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Aluminium and iron in the brain—prospects for chelation

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Contents

Abstract

Aluminium and iron both accumulate in the brain in the course of ageing. We first briefly review how aluminium may interfere with iron metabolism through its interaction with iron homeostatic mechanisms. Then we present comparative data on the chelation of brain aluminium and iron in appropriate animal models of loading with the two metals. With both desferrioxamine (DFO) B and hydroxypyridone derivatives, brain iron is much more difficult to chelate than brain aluminium. This probably reflects the localisation of the former in ferritin and haemosiderin, and the latter in a more labile, non-protein form. The potential of long-term chelation therapy in the prevention of a number of neurodegenerative diseases associated with ageing is discussed.

1. Introduction

The prevalence of neurological disorders such as Alzheimer's disease, Parkinson's disease, etc., will increase over the next decades with the increasingly ageing population of Western society. However, the role played by metals such as iron and aluminium, either in the aetiology or pathogenesis of the development of these diseases remains unclear. Accumulation of iron in brain as a function of age has been documented in both normal human subjects [1] and in animals [2]. Focal imbalances of iron have been associated with a number of neurodegenerative disorders such as Parkinson's and Alzheimer's disease [3]. An increased iron content has been found in the substantia nigra of post-mortem parkinsonian brain [4]. In a recent study of post-mortem material from normal subjects, iron content in substantia nigra was found to increase ten-fold in the first four decades of life, and it was concluded that neuromelanin, rather than ferritin, is the major form of iron storage [1]. Iron is also associated with production of reactive oxygen species, particularly the hydroxyl radical, which has been implicated in many neurological diseases [5].

Elevated levels of Al^{3+} have been reported in the brains of two groups of patients as well as in animal models of aluminium intoxication (reviewed in [6]). Firstly in post-mortem tissue from the brains of Alzheimer's patients, often associated with neurofibrillary tangles and amyloid plaques [7], and secondly in long term haemodialysis patients treated with aluminium-containing phosphate binders and/or dialysate water containing high levels of dissolved aluminium

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salts [8]. Aluminium toxicity is the likely cause of three human disorders arising from long-term haemodialysis; vitamin D-resistant osteomalacia, iron adequate microcytic anaemia and dialysis dementia [9]. The first of these conditions is consistent with interference with calcium deposition into bone, and the accumulation of aluminium in the bone matrix. Aluminium is also a possible cause of the high frequency of sclerosis and Parkinsonian dementia among the natives of certain regions of South East Asia, where the soils are high in Al^{3+} and low in Mg^{2+} and Ca^{2+}.

Considerable evidence also indicated that there was disruption of iron homeostasis in Alzheimer's disease [10]; the hypothesis has, therefore, been advanced that aluminium might exert its toxic effects by interfering with pathways involved in normal iron metabolism and iron homeostasis, e.g. by using transport and uptake systems such as transferrin and the transferrin receptor, or, as discussed below, by interfering with iron homeostasis at the level of iron regulatory proteins. We discuss below the uptake and transport of iron and aluminium across the gastrointestinal tract, their transport in serum, and their uptake by cells.

2. Iron and aluminium uptake across the GI tract and transport to cells

A normal diet contains between 12 and 18 mg Fe per day, of which about 1 mg is absorbed in man. Two important uptake systems for iron have been identified at the apical membrane of the enterocyte [5] (Fig. 1). In the first, the recently identified iron-regulated duodenal ferric reductase Dcytb will reduce dietary Fe^{3+}–Fe^{2+} [11]; this di-haem protein, a member of the cytochrome b561 family of plasma membrane reductases, is highly expressed in the brush border membrane of duodenal enterocytes. Fe^{2+} either produced by Dcyb or by non-enzymatic reduction of dietary Fe^{3+} (e.g. by ascorbate) will be transported across the brush border into the enterocyte by the proton-coupled divalent cation transporter DCT1 (Nramp2) [12]. In the second pathway, haem iron is taken up, most likely via a brush-border haem transport system, and Fe^{2+} is released within the enterocyte by the action of haem oxygenase (which releases Fe^{2+}, porphobilinogen and CO) [5]. A third possible uptake mechanism is the pathway involving the two proteins mobilferrin and integrin [13], which is

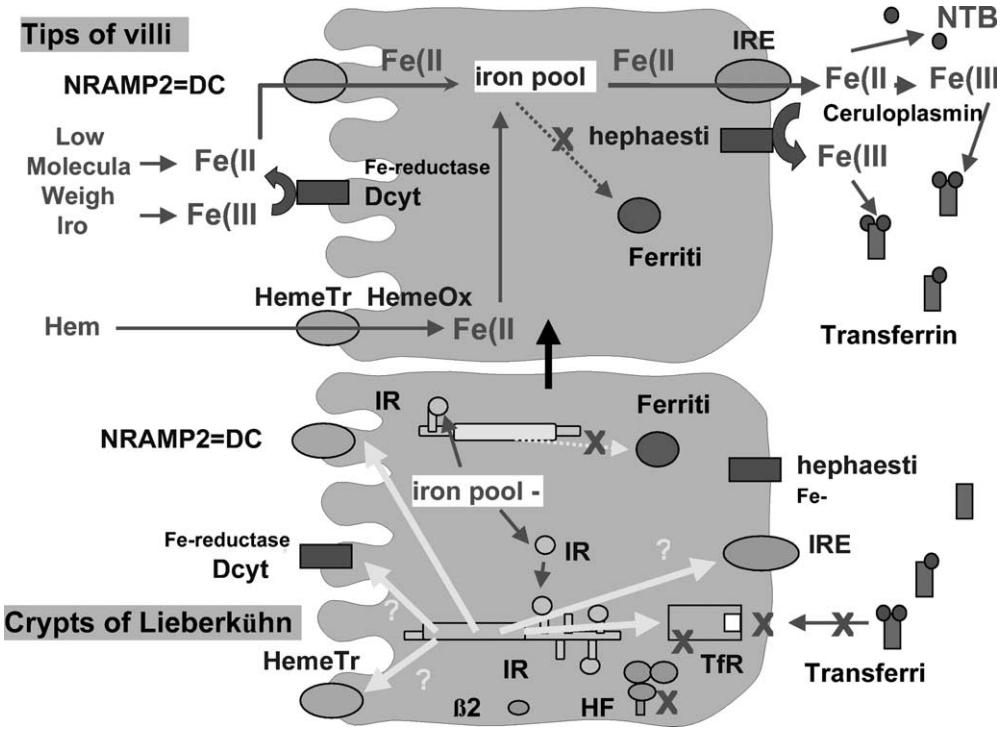

Fig. 1. Schematic representation of iron absorption in normal subjects. The panel represents a mature intestinal mucosa cell at the tip of a villus. NRAMP2, natural-resistance-associated macrophage protein 2; identical with DMT1, divalent metal transporter 1; IREG1, iron-regulated transporter 1; HemeTr, heme transporter; HemeOx, heme oxygenase I; DcytB, duodenal cytochrome B, ferric reductase. In the enterocyte as it enters the absorptive zone near the villus tips, dietary iron is absorbed either directly as Fe(II) after reduction in the gastrointestinal tract by reductants like ascorbate, or after reduction of Fe(III) by the apical membrane ferrireductase Dcytb, via the divalent transporter Nramp 2 (DCT1). Alternatively, haem is taken up at the apical surface, perhaps via a receptor, and is degraded by haem oxygenase to release Fe(II) into the same intracellular pool. The enterocyte is programmed to determine the amount of iron that is retained within the enterocyte as ferritin, and that which is transferred to the circulation. This latter process is presumed to involve IREG 1 and the GPI-linked hephaestin at the basolateral membrane with incorporation of iron into apotransferrin. Alternatively ceruloplasmin in the serum can catalyse the oxidation of iron and its subsequent incorporation into transferrin.

reputed to transport Fe^{3+} directly from the gastrointestinal tract, and requires no oxidation-reduction step. Within the enterocyte, the Fe^{2+} can either be stored in mucosal ferritin (after being oxidised by the protein to the ferric state) or traverse the cell to the basolateral membrane where it is transported across the basolateral membrane by IREG1, a transmembrane iron transporter protein [14]. The membrane-bound copper oxidase hephaestin promotes oxidation to Fe^{3+} and facilitates its incorporation into circulating apotransferrin [15]. Alternatively, the copper transport protein in plasma, ceruloplasmin, may carry out the same function [5].

The daily intake of aluminium in man is about 3 mg per day, although very little appears to be absorbed from the gastrointestinal tract; only 0.06–0.1% of an ingested dose of Al^{26} is absorbed [16], with close to 100% of the ingested dose recovered in the faeces [17]. This is consistent with studies on animals which show that it is very difficult to achieve aluminium loading via the diet [18], whereas much greater loading in a shorter time can be obtained by parenteral administration [19]. Two possible routes may be envisaged for aluminium uptake and transport, namely the mobilferrin-integrin pathway described above or alternatively, the passage of Al^{3+} between the gap junctions of the enterocytes, perhaps as aluminium citrate, thereby bypassing the apical and basolateral membranes of the enterocyte.

Serum iron is essentially bound to transferrin, the transferrin molecule has two binding sites for Fe^{3+} [5]. Since transferrin saturation is typically around 30%, this leaves substantial transport capacity available for aluminium. Once in the serum, aluminium can be transported bound to transferrin, but also to albumin and low molecular ligands like citrate. Fractionation of serum from a haemodialysis patient (with a high plasma aluminium concentration of 5 μM) gave a distribution of 60% bound to transferrin, 34% to albumin and 6% to citrate [20]. This may reflect the lower stability of the Al^{3+}-transferrrin complex compared with the Fe^{3+}-transferrin complex (a difference in formation constant of about eight orders of magnitude). However, the transferrrin–aluminium complex will be able to enter the cell via the transferrin–transferrin-receptor pathway described below.

3. Entry of iron and aluminium into cells via the transferrin–transferrin receptor pathway

Iron and aluminium, bound to transferrin, will have access to cells via the transferrin–transferrin-receptor pathway. The stability constants for iron and aluminium are very different; at pH 7.4 the stability constant, log K for the two binding sites on transferrin are 12.9 and 12.3 for aluminium and 20.7 and 19.4 for iron [21]. The

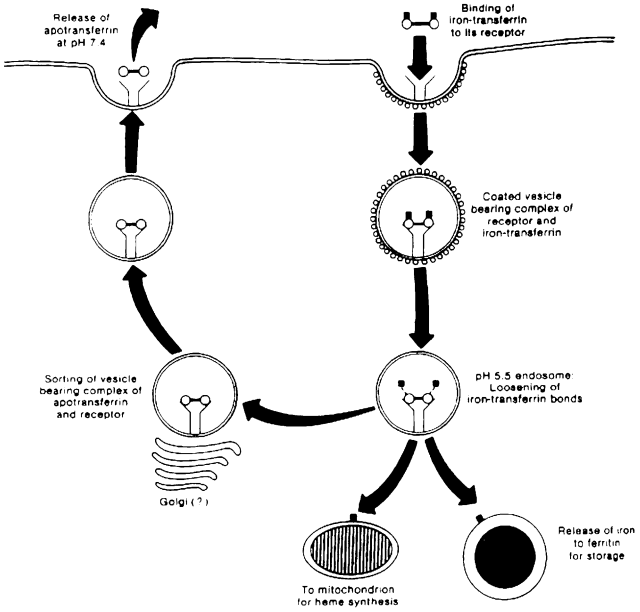

Fig. 2. The transferrin to cell cycle (from [21]).

metal, transferrin–transferrin receptor complex invaginates into a clathrin-coated pit, where the vesicle is pinched off from the membrane (Fig. 2). After budding is complete, the coat proteins are removed, resulting in the formation of smooth-surfaced vesicles, which can then fuse with the target membranes of the endosomes. Fusion of the two membranes delivers the vesicle contents into the interior of the endosome, while the vesicle membrane is added to the endosomal membrane. The interior of the endosomal compartment is maintained at a pH of ca. 5.5 by the action of an ATP-dependent proton pump in the endosomal membrane, which pumps protons into the endosomal lumen from the cytosol. It is proposed that iron is released from the $(Fe^{3+})_2$- transferrin–transferrin receptor complex [22,23] as Fe^{3+} at the acidic pH of around 5.5 within this compartment; aluminium could also be released from the transferrin at this stage. It then seems likely that Fe^{3+} is reduced by an as yet unidentified ferrireductase within the endosome and is then transported out of the endosome by the divalent cation carrier DCT1, the transmembrane iron transporter that functions with concomitant transfer of H^+, which we encountered earlier involved in iron uptake across the apical membrane of the enterocyte [24]. Apotransferrin bound to the transferrin receptor then returns to the cell surface, where the apotransferrin is released for reutilisation, completing a highly efficient cycle.

Within the acidic environment of the endosome, we assume that trivalent aluminium bound to transferrin will be released from transferrin, just as iron is. Exactly how aluminium could exit the endosome into the cytosol remains unknown, since a reduction step is once again

required prior to transport across the endosomal membrane.

4. Iron homeostasis and the role of aluminium

In mammalian cells, iron homeostasis is maintained by balancing cellular iron uptake (via the transferrin receptor) with intracellular storage (in ferritin, the principal iron storage form), and iron utilisation (the key enzyme which regulates the haem biosynthetic pathway, δ-aminolaevulinate synthase). This is carried out at the level of translation of mRNA into protein, rather than at the level of transcription (mRNA synthesis) [5,25]. In the corresponding mRNAs, highly conserved sequences of around 28 nucleotides, which form a stem loop, designated IREs (Iron Regulatory Elements) are located in the untranslated regions (UTRs) of the mRNA, at the 5′- and 3′-extremities of the coding part of the mRNA sequence. In the mRNAs for ferritin and δ-aminolaevulinate synthase, the IREs in the 5′-UTR are associated with regulating the initiation of translation, in other words ribosome binding, whereas those in the 3′-UTR of the transferrin receptor mRNA, are involved in mRNA stability and degradation, i.e. mRNA turnover. Two closely related cytosolic IRE-binding proteins (Iron Regulatory Proteins, IRP's), designated IRP-1 and IRP-2 have been identified in many mammalian cell types (Fig. 3) where they act as iron sensors, by existing in two different forms. When iron is in short supply, the apo-IRPs can bind to the IRE's (Fig. 3) with high affinity, preventing translation of ferritin and δ-aminolaevulinate synthase, while at the same time protecting the transferrin receptor mRNA from nuclease digestion. This leads to an increased cellular iron uptake and a block on synthesis of the proteins involved in intracellular iron storage and utilisation. When iron supply is increased, IRP-1 incorporates iron in the form of an Fe_4S_4 cluster, converting it to a form which is unable to bind to IREs, and which has aconitase activity (although the metabolic significance of this is not clear); in contrast IRP-2 is rapidly degraded, through a mechanism which involves ubiquitinylation and degradation by the proteosome. This results in increased ferritin and δ-aminolaevulinate synthase production, while the transferrin receptor mRNA, deprived of its protective IRP binding, is exposed to nuclease degradation and iron uptake is stopped.

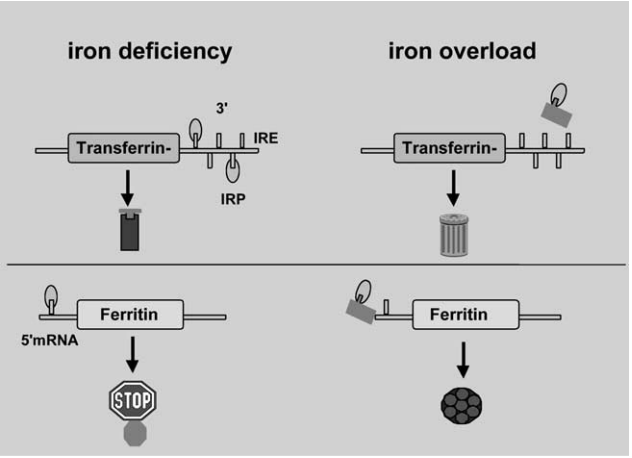

Fig. 3. Regulation of IRP-1 and IRP-2. The two IRPs are shown as homologous four domain proteins that bind to IREs (Left) In iron-replete cells, IRP-1 assembles a cubane Fe–S cluster that is liganded via cysteines 437, 503 and 506. Similar cysteines are conserved in IRP-2 (Cys 512, 578 and 581), but it is unresolved whether they also coordinate an Fe–S cluster. (Right) In iron-replete cells, IRP-2 is targeted for destruction via a specific region (shaded in black), whereas IRP-1, with a 4Fe–4S cluster, is stable and active as a cytoplasmic aconitase. Multiple signals induce IRE-binding by IRP-1 with distinct kinetics. Whether or not NO and H_2O_2 induce IRP-1 by apoprotein formation remains to be addressed directly. From [25].

We would like to know whether aluminium could interfere with iron homeostasis in the brain by altering IRP-1 and IRP-2 levels. The results to date are conflicting. Some authors report that IRP-1 levels in Alzheimer's brains were similar to controls, whereas IRP-2 showed important differences, and was found associated with intraneuronal lesions, [26] however, aluminium levels were not determined. Aluminium was found to antagonise the iron-induced decrease in IRP-2 binding to IREs in cell culture experiments, to stabilise IRP-2 against degradation and to enhance its binding to IREs [27]. This would of course result in up-regulation of transferrin receptors, resulting in increased influx of both iron and aluminium into the cells by transferrin receptor-mediated endocytosis in agreement with other studies [28,29], which also described concomitant expression of neurofibrillary tangle protein. However, other studies suggest the opposite, namely that aluminium-loading decreased IRE binding activity and transferrin receptor expression [30,31]. Clearly further studies are needed to clarify the role of aluminium in stabilising IRP2.

The possibility that aluminium might be incorporated into brain ferritin seems unlikely, consistent with our current views on the mechanism of iron incorporation into ferritin, which requires prior oxidation of Fe^{2+}–Fe^{3+}, at least in the initial stages of iron uptake [5]. In vitro studies did show that aluminium could be incorporated into ferritin in small amounts, but whether it can interfere with iron uptake into ferritin, or be incorporated into ferritin in vivo remains to be established.

It, therefore, seems likely that changes in iron homeostasis could be a major contributory factor in Alzheimer's disease, although the possible role of aluminium remains uncertain. Despite initial studies which implicated aluminium in the development of senile plaques

and neurofibrillary tangles, current results fail to consistently confirm the presence of the metal at the sites of the lesions and cast serious doubts on aluminium playing a causal role in Alzheimer's disease [6]. Nonetheless, it is clear that chronic exposure to aluminium results in cognitive impairment in animals, and dementia in man, leaving no doubt that aluminium is neurotoxic. The potential therapeutic benefit of long term chelation therapy to prevent accumulation of both aluminium and iron in brain as a function of ageing could complement a greater understanding of the role of both metal ions in the aetiology and pathogenesis of many neurological diseases.

5. Animal models of iron and aluminium overload and their use to study chelators

There has been considerable interest in the development of an animal models of iron and aluminium overload in brain which would aid the understanding of the potential neurotoxicity of these two metal ions. We have developed such models and used them to analyse the potential of chelators to mobilise brain iron and aluminium, and the results are summarised below.

There have been many attempts to find animal models, which mimic primary, and secondary iron overload in man, in order to test new classes of iron chelators, which are orally active, unlike the currently utilised desferrioxamine (DFO; Desferal®). However, when one looks for an animal model which, in addition, gives substantial iron loading in brain, there is only one option. 3,5,5-trimethylhexanoylferrocene (developed by Hoechst in the early seventies as an orally active and effective treatment for iron deficiency) is not absorbed in man, but in rodents goes directly to the liver where the iron is released and made available for haem synthesis and hepatic storage in ferritin and haemosiderin [32]. It also crosses the blood–brain barrier, possibly without being metabolised, resulting in relatively important iron loading in a number of specific locations in the brain, such as substantia nigra, cerebellum and cerebral cortex [33]. In the studies described here, Male rats (Wistar strain, 100 g) were adapted to a powder diet containing the ferrocene derivative (1 g kg^{-1} diet, with an iron content of 0.35 g Fe per kg) for 4 weeks. Subsequently they were administered an iron chelator (either, i.p. 10 mg kg^{-1}, in the case of DFO B; or by gavage, 30 mg kg^{-1} for CP24 (1-*n*-butyl-2-methyl-3-hydroxypyrid-4-one) and CP94 (1,2-diethyl-3-hydroxypyrid-4-one) every second day for 14 days (i.e. seven administrations) in combination with an iron-free diet (Fe content 0.003 g Fe per kg).

In early studies of aluminium loading, oral administration of either aluminium citrate or aluminium maltol (1 g aluminium salt per kg of diet vs. 1.52 mg aluminium per kg normal diet) fed for 3–6 months was found to induce significant levels of brain aluminium [18]. Higher concentrations of brain aluminium could be achieved by intraperitoneal injections of aluminium gluconate [19] in a much shorter time, 8–12 weeks. In the studies presented here, the latter animal model was used to compare the efficacy of different chelators in mobilising brain aluminium [19], and to establish whether perturbations of iron homeostasis occurred as brain aluminium concentration increased [34]. Male rats, (Male rats (Wistar strain, 80 g) were aluminium loaded, using aluminium gluconate, administered intraperitoneally 3 times per week at a dose of 2 mg aluminium for a period of 8 weeks. Chelator treatment was as above for ferrocene iron-loaded animals, except that CP20 (1,2-dimethyl-3-hydroxypyrid-4-one) was used instead of CP24.

At the end of chelation therapy, blood was removed by cardiac puncture, after which the animals were killed by cervical dislocation. The liver, heart, kidney and spleen were removed together with various brain regions, which were dissected over ice. Iron and aluminium content of the tissues was determined by electrothermal atomic absorption.

After iron loading with the ferrocene derivative for 4 weeks, the iron contents, expressed as μg Fe g^{-1} wet tissue were determined in the different brain regions examined, with the highest levels in cerebellum (148 μg g^{-1}), cerebral cortex (119 μg g^{-1}) and striatum (119 μg g^{-1}) followed by substantia nigra (107 μg g^{-1}), striatum (78 μg g^{-1}) and hippocampus (70 μg g^{-1}). After 2 weeks chelation with DFO, CP24 and CP94, some reduction of brain iron was seen in most brain regions (Fig. 4). The most effective result was obtained with DFO B in cerebellum, where an almost 60% decrease in iron content was observed. There was little difference between the two hydroxypyridones. Similar results were found with CP20 (deferiprone®). It should be noted that significant alterations in the levels of dopamine and its metabolites in the striatum were observed after CP94 treatment [33], which might reflect coordination of the labile active site iron of the key enzyme of dopamine metabolism, tyrosine hydroxylase. Similar effects were observed with acute administration of CP20 and DFO B at 100 mg kg^{-1} [33], but no such effects were observed with the orally active tridentate siderophore desferrithiocin and its des-methyl derivatives [35].

In the animals, which had been loaded with aluminium gluconate significant increases in brain iron content were also found, notably in various parts of the cerebral cortex (from four- to nine-fold) and the hippocampus (four-fold) compared with untreated controls [34]. Aluminium contents varied from 14 to 25 μg g^{-1} wet weight in the cerebral cortex to 37 μg g^{-1} in the hippocampus. The results of aluminium chelation (Fig. 5) were significantly greater than for iron, particularly

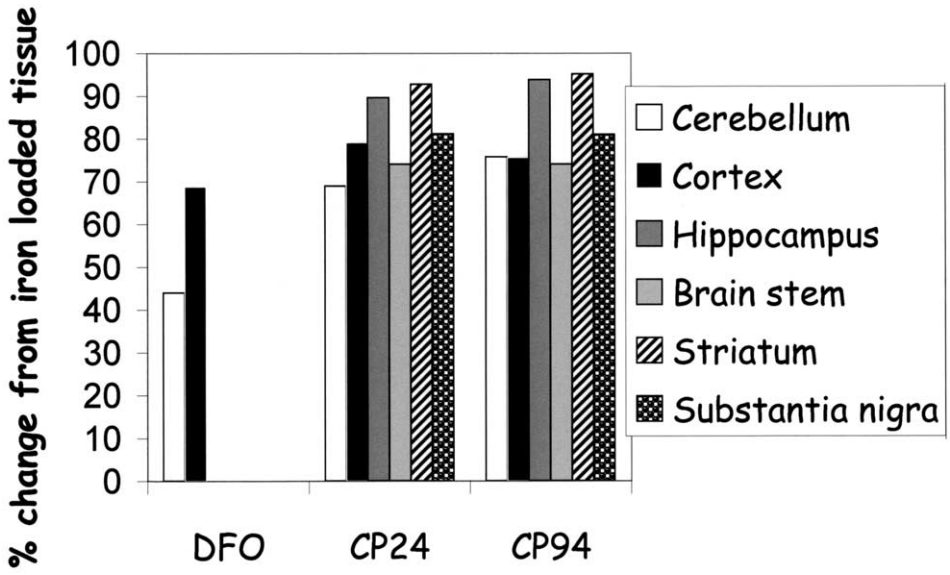

Fig. 4. Iron mobilisation from ferrocene iron-loaded brain regions by the iron chelators DFO, CP24 and CP94. Results are expressed as a percentage of the untreated iron-loaded control group. Standard errors are consistently within 5% of the mean values.

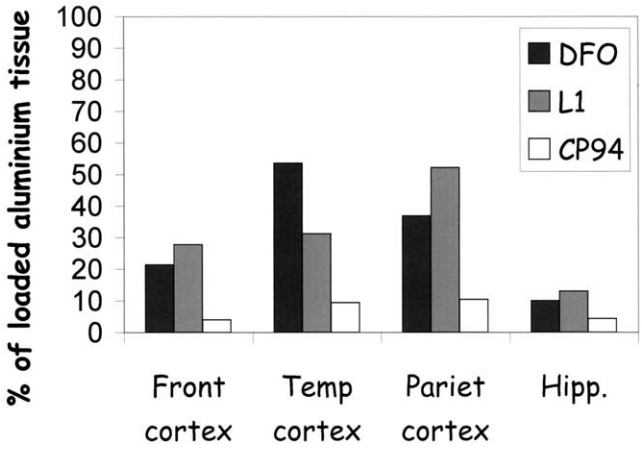

Fig. 5. Aluminium mobilisation from aluminium gluconate-loaded brain region, (1) Frontal cortex; (2) Temporal cortex; (3) Parietal cortex; (4) Hippocampus, by the chelators DFO, the hydroxypyridones, L1-CP20 and CP94. Results are expressed as a percentage of the untreated aluminium-loaded control group.

with the more hydrophobic hydroxypyridone CP94, which reduced aluminium levels in all four brain regions, examined to 10% or less.

6. Conclusions

Iron-rich areas in brain co-localise with regions where GABA neurones terminate, and are thought to be associated with ferritin, and, according to recent results, neuromelanin [1], as well as with enzymes like tyrosine hydroxylase, which are involved in neurotransmitter metabolism. High concentrations of iron in brain regions rich in dopamine pose a particular problem, since dopamine is not only an iron chelator, but both of it and its metabolites, 5-hydroxydopamine and 6-hydroxydopamine can react with molecular oxygen and 'free' iron leading to free radicals [36]. Neuromelanin is proposed to be the major storage form of iron in substantia nigra [1], and it has been suggested that interaction of 'free' iron present during the formation of neuromelanin might initiate Fenton chemistry with generation of hydroxyl radicals or ferryl species, leading to the production of cyotoxic species such as 6-hydroxydopamine, which in turn might release Fe(II) from ferritin [37]. However, we might expect that if iron was present as 'free' iron, it would be more readily chelated than either ferritin, haemosiderin, or neuromelanin iron, which is certainly not reflected in the poor chelation, we see in our animal model, particularly with the hydrophobic hydroxypyridone chelators. In human substantia nigra the high spin trivalent iron appears to be arranged in a ferrihydrite iron-oxyhydroxide cluster form [1], which from our experience would be difficult to chelate.

The unusual lability of brain aluminium towards chelation is entirely understandable if we consider the following points. While aluminium can presumably get into cells, and perhaps also cross the blood–brain barrier via the transferrin–transferrin receptor cycle, once released inside the acidic endosomal compartment (Fig. 2) by protonation of the carbonate anion, it cannot thereafter follow the path of iron. After release from transferrin as Fe^{3+}, we assume that the iron must be reduced to Fe^{2+} in order to be transported across the endosomal membrane via the divalent proton-coupled transporter DCT1. In the cytosol, it is an essential prerequisite that iron be in the divalent form to enable its incorporation into the iron storage protein ferritin.

Assuming that aluminium can exit the endosome, it would be expected to bind to appropriate chelate functions in the cytosol, possibly phosphate compounds, but certainly not to ferritin. This is probably the explanation for the lability of aluminium towards chelation when compared with iron.

In conclusion, it seems that changes in iron homeostasis are a major contributary factor in Alzheimer's disease, and that despite initial studies which implicated aluminium in the development of senile plaques and neurofibrillary tangles, its possible role in this disease remains uncertain. However, it seems clear that the potential therapeutic advantages of long term oral chelation therapy at low doses, in order to prevent possible interference with neurotransmitter metabolism, could prevent the accumulation of both iron and aluminium in brain as a function of ageing. This could be a promising approach to improving the quality of life of the ageing population and perhaps even more importantly could contribute to retarding the onset of the numerous neurological disorders associated with ageing, thus delaying the shipwreck of old age.

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