Aluminum (Al) is a toxic, nonessential metal. The accumulation of Al is most pronounced in the renally impaired human, but it can occur in those exposed to Al from occupational and numerous iatrogenic sources. Its toxicity is pronounced in the renally impaired human, but it can occur in those exposed to Al from occupational and numerous iatrogenic sources. The incidence of 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 Al 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.

Table 1

<table>
<thead>
<tr>
<th>Treatment</th>
<th>( R_1 )</th>
<th>( R_2 )</th>
<th>Dose ( ^{a,b} ) Chelator·Al Complex</th>
<th>AUC ( ^c )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Al-loaded rabbits</td>
<td>( -\text{CH}_3\text{OH} )</td>
<td>(-\text{CH}_3)</td>
<td>0.094</td>
<td>126.7</td>
</tr>
<tr>
<td>CP40 (N = 6) ( ^d )</td>
<td>(-\text{CH}_3)</td>
<td>All in aqueous</td>
<td>All in aqueous</td>
<td>126.7</td>
</tr>
<tr>
<td>CP20 (N = 7)</td>
<td>(-\text{CH}_3)</td>
<td>(-\text{CH}_3)</td>
<td>0.28</td>
<td>152.3</td>
</tr>
<tr>
<td>CP93 (N = 8)</td>
<td>(-\text{CH}_3)</td>
<td>(-\text{CH}_3)</td>
<td>0.68</td>
<td>371.3</td>
</tr>
<tr>
<td>CP52 (N = 8)</td>
<td>(-\text{CH}_3)</td>
<td>(-\text{CH}_3)</td>
<td>2.1</td>
<td>147.7</td>
</tr>
<tr>
<td>CP94 (N = 8)</td>
<td>(-\text{CH}_3)</td>
<td>(-\text{CH}_3)</td>
<td>1.5</td>
<td>217.6</td>
</tr>
<tr>
<td>CP24 (N = 3)</td>
<td>(-\text{CH}_3)</td>
<td>All in aqueous</td>
<td>All in aqueous</td>
<td>126.7</td>
</tr>
<tr>
<td>DFO (N = 9)</td>
<td>(-\text{CH}_3)</td>
<td>All in aqueous</td>
<td>All in aqueous</td>
<td>126.7</td>
</tr>
<tr>
<td>Saline (N = 8) (the Al lactate-saline group)</td>
<td>(-\text{CH}_3)</td>
<td>All in aqueous</td>
<td>All in aqueous</td>
<td>126.7</td>
</tr>
</tbody>
</table>

Non–Al-loaded rabbits

| Saline (N = 8) (the Na lactate-saline group) | \(-\text{CH}_3\) | All in aqueous | All in aqueous | 126.7 | 1.26 |

\( ^a \) Code numbers, synonyms, and Chemical Abstract Service (CAS) Registry numbers are as follows: CP40, EL1NEt2 (OH); CP20, L1 HP4A, Hdp, DMPH, CP37 391, deferiprone, CAS Reg. No. 30652-11-0; CP93, EL1; CP52, L1NEtOPr; CP94, EL1NEt; CP24, Hdp.

\( ^b \) Dose AUC. Lipophilicity of the free chelator and its complex with Al, determined as the equilibrium distribution coefficient between n-octanol and an aqueous phase at pH 7.4 (from ref. 51).

\( ^c \) AUC. Area under the concentration vs. time curve after iv dosing of 450 μmol HP/kg (from ref. 17).

\( ^d \) Number of rabbits studied.

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 weighed 3 times weekly. Food and water were provided, except for 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 calcein-labeled for histomorphometric analysis. Calcein (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 ½ 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 24 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 cabbage 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 tibia was preserved in 10% neutral-buffered formalin for evaluation of histological changes. One tibia was saved 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.

Al 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% HNO₃) 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 standards in the same matrix. Samples of CSF were compared with Al standards in the same matrix.
TABLE 2

<table>
<thead>
<tr>
<th>Organs, tissues, and fluids collected at necropsy</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adrenal\textsuperscript{a}</td>
</tr>
<tr>
<td>Brain\textsuperscript{a,b}</td>
</tr>
<tr>
<td>Bone (tibia)\textsuperscript{b}</td>
</tr>
<tr>
<td>Bone marrow</td>
</tr>
<tr>
<td>Cecum</td>
</tr>
<tr>
<td>CSF</td>
</tr>
<tr>
<td>Colon</td>
</tr>
<tr>
<td>Eyeball</td>
</tr>
<tr>
<td>Gallbladder</td>
</tr>
<tr>
<td>Heart\textsuperscript{a} (left ventricle)</td>
</tr>
<tr>
<td>Intestine</td>
</tr>
<tr>
<td>Kidney\textsuperscript{a,b}</td>
</tr>
<tr>
<td>Liver\textsuperscript{a} (right central lobe)</td>
</tr>
<tr>
<td>Lung\textsuperscript{a} (right lobe)</td>
</tr>
<tr>
<td>Lymph node</td>
</tr>
<tr>
<td>Muscle (tibialis anterior)\textsuperscript{b}</td>
</tr>
<tr>
<td>Pancreas</td>
</tr>
<tr>
<td>Spinal cord</td>
</tr>
<tr>
<td>Spleen\textsuperscript{a,b}</td>
</tr>
<tr>
<td>Stomach</td>
</tr>
<tr>
<td>Testes\textsuperscript{a}</td>
</tr>
<tr>
<td>Urinary bladder</td>
</tr>
</tbody>
</table>

\textsuperscript{a} Organs weighed at the time of necropsy.
\textsuperscript{b} Frozen for later multielemental and Al analyses.

standards prepared in an artificial CSF (21). An aliquot of each urine sample was acid-digested in 0.5 ml HNO\textsubscript{3}, H\textsubscript{2}O\textsubscript{2} (70:30) solution in a threaded Teflon vial (22), dried, reconstituted with the aforementioned Mg in HNO\textsubscript{3} solution, and analyzed compared with aqueous Al standards prepared in the same solution. Approximately 250 mg of each tissue selected for multielemental analysis was dried to constant weight, acid-digested as described for urine, and diluted with distilled/deionized (milli-Q) water to a final volume of 10 ml. An aliquot of the reconstituted tissue samples underwent multielemental (Al, Ca, Cu, Fe, Mg, Ni, and Zn) analysis. This was conducted using an ARL Direct Current Plasma Emission Spectrometer. Ni concentrations in the samples were too low to be quantitated by this method. Al was below the limit of detection in brain and CSF, and occasionally in other tissues, particularly those from Na lactate-injected rabbits. Therefore, a second aliquot of each sample was diluted with the Mg in HNO\textsubscript{3} solution and was analyzed for Al by ETAAS by comparison with aqueous standards prepared in the same solution. Standards curves were run before and after every 10 samples. An aliquot of each sample was analyzed twice by ETAAS. The sample was further analyzed when the ANOVA was significant for the comparisons among the 9 organs or the 15 histomorphometric measurements, respectively.

Histological Assessment of Soft Tissue. Selected tissues were sectioned 5-µm thick and stained with hematoxylin and eosin for evaluation under light microscopy. Grayscale-blue-stained material was interpreted as a tissue Al deposit (23). Staining with solachrome azurine (24) and laser microprobe MS was analyzed twice by ETAAS. The sample was further analyzed when the Al injections produced Al accumulation that persisted for ~40 days and some toxicity. There was significantly more

Histochemistry of bone was conducted between the Al-CP20, Al-CP94, Al-DFO, Al lactate-saline, and Na lactate-saline groups. These groups were chosen for initial evaluation, because they have been the most extensively studied HPs (CP20 and CP94), the current chelator of choice (DFO), and the controls. Bone samples were fixed in 100% ethanol, dehydrated, and embedded in methyl-methacrylate as previously described (26). Histomorphometry was conducted at a standard site, 1.5 mm below the growth plate. Six serial sections, 4 and 7 µm thick, were cut with a Microm, model HM360 microtome (Carl Zeiss, Thornwood, NY). The 4-µm-thick sections were stained with a modified Masson-Goldner Trichrome stain (26). The 7-µm-thick unstained sections were prepared for fluorescent light microscopy for analysis of calcein labeling.
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.

Macroscopic pathology revealed that the kidneys were pale in some Al-loaded rabbits, 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(7,48) = 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 emphysema (for all three tests, p < 0.0001 during saline and CP40, p < 0.0001 during CP20, and p < 0.0001 during CP24).

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, CP21, 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 CP25 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 $D_{a,b}$ 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

Al 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
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.² 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 intra-cellular 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 decouple 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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8. J. Fish


