From:
The Department of Oral Surgery,
Royal Dental College,
Aarhus, Denmark

ANTIMICROBIAL EFFECT OF TOPICAL ANAESTHETICS

by
J. E. WINTHER
M. W. KHAN

INTRODUCTION

Injections of local anaesthetics in the oral cavity are accompanied by inoculation of a varying amount of microorganisms along the path of the needle (*Streitfeld & Zinner*, 1958; *Gräf*, 1965; *Birn & Winther*, 1967; *Winther & Praphailong*, 1969). The oral microbial flora may vary in number and virulence depending among other factors on dental hygiene and concomitant infections in the nose and throat, but even under the most favourable conditions it remains a hazard to local anaesthetic injections. Disinfection of the oral mucosa at the site of injection would, therefore, seem a logical precaution to take in securing the highest possible degree of safety for the patient. While a few authors find disinfection of the oral mucosa unnecessary (*Wolf*, 1957; *Sauerwein*, 1957; *Davis*, 1961), a large number of publications favour the use of antiseptics (*Becker*, 1920; *Sivén*, 1922; *Schmidhuber & Flecker*, 1925; *Rodriquez*, 1928; *Miller & Appleton*, 1931; *Round & Kirkpatrick*, 1935; *Streitfeld & Zinner*, 1958; *Kantorowicz*, 1959; *Zinner et al.*, 1961; *Geary & Gavin*, 1963; *Knothe & Hoppe*, 1965). As most clinicians and authors of textbooks on local anesthesia recommend the application of a topical anaesthetic to prevent pain from the needle puncture, it seems a logical and rational step to combine these two procedures if possible. Various

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anaesthetic sprays (*Gräf*, 1965; *Winther & Praphailong*, 1969) and a specially prepared ointment (*Birn & Winther*, 1967) have been suggested for this combination and shown to be effective.

The present study was undertaken in continuation of the work of *Winther & Praphailong* (1969) in order to evaluate the antimicrobial effect of a number of commercial ointments and gels commonly used for surface anaesthesia. This was done under the assumption that possible bactericidal components added by the manufacturer might have an antiseptic effect on the oral microflora.

MATERIAL AND METHODS

Five topical anaesthetics were used in this experiment, Carbocain ® ointment, Leostesin ® ointment, Leostesin ® gel, Xylocain ® ointment, and Xylocain ® gel.*) The latter four contain lidocaine as the active component while Carbocain ® contains mepivacaine. These five brands were chosen because they are the ones most widely used in Scandinavia. The ointments and gels were all dispensed in tubes and were handled in the same way as in a dental practice. The experiment was divided into a clinical and a laboratory part.

Clinical investigation

Fifty dental students, all with excellent oral hygiene, participated in the study. Six different areas of the vestibular mucosa, buccal and labial fold, were selected for injection with the upper and lower premolar regions and the upper right lateral incisor region serving as test areas, while the upper left lateral incisor region served as control. In order to avoid that the test areas and the control area were prematurely affected by the topical anaesthetics, the needle punctures were carried out in the same order in all students, starting with the control area, continuing from right to left in the lower jaw and ending up in the upper jaw, going from right to left. The possible errors introduced by using different test areas were eliminated by applying the topical anaesthetics in the same order during the whole experiment, but moving clockwise the whole cyclus one step at a time for each experimental subject. In that way each anaesthetic was tried ten times in each test area (*Winther & Praphailong*, 1969).

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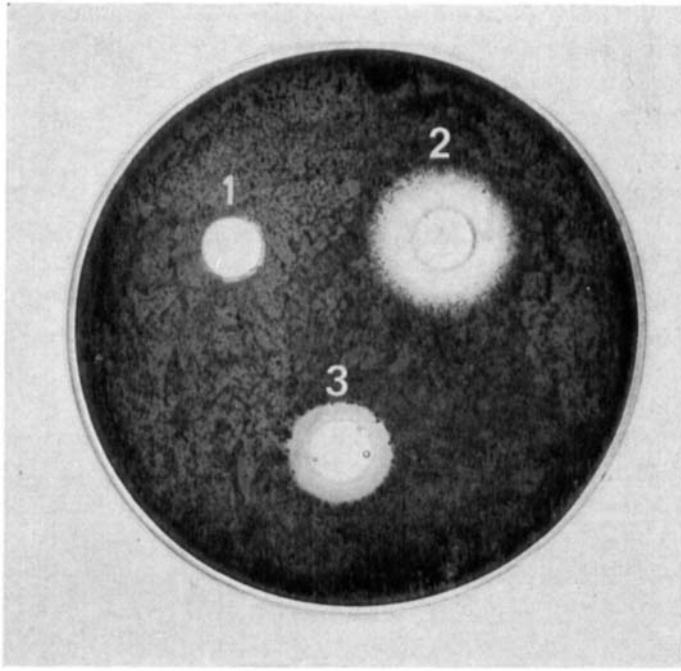

Fig. 1. Inhibition zones on a blood agar plate inoculated with a salivary microflora. 1 = Carbocair ® ointment. 2 = Leostesin® ointment. 3 = Lidocaine-chlorhexidine.

The experiment was carried out under standardized conditions at the same time of the day during a period of two weeks. The sampling technique used for evaluation of the microbial inoculation along the needle path was based on the fact that each puncture of the oral mucosa will press a certain amount of microorganisms into the needle (*Kantorowicz*, 1959; *Streitfeld & Zinner*, 1958; *Geary & Gavin*, 1963; *Gräf*, 1965; *Birn & Winther*, 1967; *Winther & Praphailong*, 1969). This plug of material was used as a relative measurement of the contamination.

The clinical test was carried out as described earlier by *Winther & Praphailong* (1969) except for the application of the topical anaesthetic. After drying the test area with a sterile swab 0.2 ccm of the ointment or gel was applied to a sterile cotton roll, which was then placed in the buccal fold to ensure the best possible contact between the medium and the mucosal surface and to keep the area dry. Disposable needles (gauge 26) was mounted on sterile syringes each filled with one milliliter of isotonic saline solution. After insertion and immediate withdrawal of the needle two drops of the

saline solution was pressed out on a blood agar plate. The liquid was spread evenly on the surface and the plates were incubated for 72 hours at 37°C under aerobic conditions. The number of colonies were counted, and the mean value for each topical anaesthetic was calculated.

Laboratory investigation

The antimicrobial activity of the five topical anaesthetics was tested *in vitro* for microorganisms. For comparison the lidocaine-chlorhexidine ointment (5 % and 0.05 % respectively) tested clinically by *Birn & Winther* (1967) was included in this part of the investigation. 0.25 ml of saliva obtained from the participants in the clinical test was spread on a blood agar plate. Three holes, each 10 mm in diameter, were punched out in the agar at adequate distances from each other. Two plates were used for each sample of saliva, and each. hole was filled to the brim with one of the six agents used in the experiment. The agar plates were incubated for 48 hours at 37°C. The total diameter of the inhibition zone plus the hole was measured in each case (Fig. 1), and the mean values calculated.

RESULTS

Clinical investigation

Of the 300 agar plates used in the clinical experiment only one from the control area exhibited a complete overgrowth of microorganisms, which made a distinction between the single colonies impossible. This plate was therefore omitted from the calculations. The results of the colony counts are shown in

Table I.

Bacterial counts according to anaesthetic used

Test medium	Number of agar plates	Total number of colonies	Mean number of colonies	Per cent reduction
Control	49	784	15.7	0
Carbocain®O.	50	415	8.3	47
Leostesin®O.	50	194	3.9	75
Leostesin®G.	50	715	14.3	9
Xylocain®O.	50	142	2.8	82
Xylocain®G.	50	838	16.8	0

(O = ointment, G = gel)

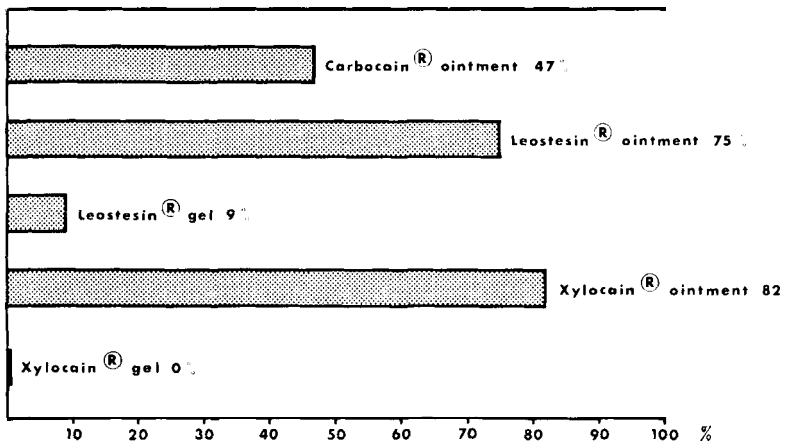

Fig. 2. Antiseptic effect of various topical anaesthetics as measured by reduction in bacterial counts (clinical trial).

Table I. The gel types of Xylocain® and Leostesin® had no or very little antiseptic effect. Carbocain® ointment had but a limited effect and only the ointments of Xylocain® and Leostesin® gave a fairly good disinfection of the oral mucosa (Fig. 2).

The results were tested with the chi square test, which showed a significant difference between the control group and the Xylocain® and Leostesin® ointments, $0.005>P>0.001$ and $0.025>P>0.02$ respectively. The difference shown between the control area and the areas treated with Carbocain® ointment was not statistically significant.

Table II.

Inhibition zones on agar plates according to anaesthetic tried

Test medium	Number of agar plates	Total sum of diameters (mm)	Mean value of diameters (mm)
Lidocaine-chlorhexidine	50	774	15.5
Carbocain®O.	50	500	10
Leostesin®O.	50	920	18.4
Leostesin®G.	50	506	10
Xylocain®O.	50	937	18.7
Xylocain®G.	50	506	10

(O = ointment, G = gel)

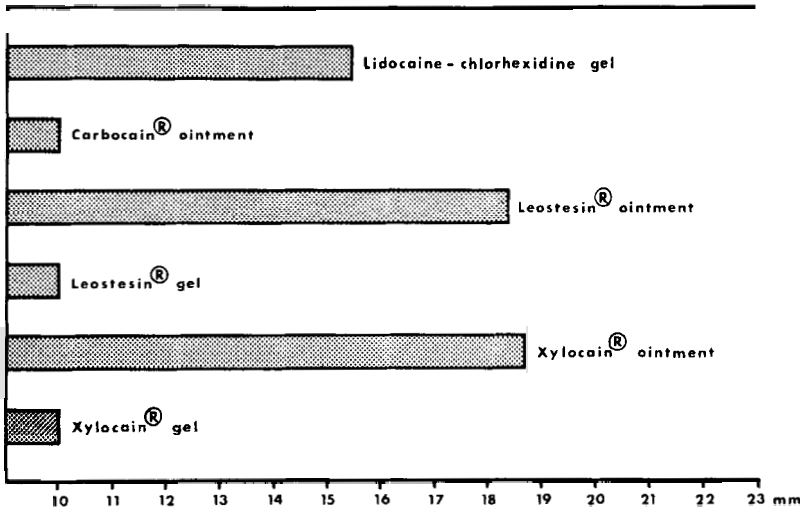

Fig. 3. Antiseptic effect of various topical anaesthetics as measured by the inhibitory effect on the growth of a salivary microflora, (laboratory test).

Laboratory investigation

The results of the laboratory investigation showed distinct differences in the antiseptic effect of the six topical anaesthetics employed (Table II). The total sum of the diameters in the third column includes the diameter of the test material (10 mm). In the last column the mean diameters of the inhibition zones (plus 10 mm) are calculated, and it is clearly shown that only Xylocain ® and Leostesin ® ointments plus lidocaine-chlorhexidine ointment had any inhibitory effect on the growth of the oral microflora. In Fig. 3 this effect is graphically outlined. The minor antiseptic effect of lidocaine-chlorhexidine is not statistically significant.

DISCUSSION

Along with a steadily progressing refinement of dental treatment and as more and more local anaesthetics are given in dental practice, the use of surface anaesthesia is gaining wider acceptance. Disinfection of the oral mucosa prior to injection has been advocated by many authors (*Streitfeld & Zinner*, 1958; *Kantorowicz*, 1959; *Zinner et al.*, 1961; *Geary & Gavin*, 1963; *Knothe & Hoppe*, 1965) and no one has ever proved it worthless. When considering the widely accepted use of surface anaesthetics, it seems logical to combine

their effect with that of an oral antiseptic in one remedy. Such combinations have been tried successfully under clinical conditions by *Gräf* (1965) and by *Birn & Winther* (1967). Antiseptics added to commercial products of topical anaesthetics for reasons of conservation have been proved, as far as spray anaesthetics are concerned, to possess an inhibitory effect on the oral microflora (*Winther & Praphailong*, 1969).

The present study deals with the same problem in commercial products of anaesthetic ointments and gels. The clinical part of the study revealed that only two of the ointments (Xylocain ® and Leostesin ®) yielded a satisfactory protection against microbial inoculation, while the gel types did not offer any antiseptic effect, and the Carbocain ® ointment did so only to a certain degree. When considering the single colony counts for the two first mentioned ointments it was found that the counts from each subject were almost all zero or one, and the total figures of 194 and 142 originated almost exclusively from one or two very large plate counts. If clinical or laboratory failures are considered to be a possible explanation of these outstandingly high figures and as a consequence the two or three highest counts from each group were discarded, the inhibitory effect of Leostesin ® ointment and Xylocain ® ointment would be even more pronounced.

To ensure the highest possible degree of uniformity in the experiment the participants chosen were in the same age group (20—30 years), the female-male ratio was 1:2 and all had an excellent oral hygiene. The test areas were protected from contamination by applying the topical anaesthetic on a sterile cotton roll. The rotational use of the anaesthetics largely eliminated the chance of errors introduced by using five different test areas with possible different quantities of microorganisms. This was proved in an earlier study (*Winther & Praphailong*, 1969) and confirmed in the present one by statistical analysis of the colony counts from the different locations.

In the present investigation a surprisingly small number of colonies were found in the control area (15.7) compared with the corresponding figure from the similar study of *Winther & Praphailong* (34.1). This difference may be due to a general improvement in the oral hygiene of the students as the experimental conditions were exactly the same. This may also explain why in this investigation only one agar plate had to be discarded on account of massive overgrowth, while the corresponding figure in the earlier report of *Winther & Praphailong* was 14.

The small amount of microorganisms in the control area, however, tends to rise the value of the results obtained, as it has been reported by *Birn & Winther* (1967) that in this kind of experiment one can expect a numerically identical final result irrespective of the number of microorganisms on the

unaffected mucosa at the start of the experiment. This means that an initial large amount of microorganisms as may be found in an ordinary outpatient material (*Gräf*, 1965) rises the percentages of reduction and in that case for instance Carbocain ® ointment would get a higher rating. However, the less the initial number of colonies in the control area, the more value can be attached to a statistically significant difference in colony count before and after application of an antiseptic.

The results of the clinical experiment was supported by the laboratory investigation, showing a definite antimicrobial effect of the anaesthetic ointments of Xylocain ® and Leostesin ® on a mixed oral flora cultivated from samples of saliva from the same group of students used in the clinical study. For the sake of comparison the non-commercial ointment of lidocaine-chlorhexidine was included. This has in a similar clinical test (*Birn & Winther*, 1967), yielded an 88 % reduction in bacterial inoculation, which means that this agent clinically is as effective as the two first mentioned ointments. The minor effect of lidocaine- chlorhexidine in the laboratory study could be expected due to the reduced diffusibility of chlorhexidine in blood agar medium (*Rindom Schiøtt*, 1970).

The laboratory results divided the test material much more definitely than did the clinical study. The gel types of Xylocain ® and Leostesin ® and the Carbocain ® ointment showed absolutely no effect on the microflora. This can only be explained by lack of bacteriostatic ingredients effective on the oral microorganisms. The names and formulas of these components in the Xylocain ® and Leostesin ® ointments were not available for publication, but apparently they have bacteriocidal properties similar to those of chlorhexidine.

It is concluded that of the five topical anaesthetics tested, only two, namely Leostesin ® and Xylocain ® ointments, gave a satisfactory disinfection both in the clinical and the laboratory studies. Carbocain ® ointment showed only a weak effect in the clinical study and none in the laboratory study. The gel types of Xylocain ® and Leostesin ® revealed no antimicrobial properties in either of the two studies. The effect of the two first mentioned ointments was comparable to the results obtained with a preparation of lidocaine-chlorhexidine and with spray anaesthetics. For preinjection purposes, however, an ointment seems more suitable than a spray, because it can be applied to and remain in small areas of the oral mucosa, wheras the spray dose affects larger areas than needed.

SUMMARY

The antiseptic effect of five topical anaesthetics, Carbocain ® ointment, Leostesin ® ointment, Leostesin ® gel, Xylocain ® gel, and Xylocain ® ointment, was tested on the oral mucosa of 50 dental students. Five test areas were selected in the vestibule using each topical anaesthetic 10 times in each area. Plugs of material, obtained by puncturing the oral mucosa with a sterile needle, were cultured and used as relative measurements of contamination. It was found that Xylocain ® and Leostesin ® ointments had a marked antiseptic effect, while the gels and the Carbocain ® ointment had no or very little effect. This result was confirmed by laboratory inhibition tests of all the anaesthetics mentioned above plus a non-commercial ointment containing lidocaine and chlorhexidine. A surprisingly small number of colonies in the control area raises the credibility of the results obtained. It is concluded that the use of topical anaesthetics with bacteriostatic ingredients effective on oral microorganisms gives an extra advantage of disinfection of oral mucosa.

RÉSUMÉ

ACTION ANTISEPTIQUE D'ANESTHÉSIQUES DE CONTACT

L'action antiseptique de cinq anesthésiques de contact, la pommade de Carbocaïne, ® la pommade de Léostésine ®, le gel de Léostésine ®, le gel de Xylocaïne ®, et la pommade de Xylocaïne ®, a fait l'objet de tests sur la muqueuse buccale de 500 étudiants en art dentaire. Cinq zones de test ont été choisies dans le vestibule, et chaque anesthésique de contact a été utilisé 10 fois dans chaque zone. Des prélèvements obtenus en introduisant une aiguille stérile dans la muqueuse buccale ont été cultivés et ont servi de mesures relatives de la contamination. Les résultats obtenus ont montré que les pommades de Xylocaïne ® et de Léostésine ® avaient une action antiseptique marquée, tandis que les gels et la pommade de Carbocaïne ® n'avaient que peu ou pas d'action. Ces résultats ont été confirmés par des tests d'inhibition faits au laboratoire sur tous les anesthésiques ci-dessus ainsi que sur une pommade non commerciale contenant de la lidocaïne et de la chlorhexidine. Un nombre étonnamment petit de colonies a été trouvé dans la zone témoin, ce qui augmente la vraisemblance des résultats obtenus. Les auteurs concluent que l'usage des anesthésiques de contact contenant des substances bactériostatiques agissant sur la flore microbienne buccale offre l'avantage de donner en supplément une désinfection de la muqueuse buccale.

ZUSAMMENFASSUNG

DIE ANTISEPTISCHE WIRKUNG VON OBERFLÄCHENANÄSTHETIKA

Die antiseptische Wirkung von fünf Anästhetika, Carbocain ®-Salbe, Leostesin ®-Salbe, Leostesin ®-Gel, Xylocain ®-Gel und Xylocain ®-Salbe wurde auf der Mundschleimhaut 50 zahnärztlicher Studenten ausprobiert. Es wurden im Vestibulum fünf Testregionen ausgewählt und zwar so, dass jedes Anästhetikum in jeder Region 10mal angewendet wurde. Durch Punktieren der Mundschleimhaut mittels einer sterilen Nadel wurde Stöpselmaterial gewonnen, das kultiviert wurde und als relativer Mass-stab für die Kontamination benutzt wurde. Es ergab sich, dass die Xylocain ®- und Leostesin ®-Salben eine ausgesprochen antiseptische Wirkung besassen, während die Gele und die Carbocain ®-Salbe keine oder aber eine sehr geringe Wirkung aufwiesen. Dieses Resultat wurde im Labor durch das Inhibitions-Testen aller obigen Anästhetika sowie einer nicht im Handel befindlichen, Lidokain und Chlorhexidin enthaltenden Salbe bestätigt. Eine überraschend geringe Anzahl Kolonien in der Kontrollzone steigert die Glaubwürdigkeit der erzielten Resultate. Es wird konkludiert, dass die Anwendung von Anästhetika mit bakteriostatischen, die oralen Mikroorganismen beeinflussenden Ingredienzen den zusätzlichen Vorteil der Desinfektion von der Mundschleimhaut ergibt.

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Address:
Department of Oral Surgery,
Royal Dental College,
Vennelyst Boulevard,
8000 Århus C,
Denmark