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Aluminum chelation principles and recent advances

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Contents

Abstract

Acute Al toxicity is not common. Toxicity from prolonged Al exposure has become much less common, but still occurs occasionally. The potential contribution of Al to Alzheimer's disease and related disorders is not resolved. Diagnosis of Al accumulation and treatment of Al toxicity can be achieved with chelators. The biology and chemistry of Al relevant to its chelation are reviewed. The approaches that have been used to assess potential Al chelators are summarized. The chemistry of desferrioxamine and the hydroxypyridinones in relation to Al chelation and the results of the more recent studies with these agents are reviewed. There is very little clinical experience with the hydroxypyridinones as Al chelators. The results of chemical and animal studies suggest they have potential to replace desferrioxamine, as orally effective Al chelators. However, adverse effects associated with the use of 1,2-dimethyl-3,4-hydroxypyridinone (deferiprone) dampen enthusiasm for the hydroxypyridinones as Al chelators. The uses of Al chelators to enhance Al removal, concentration, detection and speciation are reviewed. The role of Al chelators in the environment and their production by plants to reduce Al toxicity is summarized.

Keywords: Aluminum; Chelators; Desferrioxamine; Hydroxypyridinone

Abbreviations: AD, Alzheimer's disease; Al, aluminum; ARBD, Al-related bone disease; DFO, desferrioxamine, deferoxamine, desferrioxamine B; $D_{o/a}$, distribution (partition) coefficient between octanol and aqueous phases; EDDHA, ethylenediamine-*N*,*N*′-bis(2-dihydroxyphenylacetic acid); Fe, iron; HBED, *N*,*N*′-bis(hydroxybenzyl)ethylenediamine-*N*,*N*′-diacetic acid; HEDTA, *N*-(2-hydroxy)ethylenediaminetriacetic acid; HOPO, 3-hydroxypyridin-2-one, 3-hydroxy-2-pyridinone; HP, hydroxypyridinone; 3,4-HP, 3,4-hydroxypyridinone; iPTH, immunoreactive parathyroid hormone; o/a, octanol/aqueous.

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1. Introduction

An overview of the biology and chemistry of aluminum relevant to its chelation is presented. Details can be found in [1,2]. The use of desferrioxamine (DFO) as an Al chelator is briefly reviewed. More details of its use can be found in [2,3]. Findings published since these previous reviews are presented. Other families of Al chelators are briefly discussed as more details can be found in [2]. The approaches used to identify and develop Al chelators are presented. Most of the emphasis on Al chelator development in the past decade has focused on the hydroxypyridinones (HPs). The results of studies with this family of chelators published since the previous reviews are summarized. The adverse effects that limit the use of DFO and the development of the HPs as alternatives to DFO are reviewed. Additional topics discussed include the use of chelators in Al removal, concentration, detection and speciation as well as chelators in the environment, including those produced by plants. The review of plant-produced Al chelators focuses on results that have been reported since reviews on this topic [4,5].

2. The biology and toxicology of aluminum relevant to its chelation

Aluminum is ubiquitous. There are many sources. The major source for most humans is diet. Underarm antiperspirants, vaccines, antacids, parenteral fluids and inhaled fumes and particles from occupational exposure can be significant sources for some people. The sources and pharmacokinetics of Al were recently reviewed [6]. The skeletal system, lung, muscle, liver, brain and blood contain ca. 60, 25, 10, 3, 1 and $\ll$1% of the body burden, respectively, in the normal human [7]. Concentrations in lung are $>$ bone $>$ liver $>$ spleen $>$ heart $>$ kidney $>$ brain $>$ muscle.

Aluminum is not an essential element in the human. It has the potential to produce toxicity that has been most commonly seen in patients who have reduced or absent renal function because the kidney is the primary organ of Al elimination [6]. Acute Al toxicity can occur when the urinary bladder is irrigated with 1% alum to treat bladder hemorrhage. This acute toxicity is almost always limited to patients who have renal insufficiency [8]. Chronic Al toxicity is occasionally seen in chronic dialysis patients, termed the dialysis encephalopathy syndrome. Manifestations of chronic Al toxicity include microcytic hypochromic anemia, Al-related bone disease (ARBD), and encephalopathy. It has been suggested that Al may contribute to Alzheimer's disease (AD). It does have the potential to produce some neurobehavioral and neuropathologic changes that are seen or are similar to those seen in AD (reviewed by [9]). In the dialysis encephalopathy syndrome, Al concentrations increase ca. 10-fold in human organs, except for the lung, where the increase is less [7]. Mean serum Al concentration in normal humans has been reported to be ca. 1.6 and 5–7 $\mu g\, l^{-1}$ [10,11]. Plasma Al is bound to transferrin (91%) and citrate (7–8%) [6]. An Al chelator that does not distribute out of the vascular compartment would have to compete with transferrin and citrate for Al complexation. Dialysis does not effectively decorporate (remove from the body) significant amounts of Al. The inefficacy of dialysis is due to the extensive binding of Al to transferrin, which is not renally filtered, and the absence of ligands in dialysis fluids with a sufficiently high stability/equilibrium constant to compete with transferrin for Al binding [12]. Although intracellular Al ligands have not been positively identified, ATP and ADP are strong candidates [13].

3. Aluminum chelation chemistry

Aluminum exists only as a trivalent cation in vivo. It is too reactive to be found in its elemental state in nature. Reactions with Al are characterized by polymerization, slow ligand exchange rates, and extensive hydrolysis in aqueous solution. Its preferred coordination number is 6, producing octahedral complexes. All metals and metal ions are Lewis acids (electron pair acceptors; electrophiles) that have incomplete valence electron shells. They complex with Lewis bases (electron pair donors; nucleophiles), which are therefore effective ligands, e.g. small molecules or ions that have at least one electron pair that can be donated. Bases with valence electrons that are easily distorted, polarized or removed are soft bases whereas bases that retain their electrons much more tightly are hard bases [14]. Al is the hardest trivalent metal ion (hard Lewis acid). Its effective ionic radius is 50 pm [15]. It is highly electropositive and not easily polarized. Aluminum-ligand binding is a non-covalent interaction, generally involving ionic or electrostatic bonds. Al prefers to coordinate with hard Lewis bases such as OH^-, F^-, PO_4^{3-}, SO_4^{2-}, CH_3COO^-, ROH, RO^- and RNH_2, which donate electrons to its vacant electron orbitals. The most stable Al complexes are with multidentate ligands with negative oxygen donors [15].

The term chelator is from Greek for claw of a crab. It is a compound that binds a metal ion through at least two functional groups, where each group donates a pair of electrons to form a heterocyclic ring encompassing the metal. To form a ring, the ligand must have at least two donor atoms. In comparison to chelators, fluorine is able to complex but not chelate Al because each fluoride forms only a single bond with Al. The term denticity, from Latin for tooth, denotes the number of donor atoms the chelator uses in metal binding. Basic donor

groups, arranged in order of decreasing basicity, include aliphatic monohydroxy acid anions > catecholates > aromatic hydroxy acid anions > 3-hydroxy-4-pyridinones (3,4-HPs) > hydroxamates > 3-hydroxy-2-pyridinones (HOPOs). These donor groups, and some examples of chelators possessing the groups, are shown in Fig. 1. These form highly stable chelates with Al through dioxo interaction [15]. The affinity of multi-

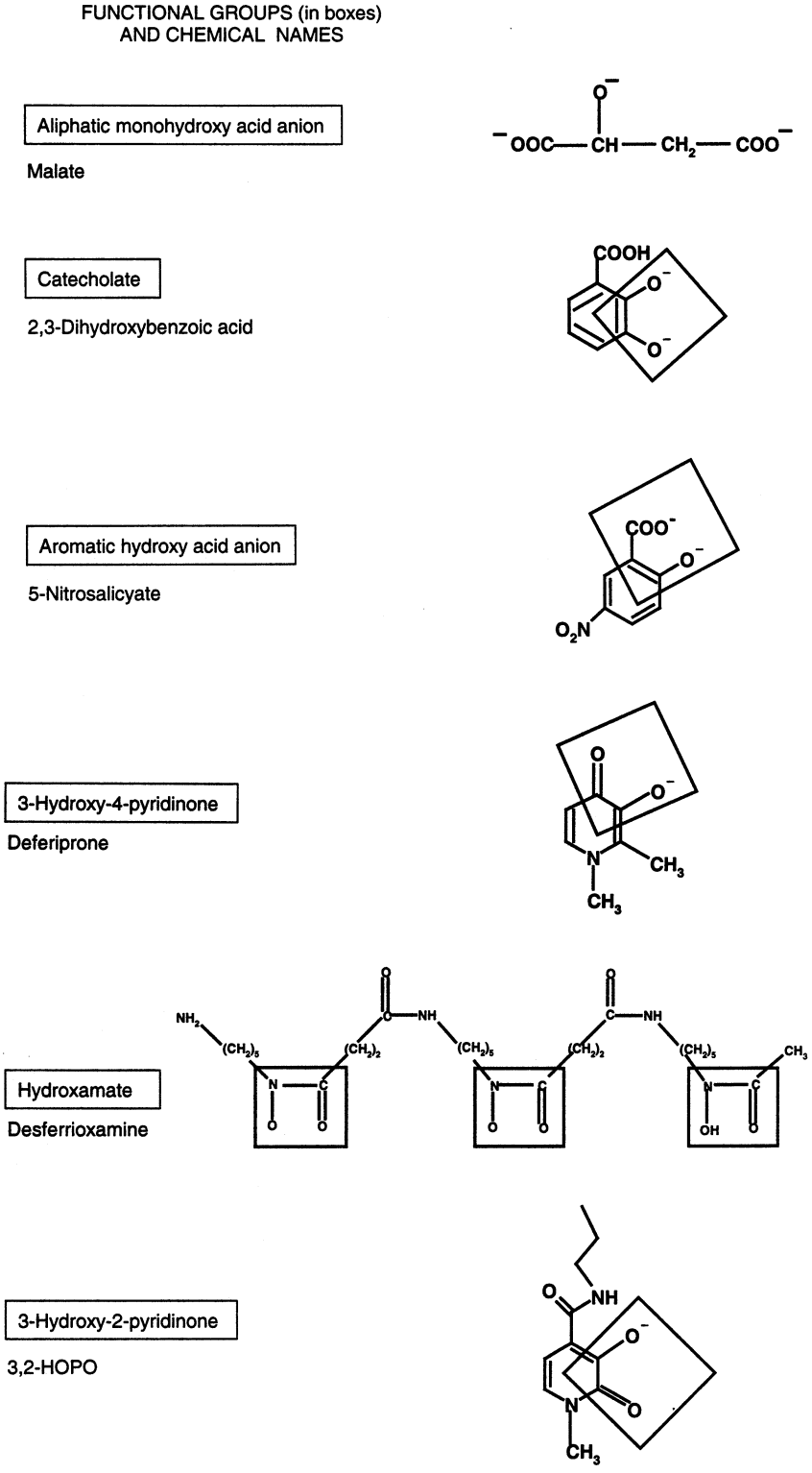
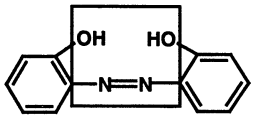


Fig. 1. Structures of functional groups that effectively chelate Al (in boxes) and some Al chelators. Some chelators are shown as examples of compounds that have the effective functional groups. For example, 2,3-dihydroxybenzoic acid has the catecholate group in the box.

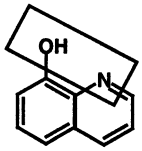
Hydroxy and azo

2,2'-Dihydroxyazobenzene

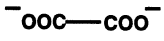


Hydroxy and tertiary N

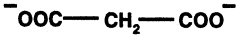
8-Hydroxyquinoline



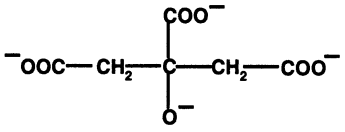
Oxalate



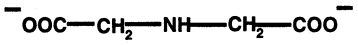
Malonate



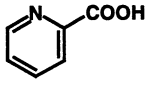
Citrate



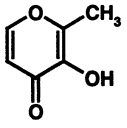
Iminodiacetic acid (Chelex®)



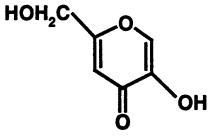
Picolinic acid



Maltol



Kojic acid



Morin, a flavonol

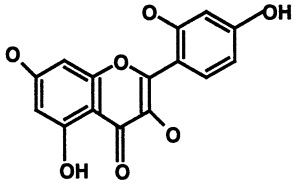


Fig. 1 (*Continued*)

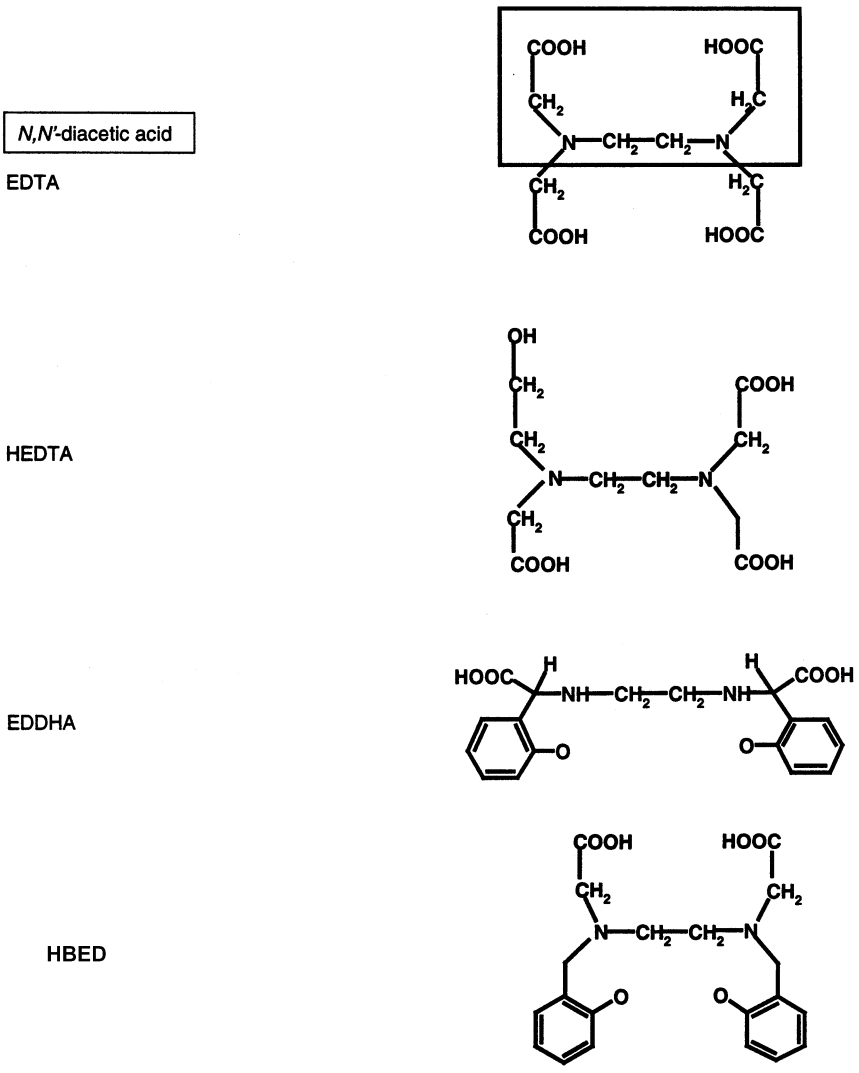


Fig. 1 (*Continued*)

dentate ligands containing these negative oxygen donor groups for Al increases as the number and basicity of the donor groups increase [15]. Results of the comparison of Al chelation activity of a series of substituted ethylenediamine-*N*,*N*′-bis(2-dihydroxyphenylacetic acid) (EDDHA) analogs are consistent with the positive correlation between the basicity of the chelator group and chelator efficacy (see Section 4.1, below). The most stable chelate rings incorporating Al are those that have six members. For examples illustrating these principles of Al chelation see [15]. Critical stability constants of many Al complexes, considered to be reliable values by Martell and Smith, were compiled, along with more recently reported values [2].

Aluminum and iron (Fe) are hard acids with similar ionic radii (54 and 64 pm). They bind to the same plasma proteins. All known Al chelators are Fe chelators. Fe complexes are more stable than Al complexes [16], due to the ability of Fe to fill its 3d orbitals during hexadentate complex formation. There are no metal-specific chelators. The selection and development of Al chelators has been greatly influenced by developments in Fe chelation, which has been much more extensively studied. However, chelation therapy that is intended to reduce Al might adversely reduce essential metals.

The goal of Al chelation is to decorporate Al. Reduction of Al accumulation and toxicity may benefit patients with end-stage renal disease, people with neurodegenerative disorders such as Alzheimer's disease (AD), and those who have neurobehavioral toxicity after prolonged occupational Al exposure. Chelation therapy of chronic hemodialysis patients might be considered when the serum Al concentration exceeds 80 μg l^{-1} [17]. Optimal treatment of these different populations may require different chelators, routes of administration and administration schedules.

Table 1
Desirable properties of Al chelators and the benefit derived from possessing the desired property

Desirable property of an Al chelator	Desired effect
Be sufficiently lipophilic to permeate membranes by diffusion or serve as a substrate for a membrane carrier	Distribute to intracellular sites of Al storage
Be a hard base	Complex with the hard acid Al
Have high affinity and reasonable selectivity for Al	Minimize depletion of Fe and other essential metals
Have a stability constant with Al that is greater than that of endogenous ligands	Favor Al:chelator complex over Al:endogenous ligand interaction to remove Al from endogenous ligands and sites
The free chelator should have a long half-life	Provide sufficient time to complex Al that might redistribute out of storage sites as free Al is complexed
Form an octahedral complex with Al	Reduce potential toxicity due to partially complexed metal which might retain its ability to promote oxidative injury
Form a water soluble complex	Enhance renal Al clearance to reduce potential toxicity
The Al:chelator complex should be stable to hydrolysis at physiological pH and resistant to metabolic degradation	Promote Al decorporation rather than redistribution
The Al:chelator complex should not be reabsorbed	Promote Al elimination
Minimal toxicity of both the chelator and the Al:chelator complex	Minimize toxicity
Good oral bioavailiability	Ease of administration, broader acceptance of use, and a safer route of drug administration providing potential application in treatment of neurodegenerative disorders

An effective Al chelator would possess the properties shown in Table 1.

4. Methods to develop and assess aluminum chelators

4.1. Structure activity relationships in Al chelation

There has not been extensive investigation into the structure activity relationships of ligand components that mediate Al chelation. Although a chelator with a greater stability constant for Al than Fe may be an impossibility, it may be possible to design chelators that favor Fe over Al to a lesser extent than those currently available. Some approaches were suggested [3]. Effective Al chelators have negatively charged oxide donor atoms. The high correlation between the formation constants of metal-ligand complexes and the formation constants of the metals with the hydroxy ion (K_1 (OH^-)), the archetypal RO^- ligand, attests to the importance of oxygen in Al chelation [18]. The greater stability constants for catecholate > 5-nitrosalicylate > kojate > malonate for comparable K_1 (OH^-) values for all metal ions suggests the catecholate moiety would have greater chelation potential than the malonate moiety. Increasing electronegativity of the Al chelating group increases Al chelation efficacy, as noted from studies conducted to determine the ability of a series of substituted EDDHA analogs to mobilize Al in an octanol–aqueous-Al borate system and to displace Al from transferrin (see Section 4.3 below). The results showed a positive correlation between chelation efficiency and the Hammett σ constant of the substituent group, a measure of its ability to attract electrons from the phenyl ring (unpublished results). The electron attracting effect increases the charge density on the oxide donor, making it more basic. These findings are consistent with the contribution of increased basicity to Al chelation.

4.2. Computer modeling

A few studies have used computer modeling to predict Al chelation activity. Clevette and Orvig [19] used published stability constants to calculate effective formation constants at pH 7.4, pM values and the ligand concentrations required to retain 20, 50 and 90% of the Al as Al:ligand in a modeled simplified human blood plasma. pM values are the negative log of the free Al concentration in the presence of ligand. Their results suggested EDTA, *trans*-cyclohexane-1,2-diamino-*N*,*N*,*N*′-*N*′-tetraacetic acid, triethylenetetraamine hexacetic acid, diethylenetriamine pentaacetic acid, 4,5-dihydroxy-1,3-benzenedisulfonic acid (Tiron), DFO and the HPs could be effective Al chelators at submillimolar concentrations. Speciation calculations suggested DFO and 1,3,5-tris(((2,3-dihydroxy-5-sulfobenzoyl)amino)methyl)benzene (MECAMS) should be able to remove Al from transferrin [20]. Calculations conducted at pH 7.4 and 5.5, the latter to model extracellular fluid inflammatory conditions, suggest the ability of a threefold higher concentration of 3,4-HPs, when present above 10^{-4} M, to more efficiently complex Al than DFO at pH 7.4 [21]. This is consistent with in vivo observations [22,23].

4.3. In vitro assessment

As an initial assessment of Al chelation potential, we utilized an octanol/aqueous (o/a) system, as typically

used to determine partitioning (distribution) coefficients ($D_{o/a}$). We modified this system by addition of Al borate, which is quite insoluble in each phase but can release Al into solution. This system enabled determination of the test compound's ability to form a stable, soluble Al complex in the absence of competing ligands. Formation of an Al:ligand complex was shown by increased Al in solution, as determined by atomic absorption spectrometry. Quantitation of Al in the octanol and aqueous phases in the absence and presence of the ligand enabled calculation of the $D_{o/a}$ of the Al:ligand complex. Similarly, using UV spectroscopy to quantitate the ligands, the $D_{o/a}$ of the ligand and the Al:ligand complex were determined. The stoichiometry of the Al:chelator complex can be modeled, based on the calculated concentrations of the free chelator and the possible metal:chelator stoichiometries. See [2] for more details. The results identified ligands that do not seem to form a complex with Al in either aqueous solution or octanol. These ligands were not considered worthy of further investigation as potential Al chelators. The results were interpreted in light of the hypotheses that: (1) an effective Al chelator is a compound with sufficient lipophilicity to penetrate membranes; but (2) after forming a complex with Al, becomes sufficiently hydrophilic to favor elimination. It was suggested that chelators with a $D_{o/a} = 0.2–1.0$ should possess a favorable benefit/risk ratio [24]. This hypothesis was tested in vivo with HPs (see Section 6 below). Other investigators developed a method to determine Al complexation with chelators based on competition between Al and cadmium or copper for chelator binding and polarographic determination of cadmium or copper [25].

An effective chelator must compete with in vivo ligands. Over 90% of plasma Al is bound to transferrin. Therefore, a method was developed to assess the ability of ligands to mobilize Al from transferrin in vitro [26]. The ability of the compounds to displace Al from transferrin correlated very well with their ability to solubilize Al in the o/a system and their stability constants with Al [26], increasing confidence in the octanol–aqueous-Al borate system as a predictor of potential Al chelators. A similar method was developed using cow serum. The ability of chelators to displace Al from serum proteins was assessed [25].

4.4. In vivo assessment

To assess Al chelation activity in vivo, we developed a rabbit model of Al accumulation and toxicity. Rabbits were given 20 subcutaneous Al injections of 0.4–0.6 mmol Al kg^{-1} as the lactate over 1 month [27]. This produced a persistent Al accumulation with an Al distribution that models the human who has Al accumulation [28]. A similar Al body burden was produced by 20 intravenous Al injections of 0.1 mmol Al kg^{-1} as the lactate, but not the citrate [22]. Ten subcutaneous injections of 0.6 mmol Al kg^{-1} as Al sulfate delivered over 2 weeks to rabbits produced an increase in Al and a pattern of Al distribution that reasonably models the human with Al accumulation [29,30]. The ability of compounds to increase urinary and biliary Al excretion, reduce tissue Al concentrations and reduce Al-induced toxicity was tested in rabbits ca. 1–7 weeks after Al loading. Similarly, mice and rats were Al loaded by intraperitoneal injections 5 days weekly for 3–5 weeks of 0.27 or 0.12–0.14 mmol Al kg^{-1} per injection as Al nitrate to mice [31] or rats, respectively [32]. Rats were Al loaded by intraperitoneal injections of 0.074 mmol Al kg^{-1} as the chloride 5 days weekly for 4 weeks [25,33], 0.074 mmol Al kg^{-1} Al as the gluconate three times weekly for 4–8 weeks [34] or 0.4 mmol Al kg^{-1} as the lactate 5 days weekly for 4 weeks [35]. Starting 1 day after the Al injections, Domingo and Gomez gave test compounds intraperitoneally three times weekly for 2 weeks, at doses equal to 25% of their LD50. The endpoints studied as measures of Al chelation potential were increased urinary and fecal Al output and reduced tissue Al concentration. Some details of studies using these methods are in [2]. Graff et al. gave the chelators by intraperitoneal injection daily for 2 weeks, beginning 1 week after the Al injections. They determined urinary Al output after the 1st, 3rd and 9th chelator treatments and tissue Al concentrations at the end of the treatments. They found increased urinary Al excretion after EDDHA, but not EDTA, DFO or *N*-(2-hydroxy)ethylenediaminetriacetic acid (HEDTA), and decreased Al concentration in one or more tissues after EDTA, DFO and HEDTA, but not EDDHA [25] [33]. The structures of these chelators are shown jn Fig. 1. Nephrotoxicity was observed with EDDHA [25], as had been previously reported (see [2]). Florence et al. [34] gave chelators every 2 days for a month before determining blood and tissue Al. Two 3,4-HPs (deferiprone and CP94) given orally and DFO given intraperitoneally decreased liver and brain but increased spleen Al concentration. Yokel conducted microdialysis 1 week after the Al injections, to determine changes in extracellular unbound Al in blood, liver and brain. Four 3,4-HPs increased blood Al slightly but increased liver extracellular unbound Al considerably, suggesting liver is a primary site of Al chelation by these agents. Addition of Al (36.4 μmol g^{-1} diet) as the hydroxide for 8 months increased Al in rat serum, tibia and kidney 2.2-, 1.25- and 1.5-fold, respectively [36]. A single 100 mg kg^{-1} DFO dose significantly increased urinary Al excretion, consistent with clinical reports suggesting DFO test dose results correlate with body Al burden. Rats were Al-loaded by addition of 50 mg Al kg^{-1} per day as Al nitrate nonahydrate and 178 mg^{-1} kg^{-1} per day of citric acid to the drinking water for 2 weeks followed by 100 mg Al kg^{-1} per day and 356 mg citric acid kg^{-1} per day

for 100 days [37]. This procedure significantly increased the kidney Al concentration in old but not young rats. Brain, bone, liver and spleen Al concentrations were not increased.

A model of moderate chronic uremia can be produced by 5/6 nephrectomy in the rat. Beginning 3 weeks after this procedure, rats were Al-loaded by 45 mg Al kg^{-1} per day, 5 days weekly of Al nitrate nonahydrate given intraperitonealy for 3 or 5 weeks, resulting in significant elevation of Al in several tissues [32,38]. Another group Al-loaded uremic rats by gastric intubation three times weekly for 9 weeks of 25 mg Al kg^{-1} as Al hydroxide plus citric acid, beginning 4 months after 5/6 nephrectomy [39]. Serum and bone Al concentrations were elevated ca. 2.5-fold over normal control rats. DFO partially reversed the elevated bone Al concentration.

The efficacy of chelators in animals that received a single Al injection has also been studied. The ability of compounds to protect against Al-induced lethality, to reduce tissue accumulation and to increase Al elimination was studied in mice. Test compounds were given intraperitoneally as a single dose that was 25–33% of their LD50. The test compound was given 0–20 min after a single intraperitoneal injection of 3–7.5 mmol Al kg^{-1} as Al nitrate, which approximates its LD50 of 4.6 mmol kg^{-1} [40]. The results using this method are summarized in [2]. Similarly, rats were given a single intraperitoneal dose of 0.22 mmol Al kg^{-1}, as the chloride, 30 min before chelator administration [41]. The results are discussed below.

5. DFO and other aluminum chelators

DFO is a trihydroxamic acid, hexadentate, Fe siderophore, shown in Fig. 1. Siderophores are low molecular weight Fe chelating agents secreted by microorganisms that complex environmental Fe. The metal:siderophore complex is apparently recognized by the microorganism and internalized, providing essential Fe. The fully protonated form of DFO predominates in the absence of metal at physiological pH [42]. It forms stable 1:1 octahedral complexes with Fe (ferrioxamine) and with Al (aluminoxamine) involving six oxygen donors that form three five-membered chelate rings. The $[Al–DFO–H]^{+}$ complex predominates at physiological pH [42]. DFO was initially investigated as an Al chelator in hemodialysis patients suffering from apparent chronic Al intoxication. The choice of DFO was based on its ability to remove Fe from the liver of dialysis patients. It was found that DFO can reduce Al-induced mortality associated with the dialysis encephalopathy syndrome, can reduce trabecular bone Al, and can improve bone histomorphometry and ARBD. Studies in animals have shown that DFO can increase Al clearance, decrease tissue Al concentrations and reduce measures of Al-induced toxicity in Al-loaded rats and rabbits. For further discussion see [2]. DFO appears to have been beneficial in the treatment of some cases of acute Al intoxication associated with massive contamination of the dialysis fluid and irrigation of the urinary bladder with 1% alum [8,43,44].

Plasma ultrafilterable Al increases after DFO treatment [45]. In the presence of serum concentrations of DFO that would be achieved in vivo with ca. 2.5–40 mg DFO kg^{-1}, ca. 80% of serum Al was found to be aluminoxamine [46]. However, two studies by another group found only 15–40% of the Al to be aluminoxamine. The remainder was attributed to other <5 kDa, therefore ultrafilterable, unidentified species [47,48]. DFO appears to be able to remove Al from tissues, based on the comparable increase of ultrafilterable and total serum Al following DFO therapy. Using an anti-desferrioxamine antiserum, it was concluded that DFO does not enter the brain [49]. DFO appears to enter cells only by endocytosis whereas a 3,4-HP (CP94) rapidly enters cells [50]. The in vivo sites of Al chelation by DFO are not well understood.

A twofold increase of cerebrospinal fluid Al was observed after DFO treatment. The Al was primarily present as aluminoxamine. In light of the hydrophilicity of aluminoxamine, which presumably prevents its distribution across the blood-brain barrier, it was concluded that DFO does distribute across this barrier to chelate/redistribute Al from intracerebral sites [51]. Although DFO appears to reduce brain Al, discussed below, it is not clear how much of its beneficial effects are due to reduction of Al, Fe and other metals; reduction of free radical-mediated toxicity due to metal chelation; or other mechanisms.

Steady state plasma Al was achieved ca. 7.5 h after 40 mg kg^{-1} DFO in anephric patients, demonstrating the benefit of prolonged DFO presence to maximally form aluminoxamine in vivo [46]. Aluminoxamine is stable in plasma. Its biliary elimination is negligible [52]. In the absence of renal function aluminoxamine persists for days until cleared by dialysis [3,46,53]. However, prolonged residence of aluminoxamine in plasma may provide the opportunity for Al redistribution to extravascular sites such as the brain, and associated toxicity. Evidence for this possibility was presented [3,53]. When DFO was administered near the end or after a dialysis session toxicity was observed between the DFO dosing and the next dialysis session, when most of the free DFO and aluminoxamine are cleared by dialysis. This toxicity was attributed to aluminoxamine [43]. To address the concern about these side effects that were thought to correlate positively with the DFO dose, a Consensus Conference in 1992 recommended the use of low dose DFO to diagnose and treat Al overload and toxicity [54]. Observations suggested a test dose of 5 mg kg^{-1} DFO had comparable ability to mobilize Al as a 40 mg

kg^{-1} dose in patients with serum Al below as well as above 50 μg l^{-1} [55]. This is consistent with the calculated ability of 10 μM DFO to completely bind 3 μM (81 μg Al l^{-1}) [16] as 5 mg kg^{-1} DFO should achieve a serum concentration of ca. 12 μM, based on its volume of distribution of 63% of body weight [56].

The efficiency of Al decorporation by DFO (moles of Al cleared into dialysate/moles of DFO administered) was $<10\%$ when 2000–6000 mg (ca. 30–100 mg kg^{-1}) of DFO was given intravenously in the first 2 h of hemodialysis. The low efficiency may reflect the limited pool of Al available for chelation during the short residency of DFO before it is cleared by dialysis. Therefore, another variable to consider in DFO therapy is the timing of its administration. If DFO is given at the end of a dialysis session the persistence of aluminoxamine until the next dialysis session may contribute to toxicity whereas if given during or too soon before dialysis its potential to chelate Al may not be achieved. Greater efficiency (13%) resulted after a lower DFO dose (14.25 mg kg^{-1}) [57] than generally used at that time (30–80 mg kg^{-1}), suggesting low dose DFO therapy may reduce toxicity and excess DFO administration.

The influence of timing of DFO administration in relation to dialysis on safety and efficacy was assessed by comparing the administration of 15 mg DFO kg^{-1} 1 h before dialysis to the same dose in the same patients 2 weeks later, given 44 h before dialysis [58]. Al clearance was comparable, representing ca. 5% efficiency. Peak serum Al was higher during the 44 h-interdialytic period than 1 h period after DFO dosing, creating the potential for greater toxicity with the former dosing regimen.

The DFO test, a single DFO injection, has been used to assess Al body burden, as evidenced by an increase in serum Al. The test dose is used because steady-state serum Al concentrations do not correlate well with the Al deposition in bone and soft tissues that results from long-term Al exposure. Five and 10 mg DFO kg^{-1} doses were randomly compared in the same subjects to assess their ability to predict Al overload and ARBD [59]. Serum Al concentrations 44 h after these two doses were not different. When paired with serum immunoreactive parathyroid hormone (iPTH) determination, the increase in serum Al concentration after the 5 mg kg^{-1} DFO test dose was quite predictive to differentially diagnose aluminum overload and ARBD. The authors provide a strategy for monitoring and diagnosing Al overload [59]. The use of a 500 mg DFO test dose versus 30 mg DFO kg^{-1} resulted in less toxicity [60], supporting the use of this lower dose. Observations in one subject with a cadaveric renal transplant showed elevated urinary Al excretion subsequent to this DFO test dose [61]. The 5 mg DFO kg^{-1} dose was also shown effective in treatment of Al accumulation and toxicity, when given weekly, reducing serum Al, reversing some manifestations of Al toxicity and increasing serum iPTH. Fewer side effects were seen when the dose was given 5 h before dialysis than during the last hour of dialysis [43]. DFO was withheld from patients whose basal serum Al concentration exceeded ca. 200 μg l^{-1} to avoid increasing the risk and exacerbating Al toxicity. The authors provide a strategy for treatment of Al overload in dialysis patients [43]. Recent studies have shown efficacy of 0.5 and 2.5 mg DFO kg^{-1} [45]. Although these lower doses did not increase total and ultrafilterable serum Al as much as 5 mg DFO kg^{-1}, the efficiency of the 0.5, 2.5 and 5 mg kg^{-1} doses, assessed by the increased ultrafilterable serum Al/DFO dose, was 37, 16 and 10%, respectively. Clearance of Al into the dialysate after a 2 mg kg^{-1} DFO dose demonstrated an efficiency of 22% [58]. The 0.5 mg DFO kg^{-1} dose might be useful in cases of severe Al accumulation to avoid great increases in serum aluminoxamine [45]. An uncontrolled study suggests it is safe and useful in the long-term treatment of Al accumulation [62]. Aluminoxamine clearance from blood may be enhanced by use of a charcoal hemoperfusion column or high-flux polysulphone membranes compared to conventional dialysers.

DFO has been immobilized in a hollow-fiber device for extracorporeal removal of Al from blood [63]. Further studies are needed to assess the long-term clinical safety and efficacy of immobilized chelators versus systemic treatment. Immobilization of chelators creates the possibility of using chelators other than DFO, some of which are discussed in Section 8 below.

To assess the potential for DFO to benefit patients with AD, 25 probable AD patients were given 125 mg (0.19 mmol) DFO intramuscularly twice daily 5 days weekly for 2 years [64]. Control groups of probable AD patients received oral lecithin or no treatment. Lower mortality and a slower rate of deterioration of a home behavioral assessment of activities of daily living were seen in the DFO-treated group. Further studies are necessary to confirm these results and to determine if the beneficial effect of DFO in AD patients is mediated by Al and/or Fe chelation, by reduction of oxidative injury or via some other mechanism. Average cortical brain Al at autopsy in three humans with AD who received 23.5–54 g of DFO was 2.69 μg Al g^{-1} compared to 4.09 in three AD subjects who received <3.5 g DFO [65], suggesting DFO-mobilization of Al from the brain might have contributed to the beneficial effects seen in the AD patients. Intracisternal Al maltolate injections to rabbits on days 0 and 7 raised the average Al concentration in seven brain and spinal cord regions to 20-fold. Twice daily DFO injections from days 4 to 14 reduced the average Al concentration to twofold above the controls [66]. Three weekly DFO injections to rats that had been given a single intravenous ^{26}Al injection reduced the brain ^{26}Al half-life to ca. 55 days, compared

to ca. 150 days in non-DFO treated rats [67]. These studies demonstrate the ability of DFO to accelerate Al clearance from the brain. It is not known if DFO enters the brain to chelate and reduce brain Al or if the reduction of brain Al was due to redistribution of Al out of the brain to maintain whole body Al equilibrium after Al chelation from peripheral sites. Similarly, DFO can reduce bone Al, although it is not known if DFO distributes into bone. The skeletal system is a candidate site of Al chelation as it contains $>50\%$ of the Al body burden. At equilibrium bone Al presumably greatly influences the Al concentration in all tissues. Alternatively, the liver may be a significant site of Al chelation. Evidence for this was presented [3,53]. Reduction of brain and bone Al by DFO might result from Al decorporation from any site and the subsequent re-equilibration of Al among tissues.

DFO administration has been shown to reduce Al-induced effects in animals. It partially reversed the neurofibrillary degeneration produced by lateral cerebral ventricular and intracisternal Al injection [66,68,69]. Al-induced lipid peroxidation, probably a product of Al-enhancement of iron-induced oxidative injury, was decreased by DFO [70]. Hydroxypyridinones also can reduce Al-induced oxidative injury (see Section 6 below).

As DFO is not an ideal chelator, particularly for long-term prevention or treatment of Al accumulation, there has been a concerted effort to identify orally-effective alternatives.

Aluminum chelation by fluoride, carboxylic acids, amino acids, catechols, polyamino carboxylic acids, phenyl carboxylic acids, maltol and hydroxamic acids, was reviewed [2]. There has been considerable interest in citrate, which effectively complexes Al. Mononuclear Al:citrate complexes predominate in blood plasma conditions [71]. Citrate has not been shown to be a safe and effective alternative to DFO as a single agent Al chelator [32]. Its co-administration with Al often exacerbates Al toxicity [2,72]. Significant additions to the published literature on Al chelation have only been achieved with the HPs during the past 5 years. These are discussed below. Preliminary results suggest bisphosphonic acids may be effective Al chelators [73], consistent with previous reports showing a reasonably high stability constant between a diphosphonic acid and Al [2,74]. A tris-hydroxamate with good Al complexing properties was described [75], consistent with the expected benefit of hexadentate ligands, discussed in Section 6, below.

Some mammalian cells, including human phagocytes and myeloid cell lines, have an inducible ability to acquire Fe from Fe:siderophore complexes. This process appears to involve separation of the Fe from the siderophore [76]. The mechanism is unknown. It suggests the possibility that these cells possess a chelating agent that more efficiently binds Fe, and perhaps Al, than siderophores. Alternatively, the cells may cause Fe-siderophore dissociation by creating an environment with a sufficiently low pH.

6. The hydroxypyridinones as aluminum chelators

Due to the lack of oral efficacy of DFO, its side effects (discussed in Section 7) and its relatively high cost, there has been an effort for several decades to identify orally-effective Fe chelators. Recently, leading candidates from this effort have been the HPs. Two structural families of HPs have been investigated; the 3,4-HPs and the 3-hydroxypyridin-2-ones (3-hydroxy-2-pyridinones; HOPOs), shown in Fig. 1. The bidentate 3,4-HPs are orally effective chelators. They are structurally quite simple and therefore relatively inexpensive to synthesize. They form 1:3 Al:HP complexes with overall log stability constants $>10^{30}$ [77,78] that are greater than Al:DFO (ca. 10^{22}–10^{24} [15,18,79]). They were shown to effectively mobilize Al in the octanol–aqueous-Al borate system [26]. Deferiprone more rapidly and more effectively displaced Al from human serum proteins than DFO [80]. The first in vivo studies conducted with the HPs were with 1,2-dimethyl-3-hydroxypyridin-4-one (a.k.a. deferiprone, L1, CP20, HP4A, dpp). Deferiprone significantly increased urinary Al output and reduced liver Al concentration in Al-loaded normal renal or uremic rats after oral, intraperitoneal or subcutaneous administration [32,81] as well as Al-loaded mice [82]. Oral and subcutaneous deferiprone efficacy were comparable [32]. However, most of the renal-impaired rats died after 3 days of once daily deferiprone treatments [32]. As neither the Al-loading nor repeated deferiprone treatments were lethal [32,83], lethality may have been due to the deferiprone:Al complex, as suggested by Gómez et al. [32]. Oral deferiprone dosing of Al-loaded rabbits increased serum Al concentration and urinary Al output and decreased bone and kidney Al but increased liver Al, generally demonstrating Al chelation efficacy [29].

An uncontrolled study in human patients who were receiving dialysis demonstrated the ability of deferiprone to increase plasma Al concentration and Al clearance into peritoneal dialysis fluid [84].

Over 100 3,4-HP analogs have been synthesized, enabling the conduct of studies to assess relationships between structure, physico-chemical properties, pharmacokinetics, efficacy and safety. We utilized 3,4-HPs to test these relationships. Based on studies of the ability of 3,4-HPs to release Fe from hepatocytes in relation to their partition coefficients ($D_{o/a}$), Porter et al. suggested that a $D_{o/a}>0.2$ was optimal for chelators to penetrate membranes [85]. Studies with Fe-loaded animals suggested that chelators with a $D_{o/a}$ between 0.2 and 1

should be sufficiently lipophilic to penetrate membranes to enable intracellular metal chelation, yet sufficiently hydrophilic to distribute out of the cell to enable reduction of intracellular Fe [24]. To test whether these hypotheses applied to Al chelation, we determined the $D_{o/a}$ of eight 3,4-HPs and their oral bioavailability. We also determined their ability to promote urinary and biliary Al elimination for 24 h after single and repeated administration in the Al-loaded rabbit. The distribution coefficients of the eight 3,4-HPs selected for study ranged from <0.001 to 2.1, whereas the $D_{o/a}$ values of their Al complexes ranged from <0.001 to 17 [2]. Oral bioavailability ranged from 30 to 74% in the rabbit. It did not correlate with lipophilicity. The most hydrophilic 3,4-HPs tested were among the most bioavailable. A distribution coefficient $\ll 0.2$ did not prevent their oral absorption [86]. As oral absorption of the 3,4-HPs is not prevented by hydrophilicity, this presents the opportunity to further develop analogs that are very hydrophilic, rather than being limited to those that have intermediate hydrophilicity/lipophilicity. A recently synthesized glucopyranose carbomyl-3,4-HP, which was shown to complex Al, would be expected to be quite hydrophilic [87]. Mean absorption times of the eight 3,4-HPs were 0.5–1.5 h, suggesting absorption from the upper intestine [86]. Their volumes of distribution, 1–2.6 l kg^{-1}, suggest extensive extravascular distribution, consistent with reports suggesting their ability to penetrate cell membranes, including the blood-brain barrier [88]. There is evidence that Al:3,4-HP complexes are transported across the blood-brain barrier, from brain to blood, which should enhance their ability to mobilize Al out of the brain [89]. Glial fibrillary acidic protein, an indicator of chemical-induced damage of the central nervous system, was elevated in Al-loaded rabbits. It was significantly reduced in the frontal cortex following repeated treatment of three 3,4-HPs, one of which also reduced the Al concentration [90]. These results are consistent with observations suggesting the 3,4-HPs can cross the blood-brain barrier. The results further suggest that the 3,4-HPs can reduce Al-induced neurotoxicity. A mechanism by which the 3,4-HPs might reduce neurotoxicity, in addition to Al chelation, is Fe chelation. Iron-induced oxidative injury, which can be enhanced by Al, was reduced by deferiprone, Al:deferiprone and the complex of Al with another 3,4-HP [91]. This effect was probably mediated by Fe chelation by the 3,4-HPs. Al would be released in this process.

To test the hypothesis that there is an optimal lipophilicity for Al chelation efficacy, blood Al concentration and urinary and biliary Al elimination were determined after single dose intravenous administration of eight 3,4-HPs to Al-loaded rabbits. Each of the HPs rapidly increased serum Al, suggesting Al mobilization from the erythrocyte, and/or extravascular sites such as the Kupffer cells of the liver [23,35]. Each of the 3,4-HPs significantly increased the sum of biliary and urinary Al output above that obtained with saline. Biliary Al clearance accounted for 6% of total Al clearance after DFO and ca. 2–20% after the 3,4-HPs. Chelation efficiencies of the 3,4-HPs ranged from 2.8 to 11.7%, greater than the 2.1% efficiency of DFO [22]. Although there were no significant correlations between the lipophilicity of the 3,4-HPs or the Al:3,4-HP complexes and biliary, urinary or total Al output, the fraction of total Al in the bile positively correlated with 3,4-HP and Al:3,4-HP lipophilicity. The results suggest that lipophilic HPs that promote clearance of a significant fraction of the eliminated Al via the bile might be useful in anephric patients.

To test the hypothesis that the optimal lipophilicity of oral Al chelators is a $D_{o/a}$ between 0.2 and 1, six 3,4-HPs were repeatedly administered orally to Al-loaded rabbits, compared to repeated intravenous DFO and oral saline [23]. All treatments were given three times weekly for 4 weeks. Each of the 3,4-HPs and DFO significantly increased total Al elimination after their last dose, demonstrating efficacy after the 12th dose that was comparable to that seen after a single intravenous dose. The sum of urinary and biliary Al output was greater after each of the 3,4-HPs than DFO. Overall, there was no correlation between 3,4-HP lipophilicity and efficacy. With the exception of the most lipophilic 3,4-HP, which produced seizures, there was no correlation between lipophilicity and toxicity.

Domingo and coworkers used two methods to assess the Al chelation efficacy of numerous 3,4-HPs. Nine 3,4-HPs, 2,3-dihydroxybenzoic acid, picolinic acid, EDDHA and methylmalonic acid were given orally, and DFO given subcutaneously, as 1.79 mmol kg^{-1}, 10 min after a single intraperitoneal dose of Al nitrate [92]. When corrected for the bidenticity of the 3,4-HPs versus the hexadenticity of DFO, considering that the dose of the 3,4-HPs was effectively 1/3 of the DFO dose, only one 3,4-HP (1-[3-hydroxy-2-methyl-4-oxopyridyl]-2-ethanesulfonic acid; L6) enhanced 3-day cumulative urinary Al excretion as effectively as DFO. Deferiprone was ca. 60% as effective as DFO. Only two compounds decreased Al in all five tissues studied. These two compounds significantly decreased urinary Al excretion. One interesting but untested explanation is the enhancement of biliary Al excretion. Although it was a stated objective of this study to determine whether the lipophilicity of the tested compounds influences chelation efficacy, this was not discussed nor are the lipophilicity values reported that would enable this assessment. Four of the 3,4-HPs that were effective in this study were subsequently administered orally for five consecutive days, as 0.89 mmol kg^{-1}, to rats that had been Al-loaded by daily intraperitoneal injections of Al for 2 months [93]. DFO was similarly given by

subcutaneous injection. When 5-day urinary Al output in 3,4-HP treated rats was multiplied by 3, to correct for bi- versus the hexadenticity of DFO, three of the 3,4-HPs, including deferiprone, were 75–105% as efficient as DFO. DFO significantly decreased bone and brain Al. Two 3,4-HPs decreased bone and three decreased brain Al, but deferiprone did not. Oral deferiprone given for 5 days significantly increased urinary Al output in young and old rats more efficiently than the same molar dose of DFO given subcutaneously [37].

Computer-aided speciation studies comparing the ability of several chelators to mobilize Al in normal blood plasma and extracellular fluid in inflammatory conditions suggests deferiprone and CP94 have greater efficiency than DFO [21], consistent with some of the above in vivo observations. Therefore, the further development of quite hydrophilic 3,4-HPs as orally effective Al chelators, which may have less toxicity than more lipophilic analogs after long-term administration, seems warranted.

Some of the 3,4-HPs are metabolized by glucuronide conjugation of the 3-hydroxy group, preventing metal chelation with this functional group. This is a disadvantage of deferiprone.

One of the concerns of an oral chelator is the potential that it will chelate metal in the gastrointestinal tract and increase metal absorption. However, several studies did not show this to be a significant problem (reviewed in [3,53,94]).

Most of the HPs studied to date have been bidentate ligands. As noted above, the preferred coordination number of Al is 6. Increasing the denticity of the ligand from 2 to 6 increases the stability of the metal-ligand complex. Comparison of the efficiency of Al chelation, assessed by Al mobilization in the octanol–aqueous-Al borate system, of monohydroxamic acids, a dihydroxamic acid (rhodotorulic acid) and a trihydroxamic acid (DFO) [26], reveals much greater efficacy of the di- and trihydroxamic acids. The advantages and disadvantages of oligodentate over bidentate ligands as Fe chelators have been discussed [95,96]. Most siderophores are hexadentate ligands, attesting to the inherent advantages of hexadentate molecules as Fe chelators. The major advantage of a hexadentate ligand should be the necessity of only one Al atom and one chelator molecule to come together to form the preferred coordination number of Al. Formation of the 1:1 Al:chelator complex is therefore first-order dependent on the hexadentate chelator concentration, whereas formation of the 1:3 Al:chelator complex is third-order dependent on the bidentate chelator concentration. The effective concentration of a hexadentate chelator should be less than that of a bidentate chelator. Furthermore, the resultant 1:1 Al:hexadentate chelator complex may reduce Al toxicity compared to a 1:1 or 1:2 Al:bidentate chelator complex. Incompletely complexed metal, which provides a coordination site available for reaction with H_2O_2, may be Fenton reactive, contributing to side effects through hydroxyl radical catalyzed oxidative injury. The major disadvantage of hexadentate ligands is their larger molecular size that reduces their ability to diffuse through cell membranes. This is thought to limit oral bioavailabilty and blood-brain barrier permeation. The latter may or may not be a disadvantage. In vitro studies demonstrated greater efficacy of hexadentate than bidentate ligands to mobilize Fe from hepatocytes and human erythroleukemia K562 cells [97,98] and to protect against free radical damage [99]. A direct comparison of the ability of a bidentate HOPO to its hexadentate analog to enhance Fe excretion in iron-loaded rats supported the hypothesis that greater Fe chelation efficiency can be achieved with hexadentate ligands [94]. More than 90% of the eliminated Fe appeared in the bile. The ability of the same bidentate and hexadentate HOPOs to enhance Al excretion in Al-loaded rats was assessed (unpublished results). Oral and intravenous administration of the ligands increased biliary Al clearance. Intravenous administration of the hexadentate ligand increased urinary Al clearance and more efficiently increased biliary Al clearance than did the bidentate analog, supporting the hypothesis of the benefit of hexadentate ligands. However, oral administration of the bi- and hexadentate ligands and intravenous administration of the bidentate ligand decreased urinary Al clearance. As $>85\%$ of Al elimination was urinary this resulted in a net reduction of Al clearance. The Al:chelator complexes of both the bi- and hexadentate ligands were considerably more lipophilic than the free ligands, probably contributing to the enhanced biliary, but reduced urinary, Al clearance. There appears to be merit in hexadentate HPs as Al chelators but the design of agents that form hydrophilic complexes with Al may be necessary to generate orally-effective agents.

An approach to Al chelation that has not been extensively investigated is the addition of an adjuvant or second chelating agent to the therapy. An adjuvant might mobilize Al from storage sites as a complex from which the Al might be removed by the primary chelator for decorporation. Chelators may mobilize Al from different compartments. This approach may enhance the efficacy of a chelator that does not distribute out of the vascular compartment. Candidate adjuvants are fluoride, silicic acid and citrate. However, addition of 100 mg 2-mercaptoethane sulfonate kg^{-1} or 100 mg ascorbic acid kg^{-1} to oral 1 mmol deferiprone kg^{-1} did not enhance 6-h urinary Al excretion in Al-loaded rabbits over that seen with deferiprone alone [30]. Co-administration of oral deferiprone and subcutaneous DFO decreased brain Al in young Al-loaded rats to a greater extent than either treatment alone, but attenuated the deferiprone reduction of bone Al. Overall, this combination provided no advantage over single-chelator

treatment [37]. Similarly, co-administration of 100 or 200 mg kg^{-1} of DFO and deferiprone was not more effective than DFO alone [41]. When deferiprone and DFO were co-administered to uremic rats that had greater tissue Al concentrations than the rats in the study of [37], the combination more effectively reduced Al concentration in some tissues than produced by twice the dose of either DFO or deferiprone alone [38]. However, the combination less effectively enhanced urinary Al excretion than either agent alone. The results obtained to date do not suggest a benefit of the combined use of DFO and deferiprone.

7. Adverse effects of aluminum chelators

Studies in Al-loaded animals suggest chelators that are quite lipophilic have a greater potential for toxicity. Administration of *N*,*N*′-bis(hydroxybenzyl)ethylenediamine-*N*,*N*′-diacetic acid (HBED) was fatal to all Al-loaded rabbits studied although it was not fatal to non-Al-loaded rabbits [100]. The Al:HBED complex was the most lipophilic of the effective Al chelators assessed in that study. Of the eight 3,4-HPs studied, the most lipophilic produced seizures when given by intravenous injection to non-Al-loaded rabbits [86].

Due to the much more extensive use of DFO as an Fe than Al chelator, the understanding of its toxicity is primarily from its use in patients with Fe intoxication. The adverse effects of DFO, in relation to its use as an Al chelator, have been reviewed [101]. Increased susceptibility for infectious diseases, such as mucormycosis, may result from its ability to act as an Fe siderophore for pathogens. This may not occur, and has not been reported, in the treatment of Al intoxication. Ocular toxicity, including retinal abnormalities and cataracts, has usually been associated with long-term treatment or high doses, and has been suggested to be a product of chelation of an essential metal. With the movement toward lower-dose DFO therapy of Al accumulation and toxicity, adverse effects may be reduced. There are reports of the onset or exacerbation of encephalopathy and seizures during or within hours after dialysis in Al-loaded humans, which were attributed to both the mobilization of Al by DFO and to DFO. Gastrointestinal side effects were observed in some subjects during the 2-year study of DFO in AD patents [64].

The benefit-risk ratio of deferiprone has been much debated [3,53,101]. A comparison of its toxicity in normal and uremic rats suggested the no-observed-adverse-effect-levels were 20 and 40 mg kg^{-1} [83,102]. Compared to effective doses of 50–100 mg kg^{-1}, these results suggested that its use would not be advisable. Reversible agranulocytsis and neuropenia, arthralgias, joint pain, and musculoskeletal and joint pain, reversible nausea and vomiting, elevation of ALT and zinc deficiency have been observed in β-thalassemia patients treated for Fe intoxication [101,103]. The toxicity of deferiprone led Ciba–Geigy to withdraw it from further study. However, its clinical use and evaluation continued with the consideration that its use might be warranted in those who could not afford DFO. Further study revealed hepatic fibrosis [104]. The authors of this study concluded that it was neither safe nor effective as an Fe chelator in β-thalassemia patients. Deferiprone (Ferriprox®) is authorized for use in the treatment of Fe overload in thalassemia in the European Union, Switzerland, and Cyprus (personal communication, Fernando Tricta, Apotex Inc.).

There are no reports of studies assessing the potential toxicity of the 3,4-HPs in Al-intoxicated humans. As the majority of any given dose of chelators is not associated with metals in vivo, adverse effects due to the free chelator might be expected irrespective of the target metal of the chelation therapy. Tissue histopathology, blood biochemistry, hematology, and ophthalmoscopic examinations for cataracts did not reveal evidence of chelator-induced toxicity following 12 treatments of 3,4-HPs to Al-loaded rabbits [23]. However, Fe decreased in a number of tissues. Adrenal weights increased and testes weights decreased. These organ weight changes are consistent with results reported for deferiprone in non-metal-loaded rats ([105]; H.P. Schnebli, personal communication). The decrease in testes weight supports the suggestion that the 3,4-HPs have antiproliferative effects [105], which are thought to be due to Fe depletion from ribonucleotide reductase, consistent with the ability of the 3,4-HPs to distribute into cells [50,85].

The requirement for higher molar doses of the bidentate 3,4-HPs than hexadenate DFO to achieve Al and Fe chelation may contribute to their toxicity. It has been suggested that the toxicity of bidentate 3,4-HP chelators, such as deferiprone, is partly due to formation of 1:1 and 1:2, rather than 1:3 metal:deferiprone complexes and the possibility that this enables redistribution of a toxic species of the metal. If true, doses of the bidentate HPs that are sufficient to form the 1:3 complex may be necessary to minimize this adverse effect.

8. Application of chelators in non-clinical Al removal, concentration, detection and speciation

Chelators have been utilized as sorbents to remove Al from solution, to separate and concentrate Al to facilitate its detection, to determine its localization, and to speciate Al based on reaction kinetics. In some applications the Al:chelator complexes are dissociated to enable Al quantitation by atomic absorption spectrometry or related methods. In many methods, Al

quantitation is based on the spectrophotometric, fluorescent or similar property of the Al:chelator complex. There has been only limited application of immobilized DFO for this purpose [106]. 8-Hydroxyquinoline (oxine; 8-quinolinol) and derivatives have been extensively used to speciate Al in aqueous solutions. The methods have been reviewed [107] and modified [108]. The Al that rapidly reacts with 8-hydroxyquinoline is considered to be chemically reactive Al forms. This chelator has also been used to quantify Al, e.g. see [109]. 8-Hydroxyquinoline has a hydroxy and a tertiary nitrogen group, enabling formation of a five-membered chelate ring with Al. Chelators having hydroxy and azo groups that complex Al include 2,2′-dihydroxyazobenzene; Lumogallion; Acid Orange 7, 8 and 12; Acid Red 88; Eriochrome Blue SE; Eriochrome Blue Black R; and Mordant Red 19. They have been used as Al sorbents and in the fluorometric detection of Al. Catecholates including Alizarin Red S, Alizarin Violet N and 3-(3′,4′-dihydroxyphenylazo-1′)-1,2,4-triazole and chelators having two hydroxyl groups separated by a carbon, including chromotropic acid and its derivatives, have been used in similar applications. The aromatic hydroxy acid anion Chromazurol S has been used for Al quantitation. Compounds that have the α-hydroxyketone functional group can form an enol. They complex many metals. This family includes the 3,4-HPs and the HOPOs, which have been developed for clinical use, as well as the flavonols, such as 3-hydroxyflavone, morin and quercetin, that form fluorometric complexes with Al [110,111]. Flavonols have a maltol-like functional group. A method to separate and quantify the flavonols using HPLC and fluorescence based on formation of the Al:flavonol complex has been developed [110]. Iminodiacetic acid (Chelex 100) has been extensively used as an Al sorbent. Secondary and tertiary *N*,*N*′-diacetic acids also chelate Al. EDTA, HBED, xylenol orange and 3-carboxy-2-naphthylamine-*N*,*N*′-diacetic acid are examples of this family. It appears that Al forms a chelate ring with either the nitrogen or oxygen of these compounds. The stability constants of many of the Al:ligand complexes have been reported [2]. The functional moieties of these families of Al chelators are shown in Fig. 1.

9. Aluminum complexation in the environment and by plants

Aluminum solubility increases as the pH moves away from the nadir of Al solubility, which is ca. pH 6.2. In acidic water and soils, the Al ion is toxic to organisms and plants. The humic substances, which derive from plant material, are poly functional. Each molecule may contain a number of different complexing groups, such as catechol, quinone, phthalate and salicylate that react strongly with Al [112]. The major humic substances that occur naturally in water and soils are humic acids and fulvic acids. They represent the major fraction of dissolved organic compounds in freshwaters. In acidic waters and soils, humic acids are thought to generally reduce Al toxicity to plants and animals by reducing Al bioavailability of the Al ion. However, they may maintain Al in bioavailable species as Al:humic/fulvic acid complexes [112]. Therefore, they do not always reduce Al toxicity. Humic acid increased Al toxicity in a ciliated protozoa, perhaps due to enhanced phagocytosis of the Al:humic acid complex [113]. Fulvic acids are the lowest molecular weight humic acids. They are complex mixtures that have multiple Al binding sites described as structurally similar [114] and as two distinct classes of binding sites [115]. Dissociation kinetics show an increased rate of Al release from fulvic acid as the Al to fulvic acid ratio increases, suggesting less ability of fulvic acid to complex Al, and therefore prevent Al toxicity. Condensed tannins, which are polymeric phenolic compounds that occur in plants, complex Al at vicinal hydroxy groups.

The site of Al toxicity in plants is primarily root cells. The Al ion apparently inhibits cellular expansion and therefore root elongation, perhaps by displacement of calcium from negatively charged binding sites on the cell wall [5]. Some barley, maize, sorghum, snapbean and wheat cultivars are much more tolerant to Al than other cultivars. Two strategies that plants employ to achieve Al resistance are to exclude Al from the root tip and to tolerate the Al that does enter the plant. There are mechanisms for both of these strategies that utilize Al chelation. One mechanism of Al tolerance is Al-induced secretion by the root tip of the di- and tricarboxylic acids oxalic, malic, and citric acids, that complex the Al in the soil, forming chelates that are apparently less toxic, and perhaps less well taken up, than the Al ion [5,116]. Their structures are shown in Fig. 1. It has been hypothesized that phenolic compounds may be similarly released [117]. An additional mechanism to limit Al uptake appears to be secretion of phosphate by the root tip. Phosphate complexes Al and may increase the pH in the soil surrounding the root tip to reduce the Al ion concentration [118]. Some plants, such as tea, hydrangea and buckwheat, accumulate Al. For example, older tea leaves can contain as much as 3% Al on a dry weight basis [119]. This accumulation may involve a chelation-mediated detoxification mechanism. Most of the Al in tea leaves appears to be associated with organic complexes that have molecular weights >2000 Da [120], perhaps polyphenols. Al concentrates in the cell sap of hydrangea and buckwheat, complexed with citrate and oxalate, respectively [116].

10. Conclusions

Chelation therapy is useful in the treatment of renal impairment-associated Al accumulation and toxicity. It might be useful in the treatment of Alzheimer's disease and related disorders. Al chelation may have utility in determining the role of Al in neurological diseases. Although DFO is an effective Al chelator, its practical limitations encourage the discovery of orally effective alternatives. Some carboxylic acids have been suggested as alternatives to DFO, however, they can increase Al absorption and the Al body burden. The 3,4-HPs have Al-chelation efficacy that is comparable to DFO. They are orally effective. However, toxicity observed during their use as Fe chelators dampens enthusiasm for this family. As the bioavailability and efficacy of the 3,4-HPs is not influenced by their lipophilicity, perhaps quite hydrophilic HPs can be developed that might retain Al chelation activity but have less toxicity. The advantages of hexadentate ligands as Al chelators suggest development of hydrophilic hexadentate ligands might be worth pursuing. Chelators can be effectively used to enhance the removal, concentration, detection and speciation of Al. Al chelation occurs in natural environments, reducing Al bioavailability. Plants protect themselves from Al toxicity by releasing Al chelators.

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