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Aluminum-Associated Hepatobiliary Dysfunction in Rats: Relationships to Dosage and Duration of Exposure[1]

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ABSTRACT. Aluminum may contaminate parenteral nutrition solutions and accumulate in bone and liver of patients receiving this therapy. Although aluminum exposure is associated with low-turnover osteomalacia, there are few studies of hepatotoxicity. We therefore studied the effects of aluminum given to rats on total serum bile acid concentration and bile flow to determine if aluminum administration could produce abnormalities. Aluminum was given intravenously as follows: 5 mg/kg daily for 7 or 14 days and 1 mg/kg for 14 days. Hepatic aluminum was high in treated rats and undetectable in controls. Total serum bile acid concentrations were significantly higher in treated rats than in pair-fed controls with higher concentrations after 14 days than after 7 days. Bile flow was reduced by 33% in rats given 5 mg/kg but not in rats given 1 mg/kg. Hepatic aluminum correlated inversely with bile flow but not with serum bile acid concentration. Aluminum exposure in rats is associated with elevated serum bile acid concentration and diminished bile flow and may play a role in the pathogenesis of parenteral nutrition-induced hepatobiliary dysfunction. (*Pediatr Res* 23:275–278, 1988)

Abbreviations

Al, aluminum
TPN, total parenteral nutrition
PE, polyetheylene

Al is a known contaminant of both dialysis solutions (1, 2) and components of TPN solutions, including casein hydrolysate (3), calcium and phosphate salts (4, 5), albumin (4, 6), and heparin (4). Elevated Al content in both liver and bone has been documented in patients with chronic renal failure (7) and in those with severe gastrointestinal disease receiving TPN therapy (3, 8, 9). Al accumulation in both groups of patients has been linked to a low-turnover vitamin D-resistant osteomalacia (9). However, until recently there has been no evidence to suggest that Al accumulation in the liver is harmful. Preliminary studies in rats have demonstrated a depression of hepatic mixed function oxidase activity with parenteral Al loading (10) whereas total serum bile acid concentrations were elevated in piglets chronically loaded with Al when compared to pair-fed controls (11). We investigated the effects of chronic intravenous administration of Al to rats in order to determine whether Al could produce hepatic or biliary dysfunction.

METHODS

The use of rats to study Al-associated hepatobiliary dysfunction was based on their prior use in studying TPN-associated liver disease (12–14) as well as the previous demonstrations of Al toxicity to bone in rats (15, 16). Male Wistar rats were prepared with chronic indwelling venous cannulas as follows: under pentobarbital anesthesia (35 mg/kg intraperitoneally) a medical grade silastic catheter 0.037 inches external diameter attached to PE tubing (PE 50) was inserted through the jugular vein a distance of 2.5 cm entering the inferior vena cava. The opposite end of the PE 50 tubing was connected to surgical grade Tygon tubing, 3/32 inches external diameter, and tunneled through the skin. The tubing emerged and was anchored between the scapulae. The animals were allowed to recover for 24 h. Patency of the cannula was maintained by daily injection of 0.4 ml of heparinized saline (10 U/ml).

Rats, maintained on standard laboratory rat Chow (Purina 5001, Ralston-Purina Co., St. Louis, MO) and water *ad libitum* and weighing 175 ± 11 g (SD) at the start of the study, were divided into three experimental groups. Littermate control animals for each experimental group (weighing 174 ± 10 g), were pair-fed to eliminate changes brought about by altered nutritional status. Experimental animals received daily intravenous injections of Al chloride, providing either 5 mg/kg/day (5 mg × 14) or 1 mg/kg/day of elemental Al (1 mg × 14) for 14 days. A third group received 5 mg/kg/day of elemental Al for 7 days only (5 mg × 7). Control animals received daily injections of the vehicle, 0.85% saline.

After the experimental loading period, animals again received pentobarbital anesthesia and bile duct and bladder cannulas were placed using PE tubing (PE 10 and PE 90, respectively). Bile and urine were collected for 3 h. At the conclusion of this 3-h period, serum was collected by carotid artery cannulation. After blood withdrawal the rats were killed by cervical dislocation while still under pentobarbital anesthesia.

Serum and biliary bile acid concentrations were determined fluorometrically using the Sterognost 3-α Flu enzymatic method (Nyegaard and Co., Diagnostics Division, Oslo, Norway) (17–19). This technique accounts for all bile acids including muricholates that also have 3-α hydroxyl groups (19). Al was analyzed

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in bile, serum, urine, and liver by flameless atomic absorption spectroscopy (20).

Livers from rats receiving Al 5 mg × 14 and pair-fed controls were prepared for electron microscopy. Specimens were fixed in a mixture of 2% paraformaldehyde and 5% glutaraldehyde in casodylate buffer for 1 h, washed in phosphate buffer, and fixed in 1% osmium tetroxide. Each specimen was washed in phosphate buffer, dehydrated in graded alcohol, and embedded in Spurr low viscosity embedding medium. Ultra-thin sections were cut on an LKB ultratome III, counterstained with uranyl acetate and lead citrate, and viewed under a Philips EM 301 electron microscope at an acceleration voltage of 80 kv (11). Light microscopy was not performed on livers from animals in this study.

This study was reviewed and approved by the Research Animal Care Committees of the City of Hope and the University of Texas Medical Branch.

RESULTS

At the time of death, the combined mean weights of all Al-loaded groups were not different from those for all control groups, 211 ± 18 g compared to 212 ± 19 g, respectively. In addition, no differences in weight gain were present among the various subgroups of experimental animals.

Hepatic Al content was elevated significantly in all three experimental groups compared to their respective pair-fed controls (Fig. 1). Hepatic Al content did not differ in rats given Al 5 mg/kg/day for 7 *versus* 14 days. Mean hepatic Al content in the rats given Al 1 mg × 14 was 35–40% of mean hepatic Al content of rats receiving either 5 mg/day regimen. Hepatic Al content never exceeded 1 mg/kg dry weight in any of the controls. Mean serum levels of Al in rats given 1 mg × 14 were approximately 50% of those of the group given 5 mg × 14 (Table 1).

Total serum bile acid concentrations were substantially higher in rats from all treatment groups than in pair-fed controls (Fig. 2). The elevation of bile acids was more pronounced after 14 days than after 7 days of Al (Fig. 2). Total serum bile acid concentrations, although clearly elevated compared to controls, were not different between rats given 1 mg × 14 or 5 mg × 14 (Fig. 2).

In the groups given 5 mg/kg bile flow was significantly reduced compared with controls, whereas bile flow was not different in rats given 1 mg/kg/day compared with controls (Fig. 2). Bile flow in rats given 5 mg × 14 was reduced by 15% compared with rats given 5 mg × 7 and by approximately 33% compared with rats given 1 mg × 14 (Fig. 2).

Regression analysis for all three groups combined revealed an inverse correlation between hepatic Al and bile flow ($r = 0.52$, $p < 0.025$) but no relationship between hepatic Al and total serum bile acid concentration. It should be noted that hepatic Al was higher in rats given 5 mg × 7 than 1 mg × 14 while serum bile acids were higher in the 1 mg × 14 group. However, the effects of both dose and duration of Al administration may have interacted in some way to influence these results.

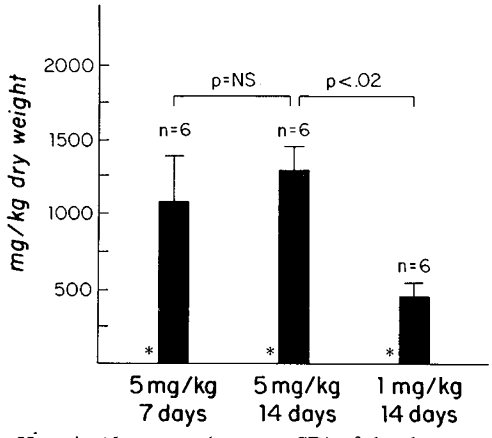

Fig. 1. Hepatic Al content (mean ± SD) of the three groups of rats receiving Al. * represents values for hepatic Al content of the pair-fed controls; all were <1 mg/kg dry weight.

Biliary excretion of bile acids and Al calculated from biliary bile acid concentration, Al concentration, and bile flow are shown in Table 1 along with serum Al levels. Calculated biliary bile acid excretion was not significantly different between Al and control rats in any of the three groups. Biliary Al excretion was substantially greater in the experimental animals compared to controls. However, it accounted for only 3–7% of the quantity of Al excreted in the urine. Analysis of covariance revealed that within each of the three groups and with all three groups combined, a direct relationship exists between biliary/urinary Al excretion and hepatic Al content, with $p = 0.033$.

Electron microscopic examination of the liver from Al-loaded rats and controls failed to reveal any abnormalities. Specifically, there were no inclusions or membrane changes seen.

DISCUSSION

Our data show that parenteral administration of Al to rats can result in the production of elevated total serum bile acid concentrations alone or in combination with decreased bile flow. These effects are both dose dependent and time dependent. Moreover, they occurred despite continued enteral stimulation provided by the pair-feeding design.

The mechanism for these cholestatic effects associated with Al loading remains unclear. The present data fail to demonstrate abnormalities in hepatic ultrastructure or a measurable decrease in biliary bile acid excretion, which is a phenomenon observed with infusions of TPN solutions into rats (14). The inverse relationship between hepatic Al content and bile flow would suggest that accumulation of Al in the liver may in some way impede bile flow. However, unchanged bile flow and biliary bile acid excretion in rats given Al 1 mg/kg/day compared to controls, despite the elevations of serum bile acid concentrations, may be due in part to the low circulating bile acid concentrations (μmol/liter) compared to the relatively high levels of biliary bile acid (mmol/liter). For example, very small decrements in hepatic bile acid clearance are reflected in large changes in total serum bile acid levels. Herein we did not determine biochemical measurements of hepatocyte injury such as serum transaminase levels. Thus, despite lack of histologic evidence of hepatocyte injury by Al we cannot exclude the possibility that Al may damage hepatocyte plasma membranes, interfering with bile acid uptake. Also the reduction in bile flow without a decrease in biliary bile acid excretion suggests an effect of Al on bile acid independent bile flow. However, additional studies would be required to support or refute this speculation (21).

Al is known to have other effects on the liver including reduction of mixed function oxidase activity in rats, a phenomenon also reported in rats receiving TPN solutions (23) and in cholestatic patients (23). However, the mechanism by which Al raises serum bile acids or impairs bile flow is unknown.

Another metal, manganese, has been shown to cause cholestasis in rats when given in combination with bilirubin (24–26). Study of this phenomenon has demonstrated that there are ultrastructural changes, including a loss of bile canalicular membrane microvilli and pericanalicular vacuolization occurring with a 20% decrease in bile flow; when bile flow returned to normal the histologic changes resolved (24, 25). No data are available regarding the effects of giving bilirubin to Al-loaded rats. Furthermore no ultrastructural changes have been associated with a 33% decrease in bile flow seen with Al-loaded rats. In addition, manganese is excreted through the bile while there is very little biliary excretion of Al. Moreover, the liver may handle a trivalent cation such as Al differently from a divalent cation such as

Table 1. *Biliary excretion of bile acids and Al [mean ± SD (range)]*

Group	*n*	Biliary bile acid: Concentration (μmol/liter)	Biliary bile acid: Excretion (μmol/h/g liver)	Biliary Al excretion (μg/h/g liver)	Biliary/urine Al excretion (μg/h/μg/h × 100)	Serum Al (μg/liter)
5 mg/kg/day		20.8 ± 4.5	1.77 ± 0.83	0.06 ± 0.04*	3 ± 2	653† ± 296
×14 days	7	(12.7–26.5)	(0.98–3.38)	(0.02–0.13)	(1.2–5.7)	(312–842)
Control		25.4 ± 7.4	3.07 ± 1.29	0.002 ± 0.001	‡	
		(15.8–32.9)	(2.21–5.82)	(0.001–0.004)		<10
5 mg/kg/day		22.9 ± 3.9	2.29 ± 0.35	0.07 ± 0.07†	4 ± 5	
×7 days	8	(16.0–26.9)	(1.65–2.74)	(0.03–0.25)	(0.2–13)	ND§
Control		20.0 ± 5.9	2.34 ± 0.63	0.001 ± 0.0004	‡	
		(14.3–31.3)	(1.52–3.45)	(0.0005–0.0013)		
1 mg/kg/day		20.8 ± 1.6	2.64 ± 0.74	0.04 ± 0.02*	7 ± 3	341 ± 37
×14 days	6	(19.0–23.1)	(1.19–3.19)	(0.02–0.07)	(2.1–11.6)	(198–582)
Control		19.2 ± 3.1	2.33 ± 0.41	0.001 ± 0.0001	‡	
		(15.1–23.2)	(1.84–3.01)	(0.0005–0.0007)		<10

* $p < 0.02$ *versus* controls by sign test. ND = not determined; ++ n = 3 only
† $n = 3$ only.
‡ Ratio not given for controls due to the very small quantities of Al measured in controls.
§ Not determined.

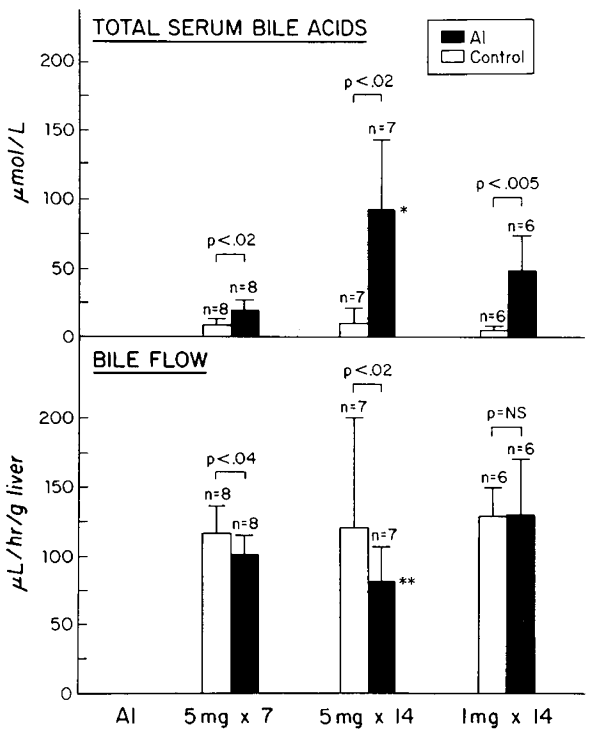

Fig. 2. The effect of duration and dose of Al on total serum bile acid concentration and bile flow. Values depicted represent mean ± SD. Five mg × 7 represents rats receiving 5 mg elemental Al/kg/day for 7 days; 5 mg × 14 represents rats receiving the same amount of Al for 14 days; 1 mg × 14 represents rats receiving 1 mg elemental Al/kg/day for 14 days. * represents a significant elevation of serum bile acids ($p < 0.05$) in the 5 mg × 14 *versus* the 5 mg × 7 group. ** represents a significant depression of bile flow ($p < 0.05$) in the 5 mg × 14 *versus* the 5 mg × 7 group and the 1 mg × 14 group.

manganese. These differences make it difficult to postulate a similar mode of action of these two metals on the liver.

Our data indicating that biliary excretion of Al accounts for a small proportion of overall Al excretion during chronic Al administration is similar to data obtained by Kovalchik *et al.* (27) in dogs given a single large bolus of Al and to enterostomal Al excretion in TPN patients who were chronically loaded with Al (28). The present data are the first to our knowledge to be obtained by direct bile duct cannulation in animals given large doses of Al for an extended period of time.

Although it appears that biliary Al excretion may be of little importance after parenteral administration, biliary Al concentration was recently reported to exceed urinary Al concentration in candidates for liver transplantation with normal renal function who ingested Al-containing antacids (29). Even though excretion rates of Al via both routes were not compared, this observation raises the possibility that Al absorbed after oral intake may be handled differently by the liver than Al administered intravenously.

The Al content of today's noncasein-containing TPN solutions currently given to infants can range from 30 to 300 μg/liter (30). At the upper range this could represent a load of 30 μg/kg/day given to premature infants often for several months. Although the daily load of Al is clearly less than in the rats we report, the duration of the TPN treatment could result in a significant amount of Al accumulation. Thus, the hepatic Al content of the rats given 1 mg/kg/day for 14 days is similar to that observed in Al-intoxicated chronic dialysis patients (31) and approximately 2-fold greater than the quantities determined in infants receiving chronic TPN (8). Thus, although it is not reasonable to extrapolate from rats to humans, chronic Al loading may be an additional factor contributing to TPN-associated liver disease. A prospective study of premature infants receiving parenteral nutrition in which the Al concentration of TPN solution, serum, and urine can be related to liver function would provide indications of whether Al may be an additional factor in the pathogenesis of TPN-associated hepatobiliary dysfunction.

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