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Original Article

Antibacterial activity of local anesthetics against multidrug-resistant bacteria in vitro

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Abstract: Objectives: Local anesthetics are widely used in clinical settings for pain management. In addition to their analgesic effects, they may also exhibit antimicrobial properties. However, data on their activity against multidrug-resistant (MDR) pathogens are limited. This study aimed to evaluate the in vitro antibacterial activity of lidocaine, levobupivacaine, and bupivacaine against MDR bacteria, including Pseudomonas aeruginosa (MRPA), carbapenem-resistant Enterobacterales (CRE), and Acinetobacter baumannii (MRAB). Methods: The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of each anesthetic were determined using the standardized broth microdilution method according to CLSI guidelines. Clinical isolates of MRPA, CRE, and MRAB were tested. Identification was performed using MALDI-TOF MS and VITEK II systems. The local anesthetics were diluted to clinically relevant concentrations and tested in duplicate. Results: Bupivacaine exhibited the strongest antimicrobial activity, with MICs of 1.6 mg/mL for MRAB and 3.2 mg/mL for CRE. Lidocaine showed limited activity, with an MIC of 16 mg/mL for MRPA. Levobupivacaine showed intermediate effects. In all cases, MBCs were higher than the corresponding MICs. These findings suggest differential antibacterial efficacy among the agents. Conclusions: Local anesthetics demonstrated measurable antibacterial effects against MDR pathogens in vitro. Bupivacaine showed the strongest activity but has a lower clinical dosage limit due to its cardiotoxicity. While lidocaine has weaker antibacterial potency, its widespread use and safety profile make it a practical option. These results suggest local anesthetics may play a complementary role in infection-prone procedures but require cautious interpretation for clinical application.

Keywords: Local anesthetics, lidocaine, bupivacaine, levobupivacaine, multidrug-resistant bacteria

Introduction

Lidocaine, levobupivacaine, and bupivacaine are amide-group local anesthetics that exert analgesic action in distinct body regions by blocking voltage-gated sodium channels. Amide-group local anesthetics are widely used in general anesthesia, regional anesthesia, and invasive procedures because of their safety [1, 2]. Aside from pain management, the supplemental role of local anesthetics as antimicrobial agents has been documented in several studies [3-6]. However, most of these studies are limited to antibiotic-sensitive microorganisms. Studies on multidrug-resistant (MDR) microorganisms, which have increased in number in recent years, are insufficient.

The incidence of MDR bacterial infections has grown significantly in Korea, not only in nosocomial infections but also in community infections [7-9]. This increase in MDR microorganisms increases the risk of infection in patients undergoing regional anesthesia and invasive procedures [10, 11]. Furthermore, despite the necessity of regional anesthesia or invasive procedures, the risk of aggravation of MDR infections can lead to delays or cancellations of proper management. This may aggravate the disease and pain in patients, thereby lowering their quality of life. Therefore, the authors conducted this study to quantify the minimum inhibitory and bactericidal concentrations of various local anesthetics against selected MDR microorganisms. Moreover, this study aimed to

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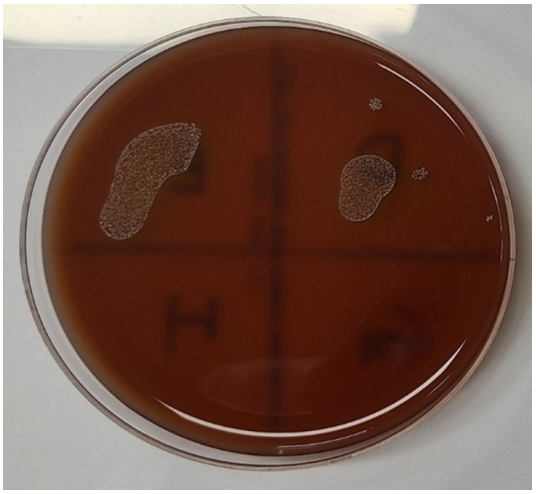

Figure 1. A blood agar plate (BAP) was used to observe bacterial growth. Upper right: VRE A; Upper left: VRE B; Lower right: MRPA D; Lower left: MRPA E.

confirm the safety of the procedures using local anesthetics in patients with MDR pathogenic infection.

Materials and methods

Bacterial strains and characterization

Clinical isolates of carbapenem-resistant *Enterococcus* (CRE), multidrug resistant *Acinetobacter baumannii* (MRAB), and multidrug resistant *Pseudomonas aeruginosa* (MRPA) from patients admitted to Chungbuk National University Hospital were included in the study for investigation of the antimicrobial effects of local anesthetics. The experiment was conducted by dividing the samples into three groups (Supplementary Tables 1, 2, 3): Group A, B, and C for MRPA, CRE, and MRAB isolates, respectively. For Group B, three experimental isolates were used, whereas ten isolates were tested in Groups A and C. However, two samples in Group C failed to grow bacteria and were therefore excluded from the analysis. Bacteria were characterized by identification and susceptibility testing using MALDI-TOF MS (Bruker Biotyper) and VITEK II system (BioMérioux, USA).

Media and local anesthetics

Cation-supplemented Mueller-Hinton broth (MHB) (BBL; BD, Sparks, MD, USA) was used to determine the minimal inhibitory concentration (MIC) of the local anesthetics. A blood agar plate (BAP) was used to observe bacterial growth and determine the minimal bactericidal concentration (MBC) (**Figure 1**).

Three local anesthetics, lidocaine, bupivacaine, and levobupivacaine, were examined for their antibacterial effects. Lidocaine (Lidocaine HCl Daihan 2% injection, Daihan Pharm Co., Ltd.), 20 mg bupivacaine in the form of a heavy injection (Marcaine 0.5%, Mitsubishi Tanabe Pharma Korea Co., Ltd.), and levobupivacaine (Chirocaine 0.75%, Abbott Laboratories) were administered.

For lidocaine, concentrations of 18, 16, 14.4, 10, 5, and 2.5 mg/mL were prepared by dilution with MHB. For levobupivacaine, concentrations of 6.75, 6.4, 5.4, 4.32, 2.7, and 2.16 mg/mL were used. For bupivacaine, concentrations of 4.5, 4, 3.6, 3.2, 1.8, and 1.6 mg/mL were applied.

Determination of MIC and MBC of local anesthetics

The MIC and MBC of the local anesthetics were determined by standardized broth microdilution methods with inoculums of 5×10^5 CFU/mL, according to the Clinical Laboratory Standard Institute (CLSI) guidelines (2019). *P. aeruginosa* (ATCC 27853) was used as a reference strain for the quality control of the microbroth dilution methods. All strains were cultivated in a non-CO_2 incubator at 35°C for 16 to 18 hrs. The MIC was defined as the lowest concentration of each anesthetic that was able to inhibit visual growth. All tests for each strain were performed in duplicate.

To determine MBC, 25 uL of each well of a microwell plates in which MIC was measured was taken and inoculated into a BAP medium. After the medium was cultured at 35°C for 16 to 18 h, the lowest concentration without visible growth was determined as the MBC.

Results

MIC and MBC values of the three local anesthetics against MDR organisms are summarized in **Figure 2**. Among the tested agents, bupivacaine demonstrated the strongest antimicrobial effect across all three pathogen groups. Notably, its MIC values were 1.6 mg/

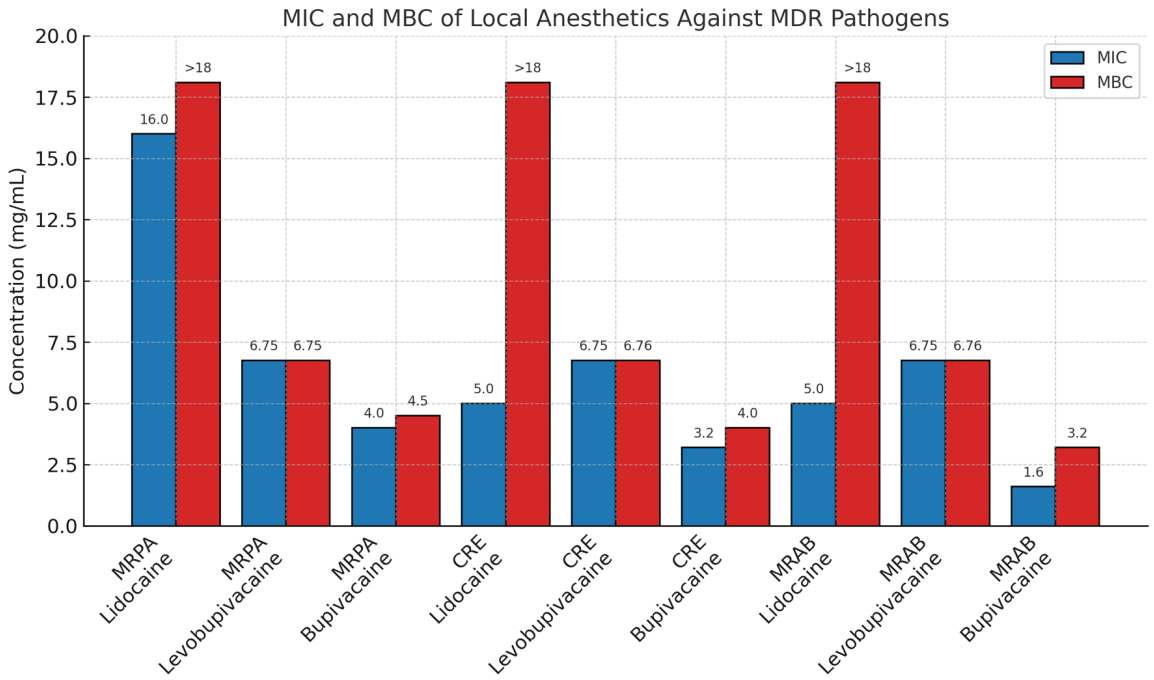

Figure 2. MIC and MBC of Local Anesthetics Against MDR Pathogens. Bar graph comparing the minimum inhibitory concentrations (MIC, in blue) and minimum bactericidal concentrations (MBC, in red) of lidocaine, levobupivacaine, and bupivacaine against multidrug-resistant pathogens: MRPA, CRE, and MRAB. MBC values exceeding the tested range (>18 mg/mL) are represented as 18.1 for visualization purposes.

Table 1. Minimum inhibitory and bactericidal concentrations of local anesthetics against multidrug-resistant pathogens

Pathogen	Local Anesthetic	MIC (mg/mL)	MBC (mg/mL)
MRPA	Lidocaine	16.0	>18.0
MRPA	Levobupivacaine	6.75	6.75
MRPA	Bupivacaine	4.0	4.5
CRE	Lidocaine	5.0	>18.0
CRE	Levobupivacaine	6.75	>6.75
CRE	Bupivacaine	3.2	4.0
MRAB	Lidocaine	5.0	>18.0
MRAB	Levobupivacaine	6.75	>6.75
MRAB	Bupivacaine	1.6	3.2

Values represent MIC and MBC (in mg/mL) of lidocaine, levobupivacaine, and bupivacaine against MRPA, CRE, and MRAB clinical isolates. ">" indicates that the MBC exceeded the highest tested concentration.

mL for MRAB, 3.2 mg/mL for CRE, and 4 mg/mL for MRPA, with corresponding MBCs of 3.2, 4, and 4.5 mg/mL, respectively (**Table 1**).

Levobupivacaine exhibited MIC values of 6.75 mg/mL against all pathogens, with MBCs ranging from 6.75 to above 6.75 mg/mL. In contrast, lidocaine showed relatively weaker antibacterial activity, with an MIC of 16 mg/mL for MRPA and 5 mg/mL for both CRE and MRAB. The MBCs for lidocaine exceeded the highest tested concentration of 18 mg/mL in all cases.

These findings indicate that bupivacaine has the most potent bacteriostatic and bactericidal effects in vitro, particularly against MRAB and CRE. Lidocaine, although widely used clinically, demonstrated limited antibacterial efficacy within the tested concentration range. The antimicrobial efficacy of each anesthetic agent varied depending on both the bacterial strain and the drug concentration, suggesting a complex interaction between drug-specific properties and pathogen susceptibility.

Discussion

Local anesthetics such as lidocaine, levobupivacaine, and bupivacaine are widely used in pain management through regional anesthesia and nerve blocks. These agents act by blocking voltage-gated sodium channels, providing effective analgesia during various surgical and

interventional procedures [1, 2] Recent research has highlighted their potential antimicrobial effects [3-6], which may be clinically relevant, especially in procedures involving patients at risk for infection with multidrug-resistant (MDR) pathogens.

In the present study, the in vitro antimicrobial activity of three commonly used local anesthetics was evaluated against MDR strains of *Pseudomonas aeruginosa* (MRPA), *Enterobacterales* (CRE), and *Acinetobacter baumannii* (MRAB). Among the tested agents, bupivacaine demonstrated the most potent activity, with MIC values of 1.6 mg/mL and 3.2 mg/mL against MRAB and CRE, respectively. In contrast, lidocaine showed relatively limited efficacy, with an MIC of 16 mg/mL for MRPA. Levobupivacaine showed intermediate results. These findings are consistent with previous studies suggesting agent- and organism-specific differences in antibacterial efficacy [12-15].

Importantly, these in vitro results must be interpreted within the context of clinical practice. Bupivacaine, while exhibiting strong antibacterial activity, has a narrow therapeutic index and is associated with significant cardiotoxicity, especially at higher doses. In contrast, lidocaine has a well-established safety profile and can be administered at higher concentrations - up to 400 mg total dose - without significant systemic toxicity. Clinically, lidocaine is typically used at 2% (20 mg/mL) and bupivacaine at 0.75% (7.5 mg/mL). The MIC values observed for bupivacaine fall within this achievable range, but its toxicity profile limits aggressive dosing. Conversely, lidocaine's higher MICs (e.g., 16 mg/mL against MRPA) are still within clinically deliverable concentrations and may offer practical benefit, particularly for superficial or localized infections.

Moreover, lidocaine's widespread use in epidural, spinal, and peripheral nerve blocks underscores its practicality. Although its antimicrobial activity is modest, its use may still help reduce microbial burden in MDR-colonized tissues during invasive procedures [16, 17]. Additionally, its lower cardiotoxicity and faster onset time compared to bupivacaine make it more favorable in high-risk patients.

The potential mechanisms of antimicrobial action for local anesthetics include disruption of bacterial membranes, interference with DNA replication, and inhibition of cell wall synthesis [18-20]. These nonspecific actions may explain their broad-spectrum effects, but further studies are needed to elucidate these mechanisms, especially under in vivo conditions.

This study has several limitations. First, it was conducted in vitro, and the drug concentrations do not account for tissue penetration, protein binding, or systemic absorption in actual clinical settings. Second, we did not perform synergistic or time-kill assays to evaluate dynamic antibacterial effects. Lastly, the lack of statistical comparison due to small sample size may limit generalizability.

Nevertheless, this study highlights that local anesthetics, particularly bupivacaine, possess significant in vitro antimicrobial activity against MDR organisms. While lidocaine may not demonstrate strong bactericidal effects, its clinical applicability, safety profile, and practical dosing make it a viable adjunct in managing patients at risk for MDR infections. These findings suggest that local anesthetics could serve not only as analgesic agents but also as complementary tools to reduce infection risk in susceptible patients undergoing invasive procedures.

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Disclosure of conflict of interest

None.

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Supplementary Tables

The following supplementary tables present patient demographic information and sample collection sites for each MDR pathogen group analyzed in the study.

Supplementary Table 1. Group A - MRPA

No.	Sex	Age	Site of Specimen Collection
1	M	60	Bronchial
2	M	73	Urine
3	F	58	Urine
4	M	78	Transtracheal
5	M	71	Transtracheal
6	M	83	Abdomen wound
7	M	84	Sputum
8	M	71	Urine
9	M	19	Urine
10	M	82	Urine

Supplementary Table 2. Group B - CRE

No.	Sex	Age	Site of Specimen Collection
1	F	76	Whole blood
2	M	87	Rectal
3	F	82	Transtracheal

Supplementary Table 3. Group C - MRAB

No.	Sex	Age	Site of Specimen Collection	Remark
1	F	62	Sputum	
2	M	71	Transtracheal	not cultured
3	M	76	Whole blood	
4	M	80	Transtracheal	
5	M	98	Transtracheal	
6	M	51	Bronchial	
7	F	71	Sputum	
8	M	62	Bronchial	not cultured
9	M	51	Bronchial	
10	M	63	Transtracheal	