Drug Development and Industrial Pharmacy, 25(5), 691–694 (1999)

COMMUNICATION

Comparison of In Vitro Dissolution and Permeation of Fluconazole from Different Suppository Bases

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ABSTRACT

Fluconazole suppositories were prepared in hydrophilic, lipophilic, and amphiphilic bases. In vitro evaluation was conducted to compare the effect of different bases on the release and permeation of fluconazole. Four types of suppository bases were evaluated: hydrophilic (polyethylene glycol, PEG), lipophilic (cocoa butter, CB; Witepsol W45® WW45), and amphiphilic (Suppocire AP® SAP, a polyglycolized glyceride). The uniformity of dosage units prepared with each base was determined by ultraviolet (UV) spectroscopy. The influence of suppository base on the release of fluconazole was studied using USP dissolution apparatus I. Rate constants for each release pattern were determined and compared using a one-way analysis of variance (ANOVA) on ranks. The order of in vitro dissolution of fluconazole from the bases was as follows: PEG > (SAP = WW45) > CB. Results suggest that in vitro release of fluconazole is greater from a hydrophilic base (PEG). Preliminary permeation studies were conducted on each type of base using Franz diffusion cells. Permeation was studied through the rat rectal membrane, and normal saline was used as the receptor medium. A modified reverse-phase high-performance liquid chromatography (HPLC) method was used and validated for analyzing fluconazole. Flux values ($\mu g/cm^2/hr$) were calculated and compared using a one-way ANOVA ($p < .001$). The order of permeation was as follows: SAP > (PEG = WW45) > CB. The increased permeation characteristics seen with the SAP base are probably due to an alteration of the membrane characteristics due to the surface active properties of the base.

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INTRODUCTION

Fluconazole is a triazole marketed in oral and parenteral dosage forms. It is used for mucosal and systemic candidiasis, central nervous system cryptococcosis, and coccidiomycosis (1). Although the rectal route is less commonly used, it represents a convenient and alternative route of drug delivery when oral and parenteral administration are not feasible. The delivery of fluconazole in a suppository dosage form would offer advantages for the following: (*a*) in oropharyngeal or esophageal mycoses, when patients might have difficulty swallowing; (*b*) anticandidal therapy when used in the surgical intensive care unit as an interesting alternative to intravenous application (2); (*c*) in patients suffering from malignancies, when therapy is hampered by iatrogenic peripheral venous destruction or nausea (cytostatics) (3); (*d*) administration to children (4); (*e*) treatment of vaginal candidiasis for which reinfection of the vagina very often occurs by rectal contamination; (*f*) physical and chemical incompatibilities of fluconazole with commonly used injectable drugs can be minimized when fluconazole is administered as a suppository dosage form (5).

MATERIALS AND METHODS

Drug, excipients, and solvents were used as received and were not purified: fluconazole (B. D. H. Industries, Mumbai, India); Suppocire AP (Gattefose Corp., Sanntpriest, France); Witepsol W45 (Huls America); polyethylene glycol (PEG) 600, PEG 900, and PEG 3350 (Union Carbide Corp); cocoa butter; chloroform (Spectrum); ammonium phosphate dibasic; acetonitrile, high-performance liquid chromatography (HPLC) grade; hydrochloric acid; *n*-octanol; *o*-phosphoric acid, 85% HPLC grade; sodium hydroxide pellets (Fisher Scientific).

Partition Coefficient

The partition coefficient of fluconazole was determined using *n*-octanol as the nonpolar phase and distilled water as the polar phase. Fluconazole was dissolved in the water phase and agitated with *n*-octanol presaturated with water for 30 min at room temperature. A modification of previously published HPLC methods was used to analyze fluconazole (6,7).

Preparation of Fluconazole Suppositories

All suppositories contained 50 mg fluconazole and were prepared by the fusion method (8). Preformed plastic shell containers (3 ml capacity), were calibrated for each base. The molten mixture of the base with fluconazole was cooled to 36°C–38°C with gentle stirring, poured into the shell, and allowed to solidify at room temperature (25°C ± 0.5°C). The molds were sealed and stored under refrigeration (4°C) until further tests were carried out.

Weight Variation and Content Uniformity

These tests were carried out per USP XXII. Absorbances were measured on a spectrophotometer (Spectronic® Genesys™5, Milton Roy) at 260 nm against a blank. The concentration of fluconazole was calculated from the standard curve.

Drug Release

The USP dissolution apparatus I was used for the determination of release rates. Each suppository was placed in a basket in a flask containing 500 ml phosphate buffer (pH 7.0). The basket was rotated at 100 rpm at a constant temperature of 37°C ± 0.5°C. After 5, 10, 20, 30, and 45 min, 10-ml samples were withdrawn. To compensate for sampling, 10 ml buffer was added to the dissolution flask. The absorbances of these solutions were measured at 214 nm on a spectrophotometer against a blank, and drug concentrations were measured from a standard curve.

Analytical Method

A stability-indicating HPLC method was used. A C_{18} column (Novapac 3.9 × 150 mm, 4 μm, Waters, MA), mobile phase consisting of buffer:acetonitrile 87:13. The buffer used was 0.02 M dibasic ammonium phosphate, with pH adjusted to 7.0 with *o*-phosphoric acid, a flow rate of 1 ml/min, and an injection volume of 20 μl (HP 1100 series isocratic pump, manual injector, thermostated column compartment, variable wavelength detector). The detection wavelength was set at 214 nm (HP Integrator 3395, Hewlett Packard), and the column compartment was maintained at 40°C.

Permeation Studies

An in vitro model using rat rectal membrane and Franz diffusion cells was used to predict fluconazole permeation through human rectal membrane. The permeation study was conducted based on a previously reported study using a rabbit rectal membrane (9). The rectal

membrane was excised from freshly sacrificed CD4 male rats weighing about 500gm. It was allowed to soak in saline at 37°C ± 0.5°C for 30 min. The rectal membrane was sandwiched between the donor and the receptor chambers of the cells. The receptor phase was saline (0.9% w/v NaCl). An accurately weighed amount of the suppository was placed in the donor chamber on top of the membrane.

The cell was maintained at 37°C ± 0.5°C, and samples were taken from the receptor cells after 2, 4, 8, 16, and 24 hr and replaced with an equal volume of fresh saline. Samples were analyzed for drug content by HPLC. A graph of amount permeated/surface area versus time was plotted. The slope of the straight-line portions for each plot was calculated. Values for flux were thus determined in micrograms/square centimeters/hour. To compare the flux values for fluconazole in the presence of different bases, a one-way analysis of variance (ANOVA) was used. All pairwise multiple comparisons were conducted at $P < .05$ using the Student-Newman-Keuls method.

RESULTS AND DISCUSSION

The octanol/water partition coefficient obtained was 2.73, which indicated that fluconazole has a greater affinity to the nonaqueous phase. When in a suppository, the high affinity between fluconazole and the lipophilic bases would result in lower release rates of fluconazole into the aqueous media. Passage of drugs through lipid membranes sometimes correlates with the octanol/water partition coefficient. The results of weight variation and content uniformity conformed to USP specifications.

The results of percentage of fluconazole released at various time intervals are shown in Fig. 1. Different suppository bases influence the release characteristics of fluconazole. Each suppository base contained 50 mg of fluconazole with slightly varying quantities of bases, depending on their displacement values. The rate constants were measured in each case by plotting a graph of percentage log remaining to be released versus time and are reported in Table 1. The rate constants were compared using a one-way ANOVA on ranks using a Sigmastat® statistical software. All pairwise multiple comparisons using the Student-Newman-Keuls method ($P < .05$) showed the following order of release: PEG > (SAP = WW45) > CB. The rate of fluconazole released from the dissolved or softened suppositories and the diffusion rate of the dissolved drug molecules across the mucosal membrane are rate-limiting factors for drug absorption. The almost complete release of fluconazole from a PEG base when placed in the environment of the gastrointestinal tract would be of fundamental concern.

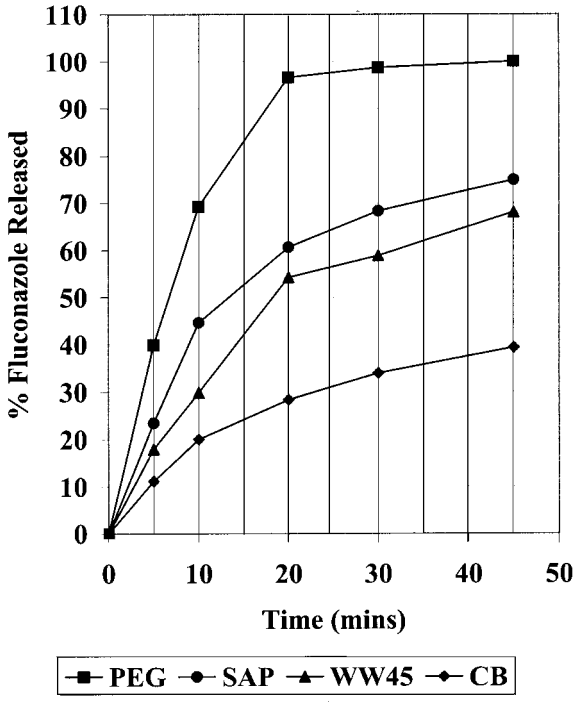

Figure 1. In vitro dissolution profile of fluconazole in different suppository bases.

A graph of the amount of fluconazole permeated/surface area versus time was plotted (Fig. 2). After 8 hr of permeation of fluconazole in the Suppocire AP base suppository, there was a visible alteration in the membrane, which is likely to be due to the surface active properties of the base. The membrane appeared to exhibit swelling in comparison with the membranes treated with the

Table 1

Rate Constants and Flux Values for Fluconazole in Different Suppository Bases

Type of Base	Rate Constant[a] ± SD	Flux[b] ± SD (μg/cm²/h)
SAP	0.032 ± 0.009	17.12 ± 2.80
WW45	0.026 ± 0.002	9.96 ± 2.03
PEG	0.098 ± 0.020	8.69 ± 2.27
CB	0.011 ± 0.001	5.00 ± 1.36

[a] Mean ± SD of 5 determinations of rate constants.
[b] Mean ± SD of 3 determinations of flux values.

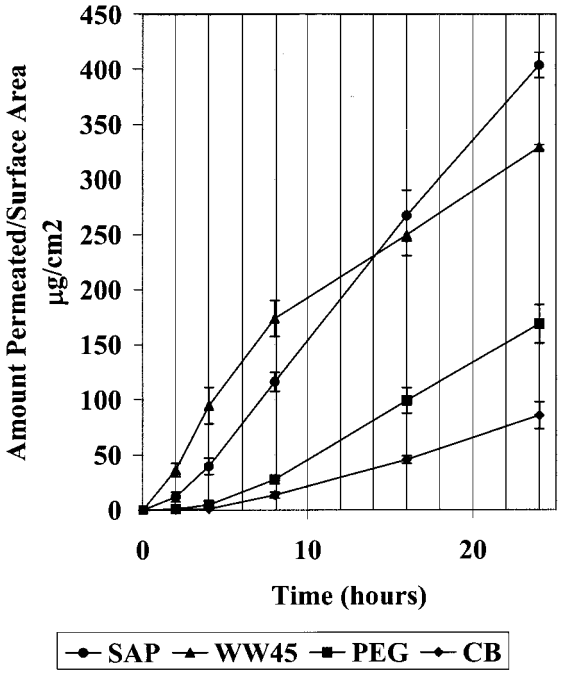

Figure 2. Permeation of fluconazole from different bases through rat rectal membrane in amount permeated/surface area ($\mu g/cm^2$) versus time (hr).

other bases. However, a straight-line plot of amount permeated/ surface area versus time ensures that the membrane has not ruptured.

The steady-state flux values were used as a measure to compare the influence of fluconazole/base properties on the permeability characteristics (Table 1). It is important at the same time to consider the complex nature of the mucous membrane and its interaction with the vehicle. The general trend observed could prove to be a useful model for in vitro evaluation of suppositories.

There is a statistically significant difference at $\alpha = .05$ ($p = 8.07E\text{-}012$). To determine the group/groups that differed, all pairwise multiple comparisons were conducted using the Student-Newman-Keuls test ($P < .05$). The order of the permeation properties was as follows: SAP > (PEG = WW45) > CB.

CONCLUSION

The influence of suppository bases on in vitro availability of fluconazole was studied. Results from the in vitro release using the USP dissolution apparatus I showed that the percentage of fluconazole released from PEG suppositories was significantly higher than the other types. This indicated that fluconazole was released faster from a hydrophilic base than from lipophilic bases.

An in vitro model was designed to evaluate the permeation characteristics of fluconazole from four different bases. A comparison of Suppocire AP, Witepsol W45, PEG, and cocoa butter, showed that the permeation of fluconazole through the rat rectal membrane is maximum when in an SAP base. A suppository with a PEG base and surfactant might give enhanced absorption of fluconazole.

ACKNOWLEDGMENT

This work was abstracted from a dissertation presented to the Division of Pharmaceutical Sciences, Massachusetts College of Pharmacy and Allied Health Sciences, Boston, Massachusetts.

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