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ANTIMICROBIAL EFFECT OF ANESTHETIC SPRAYS

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INTRODUCTION

The disinfection of oral mucosa prior to injection has always been a controversial subject. The moist surface with an abundant flora of different microorganisms continuously circulating with the flow of saliva makes the use of all antiseptics ineffective in a short time. Working in a contaminated area is the conditions faced with in dentistry, and up till now no satisfactory solution of the problem has been proposed. Fortunately the resistance of oral tissues to microbial infection enables surgical procedures, which in other locations might be hazardous or even disastrous. This clinically proved fact does not permit us, however, to disregard accepted surgical principles. All possible precautions to protect the patient from infection due to surgical intervention should be taken. Intraoral injection of local anesthetics is a minor routine surgical procedure, which in all respects is comparable to cutaneous injection. While disinfection in the latter case is a generally accepted principle a similar prophylactic measure is not in common use in connection with intraoral injections (*Zinner & Streitfeld*, 1958; *Winther & Birn*, 1967).

A few authors find disinfection of the oral mucosa an unnecessary precaution (*Wolf*, 1957; *Sauerwein*, 1957; *Davis*, 1961), while a large number

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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).

Whereas disinfection of the oral mucosa prior to injection is far from being a routine procedure, the application of a topical anesthetic to prevent pain during intraoral injections is a well accepted principle. In combining topical anesthetics with bactericidal compounds a double effect is accomplished (*Gräf*, 1965; *Birn & Winther*, 1967). *Gräf* investigated clinically the antiseptic effect of Pantocain® (Gingicain®), which besides the topical anesthetic tetracaine contains alkyl-dimethyl-benzyl-ammoniumchloride as a disinfectant. The effect was proved by a 97 per cent decrease in the relative number of bacteria inoculated into the tissues. Another investigation with a similar technique (*Birn & Winther*, 1967) using an ointment of lidocaine and chlorhexidine as the active components showed a 88 per cent reduction in the relative number of inoculated bacteria. *Knothe* and *Hoppe* (1965) showed in their experiment on dogs that the antiseptic effect of three anesthetic sprays, Gingicain®, Xylostesin®, and Xylocain® was equal to that of iodine.

The present study was undertaken in order to evaluate the antimicrobial property of different anesthetic sprays. This was done under the assumption that the components added by the manufacturer in order to inhibit microbial growth in the spray liquid might have an antiseptic effect when administered to the oral mucosa.

MATERIAL AND METHODS

Four topical anesthetics were used in this experiment. They are listed below with the generic names in brackets.*)

Carbocain® (mepivacaine)	Pantocain® (tetracaine)
Leostesin® (lidocaine)	Xylocain® (lidocaine)

All were contained in spray bottles with various antiseptics added to inhibit microbial growth. The formulas and exact concentrations of these components were not accessible for publication. Pantocain® had previously been tested by *Gräf* (1965).

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*) The material was kindly provided by Biofarma A/S, Lövens kemiske fabrik, Hoechst Danmark A/S, ASTRA A/S.

Clinical investigation

70 dental students all with excellent oral hygiene volunteered in the experiment. Five different areas of the vestibular mucosa were selected for injection, with the upper and lower premolar buccal fold serving as test areas and the labial fold of the upper left lateral incisor region as control. The five

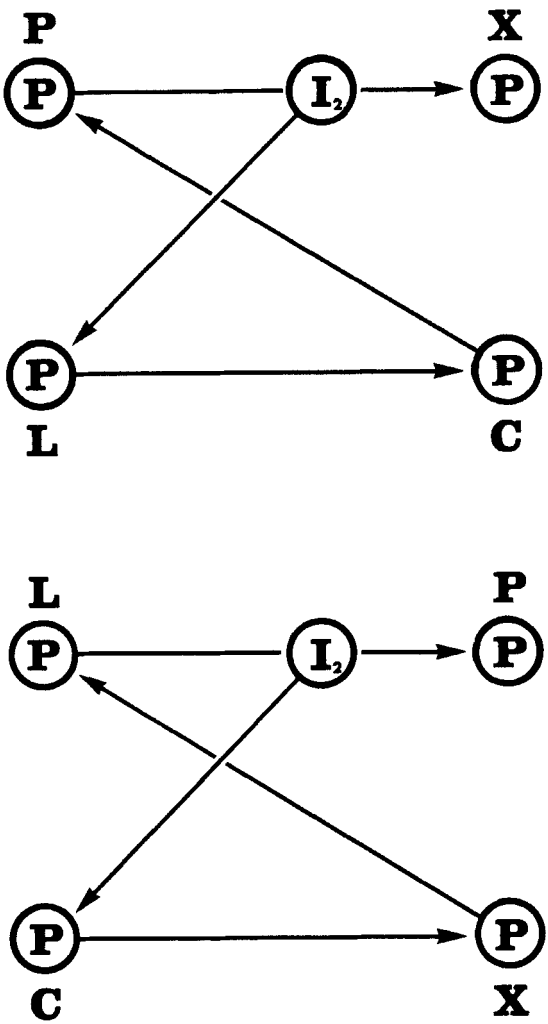

Fig. 1. The test-sequence of the five mucosal areas (upper left incisor region and the four premolar regions). Rotation of the anesthetics clockwise and in a fixed order is outlined. Two out of four combinations are shown (C = Carbocain®, L= Leostesin®, P = Pantocain®, X = Xylocain®).

locations were injected in the same order from student to student starting with the control area and continuing after the pattern outlined in Fig. 1. This sequence was chosen to keep the test areas from being prematurely affected by the anesthetic sprays.

To avoid any possible errors introduced by using different test areas the topical anesthetics were applied in the same order during the whole experiment, but were moved clockwise one step at a time for each experimental subject. Two of the four possible combinations are shown in Fig. 1.

The experiment was carried out daily for two weeks in the hour before lunchtime. No measures, such as toothbrushing or mouthrinsing, were taken in advance to alter the microbial flora. The amount of fluid per spray dose varied from one anesthetic to another. All test areas, however, were adequately covered with the anesthetic spray due to the pronounced surface-active property.

The technique used for testing the antiseptic effect was similar to the one outlined earlier (*Gräf*, 1965; *Birn & Winther*, 1967). They, among others (*Kantorowicz*, 1959; *Streitfeld & Zinner*, 1958; *Geary & Gavin*, 1963) proved that each puncture of the oral mucosa would press a certain amount of microorganisms into the needle. This plug of material from the mucosal surface was used as a relative measure of the microbial inoculation.

Each of five disposable syringes was filled with one millilitre of isotonic saline solution under sterile conditions. The syringes were mounted with disposable needles (gauge 26) for injection. Each test area was in turn dried with a sterile swab before applying one spray dose of the topical anesthetic. Due to the surface-active property a large area was overflowed. After waiting 20 seconds and keeping the area free from saliva with more sterile swabs the needle was inserted only bevel deep and then withdrawn without any injection. Two drops of the saline solution were then pressed out through the needle and spread on a blood agar plate. A similar procedure was carried out in the control area except for the spray application. The agar 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 anesthetic was calculated. The anesthetic effect was not evaluated in this context.

Laboratory investigation

The antimicrobial activity of the anesthetic sprays was tested on seven strains of oral microorganisms.*) Five of the strains were freshly isolated from the

*) The strains were kindly provided by the Department of Microbiology, Royal Dental College, Århus, Denmark.

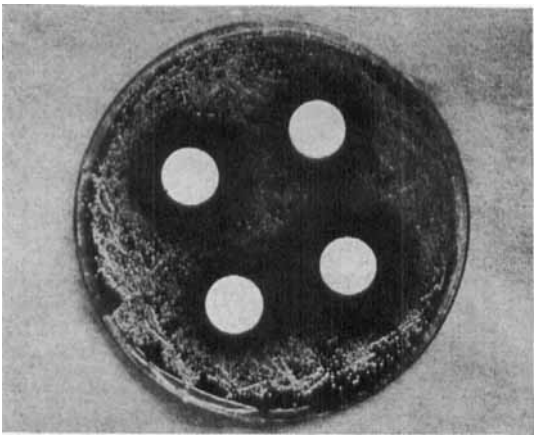

Fig. 2. Inhibition zones of the four anesthetic sprays on a blood agar plate inoculated with Candida albicans.

oral mucosa of one of the experimental subjects and represented the predominant organisms aerobically cultivable from this sample. The microorganisms were classified as follows: one strain of Candida albicans, one strain of Neisseria, one strain of Micrococcus, and four strains of Streptococcus. Each strain was inoculated on 20 blood agar plates. The plates were then divided into four zones and a sterile paper disc, 13 mm in diameter, saturated with 0.5 ml of one of the topical anesthetics, was placed in each quadrant. All four anesthetics were thus tested 20 times per strain of microorganism. The agar plates were incubated for 48 hours at 37°C. The total diameter of the inhibition zone and paper disc was measured in each case (Fig. 2), and the mean values calculated.

RESULTS

Clinical investigation

Of the 350 agar plates used in the clinical experiment 14 exhibited a complete overgrowth of microorganisms, which made a distinction between the single colonies impossible. Six of these agar plates represented the control area and eight the test areas. They were all omitted from the calculations. The results of the bacterial counts are shown in Table I.

The second column shows the total number of microorganisms isolated from all needle plugs. The next column represents the mean number of microorganisms per sample calculated from the figures mentioned above. In com-

Table I.

Bacterial counts according to anesthetic used

Test medium	Number of agar plates	Total number of colonies	Mean number of colonies	Per cent reduction
Control	64	2177	34,1	0
Carbocain®	69	458	6,6	81
Leostesin®	67	650	9,6	72
Pantocain®	69	109	1,6	95
Xylocain®	67	60	0,9	97

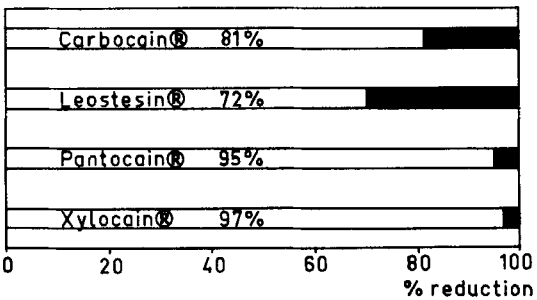

Fig. 3. Antiseptic effect measured by reduction in bacterial counts (Clinical trial).

parison with the control area the figures from the test areas show a 72—97 per cent reduction in bacterial contamination (Fig. 3).

The results were tested with the $\varkappa^2$-test, which shoved a significant difference between the control group and each of the four test groups ($P<0.001$ for Carbocain®, Pantocain®, and Xylocain®, and $0.02<P<0.025$ for Leostesin®. There was no significant difference between the effect of the four topical anesthetics tested under these conditions.

The possible errors introduced by using four different locations were evaluated by calculating the number of microorganisms isolated from each test area (Table II). Statistical analysis using the $\varkappa^2$-test showed no significant difference in bacterial counts between the four test areas, P ranging from 0.5 to 0.995.

During the clinical trial a large number of students complained of unpleasant taste and burning sensation for a short period after application of the spray dose. Another disadvantage encountered was the ready spreading of the liquid, giving anesthesia in much larger areas than needed for injection.

Table II.
Bacterial counts according to test area

Test area	Number of agar plates	Total number of colonies	Mean number of colonies
Control	64	2177	34,1
Lower right premolar	67	326	4,9
Lower left premolar	67	530	8,0
Upper right premolar	69	198	2,9
Upper left premolar	69	223	3,2

Laboratory investigation

The results of the laboratory investigation showed a distinct difference in the antiseptic effect of a topical anesthetic on different microorganisms as well as a more or less pronounced difference between the effect of the four anesthetics on the same strain of microorganisms. A pronounced effect, however, of one or more of the anesthetics on all seven microorganisms was demonstrable (Fig. 4). The dispersion of the maximum and minimum values show that the range is small in most of the groups. The zones of inhibition indicating the effect of each anesthetic on all microorganisms make up frequency distributions, which give a more detailed picture of the antimicrobial effect than did the clinical trial (Fig. 5). Apparently Carbocain® under these conditions has the most reliable bactericidal effect. It must be emphasized, however, that the results only apply to the seven strains of microorganisms tested.

DISCUSSION

Topical anesthetics are in considerably more common use than are antiseptics prior to oral injections of local anesthetics. The statewide surveys among practising dentists in Florida (*Zinner & Streitfeld*, 1958) and Denmark (*Winther & Birn*, 1967) gave the following results: In the former survey topical anesthetics were used regularly by 72 per cent and antiseptics by 43 per cent of the dentists questioned, while the figures in the latter survey were 15 per cent and 5 per cent respectively. It must be added that 54 per cent in the Danish survey used topical anesthetics occasionally, while the corresponding figure in the American survey was 6 per cent.

If these results represent a general trend, the idea of disinfection is likely to be more widely accepted when combining topical anesthetics and anti-

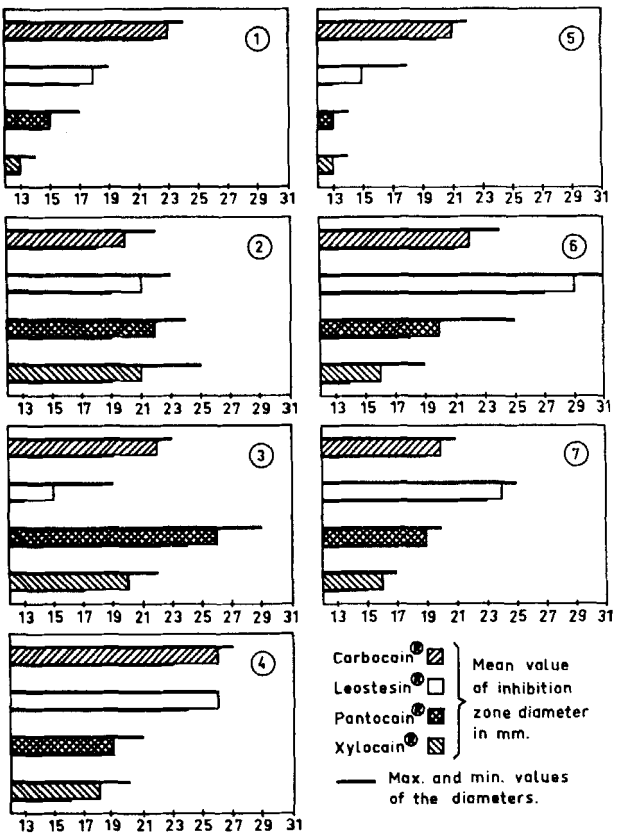

Fig. 4. Graphical outline of the diameters of the inhibition zones (in mm) produced by the four topical anesthetics. No 1: Streptococcus; No 2: Neisseria; No 3: Candida albicans; No 4: Streptococcus; No 5: Streptococcus; No 6: Streptococcus; No 7: Micrococcus.

septics in one remedy. Such combinations have been suggested earlier by *Gräf* (1965), and *Birn & Winther* (1967), who obtained a 97 and 88 per cent reduction respectively in the number of microorganisms introduced in the tissues by the injection needle.

In the present investigation the assumption that this combination already existed in some anesthetic sprays proved to be correct. In comparing each test group with the control group the difference in bacterial inoculation was found to be significant, thus proving a definite antiseptic effect of all four anesthetics. In this study the clinical results using Pantocain® (95 per cent reduction in bacterial inoculation) were in good accordance with those ob-

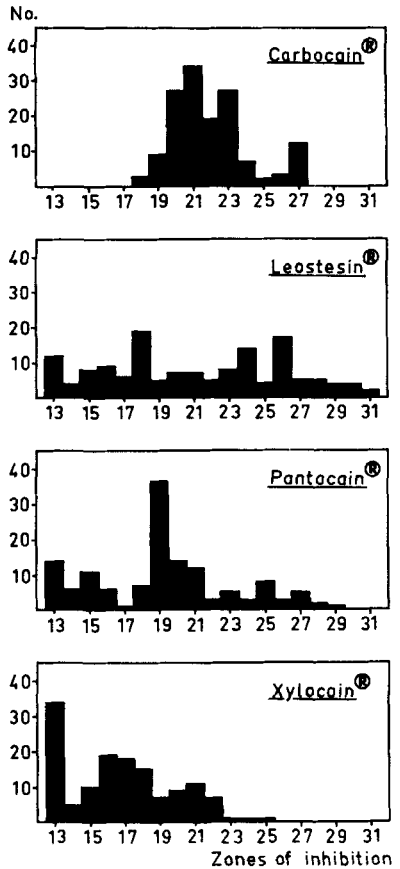

Fig. 5. Frequency distributions of the inhibition zones produced by each anesthetic for all microorganisms used in the laboratory investigation.

tained by *Gräf* (1965) (97 per cent reduction). Likewise Xylocain®, Carbocain®, and Leostesin®, which had not previously been subjected to similar tests, showed a reduction in bacterial inoculation comparable to the results of Pantocain® (97, 81, and 72 per cent respectively). The difference in the reduction effect was not statistically significant. The results in this study were not comparable with those of *Knothe* and *Hoppe* (1965) on account of the differences in experimental conditions.

In planning the experiment the highest degree of uniformity was attempted. The experimental subjects all had excellent oral hygiene, and the

control areas contained low and comparatively even numbers of microorganisms. *Gräf* (1965), who examined an outpatient material, found much higher and more uneven bacterial counts before applying the anesthetic spray. The test areas were all protected from the flow of saliva by use of gauze swabs to avoid contamination from adjacent mucosal areas. The rotation of the anesthetic sprays as outlined in Fig. 1 largely eliminated the chance of errors introduced by using four different test areas with possible different quantities of microorganisms. This was confirmed by statistical analysis of the colony counts according to location, showing no significant difference between the test areas. The massive growth on the 14 agar plates discarded in the clinical investigation was probably caused by the needle penetrating a microcolony of bacteria on the oral mucosa. As expected this was especially conspicuous in the control group, where 6 of the 14 discarded plates were found. Only aerobic cultures were done due to the findings of *Blake* and *Forman* (1967), who concluded that, although a difference between aerobic and anaerobic culturing could be shown, the differences did not appear to be sufficient to justify the use of anaerobic cultures in comparative studies of this kind.

In this study the control area was dried with sterile gauze prior to the needle puncture. It has been proved earlier that this procedure alone reduces the bacterial inoculation considerably (*Streitfeld & Zinner*, 1958; *Kantorowicz*, 1959; *Geary & Gavin*, 1963; *Birn & Winther*, 1967). It is interesting to note that the mean value of the bacterial counts obtained from the control area in the present study (34 colonies) is higher than the corresponding figure in the experiment by *Birn & Winther* (1967) (9 colonies). In the earlier study the syringes contained a standard solution of local anesthetic (Carbocain-Noradrenalin, 2 per cent), while in the present study an isotonic saline solution was used. The minute addition of a bacteriostatic (methyl-para-oxybenzoate) to the local anesthetic may well explain the lower number of colonies, since the two drops of anesthetic solution spread on the agar plate may act as an inhibitor of microbial growth.

The results of *Kantorowicz* (1959) support this concept. In his study the effect of drying the oral mucosa was tested in two ways. Partly by taking swabs for agar plate culture before and after drying and partly by injections with a local anesthetic (2 per cent Xylotox®) and subsequent discharge of two drops of the solution into glucose broth. The bacterial reduction in the first instance was less than 50 per cent, while in the last instance, where an effect of the bacteriostatic ingredients in the anesthetic solution could be expected, the reduction was 100 per cent. More studies are needed to clarify this problem further, but it seems justified to recommend that all

solutions used in experiments along this line should be completely bacteriologically inert.

In the experiment by *Gräf* (1965) drying of the mucosa was not attempted. The mean value of the colony counts from the control area (upper first molar) was therefore comparatively high (752) presumably also due to the poor oral hygiene of the outpatients. After spraying with Pantocain® the mean number of microorganisms was reduced to 23. In the present study the corresponding figure was 1.6. This difference in the final results is probably due to the fact that the mucosal layer of saliva, food debris, etc. constitutes a barrier to the antiseptic and thereby diminishes its effect. The large number of microorganisms to begin with is in itself no obstacle to efficient mucosal disinfection (*Birn & Winther*, 1967). These authors reported numerically identical final results irrespective of the number of bacteria on the unaffected mucosa in the start of the experiment. In drying the oral mucosa before application of antiseptics two objects are therefore achieved: a considerable amount of the microbial flora is removed instantly, and the effect of the antiseptic is enhanced.

The result of the clinical experiment was supported by the laboratory investigation, showing a definite antimicrobial effect of the topical anesthetics on selected strains of the oral flora. A difference in the effect of the individual anesthetics on different strains of microorganisms was also demonstrated. An exact evaluation of the actual effect in vitro of the anesthetic sprays cannot be accomplished, as only 7 strains of microorganisms out of an oral flora containing maybe 20 or 30 different species were tested. Further studies on oral microorganisms along this line are needed.

The anesthetic sprays presently tested have about the same antiseptic effect as the ointment of lidocaine and chlorhexidine tested by *Birn* and *Winther* (1967). 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, whereas the spray dose affect larger areas than needed. Ointments of different local anesthetics may through their content of bacteriostatic components have antiseptic properties too. Further studies on this problem are in progress.

It is concluded that the antiseptics added to anesthetic sprays in order to prevent microbial growth in the liquid are also capable of disinfecting the oral mucosa. Before application of the spray dose the mucosa must be dried with sterile gauze or cotton rolls, thereby removing instantly a considerable amount of the microbial flora and enhancing the effect of the antiseptic as well as of the anesthetic. Thus, in one application, surface anesthesia can now be combined with disinfection of the oral mucosa.

SUMMARY

The antiseptic effect of four anesthetic sprays, Carbocain®, Leostesin®, Pantocain® and Xylocain® was tested on the oral mucosa of 70 dental students. The experiment was carried out under conditions closely resembling those prevailing under routine injection procedures in the oral cativity. Four test areas and one control area in the buccal fold were used, thereby testing each topical anesthetic 70 times. The number of microorganisms found in the needle after insertion through treated and untreated mucosa was used for comparison. The results showed a significant antiseptic effect of all four anesthetics, and were well in accordance with earlier reports. In vitro tests also demonstrated a definite antimicrobial effect of the anesthetics on selected strains of oral microorganisms. It was concluded that the use of anesthetic spray gives an additional advantage of disinfection of the oral mucosa.

RÉSUMÉ

ACTION ANTIMICROBIENNE D'ANESTHÉSIQUES DE CONTACT EN SPRAY

L'action antiseptique de quatre anesthésiques de contact en spray, Carbocaïne®, Léostésine®, Pantocaïne® et Xylocaïne® a fait l'objet de tests sur la muqueuse buccale de 70 étudiants en art dentaire. Les expériences ont été effectuées dans des conditions se rapprochant fortement des conditions existant lors des injections pratiquées habituellement dans la cavité buccale. On a utilisé quatre zones de test et une zone témoin dans le sillon gingivo-jugal, testant ainsi chaque anesthésique de contact 70 fois. On a fait la comparaison du nombre de micro-organismes trouvés dans l'aiguille après introduction à travers la muqueuse traitée et à travers la muqueuse non traitée. Les résultats obtenus ont mis en évidence pour les quatre anesthésiques une action antiseptique significative; ces résultats concordaient bien avec les comptes rendus faits précédemment. Des essais in vitro ont aussi mis en évidence que les anesthésiques avaient sur des souches sélectionnées de micro-organismes buccaux une action antimicrobienne certaine. Les auteurs concluent que l'usage d'un anesthésique de contact en spray donne en supplément l'avantage d'une désinfection de la muqueuse buccale.

ZUSAMMENFASSUNG

ANTIMIKROBIALE WIRKUNG VON AEROSOLANÄSTHETIKA

Die antiseptische Wirkung von vier Aerosolanästhetika, Carbocain®, Leostesin®, Pantocain® und Xylocain®, wurde bei 70 zahnärztlichen Studen-

ten auf der Mundhöhlenschleimhaut ausprobiert. Das Experiment wurde unter Bedingungen durchgeführt, die den bei dem üblichen Injektionsverfahren in der Mundhöhle im allgemeinen vorkommenden ziemlich gleichkamen. Vier Testfelder und ein Kontrollfeld in der Umschlagfalte wurden benutzt, so dass jedes Anästhetikum 70-fach durchprobiert wurde. Die Anzahl der nach der Einführung der Nadel durch behandelte, bzw. unbehandelte Mucosa in derselben befindlichen Mikroorganismen wurde zum Vergleich benutzt. Als Resultat ergab sich eine ausgesprochen antiseptische Wirkung aller vier Anästhetika, die mit früheren Rapporten sehr gut übereinstimmte. Auch in vitro taten die Testproben eine entschieden antimikrobiale Wirkung der Anästhetika auf ausgewählte Arten der oralen Mikroorganismen dar. Es wurde konkludiert, dass der Gebrauch von Aerosolanästhetika den zusätzlichen Vorteil des Desinfizierens der Mundhöhlenschleimhaut innehat.

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