Autonomic Function Evaluation in an Intermittent Lead Exposure Animal Model

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Abstract — Lead (Pb) is a toxic metal, and its widespread use has resulted in environmental contamination and significant public health problems. The autonomic nervous system, the main homeostatic controller, is impaired due to acute and chronic lead exposure, with a remarked sympathoexcitation, resulting in hypertension, tachypnea, alongside baroreflex and chemoreflex dysfunction. However, up to date, no studies have described the autonomic effects of an intermittent low-level lead exposure. Therefore, in the present work, we addressed *in vivo*, autonomic modulation in Wistar rats, under chronic Pb exposure and intermittent Pb exposure. Arterial blood pressure (BP) and ECG were recorded in 28 weeks old animal and low frequencies (LF) and high frequencies (HF) were determined (to estimate sympathetic and parasympathetic activities, respectively) using FisioSinal software with Wavelet module. Preliminary results: Rats intermittently exposed to lead showed a significant decrease in systolic BP (126 ± 4 vs 144 ± 3 mmHg) with no significant changes in LF, HF and LF/HF bands (2.4 ± 0.7 vs 1.5 ± 0.3 mmHg^2, 10.12 ± 7.4 vs 7.9 ± 6.1 bpm^2 and 0.9 ± 0.33 vs 1.0 ± 0.39 mmHg^2/bpm^2) when compared to chronically Pb exposed rats. Our data suggests that the autonomic dysfunction induced by lead exposure, is similar in a chronic and intermittent Pb exposure. Nevertheless, it seems that an intermittent exposure has no effect on systolic BP values, since they maintain BP values in the normotensive range.

Keywords: Lead toxicity, autonomic activity, Fisiosinal, Wavelet analysis, Heart Rate Variability

I. INTRODUCTION

Lead is the most commonly used heavy metal worldwide for over 8000 years in a large amount of industries like automobiles, ceramics, paint, plastics, on behalf of its unique properties such as high malleability, low melting point, softness, ductility and resistance to corrosion [1, 2]. Regarding this wide usage, it is obvious that there is a rise in environmental free lead and its occurrence in biological systems regardless the non-biodegradable nature of this heavy metal. Lead toxicity, due to its ingestion, inhalation or, less probably by direct contact, can evoke irreversible effects in a wide amount of body functions, affecting mainly the cardiovascular [3, 4], hematopoietic [1, 5], reproductive [6] and renal [7] systems. Being easily accumulated in bone and soft tissues, lead is also a neurotoxin, inducing neurodegeneration and cognitive changes due to nervous cells demyelization and its ability to substitute cations [1, 8, 9]. Moreover, the exposure to toxic levels of lead produce a significant increase in sympathetic activity with high blood pressure, decreased baroreflex function, increased chemoreceptor sensitivity and tachypnea [10].

However, up to date, no studies were performed to establish the autonomic effects due to an intermittent low-level lead exposure. This type of exposure is growing due to the increased migration and to the implementation of school exchange programs. Nevertheless, people tend to come back to their hometowns after some years abroad, returning to their familiar environment. Additionally, in workplaces where the use of lead is recurrent, rotation of workers to other functions without lead exposure has been performed during the past years, as a possible prevention of adverse health effects caused by occupational permanent low-level lead exposure. To our knowledge, no studies have been performed establishing an intermittent low-level lead exposure animal model and this is the first study regarding the autonomic effects due to intermittent low-level lead exposure.

II. METHODS

A. Development of the animal model of chronic and intermittent lead exposure

Seven day pregnant Wistar rats were exposed to lead during pregnancy, via water containing Lead acetate (0.2% w/v) that replaced tap water. After birth and weaning at 21 days, the rats of both sexes were exposed to the same lead solution as that of their mother and were divided into 2 groups (n = 6/group; Pb1 and Pb2). At 12 weeks, the intake of the solution ended in both groups, followed by a period without exposure that lasted up to 20 weeks. The Pb2 group underwent a second exposure which lasted 8 weeks (named intermittent lead exposure group) while Pb1 wasn't exposed to lead for the second time (named chronic lead exposure group).

B. Surgical protocol

At 28 weeks, the acute experiment was carried out in both groups of animals (n=12). Animals of both sexes were anesthetized with sodium pentobarbital (60mg/kg, ip). The femoral artery and vein were cannulated for blood pressure monitoring and injection of saline and drugs, respectively. Rectal temperature was maintained between 36.5-39ºC. The electrocardiogram (ECG) was recorded with subcutaneous electrodes inserted in three of the four members and heart rate was also obtained through a registration method (Neurolog, Digitimer).

C. Data acquisition

Signals were amplified, filtered and acquired at 1 KHz (Neurolog, Digitimer; PowerLab). All signals were converted to digital form and stored for further analysis.

D. Analysis of heart rate and systolic blood pressure variability

Using our in-house software, FisioSinal [11] with Wavelet module, the LF, HF and LF/HF indexes were determined in basal conditions. Low frequencies (LF; 0.15-0.6Hz) obtained from systolic BP indicate sympathetic activity, high frequencies (HF; 0.6-2.0Hz) attained from R-R interval represent both parasympathetic and respiratory variations, and LF/HF is the ratio between sympathetic and parasympathetic systems. In this study, considering that the respiratory rate was higher in most animals, we adjusted the maximum value for the high frequencies band (HF) 2.4Hz.

E. Statistical analysis

Variables are expressed as mean ± SEM. Statistical data was obtained by comparisons between groups of animals – Pb1 and Pb2 – using t-test. Significance was considered to $p<0.05$. Data was analysed using GraphPad Prism 6 software (GraphPad Software, Inc., USA).

III. RESULTS

A. Cardiovascular variables and autonomic tone

Baseline levels of all measured physiological parameters were similar between the 2 groups (Mean Blood Pressure (BP): 125 ± 4 vs 116 ± 3 mmHg; Diastolic BP: 110 ± 5 vs 106 ± 3 mmHg; Basal Heart Rate: 371 ± 8 vs 404 ± 17 bpm), except for systolic blood pressure with significantly higher values (144 ± 3 vs 126 ± 4 mmHg, $p<0.05$) in Pb1-rats compared with Pb2-rats. The sympathetic and parasympathetic tone evaluated through LF and HF variability, respectively, as well as the LF/HF ratio, did not change significantly between the two groups of animals. LF values were 2.4 ± 0.7 $mmHg^2$ in Pb1 and 1.5 ± 0.3 $mmHg^2$ in Pb2, HF 10.12 ± 7.4 and 7.9 ± 6.1 bpm^2 (Pb1 and Pb2, respectively) and finally, the LF/HF ratio, 0.9 ± 0.33 $mmHg^2/bpm^2$ in Pb1 and 1.0 ± 0.39 $mmHg^2/bpm^2$ in Pb2.

IV. DISCUSSION

Current study is the first to address a new profile of lead exposure, intermittent low-level lead exposure, thus providing a new insight into the association between the autonomic function and lead intoxication. Indeed, our preliminary results reveal an association between intermittent low-level lead exposure and sympathetic hyperactivity with no effect no blood pressure values.

In conclusion, the present study brings new insights into how different lead poisoning profiles may influence autonomic and cardiovascular systems during developmental phases, which can help upraise public policy strategies to prevent and control the adverse effects of Pb toxicity.

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