The objectives of the present study were to determine the efficacy and toxicity of repeated oral administration of 3-hydroxypyridin-4-one (HP) chelators in a rabbit model of aluminum (Al) accumulation and toxicity, and the influence of chelator lipophilicity on these effects. Efficacy was assessed as chelator-induced Al mobilization and excretion and reversal of Al accumulation and Al-induced toxicity. Chelator-induced toxicity was assessed by multiple measures. Six HPs were given orally 12 times over 1 month to Al-loaded rabbits, which had significant elevation of Al in most tissues and evidence of Al-induced nephrotoxicity, osteomalacia, and anemia. Intravenous desferrioxamine (DFO), the current chelator of choice for the treatment of Al-overload and toxicity, was included as a positive control.

All six HPs and DFO demonstrated efficacy evidenced by significantly greater urinary and biliary Al elimination after the twelfth dose than seen in saline-treated controls. All of the HPs were more effective than DFO. Chelator-induced urinary Al excretion accounted for 58–98% of total (urinary plus biliary) Al excretion. Chelator-facilitated Al excretion was nearly complete within 12 hr, demonstrating a fairly short duration of action in rabbits with intact renal function.

HP treatments did not consistently affect tissue concentrations of Al or other metals. However, there was a trend toward chelator-induced reduction of Al-induced nephrotoxicity. The influence of HP lipophilicity was limited to a positive correlation between HP · Al lipophilicity and biliary Al output and a negative correlation between HP and HP · Al lipophilicity and reduction of Kupffer cell Al.

Little toxicity was evident after repeated oral HP dosing. Adrenal weight increased after treatment with several HPs. There was a decrease in testes weight after several HPs, which is consistent with an antiproliferative effect. More frequent dosing and/or a longer duration of HP treatment might produce greater reversal of the Al-induced toxicity and perhaps reveal more adverse effects than seen in this study.

There was a lack of profound toxicity during this short-term study. The 1,2-dimethyl (CP20) and 1,2-diethyl (CP94) HPs, which have been the most extensively studied HPs, were the least effective of the HPs examined. These results encourage the further investigation of other HPs as oral alternatives to DFO for the treatment of Al accumulation and toxicity.
Doses were selected to yield an AUC equivalent to an iv systemic dose of 450 μmol HP/kg. This dose was obtained by dividing 450 μmol/kg by the systemic bioavailability (17). The DFO dose was 150 μmol/kg. The dose of the HPs is based on the 3:1 HP·Al complex (43), whereas a 1:1 DFO·Al complex is formed.

### Materials and Methods

**Chelators.** The six HPs shown in table 1 were selected for study because they include two with a \( D_{o/a} < 0.2 \) and two with a \( D_{o/a} > 1.0 \). HPs were synthesized as hydrochloride salts, as described (19). Their purity was >99.5%, confirmed by HPLC, NMR, and elemental analysis. DFO was a gift of Ciba-Geigy (Basel, Switzerland). Solutions of HPs and DFO were prepared in saline immediately before dosing.

**Subjects.** Male New Zealand white rabbits, initially weighing 2.4 kg, were maintained in a AAALAC-accredited facility. They were fed ad libitum, except for 6 day, except for

- **Treatment Schedules and Sample Collections.** Beginning 7 or 10 days after the last Na or Al lactate injection, Na lactate-injected rabbits received 0.9% normal saline and the Al lactate-injected rabbits received saline, DFO, or one of the HPs by an 8 French pediatric feeding tube, with the exception of DFO, which was given intravenously through a sterilizing (0.22 μm) filter. These treatments were administered 3 times weekly for 4 weeks, in a volume of 1 ml/kg.

An ophthalmoscopic examination was performed weekly to determine the presence of cataracts. Blood samples were drawn for biochemistry assays, and 24 hr after the twelfth treatment. Blood was collected in a syringe containing 0.2 ml Na4 EDTA (100 mg/ml), from which plasma was obtained.

Blood samples were drawn for biochemistry assays, and 24 hr after the twelfth treatment. Blood was collected in a syringe containing 0.2 ml Na4 EDTA (100 mg/ml), from which plasma was obtained. At these same time points, with the exception of the last sample that was collected before the twelfth treatment, a 1-ml blood sample was drawn into a syringe containing 0.1 ml heparin (1000 units/ml) for quantitation of Al in plasma.

Eight and 2 days before the twelfth treatment, the rabbits’ bones were calcine-labeled for histomorphometric analysis. Calcine (30 mg/kg in saline at pH 7.3) was given intravenously over 30 min. One day before the twelfth treatment, a femoral vein and the bile duct were surgically cannulated to enable periodic blood sampling and quantitative bile collection, as described (18). An iv infusion of 2.5% dextrose in 1/2 normal saline (25 ml/hr) was maintained from the time of surgery until euthanasia.

Two hours before the twelfth treatment, the rabbit was placed in a Nalgene rabbit restraining cage in which it was housed for 26 hr to enable repeated sample collection. Urine, bile, and blood were collected from 2 hr before to 24 hr after treatment, as previously described (18), with the exception that bile and urine were collected from 4 to 5 and from 5 to 6 hr after treatment. Bile and urine were collected to determine the time course and extent of chelator-facilitated Al excretion. Blood was collected to determine if the HPs chelated vascular or extravascular Al. An aliquot of each bile, urine, and serum sample was stored frozen for Al analysis. Three hours after the twelfth treatment, rabbits were orally administered a slurry containing 10 g homogenized cabbages in 30 ml of bile salt substitute (18, 20) to replace bile salts lost due to bile collection and to encourage bile production. The rabbit was euthanized 24 hr after the twelfth treatment, ~40 days after the last Al or Na lactate injection. Evaluation of gross pathology was performed immediately. The organs, tissues, and fluids listed in table 2 were harvested. A sample of each tissue other than the liver was preserved in 10% neutral-buffered formalin for evaluation of histological changes. One liver was preserved in 100% ethanol for histomorphometry. An additional sample of selected tissues (table 2) was stored frozen for multielemental analysis. GFAP was measured in three brain regions; these results will be reported elsewhere.\(^2\)

**AI and Multielemental Analysis.** Al analysis was conducted by ETAAS. Aliquots of serum, plasma, and bile were diluted at least 10-fold (with a solution of 2 mM Mg in 0.2% HNO\(_3\)) to bring the Al concentration within the range of the Al standards. They were analyzed by ETAAS by comparison with Al standards in the same matrix. Samples of CSF were compared with Al

\(^2\) R. A. Yokel and J. P. O’Callaghan, submitted for publication.
of 2000×. All parameters comply with the nomenclature and were calculated according to the Histomorphometry Nomenclature Committee of the American Society of Bone and Mineral Research (27).

**Data Analysis.** The volume of each bile and urine sample was determined from the weight of the total sample divided by the density of an aliquot. Bilary and urinary Al outputs during each sample collection period were calculated from Al concentration × sample volume. These were divided by the time period of collection to obtain Al output rates, which were then normalized to body weight. Chelator efficiency was calculated as total Al output (cumulative urinary and biliary Al excretion over 24-h posttreatment) in moles of Al/mg of chelator/kg body weight, as described (28, 29), minus the response to saline treatment of Al-loaded rabbits. The result was multiplied by 100%.

Microscopic evaluation of renal histology revealed microaneurysms, focal sclerosis, and Al deposition in some glomerular tufts. These changes were recorded as absent or present. Interstitial fibrosis/cystic renal tubule was observed in some rabbits and was noted as absent (0), or present and ranked on a scale of 1–3. Al presence in macrophages in stained sections of liver (Kupffer cells), bone marrow, and spleen was ranked as absent (0) or as 1–3 (for extent of deposition).

**Statistics.** Statistically significant chelator-induced differences in bile, urine, and total Al output; organ weights (normalized to body weight); and bone histomorphometric parameters were assessed with one-way ANOVAs. Significance was accepted at the p < 0.05 level for all statistical comparisons in this study, corrected for the number of tests conducted. A p < 0.0055 or 0.0033 was accepted for the comparisons among the 9 organs or the 15 histomorphometric measurements, respectively. Duncan’s test was conducted if the ANOVA was significant.

To test for statistically significant effects of Al loading on body weight and on hematological and blood biochemistry measures, two-way mixed ANOVAs were conducted to compare values obtained before, to those obtained after, Na lactate or Al lactate injections. To test for statistically significant effects of the chelators, two-way mixed ANOVAs were conducted to determine differences in body weight and hematological and blood biochemistry measures by comparing values obtained before, to those obtained after, the chelator treatments. To test for statistically significant effects of the chelators on Al and five essential metals, differences in tissue concentrations were examined using mixed ANOVAs across the nine tissues. Significance was accepted at p < 0.0002 for the 25 biochemistry assays and hematological evaluations and <0.0009 for comparisons of the six elements in each of nine tissues. A multiple comparison test was conducted when the ANOVA was significant.

Results of the microscopic evaluation that were noted as absent or present were statistically compared by Fisher’s exact tests of the Al-saline group vs. the Na lactate-saline group and each of the seven Al-chelator groups. Results of the microscopic evaluation that were ranked from 0 to 3 were statistically compared with sign rank tests. Significance for these tests was accepted at p < 0.017 for the three Fisher’s exact tests and p < 0.0125 for the four sign rank tests.

Significant relationships between lipophilicity of the HPs, and the HP · Al complexes, and the ability of the HPs to facilitate Al excretion (biliary, urinary, total Al output, and biliary Al output as a percentage of total output) were determined by correlation analyses. Similar analyses were conducted with the extent of microaneurysm, focal sclerosis, and interstitial fibrosis/cystic renal tubule in the kidney and Al in the glomerular tufts and macrophages of the bone marrow, liver, and spleen. Analyses were conducted between the D<sub>20</sub> log D<sub>450</sub> D<sub>20</sub>/D<sub>450</sub> and D<sub>20</sub> vs. mean Al output after each HP, to test for linear and nonlinear relationships. The significance of each correlation was determined by a t test. The accepted p was <0.0015 for Al output vs. lipophilicity and <0.0009 for the morphometric changes and subcellular Al localization.

The p values reported are uncorrected. Interpretation of significance is based on the Bonferroni-corrected p values, previously described, when correction for multiple comparisons was appropriate. Due to the conservative nature of this statistical approach, we have also reported values that are not significant by this criteria, but would be in the absence of the Bonferroni correction.

**Results**

**Al Effects.** The Al injections produced Al accumulation that persisted for ~40 days and some toxicity. There was significantly more
Al concentration is shown as μg/g dry weight, except for CSF that is expressed as ng/ml. Values are mean ± SE. Values not shown in control group were <1 μg Al/g. Asterisk indicates significant difference from Al-vehicle treatment. Results from kidney cortex were very similar to those shown for kidney medulla.

Al in all tissues [F(7,482) = 10.92, p < 0.0001], except brain and CSF, of Al-loaded rabbits. Tissue Al concentrations are shown in fig. 1. Urinary Al excretion by Al-loaded rabbits averaged 0.8 μmol/kg in the 24 hr after the twelfth saline injection (given ~5 weeks after completion of Al loading), whereas Na lactate-injected rabbits did not excrete measurable amounts of Al in their urine. Cu was significantly decreased [F(5,54) = 9.99, p < 0.0001] in the adrenal, liver, and renal medulla of Al-loaded rabbits. Al loading did not result in any significant changes in Fe levels.

Macroscopic pathology revealed that the kidneys were pale in some Al-loaded, but not in the Na lactate-injected rabbits. The spleen weighed less in all Al-loaded, saline and chelator-treated groups, than in Na lactate-injected rabbits [F(8,61) = 19.82, p < 0.0001].

Histological examination showed Al accumulation in the Kupffer cells and in the macrophages of bone marrow and spleen of Al-loaded rabbits (for all three tests, p < 0.00058). There were several abnormalities found at a greater but nonsignificant level in the kidneys of Al-saline than in Na lactate-saline rabbits. These included microaneurysms (p < 0.019), focal sclerosis (p < 0.019), and Al deposition in the glomerular tufts (p < 0.052), and interstitial fibrosis in the kidney (p < 0.014).

Bone histomorphometric analysis showed that Al-loaded rabbits had significant decreases in osteoid maturation time (p < 0.0004), erosion depth (p < 0.0002), and osteoid thickness (p < 0.0034), compared with Na lactate-injected rabbits. Bone surface Al was ≥2.5% of total bone surface in Al-loaded rabbits, a nonsignificant increase above non–Al-loaded rabbits.

There were no significant effects of Al loading on plasma biochemistry values. Hemoglobin was lower in Al-loaded rabbits [F(1,89) = 4.67, p < 0.033].

**Chelator Effects.** Nine Al-loaded rabbits died during treatment (N = 1 during saline and CP40, N = 2 during CP20, and N = 5 during CP24). Although none of the deaths could be directly attributed to the treatments, some of the CP24-treated rabbits demonstrated poor health, very severe bronchopneumonia and a diffuse subcapsular hepatocytic necrosis involving the entire liver, and hyperactivity that might have contributed to their death. In contrast to all other chelator-treated rabbits, CP24-treated rabbits lost weight during treatment.

Each of the chelators significantly increased total Al elimination, when compared with saline treatment. Urinary [F(7,48) = 12.37, p < 0.0001], biliary [F(8,52) = 7.35, p < 0.0001], and total Al outputs [F(7,47) = 16.39, p < 0.0001] during the 24 hr after each of the treatments are shown in fig. 2. Al output ranged from 237% of Al-saline–treated animals after DFO to 545% after CP52. Calculated efficiencies of the HP chelators ranged from 1.2% for CP20 to 2.5% for CP52, whereas efficiency was 0.8% for DFO. Total Al output was significantly greater after CP40 and CP52 than after DFO, CP20, CP93, and CP94, and was significantly greater after CP24 than DFO, CP20, and CP94. Each chelator significantly increased Al output into urine, compared with Al-saline–treated animals. Urinary Al output was significantly greater after CP52 and CP40 than after the four other HPs and DFO. Only CP24 and CP52 significantly increased biliary Al output, compared with Al-saline–treated animals. The increase with CP24 was significantly greater than with CP52.

Measured biliary Al output and biliary Al output expressed as a percentage of total Al output showed a positive correlation with HP lipophilicity that was significant for both of these biliary output.
measures vs. the $D_{600}$ of the HP·Al complex at $p < 0.005$. Urinary and biliary Al outputs over time are shown in fig. 3. Al output peaked within the first 6 hr after chelator treatment. Most Al excretion occurred within 12 hr.

Plots of serum Al concentration in the 24 hr after the twelfth treatment as a percentage of serum Al before the twelfth treatment are shown in fig. 4. Serum Al concentration transiently increased after some treatments (DFO, CP20, and CP94) then decreased below pretreatment concentrations before returning to or exceeding the pretreatment concentration.

There were no significant effects of chelators on blood biochemistry and hematology values.

Atomic absorption analysis showed some significant changes in Al ($F(8,482) = 10.92, p < 0.00001$), compared with saline treatment (as shown in fig. 1). Spleen Al content, calculated from spleen Al concentration × spleen weight, after DFO or HP treatment, was significantly greater for CP40 and CP94, compared with vehicle treatment.

Multielemental analysis showed that Cu increased after DFO treatment in the liver ($F(8,482) = 3.10, p < 0.00001$), thus reversing the Al effect previously noted. Cu increased in muscle and lung after CP94 and in the spleen after CP20 and CP93. After the repeated chelator treatments, iron was significantly reduced in a number of tissues ($F(72,474) = 1.79, p < 0.0002$); in the adrenal after all chelators except CP94; in the liver after CP20, CP24, CP52, and CP93; in the spleen after CP40; and in the heart after CP94.

Ophthalmoscopic examinations showed no evidence of cataracts in any subject. Adrenal weight was not significantly different in Al-loaded vs. Na lactate-injected rabbits, but was significantly greater in CP24-, CP40-, CP52-, and CP93-treated rabbits than in saline-treated rabbits ($F(8,62) = 4.98, p < 0.0001$). The adrenal weight from CP24-treated rabbits was also greater than in DFO-, CP20-, CP93-, and CP94-treated rabbits. There were decreases in testes weight after CP40, CP93, and CP24 ($F(8,62) = 2.96, p < 0.0071$). An increase in lung weight followed CP52 ($p < 0.032$) vs. Al-saline rabbits.

Histopathology examination suggested that bone marrow Al was significantly reduced after CP24 treatment ($p < 0.0001$). The more hydrophilic HPs (CP40, CP20, CP93, and CP52) reduced Kupffer cell Al, whereas CP94 and CP24 did not; thus creating a negative correlation between reduction of Kupffer cell Al and both free HP and HP·Al lipophilicity ($p < 0.05$). The Al-induced renal interstitial fibrosis was significantly decreased by CP24 ($p < 0.00067$). There were also several nonsignificant effects of chelators on the Al-induced kidney abnormalities. These were the absence of Al-induced focal sclerosis in CP24-treated rabbits and a decrease of this focal sclerosis after all chelators except CP94; reduced Al deposition in the glomerular tufts after CP52 and CP93; and fewer microaneurysms after CP40, CP93, CP52, and CP94. In addition to these nonsignificant trends, there was also a trend for all chelators except CP94 to decrease bone marrow Al and all chelators except CP20 to increase spleen Al. There was no histopathology evidence for chelator-induced toxicity in any organ.

**Discussion**

**AI Effects.** The presence of lesions in the kidney supports previous reports of Al-induced nephrotoxicity (18, 24, 30). In the present study, the glomerular tuft and renal tubule were the primary sites of renal

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**Fig. 3.** Rate of urinary (left) and biliary (right) Al output over time. Values are group mean rates from each sample collection interval minus the mean from the Al-saline rabbits during the same interval. HPs are graphed from top to bottom in order of increasing lipophilicity.
The Al body burden at the time of the twelfth treatment was estimated Al loading (18), presumably reflecting the lower body burden of Al elimination after saline treatment was 30% of that seen 1 week after saline treatment. At that time, baseline Al was probably due to the relatively short duration of Al loading of 6 weeks after completion of Al loading. The decrease in these young adult rabbits with normal renal function. The decrease in damage. The location of Al in the Kupffer cells is consistent with the first-pass accumulation of Al by the liver (31), and the liver as a site of considerable Al accumulation in this model (fig. 1). The decreases in bone osteoid thickness, osteoid maturation time, and erosion depth are consistent with Al-induced osteomalacia (32). However, the lack of significance of other measures consistent with Al-induced bone disease, the small increase in surface bone Al [2.5% compared with 30% in humans with Al-induced bone disease (33)] and the small increase in bone Al (fig. 1) in Al-loaded rabbits suggests minimal production of Al bone effects. The lack of profound Al-induced bone effects prevented evaluation of the ability of the chelators to influence Al-induced bone disease. The lack of profound Al-induced bone effects was probably due to the relatively short duration of Al loading of these young adult rabbits with normal renal function. The decrease in hemoglobin is consistent with Al-induced anemia.

**Chelator Effects.** The twelfth treatment in this study was given 5 weeks after completion of Al loading. At that time, baseline Al elimination after saline treatment was 30% of that seen 1 week after Al loading (18), presumably reflecting the lower body burden of Al due to Al clearance over the four additional weeks after Al loading. The Al body burden at the time of the twelfth treatment was estimated to be 4250 μg/kg, based on the total Al content of organs/tissues listed in table 2 in which Al was determined. This was calculated from organ/tissue Al concentration × their weights [assuming bone and muscle represent 3.9 and 49% of body weight, as found for the rat (34)]. Total Al output after CP52, the most effective chelator, minus that after saline, was 2.5% of the estimated Al body burden. All treatments increased total Al output, when calculated as a percentage of saline treatment, as effectively after the twelfth oral dose as after the single iv dose given to rabbits 1 week after the completion of Al loading. Al output after the twelfth chelator treatment ranged from 237% of saline treatment for DFO to 545% for CP52 in the present study, whereas it ranged from 212% of saline treatment for DFO to 456% for CP24 after a single iv dose (18). Therefore, when given orally in doses selected to produce an area under the curve comparable with an iv dose of 450 μmol/kg, the HP chelators were as effective after the twelfth oral dose as the single iv dose.

The present study was conducted in rabbits with intact renal function, which had their bile ducts cannulated before the twelfth treatment. The efficiency of Al chelation and the profile of urinary and biliary Al elimination may be different in renally impaired subjects, due to reduction of urinary clearance of the HPs and the HP ∙ Al complexes; and in non-bile duct-cannulated subjects, if there is significant enterohepatic cycling of the HPs or the HP ∙ Al complexes. The temporal profile of Al elimination (fig. 3) is consistent with the fairly rapid absorption (mean absorption times = 0.5–1.5 hr) and elimination [mean residence times = (0.4–2.6 hr)] of these HPs (17).

Changes in serum Al after HP dosing suggest the entrance of these chelators into systemic circulation in the Al-loaded mammal, supporting similar observations in the nonmetal-loaded human (35, 36) and rabbit (17) and in the Al-loaded rabbit after iv dosing (18). The initial increase in serum Al after CP94 and CP20 demonstrates Al mobilization from erythrocytes or extravascular sites. A more pronounced increase in serum Al was seen after the iv dosing of these HPs (18), perhaps due to a greater body burden of Al in those rabbits, previously discussed, and/or due to the higher HP concentrations achieved after iv, rather than oral, dosing. Serum Al decreased below pretreatment concentrations after treatment with each of the chelators. This suggests DFO and HP redistribution of Al out of serum, presumably resulting in Al excretion. Some of the serum Al had to be mobilized from transferrin, which binds >80% of serum Al (37), to account for a >20% reduction of serum Al. The present results do not support the concern that oral Al chelators might produce a net increase in systemic Al due to facilitated Al absorption. Reduction in serum Al argues against chelator-facilitated Al absorption from the gastrointestinal tract as the sole source of the Al eliminated in the bile and urine, because chelator-induced Al absorption would probably increase, and certainly not decrease, serum Al.

One extravascular site of Al chelation by the less lipophilic HPs was presumably reticuloendothelial cells, including the Kupffer cell, as shown by the reduction of Kupffer cell Al by CP40, CP20, CP93, and CP52, but not CP94 and CP24. Chelation of this Al presumably resulted in urinary Al excretion, as shown with Fe mobilized from Kupffer cells (38), whereas chelation of Al from hepatocytes and other parenchymal cells by lipophilic HPs presumably resulted in biliary Al excretion (38). The ability of the HPs, except CP94, to decrease bone marrow Al is consistent with chelation of extravascular Al. HP reduction of Al in bone marrow would be expected to improve Al-induced anemia, because bone marrow Al has been shown to be associated with this anemia (39, 40).

We also have evidence of HP reduction of Al-induced neurotoxicity. The concentration of GFAP, a marker of neurotoxicity, was determined in the frontal cortex, hippocampus, and cerebellum of these rabbits. Frontal cortical GFAP was significantly increased in Al-loaded rabbits, suggesting Al-induced neurotoxicity. Frontal cortical GFAP and Al concentrations positively correlated, supporting...
this conclusion. GFAP was significantly reduced by CP93, CP52, and CP24, thus suggesting abrogation of the Al-induced toxicity. HPs that decreased GFAP were generally the more lipophilic HPs tested.

The nonsignificant reduction of Al in many tissues after 12 treatments with DFO and the HPs may be due to elimination of ≤2.5% of the total Al body burden with each treatment. This suggests that treatment would have to be continued for a much longer period of time, or be given more frequently, to deplete the substantial Al accumulation significantly. Clinical studies using DFO to treat Al-induced toxicity were continued for months to years (reviewed in ref. 41).

The relatively short duration of action of the HPs, and the rapid elimination of the HP · Al complex, as previously shown in the rat (42) and shown in this study by the completion of most Al elimination within 12 hr and return to pretreatment serum Al concentrations, suggests dosing more than once daily may be beneficial in renally intact subjects. However, the presence of renal impairment may prolong the duration of effect and elimination of the HP · Al complex, thus reducing the potential benefit of more frequent dosing. Further study of the HPs in renally impaired subjects is needed.

Histology results suggest that chelator-induced reduction in local Al concentration in the glomerular tuft may have contributed toward the decreases in Al-induced microaneurysm and sclerosis. Increases in Al concentration in muscle after CP52 and in lung and liver after CP93 and the increase in spleen Al content after CP40 and CP94 suggest that Al may have redistributed after treatment by these chelators. An increase in spleen Al concentration was also reported after intraperitoneal DFO and oral CP20 and CP94 treatment of Al-loaded rats (43). The redistribution of Al to the lung, liver, and spleen may reduce the toxic potential of the Al to bone and brain, the primary target organs of Al toxicity. The decrease in Fe in some tissues after HP treatment is consistent with the greater affinity of the HPs for Fe than for Al (44). Presumably, the HPs were chelating the Fe in these tissues and causing its excretion as has been reported after CP20 administration (45). Iron excretion during HP therapy to reduce Al accumulation has the potential to produce Fe deficiency. Although this has been a concern with DFO treatment of Al accumulation disorders, it has not proven to be a significant clinical problem because Fe supplements are often given (46). The lack of significant effect of the HPs on tissue concentrations of most metals is consistent with the weak association between the HPs and Ca and Mg, and only moderate association with Zn, compared with the stronger association with Fe, Cu and Al (44). It is also consistent with the lack of increased urinary excretion of Cu, Zn, Mg or Ca after CP20 (45).

The increase in adrenal weight and decrease in testes weight after several HPs is consistent with results reported for CP20 in non-metal-loaded rats (47). The decrease in testes weight supports the suggestion that the HPs have an antiproliferative effect (47). The antiproliferative effect of DFO, and presumably the HPs, is thought to be due to Fe depletion from ribonucleotide reductase, consistent with intracellularity. HP distribution (10).

Although there are reports that HPs cause a decrease in white cell counts (48, 49), no HP-induced blood cell toxicity was evident in this study. This finding is consistent with the unchanged blood cell profiles seen in Fe-loaded primates after CP20, CP94, or DFO (28) and unchanged blood cell counts in Al-loaded rats after CP20 and DFO (14). This lack of blood cell toxicity may be due to the short duration of treatment. The extent of HP-induced blood cell toxicity is a major topic of current clinical investigation and warrants investigation in future long-term animal studies.

Under the short-term oral dosing regimen used, no profound toxicity was observed that could be directly attributed to the HPs. Overall, the results from this study reveal the ability of the HPs to increase Al excretion after repeated oral administration. This demonstrates their continued effectiveness with repeated dosing, as has been seen with the ability of CP20 to promote Fe elimination (50). All six HPs studied were more effective than DFO. CP20 and CP94, the most widely studied HPs, were the least effective of the six HPs in this study, suggesting that other HPs be given greater consideration as oral alternatives to DFO for treatment of Al, and perhaps Fe, accumulation disorders. The more lipophilic HPs more effectively reduced Al-induced neurotoxicity than the hydrophilic HPs (CP40 and CP20).

The latter also increased CSF Al. Lipophilic HPs may be preferable in the treatment of Al accumulation and toxicity in the nervous system. The significant biliary Al excretion following some of the more lipophilic HPs would favor their use in patients lacking renal function, providing a route of Al elimination not seen with DFO. However, CP24 may be too toxic, suggested by its ability to produce seizures after iv, but not oral, dosing (17, 18) and the higher incidence of death and the weight loss seen in the present study than with other HPs. A longer term and/or more aggressive dosing study would better reveal toxic effects of the HPs. Increased HP lipophilicity did not produce greater total Al elimination, due to less urinary excretion after lipophilic HPs. Efficacy of CP40 illustrates the ability of very hydrophilic HPs to chelate and decoporate Al, suggesting that long-term reduction of Al body burden can be achieved with quite hydrophilic HPs, perhaps with less potential for toxicity than lipophilic HPs. The predominance of urinary Al excretion produced by the hydrophilic HPs suggests their use in subjects with renal function. The HP · Al complex seems to be cleared by dialysis (15). Further investigation of HPs as Al chelators should focus on the efficacy/safety profile of individual agents, and the specific endpoints of Al accumulation and toxicity that are to be treated, such as reduction of Al and Al-induced toxicity in brain and bone.

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