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Research Article

Haematological and Ponderal Changes Connected to Administration of Lead Acetate and Efavirenz in Wistar Rats

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Abstract: Heavy metals and antiretroviral drugs are both types of xenobiotics that induce disturbances in the body. This study aims to assess the hematological and body weight changes connected to simultaneous exposure to lead and Efavirenz. Twenty-eight rats equally divided into four groups were force-fed once daily with distilled water (G_{Ctrl}), lead acetate at 10 mg/kg (G_{Pb}), Efavirenz at 20 mg/kg (G_{Efv}) and lead acetate + Efavirenz (G_{Pb+Efv}). On Day 0, Day 14 and Day 28, the rats were weighted and retro-orbital blood collection was carried out for haemogram analysis. Results showed a significant decrease in hemoglobin level, haematocrit, erythrocytes and thrombocytes in G_{Pb} and G_{Pb+Efv}. This anaemia has tended to be normocytic hypochromic. There was no significant difference in the rates of decrease between G_{Pb} and G_{Pb+Efv}. No significant change was observed in the erythrocyte parameters in G_{Efv} and $G_{Ctrl.}$The mean body weight of rats was significantly increased in G_{Ctrl} already from Day 7 while in G_{Efv}, it is at from 21 day that, the weighty growth reached a significant level. There was no significant increase in body weight growth in G_{Pb} and $G_{Pb+Efv.}$ In conclusion, contrary to lead, Efavirenz has not induced anaemia but it has slowed the body growth with a less intensity than lead. The co-administration of lead and Efavirenz did not induced significant additive effects comparatively to lead administration alone. Further studies are necessary for verifying whether co-exposure to these two xénobiotics for a longer period would not be likely to induce a significantly higher toxicity.

Keywords: Body weight, Efavirenz, haematological parameter, lead, wistar rat

INTRODUCTION

Several recent studies worldwide evoke an outbreak of the environmental factors having negative impacts on the human health. Among the latters, heavy metals including lead appears top on the list (Chinwe *et al*., 2010; Elégbédé *et al*., 2012; Guédénon *et al*., 2012).

Lead is object of big concerns because of its high potential of bioaccumulation and bioamplification in the trophic chain (Vighi, 1981; Dallinger *et al*., 1987). It was found at higher levels than maximum allowable limits in foodstuffs such as cereals (Fangnon *et al*., 2012), vegetables (Koumolou *et al*., 2013), halieutic products (Guédénon *et al*., 2012) as well as in drinking water (Elégbédé *et al*., 2012) and herbal preparations (Arpadjan *et al*., 2008; Annan *et al*., 2010).

Besides, lead exposure is associated with several organic disturbances including at neurological (Larroque and Marret, 2000), haematological (Schwartz *et al*., 1990), reproductive (Sokol *et al*., 2002) and growth levels (Ronis *et al*., 1998; Schwartz, 1992). Its toxicity is more pronounced among people vulnerable to deficiencies in antioxidant (Saka *et al*., 2011), thus probably in People Living with Human Immunodeficiency Virus who are endlessly exposed to antiretroviral drugs (ARVs) with their toxic effects (Adikwu *et al*., 2013; World Health Organization, 2010).

That is why, the present study was led to assess specifically the haematological and body weight disturbances induced by the lead acetate and an antiretroviral drug (Efavirenz), taken alone or simultaneously in Wistar rat. Efavirenz is chosen because of its strong recommendation for HIV treatment in low income countries such as those of sub-Saharan Africa (World Health Organization, 2013).

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MATERIALS AND METHODS

Experimental design: Twenty-eight Wistar rats aged 2 to 4 weeks were acclimated for 8 weeks in a well aerated room at Research Laboratory of Applied Biology, located to the University of Abomey-Calavi. After this period, they were weighed then distributed in a random way in 4 groups consisted of 7 animals each.

They were given free access to standard rat's diet and water. The light and darkness was alternated by 12-h cycle. Cages were identified according to the following different exposure regimes:

- Group G_{Ctrl} = Control group where rats received 0.5 mL of distilled water
- Group G_{Pb} = Rats treated with 10 mg/kg body weight (bw) of lead acetate
- Group G_{Efv} = Rats treated with 20 mg/kg bw of Efavirenz
- Group G_{Pb+Efv} = Rats treated with 10 mg/kg bw of lead acetate and 20 mg/kg bw of Efv.

Forced-feeding and observation of animals: A lead acetate solution at 10 mg/mL and Efavirenz (Aviranz 200 mg) diluted daily in distilled water so as to obtain a 5 mg/mL solution were used. Exposure of rats to these xenobiotics was carried out by force-feeding every morning between 7:00 am and 8:30 am for 28 days. Animals were every day observed at the time of the force-feedings but at the end of each week, a more thorough clinical examination preceded by a measurement of weight was conducted in order to detect possible signs of physical or behavioural disorders.

Blood collection and haematological analyses: on Day 0, Day 14 and Day 28, all the rats were anesthetized and weighted then blood samples were collected from retro-orbital plexus of each. Hematological analyzes were performed on non-coagulated blood (containing EDTA) using haematology auto analyzer Horiba ABX micros ES 60.

Statistical analyses: haematological and ponderal values were expressed as mean ± SD after an initial checking of normality and homogeneity of variance thanks to the Levene's test performing with SPSS 16.0 software. Differences between treated rats and control rats at different periods were evaluated using a One-Way Analysis of Variance (ANOVA) followed by the Bonferroni multiple comparisons test. The rate of change (decrease or increase) for each parameter was calculated in percentage (%). All the differences or changes were considered as significant when the p value is lower than 0.05.

RESULTS

Haemoglobin level: 28 days after exposure, the mean of haemoglobin decreased significantly by 23.1 % in G_{Pb} ($p = 0.00087$) and by 22.5 % in G_{Pb+Efv} ($p = 0.00028$). This decrease was perceptible on Day 14 when it reached respectively 11.5 % ($p = 0.04868$) and 9.6 % ($p = 0.03877$) in these two groups (Table 1). The difference between the rate of decrease in G_{Pb} compared to $G_{Pb+Efv}$was not significant.

Haematocrit: The mean of haematocrit was significantly decreased by 12.8% in G_{Pb} ($p = 0.00204$) and by 13.0% in G_{Pb+Efv} ($p = 0.00246$). There was no significant difference in the rate of decrease between G_{Pb} and G_{Pb+Efv}. No significant change was observed in G_{Efv} and G_{Ctrl} (Table 1).

Erythrocytes number: The mean of Erythrocytes number decreased significantly by 24.5% in G_{Pb} ($p = 0.00288$) and by 26.9% in G_{Pb+Efv} ($p = 0.00499$). The

Table 1: Changes in haemoglobin level, haematocrit and erythrocytes number in control rats and in rats exposed to xenobiotics

Parameters		Period	G_{Ctrl}	G_{Pb}	G_{Efv}	G_{Pb+Efv}
Haemoglobin (g/dL)	m±SD	D0	$13.9±0.8^{a}$	$13.7±0.9^{a}$	$13.1±0.8^{a}$	$14.1±0.8^{a}$
		D14	$13.6±1.0^{a}$	$12.2±0.9^{b*}$	$12.2±1.3^{a}$	$12.7±0.6^{b*}$
		D28	$13.3±0.9^{a}$	$10.6±1.0^{b***}$	$12.2±1.0^{a}$	$10.9±0.9^{b***}$
	% de Δ	D0-D14	-2.5%	-11.5%	-6.5%	-9.6%
		D14-D28	-1.8%	-13.1%	-0.7%	-14.3%
		D0-D28	-4.3%	-23.1%	-7.1%	-22.5%
Haematocrit (%)	m±SD	D0	$39.2±1.9^{a}$	$38.7±2.8^{a}$	$37.0±2.6^{a}$	$37.8±2.6^{a}$
		D14	$39.6±3.0^{a}$	$36.1±3.3^{b*}$	$36.3±4.1^{a}$	$35.8±4.9^{a}$
		D28	$39.3±2.5^{a}$	$33.7±2.0^{b**}$	$35.8±3.2^{a}$	$32.9±3.7^{b**}$
	% de Δ	D0-D14	1.0%	-6.6%	-1.8%	-5.1%
		D14-D28	-0.7%	-6.6%	-1.5%	-8.3%
		D0-D28	0.3 %	-12.8%	-3.3%	-13.0 %
Erythrocyte (T/L)	m±SD	D0	$7.52±0.73^{a}$	$7.23±1.25^{a}$	$6.98±0.85^{a}$	$7.01±0.51^{a}$
		D14	$6.94±0.96^{a}$	$6.72±1.10^{a}$	$7.02±1.30^{a}$	$6.24±0.46^{b*}$
		D28	$7.05±0.48^{a}$	$5.45±0.95^{b**}$	$6.15±0.92^{a}$	$5.12±0.70^{b**}$
	% de Δ	D0-D14	-7.7%	-6.9%	0.6%	-11.0%
		D14-D28	1.5 %	-18.9 %	-12.5 %	-17.9 %
		D0-D28	-6.2 %	-24.5 %	-11.9 %	-26.9 %

$^{*}p<0.05$; $^{**}p<0.001$; $^{***}p < 0.001$; Values of Day 0 with the same letter (^{a}) are not significantly different. Values of Day 14 and Day 28 with letter (^{b}) are statistically different from those of Day 0 in the same column; D = Day; % of Δ = rate of change; m±SD = mean±standard deviation

Table 2: Changes in red blood cell indices in control rats and in rats exposed to xenobiotics

Parameters		Period	G_{Ctrl}	G_{Pb}	G_{Efv}	G_{Pb+Efv}
MCV (fl)	m±SD	D0	52.3±2.9[a]	53.9±3.2[a]	53.3±3.7[a]	54.1±5.1[a]
		D14	57.4±4.7[a]	54.3±5.4[a]	52.4±6.3[a]	57.7±9.8[a]
		D28	56.0±5.7[a]	63.2±10.3[a]	58.7±4.7[a]	65.8±16.6[a]
	% de Δ	D0-D14	9.9%	-0.8%	-1.7%	6.7%
		D14-D28	-2.8 %	16.3%	11.9 %	14.0%
		D0-D28	7.1 %	17.2%	10.2 %	21.7%
MCH (pg)	m±SD	D0	18.7±2.4[a]	19.3±2.4[a]	18.9±2.1[a]	20.1±1.5[a]
		D14	19.8±2.7[a]	18.5±3.7[a]	17.9±3.4[a]	20.5±2.3[a]
		D28	19.0±2.3[a]	19.9±4.1[a]	20.0±2.0[a]	21.4±1.7[a]
	% de Δ	D0-D14	6.1%	-4.3%	-5.3%	1.9%
		D14-D28	-4.0 %	7.7%	11.5%	4.5%
		D0-D28	1.7%	3.1%	5.6%	6.4%
MCHC (g/dL)	m±SD	D0	35.6±2.7[a]	35.6±1.7[a]	35.5±2.8[a]	37.4±3.2[a]
		D14	34.4±3.1[a]	33.9±4.2[a]	34.0±4.2[a]	36.0±4.7[a]
		D28	34.0±1.5[a]	31.4±2.4[b**]	34.0±1.5[a]	33.6±4.8[a]
	% de Δ	D0-D14	-3.4%	-4.7%	-4.2%	-3.8%
		D14-D28	-1.3%	-7.2%	0.1%	-6.4%
		D0-D28	-4.7%	-11.9%	4.1%	-10.2%

**p<0.01; Values of Day 0 with the same letter ([a]) are not significantly different. Values of Day 14 and Day 28 with letter ([b]) are statistically different from those of Day 0 in the same column; D = Day; % of Δ = rate of change; m±SD = mean±standard deviation

Table 3: Changes in thrombocytes number and leukocytes number in control rats and in rats exposed to xenobiotics

Parameters		Period	G_{Ctrl}	G_{Pb}	G_{Efv}	G_{Pb+Efv}
Thrombocytes (G/L)	m±SD	D0	659±143[a]	638±82[a]	611±79[a]	621±138[a]
		D14	716±110[a]	533±111[b*]	603±49[a]	518±99[b**]
		D28	692±85[a]	413±66[b**]	578±100[a]	444±84[b**]
	% de Δ	D0-D14	8.6%	-16.3%	-1.2%	-16.7%
		D14-D28	-3.6%	-22.6 %	-4.3 %	-14.2 %
		D0-D28	5.0%	-35.3 %	-5.5 %	-28.5 %
Leukocytes (G/L)	m±SD	D0	8.4±2.2[a]	8.3±3.0[a]	10.2±1.9[a]	9.8±2.2[a]
		D14	10.7±1.6[a]	14.6±3.5[b**]	8.0±3.7[a]	6.7±1.5[b*]
		D28	11.1±1.4[a]	5.4±2.2[a]	10.3±1.4[a]	13.8±5.0[a]
	% de Δ	D0-D14	27.7%	76.4%	-21.4%	-31.9%
		D14-D28	4.3%	-62.9%	28.6%	105.5%
		D0-D28	33.1%	-34.5%	1.0%	40.0%

*p<0.05; **p<0.01; ***p<0.001; Values of Day 0 with the same letter ([a]) are not significantly different. Values of Day 14 and Day 28 with letter ([b]) are statistically different from those of Day 0 in the same column; D = Day; % of Δ = rate of variation; m±SD = mean±standard deviation

Table 4: Changes in body weight in control rats and in rats exposed to xenobiotics

Body weight (g)	Period	G_{Ctrl}	G_{Pb}	G_{Efv}	G_{Pb+Efv}
m±SD	D0	149±16[a]	145±15[a]	152±13[a]	145±13[a]
	D7	159±18[b**]	152±16[a]	154±10[a]	146±13[a]
	D14	171±15[b***]	151±15[a]	164±10[a]	147±13[a]
	D21	180±10[b***]	155±13[a]	168±11[b*]	149±15[a]
	D28	190±14[b***]	159±13[a]	174±10[b**]	151±14[a]
% de Δ	D0-D7	6.4%	4.8%	1.7%	0.9%
	D0-D14	14.5%	4.2%	8.2%	1.5%
	D0-D21	20.7%	6.4%	10.6%	3.1%
	D0-D28	27.1%	9.5%	14.8%	4.0%

*p<0.05; **p<0.01; ***p<0.001; Values of Day 0 with the same letter ([a]) are not significantly different. Values of Day 14 and Day 28 with letter ([b]) are statistically different from those of Day 0 in the same column; D = Day; % of Δ = rate of variation; m±SD = mean±Standard Deviation

difference between the rate of decrease in G_{Pb} compared to G_{Pb+Efv} was not significant. No significant change was noted in G_{Ctrl} and G_{Efv} (Table 1).

Red blood cell indices: a significant decrease in the Mean Corpuscular Heamoglobin Concentration (MCHC) was observed in G_{Pb} ($p = 0.00141$). All others changes especially in the Mean Corpuscular Volume (MCV) and in the Mean Corpuscular Hemoglobin (MCH) were not significant (Table 2).

Thrombocytes number: the mean of thrombocytes number decreased significantly by 35.3 % in G_{Pb} ($p = 0.00101$) and by 28.5 % in G_{Pb+Efv} ($p = 0.00328$). The difference between the rate of decrease in G_{Pb} compared to $G_{Pb+Efv}$was not significant. In G_{Ctrl} and G_{Efv}, no significant change was observed (Table 3).

Leukocytes number: Significant changes in the mean leukocytes number were recorded on Day 14 in G_{Pb} ($p = 0.00014$) and G_{Pb+Efv} ($p = 0.00126$). But on Day 28, a

tendency of recovery followed (Table 3). No significant change was observed in G_{Ctrl} and G_{Efv}.

Body weight: From Day 0 to Day 28, the mean body weight of rats Day 0 to Day 28, the mean body weight of rats was significantly increased by 27.1 % in G_{Ctrl} ($p<0.0001$). This increase was already significant on Day 7 ($p = 0.00166$). In G_{Efv}, it is from day 21 that the weighty growth reached a significant level with a final gain of 14.8 % at Day 28 ($p = 0.00846$). The total increase of weight in G_{Pb} and G_{Pb+Efv} was not significant (Table 4).

DISCUSSION

In this study, the daily administration of 10 mg/kg bw of lead acetate for 28 days caused a significant decrease in haemoglobin level, haematocrit and erythrocyte number. This indicates an anemia in accordance to many previous studies focusing on sanitary impacts of lead (Owolabi *et al*., 2012; Ashour *et al*., 2006; Garnier, 2005; Gürer *et al*., 1998; Schwartz *et al*., 1990).

This saturnine anaemia may be explained by the lead ability to decrease heme biosynthesis by inhibiting enzymatic activity of aminolevulinic acid dehydratase, ferrochelatase (Saka *et al*., 2011; Ashour *et al*., 2006; Gürer *et al*., 1998) and aminolevulinic acid synthetase (Saxena and Flora, 2004; White and Harvey, 1972). Furthermore, the anaemia could be due to an inhibition of the synthesis of globin (Piddington and White, 1974) and an inappropriate renal erythropoietin production (Osterode *et al*., 1999). Besides, lead exposure causes oxidative stress (Ercal *et al*., 2001) which leads to chronic haemolysis (Ashour *et al*., 2006; Osterode *et al*., 1999; Valentine *et al*., 1976) and decreasing of life span of circulating erythrocytes (Kempe *et al*., 2005) as well as a decrease in the ability of blood formation (Kaminsky *et al*., 1993; Grandjean *et al*., 1989).

The anemia induced by lead exposure is usually normocytic normochromic or microcytic hypochromic (Ahmad *et al*., 2014) but there are sometimes cases of macrocytosis (Waldron, 1966). In this study, the trend of anemia found in rats exposed to lead is normocytic hypochromic.

Regarding Efavirenz exposure, the dose of 20 mg/kg bw which corresponds to a child dose reported to the weight of rat, did not cause anaemia at the end of 28 days. This is in accordance with data from World Health Organization (2010) who reported no erythrocyte disorders linked with the intake of this antiretroviral drug. Moreover, under the conditions of our experience, administration of Efavirenz simultaneously with lead did not induce a significant additive effect on the anemia generated by this metal taken alone.

As reported by Descat (2002), apart from dyserythropoiesis, the repeated ingestion of lead induce a dysthrombocytopoiesis and a dysmyelopoiesis. In our experiences, the thrombocytopenia observed in the presence of lead alone or in combination with Efavirenz is a proof of these disorders. But concerning the leukocytes number, our finding were not able to prove the real direction of its change contrary to Kayode *et al*. (2011) who found that Efavirenz increases the leukocytes number in rats.

According to this study, lead induced a significant decrease in the weight. This result is in concordance with observations of body weight loss and growth delay reported among laboratory animals (Ronis *et al*., 1998) or in children exposed to lead (Shen *et al*., 1996). Some authors (Motao *et al*., 2010; Andrews *et al*., 1994) reported a positive correlation between lead exposure in pregnant women and the delay of foetal growth as well as the low body weight of newborns. Indeed, the lead may induce anorexia in rats (Sundström *et al*., 1984) and thus their enthusiasm to eat. According to Ronis *et al*. (1998), mechanisms by which lead induces deficit in ponderal growth are complex and would be linked to endocrine disturbances on the hypothalamic-pituitary-gonadal axis.

Efavirenz has also slowed the normal growth of rats but with less intensity than lead. Nevertheless, during our experience, its administration in presence of lead acetate has not significantly intensified the ponderal growth failure generated by this latter, taken alone.

CONCLUSION

This study aimed to evaluate some haematological and body weight disturbances associated with lead and Efavirenz exposure. According to the results, lead acetate induced anaemia but not Efavirenz. The two xenobiotics induced a weight loss but the impact of lead was more severe. The administration of Efavirenz did not induced significant additive effects in the rats exposed to lead. However, further studies are necessary for verifying whether co-exposure for a longer period would not be likely to induce a significantly higher toxicity. With these results, the fight against human exposure to heavy metals should be part of challenges to improve the safety of patients receiving antiretroviral therapy. Also, it is important to find and test natural solutions that can prevent the toxic effects of xenobiotics.

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