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Excessive ethanol consumption under exposure to lead intensifies disorders in bone metabolism: A study in a rat model

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ABSTRACT

It was investigated whether ethanol (Et) modifies the damaging impact of lead (Pb) on bone metabolism in a rat model reflecting excessive alcohol consumption by humans exposed to relatively high levels of this metal. For this purpose, markers of bone formation (osteocalcin, procollagen I, osteoprotegerin, alkaline phosphatase) and resorption (telopeptides of collagen I, soluble receptor activator of nuclear factor-κB ligand), calciotropic hormones (parathormone, calcitonin, 25-hydroxyvitamin D and 1,25-dihydroxyvitamin D) in the serum, and the femur content of mineral (including calcium – Ca and inorganic phosphorus – P_i) and organic components were estimated in the rats exposed to 500 mg Pb/l (in drinking water) or/and Et (5 g/kg b.wt./24 h, by oral gavage) for 12 weeks. Moreover, Ca and P_i in the serum and urine, alkaline phosphatase in the bone tissue and Pb in the blood and femur were determined. The exposure to Pb or/and Et decreased bone formation and increased its resorption resulting in the bone demineralization. These effects were accompanied by destroying the hormonal regulation of mineral metabolism, and Ca and P_i imbalance. The co-exposure to Pb and Et-induced disorders in bone metabolism were more advanced than those caused by Pb alone. Et co-administration increased Pb concentration in the blood and decreased its accumulation in the bone. This paper is the first report providing evidence that consumption of Et under exposure to Pb intensifies disorders in bone metabolism and that destroying of the receptor activator nuclear factor-κB (RANK)/RANK ligand/osteoprotegerin system is involved in the mechanisms of interactive action of these xenobiotics on the skeleton. The modifying impact of Et may be an effect of its independent osteotropic action and interaction with Pb. Based on the results it can be concluded that alcohol abuse by subjects excessively exposed to Pb considerably increases the risk of bone damage.

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1. Introduction

Lead (Pb) belongs to the most toxic and widely distributed heavy metals [1–4]. Due to its numerous technical uses leading to pollution of the natural and occupational environment inhabitants of industrialized countries are exposed to this metal during the lifetime [1,2,4,5]. The main source of the general population exposure to Pb is food [4,6] and tobacco smoking [7,8]. Moreover, numerous workers all over the world are exposed to this metal also occupationally [5,9–15]. Pb toxicity for human has been relatively well known and widely reported [1–4,10,11,14–22]. Chronic exposure to this toxic metal causes a broad range of various disorders and dysfunctions, including anemia, damage to the nervous (especially central nervous system among children), immunological and cardiovascular systems, destroying of reproduction and pregnancy as well as kidney and skeleton damage [10,11,14–22]. Early life exposure to Pb can result in cognitive and behavioral deficits in children and adults [18]. Epidemiological studies provide evidence that this metal creates a risk for the health even at a relatively low

Abbreviations: ALP, alkaline phosphatase; AW, ash femur weight; AW/DW, ratio of ash to dry defatted femur weight (fraction of the dry defatted femur weight formed by minerals); AW/OW, ratio of mineral and organic components in femur; b_C-ALP, ALP in the bone tissue at the femoral diaphysis (cortical bone region); b_T-ALP, ALP in the bone tissue at the distal femoral end (trabecular bone region); Ca, calcium; Ca/DW, ratio of the femur Ca content and dry defatted femur weight (fraction of the dry defatted femur weight made up of Ca); Ca/AW, ratio of the femur Ca content and ash weight (fraction of the femur minerals made up of Ca); Ca/P_i, molar ratio of Ca and inorganic phosphorus content in femur; CT, calcitonin; CTX, carboxy-terminal cross-linking telopeptides of type I collagen; DW, dry defatted femur weight; Et, ethanol; OC, osteocalcin; OPG, osteoprotegerin; OW, femur organic components weight; Pb, lead; PINP, amino-terminal propeptides of type I collagen (procollagen I); PTH, parathormone; s-ALP, ALP in serum; sRANKL, soluble receptor activator of nuclear factor-κB ligand; WW, wet femur weight; 1,25$(OH)_2$D, 125-dihydroxyvitamin D; 25OHD, 25-hydroxyvitamin D; % mineral comp., percentage content of mineral components in femur; % Ca, percentage Ca content in femur; % organic comp., percentage content of organic components in femur; P_i, inorganic phosphorus; % P_i, percentage P_i content in femur.

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exposure [2,18–22]. Moreover, increased mortality associated with exposure to Pb has been noted [3,10,15].

Bone is the main place of Pb storage in the body [17,23]. This metal accumulates in the skeleton during the lifetime and affects bone tissue directly via influencing hydroxyapatite formation and bone cells activity as well as indirectly via disturbing mineral metabolism [16,23–29]. Bone damage is one of the main adverse health effects of chronic intoxication with Pb in both human [10,11,16,17] and experimental animals [23,24,26,29]. It has been known that occupational exposure to this heavy metal is associated with the risk of osteopenia and osteoporosis [10,11]. Moreover, Pb has been recognized as an environmental risk factor for osteoporosis [16]. Thus, growing interest has been focused on the bone effects of this metal. However, the damaging impact of Pb on the skeleton, including mechanisms of this action, risk of bone injury and factors modifying the bone effect of this metal are still not completely recognized.

It has been known that Pb accumulation in the organism and some effects of its action may be modified by various factors, including gender, age, lifestyle and diet as well as concomitant exposure to other xenobiotics [23,26,30–32]. A possible factor capable of modifying the bone impact of Pb seems to be ethanol (ethyl alcohol; Et). The available data shows that Et influences Pb metabolism and some effects of its toxic action, including liver, kidney and brain damage [23,30–32]. Chronic Et consumption has been reported to increase the blood Pb concentration in human [9] and experimental animals [23,30,31], but the data is sparse. Both synergistic and antagonistic interactions between these xenobiotics have been reported in animals [31,32]. However, the available data [30,31] show that damage to various organs and systems is more serious at co-exposure to Pb and Et than at the treatment with Pb alone, except for the study by Lukacs et al. [32] who have reported a protective Et impact regarding Pb neurotoxicity.

Et, like Pb, is a known risk factor for bone damage [33–43]. The injurious effect of Et on the skeleton consists in an inhibition of bone formation and stimulation of its resorption via direct and indirect action on the bone cells and disturbing mineral metabolism [34,36,38–43]. Chronic excessive Et consumption has been noted to cause osteopenia, osteoporosis or osteomalacia in human [35,37,41,42] and experimental animals [38,39,41]. On the other hand, low Et consumption has been reported to be beneficial to the bone health in men and postmenopausal women [44] as well as in rats [45]. Excessive Et consumption has been noted to decrease the bone content of Pb in rats [23]; however, the impact of this alcohol abuse under exposure to Pb on the skeleton and possible mechanisms of this influence have not been investigated up to date.

Owing to the growing prevalence of bone diseases in industrialized countries [46,47], wide Pb distribution [1–4] as well as excessive consumption of alcohol beverages by marked part of the general population [48,49] and thus the very real possibility that alcohol abusers may be exposed to excessive Pb amounts from environmental and occupational sources or via tobacco smoking, it is very important to recognize the impact of co-exposure to these xenobiotics on the skeleton. Therefore, we have undertaken a complex study, on a rat model of relatively high human exposure to Pb and Et, to explain if, to what extent and via which mechanisms excessive Et consumption under the exposure to Pb may modify the risk of bone damage. Taking into account the osteotropic action of the both xenobiotics [16,23–30,33–43] we have hypothesized that this alcohol consumption under exposure to Pb could potentiate bone damage. The present paper is the first report from these studies and it was aimed to investigate the impact of Et consumption during exposure to Pb on bone metabolism. For this purpose, markers of bone formation and resorption were determined to estimate the rate of bone turnover, and mineral status of the femur, including calcium (Ca) and inorganic phosphorus (P_i) content, was evaluated. Moreover, in order to explain possible mechanisms of Pb or/and Et action on the skeleton, indices of Ca and P_i homeostasis, and calciotropic hormones as well as Pb concentration in the blood and bone tissue were determined. The involvement of the receptor activator of nuclear factor-κB (RANK)/RANK ligand (RANKL)/osteoprotegerin (OPG) system in the mechanisms of Pb or/and Et impact on the bone tissue was evaluated as well. The RANK/RANKL/OPG system plays a crucial role in the regulation of bone turnover and its disturbed balance may lead to a severe dysfunction of bone remodeling [50]. Et has been known to destroy the RANK/RANKL/OPG system [33,34,43]; however, the involvement of this system in the mechanisms of Pb impact on the skeleton and its interactive action with Et is unknown. In the case when an interactive effect of Pb and Et was revealed attempts were made to recognize possible character of the action (synergism, antagonism). According to our knowledge similar study has not been conducted up until now.

2. Materials and methods

2.1. Chemicals and reagents

All reagents and chemicals used in this experiment were of analytical grade and higher purity. Lead acetate ($(CH_3COO)_2Pb$), sodium chloride (NaCl), potassium dihydrogen phosphate (KH_2PO_4), di-potassium hydrogen phosphate (K_2HPO_4), diisopropylether, isopropanol, hexane, chloroform and methanol were purchased from POCh (Gliwice, Poland). Physiological buffered saline (PBS) was received from Biomed-Lublin (Lublin, Poland) and lanthanum chloride hydrate ($LaCl_3 \times H_2O$) from Fluka Chemie AG (Buchs, Switzerland). Rectified spirit (95% v/v) was provided by Polmos (Lublin, Poland). Vetbutal was obtained from Biowet (Pulawy, Poland) and heparin from Biochemie GmbH (Kundl, Austria). Butylhydroxytoluene (BHT) and bovine albumin were purchased from Sigma–Aldrich Chemie, Gmbh (Steinheim, Germany) and acetonitrile from Merck (Darmstadt, Germany). The commercial kits used to measure the concentrations of osteocalcin (OC), amino-terminal propeptides of type I collagen (PINP), carboxy-terminal cross-linking telopeptides of type I collagen (CTX), parathormone (PTH) and calcitonin (CT) were received from Nordic Bioscience Diagnostic (Herlev, Denmark), Uscn Life Science Inc. (Wuhan, China), Immunodiagnostic Systems Ltd (Boldon, UK), Immutopics, Inc, (San Clemente, CA, USA), and Peninsula Laboratories, LLC (San Carlos, CA, USA), respectively. The concentrations of soluble RANKL (sRANKL), OPG, 25-hydroxyvitamin D (25OHD) and 1,25-dihydroxyvitamin D ($1,25(OH)_2D$) were measured with kits by Immundiagnostik AG (Bensheim, Germany). The diagnostic kits for determination of alkaline phosphatase (ALP) and P_i were purchased from BioMaxima (Lublin, Poland). Trace-pure concentrated nitric (69% HNO_3) and hydrochloric (36% HCl) acids (Merck, Darmstadt, Germany) and stocks of standard solutions of Pb and Ca designed for atomic absorption spectrometry (AAS method; Sigma, St. Louis, MO, USA) were applied in metals analysis. Palladium nitrate ($Pd(NO_3)_2$ in HNO_3) and magnesium nitrate ($Mg(NO_3)_2 \times 6\ H_2O$ in HNO_3), used as matrix modifiers in Pb determination, were obtained from Merck. In order to check the analytical quality of Pb and Ca measurements, the following certified materials were used: Bovine Blood Reference Material BCR-195 (No. 2551; Institute for Reference Materials and Measurements, Geel, Belgium), Trace Elements Serum level 1 (No. 201405; Seronorm™, Billingstad, Norway), Standard Reference Bone Ash (No. 1400; National Institute of Standards and Technology, Gaithersburg, MD) and Trace Elements Urine level 1 (No. 2524; Seronorm™, Billingstad, Norway). Ultrapure water, received from two-way water purification MAXIMA system (ELGA, Bucks, UK), was used in all measurements.

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