

Targeting dysregulation of brain iron homeostasis in ageing

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Abstract. Brain iron is an essential nutrient for multiple functions, including gene expression, DNA synthesis, neurotransmission, myelination, oxygen transport, storage and activation, mitochondrial electron transport, numerous important metabolic processes and cofactor for several key enzymes of neurotransmitter biosynthesis. On the other hand, many investigators, among them the late Mark Smith, who was a pioneer neuroscientist and prominent investigator with regards to brain oxidative stress (OS) and iron in Alzheimer's disease, have identified iron as a highly reactive element that can promote OS processes within the brain and might increase the toxicity of environmental or endogenous toxins. It is suggested that iron accumulation in the brain is capable of initiating free-radical reactions, which subsequently induce progressive loss of neurons, followed by a decrement in neuronal function characteristic of the ageing process. Indeed, it has become apparent that iron progressively accumulates in the brain as a function of age and that iron-induced OS can cause neurodegeneration. Chelation therapy was previously introduced as a novel therapy concept and rationale for the development of metal-binding drugs for neurodegeneration. The present review will discuss the involvement of dysregulation of brain iron homeostasis in the ageing process, addressing the potential importance of iron chelating therapeutic approaches.

Keywords: Brain, iron, ageing, iron chelation, neuroprotection

1. Introduction: Iron in the brain

In the central nervous system (CNS), iron is essential for multiple functions, including gene expression, DNA synthesis, neurotransmission, myelination, oxygen transport, storage and activation, mitochondrial electron transport, numerous important metabolic processes and as a cofactor for several key enzymes of neurotransmitter biosynthesis, such as dopamine (DA) and noradrenaline [1–3]. Iron deficiency can affect infant neurobehavioral and cerebral development, due to its role in neurogenesis and differentiation of cells

and morphology in the hippocampus and striatum [3].

Brain iron homeostasis appears to rely on similar proteins and mechanisms that exist in peripheral organs. Iron (Fe) incorporation and transport in the brain is regulated by the interaction between the endothelial cells and astrocytes: in the brain, astrocytes are devoid of transferrin receptors (TfRs), suggesting that astrocytes incorporate iron by mechanism that is not related to TfR [4, 5]. The TfR1 in the luminal membrane of endothelial cells binds Fe³⁺-loaded transferrin and internalizes this complex in the endosomal compartment, where Fe³⁺ is reduced to Fe²⁺ [4, 5]. The latter is transported across the endosomal membrane into the cytosol by the divalent metal transporter-1 (DMT1), and exported into the extracellular fluid by ferroportin [4]. Alternatively, it has

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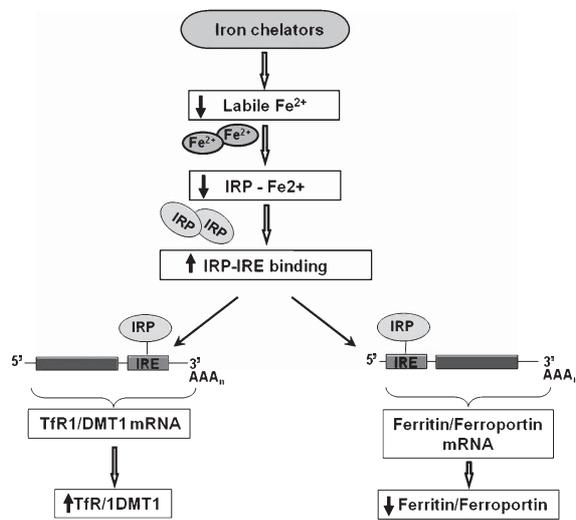


Fig. 1. Schematic illustration of the effect of iron homeostasis on the expression levels of iron-related proteins. In an environment of iron deficiency (e.g. iron chelation), iron regulatory proteins (IRPs) bind to iron-responsive elements (IREs) and facilitate the regulation of ferritin, ferroportin, transferrin receptor (TfR) and divalent metal transporter-1 (DMT1). In the case of ferritin and ferroportin, IRE's in the 5' untranslated region (UTR) of their mRNA bind to IRPs and prevent the initiation of their translation. For TfR1 and DMT1, the binding of IRPs to IREs, which are in the 3' UTR of their mRNAs, results in upregulation of synthesis of these proteins through preventing their induced degradation by nucleases; collectively, iron uptake is increased and iron storage and export are inhibited.

been proposed that transferrin-TfR1 complex may be transported from the luminal to the abluminal surface, followed by iron release [5]. Ceruloplasmin, expressed in the astrocytes, oxidizes newly released Fe²⁺ to Fe³⁺, which binds to transferrin in the brain interstitial fluid [4–6]. Fe²⁺ can also bind to ATP or citrate and be transported as non-transferrin-bound iron (NTBI), which is the source of iron for astrocytes and oligodendrocytes that do not express TfR1 [4, 5]. The control of cellular iron metabolism is a post-transcriptional regulatory action that involves two iron regulatory proteins (IRP1 and 2), which play a key role in sensing iron availability. IRPs are cytosolic RNA-binding proteins that bind to iron-responsive elements (IRE) elements, exist in mRNAs that encode the proteins involved in iron homeostasis and thus, regulate the translation or stability of these mRNAs [3]. In an environment of iron deficiency, IRPs bind to IREs and facilitate the regulation of ferritin, ferroportin, TfR1 and DMT1 [3]. In the case of ferritin and ferroportin, IRE's in the

5' untranslated region (UTR) of their mRNA bind to IRPs and prevent the initiation of their translation. For TfR1 and DMT1, the binding of IRPs to IREs, which are in the 3' UTR of their mRNAs, results in upregulation of synthesis of these proteins through preventing their induced degradation by nucleases; collectively, iron uptake is increased and iron storage and export are inhibited (Fig. 1). In conditions of iron accumulation, the IRPs are not active, thus allowing the translation of ferritin and ferroportin and reduction of TfR1 and DMT1 [3]. Ferritin comprises of two subunits: the high (H)-ferritin, a ferroxidase that is involved in rapid uptake and reutilization of iron and low (L)-ferritin, associated with long-term iron storage [6]. In all brain regions, both subunits are expressed, although H-ferritin is more widespread and the ratio of H-ferritin to L-ferritin depends on iron utilization by specific regional cells [6].

On the other hand, iron is a highly reactive element and dysfunction of iron homeostasis is accompanied by concomitant oxidation processes within the living organism. Smith and collaborators [7, 8] were among the first investigators who suggested that high concentration of reactive iron can induce oxidative stress (OS), because of its interaction with hydrogen peroxide (H₂O₂) in the Fenton reaction, resulting in an increased formation of hydroxyl free radicals in the neurodegenerative brain. Indeed, in many of the neurodegenerative diseases (e.g. AD, Parkinson's disease (PD), Huntington's disease, Friedreich ataxia and amyotrophic lateral sclerosis), as well as in the normal ageing process, excessive generation of OS and accumulation of iron levels and deposition have been observed in specific brain regions and targeted as contributing factors to the pathogenesis and pathology [8, 14–19]. Free radical-related OS causes a molecular damage that can then lead to a critical failure of biological functions, protein modification, misfolding and aggregation and ultimately neuronal death [9–12] (Fig. 2). Additionally, iron accumulation might increase the neurotoxicity of endogenous or environmental toxins; for example, iron homeostasis was reported to be linked to activation of the N-methyl-D-aspartic acid (NMDA) receptor; a signaling neurotoxicity cascade that involves nitric oxide synthase (NOS) and adaptor proteins that interact with ferroprotein [13].

The present review discusses dysregulation of brain iron homeostasis that may be an essential factor in the ageing process, and addresses the potential importance of iron binding therapeutic approaches.

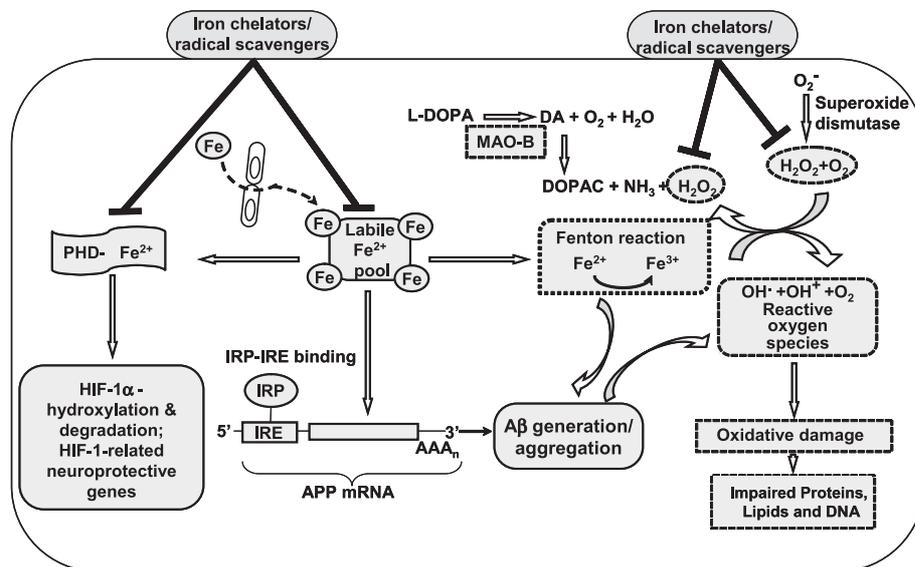


Fig. 2. Schematic illustration of the potential iron dyshomeostasis-induced neurodegenerative pathways in ageing and their prevention by iron chelators/radical scavengers. During the ageing process, high concentration of reactive iron can induce oxidative stress (OS), due to its interaction with hydrogen peroxide (H_2O_2) in the Fenton reaction, resulting in an increased formation of hydroxyl free radicals. OS may also be mediated by β -amyloid ($A\beta$)-generated reactive oxygen species (ROS). The age-related increase in cerebral oxidative damage has been mainly demonstrated by lipid peroxidation, protein oxidation and oxidative modifications in DNA. An additional possible pathway that may accompany ageing is an increase in the levels of monoamine oxidase (MAO), an enzyme that catalyzes the oxidative deamination of neurotransmitters, in which the byproducts H_2O_2 and aldehydes are subsequently generated and can form free radicals. Within the brain, a class of iron-dependent and oxygen-sensor enzymes, hypoxia inducible factor (HIF) prolyl-4-hydroxylases (PHDs) target the regulatory HIF-1 α for hydroxylation and degradation and can prevent HIF-1-adaptive target genes transcription. Iron chelators/radical scavenger can prevent generation of ROS, either through direct prevention of H_2O_2 production, or alternatively by inhibition of Fenton reaction. Iron chelators target the APP 5' UTR thus, possessing the capacity to reduce APP translation and subsequently $A\beta$ generation levels. Additionally, these compounds can restrain induction of PHDs by chelating iron, which resulted in increased transcription of HIF-1 α and HIF-1-target genes involved in the adaptation and protection of neuronal cells.

2. Iron accumulation, oxidative stress and ageing of the brain

Ageing in humans is often characterized by changes in brain volume, accompanied by a decline in motor and cognitive performance [20]. Brain ageing is associated with the progressive imbalance between antioxidant defenses and intracellular generation of reactive oxygen species (ROS) [21, 22]. Age-related increases in oxidative brain damage has been mainly exemplified by lipid peroxidation, protein oxidation and oxidative modifications in DNA [23]. Previously, age-related memory impairment has been shown to be associated with a decrease in brain and plasma antioxidants [24–26] and iron accumulates in the human brain as a function of age [17, 27]. The process of iron accumulation involves the accumulation of iron-containing molecules (primarily ferritin) in the

microglia, astrocytes and oligodendrocytes, particularly in selective brain regions (e.g. cerebellum, basal ganglia and substantia nigra (SN)) [28–30] that are targeted in ageing and age-related neurodegenerative diseases [6, 14, 17, 31–38]. Zecca et al. [18, 39] have described the brain regional selectivity of iron accumulation during human ageing, showing that the SN in old individuals (60–90 years of age) contained higher concentrations of both ferritin subunits, the H-ferritin and L-ferritin and loads of extraneuronal iron deposits, compared to locus coeruleus. In old individuals, iron deposits were observed in non-melaninized neurons of the SN, but not in neuromelanin-containing neurons of SN and locus coeruleus [18, 35, 40]. Moreover, it was proposed that increased iron levels in certain brain areas can result from alterations in brain vascularization that occur during ageing and neurodegeneration [41–43].

During ageing, brain iron storage is partially converted from its stable/soluble form, ferritin, to its degradative, high reactive form, haemosiderin and other oxyhydroxides [44]. Dysregulation of brain iron homeostasis through disruption of iron regulatory proteins may also induce neuronal vulnerability to OS [32, 45]. Consequently, the toxicity of iron as an inducer of OS in the brain has been proposed to depend on controlling iron-containing molecules in the brain [44, 46]. It is also suggested that brain iron accumulation capable of initiating free-radicals reactions, can subsequently induce a progressive loss of neurons, followed by a decrement in neuronal function characteristic of the ageing process [18, 47, 48]. Indeed, a previous study in human elderly subjects suggested a correlation between dysregulation of iron contents, observed by quantitative magnetic resonance (MR), and cognitive function [49]. In preclinical studies, it was also shown that iron load in an early stage of life induced iron accumulation in basal ganglia, accompanied by memory impairments in adulthood [50] and behavioral deficits associated with OS induction in the brain [51]. Furthermore, it was found that in aged animals, iron accumulation altered antioxidant capacity and thiol redox state [52]. Collectively, it has become apparent that age-related iron accumulation, may induce OS, and thus can cause neurodegenerative disorders associated with ageing [17, 46, 53, 54].

An additional prominent feature that accompanies ageing is an increase in the levels of monoamine oxidase (MAO), an enzyme that catalyzes the oxidative deamination of neurotransmitters, in which the byproducts H_2O_2 and aldehydes are subsequently generated [55] (Fig. 2). Given that these byproducts are compelling inductors of lipid peroxidation, it is assumed that activation of MAO is associated with age-related disturbances of the homeostasis and generation of free radicals in involution of the nervous tissue [56]. Indeed, it was shown that in the mouse brain, MAO-A activity remained stable between 2–24 months, while MAO-B activity increased significantly between 2–16 months [57]. Quantitative radiography studies also showed an age-related increase in MAO-B in various mouse brain structures [58]. Activity studies have reported high levels of brain MAO-B in aged-related neurodegenerative diseases, such as PD and AD [59–62]. The increase in MAO-B activity during ageing and aged-associated neurodegenerative diseases is exceptional, compared to most of enzyme activities that are usually decreased, and together

with the generation of highly toxic byproducts, it was indicated as being detrimental to neurons [63–65].

3. Iron chelation therapeutic approaches

From the above overview, it may be speculated that the regulated suppression of labile iron levels in the brain would decrease the rate of accumulation of over-oxidized materials inside neurons and in this way, favorably influence deteriorated ageing processes. Chelation therapy was previously introduced as a novel therapy concept and rational for the development of metal-binding drugs for neurodegeneration (see reviews: [66–69]). The next two sections will discuss the potential benefits of selective natural and hybrid iron-chelating compounds to retard/prevent the primary or secondary iron neurotoxicity in ageing (Fig. 2).

3.1. Natural iron-binding compounds

Particular attention has been focused on studying the potential beneficial effects of naturally occurring (iron chelating/antioxidant) flavonoid-type compounds on ageing and aged related neurodegenerative diseases (see review: [70]). Flavonoids are the largest polyphenol group present in green tea, in particular a subclass known as flavanols, namely catechins [71]. The principal flavanols found in green tea are: (+)-catechin (C), (–)-epicatechin (EC), (+)-gallocatechin (GC), (–)-epigallocatechin (EGC), (+)-catechin-3-O-gallate (CG), (–)-epicatechin-3-O-gallate (ECG), (+)-gallocatechin-3-O-gallate (GCG) and (–)-epigallocatechin-3-O-gallate (EGCG). Among these sub-subclasses, EGCG (Fig. 3a) is the major constituent of green tea, accounting for more than 10% of the extract dry weight, whereas the others are found in lower abundance [72, 73]. Extensive research has indicated that green tea catechins are potent antioxidants, due to their ability to directly scavenge ROS, to induce endogenous antioxidant enzymes and to bind and chelate excess divalent metal ions (e.g. Fe^{3+}) to form inactive complexes (see reviews: [74–76]). The phenolic hydroxyl groups on the aromatic rings of EGCG confer its antioxidant and iron chelating activities [77–80] (Fig. 3a). Recent studies examined the potency of EGCG and EC to prevent DNA damage caused by Fe^{2+} and H_2O_2 , suggesting that the bind-

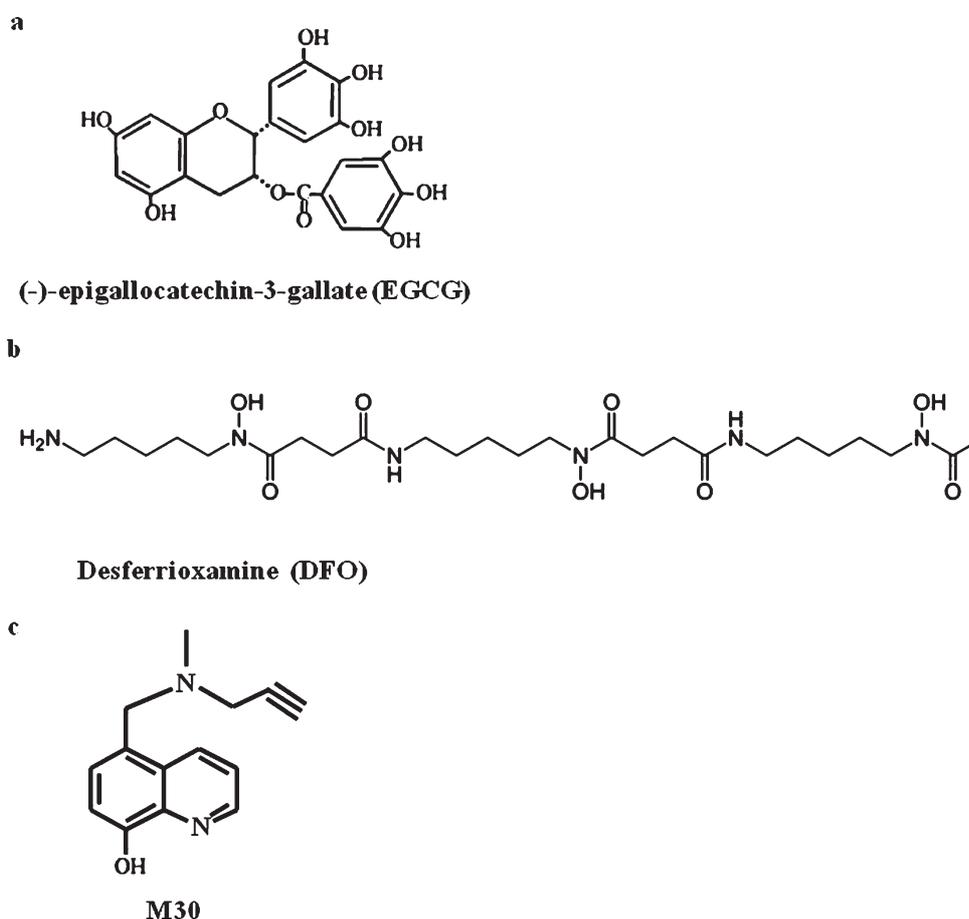


Fig. 3. Chemical structures of iron chelating/radical scavenger compounds; a. (-)-epigallocatechin-3-gallate (EGCG); b. desferrioxamine (DFO); c. M30 (5-[N-methyl-N-propargylaminomethyl]-8-hydroxyquinoline).

ing of EGCG and EC to iron is essential for their antioxidative activity [75, 79]. In this respect, green tea extract has been shown to strongly inhibit lipid peroxidation chain reactions promoted by iron-ascorbate in homogenates of mouse brain mitochondrial membranes [81] and gerbil brains [82]. EGCG has been also shown to inhibit MAO-B activity in C6 astrocyte cells [83] and adult rat brains [84].

Although the question of the precise brain permeability of green tea catechins is still to be determined, green tea ingestion does appear to inversely correlate with the incidence of brain ageing, cognition and neurodegeneration. A previous epidemiological study, aimed at investigating the association between consumption of green tea and cognitive function in elderly Japanese subjects, found that higher consumption of green tea was associated with lower prevalence of cognitive impairment [85]. A recent cross-sectional study

that assessed green tea intake in 2501 participants and 1438 cognitively intact participants over 2-years, indicated that green tea was inversely associated with cognitive impairment, but not with cognitive decline, possibly due to the small number of green tea drinkers in this cohort [86]. To add to this, more recently, green tea consumption was associated with better cognitive performance in community-living Chinese older adults group [87], as well as a lower prevalence of depressive symptoms in elderly population over 70 years old in Japan [88]. Recent findings have shown a significant improvement in Unified Parkinson's Disease Rating Scale (UPDRS) scores at 6 months for PD patients taken daily green tea polyphenols, compared with placebo patient's group [89].

Preclinical animal studies with green tea extract, or its individual catechin components have shown positive effects on cognitive and behavioral abilities during

ageing and in neurodegenerative conditions. Ageing studies have shown that the long-term administration (4–6 months) of antioxidant supplements, containing β -catechin or a green tea catechin fraction, extended the mean lifespan of senescence-accelerated mice (SAM-P8) [90–92] and C57BL/6 J mice [93]. In old Wistar rats, green tea catechins also prevented spatial learning and memory decline, as well as hippocampal and lipid modifications after chronic administration (5–7 months), possibly due to the reduction of OS-related damage [94–96]. Studies using EGCG have documented an attenuation of cognitive deficits and decline in antioxidant enzymes and apoptotic parameters in D-galactose-induced mice ageing [97]. In support, a recent study has shown that the natural prototype iron chelator/radical scavenger, desferrioxamine (DFO) (Fig. 3b) was able to reverse age-induced recognition memory deficits and reduce protein carbonylation in rat cerebral cortex and hippocampus [98]. These findings support the notion that preventing iron accumulation in the ageing brain by green tea catechins might be associated with attenuation of age-related cognitive deficits.

These preclinical studies have encouraged investigation of the molecular mechanisms underlying the effects of natural iron chelators on brain function and cognition. It was demonstrated the natural prototype antioxidant/iron chelator DFO induced the phosphorylation of mitochondrial cAMP responsive element binding protein (CREB) in primary cortical neurons through the activation of mitochondrial protein kinase A (AKT) [99]. Similarly, several studies in different cellular and animal models of neurodegeneration, demonstrated that acute and chronic green tea catechins administration, resulted in the induction of phosphorylation of neuronal pro-survival signaling pathways: phosphatidylinositol-3-kinase (PI3K)/AKT and extracellular signal-regulated protein kinase (ERK) 1/2, their downstream substrate, CREB and subsequent CREB-regulated gene expression (see reviews: [76, 100]). It was suggested that the improvement observed in cognitive performances following green tea catechins-chronic treatment is associated with increased levels of CREB phosphorylation in the hippocampus of senescence-accelerated C57BL/6J and SAM-P8 mice [92, 93, 95]. CREB is a transcription factor that binds to the promoter regions of many genes, associated with memory and synaptic plasticity that may play a crucial role in long-term potentiation (LTP) and long-term memory formation regulated by green

tea catechins, as recently addressed by Spencer and collaborators [100]. In this regards, two target genes of CREB, having regulatory roles in synaptic plasticity and survival, brain-derived neurotrophic factor (BDNF) and Bcl-2 were also increased in the hippocampus of old rats after prolonged administrations of green tea infusion [95, 100]. Further investigations to clarify the association between iron chelation and CREB activation signaling cascade are in progress.

An additional level of neuroprotection by iron-complexing compounds involves the stabilization of the transcriptional activator, hypoxia-inducible factor (HIF)-1 and expression of its target protective genes, such as erythropoietin, vascular endothelial growth factor (VEGF), TFR receptor and heme oxygenase [101, 102]. Within the cells, HIF-1 is under the control of a class of iron-dependent and oxygen-sensor enzymes, HIF prolyl-4-hydroxylases (PHDs) that target HIF-1 α for degradation by the proteasome [101] (Fig. 2). Inhibition of PHDs enzymes under iron chelating conditions results in stabilization of the HIF-1 α protein, heterodimerization and activation of nuclear translocation and subsequently binding to hypoxia responsive elements of promoters of specific HIF-1-adaptive target genes [103]. In brains of aged mice, it was demonstrated that hypoxia-induced HIF-1 α accumulation was attenuated, presumably in response to increased post-transcriptional degradation of HIF-1 that resulted from up-regulation of PHD [104]. The possibility of modulating HIF-1 activity by targeting the free-labile intracellular iron pool by iron binding molecules and inhibitors of PHDs is currently gaining recognition, posing HIF-1 as a potential therapeutic target for ageing and age-related neurodegenerative diseases [103–107]. Indeed, green tea catechins were previously shown to induce HIF-1 α protein and mRNA expression levels of HIF-1-target genes, while this effect was blocked by iron and ascorbate, indicating that these natural occurring compounds may activate HIF-1 α through iron chelation [108]. It is suggested that in the ageing brain, a reduction in the chelatable iron pool by green tea catechins may result in the inhibition of PHDs and consequently in concerted activation of HIF-1 and their respective related-adaptive genes. Future studies are required to further clarify the involvement of the HIF signaling system in the protective mechanism of action of green tea catechins on ageing-related molecular alterations and cognitive decline.

Finally, another aspect regarding the EGCG mechanism of action involves its neuroprotective effects in ageing pathology in AD. Indeed, ageing of the brain has been demonstrated to be the main risk factor for AD, with recent studies suggesting that the primary link between AD and brain ageing may be an aged related bioenergetic stress alterations in the mitochondrial electron transport chain function, resulting in increased OS, apoptosis and enhancement of the amyloid precursor protein (APP) processing to amyloid beta peptide (A β) [see review, 109]. EGCG was previously demonstrated to reduce both the immature and full-length APP in neuronal cells and mice hippocampus, as well as promote the generation of soluble APP α (sAPP α) through activation of α -secretase cleavage, accompanied by a significant reduction in cerebral A β levels and plaques in AD transgenic mice [67, 110–112]. Taken together, these findings suggest that natural iron chelating compounds, such as EGCG, possess beneficial properties in aged-related pathologies.

3.2. Hybrid iron-chelating drugs

Novel therapeutic approaches for the treatment of neurodegenerative disorders comprise candidates designed specifically to act on multiple central nervous targets [112, 113]. Based on a multimodal drug design paradigm, Youdim and collaborators have recently synthesized a multifunctional, non-toxic, brain permeable iron chelator, M30 (Fig. 3c), possessing the neuroprotective N-propargyl moiety of the anti-Parkinsonian and MAO-B inhibitor drug, rasagiline (Azilect[®], Teva, Pharmaceutical Inc.) and the antioxidant-iron chelator moiety of an 8-hydroxyquinoline derivative of the iron chelator, VK28 [113–115]. Studies have revealed that M30 has a lower affinity for iron than that of the prototype iron chelator, DFO, although M30 is a better inhibitor against iron-induced lipid peroxidation, with higher IC₅₀ value (12 μ M) comparable to that of DFO [114, 115]. It is likely that the very high iron chelating property of DFO contributes to its cytotoxicity, which precludes its application for prolonged period of time in pathological conditions unrelated to systemic iron overload. By contrast, a brain permeable compound with moderate iron chelating affinity may be a more appropriate and promising agent for age-related neurodegenerative disease therapies in which iron is selectively accumulated

in various brain regions. In addition, M30 was found to be a highly potent brain selective inhibitor of both MAO-A and -B activities [114]. Indeed, M30 was shown to possess a wide range of pharmacological activities, including neurorescue effects, induction of neuronal differentiation accompanied by induction of growth-associated protein 43 (GAP 43), BDNF and the neuronal survival pathway the serine/threonine kinase Akt [116–118]. In preclinical studies, M30 was shown to prevent the loss of mouse tyrosine hydroxylase (TH)-positive neurons, iron increase and microglial activation in the ipsilateral SN- induced by post-intranigral injection of the ubiquitin-proteasome inhibitor, lactacystin [119]. Recently, it was shown that in C57BL mice, M30 treatment significantly up-regulated a number of neuroprotective-adaptive mechanisms and pro-survival signaling pathways in the brain, associated with the iron-oxygen regulated HIF-1-system [120].

In respect to ageing, we have recently demonstrated that systemic chronic treatment of aged mice with M30 had a significant positive impact on neuropsychiatry functions and cognitive age-related impairment [121]. In particular, a significant induction of locomotor activity and higher preference of exploring the novel object recognition were observed in the groups treated with M30, compared with the vehicle-treated aged group. These beneficial responses of M30 might be attributable, at least partly, to the following mechanisms; first, given the evidence supporting a role of free radicals production and OS in brain dysfunction during the ageing process [21, 22], the neuroprotective action of M30 may be mediated by a reduction of OS, either through its iron chelating potential or its hydroxyl radical scavenging properties [114]. Additionally, the cerebral iron staining decreased after M30 treatment in aged mice, compared with the vehicle-treated aged group, indicating that the drug can prevent or attenuate the progression of neuronal degeneration by the reduction of excessive iron and its redox activity [121]. Second, M30 may beneficially influence cognitive deterioration through inhibition of brain MAO-A and -B enzymes [121].

Another interesting finding was the observation that M30 treatment reduced the levels of β -amyloid (A β) plaques in the cortex and hippocampus of aged mice, compared with vehicle-treated aged animals. It appears that this effect may be associated with the reduction in the levels of APP, previously observed with M30 [118, 122]. In this context,

previous *in vitro* studies have described the regulatory effect of M30 on APP expression/processing resulting in reduced APP expression and A β generation in SH-SY5Y neuronal cells and CHO cells stably transfected with the “Swedish” mutation [116]. It was suggested that metal chelators can reduce APP levels by modulating APP translation via IRE existed in the 5' untranslated region of the APP transcript [110, 123–125]. Recently, Duce et al. [126] have found that APP possesses a ferroxidase activity, mediated via a similar motif found in the ferroxidase active site of H-ferritin and inhibited specifically by Zn²⁺. This finding, and the reported iron-export properties of APP from the cytoplasm to the surface [126], indicate an important biological activity for this protein, whose role is still under-investigation. Indeed, M30 was found *in vitro* to suppress the translation of a luciferase reporter gene fused to the APP mRNA 5' untranslated region [118]. In addition, it was shown that M30 can activate the non-amyloidogenic pathway of APP processing and increase the amount of secreted sAPP α derivative, as also described for other propargyl-containing compounds [127, 128]. These effects may be beneficial in aged animals, in the face of previous findings demonstrating increased amyloid deposition early in the ageing process in various species [129–132]. The accumulation of A β with ageing seems to be a combination of decreased efflux transport of endogenously generated A β and increased influx transport from the vascular compartment, and it is likely that the proportions from each A β source change with time [133]. It has been demonstrated that the interaction of APP and β -site amyloid cleavage enzyme 1 (BACE1) is enhanced with ageing [134, 135]. Furthermore, increases in APP, γ -secretase and BACE1 have been observed in correlation with age in cells and *in vivo* [136, 137]. Indeed, ageing is viewed, as the most significant risk factor for AD, closely correlated with AD neuropathology and there is presumably a continuum in A β accumulation from normal ageing to AD, although the mechanism underlying this transition is not yet clarified.

4. Concluding remarks

In the past decade, the involvement of brain iron homeostasis in pathologies and cognitive deficits in ageing and aged-related neurodegenerative diseases

has been a controversial and debatable issue, mainly as iron may be defined as a “two-edged sword”, being a central nutrient but with highly reactive toxic properties in the brain [138]. It is apparent that various chronic neurodegenerative disorders (e.g. PD, AD, multiple sclerosis, amyotrophic lateral sclerosis, Friedreich's ataxia, Aceruloplasminemia, Prion's disease) exhibit changes in brain iron levels, which are linked to age-related degenerative processes [139]. Elucidation of the molecular mechanisms involved in iron toxicity is an imperative step in studying disorders that involve iron accumulation, as well as for the development of new pharmacological interventions based on iron chelation to delay neurodegeneration. Several iron-chelating compounds, approved clinically for transfusional iron overload in thalassemia, such as DFO, deferasirox and deferiprone, have been demonstrated to possess beneficial effects in pre-clinical models of aged-related neurodegenerative diseases, in particular AD [140–142]. Currently the second-generation metal chelator clioquinol, PBT2, has completed early phase clinical trials in AD patients [143]. The use of natural iron-binding compounds, or those designed to possess of multimodal properties, aimed at attenuating the deleterious effects of brain iron (Fig. 2) and addressing several aged-related central nervous system etiologies allied to iron dyshomeostasis, represent a novel preventive approach to treating the progression of neurodegeneration in ageing and aged related disorders. Adjacent to EGCG, other potential flavonoids and other natural antioxidant/iron chelating compounds, such as curcumin [144, 145], quercetin [146] and derivative of silybin, the major active component of flavonolignans [147] have been described to possess beneficial effects *in vitro* and *in vivo* models of ageing and aged related neurodegenerative diseases (e.g. AD). However, further investigation is needed to assess these for their clinical relevance. Thus far, there are only a small number of iron chelating compounds that are under investigation clinically, whilst others are in an earlier stage of development. As such, future preclinical research is vital to endorse this novel therapeutic strategy.

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References

- [1] Halliwell B. Oxidative stress and neurodegeneration: Where are we now? *Journal of Neurochemistry*. 2006;97(6):1634-58.
- [2] Benarroch EE. Brain iron homeostasis and neurodegenerative disease. *Neurology*. 2009;72(16):1436-40.
- [3] Crichton RR, Dexter DT, Ward RJ. Brain iron metabolism and its perturbation in neurological diseases. *Journal Neural Transmission*. 2011;118(3):301-14.
- [4] Moos T, Morgan EH. The metabolism of neuronal iron and its pathogenic role in neurological disease: Review. *Ann NY Acad Sci*. 2004;1012:14-26.
- [5] Moos T, Rosengren Nielsen T, Skjorringe T, Morgan EH. Iron trafficking inside the brain. *Journal of Neurochemistry*. 2007;103(5):1730-40.
- [6] Connor JR, Menzies SL, Burdo JR, Boyer PJ. Iron and iron management proteins in neurobiology. *Pediatric Neurology*. 2001;25(2):118-29.
- [7] Smith MA, Perry G. Free radical damage, iron, and Alzheimer's disease. *Journal of the Neurological Sciences*. 1995;134 Suppl:92-4.
- [8] Smith MA, Harris PL, Sayre LM, Perry G. Iron accumulation in Alzheimer disease is a source of redox-generated free radicals. *Proceedings of the National Academy of Sciences of the United States of America*. 1997;94(18):9866-8.
- [9] Halliwell B. Role of free radicals in the neurodegenerative diseases: Therapeutic implications for antioxidant treatment. *Drugs Aging*. 2001;18(9):685-716.
- [10] Sayre LM, Smith MA, Perry G. Chemistry and biochemistry of oxidative stress in neurodegenerative disease. *Current Medicinal Chemistry*. 2001;8(7):721-38.
- [11] Pratico D, Delanty N. Oxidative injury in diseases of the central nervous system: Focus on Alzheimer's disease. *Am J Med*. 2000;109(7):577-85.
- [12] Przedborski S. Programmed cell death in amyotrophic lateral sclerosis: A mechanism of pathogenic and therapeutic importance. *Neurologist*. 2004;10(1):1-7.
- [13] Cheah JH, Kim SF, Hester LD, Clancy KW, Patterson SE 3rd, Papadopoulos V, et al. NMDA receptor-nitric oxide transmission mediates neuronal iron homeostasis via the GTPase Dexas1. *Neuron*. 2006;51(4):431-40.
- [14] Bartzokis G, Sultzer D, Cummings J, Holt LE, Hance DB, Henderson VW, et al. *In vivo* evaluation of brain iron in Alzheimer disease using magnetic resonance imaging. *Archives of General Psychiatry*. 2000;57(1):47-53.
- [15] Jellinger KA. The role of iron in neurodegeneration: Prospects for pharmacotherapy of Parkinson's disease. *Drugs Aging*. 1999;14:115-40.
- [16] Berg D, Gerlach M, Youdim MB, Double KL, Zecca L, Riederer P, et al. Brain iron pathways and their relevance to Parkinson's disease. *Journal of Neurochemistry*. 2001;79(2):225-36.
- [17] Zecca L, Youdim MBH, Riederer P, Connor JR, Crichton RR. Iron, brain ageing and neurodegenerative disorders. *Nature reviews Neuroscience*. 2004;5(11):863-73.
- [18] Zecca L, Stroppolo A, Gatti A, Tampellini D, Toscani M, Gallorini M, et al. The role of iron and copper molecules in the neuronal vulnerability of locus coeruleus and substantia nigra during aging. *Proceedings of the National Academy of Sciences of the United States of America*. 2004;101(26):9843-8.
- [19] Smith SR, Cooperman S, Lavaute T, Tresser N, Ghosh M, Meyron-Holtz E, et al. Severity of neurodegeneration correlates with compromise of iron metabolism in mice with iron regulatory protein deficiencies. *Annals of the New York Academy of Sciences*. 2004;1012:65-83.
- [20] Schulze ET, Geary EK, Susmaras TM, Paliga JT, Maki PM, Little DM. Anatomical correlates of age-related working memory declines. *Journal of Aging Research*. 2011;2011:606871.
- [21] Poon HF, Calabrese V, Calvani M, Butterfield DA. Proteomics analyses of specific protein oxidation and protein expression in aged rat brain and its modulation by L-acetylcarnitine: Insights into the mechanisms of action of this proposed therapeutic agent for CNS disorders associated with oxidative stress. *Antioxidants & Redox Signaling*. 2006;8(3-4):381-94.
- [22] Harman D. Free radical theory of aging. *Mutation Research*. 1992;275(3-6):257-66.
- [23] Droge W, Schipper HM. Oxidative stress and aberrant signaling in aging and cognitive decline. *Aging Cell*. 2007;6(3):361-70.
- [24] Berr C, Balansard B, Arnaud J, Roussel AM, Alperovitch A. Cognitive decline is associated with systemic oxidative stress: The EVA study. *Etude du Vieillissement Arteriel*. *Journal of the American Geriatrics Society*. 2000;48(10):1285-91.
- [25] Perkins AJ, Hendrie HC, Callahan CM, Gao S, Unverzagt FW, Xu Y, et al. Association of antioxidants with memory in a multiethnic elderly sample using the Third National Health and Nutrition Examination Survey. *American Journal of Epidemiology*. 1999;150(1):37-44.
- [26] Rinaldi P, Polidori MC, Metastasio A, Mariani E, Mattioli P, Cherubini A, et al. Plasma antioxidants are similarly depleted in mild cognitive impairment and in Alzheimer's disease. *Neurobiology of Aging*. 2003;24(7):915-9.
- [27] Bartzokis G, Beckson M, Hance DB, Marx P, Foster JA, Marder SR. MR evaluation of age-related increase of brain iron in young adult and older normal males. *Magnetic Resonance Imaging*. 1997;15(1):29-35.
- [28] Bartzokis G, Mintz J, Sultzer D, Marx P, Herzberg JS, Phelan CK, et al. *In vivo* MR evaluation of age-related increases in brain iron. *Ajnr*. 1994;15(6):1129-38.
- [29] Youdim MB. Brain iron deficiency and excess; cognitive impairment and neurodegeneration with involvement of striatum and hippocampus. *Neurotoxicity Research*. 2008;14(1):45-56.
- [30] Benkovic SA, Connor JR. Ferritin, transferrin, and iron in selected regions of the adult and aged rat brain. *The Journal of Comparative Neurology*. 1993;338(1):97-113.
- [31] Madsen E, Gitlin JD. Copper and iron disorders of the brain. *Annual Review of Neuroscience*. 2007;30:317-37.

- [32] Connor JR, Menzies SL, St Martin SM, Mufson EJ. A histochemical study of iron, transferrin, and ferritin in Alzheimer's diseased brains. *Journal of Neuroscience Research*. 1992;31(1):75-83.
- [33] Connor JR, Snyder BS, Arosio P, Loeffler DA, LeWitt P. A quantitative analysis of iso-ferritins in select regions of aged, parkinsonian, and Alzheimer's diseased brains. *Journal of Neurochemistry*. 1995;65(2):717-24.
- [34] Bartzokis G, Cummings J, Perlman S, Hance DB, Mintz J. Increased basal ganglia iron levels in Huntington disease. *Archives of Neurology*. 1999;56(5):569-74.
- [35] Snyder AM, Connor JR. Iron, the substantia nigra and related neurological disorders. *Biochimica et Biophysica Acta*. 2009;1790(7):606-14.
- [36] Altamura S, Muckenthaler MU. Iron toxicity in diseases of aging: Alzheimer's disease, Parkinson's disease and atherosclerosis. *Journal of Alzheimer's Disease*. 2009;16(4):879-95.
- [37] Riederer P, Dirr A, Goetz M, Sofic E, Jellinger K, Youdim MBH. Distribution of iron in different brain regions and subcellular compartments in Parkinson's disease. *Annals of Neurology*. 1992;32 Suppl:S101-4.
- [38] Vymazal J, Righini A, Brooks RA, Canesi M, Mariani C, Leonardi M, et al. T1 and T2 in the brain of healthy subjects, patients with Parkinson disease, and patients with multiple system atrophy: Relation to iron content. *Radiology*. 1999;211(2):489-95.
- [39] Zecca L, Gallorini M, Schunemann V, Trautwein AX, Gerlach M, Riederer P, et al. Iron, neuromelanin and ferritin content in the substantia nigra of normal subjects at different ages: Consequences for iron storage and neurodegenerative processes. *Journal of Neurochemistry*. 2001;76(6):1766-73.
- [40] Ben-Shachar D, Youdim MB. Iron, melanin and dopamine interaction: Relevance to Parkinson's disease. *Progress in Neuro-psychopharmacology & Biological Psychiatry*. 1993;17(1):139-50.
- [41] Brun A, Englund E. A white matter disorder in dementia of the Alzheimer type: A pathoanatomical study. *Annals of Neurology*. 1986;19(3):253-62.
- [42] Snowden DA. Healthy aging and dementia: Findings from the Nun Study. *Annals of Internal Medicine*. 2003;139(5):450-4.
- [43] Faucheux BA, Bonnet AM, Agid Y, Hirsch EC. Blood vessels change in the mesencephalon of patients with Parkinson's disease. *Lancet*. 1999;353(9157):981-2.
- [44] Crichton RR, Pierre JL. Old iron, young copper: From Mars to Venus. *Biometals*. 2001;14(2):99-112.
- [45] Pinero DJ, Hu J, Connor JR. Alterations in the interaction between iron regulatory proteins and their iron responsive element in normal and Alzheimer's disease brains. *Cellular and Molecular Biology*. 2000;46(4):761-76.
- [46] Levenson CW, Tassabehji NM. Iron and ageing: An introduction to iron regulatory mechanisms. *Ageing Research Reviews*. 2004;3(3):251-63.
- [47] Sayre LM, Perry G, Harris PL, Liu Y, Schubert KA, Smith MA. *In situ* oxidative catalysis by neurofibrillary tangles and senile plaques in Alzheimer's disease: A central role for bound transition metals. *Journal of Neurochemistry*. 2000;74(1):270-9.
- [48] Floor E. Iron as a vulnerability factor in nigrostriatal degeneration in aging and Parkinson's disease. *Cellular and Molecular Biology*. 2000;46(4):709-20.
- [49] House MJ, St Pierre TG, Foster JK, Martins RN, Clarnette R. Quantitative MR imaging R2 relaxometry in elderly participants reporting memory loss. *American Journal of Neuroradiology*. 2006;27(2):430-9.
- [50] Fredriksson A, Schroder N, Eriksson P, Izquierdo I, Archer T. Neonatal iron exposure induces neurobehavioural dysfunctions in adult mice. *Toxicology and Applied Pharmacology*. 1999;159(1):25-30.
- [51] de Lima MN, Polydoro M, Laranja DC, Bonatto F, Bromberg E, Moreira JC, et al. Recognition memory impairment and brain oxidative stress induced by postnatal iron administration. *The European Journal of Neuroscience*. 2005;21(9):2521-8.
- [52] Suh JH, Moreau R, Heath SH, Hagen TM. Dietary supplementation with (R)-alpha-lipoic acid reverses the age-related accumulation of iron and depletion of antioxidants in the rat cerebral cortex. *Redox Report*. 2005;10(1):52-60.
- [53] Hunter RL, Liu M, Choi DY, Cass WA, Bing G. Inflammation and age-related iron accumulation in F344 rats. *Current Aging Science*. 2008;1(2):112-21.
- [54] Jiang H, Song N, Xu H, Zhang S, Wang J, Xie J. Up-regulation of divalent metal transporter 1 in 6-hydroxydopamine intoxication is IRE/IRP dependent. *Cell Research*. 2010;20(3):345-56.
- [55] Kumar MJ, Andersen JK. Perspectives on MAO-B in aging and neurological disease: Where do we go from here? *Molecular Neurobiology*. 2004;30(1):77-89.
- [56] Shemyakov SE. Monoamine oxidase activity, lipid peroxidation, and morphological changes in human hypothalamus during aging. *Bulletin of Experimental Biology and Medicine*. 2001;131(6):586-8.
- [57] Irwin I, Finnegan KT, Delaney LE, Di Monte D, Langston JW. The relationships between aging, monoamine oxidase, striatal dopamine and the effects of MPTP in C57BL/6 mice: A critical reassessment. *Brain Research*. 1992;572(1-2):224-31.
- [58] Saura J, Richards JG, Mahy N. Differential age-related changes of MAO-A and MAO-B in mouse brain and peripheral organs. *Neurobiology of Aging*. 1994;15(4):399-408.
- [59] Saura J, Luque JM, Cesura AM, Da Prada M, Chan-Palay V, Huber G, et al. Increased monoamine oxidase B activity in plaque-associated astrocytes of Alzheimer brains revealed by quantitative enzyme radioautography. *Neuroscience*. 1994;62(1):15-30.
- [60] Damier P, Kastner A, Agid Y, Hirsch EC. Does monoamine oxidase type B play a role in dopaminergic nerve cell death in Parkinson's disease? *Neurology*. 1996;46(5):1262-9.
- [61] Riederer P, Youdim MB, Birkmayer W, Jellinger K. Monoamine oxidase activity during (-)-deprenil therapy: Human brain post-mortem studies. *Advances in Biochemical Psychopharmacology*. 1978;19:377-82.
- [62] Riederer P, Youdim MBH. Monoamine oxidase activity and monoamine metabolism in brains of parkinsonian patients treated with l-deprenyl. *Journal of Neurochemistry*. 1986;46(5):1359-65.

- [63] Bortolato M, Chen K, Shih JC. Monoamine oxidase inactivation: From pathophysiology to therapeutics. *Advanced Drug Delivery Reviews*. 2008;60(13-14):1527-33.
- [64] Nicotra A, Pierucci F, Parvez H, Senatori O. Monoamine oxidase expression during development and aging. *Neurotoxicology*. 2004;25(1-2):155-65.
- [65] Jenner P, Olanow CW. Oxidative stress and the pathogenesis of Parkinson's disease. *Neurology*. 1996;47(6 Suppl 3):S161-70.
- [66] Van der Schyf CJ, Gal S, Geldenhuys WJ, Youdim MB. Multifunctional neuroprotective drugs targeting monoamine oxidase inhibition, iron chelation, adenosine receptors, and cholinergic and glutamatergic action for neurodegenerative diseases. *Expert Opinion on Investigational Drugs*. 2006;15(8):873-86.
- [67] Mandel SA, Amit T, Kalfon L, Reznichenko L, Weinreb O, Youdim MB. Cell signaling pathways and iron chelation in the neurorestorative activity of green tea polyphenols: Special reference to epigallocatechin gallate (EGCG). *Journal of Alzheimer's Disease*. 2008;15(2):211-22.
- [68] Bolognin S, Drago D, Messori L, Zatta P. Chelation therapy for neurodegenerative diseases. *Medicinal Research Reviews*. 2009;29(4):547-70.
- [69] Weinreb O, Mandel S, Bar-Am O, Amit T. Iron-chelating backbone coupled with monoamine oxidase inhibitory moiety as novel pluripotential therapeutic agents for Alzheimer's disease: A tribute to Moussa Youdim. *Journal Neural Transmission*. 2011;118(3):479-92.
- [70] Joseph J, Cole G, Head E, Ingram D. Nutrition, brain aging, and neurodegeneration. *J Neurosci*. 2009;29(41):12795-801.
- [71] Yang CS, Wang ZY. Tea and cancer. *Journal of the National Cancer Institute*. 1993;85(13):1038-49.
- [72] Lin YS, Tsai YJ, Tsay JS, Lin JK. Factors affecting the levels of tea polyphenols and caffeine in tea leaves. *J Agric Food Chem*. 2003;51(7):1864-73.
- [73] Wu CD, Wei GX. Tea as a functional food for oral health. *Nutrition*. 2002;18(5):443-4.
- [74] Higdon JV, Frei B. Tea catechins and polyphenols: Health effects, metabolism, and antioxidant functions. *Critical Reviews in Food Science and Nutrition*. 2003;43(1):89-143.
- [75] Perron NR, Brumaghim JL. A review of the antioxidant mechanisms of polyphenol compounds related to iron binding. *Cell Biochemistry and Biophysics*. 2009;53(2):75-100.
- [76] Weinreb O, Amit T, Mandel S, Youdim MB. Neuroprotective molecular mechanisms of (-)-epigallocatechin-3-gallate: A reflective outcome of its antioxidant, iron chelating and neurotogenic properties. *Genes & Nutrition*. 2009;4(4):283-96.
- [77] Nanjo F, Goto K, Seto R, Suzuki M, Sakai M, Hara Y. Scavenging effects of tea catechins and their derivatives on 1,1-diphenyl-2-picrylhydrazyl radical. *Free Radical Biology & Medicin*. 1996;21(6):895-902.
- [78] Kumamoto M, Sonda T, Nagayama K, Tabata M. Effects of pH and metal ions on antioxidative activities of catechins. *Biosci Biotechnol Biochem*. 2001;65(1):126-32.
- [79] Perron NR, Hodges JN, Jenkins M, Brumaghim JL. Predicting how polyphenol antioxidants prevent DNA damage by binding to iron. *Inorganic Chemistry*. 2008;47(14):6153-61.
- [80] Ji X, Huang L, Lin Q, Huang H. Characteristics and Kinetics of Iron Release from the Ferritin under the EGCG reduction. *Biological Trace Element Research* 2011. DOI: 10.1007/s12011-011-9225-4.
- [81] Levites Y, Youdim MBH, Maor G, Mandel S. Attenuation of 6-hydroxydopamine (6-OHDA)-induced nuclear factor-kappaB (NF-kappaB) activation and cell death by tea extracts in neuronal cultures. *Biochemical Pharmacology*. 2002;63(1):21-9.
- [82] Lee SR, Im KJ, Suh SI, Jung JG. Protective effect of green tea polyphenol (-)-epigallocatechin gallate and other antioxidants on lipid peroxidation in gerbil brain homogenates. *Phytotherapy Research*. 2003;17(3):206-9.
- [83] Mazzio EA, Harris N, Soliman KF. Food constituents attenuate monoamine oxidase activity and peroxide levels in C6 astrocyte cells. *Planta Medica*. 1998;64(7):603-6.
- [84] Lin SM, Wang SW, Ho SC, Tang YL. Protective effect of green tea (-)-epigallocatechin-3-gallate against the monoamine oxidase B enzyme activity increase in adult rat brains. *Nutrition*. 2010;26(11-12):1195-200.
- [85] Kuriyama S, Hozawa A, Ohmori K, Shimazu T, Matsui T, Ebihara S, et al. Green tea consumption and cognitive function: A cross-sectional study from the Tsurugaya Project 1. *The American Journal of Clinical Nutrition*. 2006;83(2):355-61.
- [86] Ng TP, Feng L, Niti M, Kua EH, Yap KB. Tea consumption and cognitive impairment and decline in older Chinese adults. *The American Journal of Clinical Nutrition*. 2008;88(1):224-31.
- [87] Feng L, Gwee X, Kua EH, Ng TP. Cognitive function and tea consumption in community dwelling older Chinese in Singapore. *The Journal of Nutrition, Health & Aging*. 2010;14(6):433-8.
- [88] Niu K, Hozawa A, Kuriyama S, Ebihara S, Guo H, Nakaya N, et al. Green tea consumption is associated with depressive symptoms in the elderly. *The American Journal of Clinical Nutrition*. 2009;90(6):1615-22.
- [89] Chan P, Qin Z, Zheng Z, et al. A Randomized, Double-Blind, Placebo Controlled, Delayed Start Study to Assess Safety, Tolerability and Efficacy of Green Tea Polyphenols in Parkinson's Disease. XVIII WFN World Congress on Parkinson's Disease and Related Disorders, Miami Beach, FL, USA, December 13-16. 2009.
- [90] Kumari MV, Yoneda T, Hiramatsu M. Effect of "beta CAT-ECHIN" on the life span of senescence accelerated mice (SAM-P8 strain). *Biochemistry and Molecular Biology International*. 1997;41(5):1005-11.
- [91] Chan YC, Hosoda K, Tsai CJ, Yamamoto S, Wang MF. Favorable effects of tea on reducing the cognitive deficits and brain morphological changes in senescence-accelerated mice. *Journal of Nutritional Science and Vitaminology*. 2006;52(4):266-73.
- [92] Li Q, Zhao HF, Zhang ZF, Liu ZG, Pei XR, Wang JB, et al. Long-term green tea catechin administration prevents spatial learning and memory impairment in senescence-accelerated mouse prone-8 mice by decreasing Abeta1-42 oligomers and upregulating synaptic plasticity-related proteins in the hippocampus. *Neuroscience*. 2009;163(3):741-9.
- [93] Li Q, Zhao HF, Zhang ZF, Liu ZG, Pei XR, Wang JB, et al. Long-term administration of green tea catechins prevents age-related spatial learning and memory decline in C57BL/6

- J mice by regulating hippocampal cyclic amp-response element binding protein signaling cascade. *Neuroscience*. 2009; 159(4):1208-15.
- [94] Haque AM, Hashimoto M, Katakura M, Tanabe Y, Hara Y, Shido O. Long-term administration of green tea catechins improves spatial cognition learning ability in rats. *The Journal of Nutrition*. 2006;136(4):1043-7.
- [95] Assuncao M, Santos-Marques MJ, Carvalho F, Andrade JP. Green tea averts age-dependent decline of hippocampal signaling systems related to antioxidant defenses and survival. *Free Radical Biology & Medicine*. 2010;48(6):831-8.
- [96] Assuncao M, Santos-Marques MJ, Carvalho F, Lukoyanov NV, Andrade JP. Chronic green tea consumption prevents age-related changes in rat hippocampal formation. *Neurobiology of Aging*. 2011;32(4):707-17.
- [97] He M, Zhao L, Wei MJ, Yao WF, Zhao HS, Chen FJ. Neuroprotective effects of (–)-epigallocatechin-3-gallate on aging mice induced by D-galactose. *Biological & Pharmaceutical Bulletin*. 2009;32(1):55-60.
- [98] de Lima MN, Dias CP, Torres JP, Dornelles A, Garcia VA, Scalco FS, et al. Reversion of age-related recognition memory impairment by iron chelation in rats. *Neurobiology of Aging*. 2008;29(7):1052-1059.
- [99] Ryu H, Lee J, Impey S, Ratan RR, Ferrante RJ. Antioxidants modulate mitochondrial PKA and increase CREB binding to D-loop DNA of the mitochondrial genome in neurons. *Proc Natl Acad Sci U S A*. 2005;102(39): 13915-20.
- [100] Spencer JP, Vauzour D, Rendeiro C. Flavonoids and cognition: The molecular mechanisms underlying their behavioural effects. *Archives of Biochemistry and Biophysics*. 2009;492(1-2):1-9.
- [101] Schofield CJ, Ratcliffe PJ. Oxygen sensing by HIF hydroxylases. *Nature reviews. Molecular Cell Biology*. 2004;5(5): 343-54.
- [102] Weinreb O, Amit T, Mandel S, Kupersmidt L, Youdim MB. Neuroprotective multifunctional iron chelators: From redox-sensitive process to novel therapeutic opportunities. *Antioxidants & Redox Signaling*. 2010;13(6):919-49.
- [103] Siddiq A, Ayoub IA, Chavez JC, Aminova L, Shah S, LaManna JC, et al. Hypoxia-inducible factor prolyl 4-hydroxylase inhibition. A target for neuroprotection in the central nervous system. *The Journal of Biological Chemistry*. 2005;280(50):41732-43.
- [104] Ndubuizu OI, Chavez JC, LaManna JC. Increased prolyl 4-hydroxylase expression and differential regulation of hypoxia-inducible factors in the aged rat brain. *American Journal of Physiology*. 2009;297(1):R158-65.
- [105] Lee DW, Andersen JK. Role of HIF-1 in iron regulation: Potential therapeutic strategy for neurodegenerative disorders. *Current Molecular Medicine*. 2006;6(8):883-93.
- [106] Weinreb O, Amit T, Mandel S, Youdim MB. Novel therapeutic approach for neurodegenerative pathologies: Multitarget iron-chelating drugs regulating hypoxia-inducible factor 1 signal transduction pathway. *Neuro-Degenerative Diseases* 2011. DOI: 10.1159/000332597.
- [107] Correia SC, Moreira PI. Hypoxia-inducible factor 1: A new hope to counteract neurodegeneration? *Journal of Neurochemistry*. 2010;112(1):1-12.
- [108] Zhou YD, Kim YP, Li XC, Baerson SR, Agarwal AK, Hodges TW, et al. Hypoxia-inducible factor-1 activation by (–)-epicatechin gallate: Potential adverse effects of cancer chemoprevention with high-dose green tea extracts. *Journal of Natural Products*. 2004;67(12):2063-9.
- [109] Swerdlow RH. Brain aging, Alzheimer's disease, and mitochondria. *Biochimica et biophysica acta*. 2011;1812(12): 1630-9.
- [110] Reznichenko L, Amit T, Zheng H, Avramovich-Tirosh Y, Youdim MB, Weinreb O, et al. Reduction of iron-regulated amyloid precursor protein and beta-amyloid peptide by (–)-epigallocatechin-3-gallate in cell cultures: Implications for iron chelation in Alzheimer's disease. *Journal of Neurochemistry*. 2006;97(2):527-36.
- [111] Rezai-Zadeh K, Shytle D, Sun N, Mori T, Hou H, Jeannot D, et al. Green tea epigallocatechin-3-gallate (EGCG) modulates amyloid precursor protein cleavage and reduces cerebral amyloidosis in Alzheimer transgenic mice. *The Journal of Neuroscience*. 2005;25(38):8807-14.
- [112] Amit T, Avramovich-Tirosh Y, Youdim MB, Mandel S. Targeting multiple Alzheimer's disease etiologies with multimodal neuroprotective and neurorestorative iron chelators. *The FASEB Journal*. 2008;22(5):1296-305.
- [113] Youdim MB, Buccafusco JJ. Multi-functional drugs for various CNS targets in the treatment of neurodegenerative disorders. *Trends in Pharmacological Sciences*. 2005; 26(1):27-35.
- [114] Zheng H, Gal S, Weiner LM, Bar-Am O, Warshawsky A, Fridkin M, et al. Novel multifunctional neuroprotective iron chelator-monoamine oxidase inhibitor drugs for neurodegenerative diseases: *In vitro* studies on antioxidant activity, prevention of lipid peroxide formation and monoamine oxidase inhibition. *Journal of Neurochemistry*. 2005; 95(1):68-78.
- [115] Zheng H, Weiner LM, Bar-Am O, Epsztejn S, Cabantchik ZI, Warshawsky A, et al. Design, synthesis, and evaluation of novel bifunctional iron-chelators as potential agents for neuroprotection in Alzheimer's, Parkinson's, and other neurodegenerative diseases. *Bioorganic & Medicinal Chemistry*. 2005;13(3):773-83.
- [116] Avramovich-Tirosh Y, Amit T, Bar-Am O, Zheng H, Fridkin M, Youdim MB. Therapeutic targets and potential of the novel brain-permeable multifunctional iron chelator-monoamine oxidase inhibitor drug, M-30, for the treatment of Alzheimer's disease. *Journal of Neurochemistry*. 2007;100(2):490-502.
- [117] Avramovich-Tirosh Y, Bar-Am O, Amit T, Youdim MB, Weinreb O. Up-regulation of Hypoxia-Inducible Factor (HIF) -1 α and HIF-Target Genes in Cortical Neurons by the Novel Multifunctional Iron Chelator Anti-Alzheimer Drug, M30. *Current Alzheimer Research*. 2010;7(4):300-6.
- [118] Avramovich-Tirosh Y, Reznichenko L, Mit T, Zheng H, Fridkin M, Weinreb O, et al. Neurorescue Activity, APP Regulation and Amyloid-beta Peptide Reduction by Novel Multi-Functional Brain Permeable Iron- Chelating- Antioxidants, M-30 and Green Tea Polyphenol, EGCG. *Current Alzheimer research*. 2007;4(4):403-11.
- [119] Zhu W, Xie W, Pan T, Xu P, Fridkin M, Zheng H, et al. Prevention and restoration of lactacystin-induced nigrostriatal

- dopamine neuron degeneration by novel brain-permeable iron chelators. *The FASEB Journal*. 2007;21(14):3835-44.
- [120] Kupersmidt L, Weinreb O, Amit T, Mandel S, Bar-Am O, Youdim MB. Novel molecular targets of the multi-functional brain-permeable iron chelating drug M30 in mouse brain. *Neuroscience*. 2011;189:345-58.
- [121] Kupersmidt L, Amit T, Bar-Am O, Youdim MB, Weinreb O. Neuroprotection by the multitarget iron chelator M30 on age-related alterations in mice. *Mechanisms of Ageing and Development* 2012.
- [122] Avramovich-Tirosh Y, Amit T, Bar-Am O, Weinreb O, Youdim MB. Physiological and pathological aspects of Abeta in iron homeostasis via 5'UTR in the APP mRNA and the therapeutic use of iron-chelators. *BMC Neuroscience*. 2008;9Suppl 2:S2.
- [123] Payton S, Cahill CM, Randall JD, Gullans SR, Rogers JT. Drug discovery targeted to the Alzheimer's APP mRNA 5'-untranslated region: The action of paroxetine and dimer-captopropanol. *J Mol Neurosci*. 2003;20(3):267-75.
- [124] Rogers JT, Lahiri DK. Metal and inflammatory targets for Alzheimer's disease. *Current Drug Targets*. 2004;5(6):535-51.
- [125] Rogers JT, Randall JD, Cahill CM, Eder PS, Huang X, Gunshin H, et al. An iron-responsive element type II in the 5'-untranslated region of the Alzheimer's amyloid precursor protein transcript. *Journal of Biological Chemistry*. 2002;277(47):45518-28.
- [126] Duce JA, Tsatsanis A, Cater MA, James SA, Robb E, Wikke K, et al. Iron-export ferroxidase activity of beta-amyloid precursor protein is inhibited by zinc in Alzheimer's disease. *Cell*. 2010;142(6):857-67.
- [127] Bar-Am O, Weinreb O, Amit T, Youdim MB. Regulation of Bcl-2 family proteins, neurotrophic factors, and APP processing in the neurorescue activity of propargylamine. *The FASEB Journal*. 2005;19(13):1899-901.
- [128] Yogev-Falach M, Amit T, Bar-Am O, Sagi Y, Weinstock M, Youdim MBH. The involvement of mitogen-activated protein (MAP) kinase in the regulation of amyloid precursor protein processing by novel cholinesterase inhibitors derived from rasagiline. *The FASEB Journal*. 2002;16(12):1674-6.
- [129] Banks WA, Farr SA, Morley JE, Wolf KM, Geylis V, Steinitz M. Anti-amyloid beta protein antibody passage across the blood-brain barrier in the SAMP8 mouse model of Alzheimer's disease: An age-related selective uptake with reversal of learning impairment. *Experimental Neurology*. 2007;206(2):248-56.
- [130] Head E, Moffat K, Das P, Sarsoza F, Poon WW, Landsberg G, et al. Beta-amyloid deposition and tau phosphorylation in clinically characterized aged cats. *Neurobiology of Aging*. 2005;26(5):749-63.
- [131] Preston JE. Ageing choroid plexus-cerebrospinal fluid system. *Microscopy Research and Technique*. 2001;52(1):31-7.
- [132] Vaucher E, Aumont N, Pearson D, Rowe W, Poirier J, Kar S. Amyloid beta peptide levels and its effects on hippocampal acetylcholine release in aged, cognitively-impaired and -unimpaired rats. *Journal of Chemical Neuroanatomy*. 2001;21(4):323-9.
- [133] Reddy PH, Manczak M, Mao P, Calkins MJ, Reddy AP, Shirendeb U. Amyloid-beta and mitochondria in aging and Alzheimer's disease: Implications for synaptic damage and cognitive decline. *Journal of Alzheimer's Disease*. 2010;20 Suppl 2:S499-512.
- [134] Li Y, Zhou W, Tong Y, He G, Song W. Control of APP processing and Abeta generation level by BACE1 enzymatic activity and transcription. *The FASEB Journal*. 2006;20(2):285-92.
- [135] Vassar R, Bennett BD, Babu-Khan S, Kahn S, Mendiaz EA, Denis P, et al. Beta-secretase cleavage of Alzheimer's amyloid precursor protein by the transmembrane aspartic protease BACE. *Science*. 1999;286(5440):735-41.
- [136] Lu T, Pan Y, Kao SY, Li C, Kohane I, Chan J, et al. Gene regulation and DNA damage in the ageing human brain. *Nature*. 2004;429(6994):883-91.
- [137] Yankner BA, Lu T, Loerch P. The aging brain. *Annual Review of Pathology*. 2008;3:41-66.
- [138] Solomons NW, Orozoco MN, Crowley C, Romero-Abal ME, Schumann K. Iron: Nutrition's two-edged sword. *Sight and Life*. 2010;2:20-4.
- [139] Sian-Hulsmann J, Mandel S, Youdim MB, Riederer P. The relevance of iron in the pathogenesis of Parkinson's disease. *J Neurochem*. 2011;118(6):939-57.
- [140] Kell, DB. Iron behaving badly: Inappropriate iron chelation as a major contributor to the aetiology of vascular and other progressive inflammatory and degenerative diseases. *BMC Med Genomics*. 2009;2(2):1-79.
- [141] Gaeta A, Molina-Holgado F, Kong XL, Salvage S, Fakhri S, Francis P, et al. Synthesis, physical-chemical characterisation and biological evaluation of novel 2-amido-3-hydroxypyridin-4(1H)-ones: Iron chelators with the potential for treating Alzheimer's disease. *Bioorg Med Chem*. 2011;19(3):1285-97.
- [142] Liu G, Garrett MR, Men P, Zhu X, Perry G, Smith MA. Nanoparticle and other metal chelation therapeutics in Alzheimer disease. *Biochimica et Biophysica Acta*. 2005;1741:246-252.
- [143] Faux NG, Ritchie CW, Gunn A, Rembach A, Tsatsanis A, Bedo J, et al. PBT2 rapidly improves cognition in Alzheimer's Disease: Additional phase II analyses. *J Alzheimers Dis*. 2010;20(2):509-16.
- [144] Zhang C, Browne A, Child D, Tanzi RE. Curcumin decreases amyloid-beta peptide levels by attenuating the maturation of amyloid-beta precursor protein. *The Journal of biological chemistry*. 2010;285:28472-80.
- [145] Dong S, Zeng Q, Mitchell ES, Xiu J, Duan Y, Chunxia L. Curcumin enhances neurogenesis and cognition in aged rats: Implications for transcriptional interactions related to growth and synaptic plasticity. *PLoS ONE*. 2012;7(2):1-12.
- [146] Richetti SK, Blank M, Capiotti KM, Piatto AL, Bogo MR, Vianna MR, et al. Quercetin and rutin prevent scopolamine-induced memory impairment in zebrafish. *Behavioural Brain Research*. 2011;217:10-15.
- [147] Yang LX, Huang KX, Li HB, Gong JX, Wang F, Feng YB, et al. Design, synthesis, and examination of neuron protective properties of alkenylated and amidated dehydro-silybin derivatives. *Journal of Medicinal Chemistry*. 2009;52:7732-7752.