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

Running title: Effect of the combination sugammadex with bupivacaine on sciatic nerve block

Omer Tasargol\textsuperscript{a}, Isfendiyar Darbaz\textsuperscript{b}, Osman Ergen\textsuperscript{e}, Feride Zabitler\textsuperscript{b}, Aziz Deniz\textsuperscript{c}, Selin Guven Kose\textsuperscript{d}, Halil Cihan Kose\textsuperscript{d}, Serkan Tulgar\textsuperscript{e}.

\textsuperscript{a}: Doctor Burhan Nalbantoglu State Hospital, Department of Anesthesiology and Reanimation, Nicosia, Cyprus.
\textsuperscript{b}: Near East University, Faculty of Veterinary Medicine, Nicosia, Cyprus.
\textsuperscript{c}: TRNC Ministry of Agriculture and Natural Resources, Guzelyurt Veterinary Department, Nicosia, Cyprus.
\textsuperscript{d}: Health Sciences University Derince Training and Research Hospital, Department of Anesthesiology and Pain Medicine, Ankara Turkey.
\textsuperscript{e}: Samsun University Faculty of Medicine, Samsun Training and Research Hospital, Department of Anesthesiology and Reanimation, Samsun, Turkey.

Corresponding Author: Selin Guven Kose

Address: Dışkapı Yıldırım Beyazıt Eğitim ve Araştırma Hastanesi, Ziraat Mah. Altındağ, Ankara, Turkey

\textit{e-mail}: selinguven89@gmail.com

Phone: +90 535 8419952

Ethical approval resitration no: 2021/129 (Local Ethical Committee of Experimental Animals Research Center of Northern Cyprus Near East University, Nicosia, Northern Cyprus)

ORCID

Omer Tasargol https://orcid.org/0000-0003-1408-5503

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Abstract

Background: Animal and other experimental studies have demonstrated increased block time and quality when alpha and beta cyclodextrin drugs are combined with the local anesthetic. However, to our knowledge there exists no study that has utilized gamma cyclodextrins in such a combination. In this present study we used an animal model to evaluate the effect of different doses of combined administration of gamma cyclodextrin (sugammadex) and bupivacaine on sciatic nerve blockage times in rats.

Methods: Sciatic block was performed with 0.20 mL mixture in all groups. This mixture consisted of 0.2ml saline in the Sham group, 0.2ml sugammadex in Group S and 0.16ml bupivacaine 0.5% and 0.04ml saline in Group B. In the experimental groups, in addition to 0.16ml bupivacaine 0.5%, 0.01ml sugammadex and 0.03ml saline was added in Group BS1 0.02ml sugammadex and 0.02ml saline was added in Group BS2 and 0.04ml sugammadex was added in Group BS4. Proprioception, nociception and motor function was evaluated until sciatic block was completely reversed.

Results: Motor, proprioceptive and nociceptive block occurred in all experimental groups within 5 minutes. In Group BS4 the duration of motor, proprioceptive and nociceptive blocks significantly increased compared with other experimental groups. However in Group BS1 and Group BS2, only the duration of nociceptive block significantly increased.

Conclusion: Combined administration of sugammadex and bupivacaine when performing sciatic nerve block in rats leads to a significant prolongation of motor, proprioceptive and nociceptive block times.

Keywords: Bupivacaine; Nerve block; Rat; Sugammadex.
Introduction

Bupivacaine is one of the most common local anesthetic agents used in regional anesthesia, due to its relatively long duration of action [1]. Prolonging the effect of regional analgesia especially if being used postoperatively 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 to the market [5], and researchers have also suggested the addition of 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 length of sensorial-nociceptive blocks without increasing the length of motor blocks is desired as part of Enhanced recovery after surgery (ERAS) protocols [9].

Recently, one experimental study has shown that inclusion complexes of alpha and beta cyclodextrins can act as carriers for local anesthetics [10]. Additionally, one animal study has reported increased block time when beta cyclodextrin was combined with bupivacaine. [11]. Considering the advantages of -cyclodextrin complexing such as decreased myotoxicity of bupivacaine it could be an alternative treatment [12]. Furthermore, in another experimental study, it has been reported that combination of the 2-hydroxypropyl--cyclodextrin and bupivacaine had both onset and duration of anaesthesia that were similar to those of the bupivacaine and epinephrine solution. [13]. However, these applications have not yet been reflected in clinical practice.

Sugammadex is a modified gamma-cyclodextrin molecule used for reversal of the effects of steroidal structured non-depolarizing neuromuscular blockers [14]. There are no studies on the interactions of gamma-cyclodextrins with bupivacaine and other types of local anesthetics, and the effects of sugammadex-bupivacaine mixture on the characteristic/effect of nerve blocks. In this
animal study we evaluated the effects of different doses of combined administration of sugammadex with fixed volume bupivacaine on the duration of sciatic nerve block in rats.
Materials and Methods

This study was conducted at the Experimental Animals Research Center of Northern Cyprus Near East University between May-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, Nicosia, Northern Cyprus on 19 March 2021 and 16 December 2021. 42 male, Wistar Albino male rats (Sham group, control group and four experimental groups with seven rats in each group) aged between 170-192 days and weighing 300-400 grams were used. All rats were kept in a controlled temperature of 23 ± 1 °C and a humidity of 50% ± 10% with a 12 hour light-dark cycle. The rats were allowed to eat and drink freely for 7-10 days prior to the start of the study.

Experimental Strategy

This animal model was designed to be controlled and double blinded. The drug mixture or saline was individually prepared by a researcher, assigned a random number and given at the application time to the researcher that performed all the blocks. Block effects were evaluated by a third researcher.

Doses and Groups

Bupivacaine was used as the local anesthetic (Marcaine vial, 0.5%, AstraZeneca, Kırklareli, Turkey). In the experimental group, local anesthetic and Sugammadex (BRIDION 200 mg/2ml, Merck Sharp Dohme, USA) were combined. To avoid confusion, from this point on, doses used in this manuscript will be stated by volume. The preservative-free forms of the drugs were not available, therefore market forms were used. 0.2 ml was chosen as the volume for each sciatic block, in accordance with a similar study [15]. 0.16 mL bupivacaine %0.5 was administered in all experimental
groups except Group S and Sham group. Sugammadex was added as 0.01 mL, 0.02 mL or 0.04 mL in their respective groups. Groups, used drugs and pH of applied solution are shown in Table 1.

**Sciatic Nerve Block**

After recording of descriptive values a 30-gauge needle was used to perform a unilateral sciatic nerve block under short acting sevoflurane anesthesia. Following identification of the greater trochanter and ischial tuberosity, 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 seconds [16].

**Neurobehavioral Examination**

Proprioception, motor function and nociception was measured at 0, 1st, 5th, 15th minutes and every 15 minutes 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 evaluation was conducted through observation of tactile placing response and hopping response, conducted at the resting position. Tactile placing response was conducted by flexing the rat’s paw until it touched the surface and observing the capability of bringing the paw back into the normal position. For hopping reflex, the front portion of the rat was elevated from the surface. One of the hind paws was lifted from the surface and the rat’s body was turned laterally. 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 block is strong. If the proprioceptive block 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, their paw gripping ability when hung upside down from their tail, and their walking ability [16,17]. Motor functions were not classified but evaluated for presence and absence only.

Nociception was evaluated by examining the withdrawal reflex (also called flexor reflex) caused by painful stimuli. Flexor reflex occurs when the flexors of the hip, knee and ankle contract. It occurs following a painful stimulus to the extremity through a polysynaptic pathway. A serrated forceps was used to perform a deep pinch at the level of the fifth metatarsal leading to painful stimuli. Response to this painful stimulus was graded from 0 to 4 with 4 being the normal reaction of an attempt to bite, vocalization and a strong paw withdrawal response. A weaker and slower withdrawal response with vocalization but no attempt to bite was classified as 3 and an even slower withdrawal response with no vocalization and no attempt to bite was classified as 2. A very weak withdrawal response or no withdrawal response was scored as 1 and 0, respectively [16,18].

Statistical Analysis

Statistical analysis was performed using SPSS 16.0 (SPSS, Chicago, IL, USA). Groups were compared using One-Way ANOVA with post-hoc Tukey utilized for determination of differences between groups. Kaplan-Meier analysis was used for comparisions and demonstrations of time to reversal of nociceptive, proprioceptive and motor blockade between experimantal groups. All values are given as average ± standard deviation. Statistical significance was accepted as p < 0.05.
Results

The study consisted of four experimental, one control and one Sham group with 7 rats included in each group. Sciatic block was not observed in any rat in the Sham group and Group S. In all other groups, motor, proprioceptive and nociceptive blocks accrued within 5 minutes. There was a statistically significant difference in the time to reversal of sciatic block between groups (Table 2).

Post-hoc analysis results are detailed in Figure 1. Figure 2 demonstrates the Kaplan-Meier analysis.

When motor and proprioceptive block times were compared between Group BS1 and other groups, there was no difference between Group BS1 when compared to Group B and Group BS1 (p>0.05). When motor and proprioceptive block times were compared between Group BS2 and other groups, there was no difference between Group BS2 when compared to Group B and Group BS1 (p>0.05). When nociceptive block times were compared there was no difference between Group BS1 and Group BS2 (p>0.05). However, nociceptive block times were significantly different between Group B when compared to Group BS1, Group BS2 and Group BS4, respectively (p<0.05). When Group BS4 was compared according to motor, proprioceptive and nociceptive block times, there was a significant difference between Group BS4 when compared to Group B, Group BS1 and Group BS2 respectively. (p>0.001)

When motor and proprioceptive block times were compared Group BS1 and Group BS2 were similar to the control group (p>0.05).

Nociceptive block times were longer in group Group BS1 and Group BS2 when compared to the control group (p<0.01). Group BS4 demonstrated statistically significantly longer blocks for all three block types when compared to all other groups (p<0.01).
Discussion

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

Regional anesthesia techniques are used in many fields of anesthesia, including pain medicine and moreover postoperative analgesia. The development of differential block - sensory block without motor block, is highly desirable in regional anesthesia. Also, prolongation of block time may require multiple injections or catheter placement.

The search is still ongoing to find new pharmacological agents with longer effect time or additives that prolong the time of current local anesthetics [5]. The results of our study have demonstrated that the combined administration of sugammadex, a gamma-cyclodextrin, with bupivacaine significantly extends block times. Should our data be confirmed in human studies, the results could mean a breakthrough regional anesthesia and pain medicine.

A recently published study by Geyik et al. [19] reported the effects of sugammadex used in conjunction with bupivacaine in sciatic nerve blocks in rats. In the study, peritoneal and perineural sugammadex administered after sciatic nerve blocks using 0.20 mL bupivacaine were reported to have no significant effect. In the study, sugammadex and bupivacaine were not combined - sugammadex was administered to the perineural area after sciatic nerve block was performed. This was confirmed by correspondence with the authors of the paper. We believe our data are far from being comparable, since the area of drug distribution will be greatly increased around the sciatic nerve, as it has been made visible by dissection and the barriers created by the surrounding tissues have been removed.
Our study is not the first regional anesthesia study in which cyclodextrins were used. Previous reports have demonstrated the effect of beta and alpha cyclodextrins on prolonging the duration of action of local anesthetic agents [10,11]. However, no previous study has reported such a large - 2.5x fold, prolongation in block durations. In our country, similar to many, there is no injectable alpha or beta cyclodextrin available, and they are generally supplied under special conditions for pharmacology studies.

Sugammadex is a licenced/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 enter clinical practice. For example, similar experimental studies have been conducted with dexamethasone, dexmedetomidine, epinephrine and many types of opioids - also easily accessible by anesthesiologists. These agents have found their place as adjuvant agents in clinical practice [15,20–22]. By all means, the effect of combining sugammadex and bupivacaine on tissues including but not limited to myotoxicity and neurotoxicity require investigation through histopathological, toxicological and pharmacokinetic studies.

The combination of bupivacaine and sugammadex may show different local anesthetic properties than bupivacaine alone. This may explain the mechanism behind the results we have reported. Beta cyclodextrins are known to prolong the block time of bupivacaine in neuraxial and nerve blocks [13,23]. The rat model showed that when bupivacaine was combined with beta-cyclodextrin, 'the block time was prolonged compared to bupivacaine alone, and the ephedrine-bupivacaine combination had similar effects [13]. Each cyclodextrin has the ability to form inclusion complexes with specific molecules. This depends on the correct fit of the molecule into the hydrophobic cyclodextrin cavity [24]. Its main indication is reversal of the effects of steroidal neuromuscular blockers. Additionally, due to its ability to form complexes, it is also reported as a promising treatment option in cases such as verapamil and digoxin toxicity [25,26]. In the perineural
area, sugammadex prolongs the action of bupivacaine, and the likely mechanism appears to be complex formation of sugammadex with bupivacaine. Although previous studies demonstrated that cyclodextrins (alpha- beta-forms) make a complex with bupivacaine (kaynak), there is no data whether sugammadex as a gamma-cyclodextrin form such a complex with bupivacaine. If this mechanism is confirmed and similar effects can be demonstrated at the intravascular-cellular level, the use of sugammadex may be considered and investigated as a life-saving option in cases such as 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 in vivo compatibility of these two molecules require investigation.

Although the combined administration of sugammadex and bupivacaine caused alkalinization in the LA solution, it is not possible to explain the results of our study with the alkalinization mechanism. Over the years, the alkalinization of local anesthetics has been the subject of many studies with the aim of shortening the time of onset of both epidural anesthesia and peripheral blocks as well as extending the block duration. However, the hypothesis that adding alkalinizing solutions such as NaHCO3 to bupivacaine in peripheral blocks in order to extend the block duration has not been proven due to insufficient evidence. Candido et al. [27] reported that adding bicarbonate to bupivacaine had no significant effect on onset time and block duration in plexus blocks. Also, in a similar rat study, beta-cyclodextrin was added to bupivacaine and the average pH of the mixture was reported as 7.01, but it was stated that it increased the nerve block time by around 30% [13]. In our study, we determined that even in the drug combination group Group BS1 with a pH value of 6.72, there was a 35% prolongation of the nociceptive block time when compared to Group B. Therefore, it is difficult to explain our results by alkalinization alone. On the other hand, there are also studies
suggesting the use of cyclodextrins for the purpose of alkalinization of bupivacaine [28]. It would therefore be beneficial to conduct new studies with adjusted pH mixtures.

Our study has some limitations. First of all we had to use market-available forms of drugs in our study. The market-available form of drugs generally contain preservatives and it is preferable to conduct animal experiments with non-commercial therefore preservative free forms. In addition, the effects of sugammadex on muscle, nerve and perineural tissues were not evaluated histopathologically. Comprehensive studies - including those that will evaluate the pharmacokinetics of perineural sugammadex administration, are required. Although nocicepion was classified, motor function was evaluated for presence and absence only. Therefore, partial impairment of motor function could be graded as a resolution of blockade and thus underestimate block duration. In our study complete block recovery was used as the indicator for “duration of block” measurements. However, as seen in some similar studies, half-recovery time could have been utilized.

We used commercially available sugammadex in our study and kept both the total injected volume and the volume of bupivacaine in the mixture constant. Therefore, we could not exceed 0.4 mL of sugammadex and therefore could not increase the concentration of sugammadex. We either had to reduce the volume of bupivacaine, or we had to use the original sugammadex molecule and obtain different concentrations that we could not achieve using the commercially available product. Lastly, previous studies demonstrated that cyclodextrins (alpha- beta-forms) make a complex with bupivacaine (kaynak), however, there is no data whether sugammadex as a gamma-cyclodextrin form such a complex with bupivacaine. This issue warrants further investigation.

Adjunctive or longer acting agents also are capable of altering the blood-brain barrier permeability to the anesthetic, thereby increasing side effects. We suggest that future studies measure the level of these drugs in CSF to provide insight on this topic. Further pharmacological studies
evaluating whether other local anesthetics have similar effects to that observed for the combination of bupivacaine and sugammadex are also required.

**Conclusion**

The combined administration of sugammadex and bupivacaine when performing sciatic nerve block in rats leads to increased duration of block and the prolongation of the nociceptive block was greater than that of motor or proprioceptive block.

**Acknowledgements relating to this article**

Assistance with the study: None.
Financial support and sponsorship: None
Conflict of interest: None
Presentation: None
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Table 1: Groups and drugs used.

<table>
<thead>
<tr>
<th>Group</th>
<th>Drugs Used</th>
<th>pH of solution</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sham</td>
<td>0.2 ml saline</td>
<td>5.69</td>
</tr>
<tr>
<td>Control (Group B)</td>
<td>0.16 ml bupivacaine %0.5 + 0.04 ml saline</td>
<td>6.40</td>
</tr>
<tr>
<td>Group S</td>
<td>0.2 ml sugammadex</td>
<td>7.84</td>
</tr>
<tr>
<td>Group BS1</td>
<td>0.16 ml bupivacaine %0.5 + 0.01 ml sugammadex + 0.03 ml saline</td>
<td>6.79</td>
</tr>
<tr>
<td>Group BS2</td>
<td>0.16 ml bupivacaine %0.5 + 0.02ml sugammadex + 0.02ml saline</td>
<td>7.22</td>
</tr>
<tr>
<td>Group BS4</td>
<td>0.16 ml bupivacaine %0.5 + 0.04 ml sugammadex</td>
<td>7.70</td>
</tr>
</tbody>
</table>
Table 2: Comparison of motor, proprioceptive, and nociceptive blocks duration according to groups. p value demonstrates significance level of one way ANOVA. Post Hoc analysis is given in figure 1.

<table>
<thead>
<tr>
<th>Duration of Blockade (minutes)</th>
<th>Group B</th>
<th>Group BS1</th>
<th>Group BS2</th>
<th>Group BS4</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean±SD</td>
<td>Mean±SD</td>
<td>Mean±SD</td>
<td>Mean±SD</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Motor Block</td>
<td>42.85±13.50</td>
<td>55.71±11.34</td>
<td>60.00±12.25</td>
<td>135.00±25.98</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Proprioceptive Block</td>
<td>75.00±19.36</td>
<td>85.71±7.32</td>
<td>79.28±22.44</td>
<td>192.85±36.35</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Nociceptive Block</td>
<td>90.00±15.00</td>
<td>122.14±13.50</td>
<td>126.42±11.80</td>
<td>248.57±19.09</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>
Figure 1: Blockade times as box plot
a: Proprioceptive blockade time
b: Motor blockade time
c: Nociceptive blockade time
Figure 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