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SYMPOSIUM

Some effects of lead contamination on liver and gallbladder bile+

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ABSTRACT Lead can cause liver damage in which free radical reactions are involved. In order to study the effect of lead on liver and gallbladder, we examined human gallbladder bile after cholecystectomy, as well as the liver and bile of broiler chickens fed with basal diet and contaminated with 400 and 600 mg/kg lead ($Pb(CH_3COO)_2$). Concentrations of lead in human bile were determined with inductively coupled plasma optical emissison spectrometer (ICP-OES). Diene-conjugate content as well as thiobarbituric acid reactive compounds in bile were determined by spectrophotometry. In the case of lead poisoning good correlation was observed between the lipid peroxidation parameters of bile and the picture of necrotised tissue observed in a histological study.High lead contamination caused mainly liver damage while cholecystitis was induced by the low concentration of lead, probably, by changing the normal biliary processes.

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KEY WORDS

lead
gallstone
bile
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Lead poisoning generally results from well-known occupational exposure *e.g.* crystal ware, glazed pottery, however, in some instances it may arise from unexpected sources *e.g.* home made wine. Lead may cause colicky abdominal pain, weight loss and elevation in liver function tests, although in 29% of these patients no complaints have been recorded (Janin et al. 1985).

In previous studies some difference was found in the metal concentration of bile between gallstone patients and control subjects. Significantly lower concentration of lead was found in bile samples from gallstone patients than from controls, although the excretion of lead was supposedly the same (Calderon et al. 2000).

The purpose of the study was to determine the effects of lead on the hepatobiliary system, especially on the liver and on the gallbladder.

Materials and Methods

Humans

Between January 1998 and December 1999, 40 patients with 60.51±14.34 years of age, and men/women ratio of 8/32 were involved in the experiments. A questionnaire was filled out to determine occupational or home lead intoxication. Gallstones and bile samples were obtained. The bile was taken by needle aspiration and the total bile volume of the gallbladder was determined. The gallbladder was prepared by routine histological methods and the degree of inflammation was determined. The fasting gallbladder volume was calculated from the sucked bile. The research was approved by the ethical committee of the institution (No.59/1996) and followed institutional guidelines for the care and use of laboratory animals (No.45/1999).

The concentration of lead in human bile and in gallstones was determined by ICP-OES. Bile fluid samples (2.0 g) and gallstones were digested with a mixture of HNO_3 (5 ml) and H_2O_2 (3 ml) in teflon vessels. After digestion the samples were diluted to 25 ml with deionised water (Szentmihályi et al. 2000).

Animals

Broiler chickens were fed with basal diet and treated with 400 and 600 mg/kg lead ($Pb(CH_3COO)_2$) ad libitum from 1 week to 6 weeks of age. At the end of the experiments the chickens were decapitated, and the liver and gallbladder bile were removed. The samples were stored at –20°C during the measurements (Blázovics et al. 2001).

The volume of liver homogenates was 0.05 ml (protein content: 10 mg/ml) measured by the method of Lowry

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+In memory of Professor Béla Matkovics

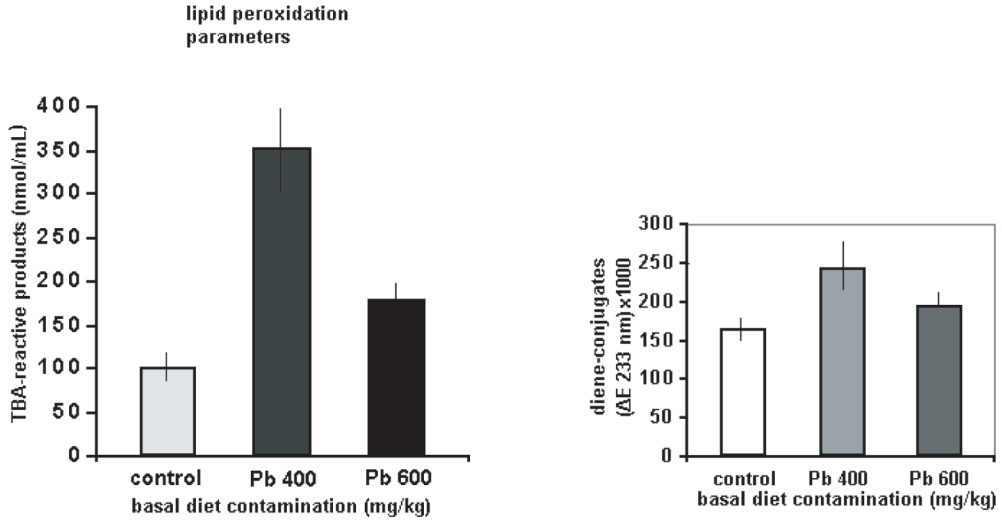

Figure 1. Lipid peroxidation processes in the bile of broiler chicken treated with lead. The decreased concentrations of thiobarbituric acid reactive compounds and diene conjugate content of bile in higher dose of lead reveal aggregation processes in the gallbladder bile.

(1951). Thiobarbituric acid reactive compounds of the bile were measured by the method of Pyles (1993) and the diene conjugate content of the bile was determined at 233 nm by spectrophotometry after fractionated separation of isooctane, then desiccated with Na_2SO_4 (AOAC 1993). Routine histopathological examinations were performed. All reagents were purchased from Reanal (Hungary). Mean and standard deviations were calculated from the results and significance was determined by Student's *t* test.

Results

Pb was detected in 9 bile samples from the above 40 patients with gallstones (23%). One patient worked with ceramic dye, one performed renovating work at home and the others were not exposed to Pb. Average lead concentration was 0.2±0.21 mg/kg. On an average, the Pb content of bile was 0.228±0.124 mg/kg, measured from 6 samples taken from 40 patients. Human gallstone Pb content was 20.55±48.69 mg/kg observed in 3 samples in very high concentration. Elevated Pb concentration in gallbladder bile and stone was detected only in samples of one patient. In the other cases, the Pb content of bile fluid and gallstone did not show parallel changes. The volume of gallbladder with lead content was significantly lower than observed for gallbladder without lead content (7.8±2.3 ml/21±12.4 ml), but histological preparation did not reveal any thickening of the gallbladder wall. The gallbladder volume with muddy bile content was always lower and could not be entirely sucked out, but the remaining volume was always lower than 10%.

In order to prove that Pb may cause lipid peroxidation injuries in the liver and gallbladder, we studied the effect of lead in broiler chicken experiments. When the basal diet of chickens was contaminated with 400 and 600 mg/kg $Pb(CH_3COO)_2$ the diene-conjugate content and concentration of thiobarbituric acid reactive substances were determined in broiler chicken gallbladder bile supernatants (Fig. 1). Similar ratio was observed between the two parameters in heavy metal poisoning. These data point to lipid peroxidation processes in the gallbladder bile. The amount of sludge was significantly higher and the volume of gallbladder was lower in highly poisoned chickens (600 mg/kg), than observed in the control animals. Histological sections confirmed the dose-dependent toxic effect of lead in the liver, treatment with 400 mg/kg lead caused lymphocyte infiltration, and 600 mg/kg lead treatment caused severe periportal inflammatory reaction, which may lead to cirrhosis (Fig. 2).

Discussion

Lead has been shown to be toxic in most of its chemical forms, either inhaled or ingested in water or food at levels humans are exposed to at the workplace as well as in the general environment. Gastrointestinal lead absorption and retention, the major pathway of lead intake, were shown to vary widely depending on the chemical environment of the gastrointestinal lumen, age and iron stores (nutritional status of the subject). Lead does not have a feedback mechanism, which limits absorption. Dietary components, such as, sodium citrate, ascorbic acid, amino acids, vitamin D, proteins, fat and lactose can bind to lead and thus enhance the absorption of lead (DeMichele 1984). When lead is ingested or absorbed in the body, blood Pb level initially rises and then falls within days. After prolonged exposure, lead is

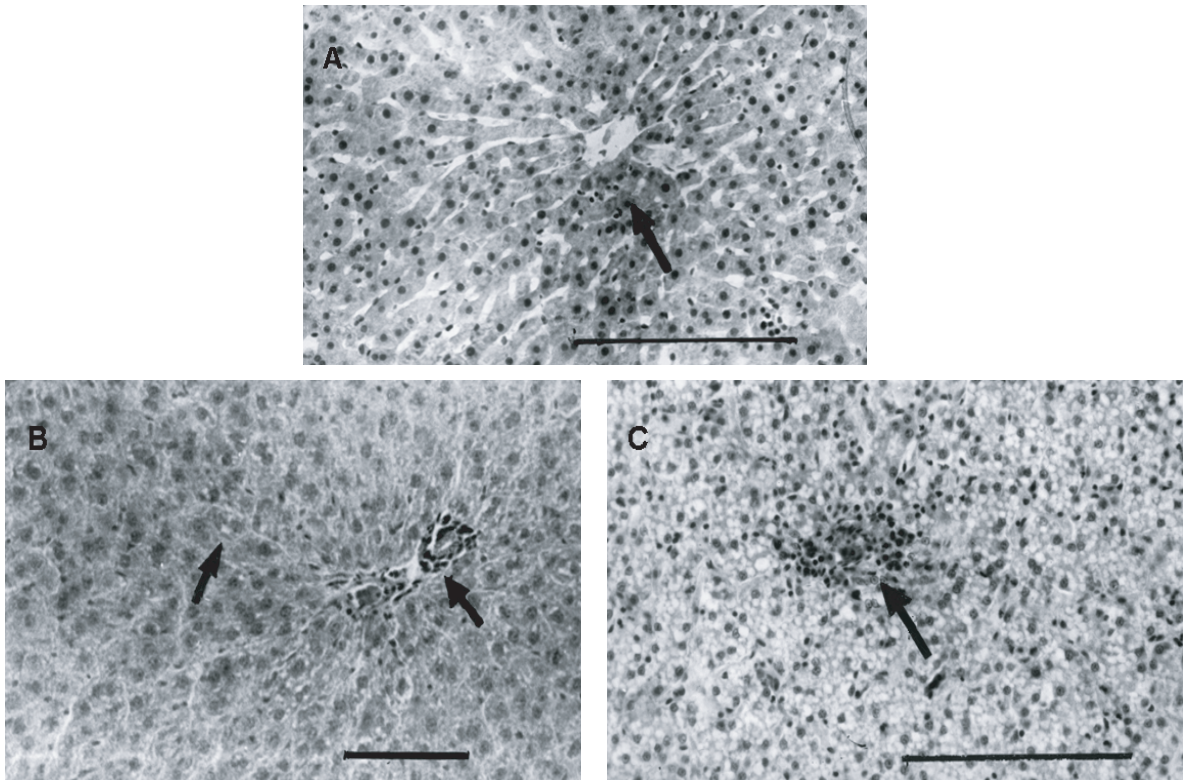

Figure 2. Light microscopic picture of the liver exposed to lead. **A**: control; **B**: 400 mg/kg lead; **C**: 600 mg/kg lead; **A**: central part of a hepatic lobule of a control animal. Bar scale = 100 mm, **B**. and **C**: parts of the hepatic lobules from a led treated chicken. Arrows show micro lipid droplets. Dotted arrows point to the periportal inflammatory reactions.

stored in the skeleton where the time of equilibration with blood and organs takes several years.

The significance of our study is that it draws attention to the fact that the cause of abdominal pain may be lead poisoning as well, which may be induced by changes in the visceral smooth muscle tone secondary to the action of lead on the visceral autonomic nervous system, as well as lead induced alteration in sodium transport in the small intestinal mucosa.

Lead can stimulate intercellular signalling between Kupffer cells and hepatocytes, which are enhanced synergistically in the presence of low lipopolysaccharide levels (Milosevic and Maier 2000) promoting thereby proteolytic activity.

Our results have shown that treatment with higher concentration of lead causes severe periportal inflammation in chicken liver, therefore, it may be assumed that long-term lead exposure might cause liver damage in humans as well.

Good correlation was found between the elevated lipid peroxidation parameters and the decreased total scavenger capacity of the liver exposed to lead (Blázovics et al. 2001).

In general, the different effects of lead in the hepatobiliary system are: catalysis of the peroxidation of unsaturated fatty acids (Yin and Lin 1995), reduction of N-oxide production (Krocova et al. 2000) and the promotion hydroxyl radical formation (Ding et al. 2000).

Each of these affects may promote stone formation. The proliferation effect of lead on smooth muscle cells (Fujiwara et al. 1995) and the rise in the tone of gallbladder may result in smaller gallbladder volume and an increase in wall thickness.

Our results show that higher concentration of lead causes mainly liver damage in which free radicals are involved, but low concentration of lead may disturb the normal biochemical process in the hepatobiliary system and the lead may precipitate into gallstones.

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