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Alveolar bone loss associated to periodontal disease in lead intoxicated rats under environmental hypoxia

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ABSTRACT

Previously reported studies from this laboratory revealed that rats chronically intoxicated with lead (Pb) under hypoxic conditions (HX) impaired growth parameters and induced damages on femoral and mandibular bones predisposing to fractures. We also described periodontal inflammatory processes under such experimental conditions. Periodontitis is characterised by inflammation of supporting tissues of the teeth that result in alveolar bone loss. The existence of populations living at high altitudes and exposed to lead contamination aimed us to establish the macroscopic, biochemical and histological parameters consistent with a periodontal disease in the same rat model with or without experimental periodontitis (EP). Sixty female rats were divided into: Control; Pb (1000 ppm of lead acetate in drinking water); HX (506 mbar) and PbHX (both treatments simultaneously). EP was induced by placing ligatures around the molars of half of the rats during the 14 days previous to the autopsy. Hemi-mandibles were extracted to evaluate bone loss by histomorphometrical techniques. TNFα plasmatic concentration was greater ($p < 0.01$) in Pb and HX animals. TBA-RS content was significantly higher in gums of rats with or without EP only by means of Pb. The SMG PGE_2 content increased by Pb or HX was higher in PbHX rats ($p < 0.01$). Pb and HX increased EP induced alveolar bone loss, while Pb showed spontaneous bone loss also. In conclusion, these results show that lead intoxication under hypoxic environment enhanced not only alveolar bone loss but also systemic and oral tissues inflammatory parameters, which could aggravate the physiopathological alterations produced by periodontal disease.

1. Introduction

More than 200 million people live at altitudes above 2500 m around the world frequently exposed to environmental pollutants. Among these, lead (Pb) is of particular interest because of its wide distribution in the environment. Previously reported studies from this laboratory suggested that chronic intoxication with Pb in immature rats under hypoxic conditions impaired growth parameters and induced negative effects on femoral and mandibular structural properties decreasing their maximal load supported at fracture and their

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energy absorption capacity so that plastic deformation and failure in bone tissue structure occurred under lower loads.[1] Chronic exposure to lead (Pb) significantly affects oral health condition among exposed workers increasing the prevalence of periodontal diseases (gingivitis and periodontitis) and in the prevalence of decay (caries), missed and filled teeth and dental abrasions.[2,3] Epidemiologic investigations in high altitude territory showed that the hypoxia environment plays an important role in the pathogenesis of periodontitis and it was demonstrated in rats that the course of periodontitis in altitude hypoxia environment is later than normal, but the degree of periodontal lesion was more severe.[4] Periodontitis is characterised by inflammation of the supporting tissues of the teeth and periodontal pocket formation that result in alveolar bone resorption and soft tissue attachment loss. It has been suggested that the submandibular gland is a key regulatory organ in the oral neuroimmunoregulatory network.[5] We recently reported in growing rats chronically intoxicated with lead (Pb) and exposed to the stress of intermittent hypobaric hypoxia (HX) deleterious effects on periodontal and dental tissues that could be due to a greater vulnerability of them to inflammatory processes.[1] Furthermore, it was demonstrated that animals co-exposed to lead and fluoride show more severe damage than lead alone,[6] which suggests the complex interaction between lead and other environmental factors and the importance of studying them together. These findings and the existence of populations living at high altitudes and exposed to lead contamination aimed us to establish in the same rat model, the macroscopic, biochemical and histological parameters consistent with a periodontal disease and whether they are or not enhanced in rats submitted to experimental periodontitis (EP) The results will try to cover the lack of information regarding the oral health in individuals living in lead contaminated high altitude areas.

2. Materials and methods

2.1. *Animals, treatments and induction of periodontal disease*

Sixty Wistar rats were randomly divided into 4 groups: C (control); Pb (intoxicated with 1000 ppm of lead acetate in drinking water for 3 months)[7]; HX (exposed to 18 h/d by placing the animals into a chamber at 506 mbar for 3 months)[8] and PbHX (both treatments simultaneously). EP was induced in half of the animals of each group under general anaesthesia with a mixture of 2% xylazine hydrochloride (5 mg/kg; i.p. König Laboratories) and 5% ketamine hydrochloride (50 mg/kg; i.p. Holliday-Scott SA) by placing a cotton thread ligature around the neck of the first lower molars (FLM) during the 14 days previous to the autopsy.[9] At the end of the experimental period, blood samples where obtained by cardiac puncture to asses hematocrit by micromethod. Pb content in bone ashes was determined using an atomic absorption spectrophotometer Varian AA 475.

All animals were treated in accordance with the guidelines and regulations for the use and care of animals of the University of Buenos Aires Ethic Committee and were carried out in accordance with the guidelines of the National Institute of Health (NIH). The use of animal tissues followed an approved animal use protocol.

2.2. *Measurement of proinflammatory and oxidative stress markers*

At the end of the experimental period blood samples were collected to asses hematocrit by micromethod and lead content using an atomic absorption spectrophotometer Varian AA 475. TNFα concentration was determined using a sandwich ELISA according to the manufacturer's instructions (BD Pharmingen, USA).[10]

Thiobarbituric acid reactive substances (TBA-RS) were evaluated in gingival tissues and SMG quantifying malondialdehyde (MDA) as the product of lipid peroxidation that reacts with trichloroacetic acid-HCl, yielding a pink-stained TBA-RS determined in a spectrophotometer (Hitachi U-2001) at 540 nm. TBA-RS were calculated as nanomoles per milligramme of tissue.[11]

2.3. *Radioimmunoassay of PGE2*

To evaluate PGE content, submandibular glands were homogenised in 1.5 ml ice cold ethanol (100%), centrifuged at 10,000 × *g* for 15 min at 4 °C, and the supernatant were collected and evaporated in a Speed-Vac. The residues were re-suspended with radioimmunoassay buffer and the Sigma antiserum was used. The PGE content was expressed as pg/mg of weight tissue.[12]

2.4. *Microscopic examination of periodontal bone loss: distance method*

After autopsy, the hemi-mandibles were resected, defleshed and stained with 1% aqueous methylene blue to delineate the cemento-enamel junction (CEJ) and the alveolar crest (AC).

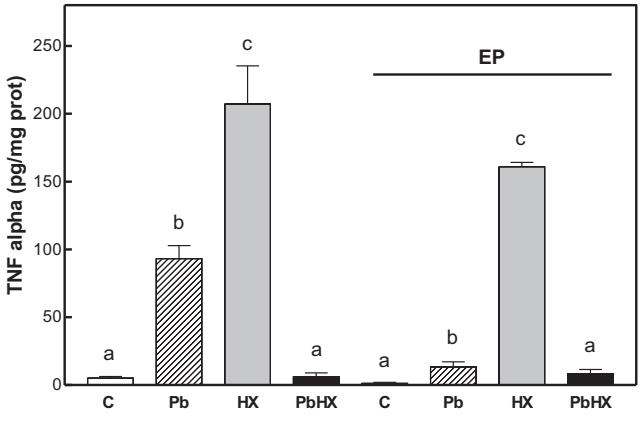

Fig. 1 – Effect of Pb (1000 ppm of lead acetate in drinking water for 3 months); HX (18 h/d at 506 mbar for 3 months) and PbHX (both treatments simultaneously) on TNFα release, in rats with and without EP. Line in graph show animals submitted to EP. Mean ± SD of 15 rats. Equal letters indicate no significant differences. A significant difference between groups was chosen as $p < 0.01$ determined by two-way ANOVA followed by Student–Newman–Keuls Multiple Comparison Test.