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Efficacy of a new ketoconazole bioadhesive vaginal tablet on *Candida albicans*

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Abstract

To develop more effective treatment for vaginal candidasis, ketoconazole (KTZ) was formulated in bioadhesive tablet formulations that increase the time of contact of drug with the vaginal mucosa. The bioadhesive vaginal tablets delivery of KTZ was prepared by direct compression of sodium carboxymethyl cellulose or polyvinylpyrrolidone or hydroxypropylmethyl cellulose (HPMC-E_{50}). Dissolution studies of bioadhesive tablets and commercial ovules were carried out with a new basket method (horizontal rotating basket). In vitro, a good sustained release action was obtained with bioadhesive tablets containing 1:1 and 1:2 drug/polymer ratio using HPMC-E_{50}. These bioadhesive tablets containing 400 mg of KTZ showed a zero-order drug release kinetic. KTZ solutions at increasing concentrations (0.16, 0.33, 0.5 and 0.66 mg/ml) were prepared for microbiological trials. These concentrations correspond to 25%, 50%, 75% and 100% of KTZ released from bioadhesive tablets, respectively. Yeast mixture was mixed with each concentration of KTZ at ratio of 1:10. One hundred microliters of this mixture was transferred in 900 μl liquid Sabouraud medium after a certain time interval for each concentration of KTZ and incubation at 37 °C for 24 h. Then this culture streaked onto Sabouraud-dextrose–agar plates, which were incubated at 37 °C for 48 h. The 0.16 and 0.33 mg/ml concentrations of KTZ showed fungistatic effect in 120 min. The 0.5 mg/ml concentration of KTZ was fungistatic in 90 and 120 min; and the 0.66 mg/ml concentration of the drug was fungistatic in 120 min as well as in 180 min. It was found that, in vitro antifungal activity of KTZ was dependent on its concentration and contact time with yeast cells. These results indicated that a new bioadhesive vaginal tablet formulations might be further developed for safe convenient and effective treatment of vaginal candidasis.

Keywords: Ketoconazole; *Candida albicans*; Bioadhesive; Minimum inhibitory concentration

1. Introduction

Vaginal candidosis is a common condition and up to 75% of all women suffer at least one episode of this infection during their lifetime. *Candida albicans* is the most important cause of vaginal candidosis, accounting for over 80% of the infection. Most patients with *Candida vaginitis* respond to topical treatment with nystatin or imidazoles [1]. Ketoconazole (KTZ) is an imidazole derivative antifungal agent developed for treatment of human mycotic infections and plays an essential role in antifungal chemotherapy [2].

Traditional vaginal drug delivery systems include solutions, suspensions, gels, foams and tablets [3]. Vaginal creams and gels provide lubrication, but tend to be messy, and are easily removed if they are water soluble. Suspensions and solutions tend to spread unevenly in the vagina. Foam producing dosage forms are preferred as excessive lubrication and leakage from the vagina are minimal and the foam adheres to the vaginal walls. Thus vaginal tablets appear to be useful dosage forms as they are easy to apply, portable and the user knows how many units remain [4,5].

During the last three decades, considerable attention has been focused on the development of novel and controlled release drug delivery systems to provide a long term therapeutic concentration of the drug following the application of a single dose [6]. Many controlled release drug delivery systems are based on hydrogels [7,8]. Bioadhesive vaginal tablet formulations that are capable of delivering the active agent for an extended period at a predictable rate have been developed and studied recently [9]. The systems are designed to give controlled delivery for 3 or more hours [10,11]. As

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sodium carboxymethyl cellulose (Na-CMC) hydroxypropylmethyl cellulose (HPMC-E_{50}) and polyvinylpyrrolidone (PVP) have excellent bioadhesive strength, they were used as a polymer material in bioadhesive tablet formulations [12,13].

In this study, the formulations of new bioadhesive vaginal tablets were developed for more effective and convenient chemotherapy against vaginal candidasis. Furthermore this study was to determine the minimum inhibitory concentration (MIC) of KTZ. For this purpose, time-dependent effect of various concentrations of KTZ on *C. albicans* was studied. We reported that KTZ bioadhesive vaginal tablet offered prolonged antifungal activity against *C. albicans* and also antifungal effect of KTZ was dependent on its concentrations as well as on contact time.

2. Materials and methods

2.1. Material

The *C. albicans* strain was isolated from the vaginal swab of a patient with vaginal candidosis who had received no antifungal therapy priorly. KTZ and magnesium stearate were supplied from Ilsan Iltas Pharmaceutical Company, Turkey, and Atabay Pharmaceutical Company, Turkey, respectively. Carboxymethylcellulose and PVP were purchased from Sigma Chemical Company, USA. HPMC-E_{50} was purchased from Colorcon, UK, and Sabouraud liquid medium and Sabouraud-dextrose–agar (SDA) from Oxoid, UK. All other chemicals were of analytical reagent grade.

2.2. Methods

2.2.1. Preparation of bioadhesive vaginal tablets

In the preparation of bioadhesive tablets, Na-CMC, PVP and HPMC-E_{50} were used as polymers. Bioadhesive vaginal tablets were prepared with 1:0.5, 1:1 and 1:2 drug/polymer ratios, and compressed under 2 tons of compression force with a hydraulic press (Perkin-Elmer). Each tablet contained 400 mg KTZ and magnesium stearate as lubricant to make 0.8% of the final tablet weight. The tablet mold was designed as special using stainless steel to prepare vaginal tablets in the shape of commercial tablets. Tablets had 2.3 cm height, 1.3 cm width and 0.9 cm thickness. To increase the adherence to the genital tract, the design of the tablet was further optimized and specially shaped to fit better to the uterine cervix.

2.2.2. In vitro dissolution study

The dissolution studies were carried out on the tablets prepared with PVP, Na-CMC and HPMC-E_{50} with 1:0.5, 1:1 and 1:2 drug/polymer ratios and commercial ovules. A new basket method was used in combination with USP dissolution apparatus I [14]. Six hundred milliliters of sodium citrate/hydrochloric acid buffers in pH 4 was used at 37 ± 0.5 °C with stirring speed of 90 rpm. One milliliter of samples withdrawn at appropriate time intervals were filtered, diluted with medium and assayed at 269 nm using a UV-1208 spectrophotometer. An equal volume of medium was returned to the system after each withdraw. All experiments were performed in triplicate.

2.2.3. Tablet swelling study

Swelling characteristics of bioadhesive tablets were evaluated dynamic swelling studies. Each sample was weighed and then placed in 10 ml sodium citrate/hydrochloric acid buffers of pH 4 in a glass vial at 37 ± 0.5 °C. The samples were periodically weighed after removing the excess water on the surface with a filter paper:

$$\text{Swelling}\ (\%) = \left[\frac{(W_t - W_i)}{W_i}\right] \text{x} 100 \quad (1)$$

where W_t is weight of the swollen sample at time *t,* and W_i is initial weight of the sample.

2.2.4. Microbial study

A turbid 48-h culture of *C. albicans* was prepared in 200 ml liquid Sabouraud medium. Then sets of KTZ solutions at increasing concentrations (0.16, 0.33, 0.5 and 0.66 mg/ml) were prepared. These concentrations correspond to the 25%, 50%, 75% and 100% of KTZ released from bioadhesive KTZ tablets, respectively. From each concentration of KTZ, a mixture of the yeast and KTZ solution was made in a sterile tube in ratio of 1:10, respectively. After 15 and 120 min 100 μl of the yeast suspension containing 0.16 mg/ml KTZ was inoculated into tubes containing 900 μl liquid Sabouraud medium. Similar transfers were made from 0.33 mg/ml suspension after 30 and 120 min, from 0.5 mg/ml suspension after 90 and 120 min, and from 0.66 mg/ml suspension after 120 and 180 min. These new suspensions were incubated at 37 °C for the 24 h. After incubation period these suspensions were streaked onto SDA plates, which were then incubated at 37 °C for 48 h. The results were noted.

As controls, tubes containing only the four concentrations of the drug for sterility control of solutions, tubes containing only liquid medium and plates of SDA for sterility control of mediums were used. The incubation of these control tubes and plates showed that they were sterile.

All the procedures described above were repeated 10 times, and each time the same results were obtained.

2.2.5. Curve fitting

Curve fitting was performed using Microsoft Excel 2000 version. The dissolution data were fitted to equation. Release exponent "*n*" was calculated [15,16].

$$M_t/M_\infty = kt^n \quad (2)$$

where M_t/M_∞ is the fraction of drug released at time *t, k* is the

Table 1
Analysis of diffusional release mechanism

Diffusional release exponent (n)	Overall solute diffusion mechanism	Time dependence of solute release rate (dM/dt)
0.5	Fickian diffusion	$t^{-0.5}$
$0.5 < n < 1.0$	Anomalous (non-Fickian) diffusion	t^{n-1}
1.0	Case II transport	Zero-order (time-independent) release
$n > 1.0$	Super Case II transport	t^{n-1}

kinetic constant of the system, and n is the exponent characteristic of the mode transport (Table 1).

2.2.6. Statistical analysis

Tests for significant differences between means were performed by Student's t-test or one-way ANOVA by using the software SPSS 10.0. Differences were considered significant at $P < 0.05$ level. Each data point represents the average of three determinations.

3. Results and discussion

The average weight of each tablets were 1.208 (±0.12), 0.8036 (±0.08) and 0.632 (±0.1) with 1:2, 1:1 and 1:0.5 drug/polymer ratio, respectively. The total amount of KTZ present in each sample was calculated by adding cumulative amount of KTZ released at the end of the dissolution study with the residual amount present in each sample. The total of KTZ present in each sample was between 395 and 400 mg.

3.1. In vitro dissolution study

The dissolution of the tablets was carried out in pH 4. Fig. 1 shows the effect of the polymer type and ratio on the dissolution of KTZ from bioadhesive tablets. The drug release characteristic of all the formulations was found to be different when compared with each other (Fig. 1, $P < 0.05$). A new basket method was used in combination with USP dissolution apparatus I [14]. This new method has advantages over the paddle and basket systems. As in the paddle system; a few turns of wire helix have to be attached to the ovules to avoid their floating on the medium surface; the ovules fall apart in an uncontrolled manner or adhere to the bottom of the vessel. In the basket system; ovules form a cake inside the baskets and the dissolution rate is very slow in spite of high speed of rotation.

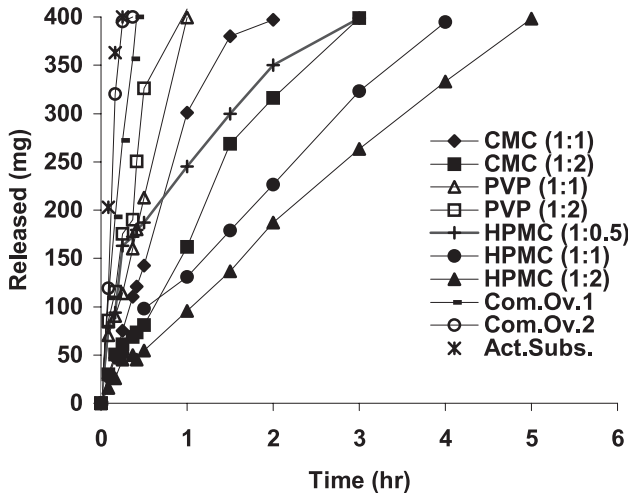

Fig. 1. Dissolution of KTZ: effect of polymer type and ratio and comparison of the dissolution rates of two different commercial ovules in Turkey and bioadhesive vaginal tablets of KTZ ($n = 3$, S.D. are not shown because they are smaller than the symbol size).

According to the comparison of the dissolution profiles of two different commercial ovules and bioadhesive tablets, the release rate of bioadhesive tablets decreased significantly ($P < 0.05$). Bioadhesive tablet forms of the drug decreased in dissolution rate and the in vitro drug release was sustained from 25 min to 5 h. Furthermore, with bioadhesive tablets containing 1:1 and 1:2 drug/polymer ratio using HPMC-E_{50}, a good sustained release effect can be seen compared to the other formulations (Fig. 1).

In bioadhesive tablets prepared with HPMC-E_{50} and Na-CMC used as polymers, when the ratio of polymer was increased, as expected, in certain time the release quantity of KTZ was decreased and release time was increased. On the other hand, the use of PVP as polymer changing the ratio of polymer in the drug has caused no change in the release profile of KTZ.

3.2. Tablet swelling study

The swelling profiles of various polymers in the physiologically relevant fluids also help to interpret the mechanism of bioadhesion [17]. The vaginal pH of women in reproductive age is acidic (pH 4–5) [9]. Therefore, swelling studies were carried out in sodium citrate/hydrochloric acid buffers (pH 4). The swelling profiles were shown in Fig. 2. The initial swelling at 15 min of all bioadhesive tablets varied between 5% and 57.3%. The equilibrium swelling of the polymers at 2.5 h varied between 40% and 86.9% except PVP. Disintegration started 15 min later with bioadhesive tablets containing PVP. At the initial stage, the swelling occurs very rapidly due to the entry of water via metastable pores in the tablets containing HPMC-E_{50} and Na-CMC. This mechanism is known as hysteresis of the swelling that is followed by swelling as a result of diffusion processes. If an intact hydrated layer can establish over the period of study, diffusion may be most important factor controlling the rate of drug release from the system diffusion [18,19]. When more swellable polymers were used into the formulations (Fig. 2) the release of decreases with time (Fig. 1). Drug release from

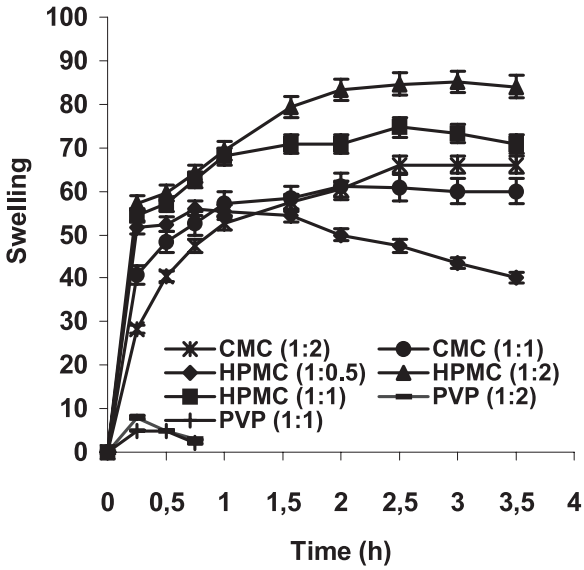

Fig. 2. Swelling profiles bioadhesive vaginal tablets of KTZ in pH 4. Each point represents the mean ± SE of triplicate experiments. Note: SE is less than the point size for some points.

hydrophilic matrix could occur both by diffusion and swelling-controlled mechanism [20].

3.3. Microbial study

In the microbiological part of this study:

- The 24-h incubation of the 1:10 suspension of *C. albicans* that had contacted the 0.16 mg/ml solution of KTZ for 15 min yielded the growth of the yeast on SDA plates.
- The 24-h incubation of the 1:10 suspension of *C. albicans* that had contacted the 0.16 mg/ml solution of KTZ for 120 min yielded no growth of the yeast on SDA plates.
- The 24-h incubation of the 1:10 suspension of *C. albicans* that had contacted the 0.33 mg/ml solution of KTZ for 30 min yielded the growth of the yeast on SDA plates.
- The 24-h incubation of the 1:10 suspension of *C. albicans* that had contacted the 0.33 mg/ml solution of KTZ for 120 min yielded no growth of the yeast on SDA plates.
- The 24-h incubation of the 1:10 suspension of *C. albicans* that had contacted the 0.5 mg/ml solution of KTZ for 90 and 120 min yielded no growth of the yeast on SDA plates.
- The 24-h incubation of the 1:10 suspension of *C. albicans* that had contacted the 0.66 mg/ml solution of KTZ for 120 and 180 min yielded no growth of the yeast on SDA plates.

In other words:

- The 0.16 mg/ml concentration of KTZ was not effective on *C. albicans* in 15 min, but showed fungistatic effect in 120 min.
- The 0.33 mg/ml concentration of KTZ was not effective on *C. albicans* in 30 min, but showed fungistatic effect in 120 min.
- The 0.5 mg/ml concentration of KTZ showed fungistatic effect on *C. albicans* in 90 min as well as in 120 min.
- The 0.66 mg/ml concentration of KTZ showed fungistatic effect on *C. albicans* in 120 min as well as in 180 min.

The quantity of KTZ in mg/ml released from the prepared bioadhesive tablets were given in Table 2. In the microbiological part of this study, 0.16 mg concentration of the drug totally inhibited the growth of *C. albicans* in 120 min. In the light of this finding, it can be stated that bioadhesive tablets that released the concentration of 0.16 mg/ml of the drug and sustained this concentration for 120 min can be effective on *C. albicans.* For this theory, when bioadhesive tablets prepared were examined according to the dissolution results, all the bioadhesive tablet formulations were fitting to the criteria stated above (Table 2). However, among the tablets prepared the best, almost ideal, sustained release was obtained with tablets containing 1:1 and 1:2 drug/polymer ratio using HPMC-E_{50}.

As HPMC-E_{50} content got greater, a straighter line was observed for a zero-order equation (Fig. 3). To investigate the mechanism of the drug release from bioadhesive tablets containing 1:1 and 1:2 drug/polymer ratio using HPMC-E_{50}, the release data were fitted to Eq. (2). The values of n were 0.809 ± 0.08 (SE) (r^2 = 0.997) and 0.908 ± 0.06 (SE) (r^2 = 0.999) for the HPMC tablet containing a drug/polymer ratio of 1:1 and 1:2, respectively, "n" showed little variation (0.908–0.931), i.e. anomalous transport or case II transport [21]. A value of n = 1 would indicate zero-order release from a planar surface [14,15].

As a conclusion, the antifungal effect of KTZ is dependent on its concentration as well as on contact time. Consequently,

Table 2
Release of KTZ quantity from bioadhesive tablets in certain time (mg/ml)

Time (min)	Na-CMC (1:1)	Na-CMC (1:2)	PVP (1:1)	PVP (1:2)	HPMC-E_{50} (1:0.5)	HPMC-E_{50} (1:1)	HPMC-E_{50} (1:2)
15	0.125 ± 0.03	0.102 ± 0.09	0.190 ± 0.09	0.292 ± 0.09	0.271 ± 0.06	0.086 ± 0.03	0.075 ± 0.06
30	0.238 ± 0.07	0.135 ± 0.10	0.355 ± 0.02	0.544 ± 0.05	0.312 ± 0.02	0.163 ± 0.09	0.096 ± 0.03
60	0.500 ± 0.09	0.270 ± 0.03	0.665 ± 0.08	0.66 ± 0.06	0.409 ± 0.09	0.238 ± 0.03	0.160 ± 0.05
90	0.633 ± 0.06	0.448 ± 0.06			0.5 ± 0.08	0.298 ± 0.03	0.228 ± 0.02
120	0.661 ± 0.07	0.527 ± 0.02			0.584 ± 0.03	0.377 ± 0.06	0.312 ± 0.07
180		0.665 ± 0.08			0.665 ± 0.09	0.539 ± 0.07	0.439 ± 0.03
240						0.658 ± 0.04	0.555 ± 0.04
300							0.663 ± 0.06

Data are presented as mean ± SE of triplicate experiments.

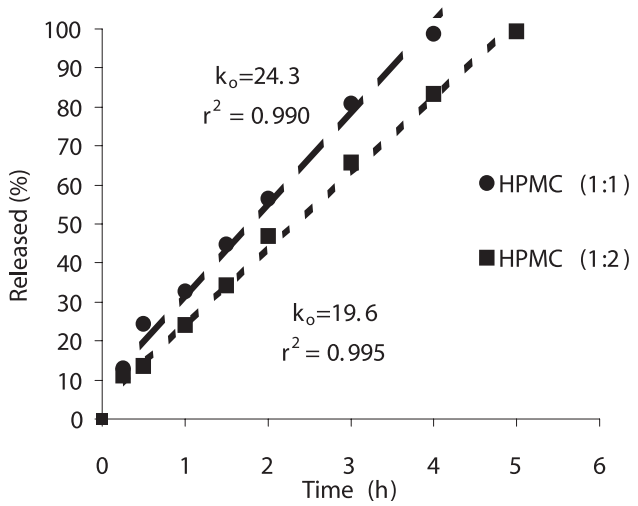

Fig. 3. A plot of the released against the zero-order release showing the release rate constants of bioadhesive tablets containing HPMC-E_{50} at pH 4.

the bioadhesive form of the drug would increase the time of contact with the vaginal mucosa and thus its therapeutic effect. In addition, the soft and rubbery nature of bioadhesive polymers will minimize mechanical and frictional irritation to the surrounding tissue.

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