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In Vitro Investigation of the Antibacterial Effects of Lidocaine and Bupivacaine Alone and in Combinations with Fentanyl

Lidokain ve Bupivakain'in Tek Başına ve Fentanil ile Kombinasyonlarının Antibakteriyel Etkilerinin İn Vitro İncelenmesi

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ABSTRACT Objective: It was aimed to investigate the in vitro antibacterial activities of the combined use of local anesthetic agents like lidocaine and bupivacaine, the antibacterial effects of which have been demonstrated, with fentanyl. **Material and Methods:** The in vitro antimicrobial activities of lidocaine, bupivacaine alone and in combination with fentanyl at different concentrations were investigated using microdilution technique. Microorganisms used in the test were *Escherichia coli* ATCC 25922, *Yersinia pseudotuberculosis* ATCC 911, *Pseudomonas aeruginosa* ATCC 10145, *Listeria monocytogenes* ATCC 43251, *Enterococcus faecalis* ATCC 29212, *Staphylococcus aureus* ATCC 25923, *Bacillus cereus* 702 Roma, *Mycobacterium smegmatis* ATCC607, *Candida albicans* ATCC 60193, and *Saccharomyces cerevisiae* RSKK 251. Antibacterial assays were performed in Mueller-Hinton broth at pH 7.3 and antifungal analyses were performed in buffered Yeast Nitrogen Base at pH 7.0. **Results**: While lidocaine, bupivacaine, and fentanyl demonstrated antibacterial activity when they were used alone, no antibacterial effect was observed when they were used in combination. **Conclusion:** The antibacterial efficacy of both lidocaine and bupivacaine is evident when both local anesthetic agents are used alone. However, the antibacterial efficacy is reduced when both agents are combined with fentanyl, which shows that the risk of infection may be more likely.

Keywords: Antimicrobial activity; lidocaine; bupivacaine; fentanyl

ÖZET Amaç: Antibakteriyel etkinliği gösterilmiş lidokain ve bupivakain gibi lokal anestezik ilaçların, fentanil ile kombine kulanımlarının antibakteriyel aktiviteleri üzerine etkisinin in vitro ortamda araştırılması hedeflenmiştir. **Gereç ve Yöntemler:** Farklı konsantrasyonlardaki lidokain, bupivakain ilaçlarının tek başına ve fentanil ile kombinasyonlarının, in vitro antimikrobiyal aktiviteleri mikro dilüsyon tekniği kullanılarak araştırıldı. Testte kullanılan mikroorganizmalar *Escherichia coli* ATCC 25922, *Yersinia pseudotuberculosis* ATCC 911, *Pseudomonas aeruginosa* ATCC 10145, *Listeria monocytogenes* ATCC 43251, *Enterococcus faecalis* ATCC 29212, *Staphylococcus aureus* ATCC 25923, *Bacillus cereus* 702 Roma, *Mycobacterium smegmatis* ATCC607, *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:** Lidokain, bupivakain ve fentanil tek başına kullanıldıklarında antibakteriyel etki gösterirken, kombine kullanımlarında antibakteriyel etki gözlemlenmemiştir. **Sonuç:** Lidokain ve bupivakainin tek başlarına kullanıldıklarında antibakteriyel etkinlik gösterdikleri açıktır; Ancak bu lokal anestezikler fentanil ile kombine edildiklerinde antibakteriyel etkinliğin azalması, kombine kullanımın enfeksiyon riskini arttıracağını düşündürmektedir.

Anahtar Kelimeler: Antimikrobiyal aktivite; lidokain; bupivakain; fentanil

It has been shown that local anesthetic agents like bupivacaine and lidocaine exhibited bacteriostatic, bactericidal, fungistatic, and fungicidal properties against a wide spectrum of microorganisms.[1-4] Infection may occur as a complication after the administration of any

technique of regional anesthesia. The use of local anesthetic agents in cases of infections of the tissues around the spinal cord and the spinal canal is dangerous.[5,6]

The addition of other agents such as preservatives, opioids, or intravenous anesthetics to the local anesthetic solutions may modify the overall antimicrobial activity through either synergistic or antagonistic action.[7] But there are not enough studies showing the use of combinations where the involved agents increase the overall antimicrobial activity and of combinations where they decrease it. The aim of this study was to investigate the in vitro antibacterial effects of fentanyl, lidocaine, bupivacaine and to compare their antibacterial efficacy with fentanyl-lidocaine and fentanyl-bupivacaine combinations.

MATERIAL AND METHODS

Five experimental groups of local anesthetic drugs (lidocaine and bupivacaine), alone and in combination with fentanyl, were constituted (Table 1).

ANTIMICROBIAL ACTIVITY ASSESSMENT

All of the tested microorganisms were obtained from the Hifzissihha Institute of Refik Saydam (Ankara, Turkey) and they were as follows: *Escherichia coli* ATCC 25922, *Yersinia pseudotuberculosis* ATCC 911, *Pseudomonas aeruginosa* ATCC 10145, *Listeria monocytogenes* ATCC 43251, *Enterococcus faecalis* ATCC 29212, *Staphylococcus aureus* ATCC 25923, *Bacillus cereus* 709 ROMA, *Mycobacterium smegmatis* ATCC607, *Candida albicans* ATCC 60193, and *Saccharomyces cerevisiae* ATCC 60193.

TABLE 1: Groups and the drugs administered.

Groups	Drug used
Group 1	Lidocaine 2%
Group 2	Bupivacaine 0.5%
Group 3	Fentanyl 50 mcg/mL
Group 4	Lidocaine + Fentanyl
Group 5	Bupivacaine + Fentanyl

DETERMINATION OF MINIMAL INHIBITORY AND MINIMAL BACTERICIDAL CONCENTRATIONS

The antimicrobial activities of the substances were tested quantitatively in broth media by using double dilution and the minimum inhibitory concentration (MIC) values (μg/ml) were determined.[1,2] The antibacterial assays were performed in Mueller-Hinton broth (MH) at pH 7.3 and the antifungal assays were performed in buffered Yeast Nitrogen Base (YNB) (Difco, Detroit, MI) at pH 7.0. Dilution of each chemical substance to be tested was prepared in 0.1 ml volumes of sterile MH and YNB broth to give concentrations ranging from 5000 μg/mL to 5 μg/mL. After preparation of the suspensions of test microorganisms in MH and YNB broth (approximately 10^6 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 again. The MIC was defined as the lowest concentration that showed no growth. The dilutions without visible growth were used for minimum bactericidal concentration (MBC) determination; 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 h. The MBC of each extract was taken as the lowest concentration that showed no growth on an agar plate. Ampicillin, streptomycin, and fluconazole were used as standard antibacterial and antifungal drugs, respectively.

MIC: The minimum effective dose: This dose may be bactericidal or bacteriostatic (inhibiting the growth and reproduction of bacteria continues when the effect of the drug ceases). MBC is determined at this state.

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

RESULTS

Lidocaine was observed to have an inhibitory effect on the growth of Gram-negative non-encapsulated bacteria *(E. coli)* and Gram-negative

encapsulated bacteria *(Y. pseudotuberculosis)* with MIC values ranging from 5000 to 10000 µg/mL (Table 2). It was observed that when used separately, both local anesthetics under study (bupivacaine and lidocaine) and fentanyl had an inhibitory effect on the growth of *B. cereus* which is a Gram-positive spore-forming bacillus in concentrations of 5000, 2500 and 25 µg/mL; but they had no inhibitory effect on the same bacilli when combined. When lidocaine, bupivacaine and fentanyl were tested against *M. smegmatis* which was an acid-fast staining bacterium, it was observed that they had an inhibitory effect on growth with MIC values in concentrations of 2500, 625 and 25 µg/mL respectively; but they had no inhibitory effect when they were used in combination (Table 2). It was determined that each of the three drugs tested had no antipseudomonal *(P. aeruginosa)* activity. Similarly, it was observed that they had also no activity against Gram-positive coccus (*S. aureus* and *E. faecalis*) and species of yeast (*C. albicans* and *S. cerevisiae*).

DISCUSSION

Local anesthetic agents are drugs blocking the transmission of nerve impulses in nerve fibers reversibly when they get in touch with nerve fibers in appropriate concentrations. It has also been determined that they have antibacterial and antifungal activities. Their antibacterial activities were discovered for the first time by Jonnesco in 1909.[8] Inhibition of growth, a decrease in the living cells, the destruction of protoplasts, changes in membrane permeability, characteristic ultrastructural changes, and inhibition of membrane-dependent enzymatic activity are the factors enabling the antibacterial activities of local anesthetic agents.[9]

Local anesthetic agents can be used together with narcotics during the administration of regional anesthesia. Prolonged use of local anesthetic agents and narcotics, especially in cancer patients via the epidural route increases the risk for infection in these patients. Therefore, antimicrobial activities of local anesthetic agents are a desired characteristic. Local anesthetic agents with antimi-

TABLE 2: Antimicrobial activity of some anesthetic drugs (µg/mL).

Drug Name	Test Concent. µg/mL	Microorganisms and Minimal Inhibition Concentration (MIC)									
		Ec	Yp	Pa	Sa	Ef	Bc	Ms	Ca	Sc
Lidocaine (20 mg/mL)	20 000	5000	10000	-	-	-	5000	2500	-	-
Bupivacaine (100 mg/20 mL)	5 000	-	-	-	-	-	2500	625	-	-
Fentanyl (0,5 mg/10 mL)	50	-	-	-	-	-	25	12	-	-
Lidocaine + Fentanyl (1:1)	10000: 25	2500: 6	5000: 12	-	-	-	-	-	-	-
Bupivacaine + Fentanyl (1:1)	2500: 25	-	-	-	-	-	-	-	-	-
Lidocaine + Bupivacaine (1:1)	10 000: 2500	5000: 1250	5000: 1250	-	-	-	-	-	-	-
Lidocaine + Bupivacaine+Fentanyl (1:1:1)	10 000: 2500:25	-	-	-	-	-	-	-	-	-
Ampicillin		2	32	>128	2	2	<1			
Streptomycin								4		
Fluconazole									<8	<8

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; **Sc:** S. cerevisiae RSKK 251; Ampicillin, Streptomycin; Fluconazole; **(-):** no activity of test concentrations.

crobial activity can be used as an adjunct to the traditional antimicrobial therapy in the clinical or laboratory setting. On the other hand, since the antimicrobial activities of local anesthetics can lead to false-negative results and inadequate culture yield, caution should be exercised in this respect.[3]

A number of cases were reported regarding the development of central nervous system infections like epidural abscess and meningitis after spinal and epidural anesthesia and analgesia. Otherwise, recent studies reveal that the development of injection complications related to the administration of neuraxial blockade has increased.[10] Moen et al. raise concern over the cases of meningitis, alpha-hemolytic streptococci and nosocomial infections after spinal blockade.[11] Additionally, in a study performed, the incidence of a spinal epidural abscess after epidural analgesia was reported to be 1/1000.[10]

In our study, it was observed that lidocaine had an inhibitory effect on the growth of Gram-negative non-encapsulated bacteria (*E. coli*) and Gram-negative encapsulated bacteria (*Y. pseudotuberculosis*). It was observed that lidocaine, bupivacaine, and fentanyl had an inhibitory effect on the growth of *B. cereus* which was a Gram-positive spore-forming bacillus, but no inhibitory effect was observed when they were used in combination since the dose was more diluted. It can be suggested that lidocaine, bupivacaine, and fentanyl were effective against *M. smegmatis*, an acid-fast staining bacterium, but no inhibitory effect was observed when they were used in combination, possibly due to the dosage. It was determined that the drugs tested had no antipseudomonal *(P. aeruginosa)* activity. Similarly, it was also determined that they had no activity against Gram-positive cocci (*S. aureus* and *E. faecalis*) and species of yeast (*C. albicans* and *S. cerevisiae*).

When other relevant studies in the literature were investigated; in a study performed by Rosenberg et al., it was observed that higher clinical concentration of local anesthetic agent bupivacaine (0.25%) had an inhibitory effect on many bacterial and fungal organisms like *Escherichia coli, S. aureus, S. epidermidis, S.pneumoniae, S. pyogenes, Enterococcus faecalis, Bacillus cereus,* and *Candida albicans.*[12] This study was performed using an agar dilution method. According to the results of the study, it was suggested that bupivacaine could exhibit a protective effect against some bacterial and fungal infections. Again in the same study, bupivacaine did not inhibit the growth of *P. aeruginosa.* On the other hand, in a study performed by Noda et al. it was reported that both bupivacaine and lidocaine at standard concentrations exhibited bactericidal activity in the colonies of *S. aureus, S. epidermidis,* and *P. aeruginosa.*[13] Moreover, when MIC values were compared, it was reported that bupivacaine had a greater antibacterial activity than lidocaine. Aydin et al. investigated the antimicrobial effects of local anesthetics ropivacaine, bupivacaine, lidocaine and prilocaine on *E.coli, S. aureus, P. aeruginosa* and *C. albicans,* and it was pointed out that lidocaine and prilocaine had more powerful antimicrobial effects than the other two local anesthetics.[14] Additionally, while both lidocaine and prilocaine at 2% concentrations had antimicrobial effects, prilocaine at 1% concentration inhibited the growth of *E. coli, S. aureus,* and *P. aeruginosa* and lidocaine at 1% concentration inhibited only the growth of *P. aeruginosa.* It was determined that bupivacaine at 0.25% concentrations inhibited the growth of P. aeruginosa and ropivacaine failed to inhibit the growth of the microorganisms tested. In another study, it was investigated whether sufentanil modified the antibacterial activity of bupivacaine and ropivacaine or not, while it was observed that when both bupivacaine and ropivacaine were used alone they inhibited the growth of *E. coli* and *S. aureus,* but they did not inhibit the growth of *E. faecalis.* When sufentanil was combined with bupivacaine, it increased the antimicrobial effect of bupivacaine but decreased the inhibitory effect of ropivacaine on the growth of *S. aureus.*[15] Consequently, it was reported that sufentanil provided a partial synergistic effect on bupivacaine and a partial antagonistic effect on ropivacaine's antibacterial activity. In a study performed by James et al. in 1976, the effect of bupivacaine on bacterial growth was investi-

gated and additionally the incidence of contamination of catheters and syringes used during epidural analgesia was studied. In this study, it was determined that syringes in 5/101 cases were contaminated by commensal skin organisms *(S. epidermidis)* and bupivacaine (0.25%) was bactericidal to both *S. epidermidis* and *Corynebacterium* spp. at 37°C but not at room temperature.[16]

When the results obtained from our study and the results of the other studies in the literature are investigated, it is seen that there are many different results regarding the spectrum and potency of antimicrobial activity. It should be emphasized that these differences might result from the concentration of the drug used, the in vitro setting, pH and temperature of the environment, and the species of the bacteria involved. The common point of all these studies is that most local anesthetics have antimicrobial activity and these activities also increase directly proportional to the increase in concentration.

CONCLUSION

Our study has shown that, while both lidocaine and bupivacaine had antimicrobial activities against several bacteria when they were used alone, this activity disappeared when they were used in combination with fentanyl. The decreased antibacterial efficacy might be attributed to dilution of the local anesthetics, which leaded a decrease in their concentration. This result confirms the importance of the concentrations of the local anesthetics regarding their antimicrobial activity. Based on our findings, it can be said that there might be an increase in the risk of infection during the combined use of lidocaine or bupivacaine with fentanyl since the combination might cause a decrease in antibacterial activity. However, it is necessary to perform further and more extensive in vitro and in vivo studies to evaluate whether the combined use of local anesthetics decrease their individual antibacterial activities.

This examination is not a human research. Laboratory animals was not used. It is an in vitro experimental study conducted in laboratory conditions. There is no patient consent and ethics committee approval.

Source of Finance

During this study, no financial or spiritual support was received neither from any pharmaceutical company that has a direct connection with the research subject, nor from a company that provides or produces medical instruments and materials which may negatively affect the evaluation process of this study.

Conflict of Interest

No conflicts of interest between the authors and / or family members of the scientific and medical committee members or members of the potential conflicts of interest, counseling, expertise, working conditions, share holding and similar situations in any firm.

Authorship Contributions

This study is entirely author's own work and no other author contribution.

REFERENCES

1. Begec Z, Gulhas N, Toprak HI, Yetkin G, Kuzucu C, Ersoy MO. Comparison of the antibacterial activity of lidocaine 1% versus alkalinized lidocaine in vitro. Curr Ther Res Clin Exp 2007;68(4):242-8.
2. Hodson M, Gajraj R, Scott NB. A comparison of the antibacterial activity of levobupivacaine vs. bupivacaine: an in vitro study with bacteria implicated in epidural infection. Anaesthesia 1999;54(7):699-702.
3. Johnson SM, Saint John BE, Dine AP. Local anesthetics as antimicrobial agents: a review. Surg Infect (Larchmt) 2008;9(2):205-13.
4. Sakuragi T, Ishino H, Dan K. Bactericidal activity of clinically used local anesthetics on Staphylococcus aureus. Reg Anesth 1996; 21(3):239-42.
5. Ready LB, Helfer D. Bacterial meningitis in parturients after epidural anesthesia. Anesthesiology 1989;71(6):988-90.
6. Ericsson M, Algers G, Schliamser SE. Spinal epidural abscesses in adults: review and report of iatrogenic cases. Scand J Infect Dis 1990;22(3):249-57.
7. Pelz K, Wiedmann-Al-Ahmad M, Bogdan C, Otten JE. Analysis of the antimicrobial activity of local anaesthetics used for dental analgesia. J Med Microbiol 2008;57(Pt 1):88-94.

8. Aarde SM, Creehan KM, Vandewater SA, Dickerson TJ, Taffe MA. In vivo potency and efficacy of the novel cathinone α-pyrrolidinopentiophenone and 3,4-methylenedioxypyrovalerone: self-administration and locomotor stimulation in male rats. Psychopharmacology (Berl) 2015;232(16):3045-55.
9. Wheatley GH 3rd, Rosenbaum DH, Paul MC, Dine AP, Wait MA, Meyer DM, et al. Improved pain management outcomes with continuous infusion of a local anesthetic after thoracotomy. J Thorac Cardiovasc Surg 2005;130(2): 464-8.
10. Wang LP, Hauerberg J, Schmidt JF. Incidence of spinal epidural abscess after epidural analgesia: a national 1-year survey. Anesthesiology 1999;91(6):1928-36.
11. Moen V, Dahlgren N, Irestedt L. Severe neurological complications after central neuraxial blockades in Sweden 1990-1999. Anesthesiology 2004;101(4):950-9.
12. Rosenberg PH, Renkonen OV. Antimicrobial activity of bupivacaine and morphine. Anesthesiology 1985;62(2):178-9.
13. Noda H, Saionji K, Miyazaki T. [Antibacterial activity of local anesthetics]. Masui 1990; 39(8):994-1001.
14. Aydin ON, Eyigor M, Aydin N. Antimicrobial activity of ropivacaine and other local anaesthetics. Eur J Anaesthesiol 2001;18(10):687-94.
15. Tamanai-Shacoori Z, Shacoori V, Vo Van JM, Robert JC, Bonnaure-Mallet M. Sufentanil modifies the antibacterial activity of bupivacaine and ropivacaine. Can J Anaesth 2004;51(9):911-4.
16. James FM, George RH, Naiem H, White GJ. Bacteriologic aspects of epidural analgesia. Anesth Analg 1976;55(2):187-90.