Effects of exposure to lead on the peripheral motor system of the rat

An ultrastructural study

Mohammed H. Badawoud, *MSc, PhD,* ***Sherif M. Hassan,*** *MD, PhD.*

ABSTRACT

الأهداف: فحص التغيرات الشكلية فى الجهاز الحركى الطرفى للفأر الناتجة عن التعرض للرصاص.

الطريقة: أجريت هذه الدراسة بقسم التشريح، كلية الطب، جامعة الملك عبدالعزيز خلال الفترة من يناير2011م إلى يناير 2012م. استخدمت أناث الفأر الأبيض الألبينوعدد=10 التى تناولت خلات الرصاص فى ماء الشرب (500مج/لتر) لمدة 30 يوم. واستخدم عدد=5 فئران من نوع ألبينو كمجموعة شاهد. كما تم ترشيح عضلات الساق وتجهيزها للفحص بالميكروسكوب الالكتروني.

النتائج: أدى التعرض لعنصر الرصاص إلى تغيرات تركيبية فى كل أجزاء الجهاز الحركى الطرفى فى الفئران، هذه التغيرات شملت تضخم خلايا شوان وتكوينها للعديد من الزوائد السيتوبلازمية، احتقان النهايات العصبية، انسحاب بعض النهايات العصبية بالإضافة إلى بعض التغيرات فى الألياف العضلية.

خاتمة: أن التسمم بالرصاص له تأثير ضار على الجهاز الحركى الطرفى فى الفأر. كما أن التغيرات المرضية التى تم رصدها تفسر بعض الأعراض الإكلينيكية للتسمم بالرصاص.

Objective: To investigate the morphological changes in the peripheral motor system of the rat induced by exposure to lead.

Methods: This study was conducted at the Anatomy Department, Faculty of Medicine, King Abdulaziz University, Jeddah, Kingdom of Saudi Arabia from January 2011 to January 2012. Female adult albino rats (n=10) were given lead acetate in their drinking water (500mg/L) for a period of 30 days. Female adult albino rats (n=5) were used as control. The soleus and gastrocnemius muscles were dissected and processed for electron microscopy.

Results: Lead administration induced morphological changes in all constituents of the peripheral motor system of the rat, including; extension of long processes by Schwann cells, engorgement of nerve terminals, withdrawal of some terminals, and muscle fiber alterations.

Conclusion: Lead toxicity is detrimental to all constituents of the peripheral motor system of the rat. The histopathological changes explain some of the clinical manifestations of lead toxicity.

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From the Department of Anatomy, Faculty of Medicine, King Abdulaziz University, Jeddah, Kingdom of Saudi Arabia.

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Address correspondence and reprint request to: Prof. Mohammed H. Badawoud, Department of Anatomy, Faculty of Medicine, King Abdulaziz University, PO Box 80205, Jeddah 21589, Kingdom of Saudi Arabia. Fax. +966 (2) 6401000 Ext. 20121. E-mail: mbadawoud@hotmail.com

Lead is a natural element, which has been recognized as a general metabolic poison. Exposure to lead, at the levels to which human beings are exposed in the general environment, continues a major public health hazard, particularly in third world nations.[1] Although exposure to lead affects virtually every system in the body, it is particularly harmful to the nervous system, specifically the developing brain.[2] Children are particularly sensitive to the hazardous effects of lead, as a greater proportion of systemically circulating lead gains access to the brain of children than of adults.[2] Many of lead's toxic effects are attributed to its ability to substitute for calcium (Ca) ions.[3] The lead-induced toxicity is also manifested in the peripheral nervous system, specifically, the peripheral motor system. Muscle fatigue, aching and tenderness of muscles and joints characterize the lead-induced peripheral neuropathy. There may also be weakness and reduced tone, and even atrophy of muscles.[4,5] It has been shown that exposure to lead affects all components of the peripheral motor system.[6] Schwann cell affection and

segmental demyelination are among the well established changes resulting from exposure to lead.[6] In addition to the lead-induced Schwann cell damage, axonal changes have been reported in lead-exposed rats. The changes include demyelination and mild axonal degeneration, in addition to reduced neurofilaments and tubules.[7] The neuromuscular junction is another target for lead poisoning. Damage to both the pre- and post-synaptic elements of the neuromuscular junction induced by lead exposure has been reported in the literature.[4,5] Lead suppresses the quantal release of neurotransmitters by blocking Ca^{2+} entry into the presynaptic nerve terminals.[8,9] In addition, exposure to lead seems to exert a detrimental effect on the neurotransmitter receptors, resulting in reducing the aggregation of these receptors at the sites of nerve-muscle contact.[5] Post-synaptically, exposure to lead blocks the nicotinic receptor-mediated response at a relative low concentration,[10] and induces various myopathic changes.[11] The above-mentioned effects on the components of the peripheral motor system are likely to induce clinical manifestations of muscle weakness, and perhaps denervation-like changes. Clinical correlates of peripheral motor neuropathy induced by lead exposure have indeed been reported.[12,13] To summarize, it is clear from the aforementioned that exposure to lead exerts harmful effects on all components of the peripheral motor system. It is logical to speculate that affection of Schwann cells induced by exposure to lead compromises the whole peripheral motor system. Schwann cells are known to exert a trophic effect on axonal growth, and to play a crucial role in the formation (and maintenance) of the neuromuscular junction.[14,15] In view of this, we speculate that the damage inflicted on Schwann cells by exposure to lead is likely to induce denervation-like changes in skeletal muscles, including withdrawal of nerve terminals and disruption of the neuromuscular junction. In the present study, we have examined the ultra-structural alterations induced in the peripheral motor system of the rat following administration of lead acetate. Our objective was to clarify whether exposure to lead-induced withdrawal of axonal terminals from muscle cells. We aimed to answer the question "would the lead-induced decreased aggregation of acetylcholine receptors lead to disruption of the neuromuscular junction and, consequently, withdrawal of nerve terminals"? In another respect, we wanted to investigate whether exposure to lead induced denervation and myopathic changes.

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Methods.

Study design. This study was conducted at the Anatomy Department, Faculty of Medicine, King Abdulaziz University, Jeddah, Kingdom of Saudi Arabia from January 2011 to January 2012. Ethical approval was obtained from the ethic approval committee in the Faculty of Medicine, King Abdulaziz University. Adult female albino rats age 60 days old (~250 gm body weight) were used (n=15). The rats were divided into treated (n=10) and control (n=5) groups. The treated rats were given lead acetate in their drinking water (500 mg/L) for a period of 30 days. The daily water intake by the adult rats was measured to adjust the dose.

Animal sacrifice and collection of specimens. Under deep Nembutal anesthesia, animals were perfused through the left ventricle with 2% glutaraldehyde. The soleus and gastrocnemius muscles were dissected, and the collected specimens processed for electron microscopy.

Electron microscopy. Longitudinal strips were taken from the dissected muscles, divided into small blocks and immediately fixed in 2% glutaraldehyde for 60 minutes. After thorough rinsing with phosphate buffer, muscle blocks were post-fixed in 2% osmium tetroxide for another hour. The blocks were then dehydrated in graded alcohol series and embedded in an Epon mixture. One-micron sections were cut from the Epon blocks, examined, and areas containing motor endplates were trimmed out. Ultrathin sections of endplate-containing areas were counterstained with uranyl acetate and lead citrate, and viewed under the electron microscope. All drugs and chemicals were purchased from Sigma Aldrich, GMBH, Germany.

Results.

Control animals. The ultrastructure of the neuromuscular junction in control animals appeared normal where the nerve terminals lay in the synaptic troughs apposed to the postsynaptic folds. The exposed parts of the presynaptic membrane were covered by Schwann cells or their cytoplasmic processes. The nerve terminals showed normal composition of synaptic vesicles and mitochondria. The covering Schwann cells also showed a normal subcellular structure. The underlying postsynaptic folds did not show any abnormalities. Also, the myofibrillar structure showed well-organized myofilaments, and the myonuclei existed peripherally, just below the plasma membrane, and were abundant near the synaptic sites. The interstitial cell profiles were normal and no cellular infiltrates could be seen.

Treated animals. Examination of muscle specimens from the lead treated animals showed a number of striking abnormalities, both pre- and post-synaptically. The observed alterations were seen to a variable degree in all animals of this group. Perisynaptic Schwann cells appeared hypertrophied, and extended many elaborate processes. In many instances, the Schwann cell cytoplasmic processes surrounded unmyelinated axons. Occasionally, Schwann cells exhibited onion-bulb formations (**Figures 1A & 1B**). The nerve terminals appeared engorged with synaptic vesicles and mitochondria, and some contained membranous inclusions (**Figures 2A & 2B**). Although the apposed postsynaptic folds appeared normal, in many instances they were denuded of their nerve terminals, where only profiles of basal lamina could be seen (**Figures 2A & 2B**). The ultrastructure of muscle fibers appeared normal in some parts of the muscle fibers, but was completely lost in other parts. Complete disorganization of the myofibrillar structure was frequently seen to a variable degree. In some areas the myofibrils disappeared completely and were replaced by an amorphous structure (**Figure 3**). The muscle membrane appeared undulated and redundant, and large mitochondrial aggregates underneath the plasma membrane were frequently encountered both near and away from synaptic sites (**Figures 4A & 4B**). In some instances, the mitochondrial cristae showed a fingerprint appearance (**Figure 4B**). Activated myo-satellite cells were frequently encountered. Satellite cells were recognized from myonuclei by their location. Satellite cells were sandwiched between the plasma membrane and the basal lamina of muscle fibers (**Figure 5A**). Some of these satellite cells extended long processes deep to the basal lamina (**Figure 5B**). In many instances the myonuclei were seen invading the center of the muscle fibers and not restricted to the muscle fiber surface. Such central nuclei appeared paler than those normally seen peripherally (**Figure 6**).

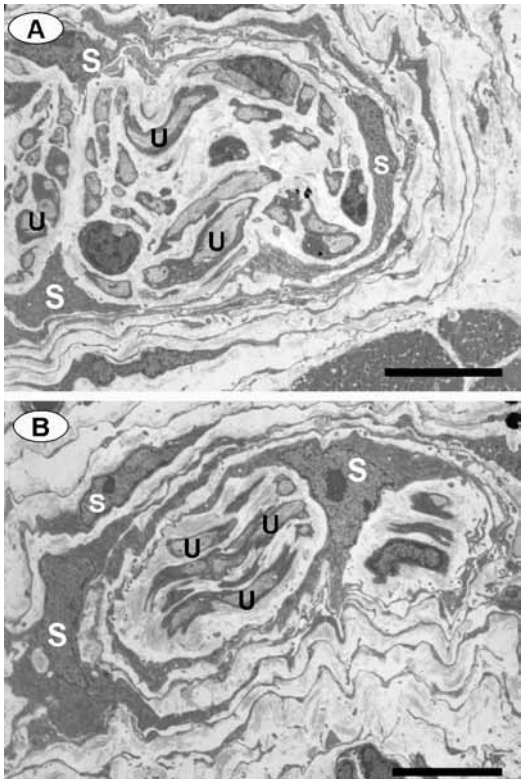

Figure 1 - Electron micrographs of a muscle specimen from lead-exposed rats. The Schwann cells (S) in **A)** and **B)** surround unmyelinated nerve fibers (u). Note the elaborate processes extending from the Schwann cells and arranged in 3 or 4 layers around the nerve fibers in an onion-bulb formation. Bars = 10 µm.

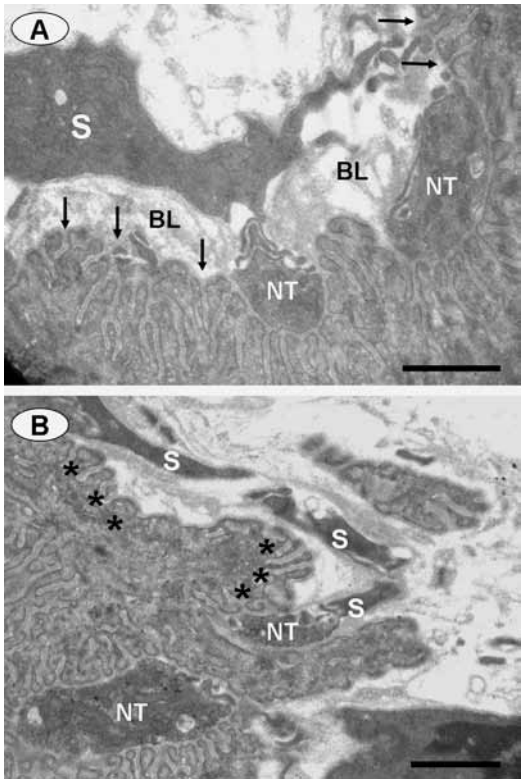

Figure 2 - Electron micrographs of neuromuscular junctions from lead-exposed rats. Schwann cell processes (S) cover the nerve terminals (NT), which are engorged with synaptic vesicles and mitochondria. Note the large expanses of postsynaptic folds in **A)** (arrows) and **B)** (asterisks), which are denuded of a nerve terminal cover. Shadows of basal lamina (BL) are seen in **A)**. Bars = 5µm.

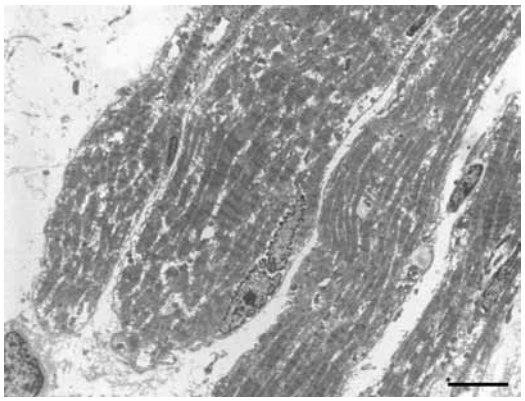

Figure 3 - Electron micrograph of a muscle specimen from lead-exposed rats. The muscle fibers appear atrophic with loss of myofibrillar architecture and widened interstitial spaces. Bar = 5µm.

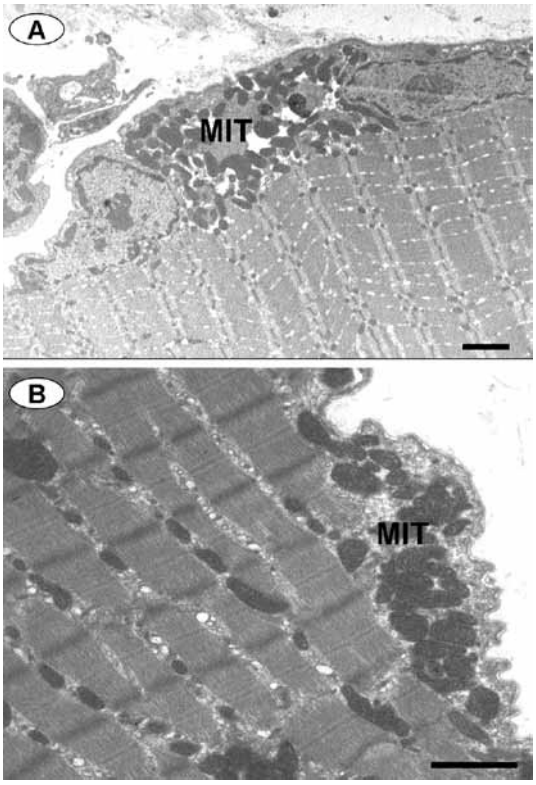

Figure 4 - Electron micrographs of a muscle specimen from lead-exposed rats. The muscle fiber in **A**) shows a mitochondrial aggregate (MIT) underneath the plasma membrane. Note the finger-print appearance of the mitochondria in **B**). Bars = 2µm.

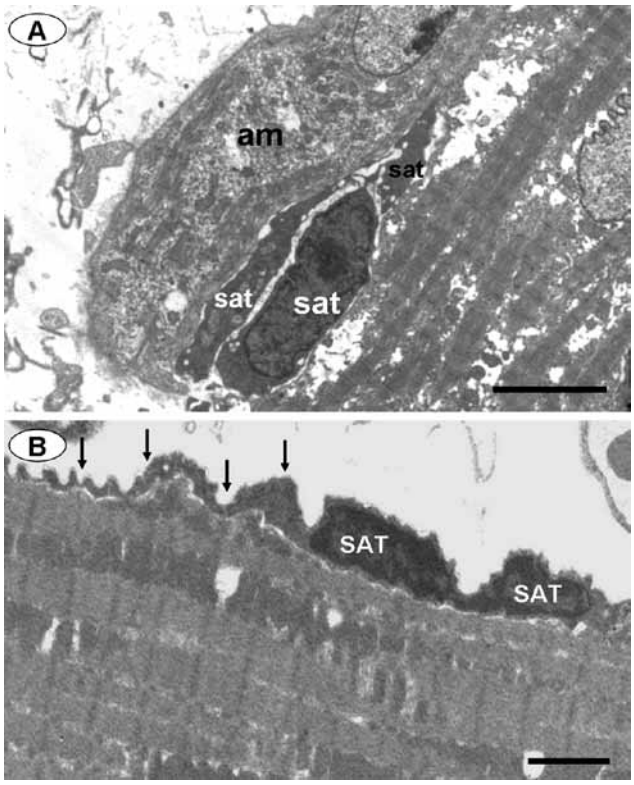

Figure 5 - Electron micrographs of a muscle specimen from lead-exposed rats. Satellite cells (sat) are seen in **A**) enveloped between the basal lamina and plasma membrane. Note the complete loss of myofibrillar architecture, which is replaced by an amorphous structure (am) and mitochondria. The muscle fiber in **B**) shows a sat cell (SAT), which has extended a long process (arrows) underneath the basal lamina. Bars = 5µm.

Discussion. In the present work, we investigated the changes induced in the peripheral motor system of the rat following oral administration of lead acetate. The results show that exposure to lead is definitely detrimental to the peripheral motor system of the rat. The alterations seen in the different constituents of the peripheral motor system were striking and inline with previous observations.

It was stated in the literature that lead is harmful to the nervous system, specifically the developing brain.[2] The toxic effect of lead was reported to be attributed to its ability to substitute for calcium ions.[3] It was also found that lead toxicity is also manifested in the peripheral nervous system, specifically, the peripheral motor system. The lead was reported to induce peripheral neuropathy.[4,5] It was found that exposure to lead caused Schwann cell damage and segmental demyelination.[6] Demyelination and mild axonal degeneration, in addition to reduced neurofilaments and tubules were also reported.[7] Damage of the pre- and post-synaptic elements of the neuromuscular junction by lead exposure has also been reported in the literature.[4,5]

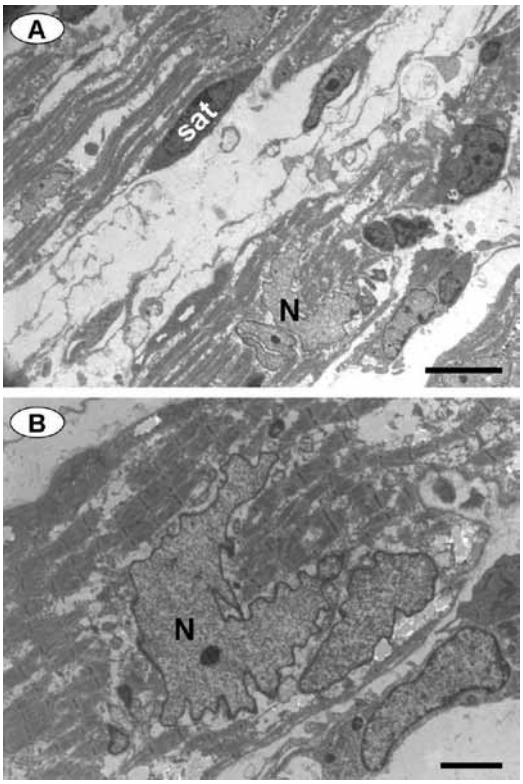

Figure 6 - Electron micrographs of muscle specimens from lead-exposed rats. The myofibrils are thinned out with loss of the normal architecture. Abnormally shaped and pale myonuclei (N) invade the center of the muscle fibers. sat = satellite cell. Bars = 5µm.

The main findings of the present work include changes in Schwann cells, nerve terminals, postsynaptic membrane, and muscle fibers. To summarize, the changes included activated hypertrophic Schwann cells which extended many long processes, onion-bulb formations, engorged nerve terminals, denuded postsynaptic folds and variable loss of myofibrillar organization.

The morphological changes seen in Schwann cells were intriguing. Extension of long cytoplasmic processes and onion-bulb formations are all indicative of nerve growth and/or repair. Schwann cells are known to surround axons, insulate them by the many layers of myelin and to provide trophic support for nerve growth and repair.[14,15] Exposure to lead appears to produce denervation-like changes. This is evident in the encountered Schwann cell changes. Similar Schwann cell alterations were reported in a number of publications.[16] However, Schwann cell damage and segmental demyelination following exposure to lead as reported by other investigators,[6] were not encountered in our study. Such a discrepancy may be attributed to the longer period of treatment, and the different route of administration of lead (intraperitoneal injections) adopted by these investigators.

The denervation-like changes reported here explain the clinical manifestations of a lead-induced peripheral neuropathy. It has been shown that such neuropathy is characterized by increased muscle fatigue, aching and tenderness of muscles, in addition to fine tremors, weakness, and reduced tone.[4] Damage to both the pre- and post-synaptic elements of the neuromuscular junction induced by lead exposure, as seen in the present work, has been reported by other investigators.[4,5] Affection of the presynaptic component of the neuromuscular junction appears to be the result of lead's effect on the presynaptic Ca^{2+} channels involved in transmitter release. Lead suppresses the quantal release of neurotransmitter by blocking Ca^{2+} entry into the presynaptic nerve terminals,[17] which leads to suppressing the activity-associated Ca^{2+}-dependent release of neurotransmitters.[9,18] Engorgement of nerve terminals with synaptic vesicles as seen in the present study is probably a morphological correlate of such suppression of the quantal release of acetylcholine. Postsynaptic troughs denuded of their covering nerve terminals as seen in the present study are indicative of elimination of such terminals. Withdrawal of nerve terminals could be the result of lead's effect on acetylcholine receptors. Chen and colleagues[5] demonstrated that exposure to lead reduced aggregation of acetylcholine receptors at the sites of nerve-muscle contact. Such aggregation of acetylcholine receptors constitutes a crucial step in the development and stability of the neuromuscular junction,[14] and hence, elimination of nerve terminals could be secondary to lack of clustering of such receptors at the synaptic sites.

Of specific interest were the alterations seen in muscle fibers, as these were striking and point to muscle stress. Undulations of the sarcolemma are indicative of muscle fiber atrophy, which could be secondary to myofiber inactivity associated with reduced quantal release of acetylcholine. Another stress-related feature is the appearance of large aggregates of mitochondria under the sarcolemma. The molecular mechanisms of such finding are yet to be established. Denervation like changes in muscle fibers included loss of myofibrillar organization and their replacement by amorphous structures.

Various myopathic changes induced by lead exposure were reported by Buchheim and colleagues.[11] It has been suggested that the lead-induced myopathic

effects may be the result of modification of Ca^{2+} release from the sarcoplasmic reticulum and increased oxygen free radical formation related to cellular energetics.[19] However, the numerous and activated myosatellite cells encountered in the present work are a sign of muscle regeneration, which probably takes place concomitant with the denervation process.

Study limitations. The limited budget available for this project was a limitation to perform more expanded study.

In conclusion, the present work demonstrates that exposure to lead is detrimental to all constituents of the peripheral motor system of the rat. The morphological abnormalities are either primary changes induced by lead toxicity or secondary to the muscle inactivity. Put together, the alterations described in the present study constitute what can be termed lead-induced neuro-myopathy. The lead-induced myopathic changes warrant closer detailed study. Immunohistochemical studies involving neurofilaments, synaptic vesicles, and acetylcholine receptors are needed to assess the damage inflicted on the axons, nerve terminals, and acetylcholine receptors.

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