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COMMUNICATION

Muco-adhesive Buccal Tablets of Clotrimazole for Oral Candidiasis

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ABSTRACT

Muco-adhesive erodible buccal tablets of clotrimazole were formulated using a combination of bio-adhesive polymers like Carbopol-974P (CP-974P) and hydroxypropylmethylcellulose-K4M (HPMC-K4M), and soluble excipients like polyethylene glycol-6000 and mannitol. Increasing the concentration of CP-974P was found to increase the in vitro adhesion time as well as the bio-adhesive strength of the formulations. A combination of 7.5% of CP-974P and 42.5% of HPMC-K4M along with the soluble excipients in the erodible matrix, was found to release the drug for up to 3 hr, in vitro without getting dislodged. The drug released was found to be microbiologically active against Candida albicans. In vivo evaluation of the buccal tablet in healthy human volunteers and its comparison with a marketed troche formulation revealed that the muco-adhesive tablet produced more uniform and effective salivary drug levels with adequate comfort, taste, and non-irritancy over a period of 6 hr.

INTRODUCTION

Recent years have seen an increasing interest in the development of novel muco-adhesive buccal dosage forms (1-6). These are useful both for the systemic delivery of drugs as well as for local targetting of drug to a particular region of the body. In an earlier study (7), an attempt was made to develop an erodible buccal tablet for local delivery of clotrimazole to the oral cavity. It was found that among the various bio-adhesive polymers, HPMC-K4M along with soluble excipients, PEG-6000 and mannitol formed the best erodible matrix, exhibiting satisfactory drug release. Since the adhesion/erosion time of the tablets was quite less, the present study was an attempt to prolong the residence time of the tablets while ensuring satisfactory drug release. CP-974P was used for the purpose. CP-974P is a newer and safer analog of CP-934P, polymerized in ethyl acetate (8). As

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studies involving CP-934P have shown it to be mildly irritating to the buccal mucosa in higher concentrations (9), CP-974P was used in concentrations not exceeding 7.5% of the total tablet weight.

MATERIALS

Clotrimazole (CLT) was a gift sample from M/s Dee Pharma Ltd. HPMC-K4M was obtained as a gift sample from M/s Unichem Labs Ltd. and CP-974P from M/s Max India Ltd. PEG-6000 (S.D. Fine chemicals) and mannitol (BDH) were obtained from commercial sources. Other solvents and materials used in the study were of reagent grade. Subculture of *Candida albicans* J1012 was obtained from Patel Chest Research Institute, University of Delhi.

METHODS

The buccal tablets were prepared using compositions as given in Table 1. The methods used for the preparation of tablets, drug release studies, analysis of samples, determination of bio-adhesive performance, and surface pH were similar to those described earlier (7).

Microbiological Evaluation of Selected Buccal Tablet

Antifungal efficacy of CLT released from the buccal tablet was determined by performing the disc agar diffusion assay on aliquots of the dissolution samples obtained during in vitro drug release studies. Sabouraud agar plates inocculated with *Candida albicans* J1012 were used for the study. 0.1 ml of the samples were carefully pipetted into uniformally spaced 7.0 mm diameter wells. These were allowed to prediffuse for 2 hr at room temperature and then incubated in the inverted position at 37°C in a B.O.D. incubator, for 18 to 24 hr. The diameter (mm) of growth inhibition surrounding each agar well was measured and the concentration of CLT was determined from the calibration curve constructed under identical conditions.

Table 1

Composition of Different Muco-adhesive Buccal Tablets

S. No.	Ingredients	Weight in Mg of X-1	X-2	X-3
1.	Clotrimazole	10	10	10
2.	HPMC-K4M	95	90	85
3.	CP-974P	05	10	15
4.	Mannitol	50	50	50
5.	PEG-6000	40	40	40

In Vivo Evaluation of Selected Buccal Tablet and Comparison of Its Activity with That of a Marketed Formulation

The selected tablets were evaluated for adhesion/erosion time and salivary concentration attained in healthy human volunteers and this was compared with that of a marketed CLT troche (Mycelex® troche, Miles Pharma, USA) containing 10 mg of CLT per troche.

Six volunteers (aged 22 to 29 years) participated in this cross-over study. Informed consent from the volunteers was taken prior to the study. In all volunteers, the muco-adhesive tablets were administered first and after an interval of two weeks, the troches were administered. The volunteers were instructed to finish their breakfast at least one hr prior to the study. Eating was restricted during the study while drinking was allowed *ad-libidum* from 60 min after administration of the muco-adhesive tablet or troche. However, no drinking was allowed 10 min before the collection of saliva.

The muco-adhesive tablets were applied by manually pressing them against the cheek for about 30 sec, without moistening before application. The volunteers were instructed to record the time of tablet application and the time and circumstances of the end of adhesion (erosion or dislodgement). They were asked to record their experiences of the tablet immediately after completion of the study.

The troches were allowed to dissolve slowly in the mouth and the time required for complete erosion was noted. A blank salivary sample was taken prior to the application of the formulations. When studying the muco-adhesive tablets, salivary samples were taken 5, 15, 30, 45, 60, 90, 120, 150, 180, 240, and 360 min after application and with troches, these were collected at 5, 15, 30, 45, 60, 90, 120, 150, 180, 210, and 240 min after administration.

Saliva samples (about 2 ml) were collected over a 2 min period (1 min before and 1 min after the given time) directly into borosilicate centrifuge tubes. The samples were centrifuged at 1500 rpm for 4 min. 1 ml of the supernatant was then extracted with 3 × 5 ml of ether and was analyzed spectrophotometrically as for in vitro samples (7).

The maximal salivary concentration (C_{max}) and the time to reach the maximal salivary CLT concentration (t_{max}) as well as the time period above the MIC value ($T^{>MIC}$) for CLT against *Candida albicans* (2.0 μg/ml) (11,12), were determined from the concentration-time curve. When the salivary concentration at the last sampling time was still above 2.0 μg/ml, the same was taken as the end point of $T^{>MIC}$. The area under the curve ($AUC_{to\text{-}tn}$) till the last sampling time (6 hr for muco-adhesive tablets and 4.0 hours for troches) was calculated using the trapezoidal rule. The $AUC_{to\text{-}tn}$ and $T^{>MIC}$ values for both the formulations were statistically evaluated using the Wilcoxon signed rank test (13).

Stability Studies

Stability studies on the final formulations were carried out to determine the effect of the presence of formulation additives on the stability of the drug and also to determine the physical stability of the formulations under accelerated storage conditions of temperature.

The muco-adhesive buccal tablets were stored in closed glass petridishes lined internally with aluminum foil. These were placed in hot air ovens maintained at 37, 45, and 60°C. Samples were withdrawn at 0, 15, 30, and 60 days and were analyzed for active drug content, bio-adhesive strength, adhesion time, hardness, friability, and weight gain/loss.

For the determination of the active drug content, a stability indicating two phase titrimetric assay method (14) was used. Bio-adhesive strength and adhesion time were determined by the methods described earlier (7). Hardness, friability, and weight gain/loss were determined in the usual manner.

RESULTS

In Vitro Drug Release Studies and Duration of Bio-adhesion

Figure 1 shows the in vitro drug release from tablets prepared using different compositions and Table 2 gives the values of the different parameters determined from the in-vitro drug release study. CP-974P, in increasing concentrations was found to increase the adhesion time, $AUC_{to\text{-}tn}$,d, and $T^{>MIC}$,d for CLT against *Candida albicans*. Formulation X-3 exhibited the maximum value for $AUC_{to\text{-}tn}$,d. It maintained the concentration of CLT in the dissolution medium above the MIC of *Candida albicans* for upto 3 hr. A maximum concentration of 17.7 μg/ml was obtained in the dissolution medium after 2 hr. Hence, formulation X-3 was selected for further study.

Bio-adhesive Strength

The bio-adhesive strength of the formulations increased with the increasing concentration of CP-974P (Figure 2) with formulation X-3 exhibiting the maximum bio-adhesive strength.

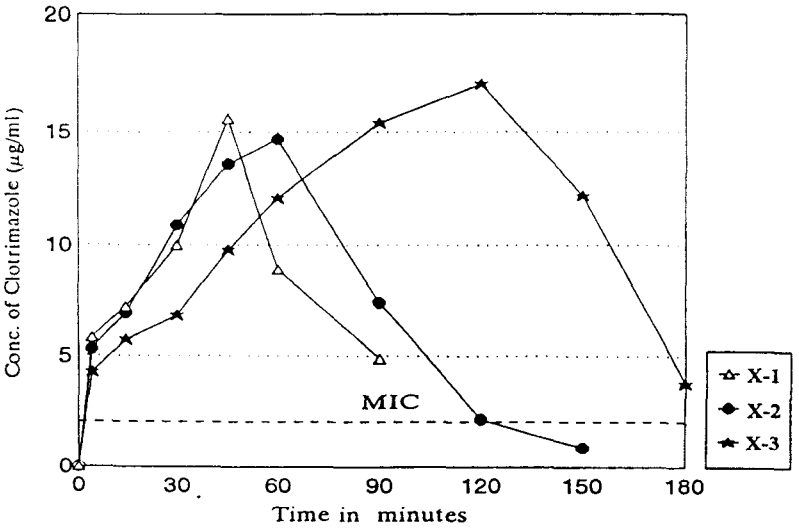

Figure 1. Drug release from different muco-adhesive buccal tablets, in vitro.

Table 2

Important Characteristics of Different Bucco-Adhesive Tablets, In Vitro

Formula Code	Bioadhesive Strength (±SD)(g)	Adhesion Time (±SD) (min)	Surface pH (±SD)	C_{max},d (μg/ml)	t_{max},d (min)	$T^{>MIC}$,d (min)	AUC_{to-tn},d ($\mu gminml^{-1}$)
X-1	20.66 (0.76)	87.3 (4.16)	6.31 (0.02)	15.6	45	89.5	791.25
X-2	36.66 (0.57)	144.0 (5.29)	5.80 (0.05)	14.7	60	123.0	1125.75
X-3	47.50 (0.50)	177.6 (7.37)	5.63 (0.02)	17.7	120	177.5	2040.75

Surface pH

The surface pH of all the formulations was within satisfactory limits (7.0 ± 1.5 units) and hence these formulations should not cause irritation in the buccal cavity.

Microbiological Evaluation

Figure 3 shows the microbiological efficacy of the aliquot samples against *Candida albicans* J1012. The drug released from the buccal tablet was able to inhibit the growth of *Candida albicans* J1012 for upto 3 hr. A maximum growth inhibition zone of 8.13 mm corresponding to a concentration of 16.21 μg/ml of CLT was obtained with the aliquot from the 2 hr dissolution sample. The results of the study corresponded well with the in vitro study using the spectrophotometric method for the analysis of samples.

In Vivo Evaluation

Figure 4 shows the mean salivary drug levels obtained with the muco-adhesive buccal tablets and Marketed troches in healthy human volunteers. Table 3 lists the important parameters determined for the formulations, in vivo. The results of the study reveal that the muco-adhesive tablet was able to maintain the concentration of CLT in the saliva for upto 6 hr as compared with the troche formulation. The maintenance of sali-

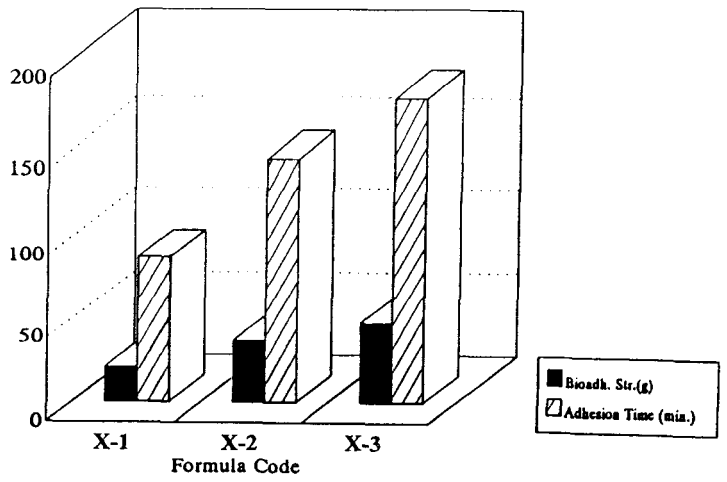

Figure 2. Bio-adhesive performance of different muco-adhesive buccal tablets, in vitro.

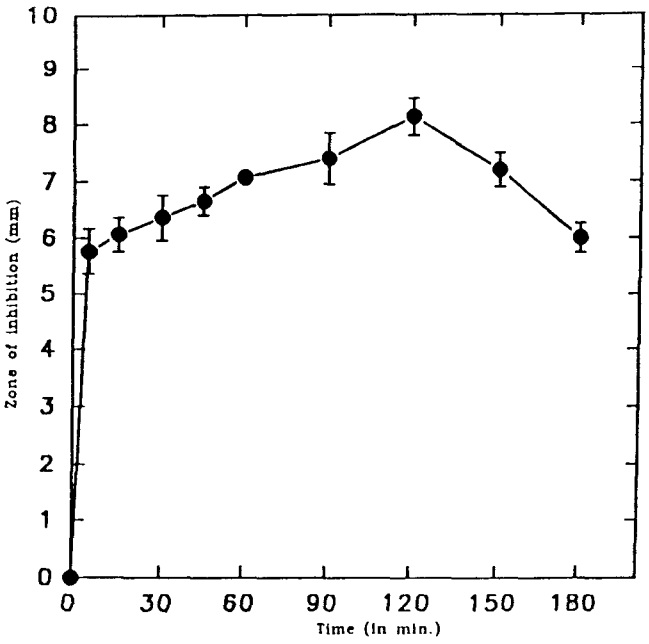

Figure 3. Antifungal activity of selected muco-adhesive buccal tablet (X-3), in vitro.

vary concentration of the drug even after complete erosion of the formulations could be attributed to reversible binding of the drug to the oral mucosa (15).

The buccal tablets did not cause any irritation or hinderance to the volunteers and their taste was acceptable. No side effects like taste alteration, dry mouth or excessive salivation were observed with the tablet.

A lot of inter-individual variation was observed for the time of erosion and salivary CLT levels obtained with both the formulations. This is probably due to the variation in the individual movement pattern of the mouth (16).

All the muco-adhesive tablets eroded completely and none had to be removed due to irritation. The mean adhesion time was 166.16 (± 34.49) min. (Range: 115–213 min). Although the salivary CLT levels with the troche formulation were higher initially, these dropped below the MIC for *Candida albicans* by the third hour. Significant salivary CLT levels were obtained with the muco-adhesive tablets and these remained uniform during the period of the study. The $AUC_{to\text{-}tn}$ and $T^{>MIC}$ values for the bio-adhesive tablet were found to be significantly higher (Wilcoxon signed rank test) than for the troche formulation.

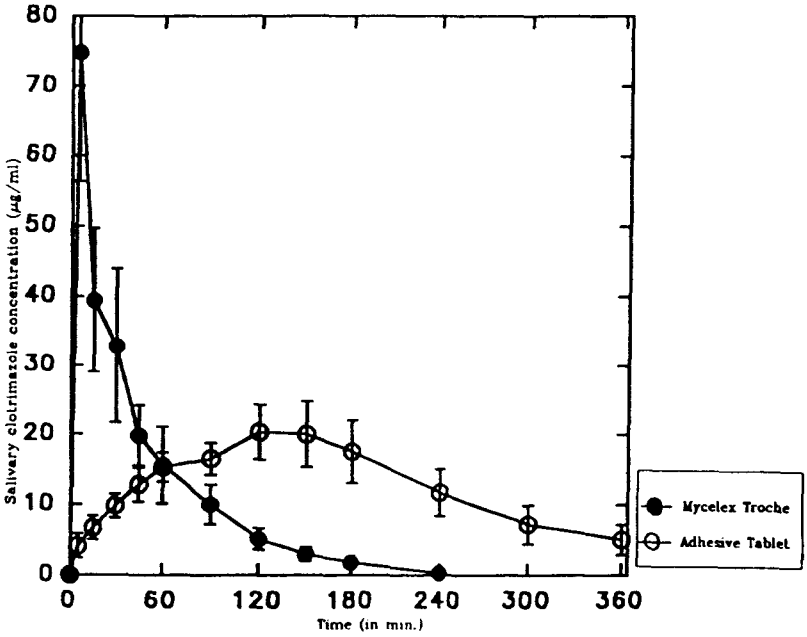

Figure 4. Drug levels in saliva obtained with muco-adhesive buccal tablets and marketed troches in healthy human volunteers.

Table 3

Certain Important Parameters of Muco-adhesive Buccal Tablets and Marketed Troches in Healthy Human Volunteers [n = 6; mean with (SD)]

Formulation	Adhesion/ Erosion time (min)	C_{max} (μg/ml)	t_{max} (min)	$T^{>MIC}$ (min)	$AUC_{to\text{-}tn}$ (μg $minml^{-1}$)
Bucco-adhesive tablets	166.2(34.5)	75.9(16.7)	125.0(22.5)	351.2(15.8)	4621.7(943.2)
Marketed troches	15.7(4.8)	21.3(4.4)	6.7(4.1)	174.5(15.9)	2721.0(498.7)

Table 4

Effect of Storage at Elevated Temperatures on the Properties of Muco-adhesive Buccal Tablets at the End of Two Months

Properties	Storage Conditions			
	Initial values	37°C	45°C	60°C
Drug content [% (± SD)]	100.73 (0.4)	100.26 (0.4)	100.03 (0.4)	98.70 (0.34)
Bioadhesive strength [g (± SD)]	47.5 (0.5)	47.16 (0.28)	47.33 (0.28)	43.50 (0.50)
Adhesion time [min (± SD)]	176.3 (5.5)	176.6 (10.0)	172.0 (8.8)	161.0 (8.18)
Hardness [Kg/cm^2 (± SD)]	5.41 (0.41)	5.33 (0.14)	5.16 (0.28)	3.58 (0.38)
Friability (%)	0.074	0.083	0.075	0.1
Weight gain/loss (% w/w)	—	-0.01	-0.17	-0.33

Each value represents a mean of three readings.

Stability Study

The stability studies of the muco-adhesive tablets revealed that no significant changes in the physical parameters occured at storage temperatures of 37 and 45°C (Table 4). However, significant changes in the physical parameters occured at 60°C. These changes could be attributed to the physical instability of PEG-6000, a low melting excipient in the buccal tablet.

No significant reduction in the content of the active drug occured over a period of two months and hence the shelf life of the formulations could be extrapolated to a minimum of two years (17). However, storage temperature not exceeding 45°C and moisture proof packing are essential to ensure stability of these formulations.

DISCUSSION AND CONCLUSION

The present study was an attempt to develop a muco-adhesive buccal drug delivery system for CLT, an antifungal drug. The main interest in such a dosage form resulted from its ability to prolong the local release of the drug in the oral cavity while maintaining the concentration of the drug above the MIC for *Candida albicans*. The advantages envisaged for such a system were:

i) Improvement in the overall therapy of Oral Candidasis.
ii) Better patient compliance due to the decrease in the frequency of administration.
iii) Reduction in dose and hence dose related side effects like nausea and vomiting.
iv) Providing an effective means of prophylaxis in high risk group patients.

The formulated tablet was found to release the drug for upto 3 hr, in vitro, without getting dislodged. The drug released was found to be microbiologically active against *Candida albicans*. In vivo evaluation of the optimized buccal tablet in healthy human volunteers and its comparison with a marketed troche formulation revealed that the muco-adhesive tablet produced more uniform and effective salivary drug levels with adequate comfort, taste and non-irritancy over a period of 6 hr. CP-974P was found to be a suitable alternative to CP-934P for the preparation of muco-adhesive formulations.

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