Research Article

Investigation of the histological changes in spinal nerve roots post intrathecal ketamine administration

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ABSTRACT

Background: Ketamine is a general anesthetic agent, administered intramuscularly or intravenously. It is generally used for induction and less frequently for the continuation of surgical anesthesia. Although ketamine was initially thought to be used as an intrathecal agent, psychomimetic side effects were observed. **Objectives:** In this study, the differences in the spinal nerve roots postintrathecal ketamine injection have been examined in the light and electron microscope. **Materials and Methods:** 30 min, 1 h, and 24-h postintrathecal ketamine (0.5 mg/kg) injection; the rabbits were sacrificed. The tissue samples taken from the injection area fixed with 1st 2% glutaraldehyde and later 1% OsO_4. Following dehydration tissue samples embedded in Araldite and sections were taken and examined in the light and electron microscope. **Results:** There were severe degenerations in the myelinated nerve fibers postintrathecal injection of ketamine. Fading on the myelin lamellae, shrinking and vacuolization of the axoplasm, endoneurial edema, organelle loss, and cystic parts were observed. The vacuolization and lack of organelles in the Schwann cell cytoplasm were clear. The degeneration of the large myelinated nerve fibers was observed more than unmyelinated fibers. **Conclusion:** The general and local anesthetics used have significant side effects such as respiratory depression or permanent neurological disorders. Therefore, new options are searched, and the effects of the drugs used in different fields of anesthesia were investigated.

KEY WORDS: Intrathecal; Ketamine; Degeneration; Histology

INTRODUCTION

General anesthesia has been used for many years in surgical cases. Furthermore, interest in spinal anesthesia is increasing, and its application is widespread. However, most of the drugs used in spinal anesthesia are not safe and can have side effects.[1,3]

Ketamine is a general anesthetic agent administered intravenous (1–2 mg/kg) or intramuscular (3–5 mg/kg) and provides dose-dependent loss of consciousness and analgesia.

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Ketamine is a phencyclidine derivative, and the chemical name is Cyclohexylamine. It is generally used for induction and more rarely for the continuity of surgical anesthesia. Dissociative anesthesia occurs during ketamine injection, although the patients appear to be awake they are transected. They do not give respond to painful stimulus, and they are in amnesia.[4-7]

Spinal anesthesia occurs by administrating the local anesthetic drug to the subarachnoid space from the lumbar area. It is a reversible nerve block in the anterior and posterior roots, posterior root ganglia, and spinal cord. It causes autonomic sensory and motor activity loss.[8,9] It is also used in surgical procedures involving the lower extremity, vaginal delivery, cesarean diagnosis, and treatment for painful procedures.[10]

Well-known local anesthetics used in spinal anesthesia have significant side effects such as respiratory depression

or permanent neurological disorders. Hence, new options are being sought, and the effects of these drugs (with different anesthetic uses) on spinal anesthesia are being investigated.[11-13]

Thus, ketamine, which is used primarily as a general anesthetic, is used in spinal anesthesia as well and detailed studies on its side effects continue today. It is known that when a drug is used intrathecally, toxic reactions such as demyelination, necrosis, arachnoiditis, vascular changes, and myelitis occur. The ultrastructural investigation is important to explain neurological disorders.[14-19]

For the indicated reasons above, this study was carried out to reveal the changes in the fine structure level that can be formed in spinal nerve fibers and roots by intrathecal use of ketamine.

MATERIALS AND METHODS

The study protocol approved by the local Animal Research Committee. The New Zealand rabbits with an average weight of 2450–2650 kg were used in this study. Rabbits were provided from "Ankara Poultry Institute Rabbit Production Center." The rabbits were divided into two groups such as control and ketamine groups. Ketamine group also divided into three groups (30 min, 1, 5 h, and 24 h groups). The rabbits were anesthetized with intravenous ketamine anesthesia (2 mg/kg) and then placed face down. The lumbal punching area was shaved. Just before the injection, rabbits held in a stabile position to prevent sudden movements and to separate the processus spinosus of the vertebrae. The cord was in flexion, and the interval between the seventh lumbar and the first sacral vertebra was the widest.[20]

About 0.5 mg/kg ketamine was intrathecally administerated to the rabbits in the ketamine group. The rabbits were sacrificed 30 min, 1 h, and 24-h post intrathecal ketamine injection. Under sterile conditions, dorsal part of the rabbit shaved, and after a straight incision, the spinal cord and nerve roots were opened and taken as soon as possible for the histological examination. The control group was administrated nothing.

For electron microscopic examination, the spinal nerve roots were fixed with 2% glutaraldehyde in 1/15 M phosphate buffer at 4°C, postfixed with 1% osmium tetroxide. Dehydrated through graded ethanol series and embedded in Araldite (CY 212). Thick sections were taken with LKB 11.800 pyramitome, and stained with toluidine blue for light microscopic examinations. Ultrathin sections were taken with LKB ultramicrotome III and stained with uranyl acetate and lead citrate.[21] Examined with JEM-100 CX II electron microscope and photographed.[22,23]

RESULTS

In this study, the differences in the spinal nerve roots have been examined 30 min, 1.5, and 24-h postintrathecal ketamine injection in the light and electron microscope.

Macroscopic findings: 30 min postketamine application, the rabbits were not awake. 1.5 h later, they were awake; however, no motor function was observed on the hind legs. Post 24 h, the rabbits were normal and moved their hind legs.

Light Microscopic Findings: In the control group, the spinal nerve roots were observed at different diameters. Myelin was mostly normal [Figure 1a].

30 min postketamine injection degenerations in myelinated nerve fibers and spinal ganglion cells were observed. The small vacuole-like cleavages on myelin sheet were seen [Figure 1b].

1.5-h postketamine injection, various types of degenerations were observed in the myelinated spinal nerve roots. In some regions, myelin sheet was shrunk, showed extraordinary linings, nodes, and cystic structures were detected [Figure 1c].

24-h postketamine injection, degeneration in myelinated nerve fibers was increased. Myelin was seen as a dense mass, the fibers were dark in color and pressed the axon [Figure 1d].

Electron Microscopy Findings: In the control group, myelin lamellae were clearly distinguished. The axoplasma was normal, mitochondria and neurofilaments were evident, and

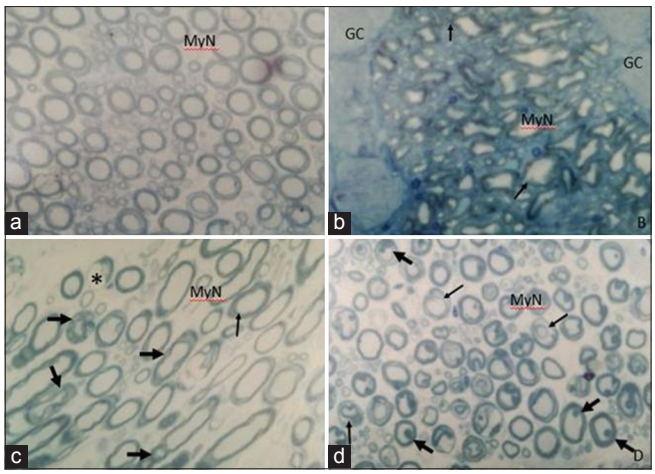

Figure 1: (a) Control group, myelinated axons (MyN). Light microscope (LM), Toluidine blue, ×40. (b) 30 min postketamine injection myelinated fibers (MyN) and spinal ganglion cells, vacuole-like structures (thin arrow). LM, Toluidine blue, ×40. (c) 1.5-h postketamine injection myelin nerve fibers (MyS), degenerated myelin (thin arrow), cystic structures (*), dense curved areas (thick arrow). LM, toluidine blue, ×40. (d) 24-h postketamine injection myelinated nerve fibers (MyS), severe myeline degeneration (thin arrows) a dense mass in the axon (thick arrows). LM, Toluidine blue, ×40

Schwann cell nucleus and nucleolus were seen. Internal and external mesaxons were apparent [Figure 2a].

30 min postketamine application shaded areas were observed in lamellae, myelin was separated by vacuoles in the spinal nerve roots taken from the needle region. The axon was normal, mitochondria and neuroflaments were prominent in axoplasm. Degenerated mitochondria and decrease of organelles were observed in Schwann cell. Nucleus and nucleolus were normal; the cytoplasm was filled with small vacuoles. The chromatine content was intense [Figure 2b].

1.5-h postketamine injection, a significant degeneration was observed in the spinal nerve roots, myelinated nerve fibers, lamellae, axoplasm, Schwann cell cytoplasm, and nucleus, in some regions, condensation was observed in the axoplasm. In myelin lamellae, cyst-like areas were enlarged. The smaller fibers were less degenerative than large ones, [Figure 2c].

24-h postketamine injection, the myelinated nerve fibers were separated from each other and form cystic structures, and Schwann cell cytoplasm was pressed. Myelin layers were blurred; degenerated myelin lamellae and swelling axoplasm were seen. The axoplasma were generally more intense than normal and filled with small vacuoles. The transverse and longitudinal sections of the neurofilaments were observed.

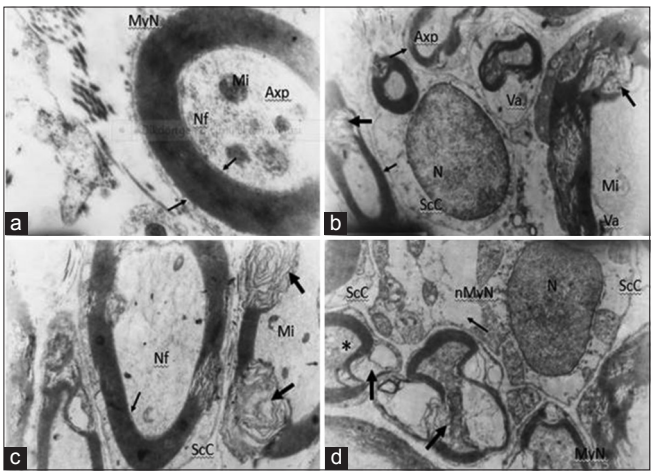

Figure 2: (a) Control group, myelinated nerve fiber (MyN), axoplasma (Axp), mitochondria (Mi), internal and external mesaxons (thin arrows), neurofilaments (Nf) in axoplasma (Axp) Electron microscope (EM), and Lead citrate-uranyl acetate, ×38.000. (b) 30 min post ketamine injection, degeneration in myelin lamellae (thin arrows), cystic structures (thick arrows), normal axoplasma (Axp), mitochondria (Mi), neurofilaments (Nf), Schwann cell (ScC), nucleus (N), vacuoles (Va), EM, and lead citrate-uranyl acetate, ×11.600. (c) 1.5 h post ketamine injection, myelinated nerve fibers (thin arrows), degeneration in lamellae (MyL), axoplasma (Akp), Schwann cell (ScC), nucleus (N), cystic structures (thick arrows). EM, and lead citrate-uranyl acetate, x11.600. (d). 24-h postketamine injection, myelinated (MyN), unmyelinated nerve fibers (nMyN), cystic structures (thick arrows), Schwann cell (ScC), neurofilaments (Nf), vacuoles (Va) EM, and lead citrate-uranyl acetate, ×11.600

There was a decrease in organelles in Schwann cell cytoplasm. However, no significant degenerations were observed in nonmyelinated nerve fibers [Figure 2d].

DISCUSSION

The phencyclidine derivative, ketamine, is a central acting drug. Ketamine causes amnesia, analgesia, cathepsis, anesthesia, and convulsions.[16,24] It was used intrathecal in short-term surgical interventions in acute war injuries in Cambodia, and good results were obtained. It was stated that the duration of the anesthesia was limited to 45 min. Changes in cardiac, vascular, and respiratory functions during anesthesia were observed.[25,26]

Davies *et al*. experimented with bupivacaine and morphine, to determine the effective dose without side effects.[2] Postsubarachnoid morphine injection complete analgesia occurred in rats. In humans, lower doses may be used intrathecally.[13] Short-term analgesic effects were observed in rats treated with 0.2, 0.4, 0.8, and 1.6 mg/kg ketamine intrathecal administration. Histopathological studies on light microscopy reveal localized toxic effects of ketamine, vacuoles were seen in posterior root ganglia.[17] The light microscopic examinations of postintrathecal injections of ketamine revealed edema, loss of axoplasmosis, and proliferation of arachnoid cells.[14] After 1month, 0.7 and 0.5 mg/kg ketamine administrations on 16 adult dogs at lumbar and sacral parts gliosis, axonal edema central chromatolysis, lymphocyte infiltration, and fibrosis thickening of dura mater were observed.[27]

In this study, complete analgesia was detected postintrathecal ketamine injection. Degenerative changes were progressing in myelinated nerve fibers at light microscopy in the spinal nerve roots postketamine application 1.5 h and 24 h. Degeneration in large diameter nerve fibers was severe, myelin folds and cystic enlargements were seen. Excessive degeneration detected 24-h ketamine injection.

Histological examinations of postintracerebral injections of prococaine, spinocaine grovocaine, and scurocaine show toxic; however, reversible changes such as inflammatory meningeal responses and secondary degenerative changes. Low concentrations of anesthetics do not cause changes in the spinal cord and meninges.[28]

Barton experimentally injured the sciatic and sural nerves in rabbits, and in the next days, changes were observed in axoplasm, Schwann cells, and myelin sheath in electron microscope level. In degenerated myelinated fibers, a large number of vacuoles and granules were observed, and the Schwann cell cytoplasm was dense.[29] According to Ho *et al*., sciatic nerve at low and high concentrations of Na and K in frog, axon and axoplasm degenerations occur and Schwann

cell organelles decreases, mesaxon widens. These were more related to Schwann cell degeneration rather than axonal degeneration.[30]

In this study, within 30 min postapplication, a significant degeneration was observed in the spinal nerve roots, especially in myelinated nerve fibers, in electron microscopy. The axon was shrunk, a few degenerated mitochondria, vacuoles, decreased organelles in Schwann cell, and dense nucleus chromatine were detected.

3-days postintratracheal application of 0.5, 1 and 2% etidocaine and 0.25, 0.5, and 1% tetracaine swelling in the axon and myelin layers and accumulation of macrophages were observed. The intensity of the degeneration varies according to the concentration. Adams *et al*.[29] observed that the postadministration of bupivacaine in sciatic nerve of the rabbits, changes at the blood–nerve barrier were dose-dependent in fluorescence microscope.[8] Watanabe *et al*. injected rabbits 0.4%, 1.25, and 2.5 gentamicin sulfate initially found severe seizures, respiratory depression, and extreme paralysis. In the upper cervical region of the spinal cord, numerous small and spongiotic lesions were observed. They reported the ultrastructural axon, astroglia and oligodendroglia degeneration, and edema in the myelin.[31]

Uğur applied four different anesthetics intrathecally (0.5% mepivacaine, 4% mupivacaine, 0.5% etidocaine, and 2% chloroprocaine) structural changes in the spinal cord were observed in the dura, pia, and arachnoid. When hyperbaric solutions were used, the myelin layers were separated, endoneurial edema and organelle loss were detected.[18]

Lee initiated the degeneration by cutting the left sciatic nerve in rabbits and rats. Post 19 h myelin loss, enlargement in the endoplasmic reticulum, swelling in the mitochondria, and clustering in the neurofilaments were observed. On day 7, 21, 28, and 31, vacuolization, degeneration in mitochondria, endoplasmic reticulum and Schwann cells, neurofilaments, and lack of organelles in the axoplasm occurred.[32]

When 0.5 mg/kg intrathecal ketamine was given to rabbits, gray and white matter mild or severe spinal cord damage was observed. Chromolysis, in small areas, necrosis in motor neurons damage around the central canal was observed. Ependymal loss, subpial necrotic lesions, loss of myelin, axonal swellings, necrosis, and reactive leukodiapedesis were seen.[33]

In our study, 24-h postapplication, myelin lamellae, axoplasma, mitochondria, neuroflamments, and Schwann cell cytoplasm were highly degenerated in both types of nerve fibers, and 24-h postapplication degeneration continued to progress.

CONCLUSION

Intrathecal administration of ketamine in the light of these findings was found to cause significant degeneration on tissue samples taken from the site of injection and peripheral areas. Degenerative changes were progressive and mostly seen in myelin lamellae, Schwann cells, axons, and a few nonmyelinated fibers. Thus, for this reason, it is necessary to investigate whether the degenerative changes are progressive or reversible, the suitability of the given drug concentration and other adverse effects. This has led to the fact that more animal experiments have to be done.

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