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A Mucoadhesive, Cyclodextrin-based Vaginal Cream Formulation of Itraconazole

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ABSTRACT

The development of vaginal medications, especially antifungal medications, requires that the drug is solubilized as well as retained at or near the mucosa for sufficient periods of time to ensure adequate bioavailability. Itraconazole is a broad-spectrum antifungal agent, which has been used for some time orally and intravenously but for which a vaginal formulation has not yet been developed. We present here a novel itraconazole formulation intended for vaginal use based on hydroxypropyl-β-cyclodextrin (HPβCD), a functional excipient that increases drug solubility and generates a mucoadhesive system in the presence of other ingredients. An aqueous phase was prepared by solubilizing itraconazole with HCl in the presence of propylene glycol and then adding an aqueous solution of HPβCD. After pH adjustment, the itraconazole/HPβCD solution was added to the oil phase (paraffin oil, trihydroxystearate, and cetyl dimethicon copolyol) and the desired cream containing 1%, 2%, and 2.5% drug obtained by homogenization. Primary irritation studies and subchronic toxicity studies using a rabbit vaginal model indicated that the formulation was safe, well tolerated, and retained in the vaginal space. Clinical investigations indicated that application of 5 g of a 2% cream was very well tolerated and itraconazole was not systemically absorbed. Additional studies in women found that the itraconazole cream was highly effective in reducing or eliminating fungal cultures with few adverse effects. These studies suggested that an HPβCD-based, emulsified wax cream formulation was a useful and effective dosage form for treating vaginal candidiasis.

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KEYWORDS

Itraconazole, vaginal, cyclodextrin, mucoadhesive, toxicity, clinical investigation, candidiasis

INTRODUCTION

Itraconazole (**Figure 1**) is an orally active antifungal agent with a broad spectrum of activity.[1-3] In addition, the drug has an interesting tissue distribution, which has made possible effective and rapid treatments of vaginal candidiasis when the drug is administered orally.[4-6] A topical itraconazole-containing formulation may be of use for several reasons including the opportunity to generate high local tissue levels, more rapid drug delivery, and lower systemic exposure. This may be especially important for treating pregnant patients. In addition, itraconazole is effective against several fungal strains such as *Torulopsis glabrate* and *Candida topicalis*, which are responsible for refractory vaginal candidiasis in more than 25% of patients suffering from this condition. Candidia-related fungal vaginitis is a common gynecological disease affecting two thirds of all women at least once during their lifetime.[7]

In addition to having an effective medication, the formulation must adhere to the mucosa in order to bring the drug in contact with the target tissue for sufficient periods of time as well as to prevent expulsion of the formulation.[8,9] Unfortunately, many of the dosage forms currently used in treating vaginal candidiasis such as gels, foams, creams, and suppositories physically break down rapidly after insertion into the vaginal cavity resulting in unwanted leakage as well as giving rise to products of limited efficacy.[10,11] Mucoadhesive systems may avoid these problems.[8,9,11] Mucoadhesive gels based on polycarbophil and carbomers have been prepared and marketed containing spermicidal agents such as nonoxynol-9 (Advantage-S) as well as progesterone

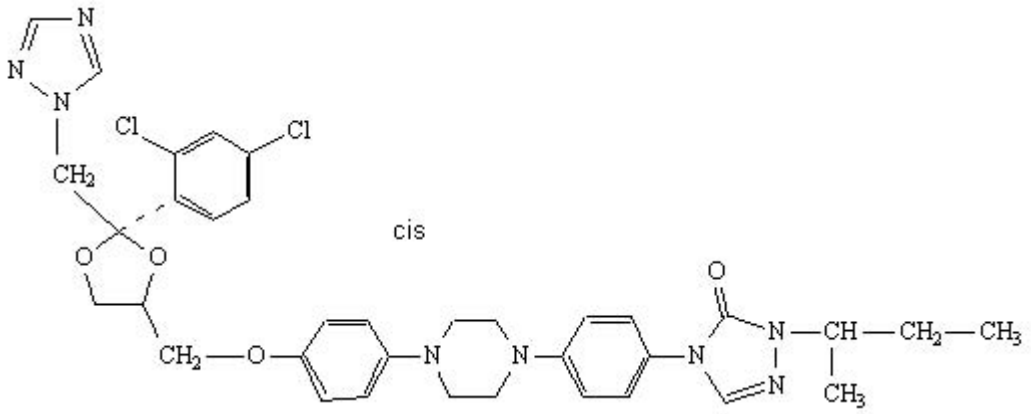

Figure 1. Chemical structure of itraconazole.

(Crinone, Columbia Laboratories, Rockville Center, NY).[12]

Finally, the drug must be in a bioavailable form. Itraconazole is associated with several properties that make it difficult to formulate, such as very poor water solubility (~1 ng/mL at neutral pH) and a high log P (>5) (**Table 1**).[13] One approach, which has been applied to producing pharmaceutically acceptable dosage forms of the drug, is the use of chemically modified cyclodextrin especially hydroxypropyl-β-cyclodextrin (HPβCD).[13,14] HPβCD solubilizes lipophiles through a dynamic inclusion complex formation in which a portion of the molecule is included in the lipophilic cyclodextrin cavity.[15] Using this technology, marketed oral and parenteral IV formulation for itraconazole have been developed (Sporanox oral solution and IV solution, Janssen Pharmaceutica, Olen, Belgium).[13]

The aim of this work was to assess the possibility of generating a mucoadhesive vaginal cream of itraconazole using HPβCD as a solubilizing and bioadhesive excipient.

MATERIALS AND METHODS

Materials

2-Hydroxypropyl-β-cyclodextrin (HPβCD) was obtained from Roquette (Lestrem, France) and was characterized by a degree of substitution of 4.2 based on a Fourier transformed infrared spectrophotometry (FT-IR) method.[16] Itraconazole was obtained from the Janssen Research Foundation, Beerse, Belgium.

Other excipients including 1,2-propanediol, paraffin oil, cetyl dimethicon copolyol and trihydroxystearate were commercially obtained with a quality consistent with the European Pharmacopeia (EP), US Pharmacopeia-National Formulary, or internal specifications.

Table 1. Physicochemical Properties of Itraconazole

Molecular weight	705.64
Partition coefficient	Log P > 5
Ionization constant	4.0
Melting point	166°C
Solubility in water (pH 7)	~ 1 ng/mL
Solubility in 0.1 N HCl	4 μg/mL

Methods

Complexes were prepared by first sonicating an excess of itraconazole in various concentrations of HPβCD ranging from 0% to 40% wt/vol prepared in an unbuffered system (pH 2, the pH was adjusted with HCl).[13] After 10 minutes of sonication, the systems were shaken for 2 days at which time the pH was checked and adjusted as necessary. The samples were then equilibrated for 3 to 6 months at 25ºC with itraconazole concentrations measured at 6 days, 2 weeks, 4 weeks, 3 months, and 6 months. At the appropriate time point, a small volume of the supernatant was withdrawn, filtered through a 0.45-μ membrane and analyzed by UV (at 254 nm). The relationship between solubilizer and drug was analyzed using the phase-solubility approach described by Higuchi and Conners.[17] Curve-fitting techniques were automated using an EXCEL spreadsheet using the Nelder-Mead nonlinear optimization technique.[13]

Creams were prepared by solubilizing itraconazole with HCl and propylene glycol. This solution was then added to an aqueous solution of HPβCD (43.5%) followed by addition of NaCl (as appropriate) and base to generate a solution with a pH of 2 to 2.7.[10] A second system was prepared by mixing paraffin oil and trihydroxystearate at 80ºC, cooling the mixture to 40ºC and then adding cetyl dimethicon copolyol. The aqueous and lipid phases were then mixed and homogenized in a cream processor for 30 minutes.

Toxicology Studies

New Zealand white rabbits were used for primary irritation and subchronic toxicity testing. All animals used in these investigations were housed singly and allowed free access to fresh tap water and pelletized rabbit chow (Pavan Service, Gent, Belgium). For irritation studies, a 2% itraconazole cream, a placebo cream, or a sham treatment were completed. For the study, 18 rabbits were used (3 groups of 6 females) in which a single vaginal application was dosed followed by 3 days of observation. The active or placebo formulations were administered using a 1 mL plastic syringe such that the material was administered at a dose of 0.25 mL/kg.

Subchronic toxicity studies were also completed using 3 doses of a 2% itraconazole cream (0.05 mL/kg or 1 mg itraconazole/kg; 0.15 mL/kg or 3 mg itraconazole/kg; 0.25 mL/kg or 5 mg itraconazole/kg), a placebo cream (dosed at 0.25 mL/kg), a sham treatment, and an untreated control group. Animals were dosed daily 6 days/week for 28 days. Each dosing group contained 6 rabbits (36 animals in total). At study termination, a number of parameters were assayed including mortality, clinical observations, body weight, hematology, serum analysis, urinalysis, organ weights, gross pathology, and histopathology. All necropsies were performed by a qualified pathologist as soon as possible after animals were killed and all macroscopic pathological changes were recorded. Special attention was paid to signs of possible irritation of the vaginal mucosa (ie, erythema and edema) and related mucosal reactions. Resorption of the formulation in the vagina was assessed. Histopathology concentrated on the ovaries, uterus, vagina, and cervix of all rabbits as well as organs that showed abnormalities on gross necropsy. Organs and tissues were trimmed, embedded in paraffin, sectioned, and stained with hematoxylin-eosin. All assessments were performed by a qualified pathologist who scored each section based on a rating of 0 (normal tissue) to 5 (severe histological change). Results were analyzed using the Mann-Whitney *U* test with calculation of the mean values.

Clinical Studies

A Phase I trial was completed with a 2% intraconazole vaginal cream in 8 healthy volunteers. Five grams of the cream were applied for 3 days after which tolerability and blood levels were assessed. Bioanalytical methods for determining the level of itraconazole in vivo were based on published literature methods.[18,19] In a phase III clinical trial, 170 patients received 5 g of a 1% itraconazole cream. The medication was dosed daily for 3 days followed by an evaluation at 1 week and 1 month after dosing initiation. Mycological cure was assessed by negative fungal cultures and microscopy.

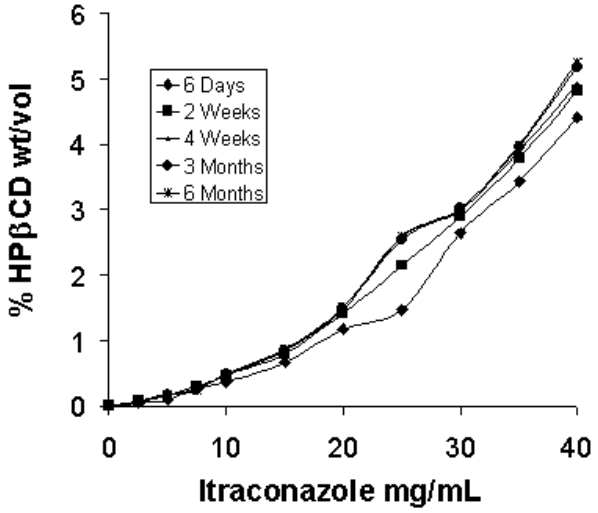

Figure 2. Solubility of crystalline itraconazole as a function of hydroxypropyl-β-cyclodextrin (HPβCD) concentration after various storage times at 25ºC at pH 2.

RESULTS AND DISCUSSION

Itraconazole Solubilization

The ability of HPβCD to solubilize itraconazole was assessed using phase-solubility techniques. At pH 2, HPβCD solubilizes itraconazole in a curvilinear manner generating an AP-type solubility profile (**Figure 2**) with equilibrium reached between 6 days and 2 weeks. A full report on the interaction of itraconazole with HPβCD at various pHs has been published.[13] The curvilinear profile suggests higher order complexation at higher HPβCD concentrations.[14] Deconvolution of the curve suggests stability constants of the following magnitude: $K_{1:1}$ of

Table 2. Composition of 1%, 2%, and 2.5% Itraconazole/HPβCD-based Cream*

Ingredient	1% Cream (mg/g)	2% Cream (mg/g)	2.5% Cream (mg/g)
Itraconazole	10	20	25
HCl	4.4	8.8	11.1
HPβCD	436	500	530
1,2-Propanediol	30	30	35
NaOH	qs pH 2.7	qs pH 2.2	qs pH 2.0
NaCl	10	10	_
Paraffin oil	200	125	45
Cetyl dimethicon copolyol	20	20	15
Trihydroxystearate	5	5	5
Purified water	qs ad 1 g	qs ad 1 g	qs ad 1 g

* HPβCD indicates hydroxypropyl-β-cyclodextrin; qs pH, qs = quantum satis; qs ad, quantum satis ad.

8929 M^{-1} and $K_{1:2}$ of 27 M^{-1}. These data are qualitatively similar to those described by Miyake et al.[14]

Formulations

Based on the solubility data of itraconazole at pH 2 obtained in the phase-solubility studies (Figure 2), a dosage form was designed whereby itraconazole was solubilized in propylene glycol and aqueous HPβCD solution. Using this as stock, creams containing 1%, 2%, and 2.5% itraconazole were prepared as indicated in **Table 2**.[10] These types of emulsified cream bases are known to possess mucoadhesive properties and were chosen for further exploration.[11,20] Other formulations including hydrophilic gels have been assessed for itraconazole but were suboptimal with regard to mucoadhesion, contact time, and/or solubilization of the drug substance (M. Francois, unpublished data, June, 2002).

In Vivo Studies and Toxicology

The ability of the itraconazole cream to be retained in the vagina as well as its primary irritation potential was assessed in the rabbit. A 2% itraconazole-containing cream or a placebo was dosed to rabbits once daily at a total volume of 0.25 mL/kg. Three days later treated animals were compared with sham control with regard to clinical observations, body weight, and gross pathology. The formulations were well retained in the vaginal space suggesting mucoadhesive properties. In addition, no drug- or vehicle-related vaginal irritation was observed at the dose administered. In an extended study to assess the mucoadhesive properties of the formulation, 3 rabbits were treated with 0.25 mg/kg itraconazole cream daily for 5 days. There was no expulsion of the formulation seen in any animal over this time course.

Subchronic studies were also carried out in which rabbits were either untreated, sham-treated, exposed to placebo, or exposed to a 2% itraconazole cream at doses of 1, 3, or 5 mg/kg itraconazole daily (6 days a week) for 28 days. The results indicated that the 2% vaginal creams (placebo or medicated) were very well tolerated at doses up to 5 mg/kg. No macroscopic vaginal irritation was noted. Leakage of the formulations from the vagina was minimal and dose-related. A comparison of the histopathological observations of the genital tract of control animals to the sham-treated, the vehicle controls, and the drug-treated rabbits found that the histological scores for the uterus were comparable among the groups. In the cervix, a small but significant increase in inflammatory changes was observed in the vehicle and high-dose drug group relative to controls; and in the vagina, a small but significant increase in inflammatory changes was observed in the vehicle, low-, and high-dose drug groups relative to controls. No other histological changes were observed.

Several clinical trials have been completed. In a phase I study, 8 women were treated with 5 g of a 2% itraconazole cream daily for 3 days after which tolerability and systemic drug absorption were measured. Itraconazole plasma levels were below the limits of detection of the high pressure liquid chromatography (HPLC) method (<1 ng/mL) in all 8 volunteers at baseline and after 3 days of treatments suggesting little or

no absorption of the drug from the vagina. No clinically relevant change in hematological, biochemical, and urinary parameters was observed. In addition, the formulations caused no noticeable erythema, edema, pain, or itching. No adverse experiences were recorded. Six of the 8 volunteers indicated that the tolerability of the formulation was very good.

A phase III clinical trial assessed the efficacy of a 1% itraconazole vaginal cream in patients suffering from vaginal candidiasis. The formulation was dosed daily for 3 days with an observation period at 1 week and at 1 month after dose initiation. At the trial end point, mycological cures were noted in 75% of patients treated with the itraconazole cream, and clinical cures were observed in 77% of patients. Symptomatic improvement was assessed using an investigator's global therapeutic impression and found to be 78% in the itraconazole group. The most frequent adverse events were headache and pruritus, and the formulation was well tolerated.

CONCLUSION

A cyclodextrin-based, emulsified wax cream containing itraconazole was found to be an effective delivery system for selective vaginal delivery. The formulation was not toxic to rabbit under either acute or subchronic exposure and was retained in the vaginal space. Clinical studies found that the formulation was well tolerated, was not systemically bioavailable, and was effective in combating vaginal candidiasis.

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