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Investigation of Antimicrobial Effects of Some Anesthetics

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lt is determinated that some anesthetics have antimicrobial properties on bacterial cells besides pain perception. Our aim was to investigate the antimicrobial effects of lidocaine 2%, bupivacaine 0.5%, levobupivacaine 0.5%, ketamine 0.5%, propofol 1%, prilocaine 2%'s commercial solutions against *Staphylococcus aureus* 25923, *Escherichia coli* 25922, *Klebsiella pneumoniae* 574, *Candida albicans* 10231, *Pseudomonas aeruginosa* 27853. EMLA® 5% and Anestol 5% creams were tested against *Staphylococcus epidermidis* 35984 and *Enterococcus faecalis* 29212 in addition to other microorganisms. All anesthetic solutions were exposed to strains for 0, 30, 60, 120, 180, 240 minutes at room temparature. The inoculums taken from diluated suspensions were reinoculated on blood agar at 37ºC for 18-24 hours, then colony forming units were counted.Most strains had their growth inhibited by prilocaine, ketamine, lidocaine, EMLA® and anestol, which corresponds to a 1/10.000 dilution of anesthetic solutions. Bupivacaine reduced the viable cells of *S.aureus and E.coli*. Propofol did not inhibit any of the microorganisms tested except *E.coli* and levobupivacaine had a poor antimicrobial effect on *P.aeruginosa*.Prilocaine, ketamine, lidocaine solutions showed inhibitory effect on bacterial growth. EMLA® and Anestol creams had significant antimicrobial properties on common wound pathogenic bacteria *in vitro.*

Key words: Anesthetics, antimicrobial effect, EMLA®.

Anesthesia, which is usually applied before surgical operations, is a process that makes a part or whole body become unreponsive to pain[1,2].Drugs that create anesthesia are called anesthetics. The various regions of the body is colonized with multiple microbial flora, some of which are opportunistic pathogens[3]. The density and composition of this microbial flora vary with anatomic localization[4]. During the process of anesthesia, normal flora can enter the tissue by minor surgical interventions following perforation or as a result of damage to the skin[5, 6, 7]. Skin is the mechanical anatomical barrier of innate immun system and prevents systemic infection from invading surface microorganisms[8]. Complications including infection have been reported due to patient's cutaneous or anesthetist's otolaryngology flora during anesthesia[9] besides these, the bacterial colonization rate of catheters and needles were investigated[10,11]. lt was thought that some general and especially local anesthetics have antimicrobial effects on microorganisms[12].The first observation of antimicrobial effects of local anesthetics is attributed to Jonnesco, who remarked that novocaine need not be sterilised since it was itself antiseptic[13]. In this study, our aim was to investigate the antimicrobial activities of two general and four local anesthetic's commercial solutions and two anesthetic creams

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against different microorganisms that responsible for nosocomial infections.

MATERIAL AND METHODS

Drugs

Commercially available solutions of bupivacaine HCl (Marcaine 0.5%, ASTRAZENECA, Turkey), levobupivacaine HCl (Chirocaine 0.5%, ABBOTT, Italy), lidocaine HCl (Jetmonal 2%, ADEKA, Turkey), prilocaine HCl (Citanest 2%, ASTRAZENECA, Turkey), propofol 1% (FRESENIUS, Germany) and ketamine HCl (Ketalar 0.5%, PFIZER, Turkey), EMLA® 5% (25 mg/ml lidocaine and 25 mg/ml prilocaine, ASTRAZENECA, Turkey), Anestol 5% (Lidocaine 5%, SANDOZ, Turkey) were used.

Microbiological assay

The following bacteria were studied all from American Type Culture Collection except *Klebsiella penumoniae*. *K. pneumoniae*was from Refik Saydam Culture Collection in Turkey.

For anesthetic solutions;*Escherichia coli*ATCC 25922, *Staphylococcus aureus*ATCC 25923,*Pseudomonas aeruginosa*ATCC 27853, *Klebsiella pneumoniae* RSKK 574 and *Candida albicans*ATCC 10231 were tested. For anesthetic creams; *Staphylococcus epidermidis* ATCC 35984 and *Enterococcus faecalis* ATCC 29212 strains were also tested besides other bacteria which were studied for anesthetic's commercial solutions.

Anesthetic creams were prepared according to European Pharmacopoeia procedures[14] and a method described by Aydin *et al*[9] was modified and used for this experiment for all drugs. Microorganisms were cultured on blood agar for 18±24 h at 37°C. For all anesthetics, 1 ml anesthetic solution was prepared according to McFarland 0.5 density separately each bacteria. 100 µl of this solution was taken at 0´, 30´, 60´, 120´, 180´, 240´, added to 900µl saline at room temperature to inactivate the antibacterial activity of anesthetics and diluted until 1/10000 concentration was reached. One group where 0.9% saline was used instead of anesthetic solution as a control group.Colonies were counted after 10 µl inoculums taken from diluated suspencions were inoculated on blood agar and incubated 18±24 hours at 37°C.

RESULTS AND DISCUSSION

Previous studies have shown that local anesthetics inhibited bacterial growth by decreasing the number of viable cells, causing lysis of protoplast and permeability changes[15].

In our study prilocaine 2% reduced the viable cells of all microorganisms at 1 hour and had no bacterial growth after 120 minute except *C.albicans*.Although prilocaine is used especially for dental anesthesia[16] and *C.albicans* is a commensal yeast normally present in the oral floraof humans, prilocaine did not have a significant effect on *C.albicans* in the present study. Pelz *et al*[3] were determinated that prilocaine was active against nearly all oral flora except *E.faecalis* and Aydin *et al*[9] determinated that prilocaine 2% decreased the *E.coli* count 100 times and *S.aureus* count 10 times at 30 min and *C.albicans* count 10 times after 120 min.

Bupivacaine hydrochloride is indicated for the production of local or regional anesthesia for dental and oral surgery procedures[18]. We investigated that bupivacaine 0.5% had an antimicrobial effect on *S.aureus* and *E.coli* strains by reducing the numbers of viable cells and other bacteria strains were resistant to this agent. Pelz *et al*[3] found that 36 bacterial and 14 *Candida* strains completely resistant to bupivacaine too. Aydin *et al*[9] said that bupivacaine 0.5% showed poor antimicrobial effectiveness, only reduced the counts of *P.aeruginosa*. Sakuragi *et al*[19] determinated that lower colony counts were observed with 3 hour or longer exposure to 0.5% bupivacaine. Rosenberg and Renkonen[20] studied the antimicrobial activity of bupivacaine with an agar dilution method and found that bupivacaine at a concentration of 5mg/ml intibited the growth of sensitive *S.epidermidis*, *S.pyogenes*, *S.pneumoniae*, *S.aureus*, *E.coli* except *P.aeruginosa*. Grimmond and Brownridge[21] remarked that bupivacaine drug showed increasing microorganism inhibition with increasing drug concentration. Hodson *et al*[22] investigated that there was no growth of *S.epidermidis, S.aureus, E.faecalis* in either 0.5% bupivacaine or levobupivacaine solution. Levobupivacaine is the S-enantiomer of bupivacaine[23]. Although levobupivacaine has similar potency to bupivacaine[24], in our study all bacteria strains except *P.aeruginosa* were resistant to

levobupivacaine 0.5%.

Lidocaine is a common local anesthetic that used for minor surgery and topically to relieve itching, burning and pain from skin inflammations[25]. The antibacterial effects of lidocaine were established[26]. In the present study we investigated that lidocaine 2% reduced the viable cells of all microorganisms tested. For *S.aureus* strain at 60th

Table 1. shown the antimicrobial effects of anesthetic solutions and creams against tested bacteria at 0, 30, 60, 120, 180, 240th minutes (colony forming units) at 10^{-4} concentration.

		Colony forming units at 10^{-4} concentration					
Anesthetics	Microorganism	0 min	30 min	60 min	120 min	180 min	240 min
Prilocaine 2%	*S.aureus*	>300	>300	19	0	0	0
	E.coli	>300	117	67	6	0	0
	K.pneumoniae	>300	66	8	0	0	0
	P.aeruginosa	>300	0	0	0	0	0
	C.albicans	111	161	73	49	58	38
Bupivacaine 0.5%	*S.aureus*	>300	95	29	0	0	0
	E.coli	>300	>300	227	176	62	16
	K.pneumoniae	>300	>300	>300	>300	>300	>300
	P.aeruginosa	>300	>300	>300	>300	>300	>300
	C.albicans	162	128	123	162	134	140
Levobupivacaine 0.5%	*S.aureus*	>300	>300	>300	>300	>300	>300
	E.coli	>300	>300	>300	259	>300	262
	K.pneumoniae	>300	>300	>300	>300	>300	>300
	P.aeruginosa	>300	>300	287	>300	164	135
	C.albicans	118	118	125	119	113	132
Lidocaine 2%	*S.aureus*	156	90	0	0	0	0
	E.coli	>300	>300	89	0	0	0
	K.pneumoniae	>300	>300	>300	56	6	3
	P.aeruginosa	>300	0	0	0	0	0
	C.albicans	41	32	33	19	16	6
Ketamine 0.5%	*S.aureus*	10	0	0	0	0	0
	E.coli	2	0	0	0	0	0
	K.pneumoniae	8	1	24	0	0	0
	P.aeruginosa	0	0	0	0	0	0
	C.albicans	1	0	0	0	0	0
Propofol 1%	*S.aureus*	>300	>300	>300	>300	>300	>300
	E.coli	>300	>300	>300	209	140	110
	K.pneumoniae	105	250	136	161	143	43
	P.aeruginosa	>300	>300	>300	>300	>300	>300
	C.albicans	37	10	25	20	20	13
EMLA® 5%	*S.aureus*	>300	>300	>300	>300	>300	>300
	E.coli	0	0	0	0	0	0
	K.pneumoniae	49	4	2	1	0	0
	P.aeruginosa	0	0	0	0	0	0
	C.albicans	>300	>300	>300	>300	>300	>300
	S.epidermidis	99	50	35	26	0	0
	E.faecalis	>300	>300	>300	203	152	124
Anestol 5%	*S.aureus*	64	28	14	7	4	1
	E.coli	0	0	0	0	0	0
	K.pneumoniae	55	10	1	0	0	0
	P.aeruginosa	9	5	3	0	0	0
	C.albicans	4	2	1	0	0	0
	S.epidermidis	25	14	8	6	3	2
	E.faecalis	69	54	33	10	6	3

minute, for *E.coli* strain at 120th minute and for *P.aeruginosa* strain at 30th minute; there were no bacterial growth detected on blood agar plates.Sedef Gocmen *et al*[4] were investigated the antibacterial effects of lidocaine 5% and lidocaine/ prilocaine 2.5% *in vitro* by the disc diffusion method and reported that lidocaine showed more activity on *S. epidermidis* and *E.coli* at standard doses, on *S.aureus* at high doses, on *S.pyogenes* and *E.faecalis* at both doses than did lidocaine/ prilocaine. Aydin *et al*[9] showed that lidocaine 5% and 2% reduced the colony counts of *S.aureus*, *E.coli*, *P.aeruginosa* and *C.albicans*. Kaya *et al*[27] reported that lidocaine 2% had bacteriostatic and bactericidal effect against *S.marcescens* and *S.aureus*. Olsen *et al*[28] demostrated that lidocaine 2% in bronchoalveolar fluid decreased the viable cells of *S.pneumoniae* but not *P.aeruginosa* and *Haemophilus influenza*.Parr *et al*[29] observed that all bacteria demonstrated a concentration-dependent inhibition of growth when exposed to lidocaine 2% and *P.aeruginosa* is susceptible to antibacterial effects of lidocaine but lesser degree than *E.coli*. They also foundthat metisillin resistant *S.aureus* and vancomisin resistant *Enterococcus* were susceptible to lidocaine. Schmidt and Rosenkranz[30] observed that Gram negative bacteria more sensitive to Gram positives to lidocaine and they reported complete susceptibility to lidocaine 2% except *P.aeruginosa*. In present study we tried one *S.aureus* strain as Gram positive and this strain was susceptible to lidocaine 2% just as much Gram negatives.

Nosocomial bacteremia or candidemia derived from vascular access reported in literatures[31]. Topical anesthetic creams used for anesthesia of skin in connection with needle insersion[32] and other invasive procedures[33] or if a tissue biopsy from a chronic wound is sampled for culture, the antimicrobial properties of anesthetics may pose a problem in producing false-negative results[34]. Topical anesthetic creams such as EMLA®5% and Anestol 5% can be applied easily into skin by nurse or patients themselves. In this study we investigated the impact of EMLA® and Anestol cream on the different bacterial strains. According to our study EMLA®5% cream had a bactericidal effect for *E.coli* and *P.aeruginosa* and a significant reduction in the viable cells of *K.pneumoniae*, *S.epidermidis* and *E.faecalis*. Batai *et al*[35] and Berg *et al*[34] confirmed our *in vitro* results. Anestol 5% reduced the number of viable cells of *S.aureus*, *K.pneumoniae*, *P.aeruginosa*, *C.albicans*, *S.epidermidis*, *E. faecalis* and had a bactericidal effect on *E.coli*.Berg *et al*[34] reported that EMLA® 5% had a rapid and powerful antibacterial effect on *S.aureus*, *E.coli*, *P.aeruginosa*, *S.pyogenes*.

We investigated that ketamine 0.5% had bactericidal effects on viable cells of all microorganisms tested. Sedef Gocmen *et al*[4] showed that ketamine had a dose dependent antibacterial activity for *S.aureus*, *S.epidermidis*, *E.faecalis*, *S.pyogenes*, *P.aeruginosa*, *E.coli* by disc diffusion and MIC method and remarked that the doses of 500 mg of ketamine had more prominent effect than the doses of 250 mg.

Sakuragi *et al*[36] reported that colony counts increased as exposure time to propofol 1% increased. In our study propofol %1 did not inhibit any of bacteria strains except *E.coli* and reduction in the number of viable cells of *E.coli* had been very low according to the number of cells by the first time that anesthetic was placed. Carr *et al*[37] remarked that microorganisms grow rapidly in propofol and that would be a source of postoperative sepsis and infection. Crowther *et al* (38) showed that the addition of thiopental to propofol reduces bacterial growth in mixture. Sakuragi *et al*[36] remarked that colony counts of *E.coli* after exposure to 0.5%, 1%, 2% and 4% lidocaine in 1% propofol were significantly lower than after exposure to 1% propofol.

CONCLUSIONS

lt is known that there may be a risk of minor bacterial infection or a contamination even if aseptic conditions are provided[9]. Nowadays researches on the antimicrobial activity of general anesthetics have limited but over the past several decades local anesthetics have substantiated an integrative role in the prevention of nosocomial infections[12]. The source of infections may be endogenous or exogenous[35]. Microbial inhibition of anesthetic solutions is important in clinic and it is not expected to see such high microorganism concentrations in anesthesia procedures[9]. If it is known that anesthetics have a sufficient efficacy on a minor infection or a contamination that may

occurs during the anesthesia provides advantages and may be a second area of use.

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