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CLINICAL RESEARCH

Antimicrobial effects of fentanyl and bupivacaine: an *in vitro* study

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KEYWORDS
Antimicrobial;
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Regional anesthesia;
Infections

Abstract
Study objective: In this study, we aimed to compare the antimicrobial effects of bupivacaine and fentanyl citrate and to reveal the impact on antimicrobial effect potential in the case of combined use.
Design: In vitro prospective study.
Setting: University Clinical Microbiology Laboratory.
Measurements: In our study, in vitro antimicrobial effect of 0.05 mg.mL^{-1} fentanyl citrate, 5 mg.mL^{-1} bupivacaine were tested against *Staphylococcus aureus* American Type Culture Collection (ATCC) 29213, *Pseudomonas aeruginosa* ATCC 27853, *Klebsiella pneumoniae* ATCC 13883, *Escherichia coli* ATCC 25922 and *Candida albicans* ATCC 10231 as Group F (Fentanyl Citrate) and Group B (Bupivacaine), respectively. *S. aureus* ATCC 29213, *P. aeruginosa* ATCC 27853, *Klebsiella pneumoniae* ATCC 13883 and *Escherichia coli* ATCC 25922 were cultured onto Mueller Hinton agar (Oxoid, UK) plates and *Candida albicans* ATCC 10231 were cultured onto Sabouraud dextrose agar (Oxoid, UK) plates for 18–24 hours at 37 °C.
Main results: In terms of inhibition zone diameters, *S. Aureus* ATCC 29213, *P. aeruginosa* ATCC 27853, and *C. albicans* ATCC10231 values obtained after 12 and 24 hours of incubation were significantly higher in Group F than Group B ($p < 0.001$). In terms of inhibition zone diameters, *E. coli* ATCC 25922, and *K. pneumomiae* ATCC 13883 values obtained after 12 and 24 hours of incubation were significantly higher in Group B than Group F ($p < 0.001$, E. coli 12ª hour $p = 0.005$).

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Conclusions: Addition of fentanyl to Local Anesthetics (LAs) is often preferred in regional anesthesia applications in today's practice owing especially to its effect on decreasing the local anesthetic dose and increasing analgesia quality and patient satisfaction. However, when the fact that fentanyl antagonized the antimicrobial effects of LAs in the studies is taken into account, it might be though that it contributes to an increase in infection complications. When the fact that fentanyl citrate which was used in our study and included hydrochloric acid and sodium hydroxide as protective agents, broadened the antimicrobial effect spectrum of LAs, had no antagonistic effect and showed a synergistic antimicrobial effect against *E. Coli* is considered, we are of the opinion that the addition of fentanyl to LAs would contribute significantly in preventing the increasing regional anesthesia infection complications.

PALAVRAS-CHAVE
Antimicrobiano;
Bupivacaína;
Fentanil;
Anestesia regional;
Infecções

Efeitos antimicrobianos do fentanil e da bupivacaína: estudo *in vitro*

Resumo
Objetivo: O objetivo do presente estudo foi comparar os efeitos antimicrobianos da bupivacaína e citrato de fentanil e revelar o impacto no potencial do efeito antimicrobiano no caso de uso combinado.
Desenho: Estudo prospectivo *in vitro*.
Local: Laboratório de Microbiologia Clínica da Universidade.
Medidas: Em nosso estudo, os efeitos antimicrobianos *in vitro* do citrato de fentanil na concentração de 0,05 mg.mL^{-1} – Grupo F e da bupivacaína na concentração de 5 mg.mL^{-1} – Grupo B foram testados em culturas de *Staphylococcus aureus* ATCC 29213 (do inglês *American Type Culture Collection* 29213), *Pseudomonas aeruginosa* ATCC 27853, *Klebsiella pneumoniae* ATCC 13883, *Escherichia coli* ATCC 25922 e *Candida albicans* ATCC 10231. As culturas de *S. aureus* ATCC 29213, *P. aeruginosa* ATCC 27853, *Klebsiella pneumoniae* ATCC 13883 e *Escherichia coli* ATCC 25922 foram semeadas em placas de ágar Mueller Hinton (Oxoid, Reino Unido), e a cultura de *Candida albicans* ATCC 10231 foi realizada em placa de ágar Sabouraud dextrose (Oxoid, Reino Unido) durante 18—24 horas a 37 °C.
Principais resultados: Com relação ao diâmetro da zona de inibição, os valores de *S. aureus* ATCC 29213, *P. aeruginosa* ATCC 27853 e *C. albicans* ATCC10231 obtidos após 12 e 24 horas de incubação foram significantemente maiores no Grupo F do que no Grupo B ($p < 0{,}001$). Os valores do diâmetro da zona de inibição das culturas de *E. coli* ATCC 25922 e *K. pneumomiae* ATCC 13883 obtidos após 12 e 24 horas de incubação foram significantemente maiores no Grupo B do que no Grupo F ($p < 0{,}001$, *E. coli* na 12ª hora $p = 0{,}005$)
Conclusões: A preferência atual e frequente pela adição de fentanil aos Anestésicos Locais (AL) para a realização de anestesia regional se deve sobretudo à possibilidade de redução da dose do anestésico local, a melhora na qualidade da analgesia e a satisfação do paciente. No entanto, ao considerar estudos em que o fentanil antagonizou o efeito antimicrobiano dos AL, pode-se pensar que esse fato contribua para aumento de complicação infecciosa. O citrato de fentanil usado em nosso estudo, contendo ácido clorídrico e hidróxido de sódio como agentes conservantes, ampliou o espectro de efeitos antimicrobianos dos AL, não teve efeito antagônico e demonstrou efeito antimicrobiano sinérgico contra a *E. coli*. Acreditamos que a adição de fentanil aos anestésicos locais traria importante contribuição na prevenção das crescentes complicações por infecção da anestesia regional.

Introduction

Bupivacaine is a Local Anesthetic (LA) agent widely used in epidural anesthesia and local surgery operations.[1,2] Fentanyl citrate is widely used in epidural anesthesia. Addition of fentanyl in epidural anesthesia reduces the LA dose and pain.[2] Addition of fentanyl citrate to LAs increases complications such as nausea-vomiting, itching, sedation, and delayed respiratory depression.[3] Still, it provides better postoperative analgesia and patient satisfaction.[2] Therefore, the addition of fentanyl citrate to LAs is controversial. Today, regional anesthesia practices are becoming more widespread while the consequent infection incidence is reported to be increasing.[4,5] For this reason, utilizing the

antimicrobial effect potential of the anesthetic agents to be used in regional anesthesia might contribute to decreasing infection incidence. While there are many studies in the literature that include results inconsistent with one another as to the antimicrobial effects of LAs, there are limited number of studies regarding the antimicrobial effects of fentanyl citrate. The number of studies on the antimicrobial effects of fentanyl + LA combinations is very low.[5,6]

In this study, we aimed to compare the antimicrobial effects of bupivacaine and fentanyl citrate and to reveal the impact on antimicrobial effect potential in the case of combined use.

Methods

Determination of *in vitro* antimicrobial effect

In our study, *in vitro* antimicrobial effect of 0.05 mg.mL^{-1} fentanyl citrate (Talinat, Vem, Istanbul, Turkey), 5 mg.mL^{-1} bupivacaine (Marcaine, AstraZeneca PLC, England) were tested against *Staphylococcus aureus*ATCC 29213 (American Type Culture Collection), *Pseudomonas aeruginosa* ATCC 27853, *Klebsiella pneumoniae* ATCC 13883, *Escherichia coli* ATCC 25922 and *Candida albicans* ATCC 10231 as Group F (Fentanyl citrate), Group B (Bupivacaine). *S. aureus* ATCC 29213, *P. aeruginosa* ATCC 27853, *Klebsiella pneumoniae* ATCC 13883 and *Escherichia coli* ATCC 25922 were cultured onto Mueller Hinton agar (Oxoid, UK) plates and *Candida albicans* ATCC 10231 were cultured onto Sabouraud dextrose agar (Oxoid, UK) plates for 18–24 hours at 37 °C. Colonies from these plates were suspended in sterile saline and a 0.5 McFarland turbidity standard suspension (corresponding to 1.5×10^8 CFU. mL^{-1}) of each isolate was prepared. Each labelled Mueller-Hinton and Sabouraud dextrose agar (Oxoid, UK) plate was uniformly seeded with a test organism by means of sterile swab rolled in the suspension and streaked on the plate surface. *In vitro* antimicrobial activity of the 0.05 mg.mL^{-1} fentanyl citrate, 5 mg.mL^{-1} bupivacaine were evaluated by disc diffusion method using with determination of inhibition zones. Each sterile disc (Merck, Germany) was impregnated with drugs (bupivacaine and/or fentanyl) and was placed and incubated on Mueller-Hinton Agar for 24 hours at 37 °C. The zone of inhibition was measured in the 12th and 24thhour. Each experiment was performed ten times.

Determination of Minimum Inhibitory Concentration (MIC) and Minimal Bactericidal Concentration (MBC)

The broth microdilution method was used to determine the Minimum Inhibitory Concentration (MIC) values on Mueller Hinton broth (Oxoid, UK) using 96-well microplates in accordance with the Clinical and Laboratory Standards Institute guidelines.[7] In this study, the anesthetic agents were prepared by two-fold serial dilution in Mueller Hinton broth, and 20, 10, 5, 2.5, 1.25, 0.625, 0.312, 0.156 and 0.078 mg.mL^{-1} concentrations of anesthetic agents of fentanyl citrate and bupivacaine were tested. The appropriate concentration of drug was added to the specific wells of a 96-well microplates containing each bacterium from overnight culture. Each test was performed three times. Microwell plates were incubated at 37 °C in a microplate incubator shaker. The OD600 (wavelength of 600 nm) was measured after 24-hour incubation by using Epoch spectrophotometer (BioTek Inst. Inc. Vermont, USA). Wells without anesthetic agents were used as growth control and wells with Mueller Hinton broth alone served as negative control. The percentage of viable cells was normalized to 100% for the growth control.[8]

To determine the Minimal Bactericidal Concentration (MBC), each well exhibiting no visible growth (viability) after 18 hours was tested for viable organisms by subculturing 10 μl samples of each well onto Mueller-Hinton agar. The plates were incubated at 37 °C to observe the growth of any colony after 24 hours.[7]

Determination of drug interaction via broth microdilution method

Fentanyl citrate (F) and bupivacaine (B) combinations were diluted in Mueller-Hinton broth with or without *S. aureus* ATCC 29213, *K. pneumoniae* ATCC 13883, *E. coli* ATCC 25922, *P. aeruginosa* ATCC 27853 and *C. albicans* ATCC 10231 which were adjusted to the turbidity of a 0.5 McFarland standard. Ten microliters of each strain were inoculated to each well. The plate was incubated overnight and microbial growth in each well was measured with Epoch spectrophotometer (Biotek, Germany) at 600 nm. Each experiment was performed ten times. The preparation of the microdilution in microwells with single agent concentrations of the analgesic to determine the Minimum Inhibitory Concentration (MIC), control wells and paired combinations of the drugs were used in unique concentrations to determine the Fractional Inhibitory Concentration (FIC). The value of the $\sum$FIC index is then used to determine whether synergism, indifference or antagonism occurred between the antimicrobial agents and it was used to interpret the nature of the interactions: synergism $\leq$ 0.5, indifference > 0.5 to $\leq$ 4, antagonism > 4 according to American Society of Microbiology.[9]

Statistical analysis

The normality of continuous variables was investigated by Shapiro-Wilk's test. Descriptive statistics were presented using mean and standard deviation for normally distributed variables and median (and minimum–maximum) for the non-normally distributed variables. Non-parametric statistical methods were used for values with non-normal. For comparison of two non-normally distributed independent groups, Mann-Whitney *U* test was used or for comparison of two non-normally distributed dependent groups Wilcoxon test was used. Statistical significance value was determined to be 0.05. *Post hoc* evaluations were made using Mann-Whitney *U* test with Bonferroni correction. Statistical significance value was determined to be 0.005. Statistical analysis was performed using the MedCalc Statistical Software version 12.7.7 (MedCalc Software bvba, Ostend, Belgium; http://www.medcalc.org; 2013).

Table 1 Distribution of inhibition zone diameters in groups against microorganisms (mm).

	Group C (Control)	Group F (Fentanyl citrate)	Group B (Bupivacaine)
S. aureus ATCC 29213			
12th hour	0.00	14.10	4.00
24th hour	0.00	15.00	4.70
P. aeruginosa ATCC 27853			
12th hour	0.00	4.40	0.00
24th hour	0.00	5.50	0.00
E. coli ATCC 25922			
12th hour	0.00	3.60	4.80
24th hour	0.00	4.40	5.80
K. pneumoniae ATCC 13883			
12th hour	0.00	0.00	5.70
24th hour	0.00	0.00	6.50
C. albicans ATCC 10231			
12th hour	0.00	4.00	0.00
24th hour	0.00	4.50	0.00

Table 2 Comparison of inhibition zone diameters between groups.

	Group C (Control) Mean ± SDMed. (min – max)	Group F (Fentanyl citrate) Mean ± SDMed. (min – max)	Group B (Bupivacaine) Mean ± SDMed. (min–max)	p^a
S. aureus 12th hour	0 (constant)	14.1 ± 0.7 14 (13–15)	4 ± 0.7 4 (3–5)	< 0.001
S. aureus 24th hour	0 (constant)	15 ± 0.8 15 (14–16)	4.7 ± 0.7 5 (4–6)	< 0.001
p^b	1.000	0.021	0.059	
P. aeruginosa 12th hour	0 (constant)	4.4 ± 0.7 4 (4–6)	0 (constant)	< 0.001
P. aeruginosa 24t^{h} hour	0 (constant)	5.5 ± 0.7 6 (4–6)	0 (constant)	< 0.001
p^b	1.000	0.015	1.000	
E. coli 12th hour	0 (constant)	3.6 ± 0.7 3.5 (3–5)	4.8 ± 0.8 5 (4–6)	0.005
E. coli 24th hour	0 (constant)	4.4 ± 0.5 4 (4–5)	5.8 ± 0.6 6 (5–7)	< 0.001
p^b	1.000	0.033	0.008	
*K. pneumoniae*12th hour	0 (constant)	0 (constant)	5.7 ± 0.9 6 (4–7)	< 0.001
*K. pneumoniae*24th hour	0 (constant)	0 (constant)	6.5 ± 0.7 7 (5–7)	< 0.001
p^b	1.000	1.000	0.005	
C. albicans 12th hour	0 (constant)	4 ± 0.8 4 (3–5)	0 (constant)	< 0.001
C. albicans 24th hour	0 (constant)	4.5 ± 0.5 4.5 (4–5)	0 (constant)	< 0.001
p^b	1.000	0.096	1.000	

[a] Mann-Whitney U test.
[b] Wilcoxon Signed Rank test.

Results

Distribution of inhibition zone diameters in groups against microorganisms (mm) were shown in Table 1.

In terms of inhibition zone diameters, *S. Aureus* ATCC 29213, *P. aeruginosa* ATCC 27853, and *C. albicans* ATCC 10231 values obtained after 12 and 24 hours of incubation were significantly higher in Group F than Group B ($p < 0.001$). Statistically, fentanyl citrate was detected to have stronger antimicrobial effect on *S. Aureus* ATCC 29213, *P. aeruginosa* ATCC 27853, and *C. albicans* ATCC 10231 compared to bupivacaine. In terms of inhibition zone diameters, *E. coli* ATCC 25922, and K. pneumoniae ATCC 13883 values obtained after 12 hours and 24 hours of incubation were significantly higher in Group B than Group F ($p < 0.001$, *E. coli* 12 hour $p = 0.005$). Statistically, bupivacaine was detected to have stronger antimicrobial effect on *E. coli* ATCC 25922, and *K. pneumoniae* ATCC 13883 compared to fentanyl citrate. From 12th hour to 24thhour, inhibition zone diameters were determined to show a statistically significant increase against *S. Aureus* ATCC 29213, *P. aeruginosa* ATCC 27853, and *E. coli* ATCC 25922 in Group F and *E. coli* ATCC 25922 and *K. pneumoniae* ATCC 13883 in Group B ($p < 0.05$). Comparison of inhibition zone diameters between groups were shown in Table 2.

According to 12th and 24th hours inhibition zone, diameters in Group F were significantly different ($p < 0.001$). Twelfth and 24thhours antimicrobial potency in Group F; *S. Aureus* ATCC 29213 > *P. aeruginosa* ATCC 27853 = *C. albicans* ATCC 10231 > *E. coli* ATCC 25922 > *K. pneumoniae* ATCC 13883. According to 12th and 24th hours inhibition zone diameters in Group B were significantly different ($p < 0.001$). Twelfth and 24th hours antimicrobial potency in Group B; *K. pneumoniae* ATCC 13883 = *E. coli* ATCC 25922. *K. pneumomiae* ATCC 13883 > *S. Aureus* ATCC 29213 > *P. aeruginosa* ATCC 27853 = *C. albicans* ATCC 10231. *E. coli* ATCC 25922 = *S.*

Table 3 ∑FIC index for drug combinations against standard strains in vitro.

	∑FIC Index (fentanyl citrate + bupivacaine)
S. aureus ATCC 29213	
12th hour	0.61
24th hour	0.64
P. aeruginosa ATCC 27853	
12th hour	0.65
24th hour	0.61
E. coli ATCC 25922	
12th hour	0.38
24th hour	0.36
K. pneumoniae ATCC 13883	
12th hour	0.75
24th hour	0.65
C. albicans ATCC 10231	
12th hour	0.51
24th hour	0.51

Aureus ATCC 29213 > *P. aeruginosa* ATCC 27853 = *C. albicans* ATCC 10231.

When the effect of fentanyl citrate + bupivacaine combination on microorganisms was examined, it was determined that according to FIC value it only had synergistic effect on *E. coli*, while it did not have any synergistic effect on other microorganisms. Fractional Inhibitory Concentration (FIC) values in Group BF were shown in Table 3.

Distribution of MIC values and the effect of different concentrations of anesthetic agents against microorganisms were shown in Table 4.

Discussion

Local and regional anesthesia practices are frequently used during or after surgery for pain management.[10] It is reported in the clinical studies in the literature that addition of fentanyl to LAs results in better postoperative analgesia, a decrease in local anesthetic dose, and an increase in patient satisfaction only if neuraxial anesthesia is performed.[2,4,11] Therefore amide LA agents such as bupivacaine, levobupivacaine, and ropivacaine are widely used in practice for pain management in spinal and epidural applications combined with opioids such as fentanyl and sufentanyl.[4] This is reported to reduce side effects related to LAs, as well as negligible side effects related to opioids.[2,4] These results in the literature increase the preference for the use of the combined drugs application in practice.

One of the complications encountered during local surgical with local anesthetics operations is wound infections. Wound infections negatively affect wound healing. This leads to an increase in treatment costs, morbidity and mortality.[1] In the literature, many cases were reported where regional (spinal, epidural) anesthesia and analgesia applications related central nervous system infections such as epidural abscess and meningitis developed.[5,12–15] It is known that central nervous system infections can result in severe morbidity that extends to paraplegia. Therefore, it is of utmost importance in the clinical practice to utilize antimicrobial effect potentials of LAs, as well as paying attention to asepsis rules in order to prevent LA application related wound infections and central nervous system infections. Thus, a decrease in morbidity, mortality, treatment costs and hospitalization durations may be obtained.[1,12]

Regional anesthesia is widely used in modern practice and some studies report an increase in the development of infections related to regional anesthesia and analgesia applications.[4,5,10,13,16] Therefore, utilization of the antimicrobial effect potentials of anesthetic agents gains importance, aside from paying attention to asepsis rules during the application. It is observed in this context that the number of studies in the literature regarding the antimicrobial effects of fentanyl + LA combinations is limited.[5,6] In these studies of limited number, it is reported that the addition of fentanyl citrate usually has a negative effect on the antimicrobial effect potentials of the LAs.[5] In our study, however, different from the results of the studies in the literature, no antagonistic effect was observed in the antimicrobial effectiveness of bupivacaine with the addition of fentanyl on five different microorganisms. It was detected that it even had a synergistic effect on *E. Coli*. Also, it was determined that when used alone, bupivacaine did not have antimicrobial effect on *P. aeruginosa, C. albicans* and fentanyl on *K. pneumoniae*, and therefore it was thought that a better contribution to decreasing the infection risk might be achieved with the combined use of these agents as the effect spectrum broadens. In the *in vitro* study by Mutlu E.,[5] lidocaine and bupivacaine were reported to have antimicrobial effect against many microorganisms while no antimicrobial effect was observed when they were combined with fentanyl. By contrast, no antagonistic effect was observed in our study for any microorganism with the addition of fentanyl to bupivacaine. In fact, a synergistic effect against *E. coli* was detected. Therefore, broadening of the antimicrobial effect spectrum of bupivacaine was contributed with the addition of fentanyl. It was concluded that this might contribute to reduction in infection complications.

The antimicrobial effect of fentanyl is not clearly known and there is a limited number of studies.[17] In the study by Feldman et al.,[17] it was reported that lidocaine and bupivacaine had antimicrobial effect against *S. Aureus*; yet, did not have antimicrobial effect when fentanyl and sufentanyl was added. In the study by Rosenberg et al.,[18] opioids were reported to not have antimicrobial effects. In the study by Tamanai-Shacoori et al.,[19] the addition of sufentanyl was reported to increase the antimicrobial effect of bupivacaine. In the study by Kampe et al.,[20] it was concluded that the antimicrobial effect increased with the addition of sufentanyl to ropivacaine. In another study in the literature, it was reported that remifentanil, which is an analogue of fentanyl, had antimicrobial effect and this effect might be due to the preservative substance in the remifentanil ampoule, glycine.[21] When the studies in the literature are analyzed, it can be seen that effects of the addition of opioids to LAs are debated and there isn't a sufficient number of studies. It is seen that opioids other than fentanyl have antimicrobial effect by themselves in some studies or have synergistic antimicrobial effect when added to LAs in some studies. However, fentanyl addition was reported to have antagonistic effect.

Table 4 Distribution of MIC values and the effect of different concentrations of anesthetic agents against microorganisms.

Fentanyl citrate (mg. mL^{-1})	0.078	0.156	0.313	0.625	1.25	2.50	5.00	10.00	20.00
S. aureus ATCC 29213	+	+	+	+	+	+	-a	–	–
P. aeruginosa ATCC 27853	+	+	+	+	+	+	+	+	-[a]
E. coli ATCC 25922	+	+	+	+	+	+	+	+	+
K. pneumoniae ATCC 13883	+	+	+	+	+	+	+	+	+
C. albicans ATCC 10231	+	+	+	+	-[a]	–	–	–	–
Bupivacaine (mg. mL^{-1})	0.078	0.156	0.313	0.625	1.25	2.50	5.00	10.00	20.00
S. aureus ATCC 29213	+	+	+	+	+	+	+	+	+
P. aeruginosa ATCC 27853	+	+	+	+	+	+	+	+	+
E. coli ATCC 25922	+	+	+	+	+	+	+	+	-[a]
K. pneumoniae ATCC 13883	+	+	+	+	+	+	+	+	-[a]
C. albicans ATCC 10231	+	+	+	+	+	+	+	+	+
Fentanyl citrate (mg. mL^{-1}) + bupivacaine (mg. mL^{-1})	0.078	0.156	0.313	0.625	1.25	2.50	5.00	10.00	20.00
S. aureus ATCC 29213	+	+	+	+	+	+	+	-[a]	–
P. aeruginosa ATCC 27853	+	+	+	+	+	+	+	+	-[a]
E. coli ATCC 25922	+	+	+	+	+	+	-[a]	–	–
K. pneumoniae ATCC 13883	+	+	+	+	+	+	+	+	-[a]
C. albicans ATCC 10231	+	+	+	+	+	-[a]	–	–	–

[a] MIC values.

In contrast with the studies in the literature, it was determined in our study that fentanyl addition did not have antimicrobial antagonistic effect on any microorganism while it had synergistic effect against *E. Coli*. The results obtained in our study suggest that the bupivacaine + fentanyl combination that is used widely due to its stronger analgesic effect and higher patient satisfaction, can be preferred safely as it will reduce the risk of infection development. The fact that fentanyl citrate had antimicrobial effect in our study in contrast with the others in the literature, whether used by itself or combined, it was regarded to be related to sodium hypochloride and sodium hydroxide that are added to fentanyl citrate as protectives because hydrochloric acid and sodium hydroxide are known to have strong antimicrobial effect.[22,23]

Conclusion

The addition of fentanyl to LAs is often preferred in regional anesthesia applications in today's practice owing especially to its effect on decreasing the local anesthetic dose and increasing analgesia quality and patient satisfaction. However, when the fact that fentanyl antagonized the antimicrobial effects of LAs in the studies is taken into account, it might be though that it contributes to an increase in infection complications. When the fact that fentanyl citrate, which was used in our study and included hydrochloric acid and sodium hydroxide as protective agents, broadened the antimicrobial effect spectrum of LAs, had no antagonistic effect and showed a synergistic antimicrobial effect against E. Coli is considered, we are of the opinion that the addition of fentanyl to LAs would contribute significantly in preventing the increasing regional anesthesia infection complications. We think that the results of the study, supported by clinical studies, will contribute greatly to the clinical practice.

Conflicts of interest

The authors declare no conflicts of interest.

References

1. Kesici U, Demirci M, Kesici S. Antimicrobial effects of local anaesthetics. Int Wound J. 2019;16:1029–33.
2. Khanna A, Saxena R, Dutta A, et al. Comparison of ropivacaine with and without fentanyl vs bupivacaine with fentanyl for postoperative epidural analgesia in bilateral total knee replacement surgery. J Clin Anesth. 2017;37:7–13.
3. Van Leeuwen L, Deen L, Helmers JHA. Comparison of alfentanil and fentanyl in short operations with special reference to their duration of action and postoperative respiratory depression. Anaesthesist. 1981;30:397–9.
4. Li B, Wang H, Gao C. Bupivacaine in combination with fentanyl or sufentanil in epidural/intrathecal analgesia for labor: a meta-analysis. J Clin Pharmacol. 2015;55:584–91.
5. Mutlu E. In vitro ınvestigation of the antibacterial effects of lidocaine and bupivacaine alone andin combinations with fentanyl. Turkiye Klinikleri J Med Sci. 2018;38:334–9.
6. Razavi BM, Fazly Bazzaz BS. A review and new insights to antimicrobial action of local anesthetics. Eur J Clin Microbiol Infect Dis. 2019;38:991–1002.
7. CLSI (Clinical and Laboratory Standards Institute): Dilution antimicrobial susceptibility tests for bacteria that grow aerobically. Approved standart. 10 ed. CLSI document; 2015. p. M7–A10.

8. Oyama LB, Crochet JA, Edwards JE, et al. Buwchitin: a ruminal peptide with antimicrobial potential against enterococcus faecalis. Front Chem. 2017;5:51.
9. Botelho MG. Fractional inhibitory concentration index of combinations of antibacterial agents against cariogenic organisms. J Dent. 2000;28:565–70.
10. Aydin ON, Eyigor M, Aydin ON. Antimicrobial activity of ropivacaine and other local anaesthetics. Eur J Anaesthesiol. 2001;18:687–94.
11. Sun Y, Xu Y, Wang GN. Comparative Evaluation of Intrathecal Bupivacaine Alone, Bupivacaine-fentanyl, and Bupivacaine-dexmedetomidine in Caesarean Section. Drug Res (Stuttg). 2015;65:468–72.
12. Coghlan MW, Davies MJ, Hoyt C, et al. Antibacterial activity of epidural infusions. Anaesth Intensive Care. 2009;37:66–9.
13. Kesici S, Demirci M, Kesici U. Bacterial inhibition efficiency of prilocaine and bupivacaine. Int Wound J. 2019;16:1185–9.
14. Reihsaus E, Waldbaur H, Seeling W. Spinal epidural abscess: a meta-analysis of 915 patients. Neurosurg Rev. 2000;23:175–204.
15. Grewal S, Hocking G, Wildsmith JA. Epidural abscess. Br J Anaesth. 2006;96:292–302.
16. Wang LP, Hauerberg J, Schmidt JF. Incidence of spinal epidural abscess after epidural analgesia: a national 1-year survey. Anesthesiology. 1999;91:1928–36.
17. Feldman JM, Chapin-Robertson K, Turner J. Do agents used for epidural analgesia have antimicrobial properties? Reg Anesth. 1994;19:43–7.
18. Rosenberg PH, Renkonen OV. Antimicrobial activity of bupivacaine and morphine. Anesthesiology. 1985;62:178–9.
19. Tamanai-Shacoori Z, Shacorri V, Van JM Vo, et al. Sufentanil modifies the antibacterial activity of bupivacaine and ropivacaine. Can J Anaesth. 2004;51:911–4.
20. Kampe S, Poetter C, Buzello S, et al. Ropivacaine 0.1% with sufentanil 1 microg/L inhibits in vitro growth of Pseudomonas aeruginosa and does not promote multiplication of Staphylococcus aureus. Anesth Analg. 2003;97:409–11.
21. Apan TZ, Apan A, Şahin S, et al. Antibacterial activity of remifentanil and mixtures of remifentanil and propofol. J Clin Anesth. 2007;19:346–50.
22. Charaibi M, Benbrahim KF, Elmsellem H, et al. Antibacterial activity and corrosion inhibition of mild steel in 1.0 M hydrochloric acid solution by M. piperita and M. pulegium essential oils. JMES. 2017;8:972–81.
23. Chavant P, Gaillard-Martinie B, Hébraud M. Antimicrobial effects of sanitizers against planktonic and sessile Listeria monocytogenes cells according to the growth phase. FEMS Microbiol Lett. 2004;236:241–8.