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Research Article

Antimicrobial effects of ropivacaine, levobupivacaine and bupivacaine at different temperatures

Ropivakain, levobupivakain ve bupivakainin farklı sıcaklıklardaki antimikrobiyal etkileri

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Abstract

Aim: Various prior studies have shown a possible antimicrobial activity of different local anesthetic (LA) drugs. The aim of this study was to assess the antimicrobial (antibacterial or antifungal) activity of commonly used commercially available LA drugs used in regional anesthesia practice such as; ropivacaine, levobupivacaine and bupivacaine on S.aureus ATCC 6538, S. epidermidis, E.coli and Candida albicans at different temperatures.

Material and Methods: Possible antimicrobial effects of ropivacaine (1% and 0.125%), levobupivacaine (0.75% and 0.125%) and bupivacaine (0.5% and 0.125%) on S.aureus ATCC 6538, S.epidermidis, E.coli and C.albicans ATCC 10231, which were isolated from patients in the microbiology laboratory were investigated at 24°C and 37°C.

Results: All three LA drugs showed antifungal activity at 37°C and 24°C in 3rd and 12th hours. Levobupivacaine's (0.75%) antibacterial effect was observed at the 12th hour. Ropivacaine (1%) had relatively more antibacterial effect at the 3rd and 12th hour at 37°C rather than 24°C whereas bupivacaine had less.

Conclusion: Potential clinical implications of antibacterial or antifungal effects of local anesthetics might be promising.

Keywords: Local anesthetics, bupivacaine, levobupivacaine, ropivacaine, Antimicrobial activity, antibacterial, antifungal

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Öz

Amaç: Lokal anestezik (LA) ilaçların, daha önce yapılan çeşitli çalışmalarla olası bir antimikrobiyal aktivitesi gösterilmiştir. Bu çalışmanın amacı, rejyonel anestezi uygulamalarında yaygın olarak kullanılan ropivakain, levobupivakain ve bupivakain gibi ticari olarak mevcut LA ilaçlarının farklı sıcaklıklarda S.aureus ATCC 6538, S. epidermidis, E.coli ve Candida albicans üzerindeki antimikrobiyal (antibakteriyel veya antifungal) aktivitesini değerlendirmektir.

Gereç ve Yöntemler: Mikrobiyoloji laboratuvarında ropivakain (%1 ve %0,125), levobupivakain (%0,75 ve %0,125) ve bupivakainin (%0,5 ve %0,125) hastalardan izole edilen S.aureus ATCC 6538, S.epidermidis, E.coli ve C.albicans ATCC 10231 üzerindeki olası antimikrobiyal etkileri 240C ve 370C'de araştırıldı.

Bulgular: Her üç lokal anestezik ilaç da, 37°C ve 24°C'de 3. ve 12. saatlerde antifungal aktivite göstermiştir. Levobupivakainin (%0,75) antibakteriyel etkisi 12. saatte gözlendi. Ropivakain (%1) 37°C'de 3. ve 12. saatlerde 24°C'ye göre nispeten daha fazla antibakteriyel etkiye sahipken, bupivakain daha az antibakteriyel etkiye sahipti.

Sonuçlar: Lokal anesteziklerin antibakteriyel veya antifungal etkilerinin potansiyel klinik sonuçları umut verici olabilir.

Anahtar Kelimeler: Lokal anestezikler, bupivakain, levobupivakain, ropivakain, Antimikrobiyal aktivite, antibakteriyel, antifungal

Introduction

In clinical practice local anesthetic (LA) drugs are used not only to provide analgesia and/or anaesthesia but also to treat arryhthmias. However, various studies have shown possible antimicrobial activity. A number of studies reported that bupivacaine (0.25%), lidocaine (1.25%) and chloroprocaine (0.75%) inhibited growth of Staphylococcus aureus (S.aureus), whereas articaine, bupivacaine and ropivacaine have shown relatively weak antibacterial effect on skin flora bacteria like Escherichia coli (E.coli), S.aureus and Pseudomonas aeruginosa (P.aeruginosa) [1-4]. Since we have not come across any comprehensive research investigating the relationship between antimicrobial activity of LA drugs and temperature until now, we hypothetize whether anesthetic and/or analgesic concentration of LA drugs might have a possible antimicrobial effect against fungus and/or bacteria at 37°C rather than room temperature which could be a potential implication in regional anesthesia practice. Therefore, we aimed to investigate the possible antimicrobial (either antibacterial or antifungal) activity of clinically used concentrations of ropivacaine, levobupivacaine and bupivacaine on S. aureus ATCC 6538, S. epidermidis, E.coli and Candida albicans at different (either room or body) temperatures. Secondly, to indicate the survivability of the possible antimicrobial effect.

Material and Methods

After obtaining approval of the institutional ethic committee for in vitro investigations, possible antimicrobial effects of ropivacaine (1% and 0.125%) levobupivacaine (0.75% and 0.125%) and bupivacaine (0.5% and 0.125%) on S. aureus ATCC 6538, S. epidermidis, E. coli and C.albicans ATCC 10231, which were isolated from patients' blood samples in the microbiology laboratory at 240C and 370C for accepted as room and body temperatures, respectively were investigated.

Experiment Protocol

1st step: Selection of LA drugs and concentration

Commercially available LA drugs (ropivacaine 1%, bupivacaine 0.5% and levobupivacaine 0.75%) were chosen and their original concentrations and 0.125% concentraions were investigated.

1. Ropivacaine (Naropin®Ampule 1%, 20 mL injektions-lösung, AstraZeneca) 1% and 0.125%.

2. Levobupivacaine (Chirocaine® Ampule, 0.75%, 10 mL, Abbott) 0.75% and 0.125%.

3. Bupivacaine (Marcaine® 0.5%, injection solution flacon 20 mL, AstraZeneca) 0.5% and 0.125%.

Step 2: Selection of Microorganisms (0.5 Mc Farland)

1. Staphylococcus aureus ATCC 6538

2. Staphylococcus epidermidis

3. Escherichia coli

4. Candida albicans ATCC 10231 were chosen.

Step 3: Procedure

Nutrient broth medium was placed in each tube. Samples of S. aureus, S. epidermidis, E. coli and C. albicans were prepared with bacterial and yeast suspensions of 0.5 McFarland each. Three bacteria strains (S. aureus, S. epidermidis and E. coli) and

1 fungus (C. albicans) and their control groups were prepared.

Total of 56 samples were studied at room and body temperatures:

Ropivacaine 2 concentrations X 4 microorganisms (n=8)

Levobupivacaine 2 concentrations X 4 microorganisms (n=8)

Bupivacaine 2 concentrations X 4 microorganisms (n=8)

Study Group 24 oC (n=24) and Control Group 24 oC (n= 4)* (24+4=28)

Study Group 37 oC (n=24) and Control Group 37 oC (n= 4)* (24+4=28)

*: 1 control sample for each microorganism

Tubes from the study and control groups were kept at 24°C and 37°C for each incubation time period (3th hour, 12 hour and 24 hour), then samples were taken from the nutrient agar medium. Petri dishes were divided into 4 sections and 5µL of each LA drug was inoculated. Each drug was studied in the incubator, which corresponds to either room or body temperature (Figure A and B).

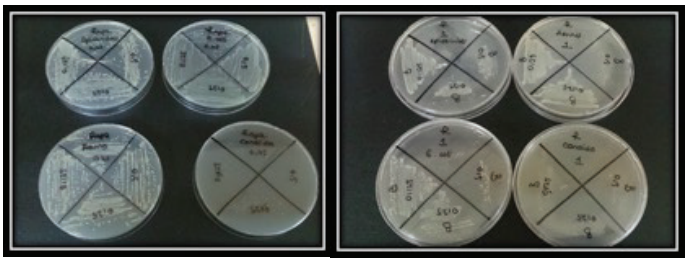

Figure 1.A-B. Incubated petri dishes containing different concentration of local anesthetics.

After incubation in incubator for 24 hours, all the plaques were evaluated whether there was a growth or not. In case of no growth which was represented as negative effect (-), antibacterial effect was considered for that local anesthetic. If there was a growth which was represented as positive (+), there was no antibacterial effect. More extensive growth was indicated with ++.

Statistical Analysis

Sample size was calculated according to the investigation of 40 bacterial isolates for 3 LA drugs as described [5]. IBM SPSS® Statistics (Statistical Package for the Social Sciences) for Windows 23 program was used. Results were expressed in numbers or percentages where appropriate. Reproductive findings were accepted as categorical variables and presence of growth were compared according to temperatures (24ºC vs 37ºC) with chi-square test using Epi info programme. A p value of less than 0.05 was considered as statistically significant.

Results

Antibacterial effects of three LA drugs; 3, 12 and 24 hours after incubation were presented at room (24°C) and body (37°C) temperatures (tables 1, 2 and 3).

Ropivacaine 1% showed antibacterial effect against S.aeurus and E.Coli at 37°C 3 hours after incubation (Table 1). Ropivacaine 1%'s antibacterial activity showed statistically significant difference between 24ºC and 37ºC (p=0.03) but that effect was not significantly different when compared to 0.125% concentration at 37ºC (p=0.083). Bupivacaine 0.5% inhibited the growth of S.aureus 3 hours after incubation at 37ºC which did not significantly differ between 24ºC and 37ºC (p=0.30)(Table 1).

After 12 hours, ropivacaine 1% at 37°C and levobupivacaine 0.75% at both 24°C and 37°C showed antibacterial effect against S.aeurus, s.epidermidis and E.coli (Table 2).

After 24 hours, antibacterial effect of ropivacaine 1% against S.aeurus and s.epidermidis was observed at 24°C and 37°C, while it was observed against E.coli only at 24°C. Levobupivacaine 0.75% at 24°C showed antibacterial effect against only S.aeurus 24 hours after incubation and this effect was not statistically significant between 0.75% and 0.125% of levobupivacaine (p=0.223). When temperatures were compared; ropivacaine, bupivacaine and levobupivacaine's antimicrobial effects were not significant between 24ºC and 37ºC (p=0.068, p>0,05 and p=0.39, respectively) (Table 3).

The three local anesthetics at studied concentrations inhibited fungus growth at 37°C and 24°C until 3 and 12 hours after incubation (tables 4A and 1B).

Discussion

We observed that antimicrobial effects of LA drugs varied according to temperature and determination time after incubation. Hereby, ropivacaine 1% at 37°C and levobupivacaine 0.75% at both studied temperatures (24°C and 37°C) showed antibacterial effect against to all studied bacteria (S.aeurus, s.epidermidis and E.coli) 12 hours after incubation. Additionally, three LA drugs both at room and body temperatures exhibited antifungal action for 12 hours independent of the concentrations used in the present study.

Various studies reported that LA drugs could be regarded as antimicrobial agents [6-11]. Bactericidal activity of preservative free bupivacaine in the skin flora and in vitro comparison of antibacterial activity of bupivacine vs levobupivacaine and ropivacaine vs bupivacaine have been demonstrated [6,7,11].

Table 1. Bacterial growth 3 hours after incubation according to temperature and concentration of local anesthetics.

	Concentration	1%		0.75%		0.5%		0.125%	
	Temperature	24°C	37°C	24°C	37°C	24°C	37°C	24°C	37°C
Ropivacaine	S. aeurus	+	-*					+	+
	S. epidermidis	+	+					+	+
	E. coli	+	-*					+	+
Levobupivacaine	S. aeurus			+	+			+	+
	S. epidermidis			+	+			+	+
	E. coli			+	+			+	+
Bupivacaine	S. aeurus					+	-	+	+
	S. epidermidis					+	+	+	+
	E. coli					+	+	+	+

*:p <0.05 No growth (-), which means antibacterial effect for that local anesthetic.
Presence of growth (+), which means no antibacterial effect for that local anesthetic.
*p=0.03 Ropivacaine 1%'s antibacterial activity showed statistically significant difference between 24°C and 37°C.
p=0.083 Ropivacaine 0.125%'s antibacterial activity did not showed statistically significant difference between 24°C and 37°C.

Table 2. Bacterial growth 12 hours after incubation according to temperature and concentration of local anesthetics.

	Concentration	1%		0.75%		0.5%		0.125%	
	Temperature	24°C	37°C	24°C	37°C	24°C	37°C	24°C	37°C
Ropivacaine	S. aeurus	+	-					+	+
	S. epidermidis	+	-					+	+
	E. coli	+	-					+	+
Levobupivacaine	S. aeurus			-	-			+	+
	S. epidermidis			-	-			+	+
	E. coli			-	-			+	+
Bupivacaine	S. aeurus					+	+	+	+
	S. epidermidis					+	+	+	+
	E. coli					+	+	+	+

*:p <0.05
No growth (-) means antibacterial effect for that local anesthetic.
Presence of growth (+) means no antibacterial effect or that local anesthetic.

Table 3. Bacterial growth 24 hours after incubation according to temperature and concentration of local anesthetics.

	Concentration	1%		0.75%		0.5%		0.125%	
	Temperature	24°C	37°C	24°C	37°C	24°C	37°C	24°C	37°C
Ropivacaine	S. aeurus	-	-					+	+
	S. epidermidis	-	-					+	+
	E. coli	-	+					+	+
Levobupivacaine	S. aeurus		-	+			+	+	
	S. epidermidis		+	+			+	+	
	E. coli		+	+			+	+	
Bupivacaine	S. aeurus			+	+	+	+		
	S. epidermidis			+	+	+	+		
	E. coli			+	+	+	+		

No growth (-) means antibacterial effect for that local anesthetic.
Presence of growth (+) means no antibacterial effect or that local anesthetic.

Table 4A. Fungal (C. albicans) growth at room and body temperatures 3 and 12 hours after incubation of varying concentrations of local anesthetics.

Concentration	0.125%		0.5%		0.75%		1%	
Temperature	24oC	37oC	24oC	37oC	24oC	37oC	24oC	37oC
Bupivacaine	-	-	-	-				
Levobupivacaine	-	-	-	-	-	-		
Ropivacaine	-	-	-	-	-	-	-	-

In case of no growth which was represented as negative effect (-), it was considered that there was an antibacterial effect for that local anesthetic. If there was a growth which was represented as positive (+), it was considered that there was no antibacterial effect.

Table 4B. Fungal (C. albicans) growth at room and body temperatures 24 hours after incubation according to concentration of local anesthetics.

	Temperature	
	24ºC	37ºC
Bupivacaine (0.5%, and 0.125%)	+	+
Levobupivacaine (0.75%, and 0.125%)	++	+
Ropivacaine (1%)	-	+
Ropivacaine (0.125%)	+	+

In case of no growth which was represented as negative effect (-), it was considered that there was an antibacterial effect for that local anesthetic. If there was a growth which was represented as positive (+), it was considered that there was no antibacterial effect.

Additionally, bacterial growth was observed with ropivacaine hydrochloride of 10 mg/mL (not with 2 mg/mL), ropivacaine 1% with sufentanil inhibited in vitro growth of p.aeruginosa [9, 10]. However, levobupivacaine 1% with sufentanil had no antibacterial effect at 25ºC in vitro [8]. Modification of the antibacterial activity of bupivacaine and ropivacaine was investigated in another study. Both bupivacaine and ropivacaine alone or with sufentanil inhibited growth of E.coli and S.aureus. The possible synergistic and/or antagonistic effect of sufentanil on the antibacterai activity of these two LA drugs could be mediated via the interaction of sufentanil with the cytoplasmic membrane where LA drugs act [12]. Therefore, we investigated local anesthetics' possible antimicrobial effect without adding any adjuvants.

In a previous study temperature and concentration dependent bactericidal activity against S. epidermidis and E.coli (skin flora bacteria) was observed with preservative free bupivacaine concentrations of 0.75%, 0.5%, 0.25% and 0.125% [6]. In another study, minimum bactericide concentration 24 hours after incubation for S. epidermidis, S. aureus and E. faecalis at 37oC for 0.125%, 0.25% and 0.5% concentrations of bupivacaine and levobupivacaine was found to be 0.25% for bupivacaine while it was 0.5% for levobupivacaine [7]. In contrast to these findings, not only bupivacaine 0.5% inhibited the growth of S.aureus at body temperature (37°C) but also ropivacaine 1% showed antibacterial effect against to S.aeurus and E.coli in our study. We chose to investigate anesthetic (0.5%) and analgesic (0.125%) concentrations to find out which concentration might produce antimicrobial effect. In regional anesthesia practice, the antimicrobial effect of the 0.5% concentration of bupivacaine against S. aureus (skin flora bacteria as well) at 37°C might have a protective role during disinfection.

Antibacterial effect of epidural infusion was studied in vitro because of the possible association with epidural abcess although it is a very rare (1 in 1000) complication [13]. It was reported that after 24 h incubation, inhibiton of growth was seen in 0.25% dilution prepared from bupivacaine 0.5% [2]. Regarding microorganisms associated with spinal/epidural abcess, inhibition of the growth of S.aureus with sufficient concentration of bupivacaine could explain very low incidence of epidural abcess as reported [2].

Despite to the early reports claming neither in vivo antimcirobial activity with bupivacaine nor in vitro antibacterial activity with ropivacaine and/or bupivacaine [11,14,15], in our study ropivacaine 1% showed antimicrobial activity 3, 12 and 24 h after incubation only at 37ºC and there was a similar antimicrobial activity pattern 24 h after incubation at both 24ºC and 37ºC as well. However, bupivacaine 0.5% inhibted growth of s.aureus at 37ºC only 3 hours after incubation. Notably, levobupivacaine 0.75% showed antimicrobial activity both at 24ºC and 37ºC 12 h after incubation.

When the possible anticandidal effects of bupivacaine and lidocaine were evaluated, 0.15% concentration of bupivacaine inhibited germ tube formation of the candida [16]. In another study lidocaine and bupivacaine at low doses showed fungistatic effect indirectly (yeast metabolic impairment) and at high doses due to direct damage to cytoplasmic membrane [17]. Currently all three LA drugs showed anticandidal effect 3 and 12 hour after incubation without being affected by temperature. However, after 24 hour, 1% of ropivacaine showed an anticandidal effect only at room temperature. The mechanism of reproduction at the 24th hour can be interpreted as improving the tolerance of yeast to the local anesthetic agent. Since this is the case in some antifungal drugs including azole group, where a similar effect was considered. Temporary and reversible local anesthetic effect may have contributed to that effect as well.

In conclusion first of all, the present study has shown the anticandidal effects of levobupivacaine and ropivacaine in addition to bupivacaine. We have also demonstrated possible

antimicrobial (either antibacterial or antifungal) activity of clinically used concentrations of ropivacaine, levobupivacaine and bupivacaine on S. aureus ATCC 6538, S. epidermidis, E.coli and Candida albicans at either room or body temperatures and partly indicated a 12-hour long survivability for antimicrobial effect. Even though not yet known fully, antibacterial and/or antifungal effects of local anesthetics might be promising for potential clinical implications.

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