LITHIUM – INDUCED CHANGES IN MUSCLES AND NEUROMUSCULAR JUNCTIONS

A. Essawy, N. Abdel-Meguid, A. Ali and A. Sedky

Department of Zoology, Faculty of Science, Alexandria University, Alexandria, Egypt

ABSTRACT

The effect of Prianil C-R (lithium carbonate) on the structure of skeletal muscles and neuromuscular functions of Swiss albino mice was investigated. with at a dose level of either 2.32 mg/mouse, daily for 30 days or 0.58 mg/mouse, daily for 90 days. Mice treated with lithium at the high dose level resulted in significant degenerative changes in the histological and subcellular structures of both myofibrillar and non-myofibrillar components of the flexor digitorum muscle. These degenerative changes were less pronounced in animals treated with low dose of lithium and were probably secondary to the nerve and motor end-plate degeneration observed in this study.

Key-words: Lithium, muscular and neuromuscular alteration, histochemical study, mice.

INTRODUCTION

Prianil C-R (lithium carbonate) is a valuable oral drug, used extensively in the treatment of manic-depressive disorders. Its use by millions of patients worldwide has resulted in the normalization of their lives and prevented many deaths from suicides (Schou, 1998; Kallner et al., 2000).

Although the efficacy of lithium as a mood stabilizer is well documented, the mechanism of its therapeutic effect associated with prolonged treatment remains unknown (Lambert et al., 1999). Among the possible mechanisms accounting for the therapeutic effect of lithium is its effect on neurotransmitters and their receptors. For example, lithium activates metabolism of brain serotonin (Sheard and Aghajanian, 1970) and dopamine (Koyama, 1987), reduces density of serotonin receptors in the hypocampus of rat (Treiser and Kellar, 1980), increases synaptosomal uptake of norepinephrine in rat brain (Young-Wuk, 1997) and increases acetylcholine synthesis and release in the brain (Millington et al., 1997).

Both clinical and experimental studies have shown lithium to be toxic to man and animals. A case of fetal malformations with bilateral agenesis of the kidneys and severe cardiac defect under continuous lithium treatment during the first trimesters was reported (Eikmeier, 1996). Lithium may directly damage thyroid follicular cells and the subsequent release of thyroglobulin into the circulation might be a cause of transient thyrotoxicosis (Mizukami et al., 1995). It may also induce acute myeloblastic leukemia (Orr and McKernan, 1979) or develop acute myeloid leukemia (Nielsen, 1980). Moreover, prolonged lithium administration exhibited degenerative testicular morphology where seminiferous tubules appeared virtually empty with the exception of some spermatogonia (Ghosh, 1991; Nandi et al., 1994; Moussa et al., 2001).

In comparison with the available data on the effect of lithium on the structure and function of different body organs, information about its pathological effect on the muscles is relatively scant and indicate certain points of conflict and confusion. Hence it could be of important to study the effect of the treatment with lithium on the histological and subcellular structures of skeletal muscles. Also the present work is a trial to evaluate the influence of lithium on the neuromuscular junctions (NMJs) of the superficial flexor digitorum muscle of the Swiss albino mice, science the effect of lithium on the peripheral nervous system of experimental animals is still controversial.

MATERIALS AND METHODS

Experimental animals:

120 adult male and female Swiss albino mice Mus musculus(20-25g ), each were used. Animals were allowed free access of feed and water throughout the experimental period (30 & 90 days). Animals were assigned randomly to one” control and “two” experimental groups and kept in large cages in environmentally controlled room [22-24°C]. The first group (control animals) is subdivided into tow sub-groups of 30 animals each and injected with 1 ml mammalian saline daily for 30 & 90 days respectively. Animals of the second group (30 mice) were injected intraperitoneally with Prianil C-R solution at 2.32 mg / mouse, daily for 30 days . This dose represents the human therapeutic dose and referred to as the high dose in the present study. Animals of the third group (30 mice) received
an intraperitoneal daily dose injection of 0.58 mg / mouse Prianil C-R for 90 days. This dose is equivalent to quarter of human therapeutic dose and referred as low dose. The equivalent doses of the given lithium were determined according to Paget and Barnes formula (1964 ). Lithium carbonate was purchased from El-Nil pharmaceutical and chemical industries Co. Cairo, A.R.E. It is available under the international trade name Prianil C-R.

**Preparation of samples for microscopical study:**

Twenty-four hours after the last injection, randomly selected animals from different groups were anaesthetized with diazepam. For demonstrating the motor endplates, pieces of the superficial flexor digitorum muscle were surgically removed and prepared to stain with gold chloride according to the method described previously by Lowit (1875). For electron microscopy small blocks of muscle from different groups were fixed with 2% glutaraldehyde buffered with 0.1M phosphate buffer (pH 7.6) at 4°C. The specimens were post-fixed in 1% O₃O₃, dehydrated in increasing ethanol concentrations, treated with propylene oxide and embedded in Epon-Araldite mixture. Sections were cut on an LKB ultramicrotome. Semithin sections were stained with toluidine blue, while thin sections were double stained with uranyl acetate and lead citrate and investigated in a JEOL 100 CX electron microscope.

**RESULTS**

**Behavioral and neurological observations:**

Injection of Swiss albino mice with lithium carbonate at a dose level of either 2.32 mg / mouse (high dose) or 0.58 mg / mouse (low dose), showed changes in the general behaviour. They became less active and their responsiveness to external stimuli was slightly decreased. Animals of high dose treated group exhibited diarrhea, hind limb dysfunction and impaired ability to balance.

**Microscopical observations recorded in the superficial flexor digitorum muscle:**

Examination of the normal superficial flexor digitorum muscle of Swiss albino mice, revealed that the muscle fibers are arranged in fascicles and showed a strictly ordered cross-striations of alternating bands of light and dark materials. The individual muscle fibers are covered with a delicate sheath of connective tissue, the endomysium and supplied with nerve side branches and blood capillaries. Sarcolemma is represented by a regular outline enclosing the muscle fiber (Fig. 1). Electron micrographs of control mice (Fig. 2), showed that the muscle fiber is composed of sarcoplasm and longitudinal arrays of myofibrils with alternating light and dark bands. The center of each dark band (A-band) is occupied by a pale area, known as H-zone “Hensen’s zone” which is bisected by an additional thin striation known as M-line “Mittelsheibe”. Each light band (I-band) is bisected by a thin dark line, known as Z-line “Zwischenschube”. Morphometric measurements showed that the average length of the sarcomere (the region between the two successive Z-lines) is about 0.38 nm.

The sarcoplasm is differentiated into peripheral sarcoplasm occupied the thin region between the sarcolemma and the nearest myofibril, interribibrilar sarcoplasm filled the spaces between myofibrils and perinuclear sarcoplasm which is found at the pole of the nucleus. The sarcoplasm contains non-myofibrilar components including nuclei, mitochondria, Golgi bodies, sarcoplasmic reticulum, ribosomes and glycogen (Fig. 3). The nucleus appeared with predominated heterochromatin and surrounded by two irregular corrugated nuclear membranes representing the nuclear envelope which is perforated by nuclear pores. Adjacent to the inner nuclear membrane, the heterochromatin appears as dense aggregates. However, smaller dense particles or patches of condensed chromatin occur frequently in the nucleoplasm. The mitochondrial appeared to be oval or nearly rounded possess distinguishable double membranes, slightly electron dense matrix and well preserved tubular cristae that traverse the whole width of the organelle. Golgi apparatus occupied a paranuclear position, consisting of large sacs and small vesicles.

Injection of mice with the high dose of lithium, induced histological alterations in the skeletal muscle. These alterations included destruction of muscle fibers, abnormal washed striation and infiltration of blood cells (Fig. 4). Electron microscopic preparations showed that ultrastructural alterations did occur in both myofibrillar and non myofibrillar components. The typical striation of the vertebrate muscle fibers became poorly defined with less distinct H-zones and affected Z-lines. Degenerative myofibril areas (lysis of myofilaments) and reducing the sarcomere length were also reported (Fig. 5).
Fig. 1. Longitudinal semithin section in control muscle, demonstrating cross striations in muscle fibers (MF), peripherally located nuclei (N), regular sarcolemma (arrow’s head) and a nerve side branch (arrow). (X 1,000).

Fig. 2. Electron micrograph of control muscle, showing A-band (A), H-zone (H), I-band (I), Z-line (Z), glycogen (g), Mitochondria (M), longitudinally oriented elements of sarcoplasmic reticulum (ISR), terminal cisterna (Tc) and transverse tubule (Tt). (x 40,000).
Fig. 3. Electron micrograph of control muscle, demonstrating nucleus (N) with heterochromatin (He), nuclear envelope (Ne) and nuclear pore (Np). G: Golgi apparatus, M: mitochondria, R: ribosomes. (x 50,000).

Fig. 4. Longitudinal semithin section of muscle fibers in a mouse treated with high dose of lithium carbonate, demonstrating thick irregular sarcolemma (arrow’s head), invested bloods cells (arrow) and washed striations (double arrows). (x 1,000).
Fig. 5. Electron micrograph of muscle fibers in a mouse treated with high dose, illustrating I-band (I) is more narrow, the H-zone (H) is less distinct and Z-line (Z) is affected. Note, the intermyofibrillar sarcoplasm contains shrunk (*) or swollen (arrow) mitochondria and increased amount of glycogen (g). Double arrow points at hypotrophied and disorganized sarcoplasmic elements and arrow’s head points at lysis of myofilaments. S: sarcomere with shorter length, M: Mitochondria (x 40,000).

Fig. 6. Electron micrograph of muscle fibers in a mouse treated with high dose, demonstrating the abnormal contour of nucleus (N) with abnormal shaped nuclear envelope (Ne). Arrow points at segmented nucleus and arrow’s head point at invagination in the nucleus. Note, the sarcolemma (Sl) became more thick and Golgi bodies (G) appeared hypotrophied. Np: nuclear pore. (x 26,000).
Fig. 7. Longitudinal semithin section in of muscle fibers in a mouse treated with low dose, demonstrating sarcolemma with abnormal shape. Mf: muscle fiber. (x 1,000).

Fig. 8. Electron micrograph of muscle fibers in a mouse treated with low dose, showing split hypertrophied myofibril, (arrow’s head), I-band (I) with shifted Z-line (Z) and small sized mitochondria (M). Note, the disappearance of the sarcoplasmic elements. (x 40,000).
Fig. 9. Electron micrograph of muscle fibers in a mouse treated with low dose, demonstrating nucleus (N) with marginated nucleolus (Nu), peripherally located heterochromatin (He) and numerous nuclear pores (arrows). Ne: nuclear envelope, A: A-band; g: glycogen granules; I: I-band; M: paranuclear mitochondria, Sl: sacrolemma, Z: Z-line. (x 26,000).

Fig. 10. Light micrograph of gold chloride preparation of muscle fibers in a control mouse, illustrating a nerve trunk (Nt) with axon terminals (At) and an obvious accessory ending (Ae). Arrow points at the last node of Ranvier. Mf: muscle fibers. (x 500).
Fig. 11. Light micrograph of gold chloride preparation of muscle fibers in a mouse treated with high dose, showing an altered end-plate, lysis of accessory ending (Ae) and degeneration at the last node of Ranvier (arrow). At: axon terminal. (x 1250).

Fig. 12. Light micrograph of gold chloride preparation of muscle fibers in a mouse treated with high dose, illustrating swollen of both accessory ending (Ae) and the last node of Ranvier (arrow) At: axon terminal, Mf: muscle fiber. (x 1250).
Fig. 13. Light micrograph of gold chloride preparation of muscle fibers in a mouse treated with low dose, illustrating slightly poorly impregnated myelinated axon terminals (At) with obvious last node of Ranvier (arrows) and more or less normal appearing accessory endings (Ae). Mf: muscle fiber. (x 1,000).

The nuclei appeared with abnormal outline. They have a bizarre shape exhibiting invaginations, the nuclear envelope became infolded and the nuclear folds entrap cytoplasmic material (Fig. 6). The mitochondria were large in size and more electron dense, the Golgi apparatus was hypotrophied, the glycogen content was variable and the sarcoplastic reticulum and T-system was damaged.

In animals treated with low dose of lithium, similar alterations were recorded in the skeletal muscle fibers but they were less pronounced (Figs. 7-9).

Microscopical observations recorded in the neuromuscular junctions:

Light microscopic examination of gold chloride preparations revealed that, the superficial flexor digitorum muscle of control mice is innervated by motor nerve trunks. Each motor nerve trunk is situated perpendicular to the muscle fibers and it contains a number of motor axons that ramify repeatedly in the vicinity of neuromuscular junctions to give many axonal terminals (Fig. 10).

The axonal terminals are found smaller in size and were less impregnated with gold chloride. They approach the muscle surface with an angle and loss the myelin sheath to form a tapering point that indicates the last node of Ranvier (Fig. 10).

The axon terminal makes a contact with the muscle fiber as a slightly elevated plaque forming the terminal bouton or accessory ending. Administration of high dose lithium induced morphological changes in the neuromuscular junctions. These alterations included degeneration of the peripheral nerves and axon terminals, swelling of the nerve terminals at the region of last node of Ranvier and degeneration of the accessory endings (Figs. 11,12). In low dose treated animals, lithium affected only the nerve fibers and axon terminals, whereas the accessory endings appeared more or less normal (Fig. 13).

DISCUSSION

Most of the previous studies were focused on the over toxicity of lithium carbonate, but relatively little progress has been made to understand the toxic impact of the low-level lithium administration.
In the current study, Swiss albino mice treated with lithium at a dose level equivalent with either the human therapeutic or quarter the therapeutic dose, induced marked changes in the structure of both muscles and neuromuscular junctions. However, the alterations produced by the therapeutic dose were more pronounced. Destruction of muscle fibers and cellular inflammatory infiltration in between the damaged fibers were recorded in mice treated with the therapeutic dose daily for 30 days. Structural damage of muscle fibers in lithium treated animals was also described by Nappi et al. (1975). Lee et al., (2001) revealed necrotic muscle fibers invaded by numerous macrophages seen in soleus muscle of rat treated with Triamcinolone acetonide (fluorinated steroid). Similar observations were reported also in the flexor digitorum muscle of mice intoxicated with acrylamide (Abdel Meguid et al., 2002).

Our electron micrographs revealed thickened sarcolemma with irregular shape, washed striation of muscle fibers and disorganized myofibrils. Such abnormalities in skeletal muscles may lead to loss of myofibrils as reported by Ghadially (1978). In addition, alteration in Z-line morphology and position was observed in the affected muscle fibers. Alterations in Z-line have been recorded in various muscle diseases (De Freitas et al., 1988) and under the effect of drug administration (Ahmed, 2000). The nuclei of some muscle fibers of lithium−treated mice showed nuclear changes involved both the shape and structure. The nuclei appeared regular, deviating from its normal corrugated pattern. The changes in the shape of the myonuclei from corrugated to regular may be an indication for alteration in the nucleoplasmic exchange and decrease in the metabolic activity which is associated with an increasing complexity of nuclear form (AbdelMeguid et al., 2002). Moreover, loss in the density of nucleoplasm and margination of the chromatin were observed in treated mice. Loss in nucleoplasm density might be attributed either to loss of nuclear protein and depolymerization of DNA or possibly to peripheral movement of chromatin granules (Latta et al., 1965). They also stated that, the marked margination of chromatin granules is probably produced largely by accumulation of chromatin at the nuclear envelope as the nucleus shrinks.

In the present work, mitochondria of treated animals appeared shrunk, swollen or having electron dense matrix reflecting ultrastructural transformation to the condensed configuration. Senger (1987) stated that, mitochondrial changes may represent a very clear demarcation of future pathological development. Novikoff and Holtzman (1976) claimed that morphological changes of mitochondria may be related to mitochondrial function. They suggested that the configurational changes reflect structural rearrangement in inner membrane proteins that are directly important for ATP formation. Also, Golgi apparatus was affected by lithium administration. It appeared hypotrophied in muscle fibers of mice treated with high dose of lithium for 30 days and disappeared in some muscle fibers of animals treated with low dose for 90 days. This indicates that, Golgi elements could not resist the continuous treatment up to 90 days, but they were reduced and destroyed. The reduced secretory activity of Golgi apparatus also discerned by Elewa et al., (1999) in guinea pigs treated with voltaren. From these results, it appears that, the Golgi elements are very sensitive to any external agent including lithium.

Results presented in this work showed variability of glycogen content among lithium treated animals, reflecting an alteration in glucose metabolism. Increased glycogen content in the skeletal muscle of treated mice may be due to the increased activity of glycogen synthesis, since previous studies had revealed that lithium acts on many enzymes related to carbohydrate metabolism (Rose and Warns, 1980).

In the neuromuscular junction, the pathological alterations induced by administration of high dose of lithium involved degeneration of nerve terminals, swelling of the axon terminals at the region of last node of Ranvier and degeneration in the accessory endings.

Similarities between the neuropathological changes induced by lithium and acrylamide (potent neurotoxin) are apparent. De Grand et al. (1990) observed widespread nerve-terminal degeneration and swollen degenerated terminal branches with paucity of synaptic vesicles in rats infected with a single large dose of acrylamide (100 mg/kg body weight). Similarly, Madrid et al. (1993) revealed swelling of the preterminal, terminal and ultraterminal axons and increase of myelinated fibers, showing axonal degeneration in the nerve supplying the extensor digitorum longus muscle of acrylamide treated rats. Furthermore, Ko et al. (1999) reported that acrylamide administration produced axonal swelling in motor nerve terminals in the distal parts of motor nerves innervating the hind foot muscles.

It must be taken into consideration that, muscle cells are entirely dependent upon their motor innervation. On this basis, an important group of muscular atrophies are encountered in peripheral nerve lesions. When the atrophy is caused by focal loss of nerve supply, the unaffected adjacent fibers may undergo compensatory hypertrophy and there may be no appreciable loss of muscle mass (Robbins, 1962).

In the present study, it becomes evident that lithium led to axonal and endplates degeneration in treated mice and this may result in muscle fiber atrophy. This atrophy required the splitting of hypertrophied muscle fibers, as seen ultrastructurally, to compensate the loss in muscle mass i.e., lithium exerts its pathological effect on the muscle fibers indirectly through altering their motor innervation.
From the results obtained in this work, it is suggested that Lithium carbonate has serious detrimental impacts on the structure of muscles and neuromuscular junction of the Swiss albino mice. The risks of adverse side effects must clearly be weighed against potential benefits whenever medical practitioners contemplate the use of lithium carbonate.

REFERENCES


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