EFFECT OF DEFERIPRONE ON LEAD-INDUCED OXIDATIVE DAMAGE AND TRACE ELEMENT LEVEL IN RATS

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Summary

Lead (Pb) exerts prooxidative effects and causes oxidative damage to various tissues by altering their antioxidant system. In the experiments male CD rats (Charles River) were treated twice a week with lead acetate at the dose of 12.5 mg/kg body weight ip for 4 weeks with and without sc *co-administration of deferiprone [Lipomed, Switzerland] (L1) at the dose molar ratio to lead acetate 10:1. Pb induced the lipid peroxidation (LPO) in both, the liver and the brain. L1 prevented this effect of lead in the liver but not in the brain. L1 alone did not change the LPO in liver whereas in the brain an elevation of LPO occured. The level of reduced glutathione (GSH) in the liver was significantly elevated in all groups compared to the control, in the brain increased by L1 treatment only. The activity of glutathione peroxidase (GSH-Px) in the liver was not influenced by Pb administration, however the combination of Pb+L1 and L1 alone decreased the activity of this enzyme. The liver, kidneys, lungs and the brain were analyzed for Pb and essential elements. Pb concentration in the liver, kidneys and brain was not changed by the L1 treatment, however Pb level increased in lungs due to L1 administration. In the liver and in the kidneys the concentration of most of the essential elements decreased due to Pb-acetate administration and were corrected by L1 co-treatment in the liver, however in the kidneys remained unaffected. The level of calcium in the liver was enhanced due to Pb administration and this effect was more expressed by L1 co-treatment. In the brain and lungs the changes in essential element concentration were minor.*

Introduction

Promotion of oxidative damage by various metals represents the leading role in the elucidation of the mechanism of their toxicity and carcinogenicity (1). Also the prooxidative effect of lead is well documented (2, 3, 4, 5).

Deferiprone - 1,2-dimethyl-3-hydroxypyrid-4-one – is an oral iron chelating drug being used in thalassaemia. It is known for its ability to inhibit the formation of toxic oxygen activated species (H_2O_2, superoxide and hydroxyl radical) catalyzed by iron (6). In our previous experiments (7) deferiprone was able to correct the cadmium induced lipid peroxidation, without any decrease in the acute toxicity of cadmium, indicating the supposed role of iron in metal induced oxidative damage.

The present study was undertaken to evaluate the effect of deferiprone (L1) on the lead induced oxidative damage.

Material and Methods

Male CD rats (body weight 130–140 g), divided into groups of 8–9 animals, were used in the experiments. The animals were treated twice a week with $Pb(CH_3COO)_2 \cdot 3H_2O$ (anal. Gr., Merck, Darmstadt) at the dose of 12.5 mg . kg^{-1} *ip* for the period of 4 weeks either with or without *sc* administration of deferiprone (L1) [Lipomed, Switzerland] at the dose molar ratio to Pb-acetate 10:1. Deferiprone was administered either alone or 1 hour before Pb-acetate administration.

Peroxidation of lipids (LPO) expressed as malondialdehyde production was estimated in liver and brain homogenates by thiobarbituric acid test (8), the level of reduced form of glutathione (GSH) was determined in liver and brain homogenates with Ellman's reagent (9). The activity of glutahione peroxidase (GSH-Px) was measured in liver homogenates according to V. A. Günzler et al. (10).

Tissues – the liver, kidneys, brain and lungs – were analyzed for lead and essential elements – calcium, magnesium, zinc, copper, iron and manganese – using flame- or graphite furnace- technique of atomic absorption spectrometry.

The results were statistically evaluated using unpaired Students *t*-test. The mean and SD values are presented. Numbers of animals (*n*) used per group are stated in the figures and tables.

Results

The analyses of the liver and brain tissues have shown that lead administration induced the lipid peroxidation. Deferiprone was able to prevent this

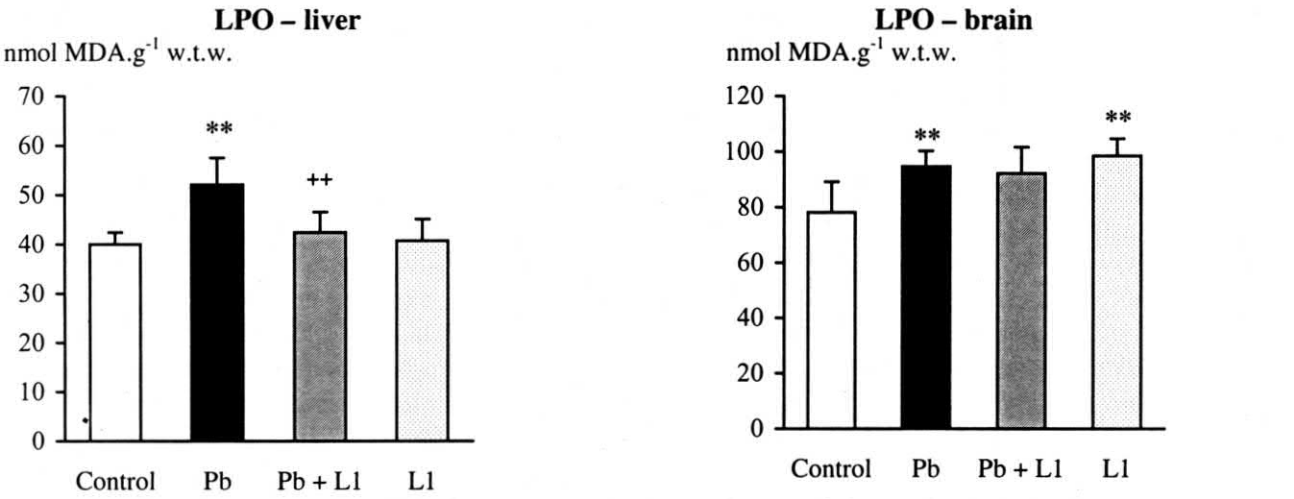

*Fig. 1: The influence of lead acetate and L1 administration on lipid peroxidation in the liver and in the brain of rats. Data represent mean ± SD; n = 8 (control, Pb), n = 9 (Pb, Pb+L1). Asterisks denote significant differences: * p < 0.05 vs. control group ** p < 0.01 vs. control group, ++ p < 0.01 vs. Pb group*

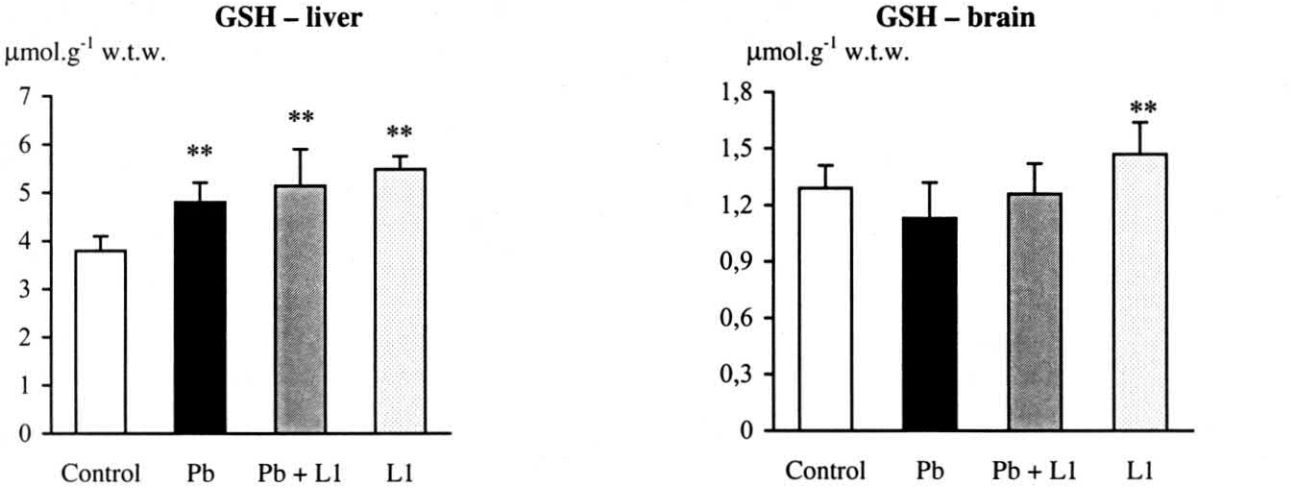

*Fig. 2: The influence of lead acetate and L1 administration on GSH level in the liverand in the brain of rats. Data represent mean ± SD; n = 8 (control, Pb), n = 9 (Pb, Pb+L1). Asterisks denote significant differences: ** p <0.01 vs. control group, ++ p<0.01 vs. Pb group*

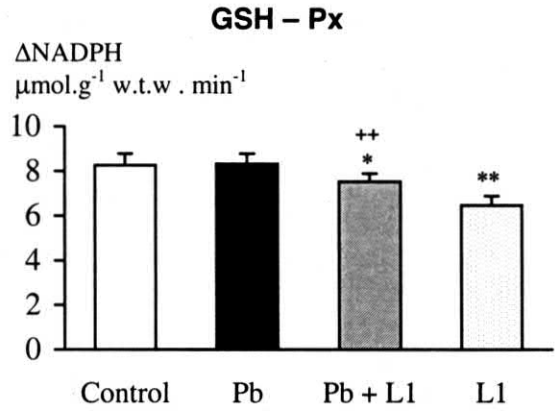

*Fig. 3: The influence of lead acetate and L1 administration on the activity of GSH-Px in the liver of rats. Data represent mean ± SD; n = 8 (control, Pb), n = 9 (Pb, Pb+L1). Asterisks denote significant differences: * p < 0.05 vs. control group, ** p < 0.01 vs. control group, ++ p < 0.01 vs. Pb group*

effect in the liver but not in the brain. L1 alone did not change the LPO in the liver whereas in the brain an elevation of LPO occurred (Fig. 1).

The level of reduced glutathione in the liver was significantly elevated in all groups compared to control group. The GSH content in the brain was increased by L1 treatment only (Fig. 2).

The activity of glutathione peroxidase in the liver tissue was not influenced by lead administration. However, L1 administered alone or in combination with lead acetate decreased significanly the activity of this enzyme (Fig. 3)

The level of lead and essential elements – calcium, magnesium, zinc, copper, iron and manganese are given in tables 1–4. Pb cummulation in the liver, kidneys and brain was not changed by L1 treatment (Tab. 1–3). An enhancement of Pb cummulation close to the level of significance occured in the lungs (Tab. 4). In the liver the concentration of most of the essential elements decreased due to Pb-acetate administration and was corrected - with the exception of Mn- by L1 treatment to the levels of control group. In the kidneys the decreased level of zinc, copper, iron and manganese caused by Pb administration remained unaffected by L1 treatment. The level of calcium was enhanced due to lead acetate administration and this effect was uncganged or even more expressed by L1 co-treatment. In the brain and lungs the changes in iron and manganese were found in Pb and Pb+L1 groups. No changes in essential elements concentration were found in the tissues animal treated with L1 alone, with the exception of decreased iron concentration in lungs.

Table 1

The influence of lead acetate and L1 administration on trace elements concentration in the liver of rats [μg . g^{-1} w.t.w.]
Data represent mean ± SD

	Ca	Mg	Zn	Cu	Fe	Mn	Pb
Control n = 9	33.7 ±1.6	191.5 ±4.2	28.9 ±1.5	3.74 ±0.15	92.3 ±11.4	1.90 ±0.13	
Pb^{2+} n = 9	41.1 ±12.3	186.4 ±3.8*	27.1 ±1.2*	3.54 ±0.13**	86.8 ±11.9	1.76 ±0.13*	7.8 ±3.3
Pb^{2+} + L1 n = 8	66.5 ±29.7*	188.4 ±5.6	28.2 ±1.7	3.65 ±0.28	93.5 ±7.4	1.66 ±0.14**	10.5 ±5.3
L1 n = 8	32.9 ±2.5	190.3 ±4.2	29.1 ±0.10	3.67 ±0.10	89.8 ±4.1	1.96 ±0.14	

** $p < 0.01$ in comparison with control; * $p < 0.05$ in comparison with control

Table 2

The influence of lead acetate and L1 administration on trace elements concentration in the kidneys of rats [μg . g^{-1} w.t.w.]
Data represent mean ± SD

	Ca	Mg	Zn	Cu	Fe	Mn	Pb
Control n = 8	54.3 ±2.2	171.9 ±7.5	22.7 ±1.5	4.95 ±0.46	40.3 ±2.3	0.83 ±0.05	
Pb^{2+} n = 8	58.5 ±6.2	176.8 ±5.1	19.7 ±0.6**	4.03 ±0.32**	32.9 ±2.7**	0.71 ±0.08**	15.6 ±6.2
Pb^{2+} + L1 n = 8	57.5 ±1.3**	177.4 ±4.2	19.5 ±0.5**	3.77 ±0.23**	34.9 ±1.9**	0.67 ±0.06**	14.9 ±2.4
L1 n = 8	57.3 ±2.5*	174.4 ±1.6	21.9 ±0.8	5.31 ±0.89	40.2 ±3.4	0.90 ±0.09	

** $p < 0.01$ in comparison with control; * $p < 0.05$ in comparison with control

Table 3

The influence of lead acetate and L1 administration on trace elements concentration in the brain of rats [μg . g^{-1} w.t.w.]
Data represent mean ± SD

	Ca	Mg	Zn	Cu	Fe	Mn	Pb
Control n = 8	44.5 ±6.4	130.7 ±4.7	30.6 ±2.8	2.21 ±0.21	16.7 ±1.5	0.38 ±0.02	
Pb^{2+} n = 8	41.0 ±3.7	128.8 ±1.8	31.7 ±2.7	2.35 ±0.24	15.1 ±0.5*	0.34 ±0.02**	0.68 ±0.12
Pb^{2+}+ L1 n = 8	41.1 ±3.3	126.6 ±3.4	30.4 ±1.5	2.23 ±0.13	15.6 ±1.2	0.33 ±0.02**	0.66 ±0.17
L1 n = 8	42.1 ±3.5	129.0 ±3.2	28.9 ±2.4	2.17 ±0.24	15.6 ±1.5	0.39 ±0.04	

** $p < 0.01$ in comparison with control; * $p < 0.05$ in comparison with control

Table 4

The influence of lead acetate and L1 administration on trace elements concentration in the lungs of rats [μg . g^{-1} w.t.w.]
Data represent mean ± SD

	Ca	Mg	Zn	Cu	Fe	Mn	Pb
Control n = 8	69.8 ±8.5	97.7 ±9.1	13.7 ±1.3	1.23 ±0.16	61.8 ±12.1	0.14 ±0.02	
Pb^{2+} n = 8	71.2 ±9.5	95.1 ±13.5	13.3 ±1.6	1.27 ±0.15	57.1 ±13.3	0.12 ±0.02	1.38 ±0.88
Pb^{2+}+ L1 n = 8	78.0 ±5.6*	102.1 ±6.7	14.5 ±0.6	1.34 ±0.09	49.2 ±8.2*	0.12 ±0.01*	3.32 ±2.66
L1 n = 8	74.1 ±4.2	103.2 ±9.0	14.6 ±1.1	1.26 ±0.09	51.6 ±2.6*	0.13 ±0.01	

** $p < 0.01$ in comparison with control, * $p < 0.05$ in comparison with control

Discussion and Conclusion

The administration of lead increased the LPO in both the liver and the brain, what confirms the pro-oxidative effect of lead. The treatment with deferiprone corrected the LPO level to the control values only in the liver but not in the brain.

In the liver the level of glutahione was elevated due to lead treatment what corresponds to the findings of other authors (11). This could be explained by the induction of glutathione S-tranferase as a response to the toxic effect of electrophiles. The elevated level of GSH represents the way of the cell defence. Deferiprone did not influence this reaction.

As demonstrated in our previous reports the activity of glutathione peroxidase can be inhibited by a long-term exposure to lead and other toxic metals (Cd, Hg,) (7, 12, 13). In lead exposure this effect was shown after the administration of thirty doses of lead acetate (0.01 mmol Pb.kg^{-1} each). Eight doses of lead acetate (0.03 mmol.kg^{-1}) administred during 4 weeks in present experiment did not change the activity of GSH-Px. The exposure time seems to be one of the main factors in this effect. Deferiprone decreased the GSH-Px activity in both cases, i.e. administered alone and also co-administered with lead. This phenomenon could not be explained without further study. This evokes a question to which extent it may be considered an adverse effect regarding to the fact that deferiprone is being used widely in the therapy of thalassaemic patients.

Deferiprone treatment did not affect lead distribution in the liver, kidneys and brain. Surprisingly an enhancement of Pb concentration occurred in the lungs and of Ca concentration in the liver after L1 co-treatment.

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