YOĞUN BAKIM ÜNİTESİNDE SEDASYON İÇİN KULLANILAN AJANLARIN ANTİBAKTERİYEL AKTİVİTELERİNİN İN VİTRO YÖNTEMLE DEĞERLENDİRİLMESİ

Evaluation of the Antibacterial Activities of the Agents Used for Sedation in Intensive Care Unit In Vitro Method

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ÖZET

Amaç: Yoğun bakımda sedasyon, anksiyetenin ve ajitasyonun tedavisinde kullanılmaktadır. Bu çalışmada, yoğun bakımda sedasyon amaçlı, infüzyon şeklinde yaygın olarak kullanılan propofol, ketamin, tiyopental, deksmedotomidin ve midazolam'ın pratikte kullanılan dozlarının antimikrobiyal aktivitesinin in vitro olarak araştırılması hedeflenmiştir.

Gereç ve Yöntem: İntravenöz anestezik ajanların in vitro antimikrobiyal aktiviteleri mikro dilüsyon tekniği ile araştırıldı. Testte kullanılan mikroorganizmalar aşağıdaki gibidir: Escherichia coli (ATCC 25922), Yersinia psödotüberküloz (ATCC 911), Pseudomonas aeruginosa (ATCC 10145), Listeria monocytogenes (ATCC 43251), Enterococcus faecalis (ATCC 29212), Staphylococcus aureus (ATCC 25923), Bacillus cereus 702 Roma, Mycobacterium smegmatis (ATCC 607), Candida albicans (ATCC 60193) ve Saccharomyces cerevisiae RSKK 251. Antibakteriyel deneyler, pH 7.3'te Mueller-Hinton sıvısında gerçekleştirildi ve pH 7.0'da tamponlu Maya Nitrojen Tabanı'nda antifungal analizler yapıldı.

Bulgular: Sadece ketamin içeren preparat antimikrobiyal aktivite gösterirken diğer ajanlar antimikrobiyal etki göstermedi.

Sonuç: Yapılan çalışma sonucunda ketamin in vitro ortamda yoğun bakım ünitesinde sık görülebilen mikroorganizmalar üzerinde antimikrobiyal etkinlik göstermiştir. Bununla birlikte, klinik kullanımda ketaminin antimikrobiyal etkinliğini, antibiyotik tedavisine potansiyel katkısını ve yoğun bakım ünitesi enfeksiyonlarının ortadan kaldırılmasına veya sepsisli hastalara ne kadar fayda sağlayacağını değerlendirmek için in vivo çalışmalara ihtiyaç vardır.

Anahtar Kelimeler: *Anestezikler; Ketamin; Yoğun bakım*

ABSTRACT

Objective: Sedation in the intensive care unit is used for the treatment of anxiety and agitation. This study aims to investigate the in vitro antimicrobial activity of propofol, ketamine, thiopental, dexmedetomidine and midazolam doses used in practice for sedation purposes in the intensive care unit.

Material and Methods: In vitro antimicrobial activities of intravenous sedative agents were investigated by using the microdilution technique. Microorganisms used in the test were as follows: Escherichia coli (ATCC 25922), Yersinia pseudotuberculosis (ATCC 911), Pseudomonas aeruginosa (ATCC 27853), Enterococcus faecalis (ATCC 29212), Staphylococcus aureus (ATCC 25923), Bacillus cereus 709 ROMA, Mycobacterium smegmatis (ATCC 607), Candida albicans (ATCC 60193), Candida tropicalis (ATCC 13803) and Saccharomyces cerevisiae RSKK 251. Antibacterial experiments were performed in Mueller-Hinton Broth with a pH of 7.3, and antifungal analyses were carried out in buffered Yeast Nitrogen Base with a pH of 7.0.

Results: Only the preparation with ketamine showed antimicrobial activity, whereas no antimicrobial activity was detected in the preparations with the other agents.

Conclusion: Ketamine has antimicrobial activity in vitro on microorganisms frequently seen in the intensive care unit practice. However, there is a need for in vivo studies to evaluate the antimicrobial efficacy of ketamine in clinical use, its potential contribution to antibiotic therapy, and how well it can eliminate intensive care unit infections or benefit the patients with sepsis.

Keywords: *Anesthetics; Ketamine; Critical Care*

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INTRODUCTION

Anxiety and agitation may develop in the intensive care unit patients due to various factors such as fear, loss of control, insomnia, pain, biochemical disorders, drugs, and fever (1–4). Lack of suppression of stress manifests as conditions such as hypertension, tachycardia, discomfort, hypoxia, and hypercapnia and struggle with a ventilator. Therefore, sedation plays an important part in the suppression of stress response. Sedation suppresses stress response, reduces anxiety, increases tolerance to ventilator support and facilitates the procedures such as aspiration, invasive procedures, and dressings (5,6). Clinical studies in the literature report that selection of the agent to be used for sedation affects the treatment results of the patient (7).

It is known that sedative agents delivered via venous catheters through continuous infusion can sometimes support the growth of microorganisms (8–10). Thus, these agents may induce systemic bacteraemias and wound infections. It is reported that some sedative agents can inhibit the growth of microorganisms (11).

Agents like midazolam, propofol, thiopental, ketamine, and dexmedetomidine are commonly used as intravenous infusions in the intensive care unit. The antimicrobial activities of midazolam, propofol, thiopental, ketamine, and dexmedetomidine may be advantageous for the infections depending on contamination. Moreover, the antimicrobial activities of these agents may provide a secondary benefit for the patients with sepsis in the intensive care unit. Therefore, of all the agents, those with antimicrobial activity may become prominent and be the primary choices by offering a secondary gain to patients with septic shock and patients in need of prolonged sedation.

The antimicrobial efficacy of the original clinical versions of these drugs on microorganisms found in the intensive care unit practice has not been adequately studied. In this study, the aim is to investigate the in vitro antimicrobial activities of propofol, ketamine, thiopental, dexmedetomidine, and midazolam, which were commonly used for sedation in the intensive care unit. Antimicrobial activity of the sedative drugs may prevent infections due to contamination and positively contribute to the treatment of the intensive care unit infections.

MATERIALS AND METHODS

In vitro antimicrobial activities of the drugs containing propofol, ketamine, thiopental, dexmedetomidine and midazolam available in the Turkish pharmaceutical market (Precedex 200 mg/mL, Ketalar 50 mg/ mL, Zolamid 5 mg/mL, Propofil 1% Fresenius 100mg/20 mL, Pental sodium 500 mg/20mL) were investigated.

Assessment of the Antimicrobial Activity

All of the tested microorganisms were obtained from the Public Health Agency of Turkey (Ankara, Turkey) and were as follows: Escherichia coli (ATCC 25922), Yersinia pseudotuberculosis (ATCC 911), Pseudomonas aeruginosa (ATCC 27853), Enterococcus faecalis (ATCC 29212), Staphylococcus aureus (ATCC 25923), Bacillus cereus 709 ROMA, Mycobacterium smegmatis (ATCC 607), Candida albicans (ATCC 60193), Candida tropicalis (ATCC 13803) and Saccharomyces cerevisiae RSKK 251.

Agar Well Diffusion Method

All bacteria were suspended in Mueller Hinton (MH) broth (Difco, Detroit, MI). The yeast-like fungi were suspended in yeast extracts (YE) broth (Difco, Detroit, MI). Then, the microorganisms were diluted to approximately 106 colony forming units (CFUs) per mL. Mueller Hinton and Brain heart infusion (BHI) agars with 0.02% Tween-80 were used for all bacteria and M. Smegmatis; respectively. For yeast-like fungi, Potato dextrose (PD) agar (Difco, Detriot, MI) was used. These were "flood-inoculated" onto the surface of MH, BHI, and PD agars and then dried. Wells 5 mm in diameter were cut from the agar using a sterile cork-borer, and 50 µL of the extracted substances were delivered into the wells (4). The plates (MHA and PDA) were then incubated at 35°C for 18 h and 48 h, respectively. M. smegmatis was grown for 3 to 5 days on BHI agar plates at 35°C. Antimicrobial activity was calculated by comparing the zone of inhibition against the test organism. Ampicillin (10 µg), streptomycin (10 µg), and fluconazole (5 µg) were used as standard drugs.

Determination of the minimum inhibitory and minimum bactericidal concentration of ketamine:

The antimicrobial effects of the substances were tested quantitatively in respective broth media by using double dilution and the minimum inhibitory concentration (MIC) values (µg/mL) were determined (4, 5). Antibacterial and antifungal assays were performed in MH broth at pH 7.3, and in YE at pH 7.0, respectively. Dilution of ketamine to be tested was prepared in 0.1 ml volumes of sterile MH, BHI and YE broths to yield concentrations ranging from 5000 µg/mL to 5 µg/mL. After preparation of suspensions of test microorganisms in MH, BHI and YNB broths (approximately 106 microorganisms per mL), one drop of suspension (0.02 ml) was added to the extract/broth dilutions. After incubation at 35°C for 18-72 h, the tubes were examined for growth. The MIC was defined as the lowest concentration that showed no growth. The dilutions without visible growth were used to determine the minimum bactericidal concentration (MBC); the samples (100 µL) were spread across the surface of dried MH and YNB agar with sterile, bent glass rods and then incubated at 35°C for 18-72 h. For each extract, the lowest concentration that showed no growth on an agar plate was defined as the MBC. Ampicillin (10 mg/mL), Streptomycin (10 mg/mL) and fluconazole (2 mg/mL) were used as standard antibacterial and antifungal drugs, respectively.

MIC (The minimum inhibitory concentration): This dose may be bactericidal or bacteriostatic (inhibition of the bacterial growth and reproduction continues when the drug activity ceases). It was found that MBC determines MIC.

MBC (The minimum bactericidal (killing) concentration): It is the lowest concentration of an antibacterial agent required to kill a particular bacterium.

RESULTS

Of all the drugs (propofol, ketamine, thiopental, dexmedetomidine, and midazolam) tested using microdilution and Agar well methods, only ketamine was determined to have antimicrobial activity. Ketamine has antimicrobial activity against all of the tested microorganisms, but it showed the highest activity against Gram-positive bacteria and M. smegmatis (Table 1, Figure 1).

Table 1: Antimicrobial activity of various sedative drugs (µg/mL).

Drug Name	**Stock Conc.**	**Test Conc. µg/mL**	**Microorganisms and Inhibition Zone (mm)**									
			Ec	Yp	Pa	Sa	Ef	Bc	Ms	Ca	Ct	Sc
Dexmedetomidine	200 mg / 2mL	5	-	-	-	-	-	-	-	-	-	-
½ dilution		2,5	-	-	-	-	-	-	-	-	-	-
Midazolam	5 mg / mL	250	-	-	-	-	-	-	-	-	-	-
½ dilution		125	-	-	-	-	-	-	-	-	-	-
Ketamine	50 mg / mL	2 500	10	10	10	18	15	15	30	8	10	8
½ dilution		1250	6	6	8	14	10	10	20	6	6	6
Propofol 1%	100 mg / 20 mL	250	-	-	-	-	-	-	-	-	-	-
½ dilution		125	-	-	-	-	-	-	-	-	-	-
Thiopental Sodium	500 mg / 20 mL	1250	-	-	-	-	-	-	-	-	-	-
½ dilution		652	-	-	-	-	-	-	-	-	-	-
Amp.		10	10	10	18	10	35	15			-	
Strep.		10							35		-	
Flu.		2								25	25	25

Ec: E. coli ATCC 35218, Yp: Y. pseudotuberculosis ATCC 911, Pa: P. aeruginosa ATCC 27853, Sa: S. aureus ATCC 25923, Ef: E. faecalis ATCC 29212, Bc: B. cereus 709 Roma, Ms: M. smegmatis ATCC607, Ca: C. albicans ATCC 60193, C. tropicalis ATCC13803, S. cerevisiae RSKK 251, Amp.: Ampicillin, Strep.: Streptomycin, Flu.: Fluconazole,(—): no activity at the tested concentration.

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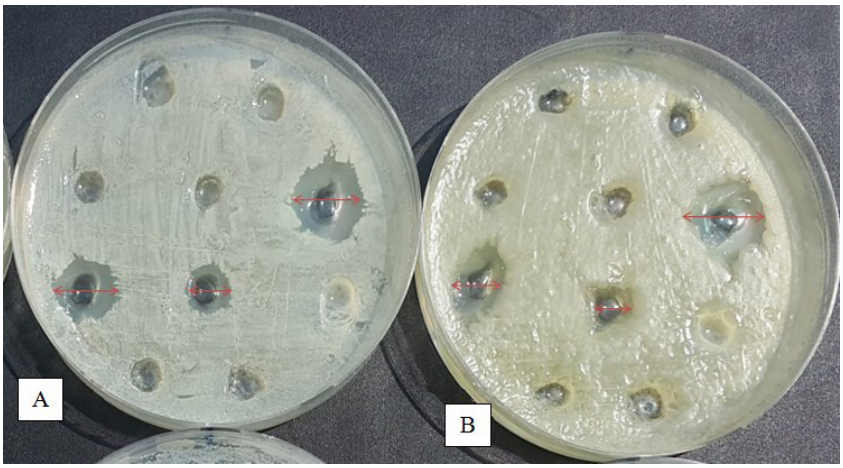

Figure 1. Antimicrobial activity of Ketamine with Agar well method. A; S. aureus, B; B. cereus, Marked areas with red arrows show the sizes of the zones of inhibition caused by ketamine in concentrations of 2500, 1250 and 650 mg-1 mL.

Antimicrobial activity of ketamine and its MBC and MIC values were determined by using the microdilution method. Ketamine's antimicrobial activity had an inhibitory effect on the growth of B. cereus, with a MIC of 15.6 mg/mL, and it had bactericidal activity against other tested microorganisms. It was observed that the concentration of ketamine required to have a bacteriostatic or fungistatic effect was between 15.6-250 mg/mL, the most sensitive microorganisms were S. aureus and M. smegmatis, and MIC and MBC values were approximately 15.6 mg/mL (Table 2).

Table 2. Antimicrobial activity of ketamine (µg/mL).

Tested Microorganisms	Ketamine (50 mg/mL)	
	Minimum Inhibitory Concentration (MIC)	Minimum Bactericidal Concentration (MBC)
E. coli ATCC 35218	250	250
Y. pseudotuberculosis ATCC 911	250	250
P. aeruginosa ATCC 27853	125	250
S. aureus ATCC 25923	7.8	15.6
E. faecalis ATCC 29212	62.5	125
B. cereus 709 Roma	15.6	-
M. smegmatis ATCC607	15.6	15.6
C. albicans ATCC 60193	125	125
C. tropicalis ATCC13803	125	125
S. cerevisiae RSKK 251	125	125

DISCUSSION

In our study, antimicrobial activities of propofol, ketamine, thiopental, dexmedetomidine and midazolam available in their original clinical forms (Precedex 200 mg/mL, Ketalar 50 mg/ mL, Zolamid 5 mg/mL, Propofil 1% Fresenius 100mg/20 mL, Pental sodium 500 mg/20mL) were investigated by using microdilution and Agar well methods. Some of the studies in the literature, which evaluate the antimicrobial efficacy of local anesthetics were included.

On the other hand, there are a small number of studies evaluating the antimicrobial efficacy of sedative agents used in intensive care.

It was observed that the clinical form of the sedative drug which contains ketamine alone exhibited antimicrobial activity against E. coli, Y. pseudotuberculosis, P. aeruginosa, S. aureus, E. faecalis, B. Cereus, M. smegmatis, C. albicans, C. tropicalis and S. cerevisiae. It can also be suggested that hydrochloric acid, which was used as a preservative in the preparations containing ketamine, plays a role in and contributes to the antimicrobial activity of the drug. However, the absence of antimicrobial activity in the dexmedetomidine and midazolam preparations containing hydrochloric acid as a preservative may indicate that the actual sedative agent itself is important for the antibacterial effect. In addition, it has been suggested that sedative agent and preservative together may have a synergistic effect.

In previous studies, antimicrobial effects of various sedative agents have been studied, but the mechanism underlying their antibacterial activity has not been fully understood. Several studies found that the antimicrobial effects of some of the sedative agents may depend on their molecular weight, pH, and thermodynamic activity (12–14). A number of researchers revealed the interaction between the cytoplasmic membrane and macromolecule, through which the agents can alter membrane functions by decreasing the number of viable cells and causing the lysis of protoplasts, thus changing the permeability (12).

In a study investigating the antimicrobial activity of dexmedetomidine and midazolam, microdilution method was used to determine the antimicrobial activities of the agents on Staphylococcus aureus, Enterococcus faecalis, Escherichia coli and Pseudomonas aeruginosa, and it was observed that midazolam exhibited inhibitory and bactericidal activity against S. aureus and E. Faecalis, while dexmedetomidine only had inhibitory activity against S. aureus, E. coli, and P. aeruginosa (15). It was suggested that midazolam's prominent bactericidal activity might be due to hydrochloric acid included in the preparation as a preservative. On the other hand, midazolam did not show any antibacterial effect in our study. Conflicting results may be attributable to the experimental conditions: bacterial strains used, methods of evaluating bacterial growth and/or drug dilutions, differences in methods, and the pH of the medium. In their study, Pelz et al. investigated the antimicrobial effects of preservatives in addition to the antimicrobial activities of anesthetics and found that this effect was not attributable to preservatives alone. They suggested that the agents and the preservatives showed a synergistic effect (16). Keles et al. showed that midazolam had antimicrobial activity on E.coli, P. aeruginosa, A. baumannii, E. coli ESBL, whereas dexmedetomidine had no antimicrobial activity on these microorganisms (17). Durak et. al reported that midazolam demonstrated an inhibitory effect on the strains of S. aureus and E. faecalis at a concentration higher than the one used in clinical practice. Antimicrobial activities of these drugs may depend on their concentration (18).

Gocmen et al. investigated the antimicrobial activity of ketamine on S.aureus, S.epidermidis, E.faecalis, S.pyogenes, S.aeruginosa and E.coli by using disc diffusion method. They determined that discs containing 500 and 250 µg of ketamine were as effective as ciprofloxacin and inhibited bacterial growth, discs containing 125 µg of ketamine had antimicrobial activity against other bacteria except for E.coli, and discs containing 6.25 µg of ketamine did not exhibit any antimicrobial activity (19). In this study, similar to our results, ketamine showed antimicrobial activity.

There are studies in the literature investigating the antimicrobial activities of the drugs that are not used as antibiotics (20). In addition, ketamine is among the drugs with antibacterial activity, but it is not used as an antibiotic (18). The results obtained in our study regarding the antibacterial activity of ketamine are similar to the results obtained in the previous studies in the literature. In addition to the inhibitory and bactericidal effects of ketamine against all bacteria except B.cereus, the results indicate that this drug may have a broad spectrum antibacterial activity and its potent bactericidal activity may be useful.

Some of the studies in the literature have found positive results regarding the antibacterial activity of midazolam and dexmedetomidine. It can be said that these differences might particularly be due to the differences in concentration. While the antibacterial activity was assessed by using dilutions of the clinical forms in our study, previous studies usually focused directly on the antibacterial activity of the active ingredient and investigated using higher concentrations of the drug. We think that, in our study, testing the doses used in the daily practice is important as it demonstrates the availability of these drugs' antibacterial activity in clinical practice.

In conclusion, our study shows that dexmedetomidine, midazolam, propofol, thiopental, and dexmedetomidine lack antibacterial properties, but ketamine has antimicrobial activity. The dose of ketamine used in the clinical practice can have antimicrobial activity and ketamine may reduce the infections that develop due to contamination. Potentially, this effect of ketamine may positively contribute to the elimination of infections in the intensive care unit. However, further animal and clinical studies are required to evaluate the usability of the antimicrobial activity of ketamine in the clinic, and the extent of its efficacy in reducing the infections in the intensive care unit.

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