



REVISTA BRASILEIRA DE ANESTESIOLOGIA

Publicação Oficial da Sociedade Brasileira de Anestesiologia
www.sba.com.br



SPECIAL ARTICLE

Perineural dexmedetomidine effects on sciatic nerve in rat



Abdulkadir Yektaş^{a,*}, Murat Çabalar^b, Mehmet Sar^b, Ayşin Alagöl^c,
Duygu Sultan Çelik^c, Vildan Yayla^b, Deniz Tolga^c

^a Bağcılar Training and Research Hospital, Istanbul, Turkey

^b Bakırköy Dr. Sadıkonuk eğitim ve araştırma hastanesi, İstanbul, Turkey

^c Bağcılar Eğitim ve Arastirma Hastanesi, Istanbul, Turkey

Received 20 May 2015; accepted 17 August 2015

Available online 28 April 2016

KEYWORDS

Paw withdrawal
latency testing;
Analgesia meter;
Sciatic nerve;
Electromyography;
Dexmedetomidine

Abstract The present study was designed to test the hypothesis that high dose dexmedetomidine would increase the duration of antinociception to a thermal stimulus in a rat model of sciatic nerve blockade without causing nerve damage. The rats were anesthetized with isoflurane. After electromyography (EMG) recordings, right sciatic nerves were explored and perineural injections were delivered: Group D ($n=7$), $40\ \mu\text{g}\ \mu\text{g}\ \text{kg}^{-1}$ dexmedetomidine administration, Group II ($n=6$), (0.2 mL) saline administration, Group III ($n=2$), only surgically exploration of the right sciatic nerve. Time to paw withdrawal latency (PAW) to a thermal stimulus for both paws and an assessment of motor function were measured every 30 min after the nerve block until a return to baseline. The compound muscle action potential (CMAP) of right and left sciatic nerves were recorded 10 times per each nerve once more after perineural injections at 14 day. After EMG recordings, right and the part of left sciatic nerve were excised at a length of at minimum 15 mm for histopathological examination. Comparison of right/left CMAP amplitude ratios before and 14 days after the procedure showed a statistically significant difference ($p=0.000$). There were no differences in perineural inflammation between the Group D, Group S, and Group E at 14 days.

© 2016 Published by Elsevier Editora Ltda. on behalf of Sociedade Brasileira de Anestesiologia. This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

PALAVRAS-CHAVE

Testes de latência de
retirada da pata;
Medidor de analgesia;
Nervo ciático;

Efeitos de dexmedetomidina perineural no nervo ciático em ratos

Resumo O presente estudo foi desenvolvido para testar a hipótese de que dexmedetomidina em dose alta aumentaria a duração da antinociceção a um estímulo térmico em modelo de rato de bloqueio do nervo ciático sem causar danos ao nervo. Os ratos foram anestesiados com

* Corresponding author.

E-mail: akyektas722000@yahoo.co.uk (A. Yektaş).

Eletromiografia; Dexmedetomidina

isoflurano. Após os registros da eletromiografia (EMG), os nervos ciáticos direitos foram explorados e injeções perineurais foram administradas: Grupo D ($n=7$) recebeu $40 \mu\text{g}/\text{kg}^{-1}$ de dexmedetomidina, Grupo II ($n=6$) recebeu 0,2 mL de solução salina, Grupo III ($n=2$) recebeu apenas exploração cirúrgica do nervo ciático direito. O tempo de latência de retirada pata (LRP) a um estímulo térmico para ambas as patas e uma avaliação da função motora foram avaliados a cada 30 minutos após o bloqueio do nervo até o retorno à fase basal. O potencial de ação muscular composto (PAMC) dos nervos ciático direito e esquerdo foi registrado 10 vezes para cada nervo, mais uma vez, após as injeções perineurais no 14º dia. Após os registros da EMG, o nervo ciático direito e parte do esquerdo foram excisados com um comprimento de no mínimo 15 mm para exame histopatológico. A comparação das proporções da amplitude do PAMC direito/esquerdo antes e 14 dias após o procedimento mostrou uma diferença estatisticamente significativa ($p=0,000$). Não houve diferenças em inflamação perineural entre os grupos D, S e E aos 14 dias.

© 2016 Publicado por Elsevier Editora Ltda. em nome de Sociedade Brasileira de Anestesiologia. Este é um artigo Open Access sob uma licença CC BY-NC-ND (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

Introduction

Peripheral nerve blocks are frequently used in surgical procedures targeted at post-operative pain and surgical anesthesia. Long-acting local anesthetics may also provide analgesia per se for 9–14 h.^{1–4} If the block is performed in the morning and afternoon, patients generally report post-operative pain at night hours. The need for opioid cause's opioid-related potential side effects and suppression of healing sleep.⁵ Using opioids results in potential airway obstruction, which causes saturation to decrease.^{6–8} Ideally, single-time peripheral nerve block should provide analgesia during the first post-operative night.

Interventions targeted increasing the duration of block in order to heal post-operative pain with many additional local anesthetics are under investigation. The efficacy of clonidine has been proven in many regional anesthetic techniques.⁹ However, long-acting local anesthetics have produced results that are not very impressive.^{10,11} In some studies, no beneficial effects of adding clonidine to long-acting anesthetics have been found.¹¹

Dexmedetomidine is a selective alpha-adrenoceptor agonist. A previously performed study showed that dexmedetomidine extends the sensorial and motor block time when combined with bupivacaine in rat models with sciatic nerve block.¹²

Studies indicated that local anesthetics result in myonecrosis; however, it is believed that the damage may not be clinically important since the muscles are regenerated in a normal way.^{13–16} Local anesthetic doses are generally reliable in healthy human beings; however, there may really be neurotoxicity present in patients with sub-clinical neuropathy diabetes or multiple sclerosis.^{17–19} An increase in inflammatory mediators was identified following the administration of perineural clonidine.^{20–25} A study reported a significant decrease in perineural inflammation at 24 h with the addition of dexmedetomidine to bupivacaine as compared with the administration of bupivacaine alone. The same study found that the perineural inflammation values at 24 h were higher as compared with the bupivacaine-only saline control group. The perineural inflammation

values of the group that received both bupivacaine and dexmedetomidine and the group that received only dexmedetomidine were similar to those of the saline group.²⁶

As discussed in previous studies, it is believed that decreased perineural inflammation with the use of dexmedetomidine is due to decreased proinflammatory products of healing immune cells and increased anti-inflammatory cytokines at the wound site.^{20–25} It is very important to determine the functional statuses of nerves. One of the functional evaluation methods for neural healing or transmission disorder is to perform electrophysiological measurements. Electromyography is a common procedure in clinical and fundamental studies in the context of *in vivo* and *in vitro* functional nerve studies. It has a widespread use in the electro-diagnostic evaluation of peripheral nerve damage of the sciatic nerve in animal models. A study performed showed that the use of a silicone tube filled with hyaluronic acid following end-to-end repair of an incised nerve could have a positive effect on latency and CMAP, and consequently on axonal regeneration.²⁷ CMAP is a valuable parameter that generally shows the time aspect of nerve regeneration and reinnervation.²⁸ A study demonstrated that stimulation single fiber electromyography (SSFEMG) was a more sensitive electrophysiological method in the detection of neuromuscular transmission block that occurs in rats with weak muscles and acute organophosphate poisoning.²⁹ These electromyographic studies showed that both nerve healing and nerve damage could be shown using electromyographic methods. In our study, we determined the effects of dexmedetomidine injection on neural transmission by using electromyographic methods.

We conducted this study in order to assess the effects of dexmedetomidine on sciatic nerves of rats through analgesimetry, histopathology and electromyography.

Method

This study was conducted at the Animal Experiments Laboratory of Bağcılar Teaching and Research Hospital upon ethics committee approval obtained during the 19th project

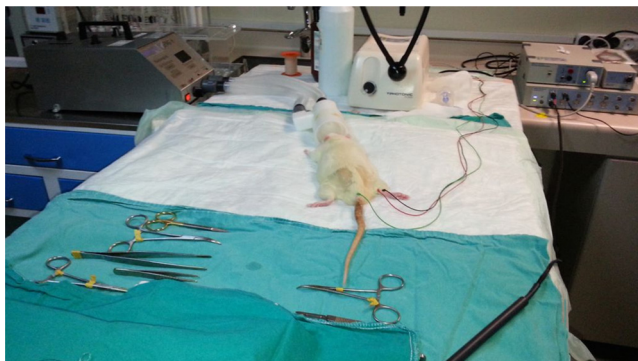


Figure 1 Induction room (Plexx Model: HNG6).

meeting issue 2012/60, number HADYEK/2012-13 held on 27.02.2012 by the Animal Experiments Local Ethics Committee of Bağcılar Teaching and Research Hospital, Bakırköy Secretariat General of the Association of Public Hospitals, Ministry of Health of the Republic of Turkey. This study was conducted in accordance with the guideline for the use and care of laboratory animals and the guideline for use and care of mammals in behavioral studies conducted in the context of neurological sciences. During the study, 15 male rats of the Sprague Dawley species weighing 400–500 g were used.

Drug preparation: Precedex IV vials (Abbott, Dexmedetomidine hydrochloride 100 µg/mL) were diluted using physiological saline solution at a ratio of 50 µg/mL.

Subfascial sciatic nerve injection

After the rats were weighed, they were anesthetized with isoflurane 2.5% in the induction room (Plexx Model: HNG6) (Fig. 1), they were rested in right lateral decubitus position and isoflurane 2.5% anesthesia continued to be provided through a mask (Fig. 1). Lateral incision was performed on the leg to receive the injection. The superficial fascia was separated and the sciatic nerve was exposed at a point proximal to the bifurcation (Fig. 2). After the sciatic nerve exposure, dexmedetomidine 40 µg/kg was injected in perineural space in Group D ($n=7$) and physiological saline solution at a volume equal to 40 µg/kg dexmedetomidine was injected in Group S ($n=6$). 30G PPD injectors were used for injection. In Group E ($n=2$), only the sciatic nerve was exposed and it was closed again. The time of injection administration was recorded. To determine the site where nerve sample would be collected, the fascia of



Figure 2 The superficial fascia was separated and the sciatic nerve was exposed at a point proximal to the bifurcation.



Figure 3 PWL test.

biceps femoris muscle corresponding to the injection site was marked using non-absorbable sutures under the skin while working on the skin preparation. The sutures were not placed around the nerve and did not touch the nerve. After the injections were made, the incision folds were closed. The isoflurane anesthesia was interrupted and the time of anesthesia interruption was recorded; the rats were placed in their cages in a supine position. Then, the times when rats returned to prone position were recorded as resumption of righting reflex (RoRR).

Paw withdrawal latency (PWL) testing

After the rats returned to prone position, a plantar analgesia meter (Series 8 model 336T, IITC Life Science Inc., Woodland Hills, CA) was placed in the plantar analgesia meter chamber in order to perform the PWL test (Fig. 3). The source of light was used as a heater at analgesia meter. After the source of light was turned on and the temperature at the point to which the rat claw corresponded reached 30°C, a waiting time of 5 min was allowed, the rat claw was moved to a point corresponding there. This procedure was repeated for both the right and left paws.

The rats with no signs of neurobehavioral abnormality were kept in lit and dark environments for periods of 12 h from the beginning until the end of the experiment (between 6.00 and 18.00 h). Before surgery, 1 h of neurobehavioral monitoring per day was performed for 3 days. A thermal PAW test was conducted and the averages of these 3 day values were taken as basal values; this procedure was repeated for both right and left claws. After the rats were taken to surgery and injection was administered, a PWL measurement was taken once every 30 min consequently and this procedure was performed for both right and left claws. All the rats were monitored for 210 min in neurobehavioral terms. Again in this period, a motor assessment of the rear extremity was performed once every 30 min. The result was recorded as motor block being present (motor score=1), normal claw position or no motor blocks (motor block=0). Once the rats returned to their basal sensorial and motor values, they were sent back to their cages. Before removing the nerve of rats on the injection site, the PWL measurements of both claws were taken 5 times each every morning as of the morning after the injection until the nerve was removed. The daily PWL values were taken as the average of these measurements.

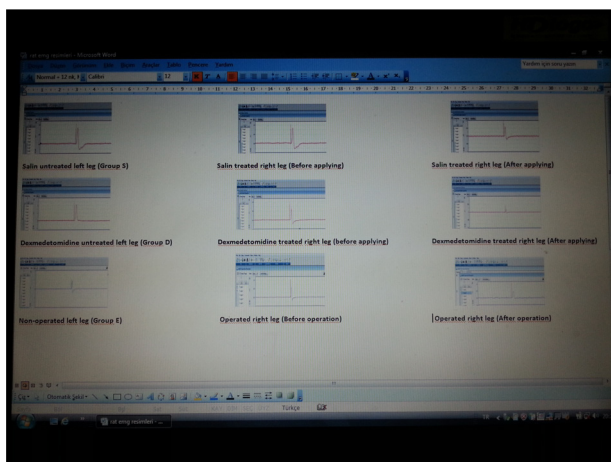


Figure 4 The peak to peak compound muscle action potential (CMAP) and distal motor latency of the right and left sciatic nerves.

Electrophysiology study

After the rats were anesthetized with isoflurane 2.5%, the tails of rats made to rest in supine position were fixed on the working platform. The needle electrode was placed in the gastrocnemius muscle and the sciatic nerve was stimulated 10 times with a surface electrode in a supramaxillary way at a suitable point proximal to the hip joint (Fig. 1). The peak to peak compound muscle action potential (CMAP) and distal motor latency of the right and left sciatic nerves were recorded, respectively (Fig. 4). For electrophysiological study, an electromyography system (Biopac systems, Inc., Model: MP150) was used and the software program LabChart 7 was used for the assessment of measurements. The same procedure was performed after administration of isoflurane anesthesia before injection to the sciatic nerve and on Day 14 after the injection into the sciatic nerve before collecting a sample from the sciatic nerve and the results were compared.

Histopathological study

On day 14 after the rats were anesthetized with isoflurane for the first electrophysiology study and drug injection into the sciatic nerve, the rats were anesthetized again with isoflurane and electrophysiology study was performed. The former incision site in the rear right extremity was opened, the marking suture was located and a sciatic nerve sample of 1.5 cm in length was collected from the corresponding site. The same procedure was repeated for the rear left extremity. The nerve samples were fixed for 3 days in glycer-aldehyde 2.5% and then embedded in paraffin blocks and cross-sections of 5 μm were prepared. These samples were stained with eosin and evaluated. The following were identified: perineural inflammation in the sciatic nerve (Figs. 5–8) (0 = no inflammation, 1 = slight edema and/or inflammation in small foci, 2 = moderate edema and/or inflammation in a locally wide area, 3 = moderate edema and/or inflammation in diffuse areas, local nerve damage findings) (0 = no lesions, 1 = damage in 1–2% of axons or myelins, 2 = damage in 2–5% of axons or myelins, 3 = damage in more than 5% of axons or

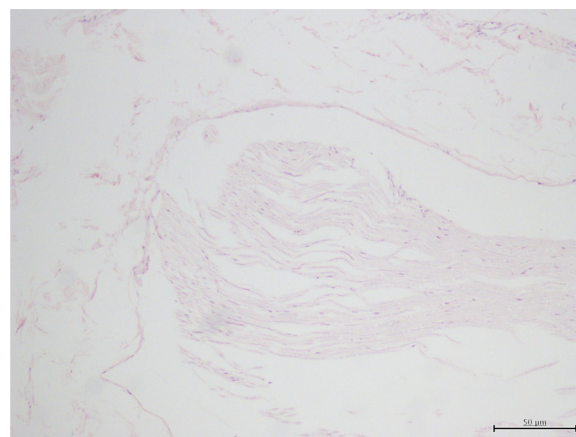


Figure 5 Microscopic images of the perineural inflammation level 0.

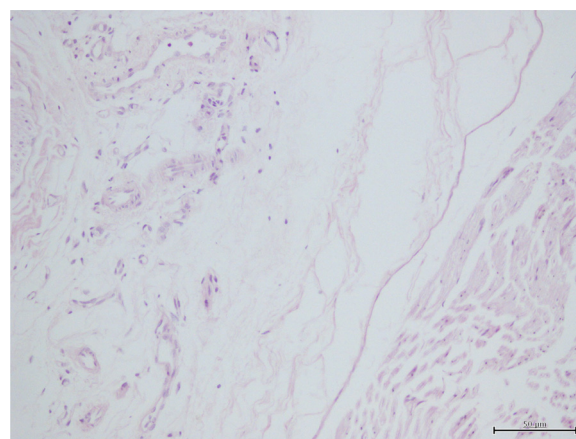


Figure 6 Microscopic images of the perineural inflammation level 1.

myelins). After the removal sciatic nerve, euthanasia was performed on rats using the cervical dislocation method.

Statistics

Statistical calculations were made using the software program SPSS 11.5 for Windows. Comparison of anesthesia

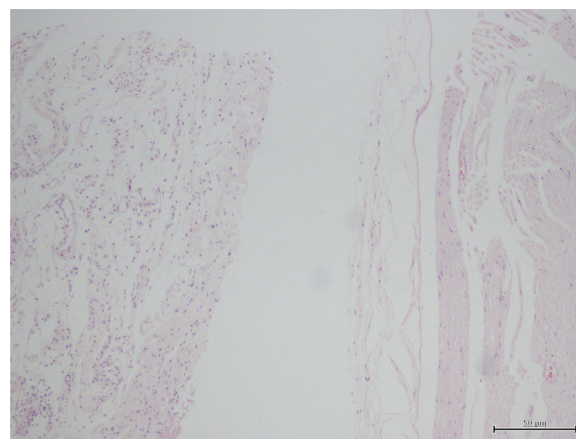


Figure 7 Microscopic images of the perineural inflammation level 2.

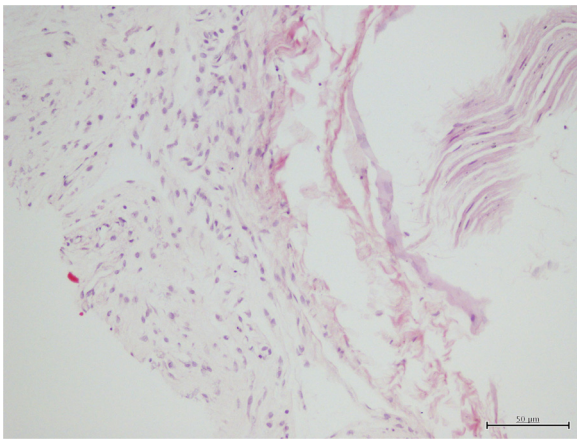


Figure 8 Microscopic images of the perineural inflammation level 3.

durations among groups, comparisons of RoRR values, histopathologically performed inflammation and local nerve damage values were made via the NPar tests Kruskal–Wallis test. In cases where there were statistically significant differences among groups, they were compared individually using the NPar tests Mann–Whitney *U* test. The rear and right left extremity basal PWL values and PWL values at 30, 60, 90, 120, 150, 180, 210min and on days 1–14 of each and every group were compared during NPar Tests Wilcoxon Signed Ranks test. The CMAP and Latency values were compared using the ANOVA test among all three groups. The comparison of electromyographic values before the drug administration and on Day 14 after the drug administration and the comparison of electromyographic values before and after drug administration among the groups were performed using the paired-*t* test. The value of $p < 0.05$ was considered statistically significant for all the data.

Results

When the durations of anesthesia provided while administering injections to rats were compared, no statistically significant differences were found ($p = 0.823$) (Fig. 9).

When the RoRR values of rats were compared as per the groups, statistically significant differences were found among groups ($p = 0.005$). When the groups were compared individually, it was seen that the RoRR values in Group D were statistically significantly different than the other two groups and the RoRR values were longer as compared to the two other groups (Group D–Group S: $p = 0.003$, Group D–Group E: $p = 0.040$) (Fig. 10).

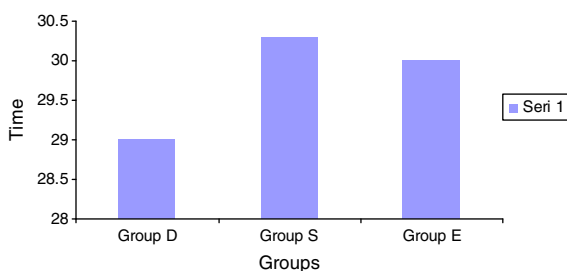


Figure 9 Comparison of duration of anesthesia in the groups.

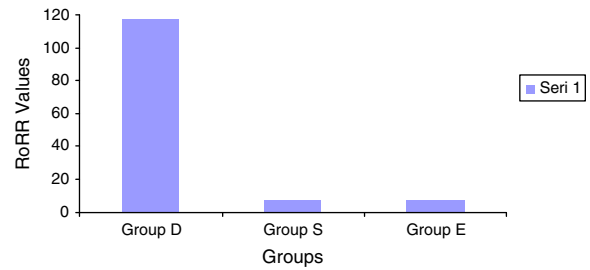


Figure 10 Comparison of RoRR in the groups.

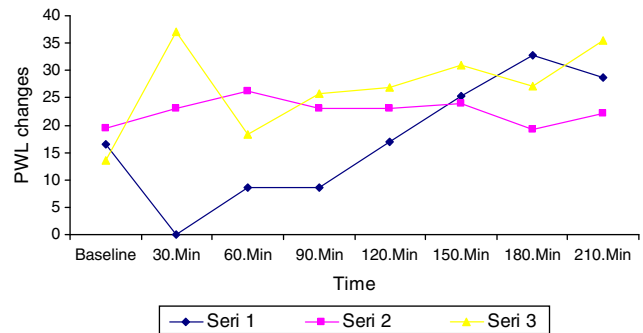


Figure 11 Time-PWL changes in the right rear limb in the groups (Seri 1, Group D; Seri 2, Group S; Seri 3, Group E).

In Group D

When the rear right extremity PWL values of the rats were compared with their basal values, it was seen that the PWL values were lower than the basal value in 30 min after drug administration and higher in 210 min. There were no statistically significant differences among values measured in 60, 90, 120, 150 and 180 min ($p = 0.018 - p = 0.237 - p = 0.237 - p = 0.735 - p = 0.499 - p = 0.091 - p = 0.028$) (Fig. 11).

When the rear left extremity PWL values of the rats were compared with their basal values, no statistically significant differences were observed in 60, 90, 120, 150, 180 and 210 min ($p = 0.044 - p = 0.138 - p = 0.067 - p = 0.54 - p = 0.128 - p = 0.098 - p = 0.056$) (Fig. 12).

When the rear right extremity PWL values of rats were compared with their basal values, no statistically significant differences were seen on Days 1, 8, 9 and 14 as compared to the basal values; however, the PWL values were statistically significantly longer on other days in comparison with the basal values (1 Day: $p = 0.735$; 2 Day: $p = 0.028$;

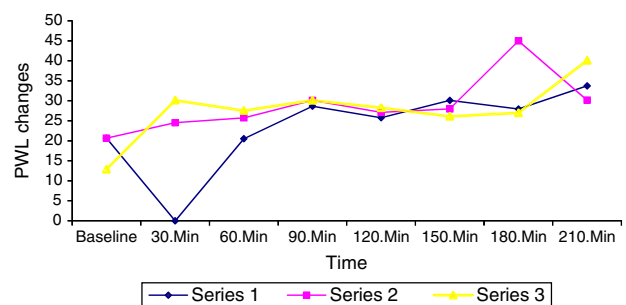


Figure 12 Time-PWL changes in the left rear limb in the groups (Seri 1, Group D; Seri 2, Group S; Seri 3, Group E).

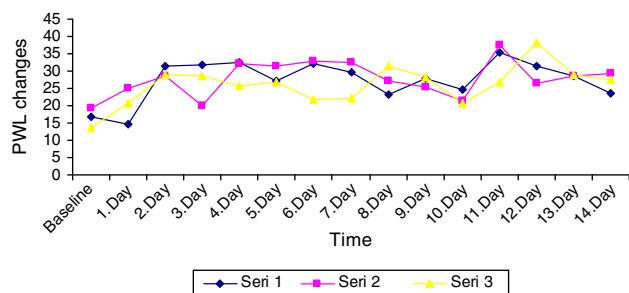


Figure 13 Time-PWL changes in the right rear limb in the groups after injection (Seri 1, Group D; Seri 2, Group S; Seri 3, Group E).

3 Day: $p=0.018$; 4 Day: $p=0.028$; 5 Day: $p=0.043$; 6 Day: $p=0.018$; 7 Day: $p=0.028$; 8 Day: $p=0.128$; 9 Day: $p=0.063$; 10 Day: $p=0.028$; 11 Day: $p=0.018$; 12 Day: $p=0.028$; 13 Day: $p=0.663$; 14 Day: $p=0.128$) (Fig. 13).

When the rear left extremity PWL values of rats were compared with their basal values, it was seen there was a statistically significant difference in terms of measurements taken on Days 4–6, 9–12 throughout 14 days and they were longer than the basal values (1 Day: $p=0.612$; 2 Day: $p=0.018$; 3 Day: $p=0.063$; 4 Day: $p=0.018$; 5 Day: $p=0.018$; 6 Day: $p=0.018$; 7 Day: $p=0.091$; 8 Day: $p=0.128$; 9 Day: $p=0.028$; 10 Day: $p=0.125$; 11 Day: $p=0.028$; 12 Day: $p=0.018$; 13 Day: $p=0.028$; 14 Day: $p=0.063$) (Fig. 14).

Motor block did not develop in any of the rats in Group D.

In Group S

When the rear right extremity PWL values of rats were compared with their basal values, no statistically significant differences were observed in 30, 60, 90, 120, 150, 180 and 210 min (Fig. 11).

When the rear left extremity PWL values of rats were compared with their basal values, no statistically significant differences were observed in 30, 60, 90, 120, 150, 180 and 210 min (Fig. 12).

When the rear right extremity PWL values of rats were compared with their basal values, there were significant differences on Days 4–7, 10, 11 and 14 and they were longer as compared to the basal values (1 Day: $p=0.345$; 2 Day: $p=0.116$; 3 Day: $p=0.600$; 4 Day: $p=0.046$; 5 Day: $p=0.046$; 6 Day: $p=0.028$; 7 Day: $p=0.028$; 8 Day: $p=0.075$;

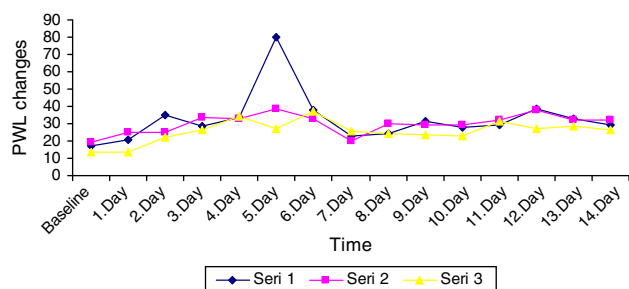


Figure 14 Time-PWL changes in the left rear limb in the groups after injection (Seri 1, Group D; Seri 2, Group S; Seri 3, Group E).

9 Day: $p=0.207$; 10 Day: $p=0.046$; 11 Day: $p=0.028$; 12 Day: $p=0.116$; 13 Day: $p=0.116$; 14 Day: $p=0.028$) (Fig. 13).

When the rear left extremity PWL values of the rats were compared with their basal values, there were statistically significant differences on Days 3–14 and they were longer than basal values (1 Day: $p=0.116$; 2 Day: $p=0.173$; 3 Day: $p=0.046$; 4 Day: $p=0.046$; 5 Day: $p=0.028$; 6 Day: $p=0.028$; 7 Day: $p=0.917$; 8 Day: $p=0.046$; 9 Day: $p=0.028$; 10 Day: $p=0.116$; 11 Day: $p=0.028$; 12 Day: $p=0.046$; 13 Day: $p=0.028$; 14 Day: $p=0.046$) (Fig. 14).

No rats in Group S developed motor block.

In Group E

When the rear right extremity PWL values of rats were compared with their basal values, no statistically significant differences were observed in 30, 60, 90, 120, 150, 180 and 210 min (Fig. 11).

When the rear left extremity PWL values of rats were compared with their basal values, no statistically significant differences were observed in 30, 60, 90, 120, 150, 180 and 210 min (Fig. 12).

When the rear right extremity PWL values of rats were compared with their basal values, no statistically significant differences were observed on Days 1–14 (Fig. 13).

When the rear left extremity PWL values of rats were compared with their basal values, no statistically significant differences were observed on Days 1–14 (Fig. 14).

No rats developed motor blocks in Group E.

When the right sciatic nerves of rats were histopathologically compared, no statistically significant differences were seen among groups with respect to inflammation and local nerve damage findings ($p=0.633 - p=0.867$).

When the left sciatic nerves of groups were histopathologically compared, no statistically significant differences were seen among groups in terms of inflammation and local nerve damage findings ($p=0.751 - p=0.668$).

When the right and left nerves in Group D were compared in terms of histopathological results, there were no statistically significant differences in terms of inflammation and local nerve damage ($p=0.891 - p=0.705$).

When the right and left sciatic nerves in Group S were compared in terms of histopathological results, there were no statistically significant differences in terms of inflammation and local nerve damage ($p=0.194 - p=0.317$).

When the right and left sciatic nerves in Group E were compared in terms of histopathological results, there were no statistically significant differences in terms of inflammation and local nerve damage ($p=1.000 - p=0.317$).

When the Group D, Group S and Group E were compared in terms of CMAP and latency values before and after the injection, there were statistically significant differences in terms of post-injection CMAP values ($p<0.001$) (Table 1). There were no statistically significant differences with respect to latency values ($p=0.618$) (Tables 1 and 2).

The post-injection CMAP measurements were statistically significantly different between Group D and Group S ($p=0.016$).

There were statistically significant differences between Group D and Group D with respect to post-injection CMAP measurements ($p=0.015$).

Table 1 CMAP amplitudes and latencies of right and left sciatic nerves before and 14 days after injection.

	CMAP amplitude mV	Latency ms
Before injection-R	0.40–1.98 0.8251 ± 0.3862	0.007–0.013 0.010 ± 0.001
Before injection-L	0.21–2.09 0.8729 ± 0.4716	0.008–0.014 0.011 ± 0.001
<i>p</i>	0.137	0.083
After injection-R	0.00–1.39 0.3285 ± 0.2640	0.006–0.031 0.013 ± 0.006
After injection-L	0.10–1.32 0.4294 ± 0.2786	0.007–0.017 0.012 ± 0.003
<i>p</i>	0.000	0.618

There was a statistically significant difference between and after injection R CMAP amplitude and after injection L CMAP amplitude, $p=0.000$.

When the post-injection CMAP measurements of Group S and Group E were compared, no statistically significant differences were found between them ($p=0.95$).

When the pre-injection and Latency values were compared among Group D, Group S and Group E, no statistically significant differences were found ($p=0.137 - p=0.083$) (Tables 1 and 2).

When the pre-injection and post-injection CMAP values of Group D were compared, there was a statistically significant difference ($p<0.001$). There were no statistically significant differences among the pre-injection and post-injection CMAP values of Group S and Group E ($p=0.126 - p=0.548$).

Discussion

This study is a placebo-controlled, randomized and blind study. It was demonstrated that high-dose dexmedetomidine caused sensorial block in sciatic nerves of rats and did not have any histopathologically toxic effects; however, their effects on neural transmission were not electromyographic studies.

It was found that dexmedetomidine inhibited paraventricular nucleus parvocellular neurons by means of I_h suppression hypothalamic nucleus neurons which are controlled by hyperpolarized-activated flows and in direct

contact with noradrenergic neurons are called I_h (H-flow) and rat hypothalamic nucleus neurons by means of the activation of G-protein pair, which internally regulates the K^+ flow.³⁰ In the *in vitro* study of rat dorsal ganglion neurons, it was found that the combination of both clonidine and dexmedetomidine with lidocaine produced an additive type of block interaction on tetrodotoxin-resistant Na flow.³¹

A previously conducted study showed that increased dose of dexmedetomidine added to ropivacaine resulted in a longer sensory motor blockage duration through thermal stimulation in rats.²⁶ The time of intensive sensorial block and time to return to normal sensorial functions increased in a dose-dependent manner with gradually increasing doses of dexmedetomidine.²⁶ Also, another study demonstrated that sensorial and motor block in the sciatic nerve blockage of rats showed a significant increase with high-dose dexmedetomidine added to bupivacaine.³² We did not use any local anesthetic drugs in our study. In Group D, we administered dexmedetomidine $40 \mu\text{g}/\text{kg}^{-1}$ only to the perineural space of the right sciatic nerve. In 30 min following drug administration in Group D, the right PWL values were statistically significantly lower than basal values and the right PWL values were statistically significantly higher than basal values in 210 min. The left PWL values were statistically significantly low in 30 min in comparison with basal values. The reason why the right and left PWL values in 30 min were lower than the basal values was that the RoRR reflex was not restored in any of the rats in the meantime, hence the PWL measurements could not be taken. When both right and left PWL values in Group S and Group E were compared with basal values, no statistically significant differences were seen. When the right PWL values on Days 1, 8, 9 and 14 were compared with basal values, no statistically significant differences were found. However, there were statistically significant differences in terms of the comparison of PWL values on the other days and they were longer than basal values. In Group D, there were statistically significant differences between left PWL values and basal values on Days 4–6, 9–12 and they were longer than basal values. In Group S, there were statistically significant differences between the right PWL values and basal values on Days 4–7, 10, 11 and 14 and they were longer than basal values. There were statistically significant differences between left PWL values and basal values on Days 3–6, 8–9, 11–14 and they were longer than basal values. In Group E, no statistically significant differences were found when the PWL values were compared with basal values on post-operative days in cases that underwent surgical opening and closure. A study conducted showed that the effect of dexmedetomidine was

Table 2 CMAP amplitude ratios of right to left sciatic nerves of the groups.

	Total	Group D	Group S	Group E
Before injection-R/L	0.37–2.26 1.0641 ± 0.3965 ^a			
After injection-R/L	0.13–3.15 0.8456 ± 0.5016	0.13–2.07 0.7297 ± 0.3608 ^{a,b}	0.27–3.15 0.9744 ± 0.6155 ^b	0.31–1.69 0.8064 ± 0.4018

Significant differences between Group D.

^a The whole group.

^b Group S.

at a significant degree only when it was added to bupivacaine and the central analgesic effects of dexmedetomidine were therefore rejected as the reason for the increased duration of sensorial block.³² Dexmedetomidine alone could produce neither sustainable motor block nor sensorial block at a significant degree.³² Clonidine failed to produce adequate analgesia when used as a single anesthetic in the human brachial plexus block.³³ Similarly, it was demonstrated that dexmedetomidine increased the sensorial and motor block duration and analgesia duration when added to bupivacaine for targeting the supraclavicular brachial plexus block.³⁴ The analgesia achieved in rats by dexmedetomidine added to ropivacaine for peripheral nerve block develops thanks to peripheral effects. In this study, the group of dexmedetomidine alone developed short and partial sensorial block.³⁵ Our study showed that dexmedetomidine resulted in PWL prolongation not only in the right sciatic nerve, where it was injected in perineural space, but also in the left sciatic nerve on specific days following drug administration. The same effect was also observed in Group S. This effect was not observed in Group E. For that reason, it would not be accurate to attribute these prolongations to dexmedetomidine; however, these effects may be attributed to interventions on the perineural space. More studies should be performed on that subject. However, the same PWL prolongations in Group D and Group S also took place in left sciatic nerves. As for the left sciatic nerves, no surgical interventions were made on them. This is one of the subjects that merits further investigation.

When high dose dexmedetomidine is added to bupivacaine for sciatic nerve block in rats, it does enhance the block.³⁶

When dexmedetomidine is intravenously administered, it generates sedation and analgesia without causing respiratory depression.^{37–40} It may change sensory perception through centrally effective analgesia and sedation.^{37–40} Contrary to the result of a previous study where all the rats received a bilateral sciatic nerve block with both bupivacaine alone and bupivacaine enhanced with dexmedetomidine,³⁶ the results of the study where a unilateral block was achieved showed that²⁶ the effects of dexmedetomidine were dominant at the peripheral nerve level.²⁶ Dexmedetomidine at a very high dose of $20 \mu\text{g}/\text{kg}^{-1}$ has very large, systemic effects that further prolong RoRR at a significant degree.²⁶ In our study, the duration of RoRR reflex in Group D was statistically significantly higher than the other groups.

Various *in vitro* and *in vivo* studies have been conducted in order to understand whether dexmedetomidine has toxic effects on nerve cells. Schoeler et al.⁴¹ exposed organotypic hippocampal slices to dexmedetomidine and create a focal, mechanical trauma in order to show that dexmedetomidine provided a neuron-protective effect in an *in vitro* traumatic brain model. Sanders et al.⁴² showed that dexmedetomidine prevented cortical apoptosis under *in vitro* and *in vivo* conditions. In another study that was conducted, the combination of bupivacaine and dexmedetomidine were compared with bupivacaine alone and it was associated with significantly less perineural inflammation at 24 h.³⁶

A study observed findings indicating that dexmedetomidine 5 or $10 \mu\text{g}$ injected into the epidural space in rabbits resulted in the demyelination of oligodendrocytes in the

white matter of the spinal cord.⁴³ However, it was reported that the neurotoxic effects of epidural dexmedetomidine could have been due to the injury of spinal cord vascular resources due to the pH effect or the direct effects of epidural catheter insertion.⁴³ In a study that was conducted,¹² it was surprising to see that the sciatic nerves did not affect myelins or axons when dexmedetomidine was used at a dose of $28\text{--}40 \mu\text{g}/\text{kg}^{-1}$; however, it had caused a significant myelin damage when administered epidurally to rabbits at a dose of $6.25 \mu\text{g}/\text{kg}^{-1}$.⁴³ The perineural inflammation values were similar to the saline control group in the group receiving bupivacaine enhanced with dexmedetomidine and in the group receiving bupivacaine alone.¹² This situation was attributed to the capacity of alpha-2 agonists to reduce inflammatory response.^{20,22,21,23,44,25,45} In the study that we carried out, there were no statistically significant differences in all three groups with respect to histopathological findings of inflammation and local nerve damage in the perineural space of the right and left sciatic nerve cross-sections.

We have not encountered any studies in the literature where electromyographic values were compared before and after the injection of dexmedetomidine in the sciatic nerve. The assessment of latency is an indication of the functional axons, number and quality of valid axons and therefore the functional status of motor neurons that are studied.⁴⁶ There were no statistically significant differences in any of the groups in terms of pre-injection and post-injection Latency values in our study. In our study, the post-injection CMAP values in Group D were statistically significantly different as compared to the other groups and they had decreased following injection. In Group D, the pre-injection and post-injection CMAP values were also statistically significantly different and decreased following the injection. With CMAP, 3 values are recorded on the basis of the muscle that is studied: 1 – size of the motor unit innervated by axons, 2 – size of the motor nerve fibers that respond to the stimulus and 3 – synchronization of their responses.⁴⁷

In conclusion, the injection of perineural dexmedetomidine to the sciatic nerve in rats prolonged the PWL value in 210 min and prolonged the post-injection PWL values on Days 2–7, 10–13. The duration of RoRR reflex had been prolonged at a statistically significant level in rats that received perineural dexmedetomidine injection. Perineural injection of dexmedetomidine into the sciatic nerve did not result in a statistically significant perineural inflammation increase or local nerve damage; however, the CMAP values on Day 14 following the perineural injection of dexmedetomidine into the sciatic nerve were found to be statistically significantly lower.

Conflicts of interest

The authors declare no conflicts of interest.

References

1. Casati A, Fanelli G, Albertin A, et al. Interscalene brachial plexus anesthesia with either 0.5% ropivacaine or 0.5% bupivacaine. *Minerva Anesthesiol.* 2000;66:39–44 [PubMed: 10736981].

2. Hickey R, Hoffman J, Ramamurthy S. A comparison of ropivacaine 0.5% and bupivacaine 0.5% for brachial plexus block. *Anesthesiology*. 1991;74:639–42 [PubMed: 2008942].
3. Hickey R, Rowley CL, Candido KD, et al. A comparative study of 0.25% ropivacaine and 0.25% bupivacaine for brachial plexus block. *Anesth Analg*. 1992;75:602–6 [PubMed: 1530173].
4. Vaghadia H, Chan V, Ganapathy S, et al. A multicentre trial of ropivacaine 7.5 mg × ml⁻¹ vs bupivacaine 5 mg × ml⁻¹ for supra clavicular brachial plexus anesthesia. *Can J Anaesth*. 1999;46:946–51 [PubMed: 10522581].
5. Lydic R, Baghdoyan HA. Neurochemical mechanisms mediating opioid-induced REM sleep disruption. In: Lavigne G, Sessle BJ, Choinire M, Soja PJ, editors. *Sleep and pain*. Seattle: International Association for the Study of Pain (IASP) Press; 2007. p. 99–122.
6. Bonafide CP, Aucutt-Walter N, Divittore N, et al. Remifentanyl inhibits rapid eye movement sleep but not the nocturnal melatonin surge in humans. *Anesthesiology*. 2008;108:627–33 [PubMed: 18362594].
7. Bowdle TA. Nocturnal arterial oxygen desaturation and episodic airway obstruction after ambulatory surgery. *Anesth Analg*. 2004;99:70–6 [PubMed: 15281506].
8. Rosenberg J, Rosenberg-Adamsen S, Kehlet H. Post-operative sleep disturbance: causes, factors and effects on outcome. *Eur J Anaesthesiol*. 1995;10:28–30 [PubMed: 7641640].
9. Eisenach JC, De Kock M, Klimscha W. Alpha(2)-adrenergic agonists for regional anesthesia. A clinical review of clonidine (1984–1995). *Anesthesiology*. 1996;85:655–74 [PubMed: 8853097].
10. Murphy DB, McCartney CJ, Chan VW. Novel analgesic adjuncts for brachial plexus block: a systematic review. *Anesth Analg*. 2000;90:1122–8 [PubMed: 10781465].
11. McCartney CJ, Duggan E, Apatu E. Should we add clonidine to local anesthetic for peripheral nerve blockade? A qualitative systematic review of the literature. *Reg Anesth Pain Med*. 2007;32:330–8 [PubMed: 17720118].
12. Brummett CM, Norat MA, Palmisano JM, et al. Perineural administration of dexmedetomidine in combination with bupivacaine enhances sensory and motor blockade in sciatic nerve block without inducing neurotoxicity in rat. *Anesthesiology*. 2008;109:502–11 [PubMed: 18719449].
13. Benoit PW, Belt WD. Destruction and regeneration of skeletal muscle after treatment with a local anaesthetic, bupivacaine (Marcaine). *J Anat*. 1970;107:547–56 [PubMed: 5492943].
14. Yagiela JA, Benoit PW, Buoncristiani RD, et al. Comparison of myotoxic effects of lidocaine with epinephrine in rats and humans. *Anesth Analg*. 1981;60:471–80 [PubMed: 7195662].
15. Zink W, Graf BM. Local anesthetic myotoxicity. *Reg Anesth Pain Med*. 2004;29:333–40 [PubMed: 15305253].
16. Zink W, Seif C, Bohl JR, et al. The acute myotoxic effects of bupivacaine and ropivacaine after continuous peripheral nerve blockades. *Anesth Analg*. 2003;97:1173–9 [PubMed: 14500177].
17. Hebl JR. Ultrasound-guided regional anesthesia and the prevention of neurologic injury: fact or fiction? *Anesthesiology*. 2008;108:186–8 [PubMed: 18212562].
18. Koff MD, Cohen JA, McIntyre JJ, et al. Severe brachial plexopathy after an ultrasound-guided single-injection nerve block for total shoulder arthroplasty in a patient with multiple sclerosis. *Anesthesiology*. 2008;108:325–8 [PubMed: 18212578].
19. Horlocker TT, O'Driscoll SW, Dinapoli RP. Recurring brachial plexus neuropathy in a diabetic patient after shoulder surgery and continuous interscalene block. *Anesth Analg*. 2000;91:688–90 [PubMed: 10960400].
20. Lavand'homme PM, Eisenach JC. Perioperative administration of the alpha2-adrenoceptor agonist clonidine at the site of nerve injury reduces the development of mechanical hypersensitivity and modulates local cytokine expression. *Pain*. 2003;105:247–54 [PubMed: 14499442].
21. Lavand'homme PM, Ma W, De Kock M, et al. Perineural alpha(2A)-adrenoceptor activation inhibits spinal cord neuroplasticity and tactile allodynia after nerve injury. *Anesthesiology*. 2002;97:972–80 [PubMed: 12357167].
22. Liu B, Eisenach JC. Perineural clonidine reduces p38 mitogen-activated protein kinase activation in sensory neurons. *Neuroreport*. 2006;17:1313–7 [PubMed: 16951576].
23. Romero-Sandoval A, Eisenach JC. Perineural clonidine reduces mechanical hypersensitivity and cytokine production in established nerve injury. *Anesthesiology*. 2006;104:351–5 [PubMed: 16436856].
24. Romero-Sandoval A, Eisenach JC. Clonidine reduces hypersensitivity and alters the balance of pro and anti-inflammatory leukocytes after local injection at the site of inflammatory neuritis. *Brain Behav Immun*. 2007;21:569–80 [PubMed: 17079113].
25. Romero-Sandoval EA, McCall C, Eisenach JC. Alpha2-adrenoceptor stimulation transforms immune responses in neuritis and blocks neuritis-induced pain. *J Neurosci*. 2005;25:8988–94 [PubMed: 16192389].
26. Brummett CM, Norat MA, Palmisano JM, et al. Perineural administration of dexmedetomidine in combination with bupivacaine enhances sensory and motor blockade in sciatic nerve block without inducing neurotoxicity in the rat. *Anesthesiology*. 2008;109:502–11.
27. Huseynoğlu N, Ozaydın I, Yayla S, et al. Electrophysiological assessment of the effects of silicone tubes and hyaluronic acid on nerve regeneration in rats with sciatic neurotomy. *Kafkas Univ Vet Fak Derg*. 2012;18:917–22.
28. Wang H, Sorenson EJ, Spinner RJ, et al. Electrophysiologic findings and grip strength after nerve injuries in the rat forelimb. *Muscle Nerve*. 2008;38:1254–65.
29. Dongren Y, Tao L, Fengsheng H. Electroneurophysiological studies in rats of acute dimethoate poisoning. *Toxicol Lett*. 1999;30:249–54.
30. Shirasaka T, Kannan H, Takasaki M. Activation of a G protein-coupled inwardly rectifying K⁺ current and suppression of Ih contribute to dexmedetomidine-induced inhibition of rat hypothalamic paraventricular nucleus neurons. *Anesthesiology*. 2007;107:605–15 [PubMed: 17893457].
31. Oda A, Iida H, Tanahashi S, et al. Effects of alpha2-adrenoceptor agonists on tetrodotoxin-resistant Na⁺ channels in rat dorsal root ganglion neurons. *Eur J Anaesthesiol*. 2007;24:934–41 [PubMed: 17568475].
32. Brummett CM, Padda AK, Amodeo FS, et al. Perineural dexmedetomidine added to ropivacaine causes a dose-dependent increase in the duration of thermal antinociception in sciatic nerve block in rat. *Anesthesiology*. 2009;111:1111–9.
33. Sia S, Lepri A. Clonidine administered as an axillary block does not affect postoperative pain when given as the sole analgesic. *Anesth Analg*. 1999;88:1109–12 [PubMed: 10320179].
34. Swami SS, Keniya VM, Ladi SD, et al. Comparison of dexmedetomidine and clonidine (α2 agonist drugs) as an adjuvant to local anaesthesia in supraclavicular brachial plexus block: a randomised double-blind prospective study. *Indian J Anaesth*. 2012;56:243–9.
35. Brummett CM, Amodeo FS, Janda AM, et al. Perineural dexmedetomidine provides an increased duration of analgesia to a thermal stimulus when compared with a systemic control in a rat sciatic nerve block. *Reg Anesth Pain Med*. 2010;35:427–31.
36. Iskandar H, Guillaume E, Dixmieras F, et al. The enhancement of sensory blockade by clonidine selectively added to mepivacaine after midhumeral block. *Anesth Analg*. 2001;93:771–5 [PubMed: 11524354].

37. Hung YC, Kau YC, Zizza AM, et al. Ephedrine blocks rat sciatic nerve in vivo and sodium channels in vitro. *Anesthesiology*. 2005;103:1246–52 [PubMed: 16306739].
38. Kau YC, Hung YC, Zizza AM, et al. Efficacy of lidocaine or bupivacaine combined with ephedrine in rat sciatic nerve block. *Reg Anesth Pain Med*. 2006;31:14–8 [PubMed: 16418019].
39. Dyhre H, Soderberg L, Bjorkman S, et al. Local anesthetics in lipid-depot formulations – neurotoxicity in relation to duration of effect in a rat model. *Reg Anesth Pain Med*. 2006;31:401–8 [PubMed: 16952810].
40. Soderberg L, Dyhre H, Roth B, et al. Ultralong peripheral nerve block by lidocaine:prilocaine 1:1 mixture in a lipid depot formulation: comparison of in vitro, in vivo, and effect kinetics. *Anesthesiology*. 2006;104:110–21 [PubMed: 16394697].
41. Schoeler M, Loetscher PD, Rossaint R, et al. Dexmedetomidine is neuroprotective in an in vitro model for traumatic brain injury. *BMC Neurol*. 2012;11:12–20.
42. Sanders RD, Sun P, Patel S, et al. Dexmedetomidine provides cortical neuroprotection: impact on anaesthetic-induced neuroapoptosis in the rat developing brain. *Acta Anaesthesiol Scand*. 2010;54:710–6.
43. Konakci S, Adanir T, Yilmaz G, et al. The efficacy and neurotoxicity of dexmedetomidine administered via the epidural route. *Eur J Anaesthesiol*. 2008;25:403–9.
44. Romero-Sandoval A, Eisenach JC. Clonidine reduces hypersensitivity and alters the balance of proand anti-inflammatory leukocytes after local injection at the site of inflammatory neuritis. *Brain Behav Immun*. 2007;21:569–80 [PubMed: 17079113].
45. Ilfeld BM, Enneking FK. Continuous peripheral nerve blocks at home: a review. *Anesth Analg*. 2005;100:1822–33 [PubMed: 15920221].
46. Korte N, Schenk HC, Grothe C, et al. Evaluation of periodic electrodiagnostic measurements to monitor motor recovery after different peripheral nerve lesions in the rat. *Muscle Nerve*. 2011;44:63–73.
47. Wolthers M, Moldovan M, Binderup T, et al. Comparative electrophysiological, functional, and histological studies of nerve lesions in rats. *Microsurgery*. 2005;25:508–19.