Dietary Lead Alters Fatty Acid Composition and Membrane Peroxidation in Chick Liver Microsomes

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ABSTRACT Inorganic Pb acetate is a pro-oxidant, and peroxidation damage to cellular membrane lipids, leading to membrane fragility and permeability, is a likely consequence of Pb poisoning. In addition to the systemic peroxidation that occurs in vivo, Pb-contaminated feedstuffs can contribute preformed peroxides. Treatments with dietary Pb that have been shown to increase tissue peroxide levels in animals may be related to the consumption of preformed peroxides from the diet. In the current study, we evaluated the possible separate effects of feed and systemic peroxides by administering equivalent doses of Pb acetate-trihydrate to chicks via either 1,500 ppm Pb in the diet or via gastric intubation. Peroxidation of lipids in hepatic microsomal membranes (assessed as malonyldialdehyde production) from birds intoxicated with Pb by either route of administration was more than double that of untreated controls. Also, both routes of Pb exposure doubled the concentration of hepatic microsomal arachidonic acid, a peroxidizable polyunsaturated fatty acid. In the data reported here, we show that tissue peroxide levels are unaffected by the method of oral Pb administration and thus, by inference, independent of peroxide content of the feed.

(Key words: lead, peroxidation, lipids, tissue peroxides, arachidonic acid)

Received for publication April 16, 1996.
Accepted for publication August 7, 1996.
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INTRODUCTION

An effect of Pb toxicity on lipid metabolism has been described in which chronic treatment with inorganic Pb increases the proportion of arachidonic acid (AA), as well as the arachidonate:linoleate (AA:LA) ratio, in the fatty acids of lipids from a variety of avian tissues. Dietary Pb (62.5 to 500 ppm) fed to chicks resulted in dose-dependent increases of AA and AA:LA in serum and liver total lipids, and in hepatic microsomes (Lawton and Donaldson, 1991). This AA is easily peroxidized (Mead, 1976), and a positive correlation between enhanced AA content and peroxidation of tissue lipids was observed. This finding suggests that peroxidation resulting from altered fatty acid composition may be responsible for some of the biological effects of Pb.

Peroxidation of highly unsaturated fatty acids can lead to membrane fragility, loss of integrity (Chvapil et al., 1972; Levander et al., 1977), and other tissue damage associated with reactive oxygen. Peroxidation by Pb has been demonstrated in brain homogenates from rats poisoned with Pb acetate in drinking water (Shafiq-Ur-Rehman, 1984), and in essential fatty acids incubated with Pb oxides in vitro (Yin and Lin, 1995). Donaldson and Knowles (1993) have reviewed further evidence implicating lipid peroxidation as a toxicity mechanism of organic and inorganic forms of Pb.

Donaldson (1991) reported that the peroxide level in 1,000 ppm Pb-supplemented feed was three times that of control feed, but peroxide level in feed with Pb plus ethoxyquin antioxidant was not different from control. Also, growth inhibition of chicks due to dietary Pb was greatly ameliorated by addition of the antioxidant to the diets. Thus, the possibility exists that some of the toxic effects of dietary Pb could be a consequence of peroxidation of dietary lipids.

The objective of this study was to determine whether the increase in tissue lipid peroxidation and AA content that occurs with dietary Pb treatment results from preformed peroxides in the Pb-supplemented feed, or from the systemic effect of chronic Pb dosing.

MATERIALS AND METHODS

Arbor Acres male broiler chicks (Gallus domesticus) were treated with either 1,500 ppm dietary Pb or an equivalent Pb dose administered by gastric intubation (gavage). The chicks were reared from hatching through 16 d in thermostatically controlled battery cages with raised-wire floors under continuous fluorescent lighting. Feed and tap water were consumed ad libitum. The feed was a soybean meal-dextrose-cottonseed oil mixture.
supplemented with adequate amounts of all nutrients (NRC, 1994). The fat content of the diets was 2% by weight and contained no antioxidant. No AA was provided in any diet.

All Pb supplements were in the form of Pb acetate trihydrate. Gavage doses of Pb were calculated based on feed consumption of paired birds. Those doses amounted to approximately 120 mg Pb/kg body weight per d. Vehicle controls received distilled water gavage. At the end of the 16-d exposure period, birds were exsanguinated by cardiac puncture and killed by cervical dislocation. Livers were perfused in situ with ice-cold 0.9% saline and then removed. Hepatic microsomal membrane fractions were prepared as described previously by Lawton and Donaldson (1991). Fatty acid compositions of total lipids from 1-mL samples of microsomal preparations were determined according to the gas-liquid chromatography method of Donaldson and Leeming (1984), and were expressed as percentages of total methyl esters. Peroxides and thiobarbituric acid (TBA) reactive substances (TBARS) in microsomal lipids were determined by the TBA reaction method (Ohkawa et al., 1979). Protein content of the microsomal fraction was estimated by the biuret method (Cleland and Slater, 1953).

All data were subjected to analysis of variance for factorial arrangements (2 x 2) with the factors being Pb dose and Pb source (diet or gavage) as outlined in Steel and Torrie (1980). The General Linear Models procedures of SAS® (SAS Institute, 1985) were used.

RESULTS AND DISCUSSION

The results of the experiment are shown in Table 1. Growth of chicks was significantly reduced by treatment regardless of the method of administration. Preparations of microsomal subcellular fraction of liver were analyzed for fatty acid composition of membrane total lipids, and the concentrations of AA and its n-6 precursor LA are shown, as is the ratio of AA:LA. Lead exposure by either route doubled the proportion of AA in microsomal fatty acids. The AA:LA ratio was also significantly increased. This ratio necessarily reflects the concentration of LA, which exhibited a mixed response to Pb. The presence of Pb, but not its form of delivery, also affected the concentrations of de novo synthesized saturated (C16:0 and C18:0) and monounsaturated (C16:1 n-7 and C18:1 n-9) fatty acids. The C18:0 content increased 32% in Pb-treated chicks, whereas C18:1 content was decreased 53% by Pb (data not shown). Microsomal peroxidation, assessed as TBARS, paralleled the concentration of membrane polyunsaturated AA, such that production of malondialdehyde in microsomes from all Pb-treated birds was more than double that of controls, regardless of the route of the Pb intoxication.

Growth inhibition due to Pb poisoning has been observed repeatedly in chicks, and is considered a sensitive measure of the extent of intoxication (Donaldson and Leeming, 1984). In those studies, the route of exposure was oral via dietary Pb. Growth response to the toxin may be mediated by changes in appetite of treated animals, or by changes in the quality of Pb-containing feed. Hammond and Succop (1995) have suggested that the reduced growth of oral Pb-treated rats is largely attributable to reduced appetite and feed intake, and can be substantially improved by force-fed calorie supplementation. Donaldson (1991) demonstrated that 80% of the reduction in chick body weight caused by dietary Pb could be restored by addition of the antioxidant ethoxyquin to the Pb-containing feed. Whether effects of Pb other than growth depression can be similarly ameliorated has not been established.
The current study clearly shows that altered tissue fatty acid composition caused by Pb is not related to the method of oral Pb administration. Consequentially, any Pb-catalyzed peroxidation of lipids in feed appears to be of only slight importance in producing this symptom of Pb toxicity. Although the nature of the metabolic mechanism by which Pb increases tissue levels of AA remains unknown, the most likely explanation is changes in the activities of the fatty acid elongation and Δ6- and Δ5-desaturation systems responsible for conversion of diet-derived LA to AA. Enhanced AA content probably accounts for the increased malondialdehyde production observed in hepatic microsomes from Pb-treated birds, because elevated levels of this highly unsaturated fatty acid increase the susceptibility of membranes to peroxidation (Tappel, 1954).

Numerous reports describing oxidative mechanisms of transition metal and Pb toxicities have been reviewed by Stohs and Bagchi (1995). Many of these studies treated animals orally with metal-supplemented drinking water or feed, but did not demonstrate that the oxidative effects were unrelated to the amount of peroxides consumed. Peroxide content of fresh, unsupplemented feed is typically very low, but addition of Pb increases peroxides substantially, by 300% in the 1,000 ppm Pb diets evaluated by Donaldson (1991). That report also showed that the antioxidant ethoxyquin prevented Pb-induced peroxide formation in the feed and ameliorated the growth depression from Pb. However, there were indications that the beneficial effects of ethoxyquin were systemic, and thus, were a reflection of reduced tissue peroxides rather than of reduced feed peroxides. In the data reported here, we have shown that tissue peroxide levels are independent of the method of Pb administration and thus, by inference, independent of peroxide content of the feed.

REFERENCES


