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α-Tocopherol Modifies Lead Induced Functional Changes at Murine Neuromuscular Junction

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Lead impacts neuromuscular junction and might induce skeletal muscle weakness. Antioxidants may prevent toxic actions of lead on muscle. In this study, resting membrane potentials, endplate potentials, miniature endplate potentials (MEPPs) and isometric twitch tensions were recorded to investigate effects of α-tocopherol (Vitamin E) on lead induced changes at murine *dorsiflexor* muscle. Moreover, levels of endplate nicotinic receptors were measured by receptor autoradiography. Forty rats were divided into four groups (lead alone, α-tocopherol, lead plus α-tocopherol and saline). Lead (1 mg/kg, i.p.), was administered daily for 2 weeks and α-tocopherol (100 mg/kg, i.p.) was given daily for 3 weeks. Lead treatment significantly reduced twitch tension (from 4.4 ± 0.4 to 2.2 ± 0.3 g) and delayed half time of decay. MEPP frequencies and quantal content were also significantly reduced after lead treatment. Pretreatment with α-tocopherol reversed twitch tension reduction (4.1 ± 0.3 g) and modified lead induced delay in half time of decay. Similarly, α-tocopherol modified the negative actions of lead exposure on MEPP frequencies and quantal content. Receptor autoradiographic studies revealed significant increase of nicotinic receptor levels at the endplate region of flexor muscle in lead treated mice. However, animals treated with lead plus α-tocopherol showed significantly decreased levels of nicotinic receptors. α-Tocopherol appears to protect against lead induced neuromuscular dysfunction. These effects of α-tocopherol are possibly mediated via a free radical mechanism or modification of calcium homeostasis.

Keywords: Lead; α-Tocopherol; Skeletal muscle; Electrophysiology; Twitch tension; autoradiography

INTRODUCTION

Heavy metals play a significant role in modern industry and exposure to metals such as lead has been continuously increasing in many countries. The harmful effects of lead extend over various body organs/systems and therefore it is crucial to control toxicity in individuals at risk.[1] Significant neurotoxic effects particularly learning difficulties and modification of memory function were observed during childhood period following prolonged exposure to lead metal.[1–3] The effects of lead toxicity are not only limited to central nervous system but peripheral nervous system might also be susceptible to toxic effects of heavy metals.[4]

Lead impacts neuromuscular junctions and modifies skeletal muscle function in the experimental animal models and this effect could in turn be noticeable as potential cause for significant muscle weakness in humans.[4,5] Various mechanisms have been proposed to explain actions of lead on the peripheral nervous system and the skeletal muscle.[6,7] These effects on skeletal muscle may be related to interference with neurotransmitters release as well as movement of ions across the cells.[8–11] For instance, it was suggested that fatigue observed in diaphragm muscle following lead treatment may be the result of modification of calcium release from the sarcoplasmic reticulum and increased oxygen free radical formation related to cellular energetics.[12]

Radical-mediated oxidative damage of skeletal muscle membranes appears to be an important mechanism implicated in the fatigue process. For instance, it was reported that α-tocopherol deficiency adversely affects muscle contractile function, resulting in a more rapid development of muscular fatigue during exercise.[13] Subsequently giving α-tocopherol as a major chain breaking antioxidant shown to reduce

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contraction mediated oxidative damage.[13] Presumably, it can be postulated that sulfur-containing compounds and antioxidants may decrease metal toxicity possibly by providing a reducing power that prevents the enzymes from undergoing oxidation.[14,15]

Therefore, free radical mechanism seems to have a potential role in mediating the effect of lead on the peripheral nervous system and skeletal muscle.[16,17] However, despite previous reports the precise biochemical and molecular mechanisms of lead induced toxicity are still poorly understood. Although α-tocopherol may exhibit some protective role in reducing potential adverse effects from heavy metals exposure on skeletal muscle, the clinical and experimental data, in the literature, in support of this theory is very much limited. The present study was designed to study the effects of lead on *dorsiflexor* muscle contraction as well as related electrophysiological parameters such as resting membrane potentials (RMPs), endplate potentials (EPPs) and miniature endplate potentials (MEPPs) in the presence of α-tocopherol. Moreover, the effect of lead on skeletal muscle endplate nicotinic receptors was evaluated. The study aimed to assess possible protective effects of α-tocopherol on lead induced modifications of skeletal muscle twitch tension, electrophysiological parameters and nicotinic receptor levels.

MATERIALS AND METHODS

Animals

All the experiments were performed on C57 BL strain male mice (29.4 ± 1.1 g body weight). Animals were housed in groups of 5 in plastic cages with a controlled light and dark cycle of 12 h each at 24–26°C. Food and water were available *ad libitum*. Forty animals were divided into four groups of $n = 10$ each. (10 control, 10 lead treated, 10 α-tocopherol treated, 10 lead treated plus α-tocopherol). Lead treatment experiments were carried out by giving daily injections of 1.0 mg/kg lead acetate in 5% glucose solution i.p. for 2 weeks before the recording day. α-Tocopherol (100 mg/kg, i.p.) was administered daily for 3 weeks before the recording day. Except for the intended treatments all groups were handled in the same manner until the time when the electrophysiological recordings were performed. Animals were treated according to the welfare procedures and were continuously observed for their safety by local institution. The present study was authorised by Animal Research Ethics Committee.

Preparation

At the time of experiments, mice were anaesthetized with urethane (2 mg/g, i.p.) and the flexor digitorum superficialis muscle was dissected out. Care was taken not to damage the muscle. For electrophysiological recording the excised flexor muscles were pinned in a Lucite chamber containing Krebs solution (pH 7.2) that was kept at 23–25°C. Because transmitter release is affected by muscle stretching the excised muscle was pinned at 1.1 times the resting length. The Krebs solution was oxygenated (95% O_2–5% CO_2) by a gas-lifting device that circulated freshly oxygenated solution, at a rate of 10–15 ml/min without agitating the recording chamber. The *dorsiflexor* muscle was chosen because it encloses predominantly fast twitch fibres and its location makes *in situ* physiological recording possible.

Electrophysiological Recording

A combination of oblique and transillumination in conjunction with a Leitz-Wild microscope was used to locate endplate regions. Glass capillary microelectrodes, filled with 3 mol/l KCl and drawn to a tip yielding 8–15 mega-ohm resistance, were inserted into muscle fibres at the endplate region. Conventional intracellular recordings of RMPs, EPPs and MEPPs were conducted. Quantal content was calculated using the direct method (EPPs amplitude/MEPPs amplitudes). Because lead precipitates bicarbonate/phosphate buffer solutions, experiments were conducted in solutions buffered with 4-(2-hydroxyethyl)-1-pipeazineethanesulphonic acid (HEPES). Exposed muscles were bathed in Krebs solution which had the following composition in mmol/l: NaCl 135, KCl 5, Ca Gluconate 2.5, $MgSO_4$ 1, HEPES 3, glucose 11 and pH = 7.2. In order to record evoked EPPs a low Ca^{2+} (0.5 mmol/l)–high Mg^{2+} (2.75 mmol/l) Krebs saline was used and the nerve was stimulated supramaximally at 0.5 Hz. In the current experiments only focal MEPPs and EPPs with rise time of <1 ms were accepted.

Measuring Isometric Twitch Tension

Isometric twitch tensions (evoked either directly by stimulation of the muscle or indirectly by stimulation of motor nerve) were measured after the tendinous insertion was attached to a force displacement transducer (Grass Model FT-03C). The output was differentially amplified and displayed on a chart recorder for analysis. Following a temperature equilibration of 37°C, twitch responses to supramaximal stimuli delivered to the *dorsiflexor* nerve at 1 Hz were recorded in *dorsiflexor* muscle. Direct muscle stimulation was accomplished by placing two wide platinum wires underneath the muscle. Twitches were evoked either directly or indirectly using a Grass (S44) stimulator delivering 5 V, 0.5 ms duration DC square wave pulses. The muscle was lengthened until

a maximum twitch response was elicited. This was achieved when the muscle was stretched by 1.1 times its resting length. Normal Krebs solution was used to irrigate the exposed muscle and nerve.

Receptor Autoradiography

Levels of nicotinic acetylcholine receptors were evaluated using quantitative receptor autoradiography. Longitudinal sections of muscle (10 mm) were obtained and incubated with ^{3}H-bungarotoxin (^{3}H-α-BTx, specific activity 70 Ci/mmol) in 0.05 M Na/K-phosphate buffer. Non-specific binding was determined in another set of slides by parallel incubation in the presence of 10^{-4} M unlabelled α-BTx. α-BTx binding was quantified in 2–3 sections from each muscle. The specific binding was expressed as fmol/mg tissue wet weight.

Plasma and Muscle Lead Levels

Plasma and muscle lead levels were measured using Inductively Coupled plasma mass spectrometry (ICP-MS, Plasma Quad 3 VG Elemental, UK). Blood, tissue samples and reference material were prepared for Pb analysis by nitric acid digestion using temperature control microwave heating (Milestone, ETHOS Labstation). Internal standard (In) was added prior to microwave digestion. Analytical standards for calibration were prepared from Pb standard solution (1 g/l) SPEX Plasma Standards, Edison, USA. All lab-ware used was polystyrene made, acid-washed and kept protected from dust. Fully quantitative three point calibration was applied for Pb analysis.

Tocopherol Levels in Plasma and Muscle

Tocopherol was assayed from plasma by HPLC using an aminoquant system (Hewlett Packard) with a waters c_{18} reverse phase column 3.9 × 300 mm, 10 μm particle size, using methanol, butanol and water (89.5: 5: 5.5%) v/v as mobile phase. Tocopherol detected using a uv detector at a wave length of 290 nm. Tocopherol and the internal standard recovered from plasma using butanol-ethyl acetate mixture (1:1) v/v concentrations worked out from a calibration curve linear from 250 ng/ml to 100 ug/ml. The method yielded a recovery of >90%, with precision c.v 6.5% (intra assay, $n = 5$) and c.v 5.4% inter assay, $n = 5$). Tocopherol from the tissues assayed by a slight modification of the method for plasma. Preweighed tissues ground, using a electric tissue homoginizer in ethyl acetate, solvent separated, after centrifugation, evaporated and reconstituted in 100 ul of extraction solvent and injected. Tocopherol content expressed in ug/mg wet tissue.

Statistical Analysis

The mean was obtained for each mouse, and then the group mean and standard deviation were calculated using means from different mice in that group. The results from various groups were compared using both *t*-test and analysis of variance (ANOVA) methods. For experiments, the sample size was 10 and the significance level of 0.05 (95% confidence) was considered as a cut off. The results of group comparison were expressed as mean ± SD.

RESULTS

Animal Weights

Mice chronically treated with lead did not differ significantly in their body weights from control groups at the time of experiment (33.1 ± 3.4 g for control vs. 31.6 ± 2.5 g for lead treated). The *dorsiflexor* muscle to body weight ratio also remained unchanged. Furthermore, lead treated mice did not exhibit signs of severe lead induced neuromuscular pathology such as ataxia or splayed gait.

Lead Levels

The concentrations of lead were measured in both plasma and muscle in all experimental groups. Lead treated animals showed significantly higher concentration in plasma compared to control. The animals treated α-tocopherol plus lead showed slightly higher plasma concentration compared to lead alone. Although this was not statistically significant α-tocopherol possibly had some role in preventing lead from getting into tissue and pushing it into plasma (Fig. 1). Muscle lead levels were significantly increased in lead treated group. Compared to lead

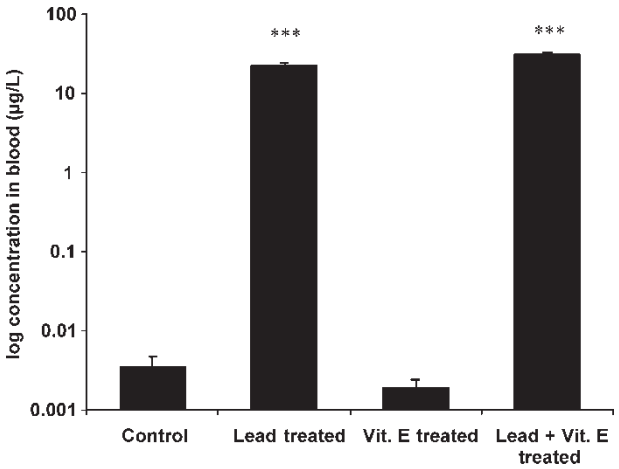

FIGURE 1 Concentration of lead in blood from controls, lead treated, vitamin E treated and lead + vitamin E treated mice. Values represent means ± SEM of 5 animals in each group. ***$p < 0.001$, significantly different from controls.

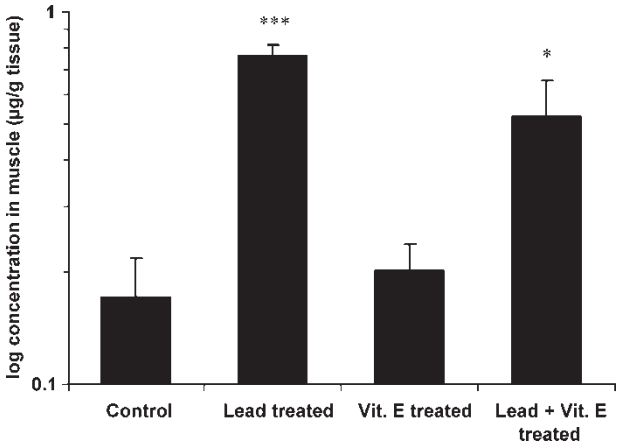

FIGURE 2 Concentration of lead in muscles from controls, lead treated, vitamin E treated and lead + vitamin E treated mice. Values represent means ± SEM of 5 animals in each group. *$p < 0.05$, **$p < 0.01$ and ***$p < 0.001$ significantly different from controls.

alone animals that received lead plus α-tocopherol showed lower concentration. This might also support the idea that that α-tocopherol may exert some effect on lead uptake by skeletal muscle. Nevertheless, contribution of this effect to the action of lead on muscle needs further investigation (Fig. 2).

α-Tocopherol Levels

α-Tocopherol concentrations were measured in both plasma and muscle in all experimental groups. The animals treated with α-tocopherol (either alone or plus lead) showed significantly higher plasma as well as muscle concentration. Pretreatment with α-tocopherol resulted in doubling the plasma concentration. Compared to control group the concentration of α-tocopherol in skeletal muscle was increased by almost 200% (Fig. 3). This might indicate that significant amount of α-tocopherol is reaching the site of action in order to produce modulator effect on skeletal muscle either through an antioxidant or a different mechanism.

RMP

There were no differences in RMP between control and lead treated mice (77.4 ± 2.1 mV for control and 76.9 ± 3.8 mV for lead treated). Pretreatment with 100 mg/kg α-tocopherol did not modify RMPs in either lead treated or control mice. RMPs values for both lead treated and control mice in the presence of α-tocopherol are shown in Table I.

Spontaneous Transmitter Release

Spontaneous transmitter release was represented as electrophysiological recording of MEPPs. Different type of MEPPs amplitude are usually present at murine NMJ. Unimodal, bimodal, small mode and large mode MEPPs amplitude were all present in both control and experimental animals. Since the bimodal amplitude was twice and the large mode was 3 standard deviations of those unimodal MEPP, we decided to set the window discriminator to exclude those kinds of MEPPs. Therefore, only unimodal MEPP were included in our analysis and hence resulted in a much more uniform MEPP amplitude with minimum SD.

Both MEPPs frequencies and MEPPs amplitudes were evaluated during the experiments. MEPPs frequencies became significantly lower with lead exposure while MEPPs amplitudes remained

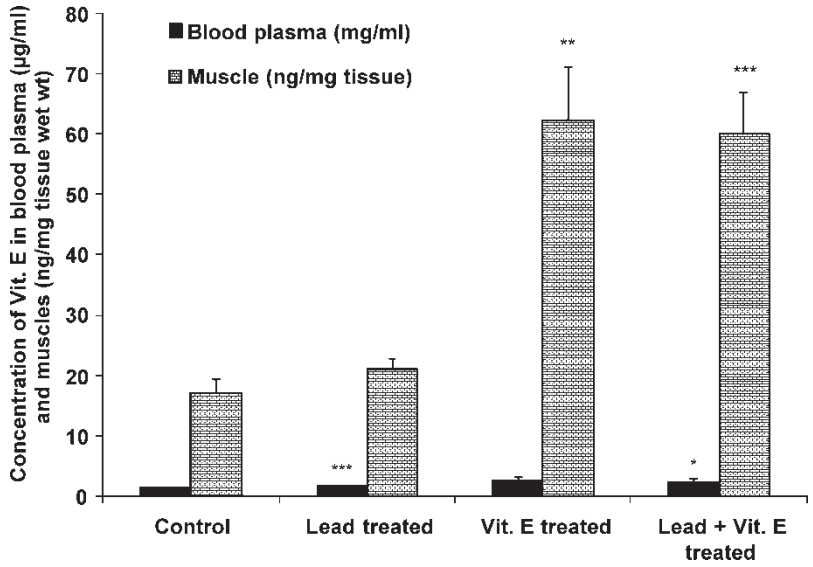

FIGURE 3 Concentration of vitamin E in blood plasma and muscles from control, lead treated, vitamin E treated, and lead + vitamin E treated mice. Values represent means ± SEM of 5 animals in each group. *$p < 0.05$, **$p < 0.01$ and ***$p < 0.001$, significantly different from controls.

TABLE I Effects of α-tocopherol (100 mg/kg, i.p.) on RMPs, MEPPs frequencies and MEPPs amplitudes in *dorsiflexor* muscle of control and lead treated mice (values represent means ± SD of 10 animals, 20 muscle fibers from each mouse)

Physiological parameters	Control	Lead	Control + α-tocopherol	Lead + α-tocopherol
RMP (mV)	77.4 ± 2.1	76.9 ± 3.8	78.2 ± 4.5	75.7 ± 5.1
MEPPs frequencies (Hz)	3.2 ± 0.2	1.8 ± 0.3*	2.8 ± 0.3	2.7 ± 0.2**
MEPPs amplitudes (mV)	0.59 ± 0.02	0.60 ± 0.01	0.58 ± 0.02	0.57 ± 0.03

*Significantly different from untreated control values ($P < 0.001$). **Significantly different from lead treated values ($P < 0.05$).

unchanged. Pretreatment with α-tocopherol (100 mg/kg) reversed lead induced alterations in MEPPs frequencies. The values of MEPPs frequencies and MEPPs amplitudes for lead treated mice and control animals in the presence of α-tocopherol are shown in Table I.

Evoked Transmitter Release

Evoked transmitter release was represented measuring quantal content. Compared to control lead treatment significantly reduced quantal content. Pretreatment with 100 mg/kg α-tocopherol reversed quantal content reduction in lead treated mice rendering it close to the control values. Changes in quantal content for lead treated mice and control animals in the presence of α-tocopherol are shown in Fig. 4.

Isometric Twitch Tension

In control mice indirect supramaximal nerve and direct muscle stimulation resulted in twitch tensions

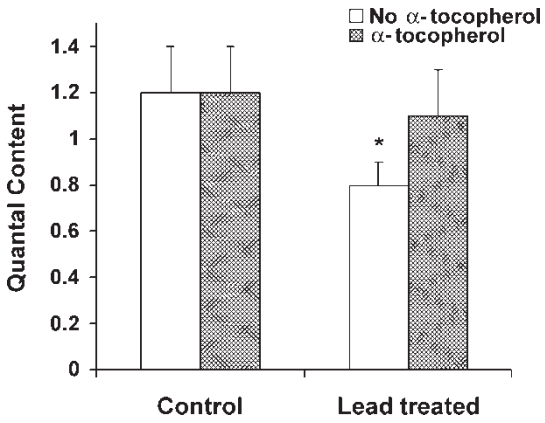

FIGURE 4 Effects of α-tocopherol (100 mg/kg, i.p.) on quantal content in *dorsiflexor* muscle of control and lead treated mice (values represent means ± SD of 10 animals, 20 muscle fibers from each mouse). *$P < 0.01$, significantly different from untreated control values.

TABLE II Effects of α-tocopherol (100 mg/kg, i.p.) on lead induced changes in *dorsiflexor* muscle contraction of control and lead treated mice (values represent means ± SD of 10 animals)

Muscle characteristics	Control	Lead	Control + α-tocopherol	Lead + α-tocopherol
Indirect nerve stimulation				
Rise time (ms)	0.42 ± 0.2	0.38 ± 0.1	0.4 ± 0.1	0.39 ± 0.1
1/2 Decay time (ms)	0.46 ± 0.2	0.58 ± 0.1*	0.47 ± 0.2	0.46 ± 0.2
Twitch tension (g)	4.1 ± 0.2	2.1 ± 0.3*	4.0 ± 0.5	3.70 ± 0.3**
Direct muscle stimulation				
Rise time (ms)	0.47 ± 0.1	0.48 ± 0.2	0.45 ± 0.1	0.46 ± 0.1
1/2 Decay time (ms)	0.50 ± 0.2	0.59 ± 0.2*	0.48 ± 0.1	0.46 ± 0.2
Twitch tension (g)	4.4 ± 0.4	2.2 ± 0.3*	4.2 ± 0.6	4.1 ± 0.3**

*Significantly different from control values ($P < 0.001$). **Significantly different from lead treated values ($P < 0.001$).

of 4.1 ± 0.2 and 4.4 ± 0.4 g, respectively. Isometric force of contraction in response to indirect supramaximal and direct muscle stimulation were both reduced in lead treated mice (indirect stimulation to 2.1 ± 0.3; direct stimulation to 2.2 ± 0.3). The reduction was proportional to the size of the muscle. Although muscles from lead treated mice generated a significantly smaller force of contraction upon stimulation, lead treatment had no effect on contractile speed. Treatment with α-tocopherol alone did not affect the force of contraction in control animals. Following treatment with α-tocopherol lead induced reduction in muscle tension was modified whether under indirect nerve stimulation or direct muscle stimulation (3.70 ± 0.3 and 4.1 ± 0.3 g, respectively). No changes were observed in latency or rise time following lead exposure and α-tocopherol treatment had no effect on latency or rise time. Half decay time was prolonged following lead treatment and α-tocopherol reduced half decay time in these animals. The results of muscle twitch tensions for both lead treated and control mice in the presence of α-tocopherol are shown in Table II.

Receptor Autoradiography

Following lead treatment significant changes in the distribution of nicotinic receptors at the postsynaptic membrane of the neuromuscular junction were found. Quantitatively, a significant increase of nicotinic receptor levels in the endplate of flexor muscle was observed in lead treated animals compared to control mice. Treatment with lead plus α-tocopherol resulted in significant decrease in levels of nicotinic receptors compared to treatment with lead alone. The changes in nicotinic receptors

TABLE III Effects of α-tocopherol (100 mg/kg, i.p.) on lead (10 mM, i.p.) induced changes in nicotinic acetylcholine receptors in the *dorsiflexor* muscle of control and lead treated mice (values represent means ± SD of 10 animals)

Receptor autoradiography	Control	Lead	Control + α-tocopherol	Lead + α-tocopherol
Level of nicotinic acetylcholine receptors (fmol/mg wet tissue weight)	18.7 ± 2.5	36.5 ± 3.5*	19.7 ± 3.1	27.5 ± 1.3**

*Significantly different from control ($P < 0.001$). **Significantly different from lead treated ($P < 0.005$).

distribution for both lead treated and control mice in the presence of α-tocopherol are shown in Table III.

DISCUSSION

Most experiments dealing with the effects of lead on the peripheral nervous system and skeletal muscle function did not address the modulating effects of antioxidants and possible implications of nutritional status on improving heavy metals exposure. The current study was designed to investigate the effects of lead treatment on skeletal muscle contraction as well as spontaneous and evoked transmitter release at mouse *dorsiflexor* muscle in the presence of α-tocopherol. Moreover, the effects of lead as well as lead plus α-tocopherol on skeletal muscle endplate nicotinic cholinergic receptors were evaluated.

Our results showed that lead treatment would not significantly alter RMP, however, MEPPs frequencies at *dorsiflexor* muscle were significantly decreased after lead exposure. Pretreatment with α-tocopherol reversed deleterious actions of lead treatment on MEPPs frequencies. This phenomenon may suggest an imbalance in antioxidative system at the skeletal muscle of mice subjected to lead induced toxicity. Lead treatment also exerted significant effects on evoked transmitter release and reduced both EPPs amplitudes and calculated quantal content. Furthermore, the *dorsiflexor* twitch tension was significantly reduced in lead treated mice compared to control. Tension decrement was observed with both nerve stimulation and muscle stimulation that elicited similar responses. The inhibitory action of lead on transmitter release resembles the effect shown by other metals like cadmium and zinc.[18–20]

Changes in physiological parameters and corresponding ultrastructural features of *dorsiflexor* muscle after lead treatment have been reported.[5] The reduction of twitch tension after lead treatment may be correlated to the morphological changes. The ultrastructural modifications after lead exposure included reduced number of synaptic vesicles, disruption of mitochondria and increased number of smooth endoplasmic reticulum and presence of myelin like figures in the intramuscular axons and neuromuscular junctions.[5] It may be possible that lead exposure compromises the skeletal muscle isometric contraction via modification of cellular mechanisms. Since the effects of lead were modified in animals pretreated with α-tocopherol this may suggest involvement of free radical systems. Lead elicited modifications in the regulation of the myoplasmic transient could form an essential part of explaining altered twitch contractile properties following lead treatment.[7] Furthermore, lead treatment may also exert some action on the process of the neurotransmitter release.[21,22]

Further studies indicated tissue specific changes following lead exposure and recorded responses to treatment with different antioxidants via monitoring parameters of oxidative damage, lipid peroxides level, antioxidant enzymes and thiol contents.[24] Interestingly, treatment with antioxidants resulted in reversal of oxidative stress even without significant initial decline in tissue lead burden. The ameliorative effects of antioxidants like α-tocopherol on parameters indicative of oxidative stress in the liver, kidney and brain of lead exposed rats pointed towards a positive value and a predictable protective role.[23,24]

Previously, we have studied the effects of ascorbic acid on lead induced modification of skeletal muscle structure and function. Lead treatment reduced twitch tension significantly and delayed half time of decay compared to controls. MEPPs frequencies became significantly lower with lead exposure while MEPPs amplitudes remained unchanged. The reduction of twitch tension after lead treatment was reversed following pretreatment with ascorbic acid. Furthermore, pretreatment with ascorbic acid modified lead induced alterations in MEPPs frequencies. It appears that ascorbic acid exerts a protective role against lead induced peripheral nerve and muscle dysfunction.[25]

Currently, similar results were obtained with α-tocopherol pretreatment. α-Tocopherol prevented twitch tension reduction in lead treated mice and reversed lead induced delay in half time of decay. The negative actions of lead treatment on MEPPs frequencies were also modified with α-tocopherol. The mechanism for the effects of α-tocopherol on lead induced changes appears to be similar to ascorbic acid and could be partly explained by free radical scavenging. However, the effects of α-tocopherol on muscle structure may not be exclusively explained by free radical scavenging.

Effects of lead treatment on skeletal muscle could result from blocking calcium entry into nerve terminals via voltage sensitive channels. This action of lead is possibly attributed either to affecting the calcium receptors located on the surface of nerve terminal or disrupting the processes involved in calcium buffering inside the nerve terminal.[7,9] Lead may have an influence on surface charge affecting the amount of calcium available to its channels in the terminal membrane or it may have an action on the internal calcium binding sites and consequently affecting the level of calcium sequestering activity.[10] There is also some evidence that demonstrate direct effect of lead on increasing calcium binding by mitochondria.[9]

Our results showed a significant change in nicotinic acetylcholine receptors distribution at the postsynaptic membrane of neuromuscular junction with formation of separate islands of receptors after lead treatment. A significant increase of nicotinic receptor levels in the end plate of flexor muscle was observed in lead treated compared to control mice. Interestingly, the lead plus α-tocopherol treated mice showed significantly decreased levels of nicotinic receptors compared to the lead treated mice. It could be speculated that lead decreases the release acetylcholine and thereby upregulation of nicotinic receptors, reminiscent of denervated muscle, leading to the observed muscle weakness.

Protective effects of antioxidant vitamins in heavy metals poisoning might occur either directly at the cellular level or indirectly by interfering with the intestinal absorption of heavy metals in other occasions.[26] For instance, the inhibition of protein kinase C was believed to be the basis for the effects of α-tocopherol on vascular smooth muscle. Additionally α-tocopherol was shown to inhibit cell proliferation, platelet aggregation and monocyte adhesion. These effects appear to be unrelated to the antioxidant activity of α-tocopherol, and possibly reflect specific interactions of α-tocopherol with enzymes, structural proteins, lipids and transcription factors.[27] Recently, several novel tocopherol-binding proteins have been cloned, that may mediate the non-antioxidant signaling and cellular functions of vitamin E and its correct intracellular distribution.[28] Similarly, the effects of vitamin E on muscle could not totally be explained by antioxidant mechanism protein kinase C dependant mechanism. The effects of α-tocopherol treatment on skeletal muscle may be related to increases in gene expression by a protein kinase C-independent, non-antioxidant mechanism. The modulation of nicotinic receptors in the current study might further support the action of α-tocopherol on gene expression.

In conclusion, lead exposure may impact nerve and muscle via affecting neuromuscular junction. The effects of lead could be extended to the modification of muscle function and alteration of nicotinic cholinergic receptors. Free radical mechanism as well as modulation of gene expression could be implicated in the effects of lead at the neuromuscular junction. Lead induced modification of neuromuscular junction and muscle function could be reversed by pretreatment with the antioxidants such as α-tocopherol

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