

## In Vitro Investigation of the Antibacterial Effect of Ketamine

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### Abstract

**Background:** Antibacterial activity of local anesthetics especially lidocaine has been shown previously. In this study, the antibacterial effect of ketamine, a general anesthetic agent was investigated.

**Methods:** The antibacterial effect of ketamine was studied using six different strains of bacteria (*Staphylococcus aureus*, *Staphylococcus epidermidis*, *Enterococcus faecalis*, *Streptococcus pyogenes*, *Pseudomonas aeruginosa* and *Escherichia coli*) with disc diffusion method. Ciprofloxacin discs (CIP, oxoid) were used as a control to verify the methodology. Minimal inhibition concentration (MIC) and minimal bactericidal concentration (MBC) of ketamine for these bacteria were also determined.

**Results:** No inhibition was evident in discs containing 62.5 µg of ketamine. Ketamine 125 µg showed activity on all the bacteria tested with the exception of *E. coli*. The inhibition rates of Ketamine were more prominent at the doses of 250 µg and 500 µg similar to the inhibition rate of CIP.

Whereas MIC and MBC values of ketamine for *S. aureus* and *S. pyogenes* were 500 µg mL<sup>-1</sup>, MIC and MBC values for *P. aeruginosa* were above 2000 µg mL<sup>-1</sup>. For other bacteria, these values ranged between these levels.

**Conclusions:** Ketamine with higher doses showed antibacterial activity. We thought that it will be proper to use ketamine hesitantly in experimental animal studies like sepsis and translocation.

## Introduction

Ketamine was synthesized in 1962 by Stevens and Mc Cartly and used clinically and qualified as ideal intravenous anesthetic agent in 1965 by Domino and Corssen. Ketamine has been used as anesthetic or analgesic agent in the patients in shock, short superficial surgical intervention, burn dressing, bronchoscopy, cardiac catheterization, radiotherapy, and radiologic investigation in pediatric patients. Intramuscular, intravenous, per oral, rectal and nasal routes have been used. The effect of intravenous ketamine 2 mg kg<sup>-1</sup> (unconsciousness) starts within 30–60 seconds. Plasma level is 640–1000 ng L<sup>-1</sup> after 10–15 minutes. Average total body clearance is equal to hepatic blood flow (1.4 L min<sup>-1</sup>). Ketamine is detoxified by cytochrome P-450 and metabolized to norketamine and hydroxyketamine in the liver. End products are excreted 91% renally, and 4% rectally, and 5% of ketamine is excreted without changing.

Ketamine is also preferred as an anesthetic agent in experimental animal studies

Key words: Ketamine, antibacterial activity

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(1). The doses used in animal studies are calculated as 40–100 mg kg<sup>-1</sup> and these doses are higher than the doses used in human clinical practice (2).

Anesthetic agents act on all cells including the neuronal cells and alter the thickness of cellular membrane and dipole potential. The electrolyte permeability of cell membrane is changed by specific and non-specific ways. They also increase lipid fluidity by 0.5 or 31% depending on the lipid system examined and the method of fluidity measurement. The change in lipid fluidity affects membrane function (3). General anesthetic agents were also shown to decrease erythrocyte sedimentation rate and change leukocyte functions (4–7).

It has long been known that local anesthetic agents, especially lidocaine, have activity on several bacteria (8). The exact mechanism of the activity of local anesthetics on bacteria has not been clearly described. Local anesthetic agents inhibit bacterial growth and membrane-bound enzymatic activities by decreasing the number of viable cells and causing to lyses of protoplasts, permeability changes, and ultra structural alterations (9). This activity shows difference among the bacteria. Besides, local anesthetics do not perform the same activity against all bacteria.

This study investigated whether ketamine, a nonvolatile anesthetic agent, has antibacterial activity against *S.aureus*, *S.epidermidis*, *E.coli*, *P.aeruginosa*, *S. pyogenes* *E. fecalis*.

## Material and methods

Firstly, antibacterial effect of ketamine was tested for the strains of *S. aureus* (American Type Culture Collection, ATCC 29213), *S. epidermidis* (ATCC 12228), *E. faecalis* (ATCC 29212), *S. pyogenes* (ATCC 19615), *P. aeruginosa* (ATCC 27853) and *E. coli* (ATCC 25922), using disc diffusion method (10).

Ketamine (Ketalor<sup>®</sup> 50 mg mL<sup>-1</sup> Pfizer) was diluted at the concentrations of 25, 12.5, and 6.25 mg mL<sup>-1</sup> with sterile saline solution. Discs containing ketamine 500, 250, 125, and 62.5 µg were prepared under aseptic conditions using 10 µL stock and diluted solutions. Ciprofloxacin discs (CIP 5µg; oxoid) were used to check the validity of the methodology.

All the bacteria were diluted to a density of 0.5 Mc Farland units (1.5X10<sup>8</sup> mL<sup>-1</sup>), and suspensions were adapted to Mueller Hinton agar. Mueller Hinton agar with 5% sheep blood was used for *S. pyogenes*.

Three discs for each different dose of ketamine and CIP were prepared for each bacterium. In addition, an empty disc used to prepare the ketamine discs was used for control. The discs were placed in the agar plates into which bacteria were inoculated. The plates were incubated at 35°C for 16–24 hours, and the inhibition zones were measured in millimeters.

The inhibition zones caused by CIP and different doses of ketamine were compared with each other.

Minimal inhibition concentration (MIC) and minimal bactericidal concentration (MBC) of ketamine for bacteria defined above were determined after antibacterial

activity of ketamine with disc diffusion method was observed. The dilutions of ketamine 125–2000  $\mu\text{g mL}^{-1}$  were studied using the methods that were described by Clinical and Laboratory Standards Institute (CLSI)-M7-A7 (11).

For these procedures, Muller Hinton culture with cation was used. The first concentration at which growth inhibition was detected with visual assessment was accepted as MIC concentration for these bacteria after incubation at 35 °C for 18 hours. Determination of the MIC and MBC was defined as the concentration at which 99.9% of growth inhibition was observed, and an aliquot from each well in the dilution series showing inhibition of visible bacterial growth was subcultured to sheep blood agar. Control cultures were performed for bacteria, broth, and drug dilutions.

Furthermore, the micro plates were measured at the wavelength of 450 nm using a micro plate reader (MicroQuant, BioTek) before and after the incubation period during MIC determination. Each absorbance value obtained from the well was compared with the results of visual assessment.

## Results

CIP affected all the six bacteria as expected. Empty discs did not reveal an inhibition zone. Discs containing 500 and 250  $\mu\text{g}$  ketamine inhibited bacterial growth resembling to that of CIP. Discs containing 125  $\mu\text{g}$  ketamine had antibacterial activity on all the bacteria with the exception of *E. coli*. No antibacterial activity was evident in discs containing 62.5  $\mu\text{g}$  of ketamine (Table 1).

No growth was detected in the control cultures of broth and drug dilutions used for MIC and MBC measurements.

MIC and MBC values of the six bacteria for ketamine were significantly high (Table 2). The most sensitive bacteria were *S. aureus* ve *S. pyogenes*, and the most resistant bacteria was *P. aeruginosa*. MIC values of *P. aeruginosa* was above 2000  $\mu\text{g mL}^{-1}$  which was the highest concentration studied (MIC values of *S. aureus* ve *S. pyogenes* was 500  $\mu\text{g mL}^{-1}$ ).

The results of turbidmetric assessment of micro plates were concordant with MIC (visual) and MBC (culturing) values (Figure 1). Thus, MBC for *E. coli* and MIC and MBC for *P. aeruginosa* were higher than the highest concentrations of ketamine tested because the decrease in measured absorbance values was parallel to increased ketamine concentrations (Figure 1).

## Discussion

The clear effect of ketamine for the six bacteria investigated was shown using disk diffusion method. However, the antibacterial activity was dose-dependent similar to the results of the study by Parr and coworkers (8).

We showed that ketamine at the doses of 500  $\mu\text{g}$  had more prominent effect than

**Table 1.** The inhibition zones of ciprofloxacin and ketamine at four different doses against six bacteria (mm)

Bacteria	Ciprofloxacin (5µg)	Ketamine			
		500 µg	250 µg	125 µg	62.5 µg
<i>S. aureus</i> ATCC 29213	30	15	9	8	-
	31	15	9	8	-
	30	14	9	8	-
<i>S. epidermidis</i> ATCC 12228	27	25	21	10	-
	27	23	21	10	-
	28	23	20	10	-
<i>E. faecalis</i> ATCC 29212	27	13	10	7	-
	28	13	10	7	-
	27	13	10	7	-
<i>E. coli</i> ATCC 25922	35	12	10	-	-
	35	12	10	-	-
	35	12	10	-	-
<i>S. pyogenes</i> ATCC 19615	24	13	11	7	-
	24	13	11	7	-
	24	13	11	8	-
<i>P. aeruginosa</i> ATCC 27853	18	8	7	7	-
	19	8	7	7	-
	18	9	7	7	-

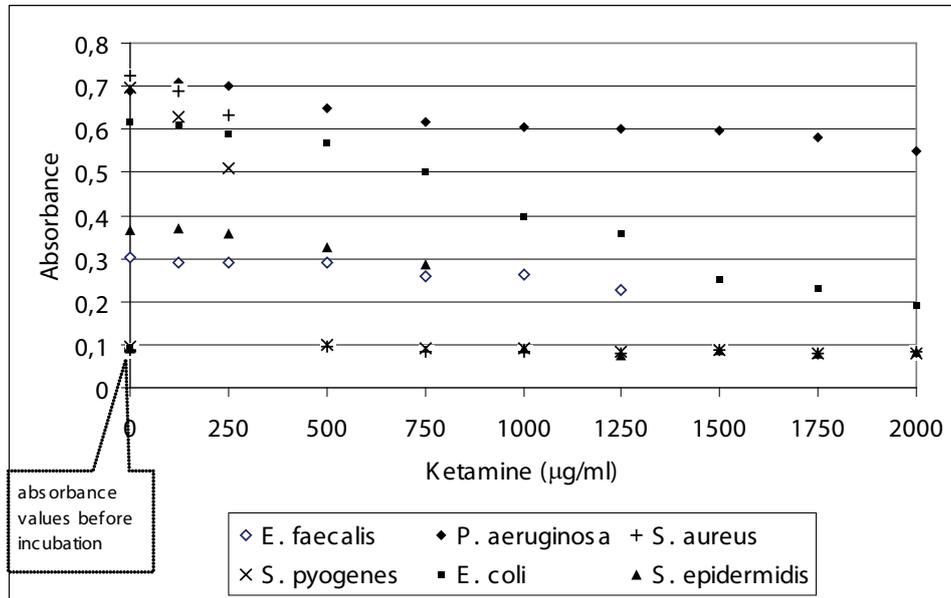
**Table 2.** MIC and MBC values of the six bacteria for ketamine (µg mL<sup>-1</sup>)

	<i>S. aureus</i>	<i>S. epidermidis</i>	<i>E. faecalis</i>	<i>E. coli</i>	<i>S. pyogenes</i>	<i>P. aeruginosa</i>
MIC	500	1000	1500	2000	500	>2000
MBC	500	1250	1750	>2000	500	>2000

the doses of 250 µg. Ketamine 500 µg caused inhibition zones with diameters of 8–25 mm. These inhibition zones were different for each bacterium. The effect on *E. coli* was as one third of CIP, whereas the effect on *S. epidermidis* was similar to CIP (Table 1). The formation of different zones for different bacteria suggested that there was a difference in bacterial resistance against ketamine similar to that seen in antibiotics.

The lowest MIC determined was significantly higher than the anesthetic blood level of ketamine. MIC was 500 µg mL<sup>-1</sup> for *S. aureus* and *S. pyogenes*, (1000,

Figure 1. Absorbance values measured in the micro plates before and after the incubation period depending different ketamine concentrations for six bacteria



1500, and 2000 µg mL<sup>-1</sup> for *S. epidermidis*, *E. faecalis* and *E. coli*, respectively) (Table 2). The growth of *P. aeruginosa* was not inhibited completely at the concentration of 2000 µg mL<sup>-1</sup> which was the highest concentration studied, but a decrease in growth was observed with the optical investigation of plaque at 450 nm. Absorbance values at wavelength of 450 nm were correlated with MIC and MBC values for all the bacteria (Figure 1)

There are many studies on the effects of ketamine on cellular metabolism and functions. These studies have shown different results related to the use of ketamine in model of sepsis in animal. While some suggest ketamine as an ideal anaesthetic, in some studies, ketamine was not considered an appropriate agent in these kinds of experiments.

Yu Y and co-workers suggested that anesthesia with ketamine was an advantage in sepsis resulting from gram-negative bacteria. They studied the effect of ketamine on activation of NF kappa B and production of TNF alpha in rat peripheral blood mononuclear cells. They showed that ketamine suppressed LPS-induced production of proinflammatory cytokines (TNF alpha and IL-6) and reduced the LPS-induced activation of NF kappa B (12). Kawasaki and co-workers also found the inhibitor effect of the ketamine on LPS-induced production of TNF alpha, IL-6 and IL-8 in a similar study (5). Sun and colleagues (13) determined the same effects of ketamine on intestinal cells. Contrary to these studies, Krumholz W and co-workers suggested that ketamine did not change the antibacterial functions of PMNL. They

studied the effects of ketamine and several anesthetics on PMNL functions in vitro and showed that the suppression of PMNL might lead to perioperative infection releasing bactericidal agents (super oxide anion, OH, and myeloperoxidase), but ketamine did not influence the PMNL functions (6). Mast cell functions were important during inflammation and auto-tissue injury. The impairment of mast cell function by anesthetics might lead to reduced defense against infection. Fujimoto and co-workers found an inhibitory effect of ketamine on mast cell exocytose in a dose-dependent manner. (7). The effects of ketamine on circulatory system in sepsis models are also under debate. Worek FS and co-workers studied the effects of ketamine or pentobarbital on hemodynamics and survival time using porcine as an animal model of *P. aeruginosa* septicemia. A minor suppressive action of ketamine on circulation in septicemia was observed and it was suggested that the studies related to gram-negative bacteria and endotoxin should be done without anesthesia or, if necessary, using ketamine (14).

Contrarily, Lüebbe AS and colleagues suggested that ketamine was an inappropriate choice in sepsis experiments in rats. They observed that during *E. coli* bacteremia, anesthetics primarily affect the reactivity of skeletal muscle small arterioles. Ketamine/xylazine anesthesia has the most pronounced effect on systemic and microcirculatory variables (15).

In conclusion, these in vitro findings showed that ketamine had prominent antibacterial activity at high doses. Because anesthetic blood level of ketamine in humans is about  $2 \mu\text{g mL}^{-1}$  (16), this antibacterial activity is not seen in humans during anesthesia. Although in experimental studies, the doses of ketamine are higher than the doses used in humans, its blood levels do not reach the lowest MIC values. However, we recommend cautious use of ketamine in experimental animal studies such as sepsis and translocation because of its prominent antibacterial activity and the activity on leucocytes and circulatory system.

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