



Effects of Adding Dexmedetomidine to Amitriptyline on Sciatic Nerve Blockage in Rats

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Abstract

Neurotoxic effects of amitriptyline which is a tricyclic antidepressant limit its use as a local anesthetic. In this study, comparing the effect of combination of different amitriptyline doses with dexmedetomidine on block initiation and termination time in sciatic nerve blockage with amitriptyline and bupivacaine alone was aimed. A total of 42 male Sprague-Dawley rats were divided into groups with 7 rats in each. These groups were randomly divided into 6 groups as amitriptyline 0.625 mg (Group 1), amitriptyline 0.312 mg (Group 2), dexmedetomidine 10 µg (Group 3), amitriptyline 0.625 mg and dexmedetomidine 10 µg (Group 4), amitriptyline 0.312 mg and dexmedetomidine 10 µg (Group 5), bupivacaine 0.5% (Group 6). In the right arm with posterior approach, 0.2 mL of local anesthetic was injected with lateral incision. Effectiveness of local anesthetic was determined in terms of motor function, proprioceptive, nociceptive sensation. In our study, amitriptyline and combination of amitriptyline-dexmedetomidine showed longer local anesthetic effect compared to bupivacaine. Mean block initiation times were shorter in bupivacaine group compared to amitriptyline group though it was not statistically significant. Adding 10 µg of dexmedetomidine to 0.625 mg amitriptyline prolonged block termination times significantly compared to 0.625 mg amitriptyline alone ($p < 0.05$). In this study, combination of amitriptyline and dexmedetomidine was shown to prolong block time in peripheral nerve blockage and additive interaction generated in increased doses was shown to be able to be more. Adding dexmedetomidine was detected to delay block initiation times.

Key Words: Sciatic nerve blockage, amitriptyline, dexmedetomidine, bupivacaine

(Rec.Date: Apr 01, 2013

Accept Date: Apr 17, 2013)

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Introduction

Regional anesthesia reduces the postoperative recovery time and duration of hospital stay associated with anesthesia, and decreases the analgesic requirement in the early postoperative period [1]. Sciatic nerve block is a regional anesthesia technique providing good muscle relaxation and pain control with minimal risk of local anesthetic toxicity for elective or ambulatory knee and foot surgeries. With the advances in algology, the use of peripheral nerve block for analgesic purposes has increased. This has led to an increased need for long-acting agents with low neurotoxic effects. In addition to amide and ester local anesthetics, opioids (fentanyl, sufentanyl), benzodiazepines (midazolam) and intravenous anesthetics (S-ketamine) are used for regional anesthesia individually or in different combinations [2].

The frequent use of regional anesthesia techniques has led to the search of new drugs that prolong the duration of block and have no neurotoxic effects. Amitriptyline, a tricyclic antidepressant drug, is a commonly used anxiolytic and antidepressant agent. In addition to these actions of amitriptyline, recent studies have suggested that amitriptyline, when used as a local anesthetic agent, is more potent and provides a longer duration of block than bupivacaine [3,4]. The use of amitriptyline in high concentrations prolongs the duration of action in relation to the increases in doses; however, this also increases the severity of neurotoxic effects [5]. Macroscopic manifestations for neurotoxic effects include color change in the nerve fiber, edema, hypervascularization of the epineural tissue, and microscopic manifestation includes variable degrees of Wallerian degeneration in a dose-dependent manner [3]. Amitriptyline may also cause chemical meningitis in addition to axonal degeneration when administered intrathecally [6]. Such neurotoxic effects limit the use of amitriptyline as a local anesthetic agent. The use of adjuvant agents is an alternative option in order to prolong the duration of block. Dexmedetomidine is a highly selective α_2 -adrenoceptor agonist and is commonly used as an intravenous sedative and a co-analgesic agent. There are animal studies that have established the analgesic effects of dexmedetomidine when used as an adjuvant to local anesthetics during regional anesthesia [7].

In regional anesthesia, amitriptyline in low doses is associated with lesser neurotoxic effects. However, short duration of action at low doses limits its use in clinical practice. In the present

study, we aimed to determine the effects of dexmedetomidine added to amitriptyline on the time to onset and resolution of motor and sensory block.

Materials and Methods

Amitriptyline (amitriptyline hydrochloride, Saroten[®], 50 mg/2 ml, Bayer, Sweden), dexmedetomidine (dexmedetomidine hydrochloride, Precedex[®], 200 µg/2 ml, Abbott, Turkey) and bupivacaine (bupivacaine hydrochloride, Marcaine[®] 0.5%, Astra Zeneca, Turkey) were purchased from the pharmacy.

The present study was carried out in Experimental Animal Research Laboratory of Inonu University, after obtaining approval from Experimental Animal Ethics Committee of Medical Faculty of Inonu University. Totally 42 male Sprague-Dawley rats were used in the present study. Rats were randomly divided into 6 groups, each containing 7 rats, as follows: Group 1, amitriptyline 0.625 mg; Group 2, amitriptyline 0.312 mg; Group 3, dexmedetomidine 10 µg; Group 4, amitriptyline 0.625 mg and dexmedetomidine 10 µg; Group 5, amitriptyline 0.312 mg and dexmedetomidine 10 µg; and Group 6, 0.5% bupivacaine. The doses to be administered were prepared by diluting amitriptyline and dexmedetomidine with 0.9% sodium chloride. Bupivacaine was used without dilution. Prior to the experiment, standard 0.2 mL local anesthetic solution was drawn into a syringe with 26-gauge needle for each rat and labeled. In the present study, 42 male Sprague-Dawley rats weighing 250 to 300 g were used. Rats were maintained on a 12 h light-12 h dark cycle 24 hours before the experiment, and their diet was not changed.

Establishment of the experimental model

The rats were closely followed-up daily for 15 days before the experiment, in order to familiarize the rats with the investigator, who performed neurological and behavioral assessments, and with the experimental environment, as well as the experimental methods such as neurological assessment. With this familiarization period, we aimed at minimizing stress contamination during the experiment and enhancing the performance of the experiment. For an accurate neurological assessment, we focused on the hind limb of the rats. Inclusion criteria for rats were as follows: the absence of certain behaviors such as biting, escaping and vocalization toward the investigator and the experimental tools; the presence of free behaviors not affecting neurological assessment; and reproducibility of results in repeated neurological assessments.

Surgical procedures of the sciatic nerve blockage

During the study period, one group of rats was studied each day. After providing a suitable environment, rats were anesthetized with ether, and using posterior approach, trochanter major and ischial tuberositas of the right leg were marked. Lateral incision was made between the marked points through the skin and subcutaneous tissue; superficial fascia and muscles were retracted. Pre-prepared 0.2 mL local anesthetic solution was injected beneath the fascia surrounding the nerve, without any damage to epineurium and perineurium, proximal to the sciatic nerve bifurcation. The superficial muscle layer was approximated and sutured with 4/0 silk. Skin was closed with metal clips. Mask anesthesia was discontinued at the end of the surgical procedure.

Measurement of blockage times

After the rats awakened from anesthesia, duration of action of the local anesthetics was evaluated using sensory and motor tests. Each rat in each group was assessed in every 2 minutes for the first 30 minutes, in every 10 minutes for the following 30 minutes, and in every 15 minutes after the first hour until the complete resolution of sensory and motor block. At the end of the study, the rats were sacrificed by decapitation under ether anesthesia. Motor function was evaluated based on the rats' ability to place weight on its hind limbs, to hop, and to grasp the cage with their paws when suspended by the tail, as well as walking ability [3,8].

The evaluation of proprioceptive sensory function was based on the resting posture and postural reaction ("tactile placing" and "hopping" responses). For tactile placing response, the rats were kept in a normal resting posture, and the hind paw toes were flexed with their dorsi placed on the table; then, the rat's ability to reposition the toes was evaluated. To evaluate the hopping response, the front half of the rats was held up. At the same time, the body of the rats was moved laterally by lifting one hind leg off the ground. During this process, rats normally hop with the weight-bearing limb in the direction of movement to avoid falling over. A predominant motor block would cause a prompt response immediately after lateral movement; however, the response is weaker than normal hopping response. In contrast, a predominant proprioceptive block would cause delayed hopping response, and a greater magnitude of passive lateral movement is required to elicit a hopping response [6,8].

Nociceptive response was evaluated by the withdrawal reflex. This reflex occurs by the contraction of flexor muscles of the hip, knees and ankles. The withdrawal reflex, which is a polysynaptic reflex, is elicited by applying painful stimulus to the extremities. The amplitude and duration of withdrawal reflex depends on the intensity of stimuli. In the present study, mechanical stimulation was used. Toothed forceps were used to apply pressure across a skin fold over the lateral metatarsus to evaluate the response to superficial pain and across the distal phalanx of the fifth toe to evaluate the response to deep tissue stimulation. A single painful stimulation was applied to the defined regions by the same investigator by exerting a force with the same intensity and duration [8].

The same order was followed to assess motor and sensory functions. First, proprioceptive tests were performed and this was followed by motor and nociceptive assessments in respective order. Motor and sensory assessments were performed by a different investigator from the one who performed sciatic nerve block injection. Contralateral extremities served as controls. The findings were evaluated as present (+) or absent (-).

Statistical analysis

The Statistical Program for Social Sciences (SPSS, Inc., Chicago, IL, USA) version 11.5 was used for the analysis of the study data. As the data were normally distributed, student t-test was used to evaluate the differences between the groups. The results were expressed as mean $\pm$ standard deviation (SD). A p value <0.05 was considered statistically significant.

Results

After injection of local anesthetic to the sciatic nerve, proprioceptive, motor and nociceptive block was not observed on the contralateral hind limb in any of the rats from the six study groups.

Based on mean times, the order of onset of block was as follows: proprioception, nociception and motor block in Groups 1 and 2; proprioception, motor block and nociception in Group 3; nociception, proprioception and motor block in Groups 4 and 5. In Group 6, proprioceptive and nociceptive blocks were achieved simultaneously which were followed by motor block.

Based on mean times, the order of resolution of block was as follows: proprioception, motor block and nociception in all groups, except for Group 6. In Group 6, the order of resolution of block was

motor block, proprioception and nociception. In all rats included in the study, complete resolution of block was observed without any clinically detectable neurological deficits. Time to onset and resolution of block are presented in Figure 1 and 2. Time to onset and resolution of block in groups receiving amitriptyline in combination with dexmedetomidine were found to be significantly longer ($p < 0.05$).

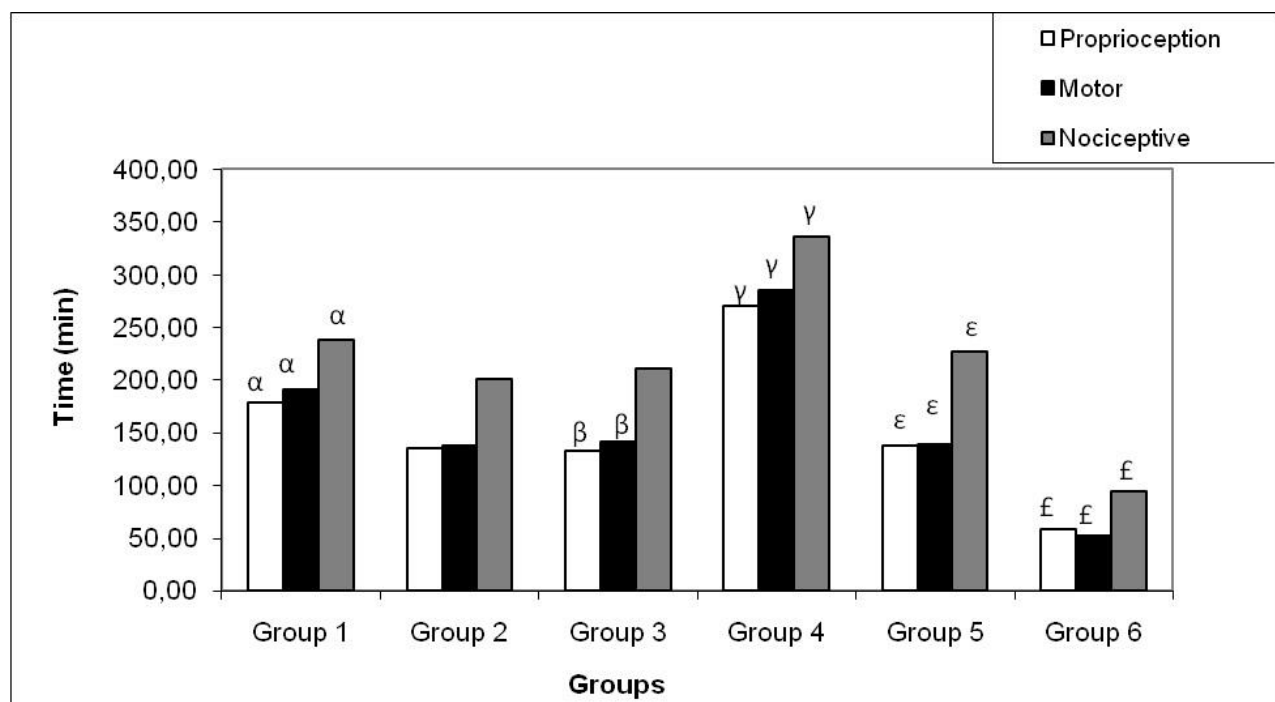


Figure 1. Block of starting times for groups: Values are given as mean $\pm$ standard deviation; α : Compared to Group 1 and 2 $p < 0.05$, β : Group 3, Group 1 and 2 compared with $p < 0.05$ δ : Group 3 and 4 compared $p < 0.05$, ϵ : Group 4 and Group 5 compared with $p < 0.05$, £ : Group 6 and Group 3, 4 and 5, compared with $p < 0.05$

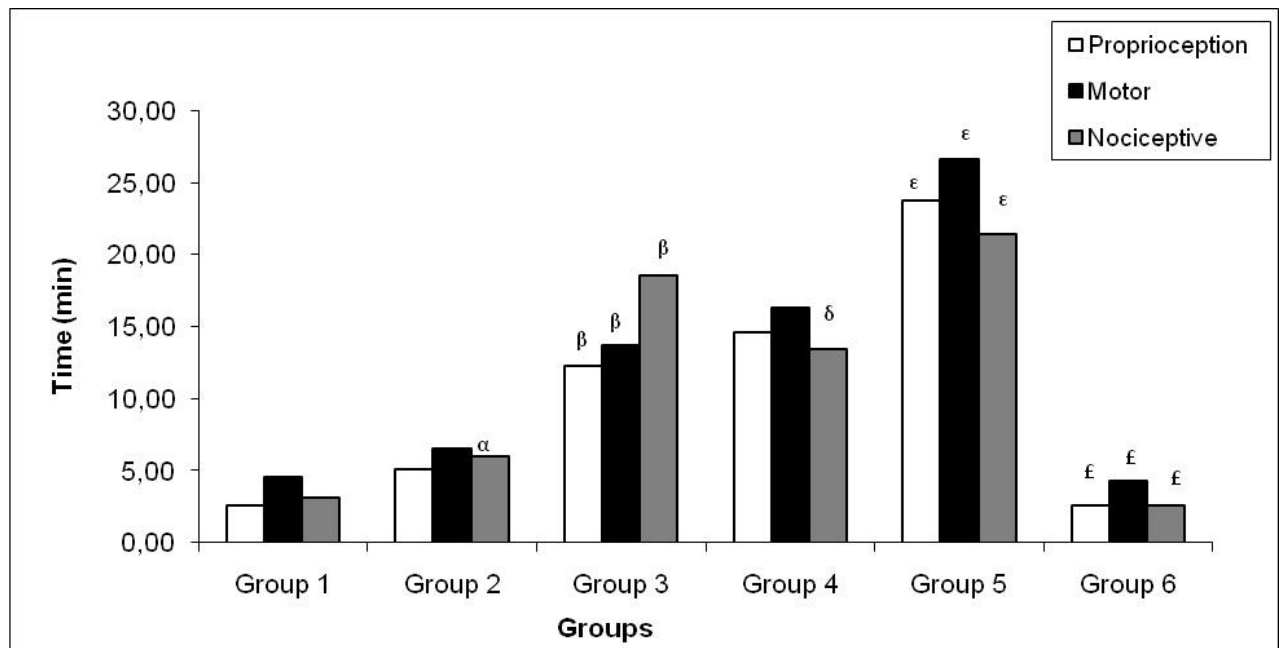


Figure 2. Block of ending times for groups. Values are given as mean $\pm$ standard deviation; α : Compared to Group 1 and 2 $p < 0.05$, β : Group 3, Group 1 and 2 compared with $p < 0.05$, δ : Group 3 and 4 compared $p < 0.05$, ϵ : Group 4 and Group 5 compared with $p < 0.05$, £ : Group 6 and Group 3, 4 and 5, compared with $p < 0.05$

Discussion

Amitriptyline is a tricyclic antidepressant commonly used orally in the treatment of chronic pain. Amitriptyline is a neurotoxic agent that causes Wallerian degeneration of nerve fibers by direct axonal injury. This effect occurs even if the drug is administered in relatively lower doses and outside the peripheral nerve bundles. Injection of increasing amitriptyline doses into the sciatic nerve has been reported to be associated with an increased neurotoxicity [3].

A percutaneous approach has been used in studies evaluating the efficacy of amitriptyline for sciatic nerve blockade [4,9]. In this approach, however, concentration of the drug near the nerve is variable and not reliable.

Estebe et al. [3] performed an injection to sciatic nerve after surgical exploration. By this method, the sciatic nerve is exposed and the drug is injected outside the perineurium. Thus, direct needle trauma to the nerve is avoided and accurate placement of the test dose of drug to the nerve is ensured. In the present study, we preferred this method for nerve blockade.

Estebe et al. [3] utilized a minimum amitriptyline dose of 0.625 mg and demonstrated a delay in the onset of block and a shorter duration of block compared to higher concentrations. Estebe et al. [3] also reported that the block induced by 0.625 mg of amitriptyline was fully reversible, whereas higher concentrations were dose-dependently associated with persistence of certain motor-behavioral disorders. In that particular study, neuropathological effects caused by amitriptyline 0.625 mg were qualified as partially moderate and reversible, and this dose was suggested to be the lowest possible dose that could achieve motor block.

In the present study, the highest amitriptyline dose was determined to be 0.625 mg. Thus, we evaluated the durations of sciatic nerve blockade with minimum and half of the doses reported by Estebe et al. [3] as well as in combination with dexmedetomidine, as well.

Experimental studies have indicated that amitriptyline has a higher potency than bupivacaine [4,5]. Nau et al. [10] demonstrated that the receptor of amitriptyline overlaps with the binding sites of local anesthetics within voltage-gated sodium channels.

Khan et al. [11] compared bupivacaine and amitriptyline for cutaneous infiltration, and found that amitriptyline was a longer-acting anesthetic as compared with bupivacaine. Consistent with the literature, amitriptyline alone and amitriptyline in combination with dexmedetomidine was found to have longer duration of anesthetic effect compared to bupivacaine in the present study. On the

other hand, some recent studies have suggested that amitriptyline, as a local anesthetic, does not have a higher potency compared to bupivacaine [12,13]. In the study by Barnett et al. [12], in which bupivacaine and amitriptyline were compared with respect to neurotoxicity and tissue damage after sciatic nerve blockade, the amitriptyline and bupivacaine concentrations required to achieve block for 100 minutes were found to be 20 mmol/L and 3 mmol/L, respectively. They suggested that 40 mmol/L of bupivacaine would be required to cause the same degree of toxicity caused by 20 mmol/L of amitriptyline and hence, bupivacaine was a more potent agent as a local anesthetic.

Amitriptyline has a partially hydrophobic molecular structure [14]. Partially hydrophobic molecules pass through biological barriers more difficultly compared to moderately hydrophobic molecules such as bupivacaine [15]. Thus, injection site must be in close proximity to the nerve in order amitriptyline to be efficient. However, bupivacaine can show its effects when administered distant from the nerve [13]. Gerner et al.[4] showed that the onset of block was delayed with amitriptyline compared to bupivacaine and that time to onset of block was shorter with increasing doses of amitriptyline. In the present study, although statistically not significant, the mean time to onset of block was shorter in the group that received bupivacaine. The reason for this could be the method we preferred for the injection of local anesthetic.

Dexmedetomidine is a highly selective α_2 -adrenoceptor agonist and is commonly used as an intravenous sedative and a co-analgesic agent. Clinical studies have demonstrated that the use of intravenous dexmedetomidine significantly decreases opioid use and the need for inhalation anesthetics [16,17].

In a study on human subjects, Kanazi et al.[7] showed that intrathecal bupivacaine combined with low doses of dexmedetomidine shortens the time to onset of motor block and prolongs the duration of motor and sensory block as compared to bupivacaine alone.

It has not been fully understood by which mechanisms α_2 -adrenoceptor agonists prolong the duration of the motor and sensory blocks of local anesthetics. Local anesthetics and α_2 -adrenoceptor agonists may exhibit additive or synergistic effects through different mechanisms. Local anesthetics block sodium channels, whereas α_2 -adrenoceptor agonists exert their effects by binding to presynaptic C fibers and postsynaptic neurons in the posterior horn. When administered intrathecally, α_2 -adrenoceptor agonists produce analgesic effects by reducing the release of neurotransmitters from C fibers and causing hyperpolarization of postsynaptic dorsal root neurons [18,19]. This effect could explain the prolonged duration of sensory block in spinal anesthesia by addition of dexmedetomidine to local anesthetics. Prolonged duration of motor block with spinal anesthetics could be resulted from binding of α_2 -adrenoceptor agonists to the neurons in the posterior horn [20]. Yaksh [21] reported that intrathecal administration of α_2 -adrenoceptor agonists in animals could cause dose-dependent motor strength loss Poree et al. [22] demonstrated that dexmedetomidine plays an analgesic role in neuropathic pain by affecting α_2 -adrenoceptors. To the best of our knowledge, there is no study in the literature evaluating the effects of local anesthetics in combination with an adjuvant dexmedetomidine in sciatic nerve blockade. Thus, the results of our study are preliminary data.

There are studies investigating the addition of dexmedetomidine to regional intravenous anesthesia (RIVA) with lidocaine in patients undergoing hand surgery [23,24]. Memiş et al.[23] compared the addition of 0.5 $\mu\text{g/kg}$ of dexmedetomidine to lidocaine with lidocaine alone in RIVA and reported shorter time to onset of sensory and motor block, prolonged motor and sensory recovery and better anesthesia conditions in lidocaine-dexmedetomidine group. Esmaoğlu et al.[24] also compared lidocaine combined with 1 $\mu\text{g/kg}$ of dexmedetomidine with lidocaine alone in RIVA and reported that, in contrast to a forementioned study, lidocaine in combination with dexmedetomidine did not affect time to onset of motor and sensory block and recovery times.

In the present study, the addition of 10 µg dexmedetomidine to 0.625 mg of amitriptyline significantly prolonged the time to resolution of blockade compared to 0.625 mg of amitriptyline alone. We considered that dexmedetomidine prolonged the duration of block by affecting α₂-adrenoceptors in the peripheral nerves and showed additive effect with amitriptyline in peripheral nerve blockade [22]. Furthermore, dexmedetomidine can compete with amitriptyline which antagonize α₂-adrenoreceptors as well. Thus, higher concentrations of amitriptyline will be available for the blockade of sodium channels. In this mechanism, amitriptyline may have an additive effect with dexmedetomidine. Combination of the same dose of dexmedetomidine with 0.312 mg of amitriptyline minimally prolonged the time to resolution of block, which was not statistically significant. This suggested that very low concentrations of amitriptyline in combination with dexmedetomidine showed minimal additive effect.

Consistent with the study hypothesis, prolonged time to resolution of block observed in the present study was an expected finding. However, time to onset of block was significantly delayed in combination groups. This could be attributed to the changes in pH of the combination solutions, changes in electrical charges of amitriptyline and dexmedetomidine, drug combinations restricting penetration of drugs through the barriers surrounding the nerve, and thus time elapsed for the drug to reach effective concentration.

To sum up, we concluded that amitriptyline, in the doses used for direct injection to sciatic nerve, is a more potent local anesthetic than bupivacaine, and that amitriptyline provided a similar time to onset of anesthesia to that of bupivacaine and a longer duration of block compared to bupivacaine. Moreover, it was concluded that amitriptyline in combination with dexmedetomidine significantly prolonged the duration of block in peripheral nerve blockade and additive interaction might increase as doses increase; however, the addition of dexmedetomidine prolonged the time to onset of block.

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