0148-6071/87/1103-0293$02.00/0
JOURNAL OF PARENTERAL AND ENTERAL NUTRITION

Vol. 11, No. 3
Printed in U.S.A.

Hepatic Abnormalities Associated with Aluminum Loading in Piglets

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ABSTRACT. Cholestasis is a common complication of total parenteral nutrition (TPN) in infants. A contributing factor to the hepatic dysfunction may be a contaminant of the TPN solution, such as aluminum, that accumulates in liver and may act as a hepatotoxin. To study the hepatic effects of aluminum, growing piglets were given daily intravenous injections of aluminum, 1.5 mg/kg, for 50 days; pair-fed controls were given heparinized saline. At sacrifice, liver and serum were obtained. Liver was analyzed for histopathology and for aluminum content and localization. The hepatocyte lysosomes of the experimental group showed aluminum peaks by x-ray microanalysis, whereas the control group did not. No differences in ultrastructure were noted between the two groups when examined by electron microscopy. Mean serum total bile acid levels (27.8 ± 15.9 SD *vs* 6.3 ± 1.5 μmol/liter, $p < 0.05$), mean alkaline phosphatase (309 ± 108 *vs* 180 ± 27 IU/liter, p = NS), and mean hepatic copper content (24.8 ± 4.5 *vs* 14.4 ± μg/g dry weight, $p < 0.01$), were elevated in the aluminum-loaded piglets, indicating that cholestasis may have been produced. Also, a small but significant reduction in serum levels of 25 hydroxyvitamin D was found in the aluminum-loaded piglets, suggesting that vitamin D hydroxylation may be impaired. Inasmuch as lysosomal contents are excreted into the bile, aluminum accumulation in lysosomes may alter lysosomal function and possibly affect bile flow or content. (*Journal of Parenteral and Enteral Nutrition* **11**:293–297, 1987)

Cholestasis and progressive liver disease are common complications of treatment with prolonged total parenteral nutrition (TPN), especially in infants and young children.[1–4] The etiology of the hepatic dysfunction is unclear and may be multifactorial. One possibility is that a component of TPN solution may act as a hepatotoxin.[1]

Aluminum has recently been identified as a contaminant of TPN solutions.[5] It was initially found in the casein hydrolysate used as the protein source,[5] and, subsequently, has been identified in calcium[6] and phosphate[6,7] salts, heparin,[6] and albumin.[6,8] It is also known to be a contaminant of some solutions used in hemodialysis.[9] Its accumulation in bone[9–11] and brain[12,13] has been linked to disease in those tissues. Infants and children treated with TPN for several months or years may accumulate significant quantities of aluminum in the liver.[14] They may also develop rickets responsive only to large doses of vitamin D.[15]

This pilot study was designed to determine whether hepatic aluminum accumulation could contribute to the pathogenesis of cholestasis or to alterations in vitamin D metabolism.

METHODS

Animals

Eight piglets (Amo Farms, Ann Arbor, MI), 6 weeks old, were studied. Piglets were selected because the study of aluminum bone toxicity in lower animals, such as rats, has not consistently produced changes closely analogous to human bone disease.[16] The piglets were divided into experimental and control groups of four each. Starting weights of the piglets in the experimental group were 9.7 ± 2.1 (SD) kg, range 8.2–12.8; starting weights for the control group were 9.2 ± 1.6 kg, range 8–11.4. Each underwent left external jugular venous catheterization following general anesthesia with 1.5 g ketamine, 0.4 mg atropine, halothane, and nitrous oxide. The Tigon catheter was tunnelled under the skin and brought out onto the back. Its patency was maintained by twice daily flushing with 1–2 ml of heparinized saline.

The four experimental animals were given a daily intravenous bolus injection of 1.5 mg/kg of elemental aluminum as aluminum chloride (MCB Reagents, Darmstadt, West Germany) for 50 days. This dose of aluminum was selected as being large enough to produce changes in renal function in dogs.[17]

Bolus injection was chosen over continuous intravenous infusion of aluminum because both long-term TPN and hemodialysis treatments are given periodically, rather than continuously; thus, aluminum administration as a contaminant of those solutions is periodic. The four controls were given an equivalent volume of heparinized saline over the same time period. Both groups of animals were pair-fed a diet of Purina Pig Growena Chow T20 during the course of the study.

At the conclusion of the study period, the piglets were fasted for 12 hr and then were killed by intravenous administration of a lethal dose of sodium pentobarbital. Sections from the right lobe of the liver were then obtained in aluminum-free containers[14] for ultrastruc-

Received for publication, April 7, 1986.
Accepted for publication, September 4, 1986.
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tural examination and aluminum and copper quantification; aliquots of serum were also obtained in plastic containers for quantitative aluminum, copper, total bile acids, alkaline phosphatase, and 25(OH) vitamin D [25(OH)D] determinations.

Tissue Preparation

Tissue submitted for electron microscopy was cut into 1-mm cubes and fixed in a mixture of 2% paraformaldehyde and 2.5% glutaraldehyde in casodylate buffer for 1 hr. It was then washed in phosphate buffer (pH 7.3) and fixed in 1% osmium tetroxide in the same buffer for 1 hr. Each specimen then was washed several times with phosphate buffer, dehydrated in graded alcohol, and embedded in Spurr low-viscosity embedding medium. Ultrathin sections were cut on a LKB ultratome III, counterstained with uranyl acetate and lead citrate, and observed under a Phillips EM 301 electron microscope at an acceleration voltage of 80 kV.[18]

X-ray Microanalysis

Blocks of piglet hepatic tissue 0.3 μm thick and not fixed in osmium were mounted on copper grids and analyzed for the presence of aluminum. Despite the lack of osmium fixation in these particular samples, it was determined that organelle resolution was adequate to proceed with microanalysis.[19] All samples were stained with lead citrate and uranyl acetate.

For microanalysis, a Phillips 420T scanning transmission electron microscope (STEM) was used in conjunction with an Edax energy dispersive x-ray spectrometer controlled by Tracor Northern TN-2000 automation. Analysis was performed in the STEM mode of operation; a low background sample holder was employed.

This apparatus is capable of detecting all elements of atomic number greater than sodium by means of characteristic x-ray emission resulting from electron beam bombardment of a region defined by the beam, which can be focused to a diameter as small as 2 nm. The accelerating voltage used was 80 kV, and the beam current intensity was approximately 0.5 nA. All areas of visualized hepatocytes were scanned by the electron beam, and characteristic x-ray emission spectra for aluminum were recorded, wherever detectable, as peaks on a computerized tracing.

A Tracor software background fitting and subtraction program (+CS) was applied to the peaks to enhance spectral display. Peaks from the aluminum-loaded piglets and their pair-fed controls were displayed in such a way as to compare the curves in the pertinent regions.

Biochemical Determinations

Quantitative determinations of aluminum and copper in liver and serum were carried out by atomic absorption spectroscopy as previously described.[20,21]

Total serum bile acid concentrations were determined fluorometrically by the Sterognost 3 alpha Flu^R enzymatic method (Nyegaard and Co., Diagnostics Division, Oslo, Norway.[22,23] Serum alkaline phosphatase determinations were carried out by standard autoanalyzer techniques. Serum levels of 25(OH)D were determined by the competitive protein binding assay.[24]

Statistical analyses were carried out using the Wilcoxon rank-sum test and paired t-test where appropriate.

RESULTS

The final weights of the pair-fed animals were similar: 17.6 ± 2.8 (SD) kg for the experimental group and 17.9 ± 2.0 kg for the controls.

Biochemical Analysis

The biochemical effects of aluminum administration are shown in Table I. Aluminum loading in the experimental group of piglets resulted in hepatic and serum aluminum levels significantly greater than those for the control group.

Cholestasis, as manifested by markedly elevated serum total bile acid levels occurred in three of the four aluminum-loaded piglets ($p < 0.05$ compared to controls by

TABLE I
Biochemical effects of aluminum administration

Group	Aluminum		Serum 25(OH)D (ng/ml)	Serum total bile acids (μmol/liter)	Copper	
	Hepatic (mg/kg dry wt)	Serum (μg/liter)			Hepatic (μg/g dry wt)	Plasma (μg/dl)
Experimental						
Piglet 2	1269	7020	10.91	**42.6**	25.74	139
4	1392	1684	9.69	**5.6**	24.56	162
6	1944	5928	9.97	34.5	29.93	
7	1438	2340	8.73	28.5	19.00	152
Mean ± SD	1511 ± 298	4243 ± 2728	9.8 ± 0.9	27.8 ± 15.9	24.8 ± 4.5	151 ± 11.5
Control						
Piglet 1	**0.5**	<2	13.91	**5.3**	12.52	124
3	**0.6**	<2	11.32	**6.8**	14.06	130
5	**0.3**	<2	10.17	**8.1**	15.58	164
8	**1.5**	<2	12.93	**4.8**	15.41	117
Mean ± SD	0.7 ± 0.5	<2	12.1 ± 1.6	6.3 ± 1.5	14.4 ± 1.4	133.8 ± 20.9
p (experimental *vs* control)	$p < 0.001$	$p < 0.001$	$p = 0.05$	$p < 0.05$	$p < 0.01$	NS

Wilcoxon rank-sum). Mean serum alkaline phosphatase levels were higher in the aluminum-loaded group, 309 ± 108 *vs* 180 ± 27 IU/liter, although the difference was not significant. The mean hepatic copper content was nearly 2-fold greater ($p < 0.01$), and the mean serum 25(OH)D levels were lower ($p = 0.05$) in the aluminum-loaded piglets, compared to pair-fed controls.

Ultrastructural Analysis

On routine electron microscopic examination, no qualitative differences in hepatocyte glycogen deposition, organelle size, or shape were apparent between the two groups. Similarly, no differences in bile ductular width, patency, or number were seen. Neither bile pigment nor inflammatory changes were detected in either group.

X-Ray Microanalysis

Figure 1 shows x-ray emission peaks from a lysosome of an aluminum-loaded piglet hepatocyte and, for comparison, from a lysosome of a pair-fed control. Peaks for aluminum and phosphate are prominent in the lysosome of the hepatocyte from the aluminum-loaded piglet. Similar patterns were detected in the hepatocytes of the other three aluminum-loaded piglets. This suggests that aluminum may be found in the lysosomes of the hepatocytes in the experimental group. However, not all lysosomes within a given hepatocyte were shown to contain aluminum; neither was aluminum detected in every hepatocyte examined. No aluminum peaks were detected after scanning the entire grid in any of the controls.

DISCUSSION

Our data indicate that growing piglets receiving a chronic parenteral aluminum load accumulate large amounts of aluminum in the liver and blood. Since serum creatinine was not different between experimental and control groups, decreased renal function cannot explain the large discrepancy in aluminum content existing between the two groups. The small quantity of aluminum, less than 1 μg/day, administered to controls with the heparinized saline clearly had no effect on hepatic or serum aluminum levels. Cholestasis manifested by elevated serum bile acids developed, despite continued enteral stimulation of bile flow by means of feeding.

The elevation of serum alkaline phosphatase levels and hepatic copper[25] in the aluminum-loaded group are consistent with the observed cholestatic effect. However, because osteomalacia was also produced in these piglets,[26] it is not clear whether the alkaline phosphatase is primarily from liver or bone. The mechanism of the elevated serum bile acid levels is unknown. However, another trace element, manganese, can initiate cholestasis by affecting canalicular membrane structure and cellular microfilaments.[27]

The failure to detect ultrastructural histopathology suggests that the elevation of serum bile acids may precede or be independent of the appearance of other hepatic pathology, as has been suggested to occur in TPN-associated liver disease.[28] It has also been shown in rabbits[29] and rats[30] that infusions of glucose and amino acid solutions can acutely decrease bile flow without altering hepatic histology.

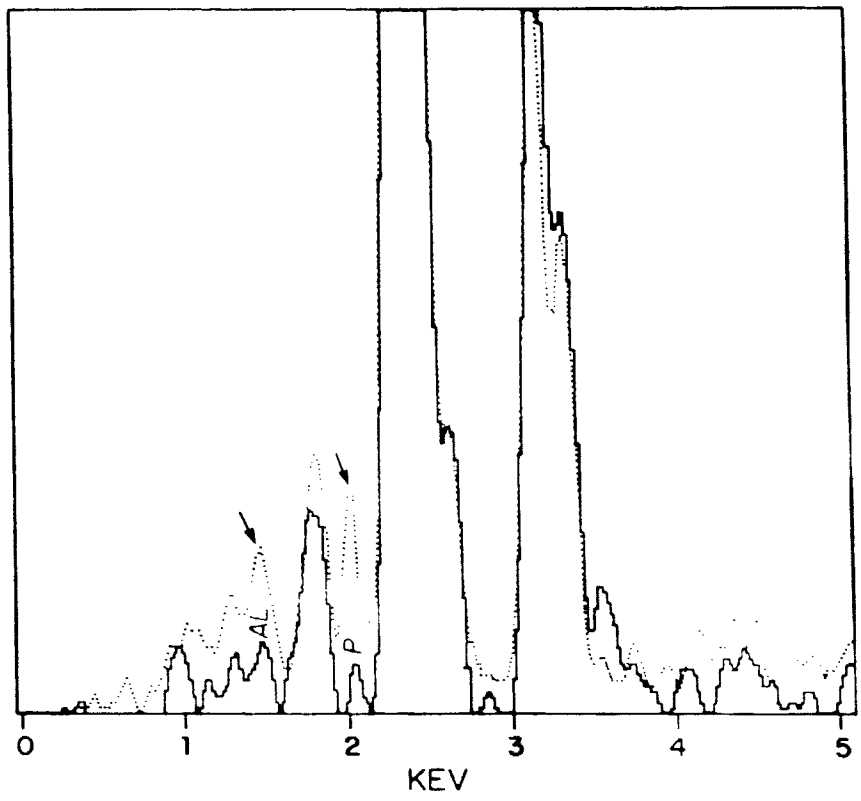

FIG. 1. Computerized tracings of element peaks from lysosomes of aluminum-loaded (broken line) and control (solid line) piglet hepatocy[illegible]s designate peaks for Al and P in the he[illegible]sosome of the aluminum-loaded piglet (keV = thousand electron volts).

It is unlikely that halothane anesthesia alone produced the cholestasis, since the control group also received halothane. Whether the halothane in some way predisposed the aluminum-loaded piglets to cholestasis cannot be determined at present.

Like most reagent grade crystals of this compound, the aluminum chloride crystals contain some lead; the lead content of the brand used was 5 ppm. Each piglet received an average of less than 1 μg/day, an insignificant quantity in relation to the amount of aluminum given each day.

A previous report by Galle and Giudicelli[31] described aluminum deposition in the fibrotic liver of a woman who had died of dialysis encephalopathy. However, the possibility of underlying chronic hepatic disease, such as non-A, non-B hepatitis, was not excluded. The same report describes changes in hepatocyte lysosomal size and shape in rats given chronic intraperitoneal injections of large doses of aluminum. However, no controls were used in the above-mentioned study, and our animals did not show these changes.

We can confirm the finding of aluminum in the lysosomes seen by Galle and Giudicelli,[31] as well as by Verbueken et al.[32] The significance of lysosomal aluminum accumulation is uncertain. However, since lysosomes may be a major route of biliary lipid excretion; the presence of aluminum may alter lysosome-to-bile excretion and contribute to an alteration of either bile composition or bile flow.[33] Lysosomal aluminum accumulation may also represent a possible excretory route of aluminum from the liver via the bile ductules. However, the biliary route of aluminum excretion was shown to be insignificant in acutely loaded dogs[34] as was enterostomal aluminum excretion in chronically loaded humans with normal renal function.[35] In our study, the bile ducts of the piglets were not cannulated; thus, the contribution of this route to aluminum elimination, in the specific case of the chronically loaded piglet liver, cannot be determined.

The lower serum levels of 25(OH)D in the aluminum-loaded piglets were still within the normal range.[36] However, since the half-life of 25(OH)D in the circulation, 2–4 weeks,[37] is long, changes in hepatic vitamin D 25-hydroxylase activity may not be readily reflected in the circulating concentration of the sterol. However, the lower levels of 25(OH)D in the serum of the aluminum-loaded piglets in consistent with the hypothesis that 25-hydroxylase activity may be decreased with aluminum loading. Data demonstrating a decrease in hepatic microsomal cytochrome P450 with aluminum loading in rats are also consistent with this hypothesis,[38] as 25-hydroxylation of vitamin D in the liver is a cytochrome P450-dependent step.

Finally, the quantities of aluminum deposited in the livers of the experimental group of piglets are at least twice those found in patients with dialysis encephalopathy[39] and at least 6-fold greater than those found in chronically loaded infants receiving aluminum-contaminated TPN solutions.[14] Thus, caution must be applied in extrapolating the results from our study to clinical situations. Further studies are necessary to determine whether these changes in liver function are still present when hepatic aluminum content is adjusted to the level seen in aluminum-loaded patients.

ACKNOWLEDGMENTS

This study was supported in part by U.S. Public Health Service Grant 15.1-973, by a grant from the John Tung-American Cancer Society Nutrition Education Center, and by funds from the Veterans Administration. Valuable technical assistance was provided by Alina Fernandez, Hsein-Chen Tseng, and Robert Dick. Valuable secretarial assistance was provided by Peggy Berg, Pat Nichols, and Melody Wilkey. The authors would also like to thank John F. Rosen, M.D., for his helpful comments.

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