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# Effects of Chronic Lead Exposure on Aminoglycosides-induced Changes in Guina Pig Auditory Brainstem Responses

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**Abstract:** The effects of chronic lead acetate treatment on alterations of Auditory Brainstem Response (ABR) induced by gentamicin and amikacin were tested in guinea pigs. This study was designed to investigate the hypothesis that aminoglycoside antibiotics and lead via their effects on phosphoinositide pathway and calcium channels may influence ototoxicity mechanisms as tested on the auditory brainstem response. Intramuscular injection of different doses of gentamicin (5, 10 mg/kg/day) and amikacin (150, 300 mg/kg/day) for three weeks induced hearing loss. Administration of lead acetate (0.05%, 35 days) in drinking water changed ABR (absolute wave latency). The gentamicin-induced alteration of ABR were attenuated by chronic lead acetate pretreatment in guinea pig. Obtained data suggest the interactions between chronic lead acetate and aminoglycoside antibiotics in alterations of auditory functions which could be accounted for, at least partially, by perturbations of the phosphoinositide and calcium cascades within the inner ear.

**Key words:** Lead acetate, gentamicin, amikacin, auditory brainstem response, phosphoinositides

## INTRODUCTION

Theoretically metal ions can interfere with most biological processes and affect many kinds of cellular activities. Although the hazardous effects of exposure to lead (Pb) have been recognized, relatively little is known regarding the cellular and subcellular mechanisms involved, especially in nerve membrane. Lead is recognized as an environmental and occupational hazard that has a significant impact on the health and development of many species. The toxicity of Pb to various components of the central nervous system as well as the increased susceptibility of the developing brain has been well documented[1,2]. The sensitivity of the auditory system to lead exposure has been recognized in occupational medicine[3]. Increase in hearing threshold was found in environmentally exposed children[4]. Impaired hearing ability is associated with alterations of the brainstem auditory evoked potential like latency shifts and amplitude decreases[5,6]. In the inner ear of guinea pigs whole nerve action potentials were affected by lead[7] and in chronically exposed monkeys lead (Pb) exerted an influence on the processing of complex sounds[8,9].

Nephrotoxicity and ototoxicity are well known side effects of aminoglycoside antibiotics, but the mechanisms of ototoxicity are not clearly full understood. The development of ototoxicity as well as nephrotoxicity limits the use of these drugs. While renal damage is reversible and well controlled by medical treatment, ototoxic side effects may result in permanent hearing loss as well as vestibular dysfunction and represent a main negative issue in the clinical use of these antibiotics in humans. It has been shown that polycationic drugs such as aminoglycosides can interact with the anionic membrane phosphoinositides and prevent the formation of inositoltrisphosphate ($IP_3$) and diacylglycerol (DAG), the second messengers of phosphoinositide cascade in stimulated cells[10,11]. The present study was carried out to clarify the possible involvement of second and third messenger systems in alterations of Auditory Brainstem

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Response (ABR) induced by aminoglycoside antibiotics[12,13] and chronic lead acetate[3].

## MATERIALS AND METHODS

**Experimental groups:** Pigmented adult male guinea pigs with preyer's reflex, weighing 250-450 g, were used in this study. The animals were housed in wire cages in group of four under controlled conditions of temperature (20±2°C) and light (12/12 h light-dark cycle). The study consisted 11 groups which in each group 6 animals were used as follow:

A: Saline alone, B: Sodium acetate alone, C: Lead acetate alone in drinking water, D: Gentamicin low dose alone, E: Gentamicin high dose alone, F: Amikacin low dose alone, G: Amikacin high dose alone, H: Lead plus gentamicin low dose, I: Lead plus gentamicin high dose, J: Lead plus amikacin low dose, K: Lead plus amikacin high dose.

Aminoglycosides were given for 21 days. Control groups were received saline. Sodium acetate was administrated as a control for determination of involvement of acetate ion in ABR in animals pretreated with lead acetate. In groups of H, I, J and K lead acetate were given for 35 days and aminoglycoside antibiotics were injected from 15th to 35th day.

Dosage and routes of administration are listed in Table 1. Body weight of animals was monitored daily and the administrated dose was adjusted accordingly.

**Evaluation of auditory function:** The Auditory Brainstem Responses (ABR) measures the electrical activity of the hearing nerve pathway from the inner ear to the brain. In this test, a clicking sound is presented to one ear at a time. The electrical activity of this signal is recorded by electrodes. The average response is displayed as a waveform that contains peaks and troughs, which correspond to various points along the hearing nerve. The time between these peaks is measured and compared to normal data. A delay in a response might indicate an abnormality on or near the hearing or balance nerve. In the present investigation ABR were measured for each animals prior to the start of the study and then at the end of the treatment. Animals were anesthetized with an intraperitoneal injection of sodium pentobarbital 15 mg $kg^{-1}$. Needle electrodes were placed subcutaneously below the ipsilateral right pinna (reference electrode) and the contralateral pinna (ground electrode). The active electrode was located at the vertex. The latency of the waves was recorded with a 4 kHz click stimulus and intensities of 80,100 and 120 dB p.e.SPL. Auditory brainstem responses measurements at each intensity were repeated twice. Wave's latencies posttreatment were compared with pretreatment wave latencies for each animal at the 100 dB p.e.SPL intensity.

Table 1: Routs of administration and dosage of drugs

| Drug | Dose |
|---|---|
| (A) Saline | 1 mL/kg/day (S.C.) |
| (B) Sodium acetate | 0.10% solution (P.O.) |
| © Lead acetate | 0.05% solution (P.O. 35 days) |
| (D) Gentamicin | 5 mg/kg/day (I.M.) |
| (E) Gentamicin | 10 mg/kg/day (I.M.) |
| (F) Amikacin | 150 mg/kg/day (I.M.) |
| (G) Amikacin | 300 mg/kg/day (I.M.) |
| (H) Lead and Gentamicin | 0.05% (P.O.) and 5 mg/kg/day (I.M.) |
| (I) Lead and Gentamicin | 0.05% (P.O.) and 10 mg/kg/day (I.M.) |
| (J) Lead and Amikacin | 0.05% (P.O.) and 150 mg/kg/day (I.M.) |
| (K) Lead and Amikacin | 0.05% (P.O.) and 300 mg/kg/day (I.M.) |

Aminoglycoside antibiotics were administered intramuscularly for 21 days. Control groups were received saline. Sodium acetate was administrated as a control for determination of involvement of acetate ion in auditory brainstem response in animals pretreated with lead acetate. In groups of H, I, J and K lead acetate (0.05%) were given for 35 days and aminoglycoside antibiotics were injected from 15th to 35th day

**Determination of serum lead level:** In the handling of samples from the collection and storage to analysis, great care was taken to prevent contamination. All containers used for collection and storage of samples were tested and found to be free from lead. All glassware used for analysis was washed thoroughly, rinsed with 10% nitric acid and then rinsed with deionized water. Sample cups and caps were soaked in 10% nitric acid and rinsed in deionized water prior to use. All samples were analyzed for lead using atomic absorption spectrophotometer (Shimadzu Model 680-A, Japan) equipped with deuterium back-ground correction after specific preparation, which varied with the nature of the samples and that have been reported previously by Abdollahi *et al.*[14].

**Statistical analysis:** Statistical analysis of the data was performed with one way analysis of variance (ANOVA) followed by the Newman-Keuls test. Differences with $p<0.05$ was considered statistically significant.

## RESULTS

**Alterations of auditory brainstem response induced by gentamicin and amikacin:** Intramuscular injection of different doses of gentamicin (5 and 10 mg/kg/day) and amikacin (150 and 300 mg/kg/day) for three weeks, caused prolongation of absolute $P_1$, $P_3$, $P_4$ and $N_4$ waves latencies in guinea pig. Animals receiving either gentamicin or amikacin showed the expected progressive hearing loss. When different doses of either gentamicin (Table 2) or amikacin (Table 3) were administered, significant changes were observed in absolute waves latencies.

**Effects of chronic lead acetate administration on auditory brainstem response:** Administration of lead acetate (0.05%) in drinking water for 35th day induced significant prolongation of absolute $P_1$ and $P_3$ waves latencies in pretreated animals (Table 2 and 3).

**Effects of chronic lead acetate pretreatment on the changes of ABR induced by gentamicin and amikacin:** Pretreatment of animals with chronic lead acetate decreased the prolongation of waves latencies ($P_1$, $P_3$, $P_4$ and $N_4$) induced by gentamicin (5 and 10 mg/kg/day) significantly, but did not show any protective response on amikacin (150 and 300 mg/kg/day) effects.

**Serum lead concentration:** In this experiment, total lead serum concentration after 35 days was 546.8±16.4 μg $dL^{-1}$ (mean±SEM).

Table 2: Effects of chronic lead acetate and different doses of gentamicin on absolute waves latency in guinea pig

| Treatment (mg $kg^{-1}$) | Absolute wave latency (ms) | | | |
|---|---|---|---|---|
| | $P_1$ | $P_3$ | $P_4$ | $N_4$ |
| Control | 2.09±o.013 | 3.23±0.031 | 4.19±0.026 | 4.63±0.024 |
| Chronic (Pb) | 2.18±.021** | 3.51±.035** | 4.20±0.012 | 4.65±0.021 |
| Genta (5) | 2.18±.018** | 3.33±.010** | 4.25±0.015 | 4.72±.012** |
| Genta (10) | 2.22±.015** | 3.42±.013** | 4.36±.031** | 4.88±.015** |
| Chronic (Pb)+ Genta (5) | 2.10±0.015 | 3.20±0.015 | 4.19±0.012 | 4.64±0.021 |
| Chronic (Pb)+ Genta (10) | 2.11±0.012 | 3.24±0.010 | 4.25±0.015 | 4.80±.017** |

Animals were injected intramuscularly with gentamicin (Genta, 5 and 10 mg/kg/day) for 21 days. Control animals received saline. Chronic lead pretreated animals received lead acetate (0.05%) for 35 days. Gentamicin (5 and 10 mg/kg/day) was injected simultaneously from 15th to 35th day in animals pretreated with chronic lead. Each point is the mean±SEM of 6 animals. **p<0.01 different from control groups

Table 3: Effects of chronic lead acetate and different doses of amikacin on absolute waves latency in guinea pig

| Treatment (mg $kg^{-1}$) | Absolute wave latency (ms) | | | |
|---|---|---|---|---|
| | $P_1$ | $P_3$ | $P_4$ | $N_4$ |
| Control | 2.09±o.013 | 3.23±0.031 | 4.19±0.026 | 4.63±0.024 |
| Chronic (Pb) | 2.18±.021* | 3.51±.035** | 4.20±0.012 | 4.65±0.021 |
| Amika 150 | 2.16±0.015 | 3.29±0.015 | 4.28±0.020 | 4.79±.031** |
| Amika 300 | 2.37±.031** | 3.46±.015** | 4.40±.012** | 4.90±.025** |
| Chronic (Pb)+ Amika 150 | 2.14±0.021 | 3.26±0.021 | 4.22±0.026 | 4.78±.012** |
| Chronic (Pb)+ Amika 300 | 2.35±0.015 | 3.44±.015** | 4.36±.021** | 4.92±.021** |

Animals were injected intramuscularly with amikacin (Amika, 150 and 300 mg/kg/day) for 21 days. Control animals received saline. Chronic lead pretreated animals received lead acetate (0.05%) for 35 days. Amikacin (150 and 300 mg/kg/day) was injected simultaneously from 15th to 35th day in animals pretreated with chronic lead. Each point is the mean±SEM of 6 animals.*p<0.05, **p<0.01 different from control groups

## DISCUSSION

Toxic metal ions can harm cells in a variety of ways, including inactivation of enzymes and catalysis of the oxidative damage of lipids, proteins and nucleic acids[15]. To understand the mode of neurotoxic action of metals, it is important to know the precise cellular site of their action. Intramuscular injection of aminoglycoside antibiotics gentamicin and amikacin induced the hearing loss. It seems that the binding of aminoglycosides to plasma membrane represents the first step of their toxic action. This binding is thought to involve charge interactions between the polycationic drug and the anionic membrane phospholipids such as phosphoinositides[10,16] which can result in inhibition of inositol triphosphate formation, the second messenger of the phosphoinositide cascade[11]. There is evidence that hair cells modulate auditory transduction which presumably are controlled by efferent neurons and regulated by the levels of intracellular calcium[17]. In a number of biological systems such as hair cells, these calcium levels are controlled by inositol triphosphate. There is evidence that aminoglycoside antibiotics are capable of altering membrane permeability and this effect is most pronounced if phosphatidyl inositol bisphosphate ($PIP_2$) is present in the bilayers[18]. The correlation between toxicity of the drugs and altering of membrane permeability further establishes the specific roles of $PIP_2$ metabolism of aminoglycoside-induced hearing loss. Thus the alterations of auditory function induced by gentamicin and amikacin (prolongation of absolute $P_1$, $P_3$, $P_4$ and $N_4$ wave latencies) may be related to their interactions with phosphoinositides cascade.

The other finding of the present study was that chronic lead acetate administration increased absolute $P_1$ and $P_3$ wave latencies. Experimental studies suggested that the auditory pathways may be unusually sensitive to the toxic effects of lead[5]. It has been reported that acute lead toxicity affected the 8th nerve compound action potential of adult guinea pigs[7]. Other researchers have also reported that lead toxicity affects synaptic transmission in a number of biological systems[19].

Lead also blocked voltage-activated calcium channel current by binding to different sites of the channel[20,21]. There is evidence that the site of Pb action is intracellular, where protein kinase C could be activated[22]. Therefore alterations of neural transmission and calcium current may be involved in the Pb induced prolongation of waves latencies ($P_1$ and $P_3$). The other important finding of the present study is that the chronic lead acetate prevents the prolongation of $P_1$, $P_3$, $P_4$ and $N_4$ waves latencies induced by gentamicin. This finding is similar to the results in which chronic lithium pretreatment showed the protective effects on gentamicin-induced ototoxicity[23] and salivary glands[24].

Metal ions such as Pb may exert their action through an intracellular messenger system like protein kinase[22,25] or adenylyl cyclase[26]. It is also shown that Pb can penetrate the membrane[27], causing additional (secondary) changes in cell functions which is associated with change of intracellular calcium concentration[20]. Increase of $IP_3$ level by lead was also shown[28]. In view of lead ability to increase $IP_3$ level and because intracellular metal ions substantially influence the calcium homeostasis of the cells[29], thus chronic lead acetate may counteract with gentamicin action on phosphoinosiotides cascade via increase in $IP_3$ and intracellular calcium and show protective effects on gentamicin response. Lead did not affect the amikacin's effect in the guinea pig *in vivo*. Some studies have shown that aminoglycoside antibiotics act as N-type calcium channel blockers with different affinities[30], therefore the contradictory effects of gentamicin and amikacin on auditory brain stem response in animals pretreated with chronic lead acetate may be related to more inhibitory effect of amikacin on neuronal calcium availability. In addition calcium release from intracellular stores induced by $IP_3$ exhibits a threshold requirement for $IP_3$ levels, thus it is possible that Pb-induced increase of $IP_3$ is not enough to prevent the alterations caused by amikacin. Considering the different effects of lead acetate which are concentration and time-dependent, it may override the expression of auditory disorders. One may speculate that aminoglycoside antibiotics and chronic lead acetate might interfere through non-specific mechanisms.

In conclusion present results show the possible involvement of phosphoinositide and calcium cascades in alterations of ABR induced by aminoglycosides and chronic lead acetate. There are, however, a variety of other toxic mechanisms proposed for aminoglycosides and lead acetate and the interactions between these agents need not be occurred by the common pathway of the phosphoinositide and calcium cascades. Further studies require clarifying the details.

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