Rev Bras Anestesiol. 2013;**63(6)**:461–5

REVISTA BRASILEIRA DE ANESTESIOLOGIA

Official Publication of the Brazilian Society of Anesthesiology
www.sba.com.br

SCIENTIFIC ARTICLE

The antimicrobial effects of ketamine combined with propofol: An in vitro study☆

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Received 9 August 2012; accepted 3 September 2012

KEYWORDS
Antimicrobial activity;
Benzethonium chloride;
Ketamine;
Ketofol;
Propofol

Abstract
Background and objectives: Ketamine and propofol are the general anesthetics that also have antimicrobial and microbial growth-promoting effects, respectively. Although these agents are frequently applied together during clinical use, there is no data about their total effect on microbial growth when combined. In this study, we investigated some organisms' growth in a ketamine and propofol mixture.
Method: We used standard strains including *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Candida albicans* in this study. Time-growth analysis was performed to assess microbial growth rates in 1% propofol. Antimicrobial activity of ketamine, alone and in propofol was studied with microdilution method.
Results: In propofol, studied strains grew from 10^3-10^4 cfu/mL to ≥10^5 cfu/mL concentrations within 8-16 hours depending on the type of organism. Minimal inhibitory concentration (MIC) and minimal bactericidal concentration (MBC) (for candida, minimal fungicidal concentration) of ketamine were determined as follows (MIC, MBC): *E.coli* 312.5, 312.5 μg/mL; *S.aureus* 19.5, 156 μg/mL; *P.aeruginosa* 312.5, 625 μg/mL; and *C.albicans* 156, 156 μg/ml. In ketamine+propofol mixture, ketamine exhibited antimicrobial activity to *E.coli*, *P.aeruginosa* and *C.albicans* as MBCs at 1250, 625 and 625 μg/mL, respectively. Growth of *S. aureus* was not inhibited in this mixture (ketamine concentration=1250 μg/mL).
Conclusion: Ketamine has sustained its antimicrobial activity in a dose-dependent manner against some organisms in propofol, which is a strong microbial growth-promoting solution. Combined use of ketamine and propofol in routine clinical application may reduce the risk of infection caused by accidental contamination. However, one must keep in mind that ketamine cannot reduce all pathogenic threats in propofol mixture.

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doi: 10.1016/j.bjane.2012.09.004

Introduction

Propofol is a widely used sedative-hypnotic drug that is administered in the induction and maintenance of anesthesia. Propofol is considered as a good microbial growth-promoting agent due to its rich nutritional content, like soybean oil, glycerol, and egg lecithin.[1,2] Accordingly, severe infections have been reported in patients following the use of contaminated propofol.[3,4]

Ketamine is a general anesthetic that has an antagonist effect on the n-methyl d-aspartate receptors. It is characterized by rapid onset of actions, including analgesia, anesthesia, elevated blood pressure, and dilatation in lower airways. Considering its favorable effects on the cardiovascular and pulmonary system, ketamine may be particularly valuable for induction of anesthesia in a hypovolemic patient.[5,6] Additionally, some studies have documented ketamine's antimicrobial activity.[7,8]

Combination of ketamine and propofol (ketofol) is shown to be pharmaceutically compatible when applied in the same syringe. Several studies have reported that ketofol has positive regulatory activity on the hemodynamic parameters in human volunteers.[9-11] Regarding the high microbial growth-promoting effect of propofol and antimicrobial activity of ketamine, investigation of the total effect of their combination on the growth of some bacteria and yeast was thought to be valuable. Therefore, we conducted an in vitro study to determine the effect of ketofol mixture on some clinically important organisms that were the significant pathogens of hospital-acquired infections in the world.

Materials and methods

Drugs and microorganisms

We used ketamine (Ketalar® 50mg/ml Pfizer), and1% propofol (Propofol® %1 Fresenius) in this study. Drug mixtures were prepared in aseptic conditions.

In this study, we used the following standard strains: *Escherichia coli* (ATCC 25922) (RSHM/Turkey), *Staphylococcus aureus* (ATCC 29213) (Oxoid/UK), *Pseudomonas aeruginosa* (ATCC 27853) (Oxoid/UK) and *Candida albicans* (ATCC 14053) (Oxoid/UK).

Microbial-growth-promoting activity of propofol

We studied growth rates of the tested microorganisms in time-growth analyses. Briefly, we selected bacterial and fungal colonies grown on nutrient agar plates and suspended in sterile 0.9% physiologic saline at McFarland 0.5 density. These suspensions were re-suspended in propofol to adjust final concentration of organisms to 1-2x10^4 bacteria per mL, and 4-5x10^3 yeast per mL. We incubated these suspensions at 35°C for 24 hours. In 2-hour periods, subcultures to nutrient agar mediums were performed between 0 and 24 hours. We read the number of colony-forming units (cfu/mL) grown on the plates visually by single investigator.

Antimicrobial activity of ketamine

We investigated the impact of ketamine, alone and in propofol mixture, on microbial growth rates of each organism with microdilution method according to published standards for antimicrobial susceptibility tests by Clinical and Laboratory Standards Institute (CLSI),[12,13] and previously published study by Gocmen et al.[7] We accepted the ketamine concentration that was associated with 100% inhibition of yeast growth as MIC value for *C. albicans*.

Antimicrobial activity of ketamine in standard tests

Briefly, we serially diluted ketamine in 100 µL cation adjusted Mueller Hinton Broth (Himedia/India) (for bacterial strains), and in MOPS (Applichem GmbH/Germany)-buffered RPMI 1640 (Sigma/Germany) liquid medium (for candida) in sterile plysteren 96-well plates. Then, we prepared bacterial and fungal suspensions in sterile 0.9% physiologic saline at McFarland 0.5 density. We re-suspended these suspensions in their standard broths. A 100 µL volume of inoculums was distributed into each well. Final ketamine concentrations ranged from 1250 to 1.22 µg•mL^{-1} in the wells with 2.5-5x10^4 cfu•mL^{-1} inoculums of bacteria, or 1-5x10^3 cfu•mL^{-1} inoculums of candida. After 24-h incubation at 35°C, we determined minimal inhibitory concentrations (MIC) by visual reading. For candida strain, 100% inhibition was accepted as the MIC value regarding the drug-free inoculums control. We determined minimal bactericidal concentrations (for candida, minimal fungicidal concentration) (MBC) by performing subcultures from the wells showing inhibition of visible bacterial of fungal growth to appropriate nutrient agar mediums. We accepted the drug concentration in the well that had 99.9% inhibition of the tested strain as the MBC. We prepared control cultures for microorganisms, broth, and drug solution.

Antimicrobial activity of ketamine in propofol

We serially diluted ketamine in propofol solution in sterile plysteren 96-well plates. The bacterial and fungal suspensions prepared in sterile 0.9 % physiologic saline at McFarland 0.5 density were re-suspended in propofol, and distributed into each well as equal aliquots. Final ketamine concentrations ranged from 1250 to 1.22 µg/mL in the wells with 2.5-5x10^4 cfu•mL^{-1} inoculums of bacteria, or 1-5x10^3 cfu•mL^{-1} inoculums of candida. After 24-h incubation at 35°C, we determined MBCs by performing subcultures from the wells onto the appropriate agar mediums as described above. We performed control cultures for microorganisms, propofol and drug solution.

Results

Microbial growth in propofol 1%

Fig. 1 shows growth rates of the tested strains in propofol 1% suspension. At the first two hours of the incubation, we detected no significant growth for *E. coli*, *P. aeruginosa*,

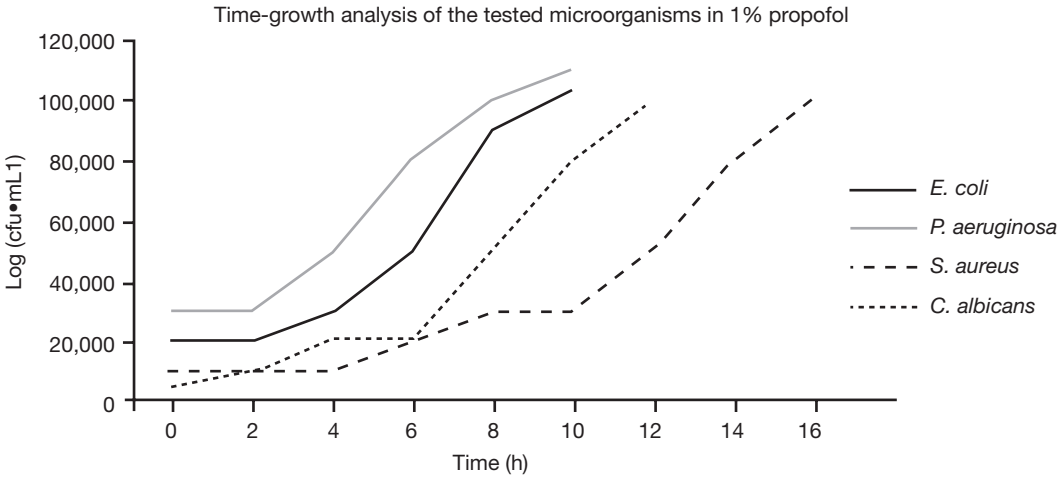

Figure 1 Growth rates of the organisms in 1% propofol solution in 2-h periods.

Table 1 Minimal inhibitory concentration (MIC) and minimal bactericidal concentration (MBC) values of ketamine alone and in propofol mixture for the studied organisms.

Organisms	Ketamine		Ketamine + Propofol
	MICs (μg•mL^{-1})	MBCs (μg•mL^{-1})	MBCs (μg•mL^{-1})
E. coli	312.5	312.5	1,250
S. aureus	19.5	156	> 1,250
P. aeruginosa	312.5	625	625
C. albicans	156	156	625[a]

[a] Minimal fungicidal concentration.

and *S. aureus* regarding time zero. But, the concentration of *C. albicans* doubled (from 5x10^3 cfu•mL^{-1} to 1x10^4 cfu•mL^{-1}) within the same interval. *E. coli* and *P. aeruginosa* reached ≥ 1x10^5 cfu•mL^{-1} concentration at 10th and 8th hours of incubation, *C. albicans* at 14th hours, and *S. aureus* at 16th hours.

Antimicrobial activity of ketamine in standard tests, and in propofol mixture

Ketamine showed an in vitro antimicrobial effect against all tested strains in standard antimicrobial susceptibility tests. We determined the lowest ketamine MIC for *S. aureus* as19.5 μg•mL^{-1}; and measured the highest MIC for *E. coli* and *P. aeruginosa* as 312.5 μg•mL^{-1}. Regarding the MBCs of ketamine, we detected the lowest value for *S. aureus* and *C. albicans*as 156 μg•mL^{-1}, and determined the highest value for *P. aeruginosa* as 625 μg•mL^{-1}.

In propofol mixture, ketamine MBCs for *P. aeruginosa* and *C. albicans*were measured as 625 μg•mL^{-1}, and for *E. coli* 1,250 μg•mL^{-1}. We could not determine MBC of *S. aurues* (MBC > 1,250 μg•mL^{-1}), as no inhibition occurred.

Table 1 shows the measured MIC and MBC values of ketamine for each organism.

Discussion

In this study, we determined that propofol was a strong microbial growth promoting solution not only for bacteria but also for funghi. Considering the organisms' types, gram negatives, *P. aeruginosa* and *E. coli*, showed the fastest growth index, reaching 1x10^5 cfu•mL^{-1} concentration within 8 and 10 hours, respectively, while *C. albicans* reached this concentration at the 14th hour. However, *S. aureus* had relatively the slowest growth rate among all tested strains (Fig. 1).

Ketamine is a drug that is primarily used for the induction and maintenance of general anesthesia. It is a core medicine which is categorized in the World Health Organization's "Essential Drug List".[14] Ketamine has a wide range of effects on humans, including analgesia, anesthesia, hallucinations, elevated blood pressure, and bronchodilation. In 2008, Gocmen et al.[7] published an in vitro study reporting that ketamine had antimicrobial activity against some streptococci, staphylococci, *E. coli*, and *P. aeruginosa* between 500-2,000 μg•mL^{-1} concentrations. As for the fact that anesthetic blood level of ketamine was about 2 μg•mL^{-1}, they state that this antibacterial activity cannot be seen in humans during anesthesia.

We observed that ketamine had a potential antibacterial and antifungal activity on the tested strains. Regarding the organisms' types, we found *P. aeruginosa* and *E. coli* to be more resistant, and *S. aureus* were the most susceptible to ketamine. We detected that *S. aureus* had 5 logs lower MIC and 2 logs lower MBC values than the gram negatives. On the other hand, MIC and MBC values of ketamine for *C. albicans* were equal in this experiment.

In this study, we investigated the antimicrobial activity of ketamine in propofol mixture. This mixture has been used successfully in different clinical situations including monitored care anesthesia, electroconvulsive therapy, procedural sedation, and analgesia in emergency patients.[9-11,15] In the ketofol mixture, we detected that ketamine sustained its antibacterial and antifungal activities at higher MBCs. In our study, we could not measure MICs of ketamine in propofol, due to fact that turbid solution was formed in the microplates following the propofol inclusion, which did not allow for clear visual evaluation. Therefore, we detected only MBCs of ketamine in the ketofol mixture. Regarding the tested strains, we observed twofold increase in the MBC values of ketofol mixture for *E. coli* and *C. albicans*. However, a certain MBC of ketofol was not determined for *S. aureus*, due to fact that its value was over the test's detection limit. Interestingly, MBC of this mixture did not change in comparison to ketamine alone, and remained stable for *P. aeruginosa* at 625 $\mu g \bullet mL^{-1}$.

Infection is a considerable concern during the clinical use of propofol. Particularly because of its lipid base, propofol provides a preferable media for many classes of microorganisms. Therefore, nosocomial postoperative infections which impose heavy burden of morbidity and mortality and have serious economic consequences can develop due to the contamination of propofol.[16] Mueller et al.[4] reported an outbreak of sepsis caused by gram negative organisms including *Klebsiella pneumoniae* and *Serratia marcescens* in 7 patients, due to contaminated propofol use in minor surgical procedures. Similarly, Henry et al.[17] reported postoperative bacteremia and wound infections caused *S. marcescens,* following the propofol use in Canada. Additionally, Bennett et al.[18] reported propofol-related infections including bloodstream infections, surgical site infection, and acute febrile episodes in 62 cases, after surgical procedures in seven US hospitals. They identified *S. aureus*, *C. albicans* and gram-negative bacteria such as morexella, enterobacter and serratia species to be responsible for these infections. In all these studies, the authors emphasized that extrinsic contamination of propofol, as a result of the lapses in aseptic preparation, handling and storage of this drug, caused these life-threatening infections.

Center of Disease Control and Prevention suggested safe medication practices, including avoiding the use of syringes on multiple patients as well as avoiding single-use medication vials for multiple patients, and strictly adhering to aseptic techniques and infection control practices during propofol application.[19] Additionally, in order to reduce the incidence of propofol-related postoperative infection, antimicrobial preservative-containing (i.e., EDTA- or Na-metabisulphide-containing) emulsions have been manufactured according to discussion with the Food and Drug Administration Agency (FDA). Such formulations are used in the United States today; nevertheless, preservative-free propofol solutions are still being marketed in Europe and in other areas of the world. Jansson et al.[16] reported that the incidence of propofol-related infection reduced from 39 to 9 infections per year in the U.S., after onset of EDTA-containing propofol use in 1996. However, as the infection problem with propofol continued despite of preservative inclusion, they have underlined that addition of EDTA is only an additional safety precaution. Hence, the practitioner must regard to good aseptic practice in any occasion during propofol medication. In this study, we tested preservative-free emulsion of propofol, which was also used in our hospital. Though antimicrobial-added propofol is also being marketed in our country, preservative-free forms are widely preferred possibly due to economic concerns.

Propofol and ketamine are unlicensedly mixed as 1:1 volumes before clinical application, achieving 5 $mg \bullet mL^{-1}$ ketamine in this mixture. Since Gocmen et al.[7] reported that blood levels of ketamine were too low to not to show any antimicrobial effect in the body, we investigated whether it could prevent the microbial growth in propofol. In this study, we observed that MBCs of ketamine in ketofol mixture were between 625 and 1,250 $\mu g \bullet mL^{-1}$ (> 1,250 $\mu g \bullet mL^{-1}$ for *S. aureus*). Therefore, we thought that ketamine might be useful to reduce growth of some bacterial and fungal pathogens in propofol before application.

In this study, we found that particularly gram-negative bacteria rapidly grew in propofol solution. These results are interestingly parallel with the data that is previously reported from the outbreak studies.[3,4,17,18] We thought that this selective promotion of propofol for the gram-negatives might explain why such bacteria would be the leading pathogens of the nosocomial outbreaks related to propofol use.

In this study, we showed that inclusion of ketamine into propofol might reduce bacterial and fungal growth in this solution and, consequently, provide a safe anesthetic medication for surgical approaches. However, ketamine's activity may vary according to type of organism. Therefore, regardless of this safeguard, we underlined that strict hygienic measurements must be taken in any occasion of propofol use, according to the recommendations of the authorities.

Conflicts of interest

The authors declare no conflicts of interest.

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