



Experimental Research Article

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Evaluation of the effects of bupivacaine combined with sugammadex on the duration of the nociceptive blockade in sciatic nerve blocks: a controlled, double-blind animal study

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Background: Animal and other experimental studies have demonstrated increased block time and quality when α - and β -cyclodextrin drugs are combined with local anesthetics. However, to our knowledge, no study has utilized γ -cyclodextrins in such a combination. In the present study, we used an animal model to evaluate the effects of different doses of the combined administration of γ -cyclodextrin (sugammadex) and bupivacaine on the duration of sciatic nerve blocks in rats.

Methods: Sciatic nerve blocks were performed with a 0.20 ml mixture in all groups. For the non-experimental groups, this mixture consisted of 0.2 ml saline (Sham group), 0.2 ml sugammadex (Group S), or 0.16 ml bupivacaine 0.5% and 0.04 ml saline (Group B). For the experimental groups, 0.16 ml bupivacaine 0.5% was administered along with 0.01 ml sugammadex and 0.03 ml saline (Group BS1), 0.02 ml sugammadex and 0.02 ml saline (Group BS2), or 0.04 ml sugammadex (Group BS4). Proprioception, nociception, and motor function were evaluated until the sciatic block was completely reversed.

Results: Motor, proprioceptive, and nociceptive blockades occurred within 5 min in all experimental groups. In Group BS4, the duration of the motor, proprioceptive, and nociceptive blockades was significantly increased compared with the other experimental groups. However, in Groups BS1 and BS2, only the duration of the nociceptive blockade was significantly increased.

Conclusions: The combined administration of sugammadex and bupivacaine for sciatic nerve blocks in rats led to a significant increase in the duration of motor, proprioceptive, and nociceptive blockades.

Keywords: Anesthetics; Bupivacaine; Nerve block; Rats; Sciatic nerve; Sugammadex; Time factors.

Introduction

Bupivacaine is one of the most common local anesthetic agents used in regional anes-

thetia, in part due to its relatively long duration of action [1]. Prolonging the effect of regional analgesia, especially if it is used post-operatively, is important for patient satisfaction and early recovery. This has led to clinicians seeking longer-acting agents and the development of catheter applications [2-4]. For this purpose, new generic medications, such as liposomal bupivacaine, have been introduced [5], and researchers have suggested adding agents such as steroids to local anesthetics [6]. Recent studies have demonstrated an increase in block time and quality when agents such as dexmedetomidine and clonidine are added to local anesthetics [7,8]. Prolonging the duration of the sensorial-nociceptive blockade without increasing the duration of the motor blockade is a goal of enhanced recovery after surgery protocols [9].

A recent experimental study showed that inclusion complexes of α - and β -cyclodextrins can act as carriers for local anesthetics [10]. Additionally, one animal study reported increased block times when β -cyclodextrin was combined with bupivacaine [11]. Considering the advantages of cyclodextrin complexing, such as reducing myotoxicity associated with bupivacaine, this combination could be a viable alternative treatment method [12]. Furthermore, in another experimental study, the combination of 2-hydroxypropyl--cyclodextrin and bupivacaine resulted in both an onset and duration of anesthesia that were similar to those of bupivacaine and epinephrine solutions [13]. However, these applications have not yet been reflected in clinical practice.

Sugammadex is a modified γ -cyclodextrin molecule used to reverse the effects of steroidal non-depolarizing neuromuscular blockers [14]. No studies have assessed the interactions of γ -cyclodextrins with bupivacaine and other types of local anesthetics and the effect of a sugammadex-bupivacaine mixture on the efficacy and characteristics of nerve blocks. In this study, we therefore evaluated the effect of different doses of the combined administration of sugammadex with fixed-volume bupivacaine on the duration of sciatic nerve blocks in rats.

Materials and Methods

This study was conducted at the Experimental Animals Research Center of Northern Cyprus Near East University between May and June 2021. Ethical approval for this study (No. 2021/129) was provided by the Local Ethical Committee of Experimental Animals Research Center of Northern Cyprus Near East University on March 19, 2021 and December 16, 2021 (ethical approval for extended methodology). For this study, 42 male Wistar Albino rats (seven rats/group in the sham group [Sham], control group [Group B], and four experimental groups [Groups S, BS1, BS2, and BS4]) aged 170-192 days and weighing 300-400 g were used.

All rats were maintained in a controlled temperature of $23 \pm 1^\circ\text{C}$ and a humidity of $50\% \pm 10\%$, with a 12-h light-dark cycle. The rats were allowed to eat and drink freely for 7-10 days before the start of the study.

Experimental strategy

The animal model was designed to be controlled and double-blind. The drug mixture or saline was individually prepared by a researcher, assigned a random number, and given to the researcher that performed all the blocks at the moment of administration. A third researcher evaluated the block effects.

Doses and groups

Bupivacaine was used as the local anesthetic (0.5% Marcaine vial, AstraZeneca, Turkey). In the experimental group, the local anesthetic and sugammadex (BRIDION 200 mg/2 ml, Merck Sharp Dohme, USA) were combined (hereafter, to avoid confusion, doses will be referred to by volume). For this study, the preservative-free forms of the drugs were not available; therefore, commercially available forms were used. In accordance with a similar study, 0.2 ml was used as the volume for each sciatic block [15]. For all experimental groups except Group S and the sham group, 0.16 ml bupivacaine 0.5% was administered. For Groups BS1, BS2, and BS4, 0.01 ml, 0.02 ml, and 0.04 ml of sugammadex were added, respectively. For Groups BS1 and BS2 0.03 ml and 0.02 ml saline were added, respectively. The group names, drug concentrations, and pH of the solutions are shown in Table 1.

Sciatic nerve block

After recording descriptive values, a 30-gauge needle was used

Table 1. Definition of the Groups in this Study according to Drugs Used and Solution pH

Group	Drugs used	pH of solution
Sham	0.2 ml saline	5.69
Group B (control)	0.16 ml bupivacaine 0.5% + 0.04 ml saline	6.40
Group S	0.2 ml sugammadex	7.84
Group BS1	0.16 ml bupivacaine 0.5% + 0.01 ml sugammadex + 0.03 ml saline	6.79
Group BS2	0.16 ml bupivacaine 0.5% + 0.02 ml sugammadex + 0.02 ml saline	7.22
Group BS4	0.16 ml bupivacaine 0.5% + 0.04 ml sugammadex	7.70

to perform a unilateral sciatic nerve block under short-acting sevoflurane anesthesia. Once the greater trochanter and ischial tuberosity were identified, the bevel of the needle was advanced towards the femoral head. After contact with the ischial tuberosity, 0.2 ml of local anesthetic was slowly injected over 5 s [16].

Neurobehavioral examination

Proprioception, motor function, and nociception were measured at 0, 1, 5, and 15 min and every 15 min thereafter until complete resolution of the sciatic block was observed. All evaluations were performed in accordance with the principles outlined by Thalhammer et al. [16].

Proprioceptive sensorial evaluations were conducted by observing the tactile placing and hopping responses of the rats at rest. The tactile placing response was conducted by flexing the rat's paw until it touched the surface and then observing the rat's capacity to return the paw to its normal position. For the hopping reflex, the front portion of the rat was elevated from the surface and one hind paw was lifted from the surface and the rat's body was turned laterally. The normal response is for the rat to use the weight-bearing paw to hop in the direction of the lateral movement. When lateral movement occurs, a fast but weak hopping reflex is observed if the motor blockade is strong. If the proprioceptive blockade is stronger, then the hopping response is delayed and increased lateral movement is required to elicit a response [15,16].

Motor function was assessed according to whether the rats were weight-bearing on their hind legs, their hopping ability, paw gripping ability when hung upside down from their tail, and walking ability [16,17]. These motor function assessments were not graded but were evaluated for presence or absence.

Nociception was evaluated by examining the withdrawal reflex (also called the flexor reflex) induced by painful stimuli. The flexor reflex occurs when the flexors of the hip, knee, and ankle contract following a painful stimulus to the extremities via a polysynaptic pathway. Serrated forceps were used to perform a deep pinch at the level of the fifth metatarsal. Responses to this painful stimu-

lus were graded from 0 to 4. A normal reaction (an attempt to bite, vocalization, and a strong paw withdrawal response) was classified as a 4. A weaker and slower withdrawal response with vocalization, but no attempt to bite was classified as a 3, and an even slower withdrawal response with no vocalization and no attempt to bite was classified as a 2. A very weak withdrawal response or no withdrawal response was scored as a 1 or 0, respectively [16,18].

Statistical analysis

Statistical analyses were performed using SPSS (version 16.0; IBM Corp., USA). Groups were compared using one-way analysis of variance (ANOVA) with Tukey's post-hoc test to determine differences between groups. Kaplan-Meier analyses were used to compare and demonstrate the time to reversal of nociceptive, proprioceptive, and motor blockades between the experimental groups. All values are presented as mean \pm SD. Statistical significance was set at $P < 0.05$.

Results

This study consisted of four experimental groups (Groups S, BS1, BS2, and BS4), one control group (Group B), and one sham group (Sham), with seven rats in each group. Evidence of sciatic blocks were not observed in the sham group or Group S. In all other groups, motor, proprioceptive, and nociceptive blockades occurred within 5 min. There was a statistically significant difference in the time to sciatic block reversal between the groups (Table 2). The post-hoc analysis results are shown in Fig. 1, and the Kaplan-Meier analysis is shown in Fig. 2.

When motor and proprioceptive blockade times were compared, no differences between Group BS1 and Group B ($P > 0.05$) or between Group BS2 and Group B or Group BS1 ($P > 0.05$) were found. When nociceptive blockade times were compared, no differences between Groups BS1 and BS2 were found ($P > 0.05$). However, nociceptive blockade times were significantly different between Group B and Groups BS1, BS2, and BS4 ($P < 0.05$). Ad-

Table 2. Comparison of the Duration of Motor, Proprioceptive, and Nociceptive Blockades according to Group

Duration of blockade (min)	Group B	Group BS1	Group BS2	Group BS4	P value
Motor blockade	42.85 \pm 13.50	55.71 \pm 11.34	60.00 \pm 12.25	135.00 \pm 25.98	< 0.001
Proprioceptive blockade	75.00 \pm 19.36	85.71 \pm 7.32	79.28 \pm 22.44	192.85 \pm 36.35	< 0.001
Nociceptive blockade	90.00 \pm 15.00	122.14 \pm 13.50	126.42 \pm 11.80	248.57 \pm 19.09	< 0.001

Values are presented as mean \pm SD. The P value shows the significance level of the one way ANOVA. The post-hoc analysis is shown in Fig. 1. For all groups 0.16 ml bupivacaine 0.5% was administered. For Groups BS1, BS2, and BS4, 0.01 ml, 0.02 ml, and 0.04 ml of sugammadex were added, respectively. For Groups BS1 and BS2 0.03 ml and 0.02 ml saline were added, respectively.

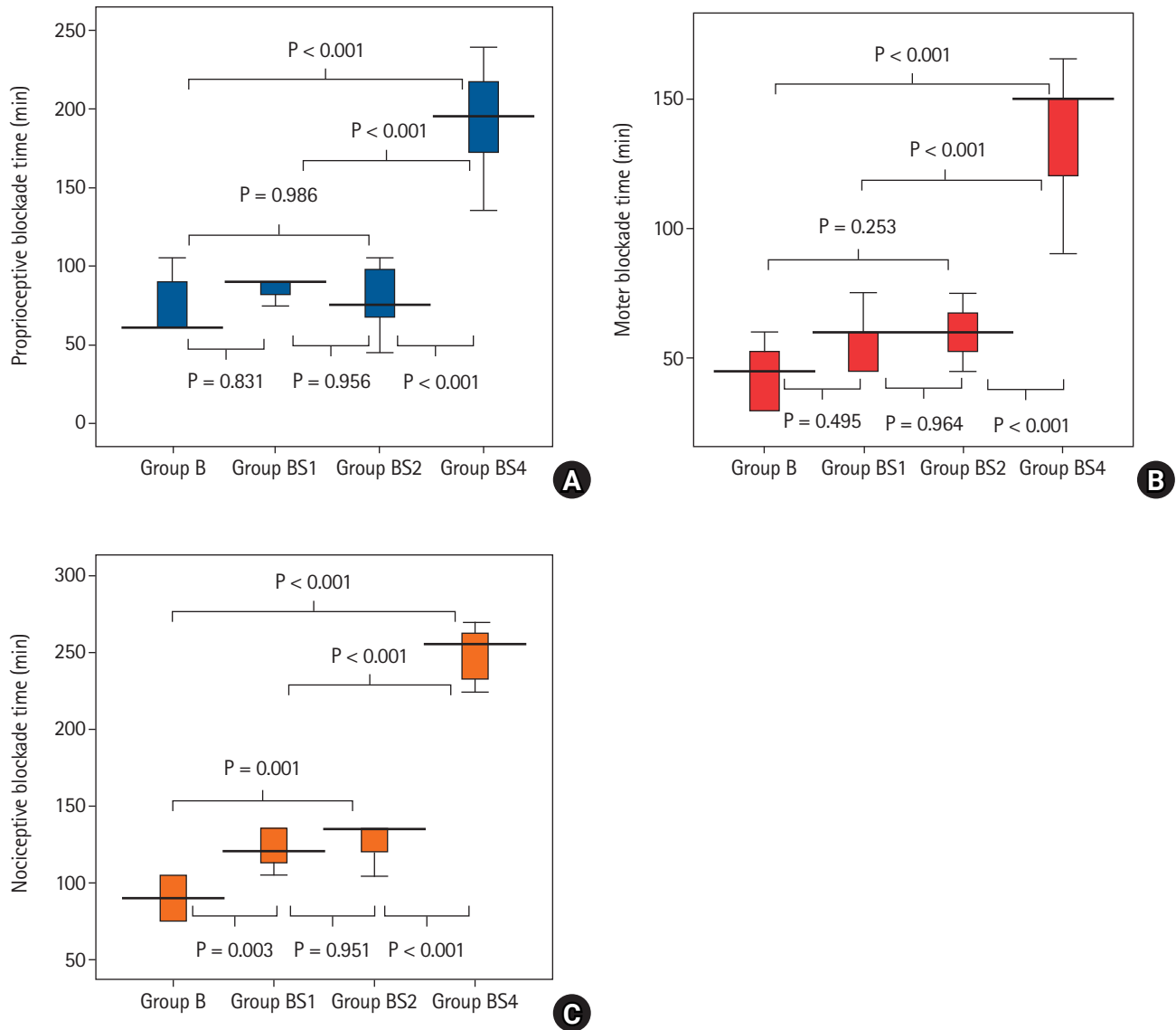


Fig. 1. Blockade times as box plot. (A) Proprioceptive blockade time. (B) Motor blockade time. (C) Nociceptive blockade time. For all groups 0.16 ml bupivacaine 0.5% was administered. For Groups BS1, BS2, and BS4, 0.01 ml, 0.02 ml, and 0.04 ml of sugammadex were added, respectively. For Groups BS1 and BS2 0.03 ml and 0.02 ml saline were added, respectively.

ditionally, significant differences in motor, proprioceptive, and nociceptive blockade times between Group BS4 and Group B, Group BS1, and Group BS2 ($P < 0.001$) were found.

When motor and proprioceptive blockade times were compared, Groups BS1 and BS2 were similar to the control group ($P > 0.05$).

The nociceptive blockade times were longer in Groups BS1 and BS2 than in the control group ($P < 0.01$). In Group BS4, motor, nociceptive, and proprioceptive blockade times were statistically significantly longer compared to all other groups ($P < 0.01$).

Discussion

Our study demonstrated that high doses (0.04 ml) of sugammadex combined with bupivacaine significantly increases the motor, proprioceptive, and nociceptive blockade times in rats undergoing sciatic nerve blocks. Relatively lower doses (0.01 ml and 0.02 ml) of sugammadex, however, significantly increased only the nociceptive blockade time.

Regional anesthetic techniques are used in many fields of anesthesia, including pain medicine and postoperative analgesia. The development of blocks with differential blockades, such as a sensory blockade without a motor blockade, is highly desirable for

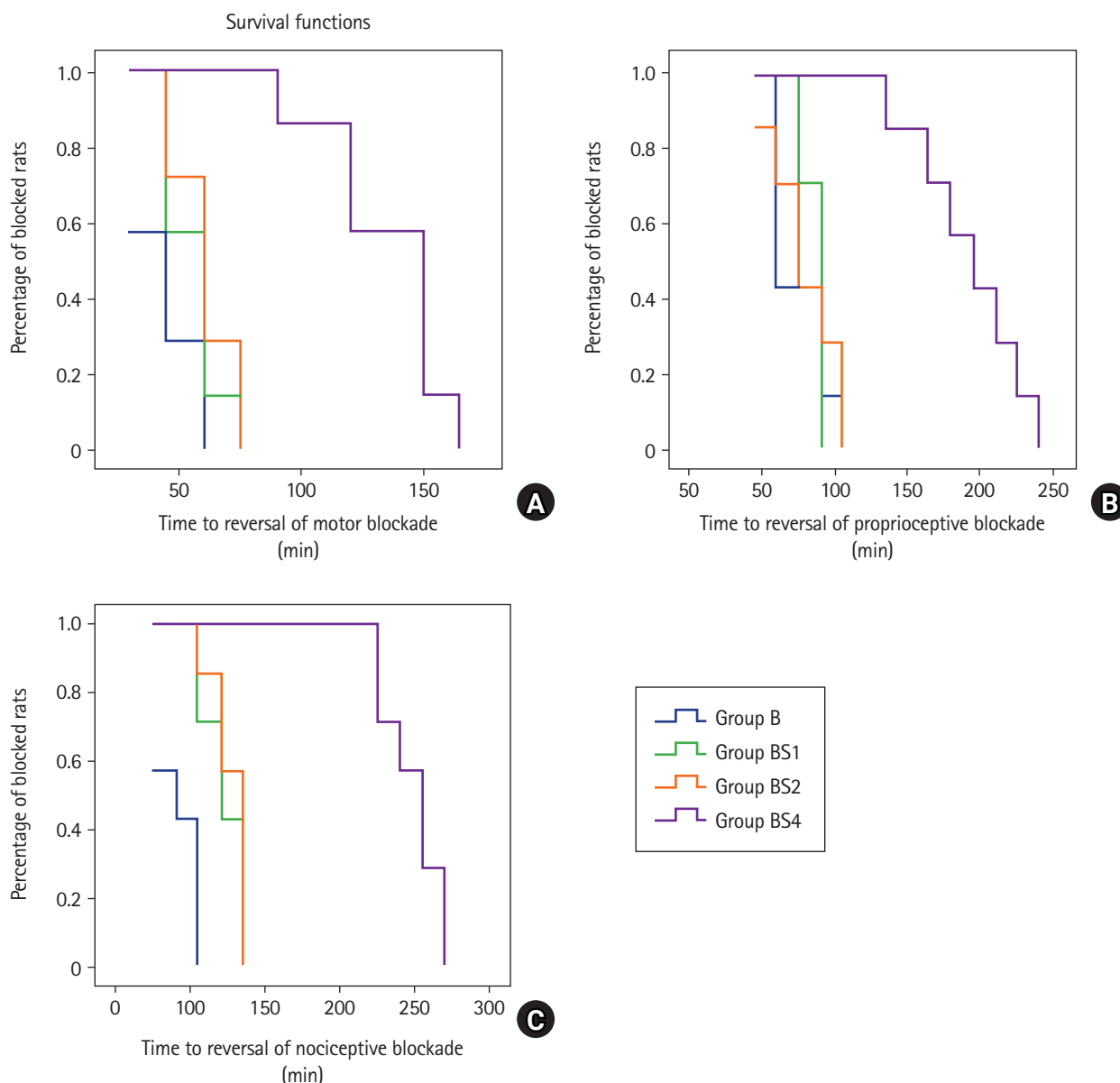


Fig. 2. Kaplan Meier curves displaying the estimated survival probability for reversal time of different blockade. (A) Motor blockade time. (B) Proprioceptive blockade time. (C) Nociceptive blockade time. For all groups 0.16 ml bupivacaine 0.5% was administered. For Groups BS1, BS2, and BS4, 0.01 ml, 0.02 ml, and 0.04 ml of sugammadex were added, respectively. For Groups BS1 and BS2 0.03 ml and 0.02 ml saline were added, respectively.

regional anesthesia. However, prolonging the block time may require multiple injections or catheter placement.

The search for new pharmacological agents with longer effect times or additives that prolong the duration of current local anesthetics is ongoing [5]. The results of our study demonstrate that the combined administration of sugammadex (a γ -cyclodextrin) and bupivacaine significantly extends block times in rats. Should our data be confirmed in human studies, these results could indicate a breakthrough in regional anesthesia and pain medicine.

A recently published study by Geyik et al. [19] also reported the

effects of sugammadex in conjunction with bupivacaine on sciatic nerve blocks in rats. In that study, peritoneal and perineural sugammadex administered after sciatic nerve blocks using 0.20 ml bupivacaine was reported to have no significant effect. In that study, however, sugammadex and bupivacaine were not combined, but rather, sugammadex was administered into the perineural area after the sciatic nerve block was performed. However, in our study, sugammadex and bupivacaine were combined. This difference was confirmed through correspondence with the authors of that study. Since the drug distribution area was greatly in-

creased around the sciatic nerve in their study, as was made evident by dissection and the removal of the barriers created by the surrounding tissues, we believe that our findings are not comparable to those of the study conducted by Geyik et al.

Our study is not the first regional anesthesia study to use cyclodextrins. Previous reports have demonstrated the effect of β - and α -cyclodextrins in prolonging the duration of action of local anesthetic agents [10,11]. However, no previous study has reported such a large (2.5-fold) prolongation in block duration. As in many countries, no injectable α - or β -cyclodextrin is readily available in our country, but will be supplied under special conditions for pharmacological studies.

Sugammadex is a licensed/approved medication in many countries and is widely available in anesthesia clinics. Therefore, if our data can be validated in experimental and human studies, the use of sugammadex in regional anesthesia may be feasible in clinical practice. Similar experimental studies have been conducted with dexamethasone, dexmedetomidine, epinephrine, and various opioids that are easily accessible by anesthesiologists. These agents have been used as adjuvant agents in clinical practice [15,20–22]. Determining the effects that combining sugammadex and bupivacaine may have on tissues (e.g., myotoxicity and neurotoxicity) requires further histopathological, toxicological, and pharmacokinetic studies.

The combination of bupivacaine and sugammadex could show local anesthetic properties that are different from those of bupivacaine alone. This may explain the mechanism underlying the reported results. β -cyclodextrins are known to prolong the block time of bupivacaine in neuraxial and nerve blocks [13,23]. A previous rat model showed that when bupivacaine was combined with β -cyclodextrin, the block time was prolonged compared to bupivacaine alone, and the ephedrine-bupivacaine combination had similar effects [13]. Each cyclodextrin type has the ability to form inclusion complexes with specific molecules, though this depends on the correct fit of the molecule into the hydrophobic cyclodextrin cavity [24]. The main indication is reversal of the effects of steroidal neuromuscular blockers. Additionally, since it can form complexes, it may be a promising treatment option in cases of verapamil and digoxin toxicity [25,26]. In the perineural area, sugammadex prolongs the action of bupivacaine, and the likely mechanism appears to be the complex formation of sugammadex with bupivacaine. Although previous studies have demonstrated that cyclodextrins (α and β forms) form a complex with bupivacaine (kaynak), no data on whether sugammadex (as a γ -cyclodextrin) forms such a complex with bupivacaine have been reported. If this mechanism is confirmed and similar effects can be demonstrated at the intravascular-cellular level, the use of

sugammadex may be a feasible life-saving option in cases of local anesthesia toxicity. In addition, physicochemical studies should be conducted to investigate chelation formation when sugammadex and bupivacaine are combined. It has been reported that bupivacaine and sugammadex do not precipitate *in vitro* [22], but the compatibility of these two molecules *in vivo* requires further investigation.

Although the combined administration of sugammadex and bupivacaine result in alkalization of the local anesthetic solution, it is not possible to explain the results of our study using the alkalization mechanism. Recently, alkalization of local anesthetics has been the subject of many studies aiming to shorten the time of onset of both epidural anesthesia and peripheral blocks as well as extend the block duration. However, the hypothesis that adding alkalizing solutions, such as NaHCO_3 , to bupivacaine in peripheral blocks to extend the block duration has not been confirmed due to insufficient evidence. Candido et al. [27] reported that adding bicarbonate to bupivacaine had no significant effect on the onset time and duration of plexus blocks. In a similar rat study in which β -cyclodextrin was added to bupivacaine, the average pH of the mixture was reported to be 7.01, which was reported to increase the nerve block time by approximately 30% [13]. In our study, we determined that even in Group BS1, with a pH value of 6.72, there was a 35% prolongation of the nociceptive blockade time compared to Group B. Therefore, it is difficult to explain our results using alkalization alone. However, studies have also proposed the use of cyclodextrins for the alkalization of bupivacaine [28]. Therefore, further studies with adjusted pH mixtures are needed.

Our study had some limitations. First, we had to use market-available forms of the drugs, which generally contain preservatives, even though it is preferable to conduct animal experiments with non-commercial, preservative-free forms. Second, the effects of sugammadex on the muscle, nerve, and perineural tissues were not evaluated histopathologically. Comprehensive studies, including those that evaluate the pharmacokinetics of perineural sugammadex administration, are needed. Third, while the nociception blockade results were graded, the motor function results were only evaluated for presence and absence. Therefore, partial impairment of motor function may still have been reported as a resolution of motor function; thus, the block time may have been underestimated. In our study, complete block recovery was used as the indicator for “duration of block” measurements. However, as observed in similar studies, the half-recovery time could have been utilized. Fourth, we used commercially available sugammadex and kept both the total injected volume and the volume of bupivacaine in the mixture constant. Therefore, we could

not administer more than 0.04 ml of sugammadex and could thus not increase the sugammadex concentration; we either had to reduce the volume of bupivacaine or use the original sugammadex molecule and obtain different concentrations that could not be achieved using the commercially available product. Lastly, previous studies have demonstrated that cyclodextrins (α and β forms) form a complex with bupivacaine (kaynak); however, no data on whether sugammadex (a γ -cyclodextrin) forms a complex with bupivacaine have been reported. This issue thus warrants further investigation.

Adjunctive or longer acting agents are also capable of altering blood-brain barrier permeability to the anesthetic, thereby increasing side effects. We suggest that future studies measure the levels of these drugs in the cerebrospinal fluid to provide further insight on this topic. Additionally, further pharmacological studies are needed to evaluate whether other local anesthetics have effects similar to those observed in this study with the combination of bupivacaine and sugammadex.

In conclusion, in this study, the combined administration of sugammadex and bupivacaine for sciatic nerve blocks in rats led to an increase in block time, with a prolongation of the nociceptive blockade that was greater than that of the motor or proprioceptive blockade. Future studies investigating the neurotoxicity and myotoxicity and all-around evaluations of the results are required to scrutinize the potential of the combined administration of sugammadex and bupivacaine for nerve blocks.

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None.

Conflicts of Interest

No potential conflict of interest relevant to this article was reported.

Data Availability

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Author Contributions

Omer Tasargol (Data curation; Formal analysis; Funding acquisition; Investigation; Methodology; Project administration; Resources; Software; Supervision; Validation)

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Aziz Deniz (Conceptualization; Data curation; Formal analysis; Funding acquisition; Investigation; Methodology; Project administration; Resources; Software; Writing – original draft)

Selin Guven Kose (Conceptualization; Project administration; Writing – original draft; Writing – review & editing)

Halil Cihan Kose (Conceptualization; Investigation; Software; Validation; Visualization; Writing – review & editing)

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