IN VITRO INVESTIGATION OF ANTIBACTERIAL ACTIVITY OF DRUGS USED IN SEDATION IN INTENSIVE CARE UNIT

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ABSTRACT

Introduction: *Sepsis is a systemic inflammatory response to infection and is a life-threatening condition. Sedation is important such as these patients. Some drugs that used for sedation may have antimicrobial effects. The aim of this study was to investigate the antimicrobial activity of propofol, ketamine, thiopental, dexmedotomidine and midazolam, which are widely used in intensive care in the form of infusion for sedation.*

Materials and methods: *In vitro antimicrobial activities of dexmedetomidine, midazolam, ketamine, propofol and thiopental sodyom drugs of different concentrations were investigated by using the agar well diffusion method. Ampicillin, streptomycin and fluconazole were used as standard antibacterial and antifungal drugs, respectively. While selecting the microorganisma, the agents which are unexpected to cause sepsis were included in the study in addition to the causative agents leading to sepsis, in order to understand the antimicrobial effect more clearly. All test microorganisms as follows: E. coli ATCC, Y.pseudotuberculosis ATCC, P. auroginosa ATCC, E. faecalis ATCC, S. aureus ATCC, B. cereus, M. smegmatis ATCC, C. albicans ATCC, C. tropicalis ATCC and S. cerevisiae ATCC.*

Results: *Ketamine was found to have antimicrobial activity against all of the microorganisms tested, but the highest efficacy was determined against S. aureus. Bactericidal (and fungocidal) activity of ketamine was determined against all other microorganisms.*

Conclusion: *We consider that it can be important to conduct the trial on the doses used in practice, and to demonstrate the usefulness of the antibacterial activity of these drugs in practice. In conclusion, in patients with sepsis followed in the intensive care unit, ketamine may be preferred for sedation. Thus, this can contribute to the patient's antibiotherapy. However, further clinical research is needed to evaluate the effectiveness of antimicrobial activity of ketamine in clinical practice and to assess the effectiveness of reducing the mortality in patients with sepsis.*

Keywords: *Antimicrobial activities, dexmedetomidine, midazolam, ketamine, propofol and thiopental sodyom.*

DOI: 10.19193/0393-6384_2019_6_552

Received *November 30, 2018;* ***Accepted*** *February 20, 2019*

Introduction

Sepsis is a systemic inflammatory response to infection and is a life-threatening condition. However, despite intensive care treatment, the mortality rate in sepsis is high. Antibiotherapy against sepsis is one of the most important parts of the treatment. A rapid, appropriate and intensive treatment approach can reduce mortality. Therefore, the treatment of patients diagnosed with sepsis should be done in intensive care[(1)]. Anxiety and agitation may develop in intensive care patients due to various factors such as fear, loss of control, insomnia, pain, biochemical disorders, drugs, and fever[(2)].

Non-suppression of stress is characterized by hypertension, tachycardia, discomfort, hypoxia and hypercapnia.

Therefore, sedation is important in suppressing stress response. Sedation suppresses stress response, reduces anxiety, increases tolerance to ventilator support, and facilitates procedures such as aspiration, invasive procedures, and dressings[(3,4)]. The selection of the drug used for sedation has been reported to affect the treatment results of the patient[(5)]. In the intensive care units, agents such as midazolam, propofol, thiopental, ketamine and dexmedetomidine are used frequently and in the form of infusion[(2,5)].

Studies on the antimicrobial effects of these drugs used in patients with sepsis in intensive care are limited.The aim of this study was to investigate the antimicrobial activity of propofol, ketamine, thiopental, dexmedotomidine and midazolam, which are widely used in intensive care in the form of infusion for sedation.

Materials and methods

Invitro antimicrobial activity of drug containing midazolam, propofol, thiopental, ketamine and dexmedetomidine was investigated.

Antimicrobial activity assessment

The microorganisms leading to sepsis such as Escherichia coli or Pseudomonas auroginosa were included in the study. In addition, the microorganisms that rarely cause sepsis such as Bacillus cereus or Saccharomyces cerevisiae were also included for understanding the antimicrobial effect more clearly. All test microorganisms were obtained from the Turkey Public Health Agency (Ankara, Turkey) and were as follows: Escherichia coli ATCC 25922, Yersinia pseudotuberculosis ATCC 911, Pseudomonas auroginosa ATCC 27853, Enterococcus faecalis ATCC 29212, Staphylococcus aureus ATCC 25923, Bacillus cereus 709 ROMA, Mycobacterium smegmatis ATCC607, Candida albicans ATCC 60193, Candida tropicalis ATCC13803 and Saccharomyces cerevisiae ATCC 60193.

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, Poteto dextrose (PD) agar (Difco, Detriot, MI) was used. These were "flood-inoculated" onto the surface of MH, BHI and PD agars and then dried. Five-millimeters diameter wells were cut from the agar using a sterile cork-borer, and 50 µL of the extract substances was delivered into the wells[6]. The plates (MHA and PDA) were then incubated at 35°C for 18 h and 48 h respectivly. 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 minimal inhibitory concentration (MIC) and minimal bactericidal concentration (MBC)

Stock solutions of Dexmedetomidine (200 mg/2mL), Midazolam (mg / mL), Ketamine (50 mg / mL), Propofol (%1, 100 mg/20 mL) and Thiopental Sodyom (500 mg / 20 mL) were used for determination of minimal inhibitory and minimal bactericidal concentration.

Ketamine

The antimicrobial effects of the substances were tested quantitatively in respective broth media using double dilution and the minimal inhibitory concentration (MIC) values (μg/mL) were determined[7,8]. The antibacterial and antifungal assays were performed in MH broth at pH7.3 and 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 give concentrations ranging from 5000 µg/mL to 5 µg/mL. After preparation of suspensions of test microorganisms in MH, BHI and YE broth (approximately 106 microorganisms per mL), one drop of suspension (0.02 mL) was added to the extract/broth dilutions. After 18-72 h at 35 °C incubation, the tubes were then examined for growth.

The MIC was defined as the lowest concentration that showed no growth. The dilutions without visible growth were used for minimal bactericidal concentration (MBC) determination; spread 100 µL samples across the surface of dried MH, BHI and PD agar whit sterile, bent glass rods and then incubated 18-72 h at 35 °C. The MBC of each extract was taken as the lowest concentration that showed no growth on agar plate. Ampicillin (10 mg/mL), Streptomycin (10 mg/mL) and fluconazole (2 mg/mL) were used as standard antibacterial and antifungal drugs, respectively.

Definitions

MIC: Minimum inhibitory concentration. This dose may be bactericidal or bacteriostatic (which stops reproduction, continues to proliferate once the effect of the drug has gone away). This is done by MBC to determine.

MBC: Minimum bactericidal concentration. That means the lowest drug concentration value that kills bacteria.

Results

It was determined that only ketamine showed antimicrobial activity among the drugs (propofol, midazolam, thiopental, dexmedotomidine and midazolam) tested by microdilution and agar well. Ketamine was found to have antimicrobial activity against all of the microorganisms tested, but the highest efficacy was determined against gram positive bacteria and M. smegmatis (Table 1, Figure 1).

Drug Name	Test Conct. µg/mL	Microorganisms and Inhibition Zone (mm)									
		Ec	Yp	Pa	Sa	Ef	Bc	Ms	Ca	Ct	Sc
Dexmedetomidine	5	-	-	-	-	-	-	-	-	-	-
1/2 dilution	2.5	-	-	-	-	-	-	-	-	-	-
Midazolam	250	-	-	-	-	-	-	-	-	-	-
1/2 dilution	125	-	-	-	-	-	-	-	-	-	-
Ketamine	2 500	10	10	10	18	14	15	30	8	12	8
1/2 dilution	1250	6	6	8	14	10	10	20	6	6	6
Propofol %1	250	-	-	-	-	-	-	-	-	-	-
1/2 dilution	125	-	-	-	-	-	-	-	-	-	-
Thiopental Sodyom	1250	-	-	-	-	-	-	-	-	-	-
	652	-	-	-	-	-	-	-	-	-	-
Amp.	10	10	10	16	10	35	15			-	
Strep.	10							30		-	
Flu.	2								25	25	25

Table 1: Antimicrobial activity of the some anesthetics drugs (µg/mL).
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 of test concentrations.

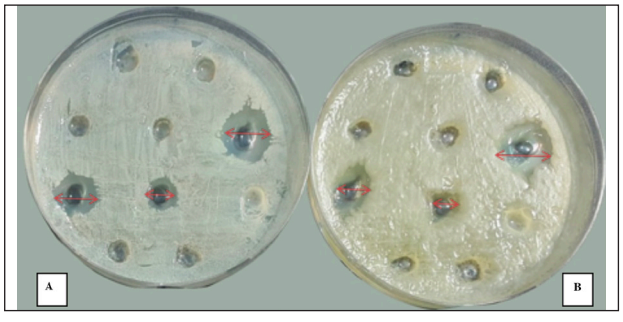

Fig.1: Efficacy of Ketamine in Agar well method. a; S. aureus, B; B. cereus, marked by red arrows, are zones of inhibition zone formed by the amount of Ketamine in concentrations of 2500, 1250 and 650 konsantrasyong / mL, respectively..

MIC value of ketamine against B. cereus was 15.6 mg/mL. Bactericidal (and fungocidal) activity of ketamine was determined against all other microorganisms. MBC values of ketamine was determined in the range of 15.6-250 mg/mL. The most sensitive microorganisms were S. aureus and M. smegmatis, and the MIC and MBC values against these bacteria were found to be 15.6 mg / mL (Table 2).

Tested Microorganisms	Ketamine 50 mg/mL	
	Minimal Inhibition Concentration (MIC)	Minimal Bactericide Concentration (MBC)
S. aureus ATCC 25923	78.125	156.25
E. faecalis ATCC 29212	625	1250
E. coli ATCC 35218	2500	2500
P. aeruginosa ATCC 27853	1250	2500
Y. pseudotuberculosis ATCC 911	2500	2500
B. cereus 709 Roma	156.25	-
M. smegmatis ATCC607	156.25	156.25
C. albicans ATCC 60193	1250	1250
C. tropicalis ATCC13803	1250	1250
S. cerevisiae RSKK 251	1250	1250

Table 2: Antimicrobial activity of the Ketamine (µg/mL).

Discussion

In the intensive care units, sedation is used to treat anxiety and agitation. In intensive care patients, sedation suppresses stress response, reduces anxiety and increases tolerance to ventilator support. Propofol, ketamine, thiopental, dexmedotomidine and midazolam are frequently used for sedation in intensive care unit[8,9].Antimicrobial activity of midazolam, propofol, thiopental, ketamine and dexmedetomidine may provide a secondary benefit in sedation patients with sepsis. Therefore, those who have antimicrobial effect from these drugs may gain a secondary benefit and may be more prominent in patients with septic shock and in patients who need long-term sedation and may be among the first choices. In a study examining the antibacterial effect of dexmedetomidine and midazolam, the antimicrobial effects against S. aureus, Enterococcus faecalis, E. coli and P. aeruginosa were assessed using microdilution method. E. coli and P. aeruginosa showed only inhibitory effect They found that midazolam had inhibitory activity against S. aureus and E. faecalis. They also reported inhibitory effect of deksmedetomidin against S. aureus, E. coli and P. aeruginosa[10]. Pelz et al.[11]. investigated the antimicrobial effects of anesthesic drugs and the preservatives they contain.

They found that the main cause of antimicrobial effect was not preservatives, and that the active

substances and preservative had a synergistic effect. Keles et al.[12] showed that midazolam had antimicrobial effect on E.coli, P. aeruginosa, A. baumannii and E. coli, but dexmedetomidine had no antimicrobial effect on these tested microorganisms. Göcmen et al.[13] investigated antimicrobial activity of ketamine against S. aureus, S.epidermidis, E.faecalis, S.pyogenes, P.aeruginosa and E.coli using disc diffusion method. They found that discs containing 500 and 250 mg of ketamine were as effective as ciprofloxacin and that they inhibit bacterial growth. They also found that 125 µg ketamine containing discs had antibacterial activity against bacteria other than E.coli, and also stated that disc containing 6.25 µg ketamine did not show antibacterial effect[13].

Of the antimicrobials included in the study, only the commercial form of ketamine showed antimicrobial affect against E. coli, Y. pseudotuberculosis, P. aeruginosa, S. aureus, E. faecalis, B. Cereus, M. smegmatis, C. albicans, C. tropicalis, and S. cerevisiae. It can be said that substances used as preservatives (i.e. benzetonium chloride) in the preparations containing ketamine might play a role in and contribute to the antibacterial effect. In our study, the antibacterial activity of ketamine was similar to the results obtained from previous studies. In addition, the fact that ketamine showed inhibitory and bactericidal effect against most bacteria in the study shows that the antibacterial activity spectrum of this drug is wide and the potency of bactericidal effect is good.

Some of the studies in the literature have positive results related to the antibacterial activity of midazolam and dexmedetomidine[10,13]. In our study, commercial forms of these drugs did not show antibacterial activity. These differences may be due to the difference in concentration. Because antimicrobial activity was evaluated using the dilutions of the commercial forms of the antimicrobials, but the previous studies tested the antimicrobial activity using the active substance directly and they used higher concentrations of the drugs.

Conclusions

In the present study, we consider that it can be important to conduct the trial on the doses used in practice, and to demonstrate the usefulness of the antibacterial activity of these drugs in practice. In conclusion, in patients with sepsis followed in the intensive care unit, ketamine may be preferred for sedation. Thus, this can contribute to the patient's antibiotherapy. However, further clinical research is needed to evaluate the effectiveness of antimicrobial activity of ketamine in clinical practice and to assess the effectiveness of reducing the mortality in patients with sepsis.

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