Analysis of the antimicrobial activity of local anaesthetics used for dental analgesia

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Seven local anaesthetics and their active anaesthetic components [Ultracaine D-S (articaine hydrochloride), Carbostesin (bupivacaine hydrochloride), Scandicaine (mepivacaine hydrochloride), Xylonest (prilocaine hydrochloride), Xylocaine (lidocaine hydrochloride), Hostacaine (butanilicaine phosphate) and Novocaine (procaine hydrochloride)] were tested for their antimicrobial activity against 311 bacterial strains from 52 different species and 14 *Candida albicans* strains. The tested pathogens were members of the oral flora, and partly members of the skin and intestinal flora. Additionally, the antimicrobial activity of methyl-4-hydroxybenzoate, sodium disulfite, adrenaline hydrogen tartrate and adrenaline (the preservative and vasoconstrictive components of the anaesthetics) was tested. For determination of MIC and minimal bactericidal concentration (MBC), the agar dilution method using Wilkins–Chalgren agar was applied. The trade preparation Ultracaine D-S showed the most prominent antimicrobial activity with regard to both MIC and MBC. Ultracaine D-S and its active substance, articaine hydrochloride, showed similar MIC values, suggesting that the antimicrobial activity is mainly caused by the anaesthetic component. Novocaine showed the lowest antimicrobial activity and did not inhibit 35 of the species tested. The MIC values of all local anaesthetics were between 0.25 and 16 mg ml⁻¹. The routinely applied concentration of Ultracaine D-S was roughly four times higher, and of Hostacaine was two times higher, than the MBC values for the tested bacteria, whereas for the other anaesthetics, the MBC values were not reached or exceeded with the concentrations used. The MIC range of the preservatives was 0.5–1.0 mg ml⁻¹ for methyl-4-hydroxybenzoate and 0.2–0.5 mg ml⁻¹ for sodium disulfite. The articaine MIC values were two to three serial dilution steps lower, and the butanilicaine MIC values one to two serial dilution steps lower, than the MIC of the preservatives. The mepivacaine mean MIC values were slightly lower for *Fusobacterium nucleatum*, *Prevotella intermedia*, *Porphyromonas gingivalis* and *Staphylococcus aureus*, but higher for *Streptococcus intermedius*, compared with the preservative methyl-4-hydroxybenzoate. The same result was found with *Streptococcus intermedius* and lidocaine. Screening of 20 MIC values of 4 pure anaesthetic substances and the corresponding preservative found 2/20 instances where the MICs of the preservatives against 5 representative species (67 strains) were lower, indicating that the antimicrobial effect was mainly due to the preservative, but 18/20 results where the pure anaesthetic component showed greater antimicrobial effects compared with the preservative. The *in vitro* results for Carbostesin, Scandicaine and especially for Novocaine indicate that a local disinfection should be done prior to injection of the anaesthetics. Due to the results obtained with nosocomial strains (*Escherichia coli*, *S. aureus* and *Pseudomonas*), disinfection of the mucous membranes should be performed routinely in immunocompromised patients, regardless of the anaesthetic used.

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INTRODUCTION

The oral cavity is physiologically colonized with multiple bacterial species, some of which are opportunistic

Abbreviation: MBC, minimal bactericidal concentration.

pathogens. During the process of dental anaesthesia – infiltration anaesthesia as well as mandibular anaesthesia – oral bacteria can enter the tissue following perforation of the mucous membrane by the needle injection (Gräf, 1965; Streitfeld & Zinner, 1958; Winther & Praphailony, 1969). Dental local anaesthesia may therefore lead to suppurative local infections or odontogenic bacteraemia, as has been shown to occur in other dental surgery interventions. These circumstances have raised the issue of routine antibiotic prophylaxis in oral surgery (Rahn, 1989; Roberts *et al.*, 1998).

The first observations that local anaesthetics inhibited bacterial growth were made in 1909 (Jonnesco, 1909). Further studies followed (Erich, 1961; Murphy *et al.*, 1955; Kleinfeld & Ellis, 1966; Schmidt & Rosenkranz, 1970). Schmidt & Rosenkranz (1970) examined the activity of lidocaine and procaine on 28 bacterial species, as well as on *Candida* and *Cryptococcus*. The bacteria investigated were all common pathogens. From their results, Schmidt & Rosenkranz (1970) concluded that specimens from patients treated with local anaesthetics were not suitable for further bacteriological or mycobacteriological analyses. In addition, in that study no oral pathogens other than '*Streptococcus viridans*' were examined and neither anaerobic bacteria nor most of the currently used anaesthetics were tested. As more than 400 bacterial species that are prevalent in the oral cavity have been described, a definitive statement on the possible antimicrobial activity of anaesthetics used in dentistry cannot currently be made. Noda *et al.* (1990) and Feldman *et al.* (1994) demonstrated bactericidal activity and the ability of lidocaine and bupivacaine to inhibit bacteria growth at clinical concentrations; however, no oral pathogens were included in these studies.

The aim of our study was to examine the antimicrobial activity of the commercially available local anaesthetics and their main components that are currently used in dental medicine. These substances were tested against a broad spectrum of commensal and opportunistic pathogenic bacteria, as well as against *Candida albicans* strains, all of which can form part of the oral flora. We addressed the question of whether the concentrations of the anaesthetics usually applied in dental surgery can inhibit the growth of bacteria that are inoculated into the soft tissue during the injection process.

METHODS

Bacterial and fungal strains. A total of 311 bacterial strains from 52 different species and 14 *C. albicans* strains was investigated in this study (Table 1). The strains tested occur in the oral flora and partly in the skin and intestinal flora. The bacterial and *Candida* strains were isolated from the subgingival plaque from patients with odontogenic abscesses or periodontitis, and only a few type strains (*Staphylococcus aureus* ATCC 25923, *Pseudomonas aeruginosa* ATCC 27853 and *Bacillus subtilis* ATCC 6633) were obtained from the Deutsche Sammlung von Mikroorganismen und Zellkulturen (Germany). Identification of strains was done by morphological and biochemical

methods, as well as by GC analysis of cellular fatty acid profiles as described previously (Otten *et al.*, 2005).

Local anaesthetics. Seven local anaesthetics and their active anaesthetic components (in parentheses) were examined: Ultracaine D-S (articaine hydrochloride; Aventis Pharma), Carbostesin (bupivacaine hydrochloride; AstraZeneca), Scandicaine (mepivacaine hydrochloride; AstraZeneca), Xylonest (prilocaine hydrochloride), Xylacaine (lidocaine hydrochloride; AstraZeneca), Hostacaine (butanilcaine phosphate) and Novocaine (procaine hydrochloride; Aventis Pharma). The trade preparations, the active anaesthetic components, and also the preservative and vasoconstrictive components, were tested.

Determination of MIC and minimal bactericidal concentration (MBC). After a series of tests with broth dilution and different media, we decided to determine the MIC by the agar dilution method using Wilkins–Chalgren agar (Wilkins & Chalgren, 1976). This method was the only one whereby we could compare all strains under the same conditions. The plates contained five dilutions of each of the original anaesthetics (based on recommended application concentrations) ranging from 16 to 1 mg ml⁻¹ for Ultracaine D-S, Xylonest and Hostacaine, from 8 to 0.5 mg ml⁻¹ for Scandicaine, Xylacaine and Novocaine, and from 2 to 0.125 mg ml⁻¹ for Carbostesin. The plates were inoculated with a multipoint inoculator, each pin delivering 10⁴–10⁵ c.f.u. µl⁻¹.

Plates without local anaesthetics were used as controls. The reference strains *S. aureus* ATCC 25923 and *P. aeruginosa* ATCC 27853 were included in each experiment to assess the reliability of the method. Facultative anaerobic strains and *Pseudomonas* were cultivated in a CO₂ incubator (6–8 % CO₂) at 36 °C for 18 h, whilst anaerobes were incubated in an anaerobic jar (Anaerocult; VWR) at 36 °C for 42 h. All tests for each strain were carried out at least in duplicate. If the MIC values of an individual strain were not identical, we calculated the mean while reading the results. Mean values of all strains of a species were then calculated. The MIC was defined as the lowest concentration of each anaesthetic that was able to inhibit macroscopic bacterial or yeast growth.

In addition to the commercially available preparations, we also determined the MICs of the active compounds of the local anaesthetics. Starting with the concentration used for injection, five serial twofold dilutions were prepared in Wilkins–Chalgren agar (40, 20, 10, 5 and 2.5 mg ml⁻¹, for articaine, prilocaine and butanilcaine; 20, 10, 5, 2.5 and 1.25 mg ml⁻¹ for mepivacaine, lidocaine and procaine; and 5, 2.5, 1.25, 0.625 and 0.315 mg ml⁻¹ for bupivacaine) (Wilkins & Chalgren, 1976). These concentrations were inoculated with representative bacterial strains (*Fusobacterium nucleatum*, *Prevotella intermedia*, *Porphyromonas gingivalis*, *S. aureus*, *Streptococcus intermedius* and *P. aeruginosa*) as described above. All MIC determinations were carried out according to Clinical and Laboratory Standards Institute guidelines.

For MBC determination, we used a variant of the agar dilution method. Inoculation spots with no visible growth were cut and top-down streaked on Columbia blood agar or yeast cysteine blood agar without inhibitory substances. The MBC was determined according to the MIC method. The lowest concentration without visible growth corresponded to the MBC.

The reproducibility was confirmed by inoculating the control strains *S. aureus* ATCC 25923 and *P. aeruginosa* ATCC 27853 in parallel on each plate with the test organisms. Each control strain was tested at least 18 times. The test was repeated if the control strains showed different MIC or MBC values. This was necessary because of the lack of standardization for this method. Moreover, the strains that could be tested in parallel with the broth dilution method showed comparable MBC values.

Table 1. Mean MICs for seven local anaesthetics determined by the agar dilution test on Wilkins–Chalgren agar

Species (no. of strains)	Anaesthetic (mg ml ⁻¹)*						
	Ultracaine D-S	Carbostesin	Scandicaine	Xylonest	Xylocaine	Hostacaine	Novocaine
Oral strains							
Gram-positive cocci							
<i>Streptococcus sanguinis</i> (10)	7.6	1.9	8.0 ^a	14.8	4.4	4.2	8.0 ^b
<i>Streptococcus oralis</i> (10)	8.8	1.9	>8	7.2	4.8	3.6	8.0 ^b
<i>Streptococcus mitis</i> (5)	8.0	1.8	8.0	9.6	4.8	3.6	8.0 ^c
<i>Streptococcus salivarius</i> (5)	6.5	1.6	6.5	13.0	6.0	3.5	8.0 ^d
<i>Streptococcus constellatus</i> (4)	5.0	1.0	5.0	4.0	3.0	0.9	>8
<i>Streptococcus intermedius</i> (6)	6.0	0.8	6.0	3.0	3.0	0.8	8.0 ^e
<i>Streptococcus mutans</i> (14)	11.4	2.0	8.0 ^d	16.0	7.4	8.0	8.0 ^b
<i>Streptococcus pneumoniae</i> (4)	2.5	1.0	2.5	5.0	2.5	1.0	2.5
<i>Streptococcus pyogenes</i> (3)	4.0	0.5	8.0	2.0	2.0	0.5	8.0
<i>Staphylococcus aureus</i> (12)	9.0	2.0	8.0	9.3	7.0	6.7	>8
<i>Peptostreptococcus micros</i> (9)	9.1	2.0 ^f	6.7	7.1	5.3	4.9	7.1
<i>Peptostreptococcus anaerobius</i> (1)	4.0	1.0	4.0	4.0	2.0	2.0	4.0
Gram-positive rods							
<i>Eubacterium lentum</i> (7)	6.3	1.0	4.3	8.9	5.4	3.3	5.1
<i>Eubacterium aerofaciens</i> (1)	8.0	2.0	8.0	16.0	4.0	4.0	8.0
<i>Actinomyces naeslundii</i> (8)	8.0	1.6	7.5	8.5	6.0	4.4	8.0 ^c
<i>Actinomyces odontolyticus</i> (2)	3.0	0.25	1.5	2.0	2.0	1.0	2.0
<i>Actinomyces viscosus</i> (4)	12.0	1.6	8.0	7.0	8.0	6.0	8.0 ^f
<i>Actinomyces denticolans</i> (4)	3.5	0.25	1.1	3.0	2.3	1.0	2.0
Gram-negative cocci							
<i>Neisseria subflava</i> (4)	9.0	1.0	3.5	4.0	3.0	5.0	5.0
<i>Neisseria mucosa</i> (5)	5.6	0.9	8.0	2.4	2.6	2.6	8.0
<i>Kingella indologenes</i> (4)	8.0	2.0	4.0	2.0	2.0	2.0	>8
<i>Veillonella parvula</i> (6)	5.3	2.0 ^d	>8	9.3	5.0	6.0	8.0 ^c
Gram-negative rods							
<i>Actinobacillus actinomycetemcomitans</i> (6)	6.7	1.7	3.3	4.7	4.0	2.7	8.0 ^c
<i>Capnocytophaga ochracea</i> (17)	3.5	0.9	4.9	2.5	2.2	1.6	>8
<i>Capnocytophaga gingivalis</i> (5)	3.7	1.7	4.2	6.0	2.5	1.7	>8
<i>Capnocytophaga sputigena</i> (12)	3.6	1.3	4.0	7.2	2.8	1.3	>8
<i>Eikenella corrodens</i> (13)	6.8	2.0	7.1	4.9	3.8	2.9	>8
<i>Haemophilus influenzae</i> (4)	5.0	1.5	5.5	9.0	4.5	3.5	8.0 ^e
<i>Prevotella intermedia</i> (6)	3.0	2.0 ^d	6.7	12.7	5.3	7.0	6.7
<i>Prevotella oris</i> (1)	4.0	2.0	8.0	8.0	4.0	2.0	>8
<i>Prevotella buccae</i> (4)	5.0	1.5	7.0	9.0	4.5	3.8	7.0
<i>Prevotella buccalis</i> (5)	3.6	1.5	7.2	6.4	4.8	1.6	6.4
<i>Prevotella oralis</i> (6)	6.7	1.4	7.0	9.3	5.7	4.0	7.0
<i>Selenomonas sputigena</i> (6)	9.3	2.0 ^c	8.0 ^c	9.3	5.3	4.7	8.0 ^e
<i>Selenomonas flueggei</i> (2)	6.0	2.0 ^e	8.0 ^e	12.0	4.0	8.0	8.0 ^e
<i>Selenomonas diana</i> (2)	2.5	0.8	2.5	3.0	2.0	4.5	6.0
<i>Selenomonas noxia</i> (2)	12.0	>2	4.0 ^e	8.0	3.0	6.0	8.0
<i>Selenomonas artemidis</i> (1)	8.0	8.0	8.0	8.0	2.0	2.0	8.0
<i>Fusobacterium nucleatum</i> (31)	6.6	1.6	7.4	6.8	4.1	4.0	8.0 ^g
<i>Campylobacter rectus</i> (9)	4.0	1.6	5.3	8.4	4.4	2.8	7.1
<i>Porphyromonas gingivalis</i> (8)	4.5	>2	8.0 ^e	13.0	4.0	6.5	8.0 ^f
<i>Leptotrichia buccalis</i> (4)	16.0	2.0	8.0	8.0	8.0	4.0	>8
Yeast							
<i>Candida albicans</i> (14)	16.0	>2	>8	12.0	>8	8.0	>8
Non-oral strains							
Gram-positive cocci							
<i>Streptococcus agalactiae</i> (1)	8.0	0.25	4.0	16.0	8.0	8.0	>8
<i>Enterococcus faecalis</i> (2)	16.0 ^e	>2	>8	>16	>8	16.0	8.0 ^e
Gram-positive rods							
<i>Bacillus subtilis</i> (6)	4.0	0.5	6.0	4.0	8.0	1.3	>8

Table 1. cont.

Species (no. of strains)	Anaesthetic (mg ml ⁻¹)*						
	Ultracaine D-S	Carbostesin	Scandicaine	Xylonest	Xylacaine	Hostacaine	Novocaine
<i>Propionibacterium acnes</i> (8)	8.3	1.5	8.0 ^h	6.5	5.0	4.0	8.0 ^b
<i>Propionibacterium avidum</i> (2)	16.0	2.0	>8	6.0	8.0	6.0	>8
<i>Clostridium perfringens</i> (3)	6.7	>2	>8	16.0	8.0	16.0	>8
Gram-negative rods							
<i>Serratia marcescens</i> (4)	16.0	2.0	8.0	16.0	8.0	8.0	>8
<i>Pseudomonas aeruginosa</i> (4)	>16	>2	>8	16.0	>8	16.0	>8
<i>Klebsiella pneumoniae</i> (3)	16.0	>2	8.0	16.0	8.0	8.0	>8
<i>Escherichia coli</i> (6)	8.0	2.0	8.0	12.0	8.0	6.0	>8

*No. of strains not inhibited: a, 9; b, 5; c, 2; d, 4; e, 1; f, 3; g, 18; h, 7.

RESULTS AND DISCUSSION

To the best of our knowledge, this is the first study of antimicrobial activities of local anaesthetics against bacteria that belong to the commensal flora of the oral cavity and/or are commonly found in dental infections. Some of the strains analysed are also constituent members of the skin or intestinal flora. In this study, antimicrobial susceptibility tests against a wide range of bacterial strains, as well as a large number of *C. albicans* isolates, were performed using seven local anaesthetics and their active components. For many years, it has been known that bacterial colonization at the site of injection of local anaesthetics can lead to the inoculation and subsequent proliferation and spread of opportunistic pathogens (Birn & Winther, 1967; Gräf, 1965; Streitfeld & Zinner, 1958; Winther & Praphailony, 1969). Usually, no local clinical manifestation is observed and an injection abscess is considered exceptional (Blake & Forman, 1967; Schuchardt *et al.*, 1964). In the past, the use of disinfection prior to injection has evoked controversial discussion (Gräf, 1965; Winther & Praphailony, 1969). Some results of previous studies on the antimicrobial activity of local anaesthetics (Erich, 1961; Kleinfeld & Ellis, 1966, 1967; Murphy *et al.*, 1955) were confirmed by the present analysis.

The mean MIC values for each of the local anaesthetics and the numbers of bacterial/fungal strains tested are shown in Table 1. In our test series, Ultracaine D-S was the most active local anaesthetic. It was active against almost all tested bacteria with a mean concentration range of 2.5–16 mg ml⁻¹. Two strains of *Streptococcus pneumoniae* were inhibited at a concentration of 1 mg ml⁻¹. Only four strains of *P. aeruginosa* and one strain of *Enterococcus faecalis* were not inhibited. The concentration of the trade preparation is 40 mg ml⁻¹, more than two times higher than the highest concentration (16 mg ml⁻¹) required for the agar dilution method. Table 2 summarizes the Ultracaine D-S MIC values for oral bacteria. The mean MIC value was between 3.0 (*Prevotella intermedia*) and 16.0 mg ml⁻¹ (*Leptotrichia buccalis*); for the opportunistic

pathogens, the mean MIC ranged from 3.0 (*Prevotella intermedia*) to 11.4 mg ml⁻¹ (*Streptococcus mutans*).

Carbostesin showed mean MIC values in the range of 0.25–2 mg ml⁻¹ (Table 1). Overall, 36 bacterial and 14 *Candida* strains were completely or partially resistant to Carbostesin. Scandicaine showed mean MIC values of

Table 2. MIC values of oral bacteria for Ultracaine D-S

Species	No. of strains	Mean MIC (mg ml ⁻¹)
<i>Actinobacillus actinomycetemcomitans</i> *	6	6.7*
<i>Capnocytophaga</i> sp.	34	3.6
<i>Eikenella corrodens</i> *	13	6.8*
<i>Haemophilus influenzae</i> *	4	5.0*
<i>Neisseria</i> sp.	9	7.1
<i>Streptococcus oralis</i> / <i>Streptococcus mitis</i>	15	8.5
<i>Streptococcus salivarius</i>	5	6.5
<i>Streptococcus sanguinis</i>	10	7.6
<i>Streptococcus constellatus</i> / <i>Streptococcus intermedius</i> *	10	5.6*
<i>Streptococcus mutans</i> *	14	11.4*
<i>Actinomyces naeslundii</i> / <i>Actinomyces viscosus</i>	12	9.3
<i>Actinomyces odontolyticus</i> / <i>Actinomyces denticola</i>	6	3.3
<i>Eubacterium lentum</i> / <i>Eubacterium aerofaciens</i>	8	6.5
<i>Leptotrichia buccalis</i>	4	16.0
<i>Peptostreptococcus micros</i> *	9	9.1*
<i>Campylobacter rectus</i>	9	4.0
<i>Fusobacterium nucleatum</i> *	31	6.6*
<i>Selenomonas</i> sp.	13	8.1
<i>Porphyromonas gingivalis</i> *	8	4.5*
<i>Prevotella intermedia</i> *	6	3.0*
<i>Prevotella buccalis</i> / <i>Prevotella oris</i> / <i>Prevotella oralis</i>	16	5.1
<i>Veillonella parvula</i>	6	5.3

*Opportunistic oral pathogens and their mean MIC values.

Table 3. Range of MIC values of 'sensitive' bacterial strains and the range of the MIC converted to the application concentration of each local anaesthetic

	Ultracaine D-S	Carbostesin	Scandicaine	Xylonest	Xylocaine	Hostacaine	Novocaine
MIC (mg ml ⁻¹)	2.5–16.0	0.25–2.0	1.1–8.0	2.0–16.0	2.0–8.0	0.5–16.0	2.0–8.0
Concn of trade preparation (%)	6–40	5–40	6–40	7–53	10–40	2–53	10–40
No. of non-inhibited strains	5	37	52	2	6	0	166

1.1–8 mg ml⁻¹. Overall, 52 bacterial strains and 14 *Candida* strains were not inhibited. The local anaesthetic Xylonest was active against nearly all strains except for *Enterococcus faecalis*, which was not inhibited. The mean MIC values were between 2 and 16 mg ml⁻¹. Xylocaine inhibited all strains except *P. aeruginosa*, *Enterococcus faecalis* and *C. albicans*. The MIC values were in the range of 2–8 mg ml⁻¹. Hostacaine inhibited all bacterial strains tested, with mean MICs of 0.5–16 mg ml⁻¹. Novocaine exerted an antimicrobial activity against only 47 % of the investigated bacterial species. The MIC values of the inhibited strains were between 2 and 8 mg ml⁻¹. Table 3 summarizes the range of MIC values and the range of the MIC converted to the application concentration for each local anaesthetic. Using the agar dilution method, the test concentration required was lower (max. 40–53 %) than the concentration of the trade preparation.

Table 4 shows the results of the MIC and MBC values of the active anaesthetic substance, as well as the MIC of the trade preparation for comparison of some selected opportunistic pathogens. The MBC values of the anaesthetic active components ranged from 3.8 to 20.0 mg ml⁻¹

(Table 4). In the following list, the anaesthetics are ordered according to their effectiveness: the MBC values for articaine were 22.5–25 % of the concentration routinely applied in dental analgesia, whereas the MBC values of butanilicaine were 22.5–50 %, of prilocaine were 16 to ≥100 %, of lidocaine were 62.5 to ≥100 %, of mepivacaine were 65 to ≥100 %, of bupivacaine were 76 to ≥100 % and of procaine were 87.5 to ≥100 % of the application concentration. Thus, Ultracaine D-S had the most potent activity of the local anaesthetics tested and it can be assumed that the initial concentration of Ultracaine D-S at the injection site was roughly four times higher than the MBC of most of the bacterial strains evaluated.

The results showed that Hostacaine was active against all of the bacterial strains investigated, as well as against *C. albicans* (Table 1). This local anaesthetic was active in a range of 2–53 % of the applied concentration (Table 3). Novocaine, in contrast, was inactive against the largest number of the tested bacterial strains, followed by Scandicaine and Carbostesin. For these local anaesthetics, we therefore recommend disinfection of the oral mucosa

Table 4. MIC and MBC values of the active anaesthetic substances tested

Species (no. of strains)		Anaesthetic (mg ml ⁻¹) [MIC of the trade preparation]						
		Articaine hydrochloride (Ultracaine D-S)	Bupivacaine hydrochloride (Carbostesin)	Mepivacaine hydrochloride (Scandicaine)	Prilocaine hydrochloride (Xylonest)	Lidocaine hydrochloride (Xylocaine)	Butanilicaine hydrochloride (Hostacaine)	Procaine hydrochloride (Novocaine)
<i>Fusobacterium</i>	MIC	8.8 [6.6]	5.0 [1.6]	13.0 [7.4]	1.5 [6.8]	8.0 [4.1]	15.0 [4.0]	17.5 [8.0]*
<i>nucleatum</i> (31)	MBC	10.0	5.0	15.0	4.9	17.0	15.0	17.5
<i>Prevotella</i>	MIC	5.0 [3.0]	4.0 [2.0]*	18.0 [6.7]	12.8 [12.7]	15.0 [5.3]	15.0 [7.0]	10.0 [6.7]
<i>intermedia</i> (6)	MBC	10.0	>5	20.0	18.0	>20	15.0	>20
<i>Porphyromonas</i>	MIC	10.0 [4.5]	>5 [>2]	15.0 [8.0]*	14.3 [13.0]	9.5 [4.0]	15.0 [6.5]	>20 [8.0]*
<i>gingivalis</i> (8)	MBC	10.0	>5	20.0	14.3	12.5	15.0	>20
<i>Staphylococcus</i>	MIC	10.0 [9.0]	2.9 [2.0]	11.5 [8.0]	17.2 [9.3]	13.0 [7.0]	7.5 [6.7]	>20 [>8]
<i>aureus</i> (12)	MBC	10.0	4.9	13.0	>30	13.0	7.5	>20
<i>Streptococcus</i>	MIC	5.5 [6.0]	3.6 [0.8]	14.0 [6.0]	13.7 [3.0]	16.3 [3.0]	6.8 [0.8]	>20 [8.0]*
<i>intermedius</i> (6)	MBC	9.0	3.8	14.0	16.7	>20	6.8	>20
<i>Pseudomonas</i>	MIC	>40 [>16]	>5 [>2]	>20 [>8]	24.0 [16.0]	>20 [>8]	30.0 [16.0]	>20 [>8]
<i>aeruginosa</i> (4)	MBC	>40	>5	>20	>30	>20	>30	>20
Concn of trade preparation (mg ml ⁻¹)		40.0	5.0	20.0	30.0	20.0	30.0	20.0

*Not all strains were inhibited.

prior to injection and surgical procedures, to protect the patient from infections. Notably, these three local anaesthetics, as well as Xylocaine, showed no inhibitory activity against *C. albicans*. Recent studies have demonstrated that lidocaine (the active component of Xylocaine) has no effect on the growth of *C. albicans*, *Haemophilus influenzae* and *P. aeruginosa* (Olsen *et al.*, 2000). We also found no inhibitory activity of Xylocaine against *P. aeruginosa*, whereas *H. influenzae* was clearly inhibited by Xylocaine (at 23 % of the application concentration).

P. aeruginosa and *Enterococcus faecalis* were the most resistant strains. It is possible that these organisms have permeability barriers for local anaesthetics or other features of the cell wall that prevent an effect of the anaesthetics (Holloway, 1969). *P. aeruginosa* was inhibited by Xylonest and Hostacaine, and *Enterococcus faecalis* by Hostacaine only. *C. albicans* was inhibited by Hostacaine (at 27 % of the application concentration), Ultracaine and Xylonest (both at 40 % of the application concentration) only.

In addition to the active components of each local anaesthetic, we also tested the antimicrobial activity of the preservatives and vasoconstrictive components of these anaesthetics: methyl-4-hydroxybenzoate (a component of Ultracaine D-S, Scandicaine, Xylocaine and Hostacaine), sodium disulfite (a component of Ultracaine D-S and Xylocaine), adrenaline hydrogen tartrate (a component of Xylocaine) and adrenaline (a component of Ultracaine D-S). Carbostesin and Xylonest do not contain preservatives.

Table 5 shows the MIC values of the individual components. Adrenaline hydrogen tartrate and adrenaline had no effect on the bacteria and yeast tested. The MIC values were 0.2 to ≥ 0.5 mg ml⁻¹ for sodium disulfite and 0.5 to 1 mg ml⁻¹ for methyl-4-hydroxybenzoate. During examination of the trade preparation, the preservatives, as well as the vasoconstrictive components, were also diluted and therefore their concentration was below the MIC with only a few exceptions. Consequently, these agents are not the main reason for the value determined for the MIC. However, there may be a synergistic effect, as seen by the higher MIC values for the active anaesthetic components (Table 4).

Comparative analyses of the antimicrobial activity of the trade preparations, their active anaesthetic components and the preservative or vasoconstrictive components revealed that the main antimicrobial effect could be attributed to the active anaesthetic components (Table 5). Only methyl-4-hydroxybenzoate and sodium disulfite exerted antimicrobial effects. The articaine MIC values were two to three serial dilution steps and the butanilicaine MIC values one to two serial dilution steps lower than the MIC of the preservatives. The mepivacaine mean MIC values were slightly lower for *F. nucleatum*, *Prevotella intermedia*, *Porphyromonas gingivalis* and *S. aureus*, and higher for *Streptococcus intermedius*, than for the preservative methyl-4-hydroxybenzoate. The same result was found with *Streptococcus intermedius* and lidocaine. Screening of 24 MIC values of 4 pure anaesthetic substances and the

Table 5. Concentration (%) of the active anaesthetic substances in comparison with the MIC values of the preservative and vasoconstrictive components of the trade preparation (as mg ml⁻¹ and %)

Species (no. of strains)	Articaine (%)	Mepivacaine (%)	Lidocaine (%)	Butanilicaine (%)	Methyl-4- hydroxy- benzoate		Sodium disulfite		Adrenaline hydrogen tartrate (mg ml ⁻¹)	Adrenaline (mg ml ⁻¹)
					(mg ml ⁻¹)	(%)	(mg ml ⁻¹)	(%)		
<i>Fusobacterium nucleatum</i> (31)	22	65	40	50	1.0	100	0.5	100	–	–
<i>Prevotella intermedia</i> (6)	12.5	90	75	50	1.0	100	0.4	80	–	–
<i>Porphyromonas gingivalis</i> (8)	25	75	47.5	50	0.9	90	0.4	80	–	–
<i>Staphylococcus aureus</i> (12)	25	57.5	65	25	0.7	70	>0.5		–	–
<i>Streptococcus intermedius</i> (6)	13.7	70	81.5	22.7	0.5	50	0.2	40	–	–
<i>Pseudomonas aeruginosa</i> (4)	>100	>100	>100	100	1.0	100	>0.5		–	–
Concn of preservative or vasoconstrictive additive of trade preparation (mg ml ⁻¹)					1.0		0.5		0.0182	0.006/0.012

–, No inhibition at the application concentration of the additives.

corresponding preservative found 2 instances where the MICs of the preservatives against 5 representative species (67 strains) were lower, indicating that the antimicrobial effect was mainly due to the preservative, but 21 results where the pure anaesthetic component showed greater antimicrobial effects compared with the preservative. One result gave identical values of 100 % (*P. aeruginosa*).

Taking the MIC values of the trade preparations into consideration, it is evident that the MICs of the trade preparations are almost always lower than the MICs of the pure anaesthetic substances. These findings lead to the suggestion that there must be a synergistic effect between the anaesthetic and the preservative, with the higher the distance between MIC values, the lower the synergistic effect, and vice versa (Table 5).

In conclusion, all of the local anaesthetics investigated in this study showed antimicrobial activity against components of the oral flora. In this respect, Ultracaine D-S represented the most favourable local anaesthetic because of the relatively high application concentration in relation to the MIC and MBC values. The weakest activity was seen with the frequently used Novocaine. The MIC values of the anaesthetic active components correlated with the efficacy of the trade preparations, which demonstrated that the antimicrobial activity could largely be attributed to the anaesthetic active components. Additionally, the additives sodium disulfite and methyl-4-hydroxybenzoate only showed antimicrobial effects greater than the anaesthetic active components in a few cases.

The *in vitro* results for Carbostesin, Scandicaine and especially for Novocaine indicate that local disinfection should be carried out prior to injection of these anaesthetics. In our opinion, due to the results obtained with nosocomial strains (*Escherichia coli*, *S. aureus*, *Pseudomonas*), disinfection of the mucous membranes should be routinely performed in immunocompromised patients, regardless of the anaesthetic used.

Further *in vivo* studies examining the local tissue concentration of anaesthetics directly after injection would be useful to determine whether the *in vivo* concentrations are comparable with our *in vitro* results. Our results also indicate that tissue samples that are taken from areas infiltrated with local anaesthetics are not suitable for diagnostic microbial analysis, as the presence of anaesthetics may lead to false-negative results.

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