ORIGINAL ARTICLE

Effects of intracisternal tramadol on cerebral and spinal neuronal cells in rat

E. A. KOSE [1], B. BAKAR [2], S. K. AYVA [3], K. KILINC [4], A. APAN [1]

[1]Kirikkale University, School of Medicine, Department of Anesthesiology and Reanimation, Kirikkale, Turkey; [2]Kirikkale University, School of Medicine, Department of Neurosurgery, Kirikkale, Turkey; [3]Kirikkale University, School of Medicine, Department of Pathology, Kirikkale, Turkey; [4]Hacettepe University Faculty of Medicine, Department of Biochemistry, Ankara, Turkey

ABSTRACT

Background. The aim was to investigate whether tramadol had toxic effect on cerebral neurons and/or spinal cord neurons when it was administered into the cerebrospinal fluid. Due to lipid peroxidation (LPO) and myeloperoxidation (MPO) levels are not specific predictors of neuronal damage, these biochemical markers of tissue damage were evaluated together with the histopathological findings of apoptosis.

Methods. Forty eight Wistar rats were anesthetized and the right femoral artery was cannulated. Mean arterial pressures, and heart rates, arterial carbon dioxide tension, arterial oxygen tension, blood pH were recorded. When the free cerebrospinal fluid flow was seen; 0.04 mL normal saline (Group Sham) or diluted tramadol in 0.04 mL volume (Group T1, T2, T0.5 and T0.1) was administered within 30 seconds from the posterior craniocervical junction of rats. For the Control Group, the free cerebrospinal fluid flow was seen but nothing was injected in it. After 7 days, following the sacrification of the rats, brain tissue, cervical and lumber segments of spinal cord were collected for the histopathological and biochemical examination.

Results: There was not a statistically significant difference among all groups regarding the brain LPO levels (P=0.485). The LPO levels of the cervical segment of spinal cord and the lumbar segment of spinal cord were also similar (P=0.146, P=0.939, respectively). The mean MPO levels of the cervical and the lumbar segments of spinal cord were similar among all groups (P=0.693, P=0.377, respectively). There were not any statistically significant difference regarding the total number of red neurons of the brain tissue and the cervical and lumbar segments of spinal cord among all groups (P=0.264, P=0.202, P=0.780, respectively).

Conclusion. Tramadol had no neurotoxic effect on brain and on spinal cord tissue when administered by the intracisternal route in cerebrospinal fluid in rats. *(Minerva Anestesiol 2014;80:904-12)*

Key words: Tramadol - Neurotoxicity syndromes - Rats.

Tramadol is an atypical opioid analgesic drug with selectivity for the μ-receptor, near 6000-fold lower than morphine.[1] Nevertheless, a non-opioid mechanism is also involved in tramadol analgesia, which consists of an enhancement of the extraneural concentration of noradrenalin and serotonin by interference with both the uptake and release mechanisms. Therefore, tramadol causes the activation of both main systems involved in the inhibition of pain: the opioid and the descending monoaminergic systems.[2] To increase the duration of analgesia produced by local anesthetics, different adjuvants have been added through the neuroaxial route. Intrathecal opioid administration has been demonstrated to provide effective postoperative analgesia, but opioids can cause increased risk of respiratory depression[3]. Tramadol, in contrast to a centrally acting opioid analgesic, has minimal respiratory depressant effect, because it has

6000-fold less affinity for μ-receptors compared to morphine.[2-4] It also rarely leads to drug tolerance observed with morphine as it interacts with fewer receptors than morphine to result in equianalgesic response. Furthermore, as it is a short-acting compound, dose adjustment is much easier than with morphine.[2-4] So, it can be said that tramadol has the potential to provide effective postoperative analgesia, with decreased risk of respiratory depression after central neuroaxial administration.

Alici *et al.* shown that tramadol administered intracisternal route in rabbit was caused to disappear of at some of the polyunsaturated fatty acids in spinal segments and in brain after drug administration. So, they speculated that intrathecal tramadol decreased membrane fluidity of blood brain barrier and could cause neurotoxicity due to loss of unsaturation and serious changes in concentrations and numbers of fatty acids.[5] On the other hand, there is a case report which was reported intrathecal granuloma formation in a patient receiving long-term spinal infusion of tramadol.[6]

Establishing the safety of neuroaxial drugs is very important before these drugs are given to humans. Despite extensive use in animal experimentation, there has not been enough data in the medical literature about the effects of this highly lipo-soluble and centrally acting opioid analgesic on cerebral neurons when administered intrathecally into the cerebrospinal fluid. The purpose of this prospective, randomized, double-blind, controlled study was to investigate whether tramadol had toxic effect on cerebral neurons and/or spinal cord neurons when it was administered in different doses centrally into the cerebrospinal fluid by intracisternal route.

Materials and methods

This experiment was performed in accordance with the guidelines for the use of laboratory animal subjects in research set by the Ethical Committee of Kirikkale University.

Forty eight male Wistar rats of 250-300 gram weight were used. The rats were placed in a temperature (21±2 °C) and humidity (60±5%) controlled room for one week before the experiment, 12 h light/12 h dark cycle was maintained, and was allowed free access to food and water before and after surgery. The animals were divided into six groups randomly which were named as follows:

— Control: No agent was administered by intracisternal route, N.=8;

— Sham: 0.9 % NaCl in a volume of 0.04 mL administered by intracisternal route, N.=8;

— Group T1: 1 mg tramadol (Contramal Amp, Abdi İbrahim, Turkey) in a volume of 0.04 mL administered by intracisternal route, N.=8;

— Group T2: 2 mg tramadol in a volume of 0.04 mL administered by intracisternal route, N.=8;

— Group T0.5: 0.5 mg tramadol in a volume of 0.04 mL administered by intracisternal route, N.=8;

— Group T0.1: 0.1 mg tramadol in a volume of 0.04 mL administered by intracisternal route, N.=8.

Anesthesia was performed with intramuscular administration of 40 mg/kg ketamine HCl (Ketalar®; Pfizer Inc, USA) and 5 mg/kg xylazineHCl (Rompun® %2; Bayer HealthCare AG, Germany). Rats were kept normothermic with thermoregulatory heating unit (Harvard Apparatus) connected the rectal probe. The right femoral artery was cannulated for continuous blood pressure monitoring (CardioCap II, Datex, Finland), and to obtain arterial blood samples. Mean arterial pressures (MAP) and heart rates (HR) were recorded during the study. Arterial blood samples were obtained at 0, 5, 15, 30 and 60 minutes to evaluate arterial carbon dioxide tension ($PaCO_2$), arterial oxygen tension (PaO_2), blood pH (ABL5, Radiometer, Denmark). When the free cerebrospinal fluid flow was seen; 0.04 mL normal saline (Group Sham) or diluted tramadol in 0.04 mL volume (Group T1, T2, T0.5 and T0.1) was administered within one minute from the posterior craniocervical junction of rats using a 26G needle. During the recovery period catheters were removed. Then the animals were returned to their home cages. All animals were re-anaesthetized with intramuscular 40 mg/kg ketamine HCl and 5 mg/kg xylazine HCl on day 8. For sacrification, the whole body blood was collected from the vena cava in-

ferior, and then craniectomy and laminectomy were performed in 30 minutes after death. The brain and spinal cord were quickly removed. The hippocampus structure was extracted from the brain. Then the spinal cord was divided into segments from two zones: midcervical and midlumbar segments. The hippocampal formations, cervical and lumber segments of spinal cord were stored in 10% buffered formaldehyde solution at room temperature for future histopathological examination. For biochemical examination, brain tissue, spinal cord tissue and the plasma of the collected whole body blood were immediately stored at -30 °C in dry air.

Biochemical analysis

Biochemical determinations were carried out by a biochemist blinded to the animal groups, and test materials. Frozen tissue samples were weighted and homogenized in 1:10 (w:v) potassium phosphate buffer (50 mM, pH: 7.4) by using a dounce homogenizer. Thiobarbituric acid reactive substances (TBARS) were measured as an index of LPO by the method of Miharaet *et al.*[7, 8] Tissue levels of lipid peroxides (as TBARS) were calculated as nanomole per gram wet tissue. Serum levels of lipid peroxides were determined by tiobarbituric acid (TBA) assay as described by Wade and Van Rij, and calculated as micromole per liter.[9] Tissue-associated myeloperoxidation (MPO) activity was measured by the modified method of Suzuki *et al.*[10] Tissue-associated MPO activity was calculated as units per gram of wet tissue.

Histopathological analysis

For histological examination, all tissue samples were fixed at 10% buffered formaldehyde and processed according to routine light microscopic tissue processing technique. Serial coronal sections of 5 μm at the level of the hippocampus formation and cervical and lumber segments of spinal cord were stained with haematoxyleneeosin. The histopathological specimens were examined and photographed by an Olympus BH-2 microscope. The presence and the number of the red neurons, which are a marker of apoptosis, were investigated in the frontal, occipital, parietal, temporal hippocampal CA1, CA2, CA3 and dentate gyrus (DG) regions of brain and in the transverse sections of medulla spinalis. A red neuron was defined as a brightly stained neuron with scanty eosinophilic cytoplasm, nuclear pycnosis, and perineural retraction spaces. The slides were examined by a pathologist blinded to the study groups. Identified red neurons were counted in all formentioned hippocampal regions separately on high power field and then were summated for each rat. Identified red neurons in cervical and lumbar segments of medulla spinalis were also counted separately for each rat.

Statistical analysis

Data were analyzed using the SPSS 11.5 (SPSS Inc. Software, Chicago, Illinois, USA) statistical software. All data were presented as mean±SD. Because tissue LPO levels, plasma LPO levels, histopathological data and the data of physiological parameters were normally distributed and the variations were homogenous among all groups, analyzed by using one-way analysis of variance (ANOVA) and with Tukey test as to determine comparison among groups, and differences among groups, respectively. The repeated measures of physiological parameters within different times were analyzed using general linear model analysis of variance for repeated measures. $P<0.05$ was considered statistically significant.

Results

Physiological parameters

Blood gas tensions, MAP, pH and rectal temperatures were similar among all groups at all times. The repeated measures of physiological parameters within different times were also similar in all groups.

Biochemical analysis

There was not a statistically significant difference among all groups regarding the brain LPO levels ($P=0.485$). The LPO levels of the cervical segment of spinal cord and the lumbar seg-

ment of spinal cord were also similar (P=0.146, P=0.939, respectively). Tissue-associated MPO activity could not be measured in the brain tissue. Additionally, the mean MPO levels of the cervical and the lumbar segments of spinal cord were similar among all groups (P=0.693, P=0.377, respectively).

Histopathological analysis

There were not any statistically significant difference regarding the total number of red neurons of the brain tissue and the cervical and lumbar segments of spinal cord among all groups (P=0.264, P=0.202, P=0.780, respectively) (Figures 1-3). The total numbers of red neurons were also similar in each of the brain region and in anterior and posterior horns of both spinal cord segments.

Discussion

Opioid analgesics produce antinociception through their action on both the peripheral and central opioid receptors in acute and chronic pain states.[11] Tramadol is a racemic mixture of two enantiomers. While (-)-tramadol inhibits noradrenalin uptake, (+)-tramadol inhibits serotonin uptake, enhances serotonin release and binds μ-opioid receptors.[12] It was suggested that tramadol may have local anesthetic effects on peripheral nerves.[13] To increase the duration of analgesia produced by local anesthetics, a number of adjuvants have been added the central neuroaxial route. Intrathecal opioid administration has been demonstrated to provide effective postoperative analgesia. The potential advantage of using combination therapy is that analgesic effects can be maximized while the incidence of adverse side effects can be minimized. Additionally, the multiplicity of mechanism involved in pain suggests that combination therapy can improve pain management.[14] Specific pain pathways can be potentially impeded by the direct application of receptor-specific therapeutics at the spinal cord, which limit systemic side effects, but this practice also carries the inherent risk of

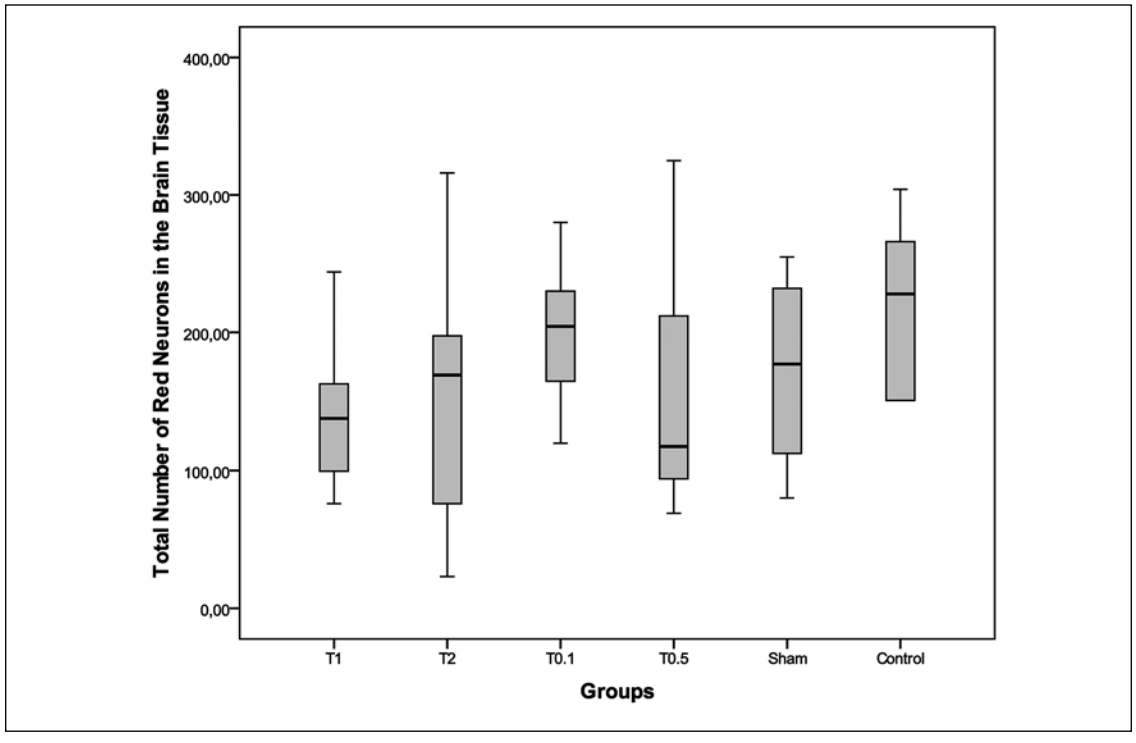

Figure 1.—The mean values of the total number of the red neurons in the brain tissue.

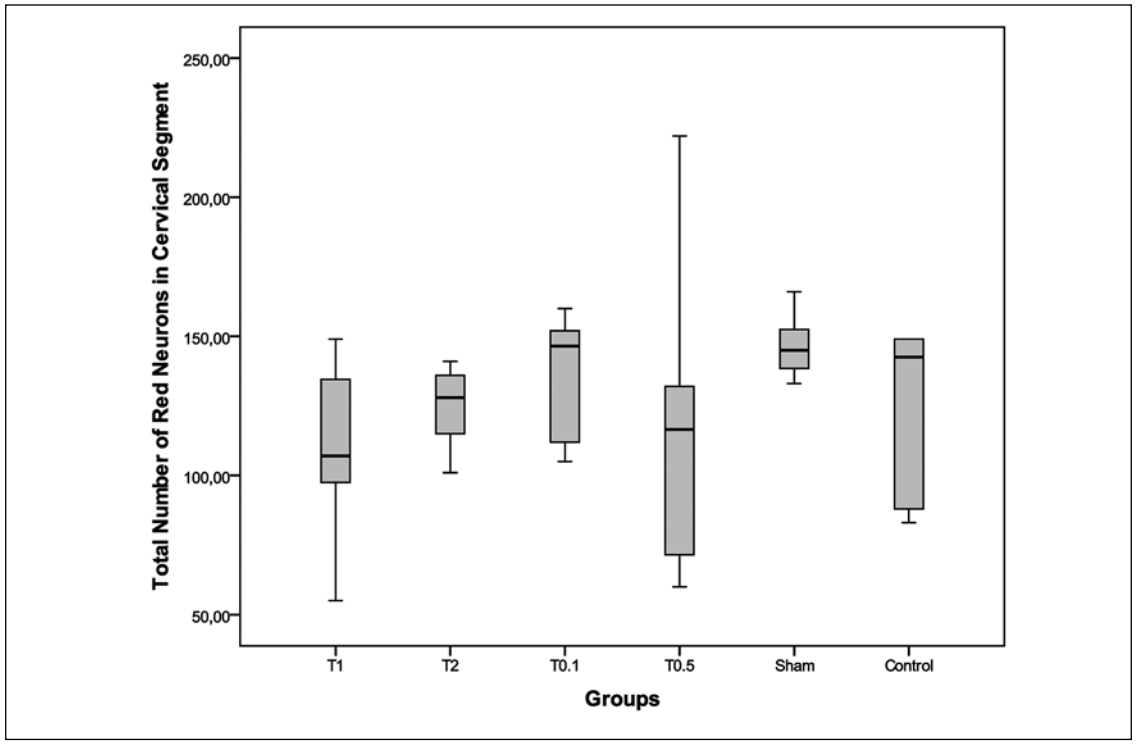

Figure 2.—The mean values of the total number of the red neurons in the cervical segment of the spinal cord tissue.

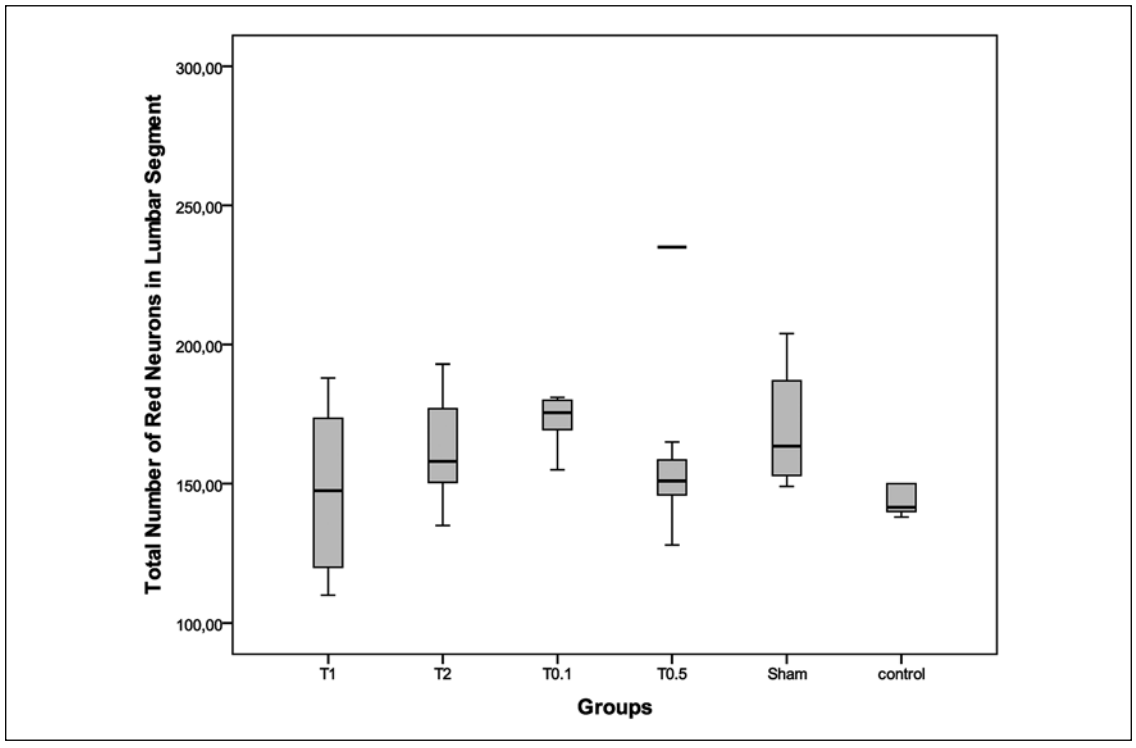

Figure 3.—The mean values of the total number of the red neurons in the thoracal segment of the spinal cord tissue.

injury to the central nervous system. Thus, the neurotoxicity of spinal drugs has become central safety issue. After exposure to a spinal drug, toxic histological changes such as apoptosis can be seen in spinal cord or nerve roots. Tramadol has been administered spinally, although this route is unsupported by toxicological data and it has not been registered to be used as analgesic.[15] The aim of this study was to investigate whether tramadol had toxic effect on cerebral neurons and/or spinal cord neurons when it was administered in different doses centrally into the cerebrospinal fluid by intracisternal route.

Tramadol administered in 1 mg and 2 mg doses through an intrathecal catheter was shown a suppressive effect on both sensory and motor neural conduction in the spinal cord.[16] In this study, we used four different doses (1 mg, 2 mg, 0.5 mg and 0.1 mg) of tramadol. Tramadol is presented in a sterile intravenous preparation with water for injection and sodium acetate as a buffering agent. Due to, neurotoxicity has never been reported to the use of sodium acetate we used the commercial intravenous form of tramadol in this study. Because, it is possible to see the free cerebrospinal fluid flow and the easiness of technique, we preferred to administer the agents used in the study into cisterna magna by an injection from posterior craniocervical junction of the rats. Due to the fact that, cerebrospinal fluid pressure of rat is very low and aspiration with using negative pressure might be harmful, we did not aspirate any cerebrospinal fluid before the administration of intracisternal drug. However, it was thought that the administered 0.04 mL additional volume within 30 seconds might produce harmful effect on neuronal structures by increasing cerebrospinal fluid pressure. And, the sham group which was 0.04 mL 0.9% NaCl given within 30 seconds by intracisternal route was added to the study for the observation of this possible additional harmful effect.

Although the neurotoxicity signs of tramadol were not mentioned in any of the experimental or clinical studies which were focused on the analgesic effect of intrathecal administration of tramadol, there was limited number of experimental studies on the neurotoxic effect of intrathecal administration of tramadol.[5, 14, 16-19] Alici *et al.* were reported in their study that tramadol administered intrathecal route caused lost of at least four polyunsaturated fatty acids in spinal cord segments, and three of those in the brain in rabbits. Therefore, they speculated that intrathecal administration of tramadol can be caused neurotoxic effect by the decreased

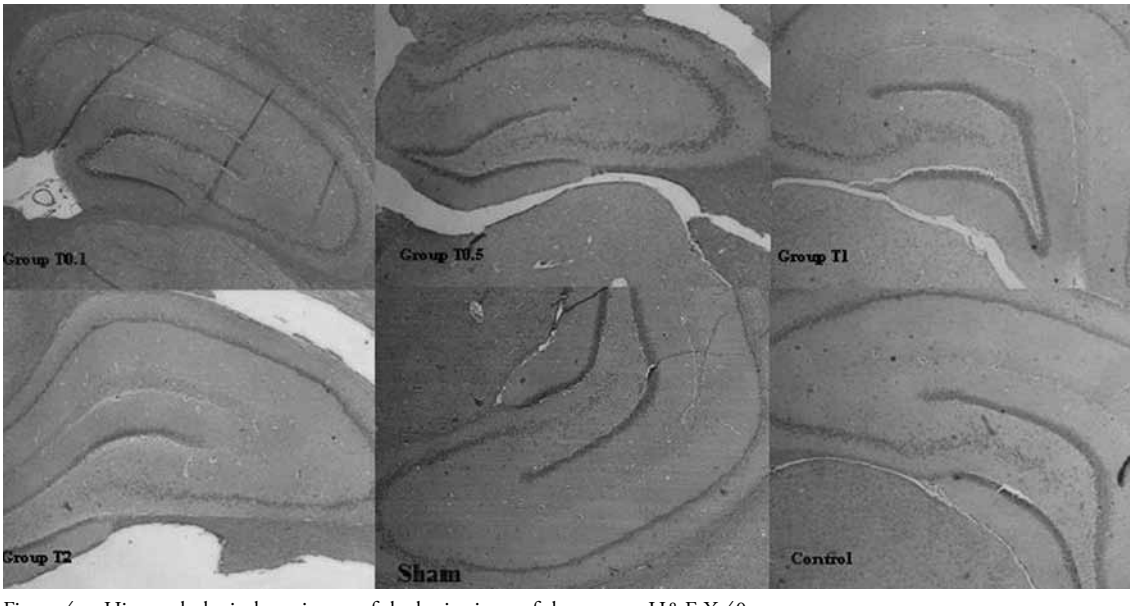

Figure 4.—Histopathological specimens of the brain tissue of the groups, H&E X 40

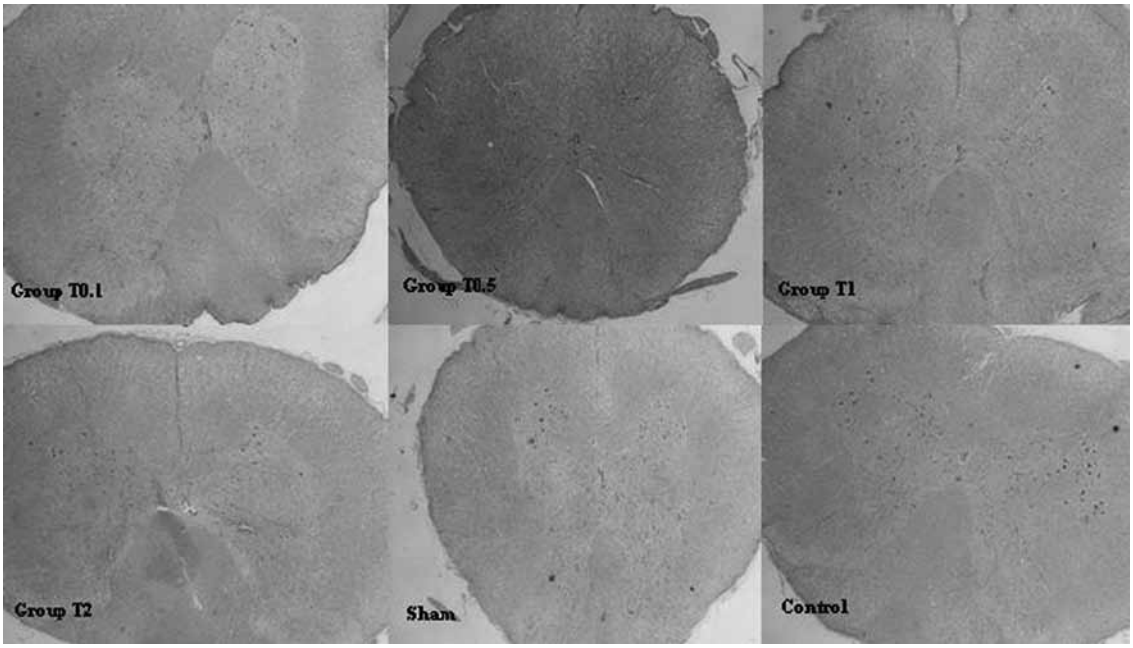

Figure 5.—Histopathological specimens of the cervical segment of the spinal cord tissue of the groups, H&E X 40.

membrane fluidity of the blood brain barrier.[5] In the present study, the red neuron numbers were similar among all groups both in the brain and in the spinal cord segments (Figures 1-3). The histopathological appearances observed by the light microscopy were similar between the control group and the other groups (Figure 4, 5). Additionally, the tissue LPO and MPO levels were similar among all groups, and an increased level of these biochemical markers which could be attributed to the tissue destruction was observed neither in brain tissue, nor in the spinal cord tissue (Table I). So, we concluded that tramadol had no neurotoxic effect on brain and on spinal cord tissue when administered by the intracisternal route in cerebrospinal fluid in rats.

Likewise the previous studies, we did not observed respiratory depression, hypoxia or hypercapnia in any of the animals. There were no significant differences between the tramadol groups and sham group based on the data of hemodynamic changes, arterial blood gases, or heart rate.[5, 19]

This prospective experimental study had some

Table I.—*Mean LPO and MPO levels in brain tissue and spinal cord segments.*

	Group T1	Group T2	Group T0.1	Group T0.5	Sham	Control
LPO Level (nmole/g)						
Brain tissue	144.2±20.7	141.9±9.5	138.9±21.4	145.6±15.5	132.6±6.9	136.2±3.2
Cervical segment of spinal cord	94.3±5.2	95.4±2.0	88.4±4.5	87.5±13.7	91.9±1.8	91.4±2.7
Lumbar segment of spinal cord	91.0±5.0	90.1±6.8	92.5±6.8	89.9±6.6	91.0±4.0	90.6±3.7
MPO level (U/g)						
Brain tissue						
Cervical segment of spinal cord	0.9±1.2	1.0±1.3	0.5±1.0	0.7±0.8	1.4±1.2	1.1±0.7
Lumbar segment of spinal cord	3.0±3.9	3.5±1.2	5.1±4.5	1.3±0.5	1.1±0.5	1.5±0.5

pitfalls. Firstly, this study could not be supported by immunohistochemical, and electron microscopic findings. Due to lipid peroxidation (LPO) and myeloperoxidation (MPO) levels are not specific predictors of neuronal damage, these biochemical markers of tissue damage were evaluated together with the histopathological findings of apoptosis. Using more specific biochemical markers of neuronal damage such as activated caspase 3, Bax/Bcl-2 ratio and electron microscopic findings could be more predictive for the detection of the neurotoxic effect. Although, we found no statistically meaningful change regarding to LPO and MPO levels and histopathological findings compatible with apoptosis at the end of the study, the possible neurotoxic effects of tramadol must be evaluated by using more sensitive markers and techniques by the future studies.

Secondly, because the aim of the study was to investigate the histopathological and biochemical changes in brain tissue and spinal cord tissue after a single intrathecal injection of tramadol, we did not insert an intrathecal catheter and effect of the repeated doses of the drug on the neuronal structures was not evaluated. Because, the acute and subacuteneuroxic effects of the agent could extend to 7 days, the animals were sacrificed on the 8th day of intracisternal injection.

Thirdly, due to there was no experimental study in the medical literature which was related to the neurotoxic effect of tramadol given in the cerebrospinal fluid by the neuroaxial route, we could not calculate the least sample size at the beginning of the study. By using the results of the study (mean values and Standard Deviations) were considered and α were accepted as 0.05, and β value was accepted as 0.20. The required minimum sample sizes of were so excessive that we could indirectly say that there were no meaningful clinical difference among different dose groups, control and sham groups.

In conclusion, there were no clinical and statistical difference among the control group, sham group and four different doses of tramadol when administered by the intracisternal route in cerebrospinal fluid in rats.

Key messages

— Intrathecal opioid administration has been demonstrated to provide effective postoperative analgesia. By using combination therapy, analgesic effects can be maximized while the incidence of adverse side effects can be minimized. Establishing the safety of neuroaxial drugs is very important before these drugs are given to humans. Thus, the neurotoxicity of spinal drugs has become central safety issue. Although, it has been administered spinally in several clinical studies, the safety of intathecal usage of tramadol is unsupported by toxicological data.

— Biochemical markers of tissue damage, LPO and MPO, and histopathological findings of apoptosis were evaluated together to investigate whether neurotoxic effect of tramadol when it was administered.

— It was concluded that, there were no clinical and statistical difference among the control group, sham group and four different doses of tramadol when administered by the intracisternal route in cerebrospinal fluid in rats.

References

1. Lewis KS, Han NH. Tramadol: a new centrally acting analgesic. Am J Health Syst Pharm 1997;54:643-52.
2. Raffa RB, Friderichs E, ReimannW,Shank R, Codd E, Vaught J. Opioid and nonopioid components independently contribute to the mechanism of action of tramadol, an "atypical" it opioid analgesic. J Pharmacol Exp Ther 1992;260:275-85.
3. Tarkkila P, Tuominen M, Lindgren L. Comparison of respiratory effects of tramadol and pethidine. Eur J Anaesthesiol 1998;15:64-8.
4. Scott LJ, Perry CM. Tramadol: a review of its use in perioperative pain. Drugs. 2000;60:139-76.
5. Alici HA, Ozmen I, Cesur M, Sahin F. Effect of spinal drug tramadol on fatty acid compositions of rabbit spinal cord and brain. Biol Pharm Buil. 2003;26:1403-6.
6. De Andres J, Vivo JT, Villanueva VL, Minguez A. Intrathecal granuloma formation in a patient receiving long-term spinal infusion of tramadol. Pain Medicine 2010;11:1059-62.
7. Mihara M, Uchiyama M. Determination of malonaldehyde precursor in tissues by thiobarbituric acid test. Anal Biochem 1978;86:271-8.
8. Ohkawa H, Ohishi N, Yagi K. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. Anal Biochem 1979;95:351-8.
9. Wade CR, Van Rij AM. Plasma thiobarbituric acid reactiv-

ity: Reaction conditions and the role of iron, antioxidants and lipid peroxy radicals on the quantization of plasma lipid peroxides. Life Sci 1988;43:1085-93.
10. Suzuki K, Ota H, Sasagawa S, Sakatani T, Fujikura T. Assay method for myelo-peroxidase in human polymorphonuclear leucocytes. Anal Biochem 1982;127:346-50.
11. Hong Y, Abbott FV. Peripheral opioid modulation of pain and inflammation in the formalin test. Eur J Pharmacol 1995;227:21-8.
12. Driessen B, Reimann W, Giertz H. Effects of central analgesic tramadol on the uptake and release of 5-jydroxitryptamine and dopamine in vitro. Br J Pharmacol 1993;105;806-11.
13. Tsai YC, Chang PJ, Jou IM. Direct tramadol application on sciatic nerve inhibits spinal somatosensory evoked potentials in rats. Anesth Analg 2001;92:1547-51.
14. Granados-Soto V, Argüelles CF. Synergic antinociceptive interaction between tramadol and gabapentin after local, spinal and systemic administration. Pharmacology 2005;74:204-8.
15. Bamigbade TA, Langford RM. Tramadol hydrochloride: an overview of current use. Pain Rev 1998;5:155-82.
16. Jou IM, Chu KS, Chen HH, Chang PJ, Tsai YC. The effects of intrathecal tramadol on spinal somatosensory-evoked potentials and motor-evoked responses in rats. AnesthAnalg. 2003;96:783-8.
17. Alhashemi JA, Kaki AM. Effect of intrathecal tramadol administration on postoperative pain after transurethral resection of prostate. Br J Anaesth 2003;91:536-40.
18. Chakraborty S, Chakrabarti J, Bhattacharya D. Intrathecal tramadol added to bupivacaine as spinal anesthetic increases analgesic effect of the spinal blockade after major gynecological procedures. Indian J Pharmacol 2008;40:180-2.
19. Lubenow TR, McCarthy RJ, Kroin JS, Ivankovich AD. Analgesic, hemodynamic and respiratory responses to intrathecal tramadol in dogs. Anesthesiology 1995;83:A822.

Conflicts of interest.—The authors certify that there is no conflict of interest with any financial organization regarding the material discussed in the manuscript.

Received on January 26, 2013. - Accepted for publication on December 9, 2013.

Corresponding author: E. A. Kose, Kirikkale University, School of Medicine, Department of Anesthesiology and Reanimation, 71100-Kirikkale, Turkey. E-mail: arzuhct@hotmail.com