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SCIENTIFIC ARTICLE

Neurotoxic effects of levobupivacaine and fentanyl on rat spinal cord

Yesim Cokay Abut[a,*], Asli Zengin Turkmen[b], Ahmet Midi[c], Burak Eren[d], Nese Yener[c], Asiye Nurten[b]

[a] *Department of Anesthesiology, Kanuni Sultan Suleyman Education and Training Hospital, Istanbul, Turkey*
[b] *Department of Physiology, Faculty of Medicine, Yeni Yuzyil University, Istanbul, Turkey*
[c] *Department of Pathology, Faculty of Medicine, Maltepe University, Istanbul, Turkey*
[d] *Department of Neurosurgery, Bakirkoy Sadi Konuk Education and Training Hospital, Istanbul, Turkey*

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KEYWORDS
Levobupivacaine;
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Abstract
Background: The purpose of the study was to compare the neurotoxic effects of intrathecally administered levobupivacaine, fentanyl and their mixture on rat spinal cord.
Methods: In experiment, there were four groups with medication and a control group. Rats were injected 15 μL saline or fentanyl 0.0005 μg/15 μL, levobupivacaine 0.25%/15 μL and fentanyl 0.0005 μg + levobupivacaine 0.25%/15 μL intrathecally for four days. Hot plate test was performed to assess neurologic function after each injection at 5th, 30th and 60th min. Five days after last lumbal injection, spinal cord sections between the T5 and T6 vertebral levels were obtained for histologic analysis. A score based on subjective assessment of number of eosinophilic neurons – Red neuron – which means irreversible neuronal degeneration. They reflect the approximate number of degenerating neurons present in the affected neuroanatomic areas as follows: 1, none; 2, 1–20%; 3, 21–40%; 4, 41–60%; and 5, 61–100% dead neurons. An overall neuropathologic score was calculated for each rat by summating the pathologic scores for all spinal cord areas examined.
Results: In the results of HPT, comparing the control group, analgesic latency statistically prolonged for all four groups.

In neuropathologic investment, the fentanyl and fentanyl + levobupivacaine groups have statistically significant high degenerative neuron counts than control and saline groups.
Conclusions: These results suggest that, when administered intrathecally in rats, fentanyl and levobupivacaine behave similar for analgesic action, but fentanyl may be neurotoxic for spinal cord. There was no significant degeneration with levobupivacaine, but fentanyl group has had significant degeneration.

* Corresponding author.
E-mail: yesimabut2000@yahoo.com (Y.C. Abut).

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PALAVRAS-CHAVE
Levobupivacaína;
Neurotoxicidade;
Fentanil

Efeitos neurotóxicos de levobupivacaína e fentanil sobre a medula espinhal de ratos

Resumo
Justificativa: O objetivo desde estudo foi comparar os efeitos neurotóxicos da administração por via intratecal de levobupivacaína e fentanil e suas misturas sobre a medula espinhal de ratos.
Métodos: O experimento compreendeu quatro grupos que receberam medicamento e um grupo controle. Os ratos foram submetidos à injeção de salina (15 μL) ou fentanil (0,0005 μg/15 mL), levobupivacaína a 0,25% (15 μL) e fentanil (0,0005 μg + levobupivacaine a 0,25%/15 μL) por via intratecal durante quatro dias. O teste de placa quente foi usado para avaliar a função neurológica após cada injeção nos minutos 5, 30 e 60. Cinco dias após a última injeção lombar, secções da medula espinal entre os níveis vertebrais T5 e T6 foram obtidas para análise histológica. Usamos um escore com base na avaliação subjetiva do número de neurônios eosinofílicos (neurônios vermelhos), o que significa degeneração neuronal irreversível. Esses neurônios refletem o número aproximado de neurônios em degeneração presentes nas áreas neuroanatômicas afetadas da seguinte forma: 1 = nenhum; 2 = 1-20%; 3 = 21-40%; 4 = 41-60% e 5 = 61-100% neurônios mortos. Um escore neuropatológico global foi calculado para cada rato pela soma dos escores patológicos para todas as áreas examinadas da medula espinhal.
Resultados: Nos resultados do TPQ, comparando o grupo controle, a latência analgésica foi estatisticamente prolongada para todos os quatro grupos.

Em investimento neuropatológico, os grupos fentanil e fentanyl + levobupivacaine apresentaram degeneração neuronal em contagens significativamente mais altas que os grupos controle e salina.
Conclusões: Estes resultados sugerem que fentanil e levobupivacaína, quando administrados por via intratecal em ratos, se comportam de forma semelhante à ação analgésica, mas fentanil pode ser neurotóxico para a medula espinhal. Não houve degeneração significativa com levobupivacaína, mas o grupo fentanil apresentou degeneração significativa.

Introduction

Increasing laboratory evidences[1–5] suggest that all local anesthetics are potentially neurotoxic, and that neurologic impairment after neuraxial blockade may result from a direct neurotoxic effect of drugs. Today, commercially available bupivacaine is a racemic mixture of S (−) and R (+) enantiomers. Its isolated S (−) enantiomer levobupivacaine has a lower potential for producing toxicity in the central nervous system and cardiovascular system than does R (+) bupivacaine in animals and humans.[6–8]

In clinical setting, the main purpose of spinoaxial administration of opioids is to reduce the local anesthetic dosage, to maximize efficacy and to minimize side effects of the involved drugs whose action sites are in the central nervous system. Lipophilic opioids such as fentanyl are commonly administered spinally in adults. There is minimal published report specifically addressing the histologic, physiologic, or clinical evidence of neurotoxicity with spinal fentanyl administration.[9,10]

In the present study we investigate that the repeated bolus intrathecal injection of fentanyl, levobupivacaine and their mixture could be neurotoxic for spinal cord on a rat model.

Methods

The protocol was approved by the Animal Research and Use Committee of Istanbul University (25/02/2010 Number: 26). All experiments were performed in DETAE (Department of Neuroscience, Institute of Experimental Medicine, Istanbul University, Istanbul). The experiment was conducted on Wistar albino rats 6–8 months old and (240–320 g). Animals were divided into five groups of 8 animals each. No drug was injected to control group (Group Control). After positioning prone and shaving, under aseptic conditions, without anaesthetizing, the following drugs were injected intrathecally once a day at the same hour, for four days, through the L4-5 intervertebal space: isotonic saline (Group Saline), fentanyl 50 μg/mL (Group Fentanyl), levobupivacaine 2.5 mg/mL (Group Levobupivacaine) or fentanyl 50 μg/mL + levobupivacaine 2.5 mg/mL (Group Fentanyl + Levobupivacaine). Solutions were prepared from fentanyl citrate(additive free) (Fentanyl-Janssen, Janssen – Cilag, Belgium) and levobupivacaine hydrochloride (Chirocaine-Sigma Chemical, Steinheim, Germany). Solutions were diluted with sterile isotonic saline (Serum Physiologique 0.9%-Galen Deva-Kocaeli, Turkey) (Table 1). All solutions were prepared and injected at room temperature (20–24 °C). Because chronically implanted intrathecal catheters can induce damage in tissue,[11,12] we prefer intrathecal injection technique instead of intrathecal catheter. Neurologic function was observed and measurements of hot plate test (HPT) were repeated 5th, 30th and 60th min and continued for 4 days after every drug administration. The hot plate response was assessed by placing the rats on a metal surface maintained at 45 °C. The test was measuring the latencies between the time of placing the animal on the surface and behavioral endpoint. In the

Table 1 Density of CSF, isotonic saline, levobupivacaine and fentanyl at 37 πC.

	37° C
CSF	1.000646 ± 0.000086
Saline	0.99951 ± 0.00001
Levobupivacaine 2.5 mg/mL	0.99985 ± 0.00002
Fentanyl	0.99333 ± 0.00002

majority of animals, licking the hind paws was observed; in remainder, jumping the end point, cut off time was 15 s. Behavioral tests were performed by a neuroscientist who was blinded the group assignment. Motor function (MF) of the posterior limbs was assessed by bilaterally grading the motor block as: 0, none; 1, partially blocked; and 2, completely blocked.[13] Motor blockade was graded as none when the rat had no visible limb weakness and normal gait; as partially blocked when the limb was able to move but not able to support the normal; and as completely blocked when the limb was flaccid, with no detectable resistance to extension of the limbs. The animals were examined 30 min before and after each injection. Rats having any problem with tail movement or motor dysfunction in the hindlimbs were not used in the experiments. The following parameters were measured and recorded over a 2 h period: sensory blocked, as determined by the response to the hemostat pinch test. After each injection, they were maintained on a 12-h light–dark schedule and housed with free access to food and water.

Histological evaluation

After the last functional examination, the rats were killed by intraperitoneal high dosages (100 mg/kg) pentobarbital. Spinal cord was excised by a neurosurgeon blinded to the group assignment and to the results of behavioral measurements. In order to discover cranial spread of local anesthetics, sections which obtained from T5-6 spinal cord were used for qualitative evaluation. Spinal cord was fixed in neutral (10%) buffered formalin for 7 days. Tissues exposed to formalin, alcohol, Xyloid and paraffin with tissue follow machine (Thermo Shandon Exelsior ES) and embedded in paraffin by routine techniques. The spinal cord was sliced at 2 μm intervals with the help of rotary type microtome (Thermo Shandon Finesse 325). Tissues stained with hematoxylin and eosin, and evaluated by light microscopy (Olympus CX31) by a pathologist blinded to the group assignment and to the results of behavioral measurements. The primary neuropathologic alteration seen in the rats was one of acute eosinophilic neuron degeneration.[14] Degrees of neuropathologic alterations within a given anatomic region were scored based on subjective assessment of number and distribution of eosinophilic neurons – Red neuron – which means irreversible neuronal degeneration. They reflect the approximate number of degenerating neurons present in the affected neuroanatomic areas as follows: 1, none; 2, 1–20%; 3, 21–40%; 4, 41–60%; and 5, 61–100% dead neurons. An overall neuropathologic score was calculated for each rat by summating the pathologic scores for all spinal cords, 10 areas examined for every preparation.

Statistical analysis

The results of the HPT were evaluated using one-way analysis of variance (ANOVA), followed by Dunnet's test for post hoc evaluation to compare all groups with control group.

For examination of tolerance, 2nd-3rd and 4th days HPT latency values were compared with the first day results of each group, and ANOVA followed by Dunnet's test were performed. MF was not analyzed because all animals have 0 motor function levels. Degrees of neuropathologic alterations within spinal cord were analyzed with Kruskal Wallis followed by Mann Whitney-*U* test. Significant difference testing was $p < 0.05$ on ANOVA.

Results

All rats completed the experiment and included the data analysis. All animals recovered fully, were awake and actively mobile and eating and drinking normally after 30 min of injection. During the experiment motor block has not been observed in any of the rats. No animals had sustained visible injury or bleeding in the spinal cord when the spinal cord excised at the end of the experiment.

HPT latencies prolonged for all groups comparing the control group without any motor block.

The result of HPT latency on the first, second, third and fourth day, at 5th, 30th and 60th min, can be seen in Table 2. In 5th min, HPT latencies – comparing to control group – were prolonged statistically significantly in saline ($p < 0.02$), fentanyl and fentanyl + levobupivacaine groups ($p < 0.05$). In 30th min, HPT latencies were statistically significantly increased – comparing to control group – in saline ($p < 0.02$), fentanyl ($p < 0.01$) and fentanyl + levobupivacaine ($p < 0.02$). It was found that HPT values for levobupivacaine ($p < 0.01$) and fentanyl + levobupivacaine ($p < 0.02$) group were increased significantly different from control values in 60th min.

The result of HPT latency on the second day, at 5th, 30th and 60th min, have prolonged in all groups when compared to control group. This was found significant for saline ($p < 0.01$) group and also fentanyl and fentanyl + levobupivacaine ($p < 0.05$) groups in 5th min. In 30th min, there was no statistically significant difference in prolonged latencies. The increment of HPT values in fentanyl + levobupivacaine group at 60th min was significantly different from control group in day 2 ($p < 0.01$).

The result of HPT latency on the third day, at 5th, 30th and 60th min, in levobupivacaine group was significantly prolonged in 5th min when compared to control group ($p < 0.05$). A significant increase in 60th min in fentanyl ($p < 0.05$), levobupivacaine ($p < 0.05$) and fentanyl + levobupivacaine ($p < 0.02$) groups was found.

The result of HPT latency on the fourth day, at 5th, 30th and 60th min, found no significant changes in 5th min values of HPT in day 4. In 30th min values, there was statistically significant increase in saline group ($p < 0.02$) and levobupivacaine group ($p < 0.05$) when compared to control group. HPT latencies in fentanyl ($p < 0.01$), levobupivacaine ($p < 0.01$) and fentanyl + levobupivacaine groups ($p < 0.05$) were significantly prolonged in 60th min when compared to control group (Table 2).

Table 2 The effect of repeated drug treatment on tolerance development.

Group	*n*	Hot plate latency (s)					
		Day 1			Day 2		
		5 min	30 min	60 min	5 min	30 min	60 min
Control	8	1.3 ± 0.3	1.3 ± 0.2	1.3 ± 0.2	1.1 ± 0.1	1.3 ± 0.2	1.4 ± 0.3
Saline	8	5.3 ± 1.3	3.9 ± 0.3	2.9 ± 0.6	3.9 ± 0.7	2.1 ± 0.3[a]	2.3 ± 0.3
Fentanyl	8	4.9 ± 0.7	4.4 ± 0.9	1.8 ± 0.3	3.1 ± 0.6[b]	2.5 ± 0.5	2.1 ± 0.4
Levobupivacaine	8	3.9 ± 1.0	3.3 ± 0.5	3.5 ± 0.8	2.8 ± 0.5	2.4 ± 0.5	2.4 ± 0.5
Fentanyl + levobupivacaine	8	4.9 ± 1.1	3.8 ± 0.7	3.4 ± 0.4	2.9 ± 0.3	3.1 ± 0.9	3.4 ± 0.5
Group	*n*	Hot plate latency (s)					
		Day 3			Day 4		
		5 min	30 min	60 min	5 min	30 min	60 min
Control	8	1.3 ± 0.1	1.4 ± 0.1	1.3 ± 0.2	1.3 ± 0.2	1.3 ± 0.2	1.4 ± 0.2
Saline	8	2.7 ± 0.6	2.1 ± 0.1[a]	2.6 ± 0.2	3.1 ± 0.6	3.6 ± 0.3	2.8 ± 0.4
Fentanyl	8	2.5 ± 0.2[c]	2.5 ± 0.4	3.5 ± 0.6[d]	2.6 ± 0.3[c]	3.0 ± 0.5	3.6 ± 0.5[e]
Levobupivacaine	8	2.8 ± 0.6	3.0 ± 1.0	3.4 ± 0.6	3.3 ± 1.2	3.3 ± 0.9	3.6 ± 0.7
Fentanyl + levobupivacaine	8	1.8 ± 0.3[b]	3.0 ± 0.5	3.8 ± 0.8	3.8 ± 0.8	2.9 ± 0.4	3.4 ± 0.4

n: number of animals.
Values are expressed in terms of mean ± SE.
[a] $p < 0.001$ compared to Day 1, 30th min value.
[b] $p < 0.02$ compared to Day 1, 5th min value.
[c] $p < 0.01$ compared to Day 1, 5th min value.
[d] $p < 0.05$ compared to Day 1, 60th min value.
[e] $p < 0.02$ compared to Day 1, 60th min value.

In Fig. 1, it could be seen that the repeated drug applications showed analgesic effects. Control group showed that there was no change on analgesic response time for four days. In saline group, we observed statistically significant short analgesic response time on second and third day at 30th min values ($p < 0.001$). In fentanyl group, analgesic response time was found shorter on second ($p < 0.02$), third ($p < 0.01$) and fourth ($p < 0.01$) day at 5th min and third ($p < 0.05$) and fourth ($p < 0.02$) day at 60th min. In levobupivacaine group, there was no change, on analgesic response time for four days. In fentanyl + levobupivacaine group, analgesic response time was found shorter at the 5th min of third day ($p < 0.02$).

Spinal cords neuropathologic analyses of all groups are shown in Fig. 2. In neuropathologic evaluation, degenerative neuron scoring was increased statistically significantly in fentanyl group in comparison to control and saline groups ($p < 0.05$). Fentanyl + levobupivacaine group showed high degenerative neuron scores than control, saline and levobupivacaine groups ($p < 0.01$).

Discussion

Since Bier and Hildebrant initially performed spinal anesthesia with cocaine in 1898, the history of spinal local anesthetic usage in humans has been followed by widespread application with little or no controlled testing for neurotoxicity.[15] In 1985, Ready et al. evaluated the neurotoxic effects of single injections of local anesthetics in rabbits. They reported that histopathological changes and neurologic deficits occurred with higher concentrations of tetracaine (1%) and lidocaine (8%).[16,17] In the past, local anesthetic solutions in clinically administered doses, rarely induced neurologic injury, and the observation of neurotoxic effects would require larger dosage of drugs. To produced injury, a rat model in which local anesthetics were continuously infused has been designed by Drasner et al. In this study, without clinically relevant, the functional impairment and morphologic damage were observed.[18] Previous studies designed by Kofke indicated that when they were given systemically, opioids can produce limbic system hypermetabolism and brain damage in quite large doses.[19–21] In 2000, the density and baricity of the mixtures used in spinal blocks were determined for the first time in Brazil.[22]

Above the light of this studies, we wanted to observe, some intrathecal drugs if they chronically penetrate spinal cord, could be neurotoxic or not. In our study, the results of the HPT have indicated that, there were no motor block but significant antinociceptive-analgesic effects in all groups.

In 5th min, HPT latencies – comparing to control group – were prolonged statistically significantly in saline ($p < 0.02$) group. Similarly after repeated injections, its analgesic response time was decreased like other opioid analgesics in saline group. We could not explain that why saline behave like an analgesic solution.

After repeated applications, fentanyl group was developed tolerance to the analgesic effect but this tolerance was not developed with levobupivacaine group.

We also tested the rostral spread of the drugs. For many years, we know that there are many factors that affect the cranial dispersion of spinal anesthesia which include

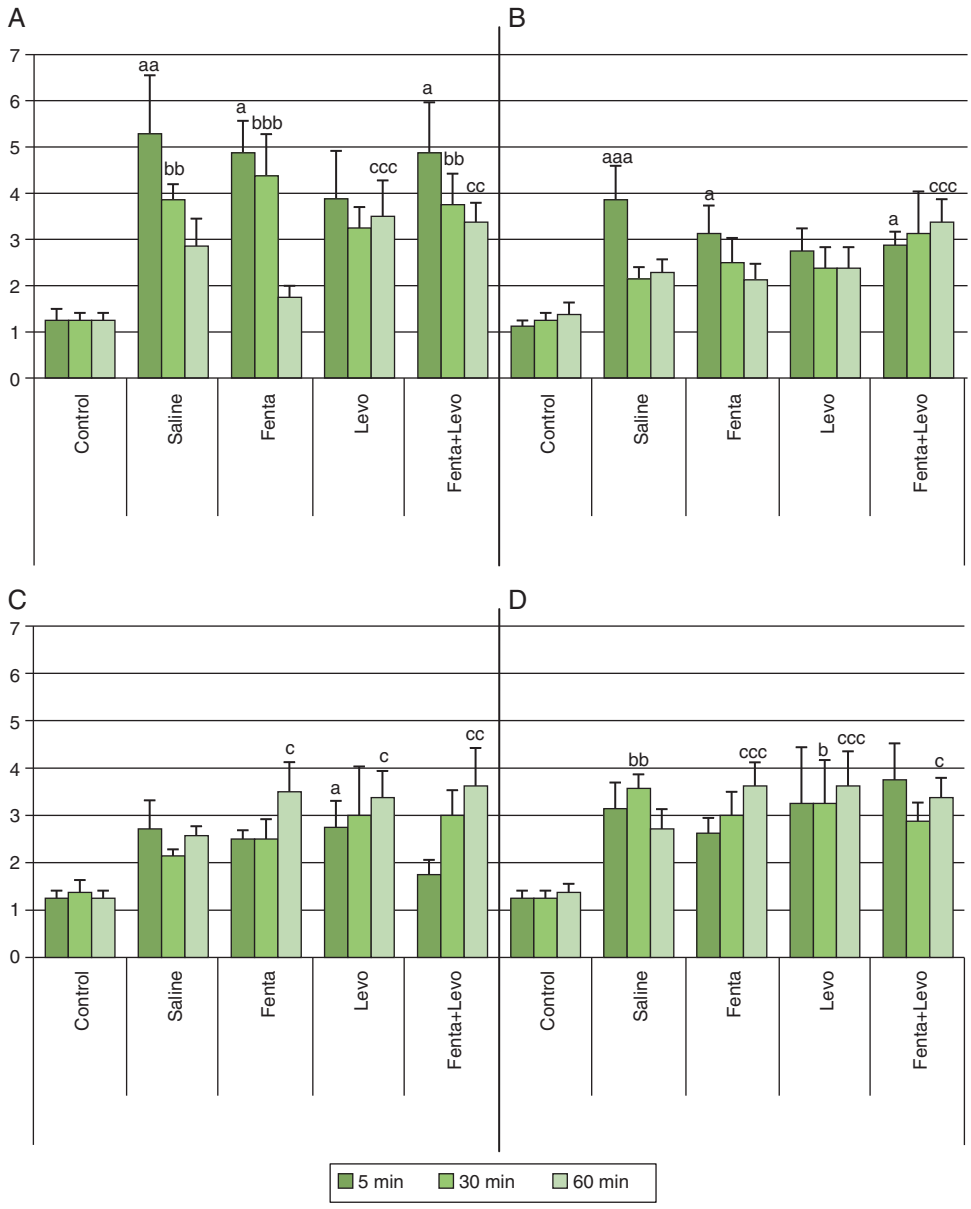

Figure 1 The effects of drug applications on the 1st (A), 2nd (B), 3rd (C) and 4th (D) day results of HPT latency. All data are presented as mean ± SE.
$^{a}p < 0.05$, $^{aa}p < 0.02$, $^{aaa}p < 0.01$ according to 5th min value to control group.
$^{b}p < 0.05$, $^{bb}p < 0.02$, $^{bbb}p < 0.01$ according to 30th min value to control group.
$^{c}p < 0.05$, $^{cc}p < 0.02$, $^{ccc}p < 0.01$ according to 60th min value to control group.

the patient posture, composition of solution, type of needle, level and speed of injection, volume, viscosity, inferior vena cava obstruction and pregnancy.[23–27] But the baricity and temperature of local anesthetics are the most important factors of the distribution of the local anesthetic into subarachnoid space.[28–30]

The density of the human CSF is not uniform, and it can vary with age, gender, pregnancy, and several diseases. The relationship between the density of the local anesthetic and the CSF, known as baricity.

Temperature changes also, affect the distribution of local anesthetics. When the local anesthetics are injected into subarachnoid space(generally in room temperature 20–24 °C) the temperature of the local anesthetic reaches an equilibrium with the body temperature (37 °C) very quickly, before being fixed at the nerve roots and at 37 °C, all isobaric anesthetics become hypobaric solutions.

Adjuvants are frequently added to local anesthetics to improve anesthesia and prolong postoperative analgesia. Opioids (morphine, fentanyl, and sufentanil) and clonidine showed to be hypobaric at 37 °C and, when added to local anesthetics, they reduce the density of the new solution, making it more hypobaric, according to some studies,[31,32] but it does not seem to have any effect on clinical practice suggesting that the change in density is very small.[27,33–35]

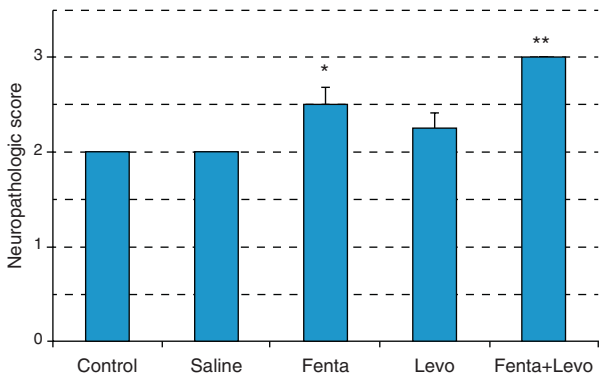

Figure 2 The effects of repeated drug applications on spinal cord.
Data are presented as mean ± SE.
*$p < 0.05$ compared with control and saline group.
**$p < 0.01$ compared with control, saline and levobupivacaine group.

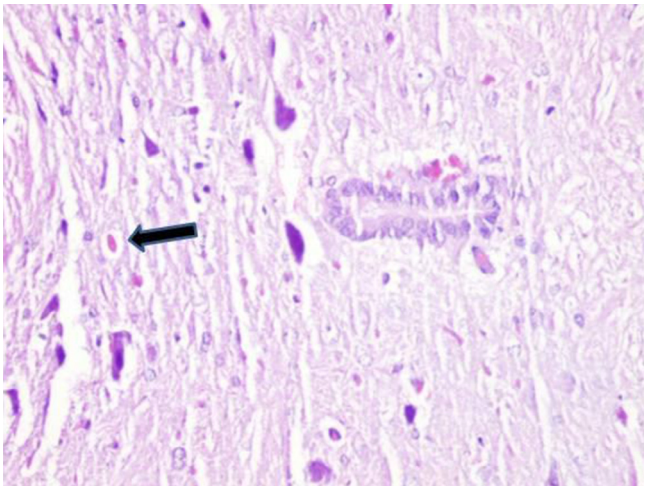

Figure 3 Arrow shows eosinophilic neuron of rat spinal cord in fentanyl group.

In our study, our solutions' temperature (in room temperature 20–24 °C) and their opioid mixture can affect the rostral spread of drugs. But this mechanism does not explain the excessive eosinofilic neuron count, especially some groups.

In clinical practice, there are many studies that local anesthetic solutions were diluted with isotonic sterile saline as we did in our study.[36–39] Rostral spread of drugs can be explained with the hypobaricity of our solutions, however, our neuropathologic results – against the results of Fukushima – showed that when the drugs were used even in very low analgesic doses (without motor block) they had permanent neurotoxic effects in thoracic spinal cord.[40] Because it shows the non-recoverable neuron damage, the eosinophylic neuron count has been used – as neurotoxicity signs in our study, instead of non-specific findings such as vacuolization, edema and invasion of macrophage, picnotic nuclei.[41] We can see eosinophylic-degenerative neuron in Fig. 3. It had been determined by Kofke before.[14]

In neuropathological investment degenerative neuron score in spinal cord was found significantly high in fentanyl and fentanyl + levobupivacaine group than control group and saline group. Also fentanyl + levobupivacaine group has significantly higher score than levobupivacaine group.

Indeed, one of the major aims of this study was to determine the neuropathologic changes on spinal cord, after it is chronically exposed to intrathecal drugs. Chronically implanted intrathecal catheters characteristically induce damage in control animals, so we prefer repeated injection technique instead of intrathecal catheter.[11,12]

Our data confirm that fentanyl and levobupivacaine can cause spinal cord damage in rats when they were injected for 4 days, even in analgesic doses.

At the end, our study tried to explain different sides of neurotoxicity. It is an animal study and has behavioral component. Neuropathologic methodology is different and may be more objective than previous studies, and also our study is based on the in vitro CSF-local anesthetic distribution-baricity studies. New studies should be planned with electron microscopy or behavioral studies may show that the long term penetration of local anesthetics may cause neuropathological degenerations on spinal cord, in future.

Conflicts of interest

The authors declare no conflicts of interest.

References

1. Sakura S, Chan VW, Ciriales R, et al. The addition of 7.5% glucose does not alter the neurotoxicity of 5,5 lidocaine administered intrathecally in the rat. Anesthesiology. 1995;82:236–40.
2. Sakura S, Bollen AW, Ciriales R, et al. Local anesthetic neurotoxicity does not result from blockade of voltage-gated sodium channels. Anesth Analg. 1995;81:338–46.
3. Hashimoto K, Sakura S, Bollen AW, et al. Comparative toxicity of glucose and lidocaine administered intrathecally in the rat. Reg Anesth Pain Med. 1998;23:444–50.
4. Sakura S, Kirihara Y, Muguruma T, et al. The comparative neurotoxicity of intrathecal lidocaine and bupivacaine in rats. Anesth Analg. 2005;101:541–7.
5. Yamashita A, Matsumoto M, Matsumoto S, et al. Comparison of the neurotoxic effects on the spinal cord of tetracaine, lidocaine, bupivacaine, and ropivacaine administered intrathecally in rabbits. Anesth Analg. 2003;97:512–9.
6. Aberg G. Toxicological and local anaesthetic effects of optically active isomers of two local anaesthetic compounds. Acta Pharmacol. 1972;31:273–86.
7. McLeod GA, Burke D. Levobupivacaine anaesthesia. 2001;56:331–41.
8. Gautier P, de Kock M, Huberty l, et al. Comparison of the effects of intrathecal ropivacaine, levobupivacaine, and bupivacaine for caesarean section. Br J Anaesth. 2003;91:684–9.
9. Allen JW, Horais KA, Tozier NA, et al. Opiate pharmacology of intrathecal granulomas. Anesthesiology. 2006;105:590–8.
10. Bahar M, Cohen ML, Grinshpoon Y, et al. An investigation of the possible neurotoxic effects of intrathecal midazolam combine with fentanyl in the rat. EJA. 1998;15:695–701.
11. Bahar M, Rosen M, Vickers MD. Chronic cannulation of the intradural or extradural space in the rat. Br J Anaesth. 1984;56:405–10.
12. Sakura S, Hashimoto K, Bollen A, et al. Intratecal catheterisation in the rat: improved technique for morphologic analysis of drug induced injury. Anesthesiology. 1996;85:1184–9.

13. Kaneko M, Saito Y, Kirihara Y, et al. Synergistic antinociceptive interaction after epidural coadministration of morphine and lidocaine in rats. Anesthesiology. 1994;80:137–50.
14. Kofke WA, Garman RH, Garman R, et al. Opiod neurotoxicity: fentanyl-induced exacerbation of cerebral ischemia in rats. Brain Res. 1999;818:326–34.
15. Bier A. Versuche uber Cocainisirung des Ruckenmarkes. Dtsch Z Chir. 1899;5151:361.
16. Ready LB, Plumer MH, Haschke RH, et al. Neurotoxicity of intrathecal local anesthetics in rabbits. Anesthesiology. 1985;63:364–70.
17. Hodgson PS, Neal JM, Pollock JE, et al. The neurotoxicity of drugs given intrathecally (Spinal). Anesth Analg. 1999;88:797–809.
18. Drasner K, Sakura S, Chan VWS. Persistent sacral sensory deficit induced by intrathecal local anesthetic infusion in the rat. Anesthesiology. 1994;80:847–52.
19. Kofke WA, Garman RH, Tom WC, et al. Alfentanil-induced hypermetabolism, seizure, and histopathology in rat brain. Anesth Analg. 1992;75:953–64.
20. Kofke WA, Garman RH, Janosky J, et al. Opioid neurotoxicity: neuropathologic effects in rats of different fentanyl congeners and the effects of hexamethonium-induced normotension. Anesth Analg. 1996;83:141–6.
21. Kofke WA, Garman RH, Stiller RL, et al. Fentanyl dose-response relation in rats. Anesth Analg. 1996;83:1298–306.
22. Cangiani LM. Determinação da densidade e da baricidade das misturas para anestesia subaracnóidea. Rev Bras Anestesiol. 2000;50:92–4.
23. Greene NM. Distribution of local anesthetic solutions within the subarachnoid space. Anesth Analg. 1985;64:715–30.
24. Stienstra R, Greene NM. Factors affecting the subarachnoid spread of local anesthetic solutions. Reg Anesth. 1991;16:1–6.
25. Carpenter RL, Hogan QH, Liu SS, et al. Lumbosacral cerebrospinal fluid volume is the primary determinant of sensory block extent and duration during spinal anesthesia. Anesthesiology. 1998;89:24–9.
26. Connolly C, McLeod GA, Wildsmith JA. Spinal anaesthesia for Caesarean section with bupivacaine 5 mg ml ± 1 in glucose 8 or 80 mg ml ± 1. Br J Anaesth. 2001;86:805–7.
27. McLeod GA. Density of spinal anaesthetic solutions of bupivacaine, levobupivacaine, and ropivacaine with and without dextrose. Br J Anaesth. 2004;92:547–51.
28. Lui AC, Polis TZ, Cicutti NJ. Densities of cerebrospinal fluid and spinal anaesthetic solutions in surgical patients at body temperature. Can J Anaesth. 1998;45:297–303.
29. Horlocker TT, Wedel DJ. Density, specific gravity, and baricity of spinal anesthetic solutions at body temperature. Anesth Analg. 1993;76:1015–8.
30. Stienstra R, Gielen M, Kroon JW, et al. The influence of temperature and speed of injection on the distribution of a solution containing bupivacaine and methylene blue in a spinal canal model. Reg Anesth. 1990;15:6–11.
31. Parlow JL, Money P, Chan PS, et al. Addition of opioids alters the density and spread of intrathecal local anesthetics? An in vitro study. Can J Anaesth. 1999;46:66–70.
32. Hare GM, Ngan JC. Density determination of local anaesthetic opioid mixtures for spinal anaesthesia. Can J Anaesth. 1998;45:341–6.
33. Patterson L, Avery N, Chan P, et al. The addition of fentanyl does not alter the extent of spread of intrathecal isobaric bupivacaine in clinical practice. Can J Anaesth. 2001;48:768–72.
34. Imbelloni LE, Moreira AD, Gaspar FC, et al. Assessment of the densities of local anesthetics and their combination with adjuvants. An experimental study. Rev Bras Anestesiol. 2009;59:154–65.
35. Nicol ME, Holdcroft A. Density of intrathecal agents. Br J Anaesth. 1992;68:60–3.
36. Faust A, Fournier R, Van Gessel E, et al. Isobaric versus hypobaric spinal bupivacaine for total hip arthroplasty in the lateral position. Anesth Analg. 2003;97:589–94.
37. Heller AR, Zimmermann K, Seele K, et al. Modifying the baricity of local anesthetics for spinal anesthesia by temperature adjustment model calculations. Anesthesiology. 2006;105:346–53.
38. Srivastava U, Kumar A, Saxena S, et al. Spina anaesthesia with lignocane and fentanyl. Indian J Anaesth. 2004;48:121–3.
39. Ben-David B, Solomon E, Levin H, et al. Intrathecal fentanyl with small dose dilute bupivacaine: better anesthesia without prolonging recovery. Anesth Analg. 1997;85:560–5.
40. Fukushima S, Takenami T, Yagishita S, et al. Neurotoxicity of intrathecally administered fentanyl in a rat spinal model. Pain Med. 2011;12:717–25.
41. Pires SRO, Ganem EM, Maqus M, et al. Effects of increasing sinal hyperbaric lidocaine concentrations on spinal cord and meninges. Experimental study in dogs. Rev Bras Anestesiol. 2006;56:253–62.