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Antimicrobial activity of topical anaesthetic preparations

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SUMMARY Eight commercial topical anaesthetic preparations were tested for their ability to inhibit microbial growth in vitro by incubating serial dilutions with each of 4 micro-organisms. In addition corneas of mice were infected with *Staphylococcus aureus*, and the effect of the anaesthetics on isolation rates of bacteria was investigated. The preparations were shown to have a wide range of antimicrobial activity, correlating both with the active agents and the preservatives. We suggest that some preparations are unsuitable for use prior to collection of specimens from human corneal ulcers.

The successful management of corneal infection depends on the early commencement of specific antimicrobial chemotherapy. Since this requires the prompt identification of causative micro-organisms, the procedure for specimen collection must ensure no loss of viability of these agents.

Material for culture from infected corneas is often collected after application of a topical anaesthetic. Usually only a small amount of material is obtained, giving a light growth of bacteria or fungi. An anaesthetic preparation with considerable antimicrobial activity might lead to a dangerous false negative culture result.

A range of topical preparations is available to ophthalmologists; multidose containers of anaesthetic and preservative and single-dose vials containing the anaesthetic agent only. The antimicrobial activities of the anaesthetic agents and preservatives have been determined separately in vitro,[1–3] and several clinical trials of anaesthetics during bronchial and odontological procedures have been reported.[4–6] We determined the antimicrobial activities of 8 commercially available topical anaesthetics in vitro, using a minimum inhibitory concentration (MIC) assay and used a mouse model of corneal infection to test the effect of the preparations on the recovery of viable bacteria.

Materials and methods

MINIMUM INHIBITORY CONCENTRATION ASSAY

Four clinical isolates each of *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Streptococcus pneumoniae*, and *Candida albicans* were selected, as these organisms are commonly found in infected corneas. Single colonies were cultured overnight in Mueller-Hinton broth (*Staph. aureus* and *Ps. aeruginosa*), Todd-Hewitt broth (*Str. pneumoniae*), or Sabouraud broth (*C. albicans*).

Eight commercial preparations were assayed. These are set out in Table 1.

The microbial suspensions were subcultured and reincubated for 3 hours. Serial 2-fold dilutions of the anaesthetic preparations were made in the appropriate broth in microtitre trays. An equal volume of suspension (50 μl) containing 10^5 organisms in the log phase of growth was added to each well, and the trays were incubated for 18 hours at 37°C. Growth of organisms was defined as the appearance in a well of an aggregate larger than 2 mm in diameter. Loopfuls from the no-growth wells were plated out on to blood agar to give the maximum microbicidal dilution.

IN-VIVO ASSAY

The animals chosen were F1 (Balb/c×C57B1) mice. A *Staph. aureus* strain (penicillin-resistant, phage type 55/71) isolated from a human corneal ulcer was used to infect the mice.

The animals were given ketamine intraperitoneally (4 mg). Both corneas of each mouse were incised with a scalpel blade and keratome, and several drops of a concentrated (10^{10}/ml) bacterial suspension were dropped onto the cornea. 24 hours later 80% of the eyes had developed keratitis, and these were used in the test. The eye was prolapsed and a sterile

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Table 1 *Topical anaesthetics*

Commercial preparation	*Active reagent*	*Preservative(s)*
Alcaine (Alcon)	Proxymetacaine 0·5%	Benzalkonium chl 0·01%
Decicain (Winthrop)	Amethocaine 0·5%	Chlorobutanol 0·42%
Novesine (Sandoz)	Oxybuprocaine 0·4%	Chlorhexidine diacetate 0·001% and boric acid 0·2%
Ophthaine (Squibb)	Proxymetacaine 0·5%	Benzalkonium chl 0·01% and chlorobutanol 0·2%
Ophthetic (Allergan)	Proxymetacaine 0·5%	Benzalkonium chl 0·01%
Xylocaine Ophthalmic (Astra)	Lignocaine 4%	Benzalkonium chl 0·004%
Minims amethocaine (Smith & Nephew)	Amethocaine 0·5%	None
Minims benoxinate (Smith & Nephew)	Oxybuprocaine 0·4%	None

Benoxinate≡oxybuprocaine. Amethocaine≡tetracaine. Proxymetacaine≡proparacaine.

platinum spatula stroked 5 times across the cornea. Collected material was transferred directly to blood agar. The spatula was stroked 3 times across the plate, which was immediately streaked out. The test anaesthetic preparation (50 μl) was dropped on to the cornea, and 5 minutes later the eye was gently wiped and the scraping procedure repeated. The plates were incubated overnight at 37°C. Eight eyes were tested in each group.

Results

The maximum inhibitory dilution (MID) and maximum microbicidal dilution (MMD) for each drug for each organism are set out in Table 2. These expressions are more suitable than concentrations when we are dealing with mixtures. Each value was derived from testing 4 different isolates in duplicate. Variation between isolates of one organism rarely exceeded one well. Novesine was generally the most lethal preparation, followed by Alcaine, Ophthaine, and Ophthetic with intermediate antimicrobial activity. The Minims, Decicain, and Xylocaine had low inhibitory power. Novesine was the only preparation with substantial anti-pseudomonas activity. The 2 Gram-positive bacteria had a similar susceptibility pattern, with *Str. pneumoniae* generally more sensitive than *Staph. aureus*. Alcaine and Ophthetic, with identical anaesthetic agents and preservatives, differed in their activity to *Str. pneumoniae* by a factor of about 3. We cannot account for this observation. From this in-vitro work we selected *Staph. aureus* as a suitable organism for the in-vivo test.

The mouse assay results (Table 3) are expressed as the mean ($\bar{x}$) of the difference in colony counts between the scrapings prior to and after application of topical anaesthetic, i.e.,

$$x = \log_{10}(\text{counts } \#1+1) - \log_{10}(\text{counts } \#2+1)$$

Novesine reduced the counts by over 1 log, saline had

Table 2 *Antimicrobial activity in vitro*

Preparation		*Micro-organism*			
		Staphylococcus aureus	*Pseudomonas aeruginosa*	*Streptococcus pneumoniae*	*Candida albicans*
Alcaine	MID	640	2	600	20
	MMD	200	2	600	18
Decicain	MID	14	<2	24	2
	MMD	13	–	20	2
Novesine	MID	1340	52	580	64
	MMD	850	15	450	60
Ophthaine	MID	240	2	280	18
	MMD	140	2	180	18
Ophthetic	MID	640	2	190	18
	MMD	90	2	180	18
Xylocaine Ophthalmic	MID	10	2	140	3
	MMD	4	2	45	3
Minims amethocaine	MID	8	2	34	3
	MMD	5	2	26	3
Minims benoxinate	MID	4	<2	18	3
	MMD	3	–	14	3

MID=maximum inhibitory dilution. MMD=maximum microbicidal dilution.

Table 3 *Change in number of viable Staph. aureus isolated from corneas of mice with anaesthetics*

Treatment	$\bar{x}$ (±0·196)
Saline	0·016
Alcaine	0·554
Decicain	0·321
Novesine	1·217
Ophthaine	0·560
Ophthetic	0·974
Xylocaine Ophthalmic	−0·170
Minims amethocaine	0·344
Minims benoxinate	0·168

Least significant difference for p=0·05 is 0·565.

little effect on the counts, and Xylocaine actually improved the recovery. To assess the significance of these differences a one-way analysis of variance was performed.[7] This gave an F ratio of 5.084, high enough to conclude that a real difference exists between 2 or more groups as compared with the variation within them. The method of least significant differences[8] resulted in 2 drugs significantly reducing the counts compared with saline; Novesine ($p<0·001$) and Ophthetic ($p<0·01$). For Ophthaine and Alcaine, $p=0·07$. The more stringent method of Dunnett's[9] results in only Novesine significantly reducing counts ($p<0·05$). The difference in the $\bar{x}$ values for Alcaine and Ophthetic is surprising but repeatable.

Discussion

The results indicate that some topical anaesthetic preparations can affect the recovery of organisms from infected corneas. This antimicrobial effect correlates with the presence of preservative. The results for Minims benoxinate (no preservative) and Novesine (benoxinate plus preservatives) show this. The chlorobutanol in Decicain is seen to be relatively ineffective at inhibiting growth or recovery of staphylococcus as compared to benzalkonium chloride at 0·01% or chlorhexidine.

In 1966 Kleinfeld and Ellis[1] found that 0·5% amethocaine, 0·4% benoxinate, 2·5% cocaine, and 0·4% chlorobutanol inhibited growth of *Staph. epidermidis, Ps. aeruginosa*, and *C. albicans* in liquid media. Four years later Schmidt and Rosenkranz[2] reported that lignocaine and procaine would inhibit a wide range of micro-organisms. In 1975 Weinstein *et al.*[3] tested 8 anaesthetics in an MIC assay and found amethocaine and hexylcaine to have the greatest inhibitory power. Our in-vitro results for the 2 Minims preparations may be compared with the results of these authors. The minimum inhibitory concentrations for Minims amethocaine and benoxinate are in agreement with those obtained by Kleinfeld and Ellis[1] using pure preparations, but our findings are at variance with those of Weinstein *et al.*[3] in several respects. Firstly, we find 0·15% benoxinate inhibits growth of *Staph. aureus* and *C. albicans* and 0·025% inhibits growth of *Str. pneumoniae*, whereas Weinstein *et al.*[3] report >1% benoxinate is required to inhibit these 3 organisms. Also our streptococci are more sensitive to amethocaine (0·015% versus 0·5% for inhibition) and our candidas less sensitive to amethocaine (0·167% versus 0·015%). These inconsistencies may reflect different strains, media, and anaesthetic preparations, since the Minims contain stabiliser.

A number of factors could influence the effect of the anaesthetics on organisms in human corneas, including total dosage, dilution by tears, the number of organisms present, and the efficiency of absorption into the diseased area of both the anaesthetic agents and preservatives. From the mouse studies we find that the time the drug is on the eye before culturing is important: counts decrease over the first 10 minutes.

Novesine significantly reduces the isolation rate of *Staph. aureus* from corneal ulcers in mice, and this preparation has a high anti-microbial activity in vitro. It would seem that Novesine should not be placed on a patient's eye prior to collecting material for microbiological study. Ophthetic, Ophthaine, and Alcaine, and any other topical anaesthetic containing benzalkonium chloride at 0·01%, also appear to have an unacceptably high level of antimicrobial activity in this instance.

From these results and previous reports it would appear that Minims benoxinate is the preferred drug for obtaining material for culture. It is also unlikely that Minims amethocaine, Decicain, containing chlorobutanol, or Xylocaine Ophthalmic, containing benzalkonium chloride at low concentration, have any effect on isolation rates of organisms from corneas if not left on the eye for more than 2 or 3 minutes.

It should be remembered that infected corneas are relatively insensitive, and collections can often be made without the use of a local anaesthetic. When this is not possible, any reduction in the number of viable organisms recovered from a cornea due to the anaesthetic agent is probably counterbalanced by the increased amount of material obtainable from an anaesthetised eye. The anaesthetic preparation we choose to use should not compromise our chance of recovering the causative agent.

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