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In vitro studies on cinchocaine-preservative combinations

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Summary

The bactericidal activity of 6 different preservatives in the presence of cinchocaine was investigated.

The antibacterial activities of cinchocaine combinations with the tested preservatives have been found to vary markedly between synergism and antagonism.

Introduction

Cinchocaine, a commonly used local anaesthetic, is employed in a wide variety of pharmaceutical preparations such as ointments, creams, suppositories and injections (Martindale, 1977).

Cinchocaine itself has been shown to possess some antibacterial activity per se (Conte and Laforet, 1962; Kleinfeld and Ellis, 1967; El-Nakeeb and Farouk, 1973).

Butacaine, another local anaesthetic, was reported to potentiate the antimicrobial activity of some preservatives and to antagonize others (El-Nakeeb and Farouk, 1976).

Since the various types of cinchocaine-containing pharmaceutical preparations are generally preserved with different types of preservatives (Martindale, 1977), it was felt necessary to investigate how far the combination of cinchocaine with such preservatives could affect the effectiveness of these agents.

Materials and Methods

Aqueous stock solutions of cinchocaine hydrochloride (Ciba Laboratories), benzalkonium chloride (Koch-Light Laboratories), benzyl alcohol, chlorocresol, methyl -*p*-hydroxybenzoate (methylparaben), phenylmercuric nitrate (British Drug Houses), and chlorhexidine digluconate (Imperial Chemical Industries) were pre-

TABLE 1

BACTERICIDAL ACTIVITY OF CINCHOCAINE, PRESERVATIVES AND THEIR COMBINATIONS

System (concentration; mg/100 ml)	*Staphylococcus aureus*		*Escherichia coli*	
	Viable cells per ml [a]	% Killing	Viable cells per ml [a]	% Killing
Control	6.75×10^{10}	00	3.45×10^{10}	00
Cinchocaine (2)	5.27×10^{10}	22	2.38×10^{10}	31
Benzalkonium (0.01)	5.74×10^{10}	15	3.11×10^{10}	10
Combination	7.43×10^{9}	89	2.76×10^{9}	92
Chlorhexidine (0.01)	5.20×10^{10}	23	2.90×10^{10}	16
Combination	2.71×10^{9}	96	1.73×10^{9}	95
Chlorocresol (0.1)	5.87×10^{10}	13	2.83×10^{10}	18
Combination	4.05×10^{10}	40	1.55×10^{10}	55
Benzyl alcohol (5)	5.62×10^{10}	17	2.73×10^{10}	21
Combination	4.93×10^{10}	27	2.04×10^{10}	41
Methylparaben (0.2)	5.40×10^{10}	20	2.90×10^{10}	16
Combination	4.39×10^{10}	35	2.31×10^{10}	33
Phenylmercuric nitrate (0.001)	5.34×10^{10}	21	2.76×10^{10}	20
Combination	4.52×10^{10}	33	2.42×10^{10}	30

[a] Average of 10–15 determinations.

pared separately and sterilized by autoclaving.

Suitable dilutions of the stock solutions were made in normal saline so that 1 ml of each of the final solutions contained the required amount of the investigated agents.

The reaction systems of the individual agents were prepared by mixing 1 ml quantities of the proper dilution of the agent in question with 8 ml normal saline. Whereas the systems of the combinations were prepared by mixing 1 ml aliquots of cinchocaine with 1 ml quantities of either of the different preservatives together with 7 ml saline.

The concentrations indicated in Table 1 represented the final concentrations expressed in mg per 100 ml of system.

Then the systems were inoculated with 1 ml aliquots of either *Staphylococcus aureus* NCTC 6571 or *Escherichia coli* NCTC 9001 suspensions, representing Gram-positive and Gram-negative bacteria, respectively. The control systems contained 1 ml of the bacterial suspension and 9 ml saline.

After inoculation, all systems were incubated for 1 h at 37°C. Each system was prepared in triplicate, and for each of the triplicates, 5 viable count determinations were performed.

The bacterial suspensions used for inoculation of the systems were obtained by growing either of the two organisms on large slants (200 ml bottles) of dextrose nutrient Agar (Oxoid) for 24 h at 37°C, and the growth was washed with 20 ml of saline. The suspension was centrifuged, supernatant discarded and the cells resuspended in 50 ml saline. The number of viable cells in the suspension thus obtained

was of the order 10^{11} cells per ml, determined by viable counting technique.

At the end of the 1-h contact time, 1 ml aliquots of each of the systems and the controls were ten-fold serially diluted in solutions of suitable neutralizing agents to inactivate any carryover of the preservative in the samples. A 1% aqueous Tween solution was used for inactivation of systems containing benzalkonium, chlorhexidine, chlorocresol or methylparaben; 0.1% sodium thioglycollate solution was used for phenylmercuric nitrate-containing systems whereas benzyl alcohol was inactivated by serial dilution in normal saline (Wedderburn, 1964; Kostenbauder, 1968).

The number of survivors in these dilutions was determined by the surface viable counting technique (Miles and Misra, 1938), using calibrated dropping pipettes. Standard drops of the different dilutions were dropped on the surface of previously dried nutrient Agar plates, and the plates were incubated for 24 h at 37°C.

All the viable count data thus obtained were statistically analyzed, using the χ^2-test, and in no case did the calculated χ^2-values exceed those tabulated at the corresponding degrees of freedom.

Results and Discussion

Table 1 shows the viable count in each of the systems studied, after one hour contact between the tested organisms and the indicated system.

It is evident that cinchocaine, in the concentration used, had some bactericidal effect on the two tested bacteria, namely *Staphylococcus aureus* and *Escherichia coli*, producing 22% and 31% killing, respectively; in agreement with previously reported findings (Conte and Laforet, 1962; El-Nakeeb and Farouk, 1973).

This killing is by no means due to pH effect as the final systems containing cinchocaine had a pH ranging between 6.3 and 7.0.

On the other hand, the preservatives, tested separately, produced a killing effect ranging between 13% and 23% against *Staphylococcus aureus* and between 10% and 21% in the case of *Escherichia coli*.

This relatively low killing effect in the case of the preservatives is due to the fact that these agents were used at concentrations ranging between 1/10 and 1/100 the usual values employed for preservation. Furthermore, the systems were challenged with relatively large inocula (10^{10} cells). But these conditions were intentionally chosen in order to clearly observe if there were any potentiation or reduction in the activity of the preservatives.

The bactericidal activity of cinchocaine combinations with the various preservatives varied between 96% and 33% against *Staphylococcus aureus*, and 95% and 30% against *Escherichia coli*.

In order to find out whether the combined activity of cinchocaine and the different preservatives was antagonistic, additive or synergistic, the activity factors and indices for the different combinations were computed and tabulated (Table 2).

The activity factor of the combination equals to the % killing exerted by the combination divided by the sum of the % killing produced by the individual agents of that system.

TABLE 2

ANTIBACTERIAL ACTIVITIES OF CINCHOCAINE–PRESERVATIVE COMBINATIONS

Combination	*Staphylococcus aureus*		*Escherichia coli*	
	Activity factor [a]	Activity index [b]	Activity factor [a]	Activity index [b]
Cinchocaine–benzalkonium	2.41	+0.382	2.24	+0.350
Cinchocaine–chlorhexidine	2.13	+0.328	2.02	+0.305
Cinchocaine–chlorocresol	1.14	+0.057	1.12	+0.049
Cinchocaine–benzyl alcohol	0.69	−0.161	0.79	−0.102
Cinchocaine–methylparaben	0.83	−0.081	0.70	−0.155
Cinchocaine–phenylmercuric nitrate	0.77	−0.114	0.59	−0.229

[a] Activity factor = $\frac{\text{\% killing produced by the combination}}{\text{Sum of the \% killing produced by the individual agents}}$

[b] Activity index = Logarithm of the activity factor. − or + indicates antagonism and synergism, respectively.

Thus an activity factor of 1.0 is indicative of an additive effect, more than 1.0 indicates a synergistic effect and less than 1.0 indicates an antagonistic effect.

For a more justifiable comparison, the activity indices were obtained. The activity index, being the logarithm of the activity factor, would be positive or negative if the combination is synergistic or antagonistic, respectively, whereas activity index of zero value indicates an additive effect.

From Table 2, it is evident that cinchocaine combination with either benzalkonium, chlorhexidine or chlorocresol was synergistic, against the two tested microorganisms. However, the potentiation of activity observed was slightly higher in the case of *Staphylococcus aureus*-containing systems than in the ones inoculated with *Escherichia coli*.

On the other hand, cinchocaine produced an antagonistic effect when combined with benzyl alcohol, methylparaben or phenylmercuric nitrate, when either of the two tested bacteria was used.

The results obtained were in agreement with the reported findings in the case of butacaine combinations with these agents (El-Nakeeb and Farouk, 1975, 1976).

It is obvious from the results obtained that the greatest synergistic effect was observed in the cinchocaine–benzalkonium combination,and the least in the cinchocaine–chlorocresol system, against the two tested organisms.

However, the antagonism observed was greatest in the case of the cinchocaine–benzyl alcohol combination and least when cinchocaine was combined with methylparaben, when the systems were inoculated with *Staphylococcus aureus*. But with *Escherichia coli*, the highest antagonism was observed in the cinchocaine–phenylmercuric nitrate system and least in the cinchocaine–benzyl alcohol combination.

From the results obtained it may be concluded that for efficient preservation of cinchocaine preparations, either benzalkonium, chlorhexidine or chlorocresol could be safely used. This conclusion is based on the fact that cinchocaine has significantly

enhanced the bactericidal activity of benzalkonium and chlorhexidine, and to a less extent that of chlorocresol. However, the killing values were not as high as they should be in a proper preservation process, particularly in the case of chlorocresol, due to the fact that these agents were employed at concentrations markedly lower than those normally used in preservation.

On the other hand, the preservation of cinchocaine preparations with benzyl alcohol, methylparaben or phenylmercuric nitrate may minimize the efficiency of these agents, reduce the usefulness of the preparations and might endanger the health of the patient using such preparations.

References

Conte, B.A. and Laforet, E.G., The role of topical anaesthetic agents in modifying bacteriologic data obtained by bronchoscopy. New Engl. J. Med., 267 (1962) 957–960.

El-Nakeeb, M.A. and Farouk, A., Antibacterial activity of some local anaesthetics. Ind. J. Pharm., 35 (1973) 158–159.

El-Nakeeb, M.A. and Farouk, A., Antibacterial activities of certain local anaesthetic-preservative combinations. Ind. J. Pharm., 37 (1975) 112–115.

El-Nakeeb, M.A. and Farouk, A., Factors influencing the antibacterial activities of some butacaine–preservative mixtures. Can. J. Pharm. Sci., 11 (1976) 58–62.

Kleinfeld, J.and Ellis, P.P., Inhibition of microorganisms by topical anaesthetics. Appl. Microbiol., 15 (1967) 1296–1298.

Kostenbauder, H.B., In C.A. Lawrence and S.S. Block (Ed.), Disinfection, Sterilization and Preservation, Lea and Febiger, Philadelphia, 1968, p. 44.

Martindale, In A. Wade (Ed.), The Extra Pharmacopoeia. Pharmaceutical Press, London, 1977, p. 857.

Miles, A.A. and Misra, S.S., Estimation of bactericidal power of blood. J. Hyg., 38 (1938) 732–749.

Wedderburn, D.L., In Bean, H.S., Beckett, A.H. and Carless, J.E. (Ed.), Advances in Pharmaceutical Sciences, Vol. 1, Academic Press, London, 1964, p. 196.