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SCIENTIFIC ARTICLE

In vitro evaluation of antimicrobial features of sugammadex[☆]

Volkan Hanci^{a,*}, Ahmet Vural^b, Sevgi Yılmaz Hanci^c, Hasan Ali Kiraz^d, Dilek Ömür^d,
Ahmet Ünver^b

^a Department of Anesthesiology and Reanimation, Medical Faculty of Dokuz Eylül University, İzmir, Turkey

^b Department of Microbiology, Medical Faculty of Çanakkale Onsekiz Mart University, Çanakkale, Turkey

^c Clinic of Microbiology, Çanakkale State Hospital, Çanakkale, Turkey

^d Department of Anesthesiology and Reanimation, Medical Faculty of Çanakkale Onsekiz Mart University, Çanakkale, Turkey

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Abstract

Background: Drugs administered by intravenous routes may be contaminated during several stages of production or preparation. Sugammadex is a modified gamma cyclodextrin. While research into the antibacterial effects of varieties of cyclodextrin is available, there are no studies focusing on the antibacterial effects of sugammadex. This study investigates the in vitro antimicrobial activity of sugammadex.

Materials and methods: The in vitro antimicrobial activity of sugammadex was investigated using the broth microdilution method. The pH of the test solution was determined using a pH meter. The test microorganisms included *Staphylococcus aureus* ATCC 29213, *Enterococcus fecalis* ATCC 29212, *Escherichia coli* ATCC 25922 and *Pseudomonas aeruginosa* ATCC 27853. In the second phase of the study 100 mg/mL sugammadex (50 µg) was contaminated with test microorganisms (50 µg), including *S. aureus* ATCC 29213, *E. fecalis* ATCC 29212, *E. coli* ATCC 25922 and *P. aeruginosa* ATCC 27853, left to incubate for 24 h and then the bacterial production in sugammadex was evaluated.

Results: The pH of the test solutions ranged between 7.25 and 6.97. Using the microdilution method, sugammadex had no antibacterial effect on *S. aureus*, *E. fecalis*, *E. coli* and *P. aeruginosa* at any concentration. In the second phase of the study bacterial production was observed after 24 h in 100 mg/mL sugammadex contaminated with the test microorganisms *S. aureus*, *E. fecalis*, *E. coli* and *P. aeruginosa*.

Conclusions: Sugammadex had no antimicrobial effect on the test microorganisms, *S. aureus*, *E. fecalis*, *E. coli* and *P. aeruginosa*. Care should be taken that sterile conditions are maintained in the preparation of sugammadex; that the same sugammadex preparation not be used for more than one patient; and that storage conditions are adhered to after sugammadex is put into the injector.

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* Corresponding author.

E-mail: vhanci@gmail.com (V. Hanci).

Introduction

Some anesthetic agents such as propofol are known to support the growth of microorganisms,¹⁻⁵ while other anesthetic agents such as morphine sulphate, thiopental sodium, fentanyl citrate, dexmedetomidine and midazolam inhibit microbial growth.³⁻⁷ Anesthetic agents may be contaminated by microorganisms at various stages during preparation for use.² It is important for this reason that the antibacterial properties, or the ability to enhance bacterial production, of anesthetic agents in a contaminated situation be known.⁸

Sugammadex is a modified gamma cyclodextrin.⁹⁻¹¹ Cyclodextrins are water soluble cyclic oligosaccharides with a lipophilic core. Sugammadex has quickly found a place in clinical use as a selective neuromuscular blockade reverser.⁹⁻¹¹ Sugammadex quickly encapsulates steroidal neuromuscular blockers, increasing the amount of encapsulated steroidal neuromuscular blockers in plasma and separating the blockers from the nicotinic acetylcholine receptors.⁹⁻¹¹

Cyclodextrins are molecules that are often used in the food and pharmaceutical industries. They are commonly used to convert lipophilic medications to hydrophilic forms. Other applications of cyclodextrins include the field of microbiology. Some cyclodextrins, such as dimethyl- β -cyclodextrin, have been used to increase production of *Helicobacter pylori*,¹² while others, like hydroxypropyl- β -cyclodextrin, have been reported to prevent bacterial production when used to coat vascular prostheses.¹³ However there are no studies evaluating the effect of sugammadex, a modified gamma cyclodextrin molecule lately being used in anesthesiology, on bacterial production.

The aim of this study was to evaluate the antimicrobial effects of sugammadex on the test microorganisms. The test microorganisms chosen were *Staphylococcus aureus* American Type Culture Collection (ATCC) 29213, *Enterococcus faecalis* ATCC 29212, *Escherichia coli* ATCC 25922 and *Pseudomonas aeruginosa* ATCC 27853.

Materials and methods

The antibacterial activity of sugammadex was investigated using the broth microdilution method according to the procedures outlined by the Clinical and Laboratory Standards Institute (CLSI).¹⁴

Briefly, sugammadex was diluted with 0.9% sterile saline to final concentrations of 512 $\mu\text{g/mL}$, 256 $\mu\text{g/mL}$, 128 $\mu\text{g/mL}$, 64 $\mu\text{g/mL}$, 32 $\mu\text{g/mL}$, 16 $\mu\text{g/mL}$, 8 $\mu\text{g/mL}$, 4 $\mu\text{g/mL}$, 2 $\mu\text{g/mL}$, 1 $\mu\text{g/mL}$ and 0.5 $\mu\text{g/mL}$. For each neuromuscular blocking drug, the pH values of all the dilutions were determined with a pH meter (Sartorius pH Meter PB-11). *S. aureus* ATCC 29213, *E. faecalis* ATCC 29212, *E. coli* ATCC 25922 and *P. aeruginosa* ATCC 27853 were used as control microorganisms. The bacteria (5×10^5 colony-forming units per milliliter; (CFU/mL)), MHB (Mueller-Hilton broth) and the sugammadex in the specified concentrations were incubated in wells on microplates at 35°C for 20h. The minimal inhibitory concentrations (MIC) were determined by observing the lowest concentration of the agent that

Table 1 The pH values of tested dilutions of sugammadex.

Dilution of sugammadex ($\mu\text{g/mL}$)	pH
512 $\mu\text{g/mL}$	7.25
256 $\mu\text{g/mL}$	7.22
128 $\mu\text{g/mL}$	7.14
64 $\mu\text{g/mL}$	7.09
32 $\mu\text{g/mL}$	7.04
16 $\mu\text{g/mL}$	7.04
8 $\mu\text{g/mL}$	7
4 $\mu\text{g/mL}$	6.99
2 $\mu\text{g/mL}$	6.98
1 $\mu\text{g/mL}$	6.97
0.5 $\mu\text{g/mL}$	6.97
Physiological serum 0.9%	6.8

inhibited visible growth of the bacterium. Haze or turbidity in the wells was an indicator of bacterial growth.

In the second stage of the study 100 mg/mL sugammadex was contaminated with the test organisms, *S. aureus* ATCC 29213, *E. faecalis* ATCC 29212, *E. coli* ATCC 25922 and *P. aeruginosa* ATCC 27853. Bacteria, 50 μL (5×10^5 colony-forming units per milliliter; (CFU/mL)), and 50 μL sugammadex (100 mg/mL) were incubated at 35°C for 24h. After 24h the bacterial production in the sugammadex was evaluated.

Results

Using the microdilution technique, sugammadex had no antibacterial effect on *S. aureus*, *E. faecalis*, *E. coli* and *P. aeruginosa* at any concentration.

In the second part of the study, after 24h incubation 100 mg/mL sugammadex contaminated with *S. aureus*, *E. faecalis*, *E. coli* and *P. aeruginosa*, bacterial growth was observed.

The pH of the test solutions ranged between 7.25 and 6.97. The pH values are listed in Table 1.

Discussion

In this study, we found that sugammadex does not have antimicrobial properties with regard to the test organisms, *S. aureus*, *E. faecalis*, *E. coli* and *P. Aeruginosa*.

Drugs manufactured for intravenous use should be prepared and administered in sterile conditions. Infectious microorganisms can be introduced into the patient through contaminated containers, rubber diaphragms, needles and infusion sets.

Anesthetic agents may be contaminated by microorganisms during preparation. For this reason, the antimicrobial effects of the used agents are important.⁸ It is known that propofol supports the growth of microorganisms.^{2-4,7,8,15-18} On the other hand, morphine sulphate, thiopental sodium, fentanyl citrate, dexmedetomidine, atracurium, rocuronium and midazolam have antimicrobial effects.^{3,5-8}

Sugammadex is a modified gamma cyclodextrin.⁹⁻¹¹ Cyclodextrins are molecules that are often used in the food and pharmaceutical industries. They are commonly used to convert lipophilic medications to hydrophilic forms. Cyclodextrins are water soluble cyclic oligosaccharides with

a lipophilic core. Other applications of cyclodextrins include the field of microbiology. Some cyclodextrins, such as dimethyl- β -cyclodextrin, have been used to increase production of *H. pylori*.¹² When added to agar gels cyclodextrins such as alpha- and beta-cyclodextrin/hexadecane are suitable foodbeds for the growth of microorganisms such as *Candida lipolytica* and *C. tropicalis*.¹⁹ Research has shown that cyclodextrin molecules, such as beta-cyclodextrin, when added to liquid cultures neutralize potential toxic combinations and increase the growth of microorganisms such as *H. pylori*.^{20–22} Solid cultures including modified cyclodextrins have been used for selective isolation of microorganisms such as *Bordetella pertussis*.^{23–26}

However other cyclodextrins, such as hydroxypropyl- β -cyclodextrin, have been reported to prevent bacterial production when used to coat vascular prostheses.¹³ Previous studies have reported methyl- β -cyclodextrins inhibiting the growth of bacillus types.²⁷ Researchers found that methyl- β -cyclodextrins crossed the cell membranes of bacillus species and caused cell lysis; however they emphasized that this activity was not observed for other gram negative and positive bacteria.²⁷ Another study found that cyclodextrin derivatives acted like antimicrobial peptid polymixin B and could inhibit bacterial proliferation.²⁸

There are no studies evaluating the effect of sugammadex, a modified gamma cyclodextrin molecule lately being used in anesthetic practice, on bacterial production. In our study, we found that sugammadex did not have antimicrobial properties with respect to the growth of *S. aureus*, *E. coli*, *P. aeruginosa* and *E. fecalis*.

Most bacteria prefer a fairly narrow pH range, between 6 and 8, for survival.^{3,17} The growth of *S. aureus* (ATCC 25923), *E. coli* (ATCC 25922) or *P. aeruginosa* (ATCC 27853) was not affected by growth conditions with a pH between 5.0 and 8.0.²⁹ The bactericidal properties of thiopental are thought to be related to its high pH.³⁰ Similarly, the pH range of midazolam was shown to be responsible for its bacterial inhibitory effect.^{5,7,31} In our study, prior to performing the recommended dilution, the pH of sugammadex was approximately 7.5. The diluted sugammadex had pH in a narrow range between 6.97 and 7.25. These pH values are within the range for proliferation of the test microorganisms *S. aureus* (ATCC 25923), *E. coli* (ATCC 25922) and *P. aeruginosa* (ATCC 27853).

In conclusion, sugammadex had no antibacterial effect on *S. aureus*, *E. fecalis*, *E. coli* and *P. aeruginosa*.

Conflicts of interest

The authors declare no conflicts of interest.

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