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Bronchoalveolar Lavage Fluid in Rats Treated Intratracheally with Lead Acetate

P. Salovsky,[1] V. Shopova,[1] V. Dancheva,[1] A. Pandurska,[2] R. Marev[2]

[1]Department of Disaster Medicine, University of Medicine, Sv. Climent Ohridski 1, Pleven 5800, Bulgaria
[2]Department of Pharmacology, University of Medicine, Sv. Climent Ohridski 1, Pleven 5800, Bulgaria

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The lead and its derivatives found a large application in industry and life. They are ones of the most wide spreated and toxic pollutants of the environment. A serious ecological problem today is the air pollution of the cities with an intensive road trafic. There are data that the lead concentration in the air of some large cities goes beyond the allowed limits (Hernberg, 1975, Friberg et al., 1979, Tsuchiya, 1986, Yakovlev, 1991). In spite of the important role of the inchalatory absorption of the lead, there are no recent studies on its pneumotoxic effect. Therefore, our aim was to investigate the influence of the lead acetate on the lung parenchyma in rats,using sensitive biochemical and cytological indices in the bronchoalveolar lavage fluid (BALF).

MATERIALS AND METHODS

The experiment was carried out on 48 male Wistar rats, 4 months old, and with a primary weight of 200 ± 20 g. These animals,received from the Research and Laboratory Animal Breeding Centre of Slivnitsa(Bulgaria) were kept in our laboratory one month in 20 - 22°C and 50 ± 10 % humidity, on normal pelleted diet and water ad libitum. The rats were divided into two groups: control and experimental. The lead acetate /$Pb(CH_3COO)_2$/ in form of water solution, was administered intratracheally with a metal probe in dosage of 100 μg for each animal and 0.2 mL volume. The animals from the control group received the same volume saline. Six rats of each group were anaesthetized with sodium pentobarbiturate and killed on days 1, 5, and 15 after the intoxication, by a cut off of the abdominal aorta.
The bronchoalveolar lavage was made in situ, by triple lavage via the trachea with a total of 5 mL saline, warmed to 37° C. The volume of the restored fluid was between 80 and 90 %(average 85%). One aliquote part was

Correspondence to: P. Salovsky

used for counting of the total number of the cells in Burcker's camera. Then, the cells were eliminated by a centrifugation x 300 g for 10 min and the cell pellet was resuspended in 1 mL saline. A cytospin preparation of the cells was stained by the method of Danos and Keebler(1977) and a differential cell counting was done. The supernatant, resulting from the centrifugation was used for biochemical analysis.
The following studies were made:
a) Lung weight coefficient (organ weight in mg/100 g body weight).
b) BALF:
- total cell number x 10^5 (mL) of the restored fluid,
- differential cell counting, including alveolar macrophages (AM), lymphocytes, and granulocytes (PMN),
- activity of the enzymes lactate dehydrogenase(LDH) alkaline phosphatase (APH) and acid phosphatase (AcPH), by the method of Bergmeyer (1974),
- content of the total protein, by the method of Lowry 1951),
- content of the glucose, using commercial kits from the firm GBS (Centre of Biogenic Stimulants, Bulgaria).
The enzyme activity is presented in units/mL. One unit was defined as 1 nmol substrate converted per minute. The total protein content is given in mg/mL,and glucose content in μg/mL.
The experimental data were statistically analysed by Student's t test ($p<0.05$). The results from the study are shown on the table as an arithmetical mean$\pm$SE. On the graphic diagrams the results are presented in percentage from the control group. On the table and the diagrams, the statistically significant differences are marked with an asterisk (*).

RESULTS AND DISCUSSION

In our experiment we applied intratracheally 100 μg lead acetate per animal. The dose is commensurable with the daily amount of lead taken with the food, which for the most countries is 100 - 200 μg(Friberg 1979) . Naturally, in life and professional circumstances the inhalatory amount of lead is depending on many factors and could vary largely.
We evaluated the changes of the lungs according to the commonly accepted in the experimental toxicology and pulmology biochemical and cytologic markers in broncho alveolar lavage fluid. In the last 10 - 15 years it was proved that the increased LDH activity in BALF is an indicator for the damage of the pulmonal cells, which can vary from an increased membrane permeability to a frank cell lysis. The increased APH activity was accepted as an indicator of one selected damage of the pneumocytes Type II, or of an increased secretion of this enzyme from them. The AcPH level in BALF was connected either with an increased phagocytic activity,

or with damage of the PMN and/or of the AM, and the influx of PMN in the bronchoalveolar spaces - with inflammatory processes in the lung parenchyma(Hook,1978, Henderson, 1984, Guth and Mavis, 1985, Smith, 1985).
The analysis of our experimental data showed that the lead acetate increased sharply the LDH, APH, and AcPH activities during the day 1. On day 5 they decreased quickly and reached the level of the control group. Only the LDH activity on day 15 showed a significant decrease in comparison with the control group (Fig. 1). According to the above mentioned literature data these results discovered that the intratracheal application of 100 μg lead acetate provokes an early, but transient toxic response of the lung parenchyma. The higher increase of APH level on day 1 (with 187% compared to the control), in comparison with the increase of the other two enzymes (LDH-135% and AcPH-119%),indicated a more severe damage of the pneumocytes Type II, the only cells which produce it in the bronchoalveolar areas. On the other hand, the decreased LDH activity on day 15 probably reflects the depletion of the enzyme in the pulmonary cells from which is depending its level in BALF.
For evaluation of the capillary-alveolar membranes permeability, we used two indices:total protein content and glucose content in the lavage fluid. It is well known that many chemical substances damage these structures and cause transudation of plasmatic proteins in the bronchoalveolar spaces (Alpert et al., 1971, Hu et al., 1982). Probably, this is the mechanism by which the lead acetate in our experiment, increased the total protein content in BALF on day 1. The dynamics of the other index, the glucose, was different.In comparison with the controls, its content was increased with 300% (139% for the total protein) and remained significantly higher on day 1, too (Fig. 2). These experimental data confirm the finding of Nambu et al., 1991, that the determination of glucose in BALF is a more sensitive marker for the evaluation of capillary - alveolar barrier's condition. In experiments with rats exposed on different ozone levels of concentration, the authors establish that the elevation of the glucose content is higher and more prolonged in comparison with the total protein.
The results obtained from the biochemical study of BALF correlated with the changes of the cytological indices (Table). The lead acetate increased the total cell number in the lavage fluid on day 1. The part of PMN reached 60.2% on day 1 but decreased significantly during next time-points. The percentage of AM showed an inverse dynamics - a decrease on day 1 and normalizing tendency on days 5 and 15. In comparison with the controls, the lymphocytes didn't show any significant deviations during the three days of observation. This finding proved the essential role of the inflammatory

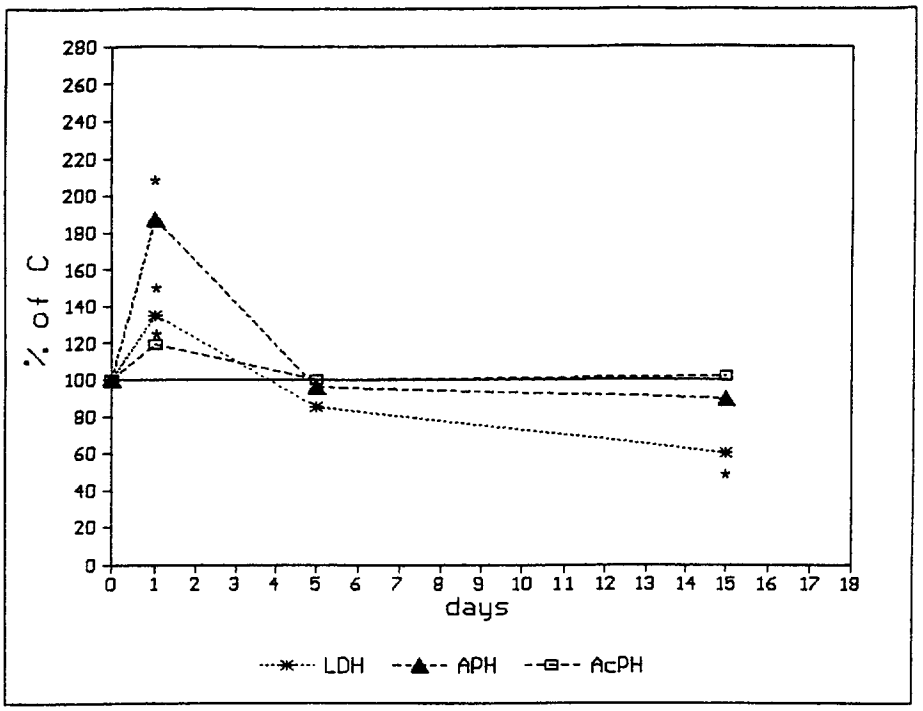

Figure 1.Time-dependent effect of lead treatment on lactate dehydrogenase (LDH), alkaline phosphatase (APH) and acid phosphatase (AcPH) activities in BALF. Each point is mean ± SE for six rats, expressed as a percentage of respective control value. The mean of control group of LDH were between 71 and 78 units/mL of APH were between 3.10 and 3.33 units/mL and of AcPH were between 2.06 and 2.46 units/mL during the 15 days study period. * Significantly different than control at $p<0.05$ by Student's t test.

component in the early toxic reaction of the lungs towards the chemical agent. The increased weight coefficient on days 1 and 5, was an evidence about the acute toxic damage of the lungs (Table).
Our experimental work doesn't give an answer about the mechanism of the lung damage caused by lead acetate. This could be object of other experiments with other methods. The modeling of a chronic lung damage with lower lead concentrations, similar to the intensively polluted atmosphere of many cities, could be of great practical importance.
In conclusion, we could accept that the single intra tracheal administration of 100 μg lead acetate per animal, provokes an acute but transitory increase of some sensitive biochemical and cytological indices in the bronchoalveolar lavage fluid.In comparison with the

Table. Effect of lead treatment on the weight coefficient of lungs and cytological indexes

	Day after treatment					
	1		5		15	
Parameter	Control	Lead	Control	Lead	Control	Lead
Weight coefficient of the lungs, mg/100g body weight	765±10.2[a]	1065±73.2*	812±27.2	1200±165*	-	-
Total cell number, x 10^5 mL/BALF	7.6±0.11	21.5±2.8*	6.9±0.22	7.8 ±1.1	7.7±0.75	8.3±2.1
AM, %	87.5	28.7	87.5	79.9	88.1	79.9
Lymphocytes, %	8.0	11.0	8.2	11.3	8.6	9.1
PMN, %	3.5	60.3	4.3	8.0	3.3	11.2

Abbreviations: BALF, bronchoalveolar lavage fluid; AM, alveolar macrophages; PMN, neutrophils

[a]Values represent mean ± SE of six animals

*Different from control at $p<0.05$, Student's t test

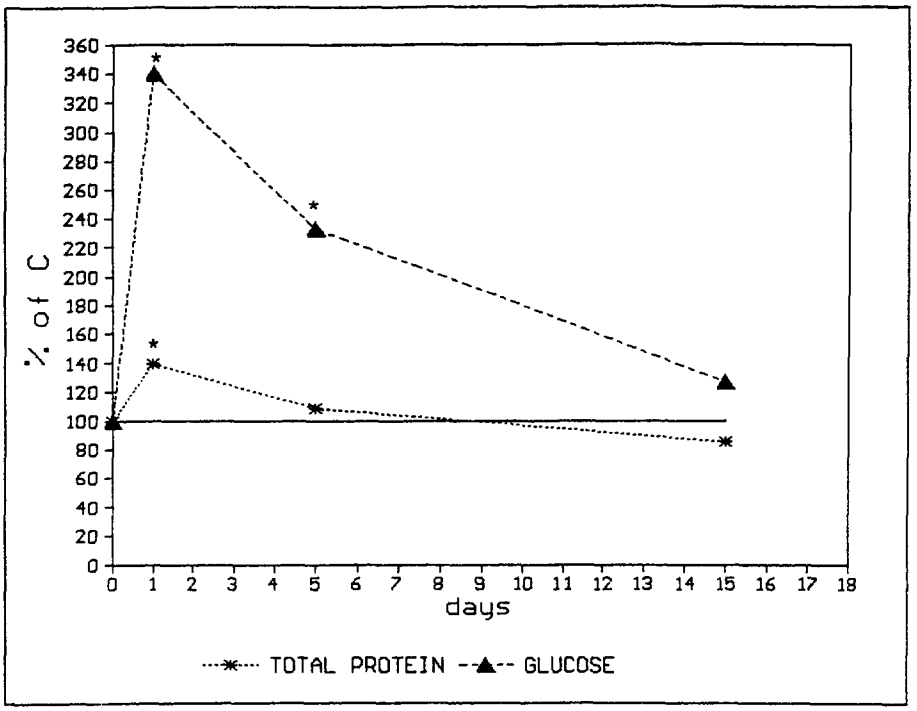

Figure 2. Time-dependent effect of lead treatment on total protein and glucose content in BALF. Each point is mean ± SE for six rats, expressed as a percentage of respective control value. The mean of control group of total protein were between 0.35 and 0.42 mg/mL and of glucose were between 12 and 14 μg/mL during the 15 days study period. *Significantly different than control at $p<0.05$ by Student's t test.

total protein, the determination of the glucose level in the lavage fluid was a more sensitive index for the evaluation of the capillary - alveolar membrane's permeability.

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