

Comparative Effects of Salinomycin and Meso-2,3-Dimercaptosuccinic Acid (DMSA) on Lead-Induced Impairment of Reproductive Function in Male Mice

Pavlova E.^{1,*}, Kamenova K.², Gluhcheva Y.¹, Petrova E.¹, Ivanova Ju.³, Atanassova N.¹

¹ Institute of Experimental Morphology, Pathology and Anthropology with Museum, Bulgarian Academy of Sciences, Sofia, Bulgaria

² Faculty of Chemistry and Pharmacy, Sofia University "St. Kliment Ohridski", Sofia, Bulgaria

³ Faculty of Medicine, Sofia University "St. Kliment Ohridski", Sofia, Bulgaria

*corresponding author: Assoc. Prof. E. Pavlova, Institute of Experimental Morphology, Pathology and Anthropology with Museum
e-mail: e_bankova@yahoo.com

Abstract

Lead (Pb) directly targets testicular spermatogenesis and also the sperms in the epididymis inducing reproductive toxicity. In our study we compare the effects of meso-2,3-dimercaptosuccinic acid (DMSA) and tetraethylammonium salt of salinomycin acid (Sal) on testis morphology, sperm count, Pb content, endogenous levels of calcium, copper, zinc and iron in testis of mice, subjected to Pb intoxication. Both chelating agents considerably reduced Pb content in the testis compared to the toxic control. The administration of Sal ameliorated Pb-induced alterations in Cu homeostasis and significantly restored endogenous Ca and Zn concentrations levels unlike DMSA. Testicular morphology of Pb-intoxicated mice recovered to control after salinomycin treatment but sperm count remained 21% lower compared to the untreated mice. The results suggest the potential application of salinomycin as antidote for treatment of lead intoxications.

Keywords: Pb intoxication, Salinomycin, Chelation therapy, essential elements, Spermatogenesis

1. Introduction

Lead targets testicular spermatogenesis and also the sperms in the epididymis inducing reproductive toxicity. By long-term exposure signaling is disrupted, thereby altering the gonadotropin-releasing hormone system in the male rat (Sokol et al., 2002). Chelation therapy is a procedure that involves the administration of chelating agents to remove heavy metals from the body (Aaseth et al., 2016). The profile of a successful chelating drug includes high affinity for the toxic metal(s) but low affinity for essential metals, minimal toxicity, lipid solubility, and preferably, good absorbability from the gastrointestinal tract (Blanus et al., 2005).

Salinomycin is a promising novel anticancer agent. Among the polyether ionophorous antibiotics salinomycin is the least toxic of all the ionophores (Oehme et al., 1999). In this study we present the first results on the effect of salinomycin on testis morphology, sperm count, Pb content and the endogenous levels of

copper, zinc and iron in testis of mice, subjected to subacute Pb intoxication. We compare the effects of the antibiotic with those of DMSA (conventional antidote to Pb-poisoning).

2. Materials and Methods

Adult male ICR mice were divided into four groups: control (ctrl) - untreated control mice obtained distilled water for 28 days, toxic control group (Pb) – mice orally subjected to Pb intoxication for 14 days (average daily dose of 80 mg/kg b.w. Pb(NO₃)₂). Pb+DMSA – mice exposed to Pb as the toxic control group followed by treatment with 20 mg/kg b.w. DMSA for two weeks; Pb+Sal – mice intoxicated with Pb(NO₃)₂ similarly to the toxic control group followed by administration of 20 mg/kg b.w. tetraethylammonium salt of salinomycin acid for another 2 weeks. On day 29 the experimental mice were sacrificed. Testes were sampled and embedded in paraffin using routine histological practice. Spermatozoa were isolated from both ductus deferens and counted using Buerker's chamber. An atomic absorption spectrometer (Perkin Elmer Analyst 400, air-acetylene flame) was used to assess Cu, Zn, Ca and Fe concentrations and an electrothermal atomic absorption spectrometer (Zeeman Perkin Elmer 3030, HGA 600) to determine the Pb concentration in the testes of the control and experimental animals. Data were statistically processed using Student's t-test.

3. Results and Discussion

Histological observation of testes in Pb-intoxicated mice revealed normal and affected seminiferous tubules with single Sertoli cell only phenotype (SCO), or incomplete spermatogenesis with partial loss of germ cells. (Fig. 1B). Detoxification with DMSA ameliorated the structure to a large extent but some SCO tubules still could be seen (Fig. 1C). Salinomycin restored the normal testicular morphology, comparable to control (Fig. 1D). Spermatozoa isolated from both ductus deferens in the toxic control were 25% lower than control value (Fig.2A). Despite histological findings in salinomycin-

and DMSA-detoxified groups the sperm counts remained 21% and 9% lower than control, respectively. There are no data in the literature that Pb affects blood-testis barrier integrity that protects the germ cells from the direct effects of high levels of Pb but chronic Pb exposure was found to reduce male fertility (Wani, 2015). Long-term low-dose Pb exposure was shown to alter the signaling system between the hypothalamus and pituitary gland of male rats (suppressed rates of serum testosterone, intratesticular sperm counts, and sperm production) (Sokol et al., 2002).

Atomic absorption analysis demonstrated that intoxication of mice with Pb salt resulted in a significant accumulation of Pb in the testes compared to the untreated control animals. Application of salinomycin or DMSA considerably decreased the concentration of the toxic metal ion compared to the toxic control (Fig. 2B). Palani (2017) summarized that intracellular Ca and Zn are essential for normal spermatogenesis and sperm motility. Cu plays an indirect role in male infertility via increasing or decreasing of other elements' content and high level of Fe may decrease sperm motility. Investigating the endogenous concentrations of essential elements in the testis we observed that Pb treatment significantly suppressed Cu and Ca concentrations in the intoxicated animals - more than 2 fold reduction of Cu and 42 % lower values of Ca compared to control (Fig. 2C). Pb intoxication did not affect significantly the endogenous concentrations of Zn and Fe in the testis compared to the normal values. The treatment of the Pb-exposed mice with DMSA or salinomycin restored the endogenous concentrations of Cu and Ca to the normal control values (Fig. 2C). Both chelating agents did not affect the endogenous levels of Fe and Zn compared to the

untreated control mice. Our previous study demonstrated significant elevation of Pb accumulation in kidneys, spleen, liver and brain in Pb-intoxicated animals. Use of salinomycin significantly reduced Pb content in the organs of toxic control animals (Ivanova, 2016). All these results suggest the potential application of salinomycin as antidote for treatment of lead intoxications.

The authors acknowledge the financial support of Sofia University "St. Kliment Ohridski" Science Fund (Grant 80.10-168/2018, PI: Julianna Ivanova).

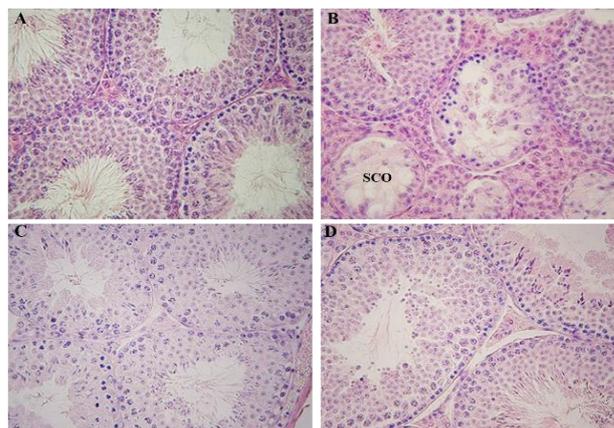


Figure 1. Morphology of the seminiferous tubules of control mice testis (A), testis subjected to intoxication with Pb(NO₃)₂ (B), Pb intoxication followed by detoxification with DMSA (C); Pb intoxication followed by detoxification with Sal. (D). HE, x 400. SCO - Sertoli cell only tubules.

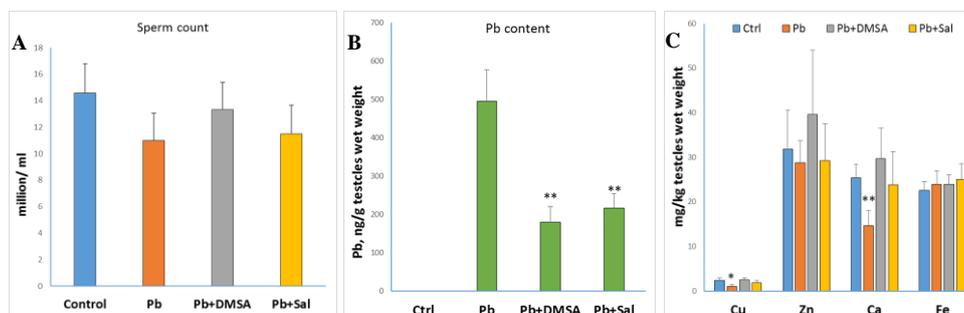


Figure 2. A - Sperm count (million/ml); B - Pb content in the testis (ng/g testis wet weight); C - Endogenous concentrations of Cu, Zn, Ca and Fe (mg/kg testes wet weight). Ctrl-control; Pb-toxic control; Pb+DMSA - Pb intoxication followed by detoxification with DMSA; Pb+Sal - Pb intoxication followed by detoxification with Sal. Data represent mean value \pm SD (* p < 0.05; ** p < 0.01).

References

- Aaseth, Jan; Crisponi, Guido; Anderson, Ole (2016). *Chelation Therapy in the Treatment of Metal Intoxication*. Academic Press. p. 388.
- Blanusa, M., Varnai, V. M., Piasek, M., & Kostial, K. (2005). Chelators as antidotes of metal toxicity: therapeutic and experimental aspects. *Current medicinal chemistry*, 12(23), 2771-2794.
- Ivanova, J., Gluhcheva, Y., Dimova, D., Pavlova, E., & Arpadjan, S. (2016). Comparative assessment of the effects of salinomycin and monensin on the biodistribution of lead and some essential metal ions in mice, subjected to subacute lead intoxication. *Journal of Trace Elements in Medicine and Biology*, 33, 31-36.
- Oehme, F.W., J.A. Pickrell, An analysis of the chronic oral toxicity of polyetherionophore antibiotics in animals, *Vet. Hum. Toxicol.* 41 (1999) 251-257.
- Palani A., Alshatteri A. (2017), Impact of Trace Elements in the Seminal Plasma on Sperm Quality in Infertile Men, *ZANCO Journal of Pure and Applied Sciences*, 29 (4), 150-156.
- Sokol R. (1989). Reversibility of the toxic effect of lead on the Male Reproductive Axis. *Reprod Toxicol* 3: 310-316.
- Wani, A. L., Ara, A., & Usmani, J. A. (2015). Lead toxicity: a review. *Interdisciplinary toxicology*, 8(2), 55-64.