclerosis will be presented. Preliminary results from trials of iron chelation therapy in these neurodegenerative diseases will be reviewed.

Introduction

The human brain gives us the power to speak, imagine and problem solve as well as the ability to perform a number of tasks, which include the control of body temperature, blood pressure, heart rate and breathing, accept information from various senses, such as visual, auditory and smell, as well as allowing one to think, dream, reason and experience emotions. However it is difficult to imagine how mental entities such as thoughts and emotions could be implemented by physical entities such as neurons, glial cells and synapses or by any other type of mechanism. For these reasons, the way in which the brain can perform such functions remains one of the greatest scientific challenges of the 21st century. Recently the Human Brain Project has been launched by the European Commission (1], which should go a long way to improving our understanding of brain function in health and disease, as well as the changes which occurs with aging. It has as its goal to lay the technical foundations for a new model of ICT-based brain research, driving integration between data and knowledge from different disciplines, and catalysing a community effort to achieve a new understanding of the brain, new treatments for brain disease and new brain-like computing technologies. Thanks to the progress of modern medicine, and to improved living standards, the life expectancy of the human race continues to increase steadily, unlike other mammals. However, the downside is that as our population ages, so the risk of contracting one of a number of neurodegenerative diseases also increases. The most common of these are dementias, characterised by decline in cognitive faculties and the occurrence of behavioural abnormalities which interfere with the capacity of the afflicted individual to carry out normal daily activities. It most often affects elderly individuals and the most common is Alzheimer's disease (AD). Dementia prevalence increases with age; in the USA whereas 5.0% of those aged 71–79 years are affected, this climbs to 37.4% of those aged 90 and older [2].

In this review, we outline some of the mechanisms underlying neurodegenerative diseases which involve the essential metal iron, and discuss some of the preliminary results which have used the therapeutic strategy of chelation to remove potentially toxic iron from the brain.

The importance of metals in the brain

A number of important biological functions in the brain require metal ions such as potassium, sodium, calcium and zinc together with the redox-active iron and copper [3]. For example; the fast transmission of electrical impulses between neurons and along their axons to muscles and endocrine tissues, the maintenance of ionic gradients and the synthesis of neurotransmitters require these metal ions. The opening and closing of gated sodium and potassium channels to generate electrochemical gradients across the plasma membranes of neurons allows the transmission of nervous impulses, not only within the brain, but also the transmission of signals from the brain to other parts of the body.

The function of proteins is often dependent on their shape and their charge, and the binding of Ca^{2+} to proteins, just like the phosphorylation of the hydroxyl groups of Ser, Thr or Tyr residues by protein kinases, can trigger changes in both shape and charge. This ability of Ca^{2+} and phosphoryl groups to alter local electrostatic fields and thereby protein conformation and function are the two universal tools of signal transduction in biology. In most cells, including nerve cells, fluxes of Ca^{2+} ions play an important role in signal transduction regulating a wide range of cellular processes through ligand-gated channels, such as the NMDA receptor, activated by the glutamate agonist N- methyl-D-aspartate or voltage-gated Ca^{2+} channels. The transient rise in cytosolic Ca^{2+} levels initiated by extracellular signals, leads to the binding of Ca^{2+} by Ca^{2+}-sensor proteins, like calmodulin and synaptotagmin 1, which in turn activate a great variety of enzymes. Two target proteins for calmodulin in mammalian brain are calcineurin, a heterodimeric phosphatase, which is be involved in synaptic plasticity [4] and the Ca^{2+}/calmodulin-dependent protein kinase CaMKII, which plays a central role in Ca^{2+} signal transduction [5], and is the most abundant protein in the postsynaptic density [6], the region of the postsynaptic membrane physically connected to the ion channels which mediate synaptic transmission.

Another metal ion that has been extensively implicated in brain function is Zn^{2+} [7]. The mammalian forebrain contains a subset of glutamatergic neurons that sequester zinc in their synaptic vesicles, which is released into the synaptic cleft during synaptic transmission. Zinc may act as a critical neural messenger through its ability to regulate NMDA receptor activity. Excessive synaptic release of Zn^{2+} followed by entry into vulnerable neurons contributes to severe neuronal cell death.

The redox-active metal ions copper and iron are both essential for normal brain function [8, 9]. Deficiency of copper during the foetal or neonatal period, will have adverse

effects both on the formation and the maintenance of myelin. Excess "free" copper is however also dangerous, due to its capacity, like iron, to participate in redox reactions, generating toxic reactive oxygen and nitrogen species. Copper serves as an essential cofactor for two key proteins involved in neurotransmitter synthesis, dopamine β-hydroxylase, which transforms dopamine to nor-adrenaline, and peptidyl-α-amidating monooxygenase involved in the amidation of neuropeptides. Iron is involved in many fundamental biological processes in the brain, including oxygen transport, DNA synthesis, nitric oxide metabolism and mitochondrial respiration, as well as as for several specific neuronal functions in myelin synthesis and neurotransmitter synthesis and metabolism [9]. Since iron is involved in many central nervous system processes [9] which might affect infant behaviour and development, iron deficiency has adverse effects on pre- and post-natal brain development. With ageing, there is an elevation of brain iron (within ferritin and neuromelanin) in specific brain regions, i.e. frontal cortex, caudate nucleus, putamen, substantia nigra and globus pallidus, with no apparent adverse effect. However, ill-placed excessive amounts of iron in specific intracellular compartments or in specific regions of the brain, lead to neurodegenerative diseases [10, 11] through mechanisms described in the next section.

Metal-based neurodegeneration

Over the last decade, it has become more and more widely accepted that inflammation, associated with dysfunction of metal ion homeostasis (Fe, Cu, Zn) resulting in concomitant oxidative stress, are key factors in a large number of neurodegenerative diseases [10, 11]. Support comes from the observation that AD, PD and many other neurodegenerative diseases are characterised by increased levels of these metal ions in specific regions of the brain.

The **'metal-based neurodegeneration hypothesis'** is briefly outlined here [10. 11]. Redox-active metal ions (Fe, Cu), present within specific brain regions, can generate oxidative stress by production of reactive oxygen and nitrogen species (ROS, RNS). The chemistry of iron with oxygen and its two-electron reduction product, hydrogen peroxide has been reviewed[12, 13]. The one-electron reduction of H_2O_2, the well-known Fenton reaction gives the hydroxyl radical, $OH^{\cdot}$ [Equation 1]:, one of the most reactive free radical species known, which can react with a wide number of cellular constituents.

$$Fe^{2+} + H_2O_2 \longrightarrow Fe^{3+} + \cdot OH + OH^- \qquad [1]$$

When ROS are generated by redox metals in proximity to membrane phospholipids they initiate peroxidation of polyunsaturated fatty acids in the phospholipids, and the lipid hydroperoxides which are generated will break down to form a variety of lipid-derived α,β-unsaturated 4-hydroxyaldehydes [14, 15], of which the most prominent are 4-hydroxynonenal (HNE) and 4-hydroxy-2-hexenal (HHE).

Both ROS and RNS play important roles in signal transduction and gene expression. The first RNS to be discovered, the free radical nitric oxide ($NO^{\cdot}$), is an omnipresent intercellular messenger in all vertebrates, modulating blood flow, thrombosis, and neural activity. Although $NO^{\cdot}$ is often described as highly toxic and reactive, it has become evident that most of its cytotoxicity is due to the much more powerful oxidant peroxynitrite ($ONOO^{-}$), formed from the reaction between $NO^{\cdot}$ and superoxide ($O_2^{\bullet -}$) [Equation2], which can oxidize methionine and nitrate tyrosine residues in proteins.

$$NO^{\cdot} + O_2^{\cdot -} \longrightarrow ONOO^{-} \qquad [2]$$

Proteins are a major target of both ROS and RNS, and numerous oxidative modifications, reversible and irreversible forms have been characterized, which may change both the structure and the function of the oxidized protein, **Figure 1**. It is clear that a distinction must be made between those, mostly reversible, at low concentrations, which play an important role in cellular signalling and the essentially irreversible modifications which generate high levels of ROS and RNS with deleterious effects. A significant portion of such ROS-induced modifications will result in the addition of reactive carbonyl functional groups to proteins, generically termed “protein carbonylation” [16, 17]. Most of these carbonyl groups are generated from lipid peroxidation, from glucose-protein or glucose-lipid interactions (glycation) or from oxidative modification of amino acids in proteins (amino acid oxidation).

The damaged proteins become misfolded, aggregate, and finally overwhelm the ubiquitin/proteasome protein degradation system, such that the aggregated, ubiquinated proteins accumulate as deposits of characteristic fibrillar, protein-containing aggregates inclusion bodies in the affected tissues and organs. The presence of such intracellular inclusions or extracellular plaques, known as amyloid, is a common hallmark of many neurodegenerative diseases, including Alzheimer's disease (AD), Parkinson's disease, and prion diseases [18]. However, for protein aggregates to be properly described as amyloid, they should have the characteristic ‘cross-β’ X-ray fibre diffraction pattern [19]. The current definition of amyloid is 'an

unbranched protein fibre whose repeating substructure consists of β strands that run perpendicular to the fibre axis, forming a cross-β sheet of indefinite length' [20]. Amyloid fibres have very special mechanical properties, are extremely resilient to proteolytic attack, and are not easily degraded. The pioneering work of Chris Dobson and his colleagues has clearly demonstrated that many, if not all, proteins can adopt an amyloid-like conformation under appropriate conditions [18, 24].

Iron chelation and neurodegeneration

The possible therapeutic use of iron chelators to remove the excess amounts of iron from specific brain regions which occurs in different neurodegenerative diseases has received considerable attention over the past few years. The iron chelator should essentially be able to penetrate cellular membranes as well as the blood brain barrier, target the region of iron accumulation without depleting transferrin bound iron from the plasma, and be able to remove the chelatable iron from the site of accumulation or to transfer it to other biological proteins such as circulating transferrin. [25] Since the majority of patients with neurodegenerative disease will have normal iron homeostasis, low doses of the chelator need to be used to minimise side effects. The MRI technique T2* has been extensively used to monitor changes in the iron content of specific brain regions, which appears at best to be semiquantitative, the results being influenced by differences in crystal structure and/or unequal ferritin clustering in these specific brain regions as well as by size of the magnetic particle [26]. The chemical structures of three iron chelators which have been approved for clinical use in the treatment of beta-thalassaemia, desferrioxamine, deferiprone and desferasirox are shown in **Figure 2**. Two of these iron chelators are orally active, deferiprone and desferasirox, which has made their use in the treatment of neurodegenerative diseases more appropriate.

Parkinson's disease

Changes in brain iron homeostasis occur in Parkinson's Disease patients, with elevated levels of iron, in the form of H-ferritin and neuromelanin, occurring in the substania nigra, SN. Furthermore in the early stages of PD, no changes in iron content of other brain regions have been noted. Neuroinflammation and neurodegeneration occurs in the SN, probably due to the presence of Lewy bodies and precipitated α-synuclein, activating microglia, which will accumulate iron as a result of up regulation of the iron transporter ferroportin. One of the

main pathological hallmarks of PD is the preferential degeneration of dopaminergic neurons, which supports a direct role of dopamine itself in promoting the disorder. The oxidative chemistry of dopamine, may ultimately lead to the formation of free radicals and reactive quinone species. Such dopamine-derived quinones may react with several cellular targets which could foster the processes involved in the pathogenesis of PD and contribute to the progression of the disorder.

Preliminary chelation studies of one PD patient (deferiprone 30mg/kg/day for 32 months), who presented with a variety of symptoms including dysarthria and orofacial dystonia, with iron accumulation in many brain regions including the dentate nuclei, substantia nigra and red nuclei as assessed by T2* MRI showed some positive rsults. After 6 months there was an improvement in many of the symptoms, after 1 year an improvement in the UPDRS score, while the T2* MRI showed a rapid onset decrease in iron accumulation in the bilateral dentate nuclei, with a milder but later decline in the substantia nigra. No significant change in the iron content of the red nuclei was reported [27]. Since this initial observation of the beneficial effect of deferiprone, two further clinical trials have investigated the efficacy and safety of deferiprone in double-blind placebo studies for the treatment of Parkinson's Diseases. Either R2* or T2* MRI sequences were utilised, UPDRS motor scores were analysed, and serum ferritin, a marker of iron stores and inflammation, was also measured. One study indicated that deferiprone, 30mg/kg/day, slightly improved motor signs after 12 months of treatment, decreased motor handicap progression (mean change in UPDRS motor score =-2) while the iron content in substantia nigra was significantly decreased, (mean change in R2* MRI sequence =0.6) after one year. Three of the 40 patients in the study developed neutropenia or agranulocytosis which resolved rapidly with cessation of the oral therapy [28,29). In the other study, a small improvement in UPDRS scores was evident after 6 months of deferiprone therapy, (either 20mg/kg or 30mg/kg), significant decreases of iron in specific brain regions

were detected by T2* MRI, and the drug was well tolerated by all of the patients apart from 2 who developed neutropenia [30].

Alzheimer's disease, AD

High levels of zinc, copper and iron are present in the insoluble amyloid plaques in post mortem AD brains, such that disequilibrium of these essential trace elements possibly plays a role in the misfolding process which occurs with amyloid, Aβ, aggregation. The toxicity of any excessive amounts of iron is exemplified by the fact that increased amounts of iron will influence furin activity, (which is important for the activation of secretases), such that low levels of furin (induced by high levels of iron) will preferentially enhance the amyloidogenic pathway. In addition, iron may modulate APP processing, by virtue of the presence of a putative iron responseive element in APP mRNA [31]. Downregulation of APP will cause a decrease in the production of APP, and possibly lead to a reduction in amyloid protein, while iron influx will reverse this inhibition, and cause iron accumulation within neurons.

Early studies [32] showed that there was a significant reduction in the rate of decline of daily living skills in the 48 AD patients who received desferrioxamine (125mg i.m.2xdaily/5times /week for 24 months) when compared to AD patients receiving placebo. Despite such positive results, there have been no other clinical studies reported where any of the iron chelators have been investigated for their clinical efficacy in this disease.

Currently only one family of metal binding agents, PBT2 (5,7-dichloro-2-(dimethylamino)-methyl)-8-hydroxyquinoline) is in clinical trials for the treatment of Alzheimer's and Huntington's Diseases. It mainly binds excesses copper and zinc and possibly iron in the brain, thereby diminishing the amount of amyoid plaque formation and relocating these metal ions to depleted cellular and neuronal compartments. AD patients who received 250mg/day showed a significant reduction in cerebrospinal fluid Aβ concentration, while some cognitive improvement (executive function) was also noted. [33].

Friedreich's ataxia. FRDA

Friedreich's ataxia is the most common of the hereditary ataxias, the occurrence being one case in 50,000 individuals in the Caucasian population, and is caused by triplet repeat extensions in the frataxin gene. The first symptoms usually develop during childhood or

puberty with a life expectancy between 40 and 50 years The most common mutation in the frataxin gene is an expanded GAA trinucleotide repeat in intron 1 of the frataxin gene which occurs in approximately 96% of frataxin patients. The severity of the disease depends on the number of repeats. Iron accumulates in the mitochondria in FRDA, and there is a deficiency of i) mitochondrial Fe-S cluster containing proteins, e.g-aconitase, ii) respiratory chain electron transporters on complex I-III, which results in oxidative stress and free radical accumulation. The highest concentration of frataxin is found in the heart, spinal cord and dorsal root ganglia. The essential role of frataxin in mitochondrial iron metabolism is only partially understood; it may regulate iron handling in mitochondria, (as an iron binding protein) and, as such, prevent iron from generating oxidative stress. However, it most likely functions as an iron chaperone for the biosynthesis of Fe-S clusters and perhaps also haem.

In ground breaking research Boddaert and colleagues [25], were the first to investigate the efficacy of deferiprone in the treatment of FRDA patients. They showed that treatment with deferiprone, either 20 or 30mg/kg/day, for 6 months in 9 FRDA patients aged between 14 and 23 years, reduced the iron content of the dendate nuclei, which was associated with significant neurological improvements e.g manipulative dexterity, speech fluency, reduction in neuropathy and ataxia gait particularly in the youngest patients. This occurred relatively rapidly, 2 months after the commencement of treatment, the larger the iron accumulation the more robust and proportional the change evoked by the chelator. In addition, the authors suggested that the form of iron chelated was the labile iron pool, with iron possibly being bound to enzymes such as hydroxylases, as well as to ferritin. Interestingly no other brain region investigated, i.e. the pallidal nuclei, the thalamus and cerebellar white matter region showed any significant changes in iron accumulation, as detected by T2* measurements over the period of the chelation therapy. In another study by Velasco-Sanchez et al., [34] deferiprone, 20mg/kg/day, together with the antioxidant idebenone was administered to 20 FRDA patients, aged between 8 and 25 years. The chelation of iron from the dentate nuclei occurred after 11 months of treatment, (as assessedby T2* MRI), which was similarly associated with a stabilising effect on neurological function, e.g. a significant recovery of kinetic functions, although gait and posture scores worsened.

Multiple sclerosis, MS

MRI and histological studies have shown global alterations in iron levels in the brains of MS patients in deep grey matter structures, which are associated with increased disability and

grey matter atrophy. In addition, increases in the iron stored by macrophages and microglia are also evident which may indicate that a pathogenic process is occurring. Iron overload is also evident in macrophages which will promote a pro-inflammatory M1 activation state (see below). Such increases in iron have generally been thought to be detrimental. The possible causes of iron accumulation and deposition in the CNS include degeneration of oligodendrocytes and myelin, infiltration of immune cells into sites of neurodegeneration, release of haem following vascular haemorrhage, dysregulation of iron transport proteins and/or other regulatory molecules, and other pathologies [35] (Reviewed by Stephenson et al., 2014) . Oligodendrocytes are the most metabolically active cells in the brain (probably owing to their role in myelination), and contain an abundance of iron-requiring enzymes that are important for oxidative metabolism. In addition, oligodendrocytes are rich in ferritin-bound and myelin sheaths in actively demyelinating MS lesions such that this is one obvious potential source of the iron accumulation observed in activated macrophages and microglia

Neuroinflammation

Neuroinflammation plays an important role in the pathogenesis of many of the neurodegenerative diseases. Such inflammation is induced possibly by the presence of misfolded proteins which act as a catalyst for the activation and sustained activity of glial cells. Glial cells, i.e. microglia, astrocytes and oligodendrocytes have important roles in the brain. Microglia play an important role in maintaining normal CNS function, as well as continually searching for alterations in brain homeostasis through their constant scanning dynamic ramifications. They are involved in the maintenance of the synapses, having receptors for a wide variety of neurotransmitters, thereby directly interacting with termini, spines, astrocytic processes and the synaptic clef [36]. Astrocytes provide biochemical and nutritional support for endothelial cells, (in the blood brain barrier) and neurons as well as being involved in the release of pro-and anti-inflammatory cytokines but the latter is at a

lesser extent than microglia. The main function of oligodendrocytes is to provide support and insulation to axons in the central nervous system.

Activated microglial cells have been implicated in both the initiation and progression of neurodegenerative diseases. When challenged microglia are capable of acquiring diverse and complex phenotypes, which permits them to participate in the cytotoxic response, immune regulation and injury resolution. This is characterised by four main phenotypes, classically activated M1 with cytotoxic properties; M2a an alternate activation which is involved in repair and regeneration; M2b with an immune-regulatory phenotype; or M2c with an acquired-deactivating phenotype. (**Figure 3**) Activated microglia, M1 phenotype, will release many pro-inflammatory mediators which induce neuronal injury and death. However to date, there have been few investigations of the microglia phenotype in the different neurodegenerative diseases, or of therapeutic strategies which are able to switch the M1 phenotype to a M2 phenotypes thereby possibly reducing disease progression.

Parkinson's Disease, PD

In the PD brain proliferation of microglia is observed early in the disease process and was reported to remain relatively static and unrelated to the extent of striatal degeneration and disease severity, [reviewed in 37]. However the advanced dopaminergic degeneration in symptomatic PD has been associated with an overproduction of cytotoxic cytokines, which could indicate that microglia are polarised to a mainly M1 phenotype in advanced PD disease, [38]. Therefore multiple phenotypes may exist in PD, such that the disease progression or retardation may shift between the different phenotypes.

Alzheimer's disease (AD)

Alzheimer's disease (AD) is characterised by a classical neuropathology: intraneuronal accumulations of hyperphosphorylated microtuble-associated protein tau known as neurofibrillary tangles (NFTs), and extracellular deposition of amyloid β-peptide (Aβ) known as amyloid or senile plaques. Age-dependent neuro-inflammatory changes may play a significant role in this process, where microglia switch from an M2 to M1 phenotype. It was originally hypothesised (the amyloid cascade hypothesis) that the abnormal processing of APP, (occurring either spontaneously or genetically) induces overproduction of Aβ42 fragments, which accumulate in the brain and activate the innate immune system, which causes AD [39]. However, there are certain observations which do not concur with this hypothesis. Firstly the removal of Aβ from the brains of animal models and humans does not halt the progression of the disease and secondly Aβ is often present in healthy brains [40]. Although there is increased inflammation in AD, both in the serum, (Il-1β and TNFα), and brain tissue (IL-6), the levels of cytokines determined may be insufficient to elicit significant neuronal damage in the AD brain. There is an increase in the number of 'activated' microglia cells (of unknown phenotype) surrounding senile plaques in the cerebral cortex, Aβ deposits are present in T cell, and there is a significant decrease in CD200 protein and mRNA in AD hippocampus and inferior temporal gyrus, but not cerebellum.[40]. Therefore a second hypothesis has been presented indicating that neuronal damage that occurs in AD is not entirely due to Aβ deposition or intracellular tau accumulation but caused by an abnormal immune response. There seems to be some controversy with respect to the functioning of the M1 and M2 phenotypes in AD brains. M1 phenotype, have been identified in some AD mouse models e.g APP + PS1, which appeared to inhibit Aβ clearance while M2a or M2c phenotype enhanced Aβ clearance [40]. In contrast Wilcock, [41] indicated that M1 phenotype lowered amyloid load but exacerbated neurofibrillary tangle pathology, while M2a

phenotype is accompanied by elevated amyloid load and appears to ameliorate neurofibrillary pathology

Multiple sclerosis

MS is an auto-immune disorder of the CNS characterised by inflammatory destruction of the myelin sheath of the long axons of motor neurons, the oligodendrocytes being the principal target of the inflammatory attack. Focal lymphocytic infiltration occurs which leads to damage to the myelin and axons. The hallmark sign is the formation of the sclerotic plaque which represents the end stage of a process involving inflammation, demyelination and remyelination, oligodendrocyte depletion and astrocytosis, neuronal and axon deheneration [42]. Initially inflammation is transient and remyeliation occurs but it is not durable. This could indicate that the phenotype of the microglia is altering, the M1 phenotype exacerbating the disease, by the production of proteases, glutamate, ROS and other cytotoxic agents thereby promoting myelin breakdown. Alternatively a switch to the M2 phenotype would assist in CNS repair through the production of neurotrophic factors and clearance of myelin debris.

Concluding remarks

Considering the importance of metal ions in the normal functions of the human brain, it is not surprising that dysregulation of metal homeostasis should have harmful effects on brain function. A growing body of data supports the view that the redox-active metals iron and copper can generate oxidative stress and inflammation, leading to protein misfolding and aggregation associated with many neurodegenerative diseases. There is increasing evidence that disruption of iron regulation plays a key role in the aetiology of Alzheimer's disease, Parkinson's disease, Huntington's disease, Friedreich's ataxia and a number of other neurological disorders. The therapeutic utilisation of iron chelators in neurodegenerative diseases is still in its infancy. However, advances in non-invasive techniques, notably MRI have enabled us quantify, albeit it approximately, brain iron content in specific brain regions, which allows us to evaluate the effects of chelation therapy in removing iron. Secondly neuroinflammation plays an important role in the progression of each neurodegenerative disease, such that identification of the microglia phenotype is clearly important, and if M1 phenotype is present, therapeutic agents to switch such a phenotype to a more protective

phenotype could be of therapeutic value. The preliminary results for iron chelation in a growing number of neurodegenerative diseases have been reviewed here, and are globally positive. In view of the relative paucity of other therapeutic possibilities the use of iron chelators as well as neuroinflammation inhibitors for the treatment of these disorders, may be encouraging portents for the future.

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Figure Legends

Figure 1: The production of protein carbonyls. From Dalle-Donne [16]. (a) This can arise from direct oxidation of amino-acid side chains (Pro, Arg, Lys, and Thr). (b) Protein carbonyl derivatives can also be generated through oxidative cleavage of proteins, via the α-amidation pathway or through oxidation of glutamine side chains, leading to the formation of a peptide in which the N-terminal amino acid is blocked by an α-ketoacyl derivative. (c) The introduction of carbonyl groups into proteins can occur by Michael addition reactions of α,β-unsaturated aldehydes, such as 4-hydroxy-2-nonenal, malondialdehyde and 2-propenal (acrolein), derived from lipid peroxidation, with either the amino group of lysine, the imidazole moiety of histidine, or the sulphydryl group of cysteine (advanced lipoxidation end products). (d) Carbonyl groups can also be introduced into proteins by addition of reactive carbonyl derivatives (ketoamines, ketoaldehydes and deoxyosones), produced by the reaction of reducing sugars or their oxidation products, to the amino group of lysine residues, by mechanisms referred to as glycation and glycoxidation. This eventually yields advanced glycation end products, such as carboxymethyllysine and pentosidine. From [16].

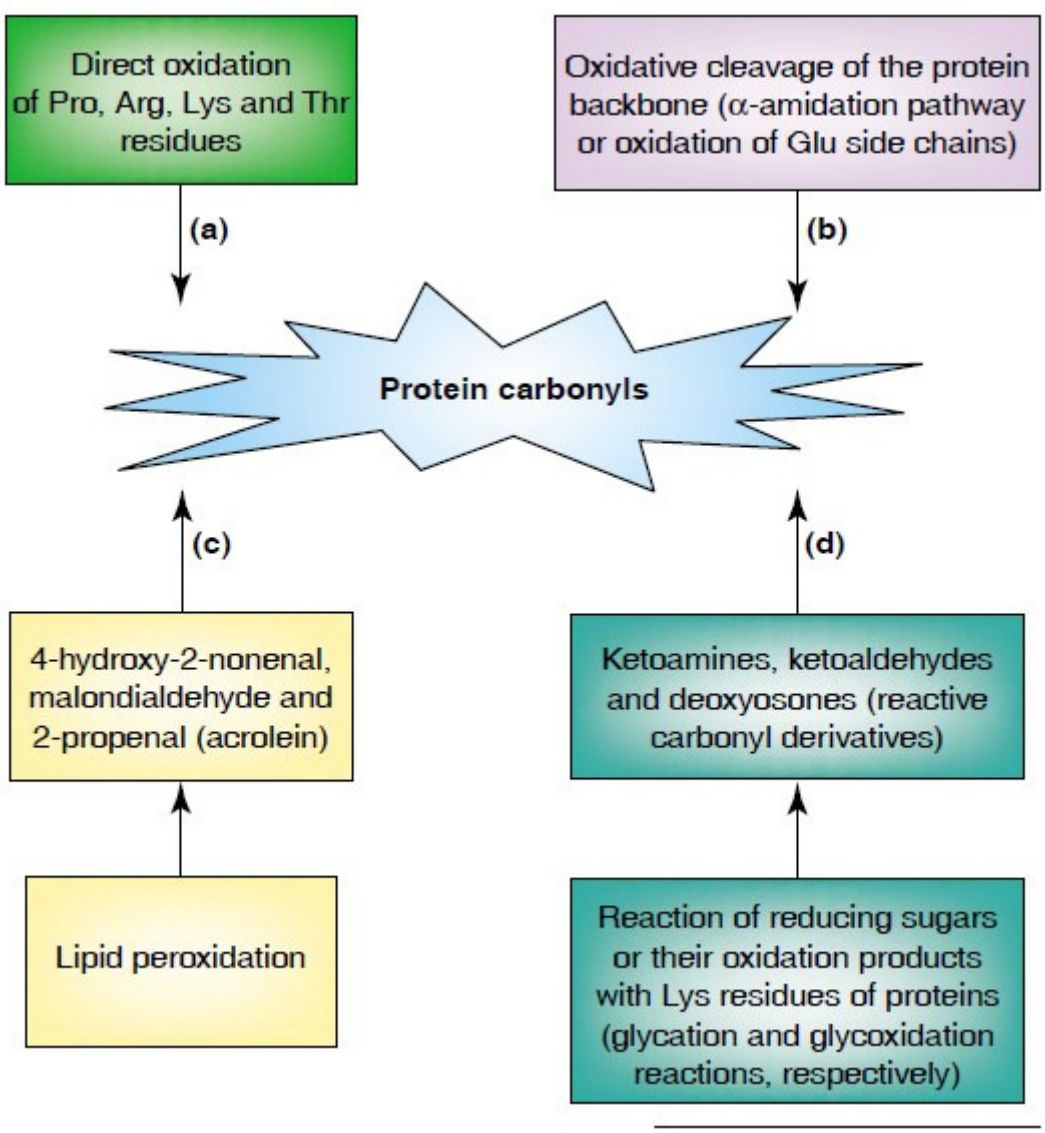

Figure 2: Chemical formulas of the iron chelators desferrioxamine, deferiprone and desferasirox currently in clinical use for iron loading syndromes and in experimental clinical trials for the treatment of neurodegenerative diseases.

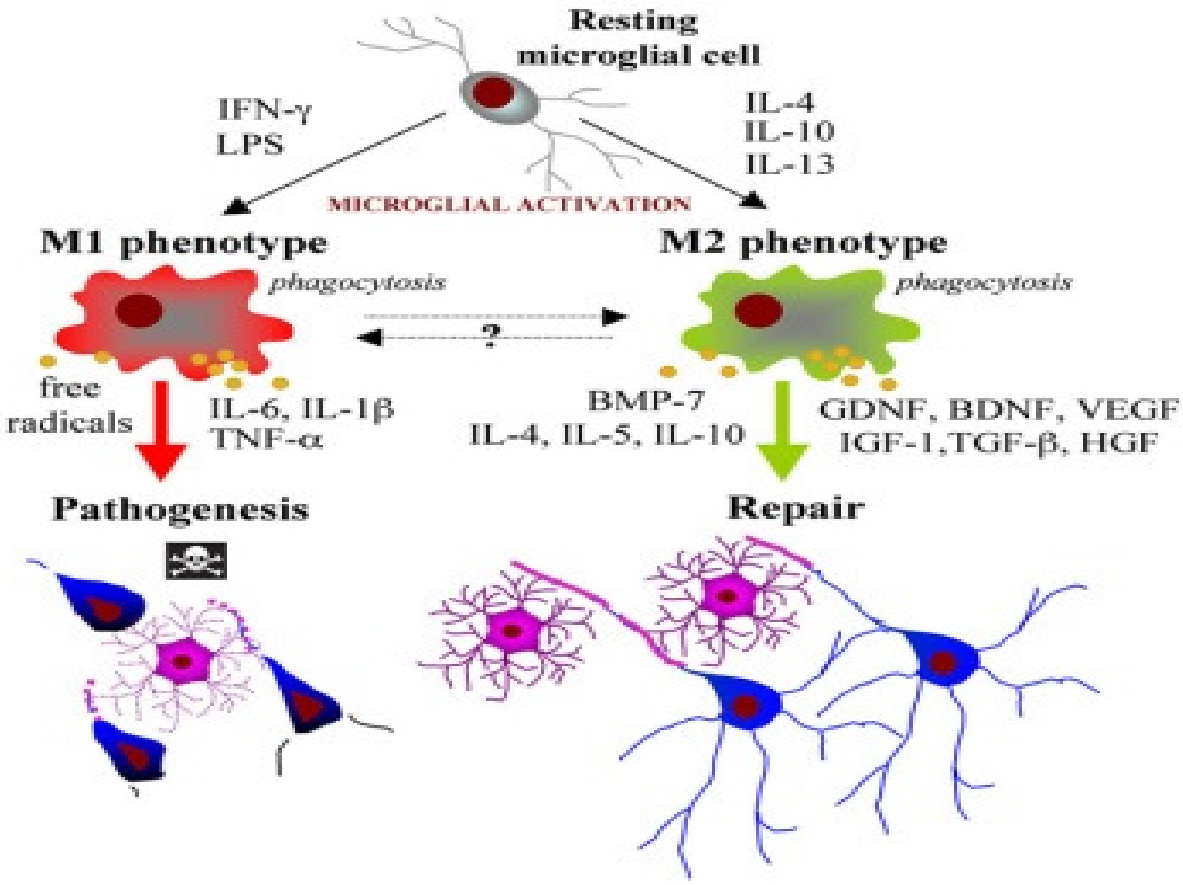

Figure 3. Microglia phenotypes. A detrimental/pro-inflammatory immune cell M1 phenotype is acquired upon classical activation of resting microglia by either LPS or INF-gamma, while IL-4, IL-10 or IL-13 typically induce an anti-inflammatory M2 phenotype (M2a, M2b, M2c) representing an alternative, apparently beneficial, activation state more related to a fine tuning of inflammation, scavenging of debris, promotion of angiogenesis, tissue remodeling and repair. M1 cells release pro-inflammatory cytokines such as IL-6, TNF-alpha, IL-1 beta and free radicals, that contribute to amplifying the neuroinflammatory response. Pro-inflammatory cytokines exert cytotoxic effects on oligodendrocytes and neurons leading to demyelination and axonal damage. M2 cells release neurotrophic factors such as GDNF, BDNF, bFGF, IGF-1, TGF-beta, HGF that provide trophic support to neurons in the injured area, in part by potentiating the recruitment, proliferation and differentiation of oligodendrocyte precursor cells.[43].