

COMPARATIVE STUDY ON THE ABILITY OF 2,3 DIMERCAPTOSUCCINIC ACID AND D-PENICILLAMINE TO ENHANCE URINARY EXCRETION OF LEAD IN RABBITS

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Summary

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The potential of 2,3 dimercaptosuccinic acid (DSMA) and D-penicillamine – heavy metal chelating drugs which enhance the urinary excretion of lead and restore altered levels of urinary δ -aminolevulinic acid (δ -ALA) and blood δ -aminolevulinic acid dehydratase (δ -ALA-D) in lead poisoned rabbits, was compared. The performance of DMSA was better than that of D-penicillamine. It was attributed to the two –SH groups as stronger metal binding sites in DMSA molecule.

Key words: 2,3 dimercaptosuccinic acid, D-penicillamine, heavy metals, lead, rabbits

INTRODUCTION

Thiol chelating agents were found to be effective for treatment of heavy metal intoxications including lead poisoning in order to prevent the binding of heavy metals to –SH groups of active biomolecules in the body (Apoishian, 1983; Klassen, 1985). However, the therapy with the well known dimercaprol (BAL) involves a series of injections (Chisolm, 1971) and many patients experience side effects such as nausea, vomiting and severe headache (Chisolm, 1968). It is contraindicated in lead poisoning. Penicillamine may cause an increase in eosinophils, haematological changes, renal damage and has been reported to be unsafe in patients allergic to penicillin (Chisolm, 1971). 2,3 dimercaptosuccinic acid (DMSA) have been considered useful in heavy metals intoxication (Liang *et al.*, 1980) and thus, deserves further evaluation.

The increase in urinary δ -aminolevulinic acid (δ -ALA) levels and the de-

creased activity of blood δ -aminolevulinic acid dehydratase (δ -ALA-D) were thought to be reliable indicators of lead poisoning for comparing the efficiency and the suitability of chelating agents in treatment of Pb intoxication (Grandjean *et al.*, 1991; Shanon *et al.*, 1988).

Our studies were therefore based on these considerations. The aim of the present experiment was to compare the potential of two important thiol chelating drugs – D-penicillamine and DMSA to enhance the excretion of lead and to reverse altered biochemical parameters in Pb poisoning as a model of experimental heavy metal intoxication.

MATERIALS AND METHODS

The trials were performed on 32 Chinchilla male rabbits weighing 1.3 ± 0.3 kg, maintained on standard pellet diet. The animals were kept in metabolic cages (one

per cage) for collection of 24 h urine. The animals were divided equally into four groups (8 animals each) and treated as followed:

- Group I – received an equal volume of unleaded water as the experimental control groups and served as non-intoxicated and untreated control group.
- Group 2 – received lead in drinking water 10 mg Pb/kg/24 h as lead acetate trihydrate¹ (Sigma, Germany) for 3 weeks and was treated with equal volume normal saline s.c. as the experimental groups on days 7, 9 and 11 after the first dose of lead and served as intoxicated and untreated control group.
- Group 3 – received lead as group 2 and D-penicillamine (Sigma, Germany) s.c. in normal saline on days 7 (1st dose – 100 µmol/kg), 9 (2nd dose – 200 µmol/kg) and 11 (3th dose – 300 µmol/kg) after the first dose of lead.
- Group 4 – received lead as group 2 and 2,3 dimercaptosuccinic acid (DMSA) (Sigma, Germany) s.c. in normal saline on days 7 (1st dose – 100 µmol/kg), 9 (2nd dose – 200 µmol/kg), and 11 (3th dose – 300 µmol/kg) after the first dose of lead.

Twenty four hour urine samples were collected for measuring the excretion of Pb and δ-aminolevulinic acid over two days in 3 consecutive intervals: on days 7–8; on days 9–10 and on days 11–12 after the first dose of lead and on day 10 after termination of the experiments.

The samples of blood were obtained from *v. auricularis* on days 9, 11, 13 after receiving the first lead dose and 10 days after termination of the treatment. Standard procedures were employed to deter-

mine blood δ-aminolevulinic acid dehydratase (δ-ALA-D) (Berlin, 1974), urinary δ-aminolevulinic acid (δ-ALA) (Davis *et al.*, 1968), lead in urine (Kopito *et al.*, 1967) and in blood (Yeager *et al.*, 1971). A spectrophotometer (Specol; Carl-Zeiss, Jena) and an atom absorption spectrophotometer (Perkin Elmer 5000) were employed. Data were statistically processed using the Student's t-test (Sepetliev, 1986).

RESULTS

The excretion of lead and δ-ALA with urine and the blood lead level increased (by 129%–261%, 123%–151% and 117–126% respectively) whereas the activity of blood δ-ALA-D decreased (by 25–44%) in the intoxicated and untreated group (Table 1). The urinary excretion of lead increased to a higher degree during the treatment with DMSA than with D-penicillamine in lead poisoned rabbits (Table 1). This was followed by a lower excretion of lead in DMSA-administered animals 10 days after termination of the treatment. The blood lead level and urinary δ-ALA excretion were not affected significantly 48 hours after the first dose of either DMSA or D-penicillamine in lead exposed animals (Table 1). At the same interval, blood δ-ALA-D activity in D-penicillamine and DMSA groups was significantly increased vs the respective values in untreated controls, more pronounced in the DMSA group. The blood lead level decreased slowly and steadily on further treatment with DMSA while a sudden fall was observed in animals administered D-penicillamine followed afterwards by increased values 48 hours after the second dose. The lead induced decreased activity of blood δ-ALA-D and increased urinary excretion of δ-ALA (vs non-intoxicated controls) recovered to

¹ Pb(CH₃COO)₂·3H₂O.

Table 1. Effect of DMSA and D-penicillamine on urinary and blood parameters (mean $\pm$ SEM) in lead poisoned rabbits

Doses of the anti-dotes/Intervals	Urine		Blood	
	Lead ($\mu\text{g/dL}$)	δ -ALA [#]	Lead ($\mu\text{g/dL}$)	δ -ALA-D ^{##}
Group I: Non-intoxicated and untreated controls (n=8)				
	15.07 $\pm$ 1.86	43.05 $\pm$ 4.00	23.46 $\pm$ 2.71	73.18 $\pm$ 2.28
Group II: Intoxicated and untreated controls (n=8)				
0/9 days ¹	34.62 $\pm$ 2.91	108.11 $\pm$ 9.15	53.18 $\pm$ 4.12	41.61 $\pm$ 1.39
0/11 days ¹	39.35 $\pm$ 3.07	97.06 $\pm$ 8.57	50.96 $\pm$ 4.07	49.28 $\pm$ 2.01
0/13 days ¹	37.48 $\pm$ 3.00	98.75 $\pm$ 8.91	47.91 $\pm$ 3.96	55.01 $\pm$ 1.25
0/last 10 days ²	36.12 $\pm$ 2.98	59.19 $\pm$ 4.29	50.26 $\pm$ 4.18	46.19 $\pm$ 1.91
Group III: Intoxicated and treated with D-penicillamine (n=8)				
100 $\mu\text{mol/kg}$ /9 days ¹	45.58 $\pm$ 3.62	81.16 $\pm$ 8.17	47.79 $\pm$ 3.87	54.29 $\pm$ 2.29*
200 $\mu\text{mol/kg}$ /11 days ¹	48.27 $\pm$ 3.96	79.28 $\pm$ 8.01	34.60 $\pm$ 4.01*	58.76 $\pm$ 3.98
300 $\mu\text{mol/kg}$ /13 days ¹	69.20 $\pm$ 6.01***	59.19 $\pm$ 5.11**	49.05 $\pm$ 5.11	49.28 $\pm$ 5.66
0/last 10 days ²	39.10 $\pm$ 2.16	78.80 $\pm$ 8.01	53.15 $\pm$ 4.86	54.36 $\pm$ 6.17
Group IV: Intoxicated and treated with DMSA (n=8)				
100 $\mu\text{mol/kg}$ /9 days ¹	40.96 $\pm$ 4.29	90.50 $\pm$ 8.65	48.29 $\pm$ 4.17	57.20 $\pm$ 4.67**
200 $\mu\text{mol/kg}$ /11 days ¹	52.29 $\pm$ 6.01	79.10 $\pm$ 7.60	47.00 $\pm$ 4.25	61.60 $\pm$ 7.51
300 $\mu\text{mol/kg}$ /13 days ¹	79.90 $\pm$ 8.26***	64.55 $\pm$ 6.55**	39.30 $\pm$ 3.15	69.50 $\pm$ 7.51
0/last 10 days ²	29.55 $\pm$ 1.65	51.52 $\pm$ 4.75	30.16 $\pm$ 2.97**	65.70 $\pm$ 7.76

[#] μmol δ -ALA/min/L erythrocyte, ^{##} mg/100 mL; ¹days after the first dose of lead; ²ten days after termination of lead treatment; * $p < 0.02$, ** $p < 0.01$, *** $p < 0.001$.

normal more effectively on treatment with DMSA than with D-penicillamine.

DISCUSSION

Lead is known to inhibit the activity of blood δ -ALA-D probably by competing with Zn for binding to SH-groups of enzyme activity sites resulting into increased urinary excretion of δ -ALA (Morgan *et al.*, 1972). The greater effectiveness of DMSA compared to that of D-penicillamine for mobilizing blood lead and reducing the toxic effect of lead may be attributed to the presence of two SH groups in DMSA that provides better binding site for lead than those of D-penicillamine having one SH and one NH_2 groups.

δ -Aminolevulinic acid dehydratase,

catalysing the synthesis of porphobilinogen from two molecules δ -ALA, is very sensitive to lead (Hernberg *et al.*, 1970). The degree of inhibition closely correlates to the concomitant level of lead in blood (Tomokuni, 1974). The faster rejuvenation of enzyme activity in the group treated with DMSA was in turn reflected by decrease in urinary δ -ALA excretion (Graziano *et al.*, 1985).

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