

Once daily bioadhesive vaginal clotrimazole tablets: Design and evaluation

GARIMA SHARMA
S. JAIN
A. K. TIWARY
GURPREET KAUR*

*Department of Pharmaceutical Sciences
and Drug Research, Punjabi University
Patiala-147002, India*

In this study, a bioadhesive dosage form of clotrimazole was designed using a combination of bioadhesive polymers Carbopol 934P, sodium carboxymethyl cellulose and sodium alginate in different ratios. The bioadhesive strength was evaluated by measuring the force required to detach the tablets from porcine vaginal mucosal membrane. The strong interaction between polymer and mucus lining of the tissue helps increase the contact time and permits localization of activity. Carbopol 934P showed maximum bioadhesion and required maximum force for detachment; the force required for detachment was directly proportional to its content. The formulations were tested for their swelling behavior using the agar gel plate method. The swelling index was a function of the concentration of the hydrophilic polymer and the formulations containing Carbopol 934P and sodium carboxymethyl cellulose were found to swell to a greater extent than those containing Carbopol and sodium alginate. *In vitro* release studies showed that the batch consisting of 2:1 ratio of Carbopol 934P/sodium alginate (batch C3) released clotrimazole over 24 h. The similarity factor showed that the dissolution profiles of fresh and aged tablets were similar, suggesting good stability of vaginal tablets prepared using a combination of Carbopol 934P and sodium alginate.

Keywords: bioadhesives, vaginal tablets, clotrimazole, Carbopol 934P, sodium alginate, sodium carboxymethyl cellulose

Accepted April 20, 2006

Incidence of *Candida albicans* vaginitis has been increasing since a significant number of women suffer from acute episodes, and recurrent infection may frequently occur after therapy (1). It has been estimated that 75% of all women will experience a vaginal candidiasis once in their lifetime, with up to 5% suffering from recurrent vaginal candidiasis (2). From such a perspective, topical treatment of candida vaginitis could be a rational choice for management of localized infection, while systemic therapy could be limited to patients proven to be nonresponsive or intolerant to intravaginally administe-

* Correspondence, e-mail: kaur_gp@rediffmail

red drugs. Although antimycotic agents such as broad spectrum imidazole derivatives are now available for topical short-term therapy (3, 4), intravaginally delivered drugs may fail to achieve high concentration at the site of infection due to their fairly prompt removal from the vaginal compartment through physiological secretions (5). Conventional delivery systems such as creams, foams, gels, tablets, irrigations or pessaries might not ensure sufficient therapeutic efficacy because of their short residence time due to the self-cleansing action of the vaginal tract (6–8). Therefore, it can be envisaged that high concentration at the site of infection could be achieved by the use of a bioadhesive polymer that can interact with the mucosa and can hold the delivery system in the vaginal tract for a few days without any toxic effects or important physiological modifications (9, 10).

Clotrimazole is a broad-spectrum antimycotic agent effective against pathogenic dermatophytes, yeasts and several species of *Candida*, *Trichophyton*, *Microsporum*, *Epidermophyton* and *Malassezia*. Oral bioavailability of clotrimazole is poor and it has a high incidence of gastrointestinal disorders and neurological reactions. However, clotrimazole is known to be very effective locally and causes no major side effects (11).

Local concentrations of econazole, miconazole, estriol or iodine in vagina are reported to be sustained using combinations of polycarbophil and semisynthetic triglycerides or polyethyleneglycol (12). Polycarbophil or carbopol have been also combined with hydroxypropyl cellulose, hydroxyethyl cellulose and HPMC (13) for modulating the bioadhesive strength and drug release from bioadhesive tablets. It is essential to partly substitute polycarbophil or carbopol by other polymers in order to decrease their inherently high bioadhesive strength that might damage the vaginal mucus membrane (14). The present investigation was aimed at formulating vaginal tablets by employing various combinations of Carbopol 934P and sodim carboxymethyl cellulose or sodium alginate and evaluating them for their bioadhesive characteristics and *in vitro* release of clotrimazole.

EXPERIMENTAL

Materials

Clotrimazole (CLT) was received as a gift sample from Ranbaxy Research Labs., India. Carbopol 934P (CP) was a gift sample from Ind. Swift Laboratories Limited, India. Sodium alginate, sodium carboxymethyl cellulose (NaCMC) and magnesium stearate were purchased from SD Fine Chemicals Ltd., India, agar powder and dioxane were purchased from Hi-Media Labs Pvt. Ltd, India and E. Merck Ltd., India, respectively. Ammonium acetate and glacial acetic acid were purchased from CDH Pvt. Ltd., India. All reagents and chemicals were of analytical grade and used as received.

Preparation of bioadhesive tablets

Each tablet contained a constant mass of CLT (100 mg) and magnesium stearate (10 mg) and a varying composition of a bioadhesive polymer mixture of either CP or NaCMC or CP and sodium alginate (Table I). All materials were passed through a 125- μ m sieve

Table I. Composition of various bioadhesive CLT vaginal tablet formulations

Batch	CLT (mg)	CP/NaCMC	CP/sodium alginate	Magnesium stearate (mg)
A	100	3:0	–	10
B1	100	0:3	–	10
B2	100	2.25:0.75	–	10
B3	100	2:1	–	10
B4	100	1:2	–	10
C1	100	–	0:3	10
C2	100	–	2.25:0.75	10
C3	100	–	2:1	10
C4	100	–	1:2	10

Total mass of CP alone, NaCMC alone, sodium alginate alone or combination of CP and NaCMC or CP and sodium alginate was 900 mg in all the formulations.

and retained on a 90- μ m sieve. CLT was first mixed with the bioadhesive polymer mixture for 10 min. Magnesium stearate was then added and mixing was continued for another 10 min. Tablets of approximate mass of 1 g were produced by direct compression of this mixture.

Evaluation of bioadhesive tablets

The formulated tablets (10 in number) of each batch were evaluated for hardness using the Monsanto hardness tester (Tab Machines, India). Friability was determined according to the procedure mentioned in *USP 28* (15). Mass variation of the formulated tablets (20 in number) was tested in accordance with the procedures given in *Indian Pharmacopoeia* (16). The swelling rate of bioadhesive tablets was evaluated by using a 1% agar gel plate at 37 °C (17).

In situ bioadhesive strength

Bioadhesive strength of the tablets was measured by a method reported by Gupta *et al.* (18). Porcine vaginal mucosa was used as a model membrane and acetate buffer, pH 6.0 as moistening fluid for measurement of bioadhesive strength. The surface of the mucosal membrane was first blotted with a filter paper and then moistened with 25 μ L of acetate buffer. The force required to detach the tablet from the mucosal surface was taken as the measure of bioadhesive strength. The thickness of the vaginal mucosal membrane was 0.01–0.05 mm and the temperature was maintained at 37 °C throughout the study. Each experiment was performed using porcine vaginal mucosa obtained from three different animals.

Vaginal mucosa was obtained within 1 h of sacrificing female pigs at the local slaughter house. The age (mean \pm SD) of female pigs was 1.5 \pm 0.5 years.

Dissolution rate

The *in vitro* dissolution studies were carried out using the USP 28 (15) type 5-paddle method. The dissolution medium contained a 65:35 ratio of 0.1 mmol L⁻¹ acetate buffer pH 6.0 and dioxane (19). The medium was maintained at 37 ± 1 °C and was stirred at 100 rpm. Samples (3 mL) withdrawn at suitable time intervals were compensated with fresh dissolution medium and assayed spectrophotometrically at 270 nm. It was made clear that none of the ingredients used in the matrix formulation interfered with the assay. Each experiment was performed in triplicate.

CLT release kinetics

The release kinetics of CLT from prepared bioadhesive tablets was evaluated by employing the Peppas and Sahlin equation (20): $M_t/M_\infty = k t^n$, where M_t is the amount of the drug released at time t , M_∞ is the amount of the drug released after infinite time, k is the kinetic constant incorporating structural and geometric characteristics of the tablet and n is the diffusional exponent indicative of the mechanism of drug release.

Stability studies

The selected formulation containing a CP/sodium alginate 2:1 ratio (batch C3) was subjected to accelerated storage conditions (40 ± 2 °C/75 ± 5% RH for 6 months). The formulation was analyzed for organoleptic characteristics, hardness and dissolution. Similarity factor f_2 was calculated to compare the dissolution profiles according to equation (21):

$$f_2 = 50 \log \left\{ \left[1 + \frac{1}{n} \sum_{t=1}^n (R_t - T_t)^2 \right] \times 100 \right\}$$

T_t and R_t are the percent drug dissolved at each time point for test and reference. Three tablets were subjected to this study.

RESULTS AND DISCUSSION

The average mass of vaginal tablets (Table II) was found to range from 975 to 995 mg exhibiting a mass of 985 ± 6 mg (mean ± SD). Tablets of all batches complied with the mass variation requirement of *Indian Pharmacopoeia* (16); tablets of no batch varied more than 5% of the average mass. Average hardness of tablets belonging to various batches (Table II) indicated good strength. Sufficient strength of all tablets was also evident since the friability was less than 1% (m/m), indicating compliance with the requirements of USP 28 (15). Vaginal tablets formulated using maximum amount of CP (batch A) required the greatest force to be detached them from the pig vaginal membrane (Table II). The force required for detaching these tablets was, respectively, 3-fold and 5-fold greater than that required for tablets containing an equivalent amount of NaCMC (batch

Table II. Characterization of various bioadhesive tablets

Batch	Tablet mass (mg) ^a	Friability (%)	Hardness (g cm ⁻²)	Drug content (%) ^b	Swelling index ^b	Bioadhesive strength (N) ^b
A	990 ± 7	0.19	5.5	98.5 ± 2.5	0.54 ± 0.06	0.30 ± 0.29
B1	980 ± 10	0.22	4.8	99.2 ± 2.0	–	0.10 ± 0.08
B2	985 ± 7	0.21	4.5	97.8 ± 3.0	0.73 ± 0.08	0.27 ± 0.02
B3	975 ± 6	0.10	6.0	99.0 ± 2.5	0.84 ± 0.09	0.22 ± 0.25
B4	985 ± 5	0.31	5.6	96.2 ± 1.5	1.53 ± 0.12	0.16 ± 0.06
C1	988 ± 6	0.20	5.2	98.4 ± 3.5	–	0.06 ± 0.28
C2	990 ± 6	0.31	4.6	98.6 ± 4.5	0.57 ± 0.01	0.22 ± 0.34
C3	995 ± 7	0.25	5.0	99.1 ± 3.0	0.74 ± 0.01	0.18 ± 0.07
C4	980 ± 7	0.31	4.8	97.5 ± 4.5	0.85 ± 0.03	0.12 ± 0.34

Each value represents mean ± SD: ^a *n* = 20, ^b *n* = 3.

– Swelling could not be measured.

B1) or sodium alginate (batch C1). This indicated a superior bioadhesive property of CP compared to NaCMC or sodium alginate. The bioadhesive property of CP is reported to be due to carboxyl groups present on its acrylic acid backbone, which possess an ability to interact with sialic acid molecules present in the mucus layer (22). Replacement of CP with increasing amounts of NaCMC in tablet formulations produced tablets that exhibited greater bioadhesive strength than those prepared with NaCMC alone. The force required to detach tablets formulated using CP/NaCMC ratios of 2.25:0.75 (batch B2), 2:1 (batch B3), or 1:2 (batch B4) was, respectively, 0.9-fold, 0.7-fold and 0.5-fold lower than that required for tablets prepared by using CP alone. Similarly, tablets prepared by using CP/sodium alginate ratios of 2.25:0.75 (batch C2), 2:1 (batch C3), or 1:2 (batch C4) required, respectively, 0.8-fold, 0.6-fold and 0.4-fold lower force *in vitro* to be detached from the porcine vaginal membrane as compared to tablets prepared with CP only. Furthermore, data summarized in Table II indicate lower bioadhesive strength of tablets prepared by using combinations of CP and sodium alginate (batches C2–C4) compared to those prepared by using similar combinations of CP and NaCMC (batches B2–B4). This seems to be due to lower bioadhesive strength of sodium alginate than that of NaCMC (23). These results indicate that the inherently high bioadhesive strength of Carbopol 934P could be modified by partly substituting its content with NaCMC or sodium alginate.

Swelling index of various vaginal formulations is summarized in Table II. Tablets containing CP alone (batch A) exhibited the lowest swelling index, suggesting the least water uptake whereas those containing NaCMC alone (batch B1) or sodium alginate alone (batch C1) gelled to such an extent that their swelling index could not be determined. This appears to be due to excessive water uptake by tablets prepared by using NaCMC or sodium alginate alone. A comparison of the tablets prepared by using 2.25:0.75, 2:1, or 1:2 ratios of CP/NaCMC (batches B2–B4) showed an increase in the swelling index. A similar trend was also found when similar ratios of CP/sodium alginate were used (batches C2–C4). However, the swelling index of tablets containing various ratios of CP/sodium alginate was lower than that of tablets containing similar ratios of CP/NaCMC.

The observed difference in the swelling behaviour of these tablets could be attributed to greater hydrophilic nature of NaCMC. The swelling index of CP is reported to be less than that of NaCMC (23). Further, the swelling index of sodium alginate is reported to be less than that of NaCMC (25). Hence, the observed swelling index of tablets appears to be predominantly governed by the presence of NaCMC or sodium alginate. Swelling of the polymer has been reported to help in the interpenetration of mucus and polymer, making bioadhesion possible (26). Therefore the formulations containing NaCMC can be expected to possess a better bioadhesive character.

It is evident from Figs. 1a and 1b that the release of CLT was slower from tablets confirming combinations of CP and NaCMC (batches B2-B4) as compared to those containing different ratios of CP and sodium alginate (batches C2-C4). An increase in the thickness of the diffusional path length due to higher relaxation of the cellulosic chains in tablets containing NaCMC seems to be responsible for slowing down the release of CLT (26). Tablets formulated using CP alone (batch A) and those prepared using a mixture of 2.25:0.75 of CP/NaCMC (batch B2) or CP/sodium alginate (batch C2) released 99% of CLT in 18 h. On the other hand, tablets prepared using a mixture of CP and NaCMC in the ratio of 2:1 (batch B3) or 1:2 (batch B4) released 94% and 85% CLT, respectively, in 24 h. Tablets formulated with 1:2 ratio of a mixture of CP/sodium alginate (batch C4) released 90% of CLT over 24 h. Complete release (100%) of CLT over 24 h was observed in tablets prepared using a 2:1 mixture of CP/sodium alginate (batch C3). It is well known that NaCMC possesses excessive swelling behaviour. Hence faster release of CLT from tablets containing a combination of CP and sodium alginate (Fig. 2) as compared to that from tablets containing combinations of CP and NaCMC can be ascribed to relatively lower swelling properties of sodium alginate (25). Therefore, dissolved CLT molecules can easily cross the narrow diffusional barrier formed due to the presence of CP and sodium alginate.

Irrespective of the ratio of CP/NaCMC or CP/sodium alginate, a zero order release of CLT was observed in all tablet formulations. Application of Peppas and Sahlin equation (20) to CLT release data of tablets prepared with various combinations of CP and NaCMC or CP and sodium alginate revealed that the value of n , the diffusional exponent of *in vitro* release ranged from 0.5 to 0.8. This indicated that zero order release from vaginal tablets was due to a combination of diffusion and chain relaxation mechanism being operative during drug release from all formulated vaginal tablets.

The tablets prepared with a 2:1 mixture of CP/sodium alginate (batch C3) released 100% of CLT over 24 h, suggesting their suitability for once a day administration. These tablets showed no change in physical characteristics and drug release profile when subjected to accelerated stability studies. A comparison of CLT release profiles of fresh and aged tablets showed a f_2 value of 66. f_2 values ranging between 50 to 100 are reported to indicate similar drug release profiles (21). Hence, the tablets prepared using a 2:1 mixture of CP/sodium alginate can be an effective and stable once a day formulation for vaginal conditions.

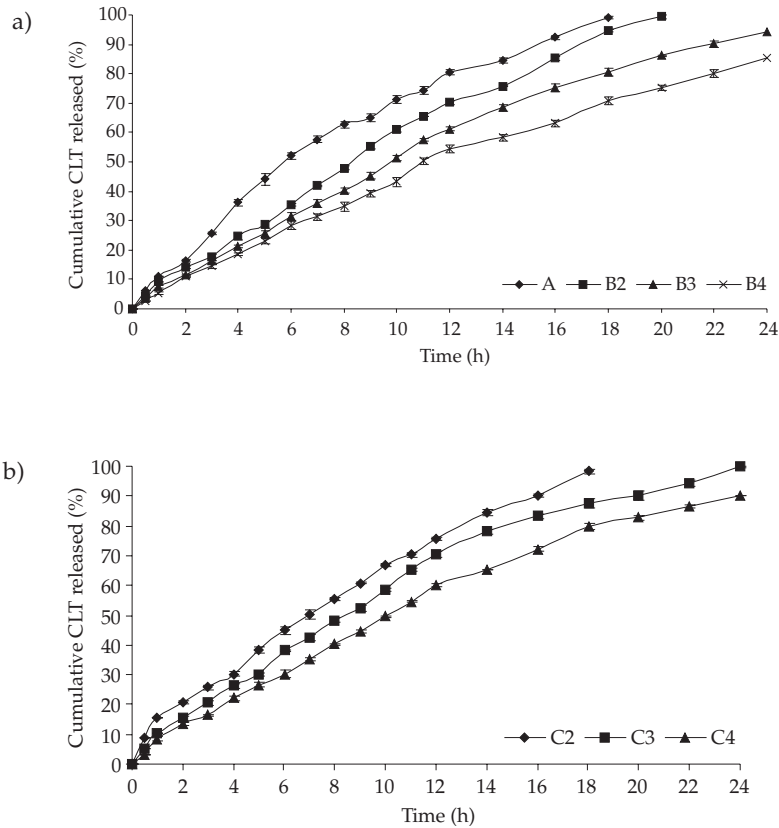


Fig. 1. CLT release profiles *in vitro* of vaginal tablets formulated using a) CP only (A), and various ratios of CP/NaCMC, (B2: 2.25:0.75, B3: 2:1 and B4: 1:2) and b) various ratios of CP/sodium alginate (C2: 2.25:0.75, C3: 2:1 and C4: 1:2). Each point denotes mean \pm SD, $n = 3$.

CONCLUSIONS

Use of bioadhesive polymers for vaginal drug delivery offers the advantage of sustained effect in the vagina. The results of the present investigation indicate that the inherently high bioadhesive strength of CP could be suitably modified by addition of sodium alginate. The tablets containing a 2:1 mixture of CP/sodium alginate (batch C3) slowly released 99% CLT over 24 h. These tablets are expected to offer a patient compliant once-a-day vaginal bioadhesive formulation for sustained local effect of CLT in vaginal candidiasis.

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S A Ž E T A K

Bioadhezivne vaginalete s klotrimazolom: priprava i evaluacija

GARIMA SHARMA, S. JAIN, A. K. TIWARY i GURPREET KAUR

U radu je opisana priprava bioadhezivnih vaginaleta s klotrimazolom, kombinacijom nekoliko bioadhezivnih polimera u različitim omjerima (Carbopol 934P, natrijeva sol karboksimetilceluloze i natrijev alginat). Bioadhezivnost je određena mjerenjem sile koja je potrebna za odvajanje tablete s vaginalne mukozne membrane svinje. Zbog jake interakcije između polimera i mukoze produljilo se vrijeme kontakta pripravka s kožom i lokaliziralo se djelovanje lijeka. Maksimalna bioadhezivnost postignuta je uz Carbopol 934P, a sila potrebna za odvajanje pripravka bila je proporcionalna njegovom udjelu. Pripravcima je ispitana sposobnost bubrenja pomoću metode s agarnim pločama. Indeks bubrenja ovisio je o koncentraciji hidrofilnog polimera. Pripravci s karbopolom i karboksimetilcelulozom jače su bubrili od pripravaka s karbopolom i natrijevim alginatom. *In vitro* ispitivanja pokazala su da se iz pripravaka s omjerom karbopola i natrijevog alginata 2:1 (pripravak C3) oslobađao klotrimazol tijekom 24 h. Profil oslobađanja bio je sličan iz svježih pripremljenih i starih vaginaleta, što ukazuje na njihovu stabilnost.

Ključne riječi: bioadhezivi, vaginalne tablete, klotrimazol, Carbopol 934P, natrijev alginat, natrijeva sol karboksimetilceluloze

Department of Pharmaceutical Sciences and Drug Research, Punjabi University, Patiala-147002, India