Excipient Interaction with Cetylpyridinium Chloride Activity in Tablet Based Lozenges

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Purpose. The purpose of the investigation was to determine the effect of tablet excipients on the activity of cetylpyridinium chloride (CPC) and the relative interaction between excipients and CPC.

Methods. An analytical assay was developed to evaluate the interaction between CPC and the excipients. *In vivo* activity was investigated using six volunteers by determining the reduction in colony forming units recoverable from the oropharynx after sucking each proprietary lozenge separately on different days. *In vitro* determinations investigated the relative antimicrobial activity of aqueous solutions of the lozenges and, the effect of pH and tablet base excipients on that activity against *Staphylococcus aureus, Streptococcus pyogenes* and *Candida albicans.*

Results. Both *in vivo* and *in vitro* results showed that the tablet based lozenges had markedly reduced antimicrobial activities compared with previous results with a candy based lozenge (*in vivo* and *in vitro*) or the same concentration of aqueous CPC (*in vitro*). Magnesium stearate suspensions in CPC 250 μg/ml indicated that magnesium stearate adsorbed CPC and at 0.4% lozenge weight and above significantly reduced the antimicrobial activity of CPC 250 μg/ml.

Conclusions. The reduced activity of CPC in tablet based lozenges resulted from a decreased availability of CPC in solution due to an adsorption of CPC on magnesium stearate. To avoid this reduction in activity tablet based lozenges containing CPC 250 μg/ml, or similar concentrations, plus magnesium stearate should contain not more than 0.3% w/w lozenge weight of the lubricant.

KEY WORDS: cetylpyridinium chloride; antimicrobial activity; tablet-based lozenges; magnesium stearate; *in vivo; in vitro.*

INTRODUCTION

Commercial lozenges consist mainly of candy-based or tablet-based lozenges although there are a few gelatin based products also. *In vivo* and *in vitro* investigations have demonstrated that lozenges containing the antimicrobial cetylpyridinium chloride (CPC) are effective in reducing the bacterial flora of the orophaynx (1–5) and have indicated that the activity of a candy based lozenge was greater than the activity of an equivalent tablet based lozenge. Different candy based formulations can also influence the antimicrobial activity of CPC (6). The purpose of this present investigation was to determine the influence of common tablet excipients on the antimicrobial activity of CPC.

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ABBREVIATIONS: CFU: colony unit; CPC: cetylpyridinium chloride; WSD: Tukey's Wholly Significant Difference.

MATERIALS AND METHODS

Staphylococcus aureus NCTC 10788, *Streptococcus pyogenes* NCTC 10867 and *Candida albicans* NCTC 3179 were obtained from the National Collection of Type Cultures, Colindale, London. Tween 80, lecithin and magnesium stearate from BDH, Poole, England; sorbitol from Thornton and Ross, Huddersfield; cetylpyridinium chloride (CPC) and xylitol from Sigma, Poole, England; Nutrient broth, Sabouraud broth, Todd-Hewitt broth, blood agar base and fabricated horse blood were obtained from Oxoid, Basingstoke, England. Two proprietary lozenges 'A' and 'B' contained CPC 2.5 mg and 1.5 mg per tablet based lozenge respectively.

Measurement of pH

The pH of each antimicrobial solution was determined at 20–22°C using a Mettler Delta 320 pH meter.

Assay for the Determination of CPC in Solution and Adsorbed in Suspension

The lozenge suspensions were prepared by dissolving 5 of each of the two brands of CPC lozenges separately in 20 ml water. In addition suspensions of CPC plus either magnesium stearate or talc were prepared in 20 ml quantities of water. The suspensions were each filtered using a 0.2 μm Acrodisc (GelmanSciences, Michigan, USA). After filtration the Acrodiscs were washed with two separate quantities of 2 ml water. The filtrate and washings were collected for each sample. The particulate matter on each Acrodisc was washed with a 2 ml volume of absolute ethanol to elute the adsorbed CPC. The alcoholic filtrate was then evaporated to dryness in a stream of air at 60°C and the dry residue reconstituted in 10 ml of water, using a vortex mixer, immediately before assaying for CPC. Twenty ml of CPC 250 μg/ml in water was used as the control.

The filtrates, washings and the alcoholic extract were all separately assayed using the USP assay for CPC Lozenges (7). In addition CPC lozenge suspensions (without filtering) and suspensions of either magnesium stearate or talc plus equivalent concentrations of CPC (without filtering) were also assayed for CPC. The sum of the CPC determined in the filtrate plus CPC determined in the washing solution was the quantity of CPC in solution in equilibrium with the CPC in suspension. The CPC in the alcoholic extract was the concentration of CPC previously adsorbed to the solid excipients in suspension.

Characteristics of the Assay

Sets of six standards covering a range of CPC concentrations from 50 to 300 μg/ml were prepared either in water, in water plus magnesium stearate 0.9 mg/ml or in water plus talc 1.8 mg/ml. These solutions and suspensions were subjected to identical filtration and titration procedures. The precision of the CPC assay was determined for five replicate filtrations. The recovery achievable with the sample filtration method described above was established by comparing the total quantity of CPC obtained from the filtrate, washings and alcoholic extraction using spiked samples of 250 μg/ml CPC, with the concentration

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of CPC determined for CPC solutions having a concentration corresponding to 100% recovery.

Determination of the Concentration of Magnesium Stearate in the Lozenge base

The B.P. assay for magnesium stearate was used to determine the concentration of magnesium stearate in the lozenges (8).

In Vivo Evaluations: Antimicrobial Activity in Oral Cavity

Two proprietary lozenges were tested on two separate days using the same six volunteers (Figure 1). A lozenge was sucked and the lozenge saliva solution collected as described previously (6). The pH value for each lozenge saliva solution was measured. The effect of sucking a lozenge on the number of microorganisms recoverable from the oral cavity before and after sucking the lozenge was determined as described previously (3,4,6).

In Vitro Evaluation: Inoculum Preparation

Inocula were prepared as described previously (6). *S.aureus* and *Str.pyogenes* were cultured at 37°C for 18 h and *C.albicans* at 25°C for 48 h using Nutrient broth, Todd Hewitt broth and Sabouraud broth respectively.

Determination of Antimicrobial Activity

The microtitre colony forming unit (CFU) counting method was validated for each test organism as described by Richards & Xing (5). A volume of 0.15 ml sterile distilled water (control) and of each of the CPC solutions was separately mixed with 0.15 ml cell suspension to give final concentrations of approximately 8×10^9, 1.76×10^8 and 2.0×10^8 CFU per ml for *S.aureus, Str.pyogenes*, and *C.albicans* respectively. All the evaluations were performed in triplicate as described previously (6). The antimicrobial activities of 10 ml aqueous solutions of the two lozenge brands 'A' and 'B' were also evaluated as previously described (6).

Effect of Tablet Base Excipients on the Antimicrobial Activity of CPC

The reduction in CFU for inocula of *S.aureus Str.pyogenes* and *C.albicans* was compared for a series of 10 ml aqueous solutions/suspensions containing CPC 250 μg/ml alone; CPC 250 μg/ml plus the tablet diluent sorbitol 0.873 g (97% lozenge weight); CPC 250 μg/ml plus the tablet lubricant magnesium stearate 9 mg (1% lozenge weight); CPC 250 μg/ml plus the tablet lubricant talc 18 mg (2% lozenge weight). Dilutions and platings out were carried out at contact times of 0, 2.5, 5, 7.5, 10 and 20 min.

The Effect of Magnesium Stearate on the Antibacterial Activity of CPC

The reduction in CFU for inocula of *Str.pyogenes* was compared for a series of 10 ml aqueous suspensions containing CPC 250 μg/ml plus 18, 13.5, 9, 4.5, 3.6, 2.7, 1.8 and 0.9 mg magnesium stearate (2, 1.5, 1, 0.5, 0.4, 0.3, 0.2 and 0.1% lozenge weight). Dilutions and platings out were performed after 10 min contact time.

The Effect of Talc on the Antibacterial Activity of CPC

The reduction in CFU for inocula of *Str.pyogenes* was compared for a series of 10 ml aqueous suspensions containing CPC 250 μg/ml plus 9, 18 and 36 mg (1, 2 and 4% lozenge weight) talc respectively. Dilutions and platings out were performed at a contact time of 10 min.

Physical-chemical Interaction Between CPC and Excipients

A possible physical-chemical interaction between CPC and magnesium stearate was determined by using the CPC assay described above with a series of 20 ml aqueous suspensions containing CPC 250 μg/ml plus magnesium stearate 36, 27, 18, 9, 7.2. 5.4, 3.6 and 1.8 mg (2, 1.5, 1, 0.5, 0.4, 0.3, 0.2 and 0.1% lozenge weight). A series of 20 ml aqueous suspensions containing CPC 250 μg/ml plus talc 18, 36 and 72 mg (1, 2 and 4% lozenge weight) were also assayed. Ten ml CPC 250 μg/ml solution without either magnesium stearate or talc was used as control. Twenty ml unfiltered suspensions containing CPC 250 μg/ml plus either magnesium stearate or talc in the same series of concentrations as before were also assayed.

The Effect of Tablet Lubricants and Diluents on the Antibacterial Activity of CPC

The reduction in CFU for inocula of *Str.pyogenes* was compared for 10 ml aqueous solutions/suspensions containing CPC 250 μg/ml alone and plus sorbitol solution together with magnesium stearate suspensions and plus xylitol solution together with magnesium stearate suspensions. CPC 250 μg/ml plus either sorbitol or xylitol solutions with suspensions of talc were also evaluated. The concentrations of lubricants and diluents used are listed in Table 1. Colony counts were performed at a contact time of 10 min.

Statistics

Tukey's Wholly Significant Difference (WSD) test described previously (6) was used to test the difference in activity between pairs of antimicrobial solutions within a particular test group for significance at the 5 percent significance level (9). The WSD was calculated as shown below (10).

$$WSD = q(k,v)\sqrt{\frac{ErrorMS}{n}}$$

WSD represents the 'Wholly Significant Differences' between means at the 5 percent level of significance; *ErrorMS* indicates the pooled error mean square; *n* indicates the number of readings in each mean; *k* represents the number of means under test; *v* represents the number of degrees of freedom associated with the ErrorMS; *q(k,v)* is the quantile of a studentized range distribution. Values of *q(k,v)* at the 5 percent level are given in Table 11 of "Statistical Tables" (9). A significant difference at the 5 percent level existed when the difference between the means in a particular group tested was greater than the calculated WSD value.

Table 1. The Concentration of Suspensions of CPC 250 μg/ml Containing Either Magnesium Stearate or Talc in Suspension and Sorbitol or Xylitol in Solution

Magnesium stearate		Talc	
Mg stearate + sorbitol[b] mg/10 ml (%[a])	Mg stearate + xylitol[b] mg/10 ml (%[a])	Talc + sorbitol mg/10 ml (%[a])	Talc + xylitol mg/10 ml (%[a])
0.9 (0.1) + 896.6	0.9 (0.1) + 896.6	9.0 (1.0) + 888.5	9.0 (1.0) + 888.5
1.8 (0.2) + 895.7	1.8 (0.2) + 895.7	18.0 (2.0) + 879.5	18.0 (2.0) + 879.5
2.7 (0.3) + 894.8	2.7 (0.3) + 894.8	36.0 (4.0) + 861.5	36.0 (4.0) + 861.5
3.6 (0.4) + 893.8	3.6 (0.4) + 893.8		
4.5 (0.5) + 893.0	4.5 (0.5) + 893.0		
9.0 (1.0) + 888.5	9.0 (1.0) + 888.5		
13.5 (1.5) + 884.0	13.5 (1.5) + 884.0		
18.0 (2.0) + 879.5	18.0 (2.0) + 879.5		

[a] % Lozenge weight; CPC: cetylpyridinium chloride; Mg stearate: magnesium stearate.
[b] The concentrations of either sorbitol or xylitol were chosen so that CPC 2.5 mg + Mg stearate or talc + sorbitol or xylitol totalled 900 mg (the weight of one lozenge).

RESULTS

Assay Characteristics

Table 2 summarises the quantitative aspects of the assay methodology for CPC either alone or after interaction with fixed concentrations of magnesium stearate or talc.

Lozenge Characteristics and In Vivo Activity

The pH of the lozenge saliva solutions and the pH of 10 ml aqueous solutions of lozenges are given in Table 3. It is seen that saliva has a slight buffering capacity modifying the pH compared with the distilled water solutions by 1.29 units for lozenge 'A' and of 0.56 units for lozenge 'B'. Both lozenges were shown by assay to consist of approximately 2.0% w/w magnesium stearate (Table 3). The activity of the lozenges against the normal aerobic bacterial flora of the oropharynx of each of six subjects is given in Figure 1. Both lozenges slightly reduced the bacterial count by approximately 1 log cycle. A sample result from previous work (6) with a candy based lozenge 'C' containing CPC 1.4 mg was included for comparison.

In Vitro Activity

Comparison of the Activity of Aqueous Lozenge Solutions

Figure 2 gives the activities of the lozenges dissolved in 10 ml sterile distilled water and the activity of equivalent aqueous concentrations of CPC. After 10 min contact the lozenges reduced the average bacterial count of *S.aureus* by 2.5 log cycles and of *Str.pyogenes* by approximately 1 log cycle but the lozenges did not reduce the count of *C.albicans*. CPC 250 μg/ml water was much more active and reduced the bacterial counts by 5–7 log cycles and the yeast count by 2 log cycles. Aqueous CPC 150 μg/ml reduced the bacterial counts and yeast counts by 3–6 log cycles and by 2 log cycles respectively. The assay for CPC showed that of the stated 2.5 mg CPC per lozenge 'A' when mixed with 10 ml water only 0.32 mg was in 10 ml solution and for lozenge 'B' containing 1.5 mg CPC per lozenge only 0.22 mg CPC was in solution. A sample result from previous work (6) with a candy based lozenge 'C' containing CPC 1.4 mg was included for comparison.

Table 2. Analytical Characteristics of the Assay Method for Either CPC 250 μg/ml Alone or in Contact with Magnesium Stearate or Talc

Analyte	Concentration μg/ml		R^2 value for calibration line	CPC Recovery at 250 μg/ml		Detection limit (μg/ml)
	Actual value	Assay value		%	RSD (n = 5)	
CPC alone	250	248.0	0.997	99.2	1.34	10
Mg stearate[a] alone	0	0				
talc alone[b]	0	0				
CPC + Mg stearate	250	241.8				
CPC in solution		31.9	0.993	96.7	2.47	10
CPC in suspension		209.9				
CPC + talc	250	242.8				
CPC in solution		241.7	0.994	97.1	1.84	10
CPC in suspension		1.1				

[a] The concentration of magnesium stearate was 2 mg/ml.
[b] The concentration of talc was 2 mg/ml.

Table 3. Lozenges Characteristics

	Lozenge 'A'	Lozenge 'B'
Lozenge Weight[a]	901 ± 2.4 mg	778 ± 2.3 mg
Composition		
CPC	2.5 mg	1.5 mg
Magnesium stearate[a]	17.84 mg (1.98% w/w)	19.64 mg (2.52% w/w)
Other known compositions	Tyrothricin 0.5 mg Benzocaine 5 mg	
pH values		
lozenge saliva solutions[b]	7.10 ± 0.24	7.04 ± 0.19
lozenge in 10 ml water	8.43	7.74

[a] Mean value of five lozenges.
[b] Mean of six subjects. All lozenges dissolved within 6.4 to 10 min and produced 8.68 to 12.4 ml of lozenge solutions.

Effect of Possible Tablet Base Constituents

Figure 3 shows the effect of two tablet lubricants talc and magnesium stearate and of the tablet diluent sorbitol on antimicrobrobial activity. The presence of sorbitol 897.5 mg, (99.72% lozenge weight) had no effect on the activity of CPC 250 μg/ml, nor did talc significantly decrease CPC 250 μg/ml activity against either bacteria or *C.albicans*. It is seen that magnesium stearate markedly reduced the kill of all three test organisms.

Effect of Magnesium Stearate Concentration

The concentrations of magnesium stearate in lozenge 'A' and 'B' are given in Table 3. Figure 4a indicates that magnesium stearate 0.1% and 0.2% lozenge weight caused only a slight reduction in the bacterial kill produced by CPC 250 μg/ml at 10 min contact time. A significant difference occurred in the kill at 10 min produced by the systems containing magnesium stearate 0.3% or below of total lozenge weight and the systems containing magnesium stearate 0.4% lozenge weight and greater. Figure 4b indicates that CPC is distributed between the aqueous solution and the magnesium stearate suspension. After the suspensions containing 0.1%, 0.2% and 0.3% lozenge weight of magnesium stearate had been filtered, thus removing the magnesium stearate and the adsorbed CPC, the percentages of the total CPC 2.5 mg still in the aqueous phase were 48, 42 and 38 respectively (Figure 4b). When the concentrations of magnesium stearate were 0.4% lozenge weight or greater then more than 85% of the total CPC present had been adsorbed.

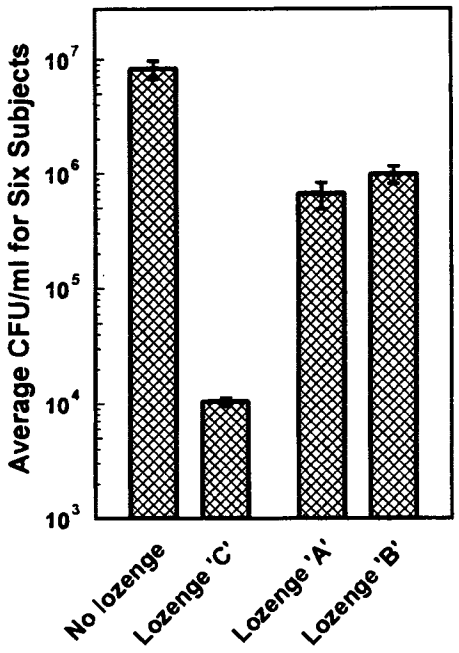

Fig. 1. Average counts with standard deviations of CFU recovered from the oropharynx of six subjects before and after sucking each of two lozenges on two separate days. Tukey's Wholly Significant Difference Analysis showed that a significant difference existed between the activity of either lozenge 'A' or lozenge 'B' and the control. A significant difference also existed between the activity of either lozenge 'A' or lozenge 'B' and candy based lozenge 'C' (Merocets data from Ref. 6).

The Effects of Talc

When suspensions of CPC and talc were tested there was no significant inhibition of CPC activity by talc at concentrations of 1%, 2% and 4% lozenge weight. That is a reduction of 4.5 log cycles of *Str.pyogenes* still occurred at 5 min contact time. On analysis no significant adsorption of CPC was detected with talc. After removing the talc and the adsorbed CPC the percentages of the total CPC 2.5 mg remaining in solution in the suspensions containing talc at concentrations 0.1%, 0.2%, and 0.3% lozenge weight respectively were 99.0, 98.8, and 98.7.

The Effects of Lubricants Plus Diluents

A significant difference in the activity against *Str.pyogenes* within 10 min of CPC 250 μg/ml in aqueous solutions of sorbitol compared with xylitol (in the concentrations given in Table 1) plus magnesium stearate occurred when the percentage of magnesium stearate equalled or exceeded 0.2% lozenge weight (Figure 5). That is magnesium stearate in the presence of xylitol is less inhibitory to the activity of CPC than it is in presence of sorbitol.

Neither sorbitol or xylitol had an inhibitory effect on the antibacterial activity of 250 μg/ml CPC solutions in the presence of talc in suspension at concentrations equivalent to 1, 2 and 4% lozenge weight. CPC in the presence of all three talc concentrations reduced the inoculum of 1.8×10^8 *Str.pyogenes* by 4.5 log cycles in 5 min similar to CPC 250 μg/ml aqueous solution containing no talc.

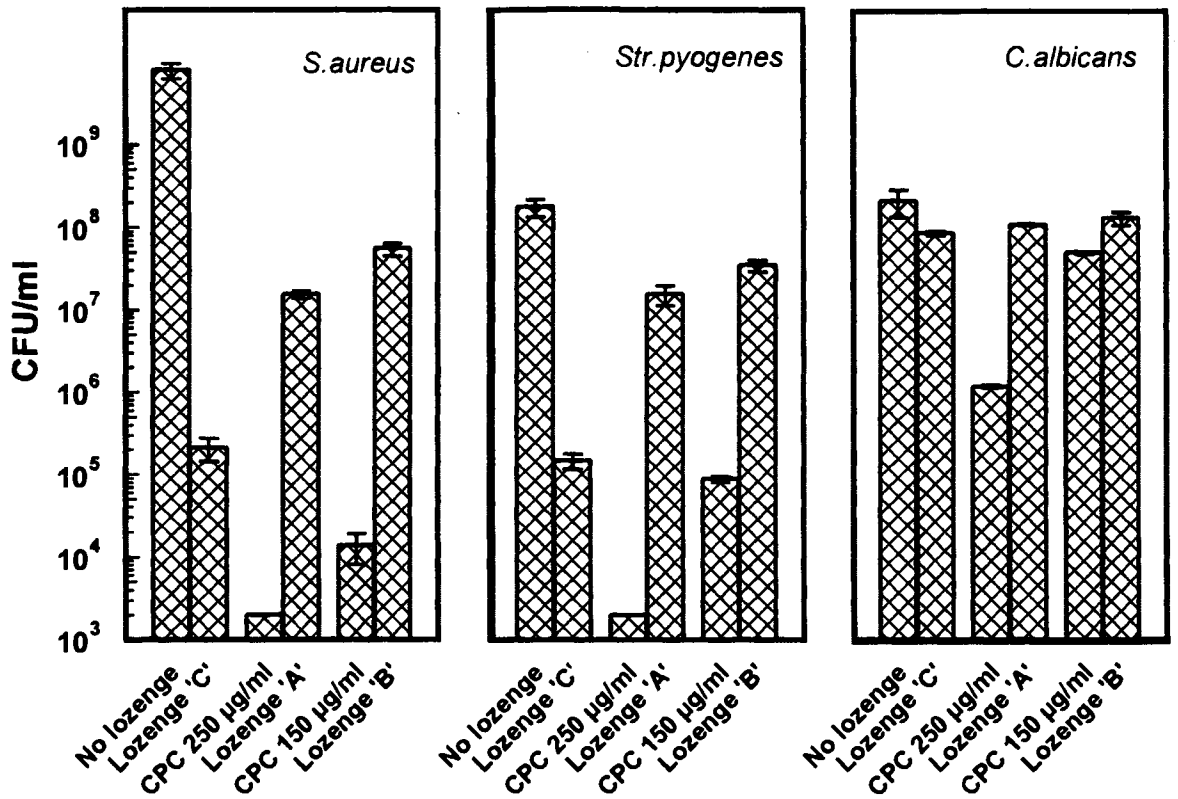

Fig. 2. Kill produced by 10 ml lozenge solutions in distilled water after 10 min contact at 20–22°C with bacterial cell suspensions. Tukey's Wholly Significant Difference Analysis showed that a significant difference existed between either lozenge 'A' or 'B' and the corresponding equivalent concentrations of CPC aqueous solutions against bacteria. A significant difference existed between CPC 250 µg/ml and lozenge 'A' against *C.albicans*. A significant difference also existed between the activity of either lozenge 'A' or lozenge 'B' and candy based lozenge 'C' (Merocets data from Ref. 6) against two strains of bacteria.

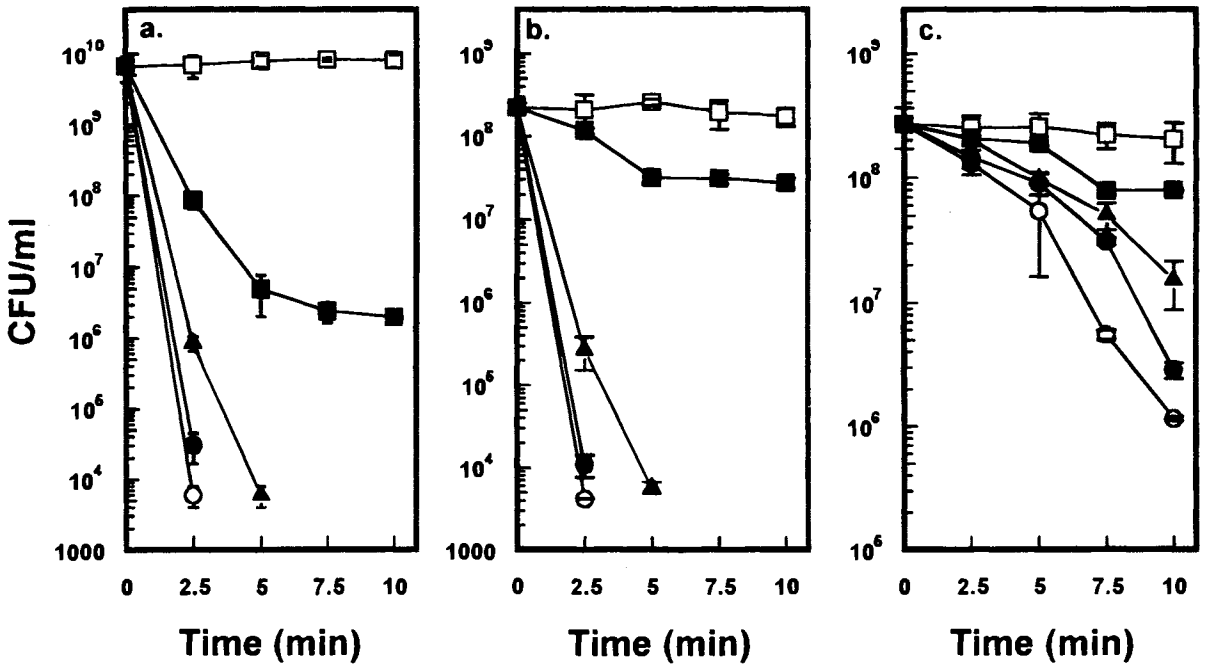

Fig. 3. Killing curve at 20–22°C of a. *S.aureus*, b. *Str.pyogenes* and c. *C.albicans* suspensions over 10 min contact with 10 ml aqueous solutions containing ○ CPC 250 µg/ml; ● CPC 250 µg/ml plus 0.94 g sorbitol (94% lozenge weight); ■ CPC 250 µg/ml plus 10 mg magnesium stearate (1% lozenge weight); ▲ CPC 250 µg/ml plus 50 mg talc (5% lozenge weight). □ Aqueous controls with no added CPC.

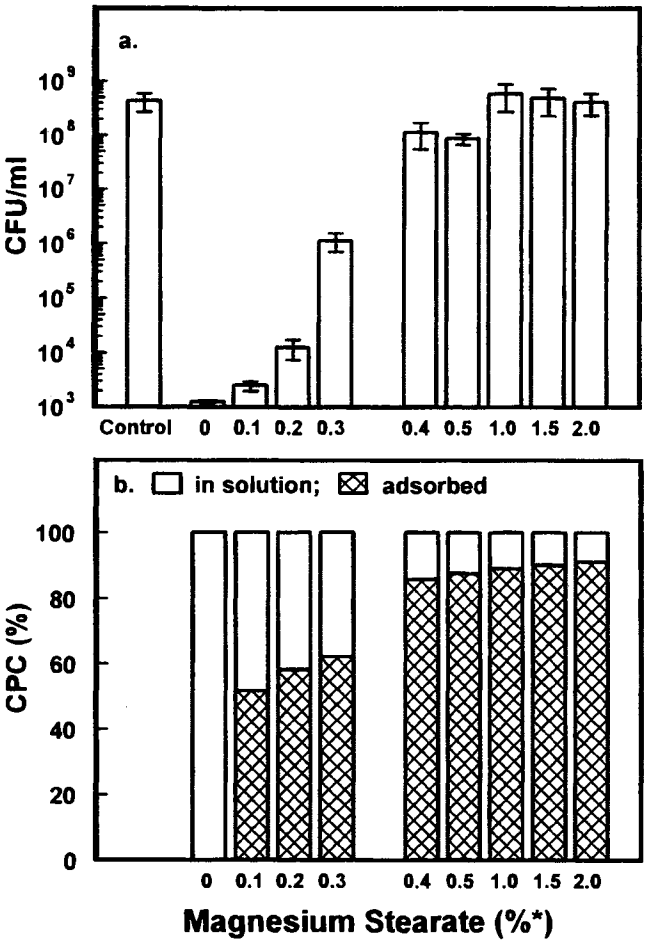

Fig. 4. a. Kill at 20–22°C of *Str.pyogenes* suspension after 10 min contact with 10 ml aqueous solutions containing CPC 250 μg/ml plus different concentrations of magnesium stearate. b. The distribution of CPC in solution and adsorbed to the magnesium. * % lozenge weight. Tukey's Wholly Significant Difference Analysis showed that a significant difference existed between: CPC alone or plus the lower magnesium stearate concentrations of 0.1, 0.2 and 0.3% lozenge weight on the one hand and CPC plus magnesium stearate 0.4, 0.5, 1, 1.5 and 2% lozenge weight on the other hand.

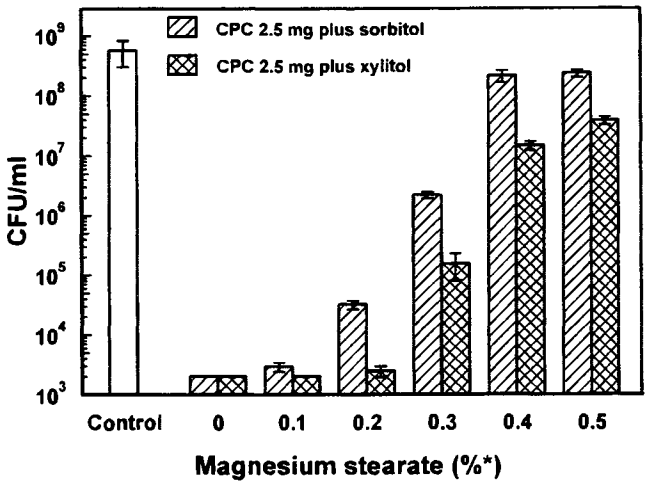

Fig. 5. Kill at 20–22°C of *Str.pyogenes* suspension after 10 min contact with CPC 250 μg/ml in 10 ml aqueous solution containing either sorbitol or xylitol (concentrations of sorbitol and xylitol were as given in Table 1) plus different concentrations of magnesium stearate. * % lozenge weight. Tukey's Wholly Significant Difference Analysis showed that a significant difference in CPC activity existed between CPC in solution with sorbitol compared with CPC in solution with xylitol when the magnesium stearate present as a suspension ≥ 0.2% lozenge weight.

DISCUSSION

The CPC assay method for aqueous solutions and for suspensions resulted in high recoveries. At a CPC concentration of 250 μg/ml more than 95% CPC was recovered from the CPC solutions and from the CPC magnesium stearate or CPC talc suspensions (Table 2).

Both lozenge 'A' and 'B' had significantly less *in vivo* and *in vitro* activity than equivalent CPC solutions (Figures 1 and 2). These lozenges were much less active than candy based CPC lozenges (6). The results presented here support previous findings that lozenge base composition had an effect on antibacterial activity and indicated that the tablet based lozenges were less active than candy based lozenges (3–5). This work shows clearly that magnesium stearate is responsible for this reduction in CPC activity.

Figure 3 indicates that magnesium stearate at a regular percentage of the tablet lozenge weight had a significant inhibitory effect on the killing activity of CPC 250 μg/ml against all three microorganisms when compared with either talc or sorbitol. Figure 4a indicates that with increasing concentrations of magnesium stearate an increased inhibition of the antibacterial activity of CPC occurs. A significantly greater bacterial kill within 10 min was produced by CPC in the presence of magnesium stearate at 0.1%, 0.2% and 0.3% lozenge weight than occurred in the presence of magnesium stearate 0.4% lozenge weight and above.

Magnesium stearate is hydrophobic and it has been reported that it may retard the dissolution of a drug from a solid dosage form (11–13). The results in Figure 4 indicate that the activity of CPC was related to the quantity of CPC in solution and not to the total concentration of CPC distributed between solution and the magnesium stearate suspensions. The reduction of activity of CPC was directly related to the concentration of CPC adsorbed on the magnesium stearate (Figure 4). It would appeared that the reduction of CPC activity is due to the CPC cations in solution interacting with the surface stearate anions on the magnesium stearate particles.

Talc did not exhibit the same inhibitory action as magnesium stearate. Less than 2% CPC was adsorbed on to the talc when CPC 250 μg/ml aqueous suspension was prepared using 9–36 mg quantities (1–4% lozenge weight) of talc.

Aqueous CPC 150 μg/ml produced no significant kill within 10 min against an inoculum of *C.albicans* 2.0×10^8 cfu/ml (Figure 3c). This is similar to previous findings (6).

Ideally, a diluent is an inert substance acting purely as a bulking agent and having no influence on the efficacy of the active ingredient. Sorbitol and xylitol are relatively chemically inert and compatible with most excipients (14,15). The suitability of sorbitol and xylitol as diluents for lozenges which incorporate CPC is indicated by the results in Figures 3 and 5.

Magnesium stearate plus xylitol is less inhibitory to CPC than magnesium stearate plus sorbitol (Figure 5).

CONCLUSIONS

The findings of this investigation show that magnesium stearate, in the concentration currently incorporated into lozenges, has an inhibitory effect on the quaternary ammonium antimicrobial CPC. The inhibition of the activity of CPC resulted from CPC being adsorbed on to the magnesium stearate. Magnesium stearate is used as a lubricant and was present in both proprietary tablet based lozenges investigated. This greatly reduced the antimicrobial activity of the lozenges. Magnesium stearate is very effective as a tablet lubricant but it must be used at less than 0.3% w/w of the lozenge weight when it used with CPC at the concentrations tested. Talc is not such an efficient lubricant but it may be used without inactivating CPC.

REFERENCES

1. R. M. E. Richards. Inhibitory activity of lozenges on oral bacteria. *Pharmacotherapy.* **8**:52, (1988).
2. R. M. E. Richards and G. Cowie. Evaluation of the number of viable bacteria recovered from the mouth after sucking lozenges. *Pharm. J.* **241** (Practice Res. Suppl.): R26–27, (1988).
3. R. M. E. Richards, G. Cowie and G. J. McCague. In vivo investigations of the antibacterial activity of lozenges and mouthwashes on the aerobic bacterial flora of the mouth and throat. *Pharm. J.* **242**:659–663, (1989).
4. R. M. E. Richards and L. M. Rochester. Influence of formulation on the effectiveness of antimicrobials in the oropharynx. 49th. FIP, *Int. Cong. Abstracts,* Abstr. 120, 1989.
5. R. M. E. Richards and D. K. L. Xing. In vitro evaluation of the antimicrobial activities of selected lozenges. *J. Pharm. Sci.* **82**:1218–1220, (1993).
6. R. M. E. Richards, J. Z. Xing and L. Weir. The effect of formulation on the antimicrobial activity of cetylpyridinium chloride in candy based lozenges. *Pharm. Res.* **13**:581–585, (1996).
7. Cetylpyridinium chloride, In *The United States Pharmacopeia XXII.* United States Pharmacopeial Convention, Inc., United States, 1990, pp.268.
8. Magnesium stearate, In *British Pharmacopoeia.* London HMSO, United Kingdom, 1993, pp. 397–398.
9. J. Murdoch and J. A. Barnes. *Statistical Tables,* Macmillan Education Ltd., London, 1991.
10. P. R. Nelson. Design and analysis of experiments. In H. M. Wadsworth, Jr. (ed.), *Handbook of Statistical Methods for Engineers and Scientists.* McGraw-Hill Publishing Company, United States, 1990, pp.14.21–14.23.
11. G. Levy and R. H. Gumtow. Effect of certain formulation factors on dissolution rate of the active ingredient III: tablet lubricants. *J. Pharm. Sci.* **52**:1139–1144, (1963).
12. H. C. Caldwell. Dissolution of lithium and magnesium from lithium carbonate capsules containing magnesium stearate. *J. Pharm. Sci.* **63**:770–773, (1972).
13. M. S. H. Hussain, P. York and P. Timmins. Effect of commercial and high purity magnesium on in-vitro dissolution of paracetamol DC tablets. *Int. J. Pharm.* **78**:203–207, (1992).
14. R. A. Nash. Sorbitol. In A. Wade and P. J. Weller (ed.) *Handbook of Pharmaceutical Excipients,* (2nd. Ed.) The Pharmaceutical Press, London, 1986, pp.477–480.
15. H. E. C. Worthington and P. M. Olinger. Xylitol. In A. Wade and P. J. Weller (ed.) *Handbook of Pharmaceutical Excipients,* (2nd. Ed.) The Pharmaceutical Press, London, 1986, pp.564–567.