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The Release of Isoconazole Nitrate from Different Suppository Bases: In-vitro Dissolution, Physicochemical and Microbiological Studies

M. ASIKOGLU, G. ERTAN AND G. COSAR*

*Ege University, Faculty of Pharmacy, Pharmaceutical Technology Department, and *Microbiology Department, Izmir, Turkey*

Abstract

The influence of the suppository base on the in-vitro release of isoconazole nitrate was studied by dissolution, physicochemical diffusion and microbiological disk-diffusion methods. Vaginal suppository formulations containing 25 mg isoconazole nitrate for local treatment of vaginitis were prepared by a fusion method, using different hydrophilic and lypophilic suppository bases (PEG 6000, PEG 4000, PEG 1500, Witepsol H15, Novata BD and Cremao). In-vitro release rates were examined by dissolution, physicochemical diffusion and microbiological disk-diffusion methods. In the physicochemical investigations the pH indicators (pH 1-14) erythrosin B, thymol blue, bromocresol green, chlorophenol red, phenol red, and alkali blue were added to agar gels. The discs were placed onto the agar gels and 21 h later, the coloured zone diameters were measured. In the microbiological investigations, the discs were put on the inoculated plates with the suspension of *Candida albicans* (Institute Pasteur 628). The inoculated plates were incubated at 37°C for 3 days, then the diameters of inhibition zones were measured.

In the dissolution investigations release rates were in the order PEG 6000 > PEG 4000 > PEG 1500 > > Witepsol H15 > Cremao > Novata BD. The diffusion distance of isoconazole nitrate in the physicochemical investigation was in the order polyethylene glycols > Witepsol H15 > Novata BD. In the microbiological studies release rates were found with polyethylene glycols > Witepsol H15 > Novata BD > Cremao.

The findings in the in-vitro studies suggested that polyethylene glycols are suitable bases for vaginal suppositories.

Isoconazole nitrate is an antifungal agent with a range of antimicrobial activities (Martindale 1989). It is used in the treatment of vaginal mycoses, particularly due to *Candida* species, in a single dose of 600 mg in the form of pessaries (Hoffken et al 1983; Bradbeer & Thin 1985). It is often used in conjunction with the topical application of a 1% cream and some local reactions including burning and itching have been reported (Fechner & Gross 1981). During the study of healthy subjects it was observed that the slight amount of drug that was absorbed, was rapidly inactivated and excreted in urine (Martindale 1989). On intravaginal application, the hard surface of tablet and the number of ingredients may lead to irritation of the vaginal epithelium. The slippery and smooth surface of suppositories may facilitate application, and the irritation and burning in the vulvo-vaginal region will be less. The release of drug from a suppository is greatly dependent on the suppository formulation (Florence & Attwood 1981). The methods used for testing drug release rate characteristics of suppositories in-vitro can be classified in terms of five general types. The first type consists of simple placement of the suppository in a flask or beaker (Whitworth & LaRocca 1959; Pagay et al 1974). The second type utilizes an existing tablet dissolution apparatus with a wire-mesh basket for holding the sample (Kellaway & Marriott 1975; Parrott 1975). The third and fourth types employ a membrane; the third consists of a sample chamber separated from a reservoir by a membrane (Kapas et al 1979), whereas the fourth employs dialysis tubing or a natural membrane (Plaxco et al 1967). The fifth type involves a flow system in which the sample is placed on cotton or a wire screen (Puffer & Crowell 1973; Roseman et al 1981). According to the second type, dissolution-controlled drug release was investigated using a British Pharmacopoeia dissolution apparatus in this study. In addition, physicochemical diffusion and microbiological methods, which are applied for controlling the release of the active substance from the ointment bases, were applied to the suppositories. The purpose of this study was to investigate in-vitro release of isoconazole nitrate from different suppository bases to provide a formulation which would remain at the application site for sustained local action.

Correspondence: M. Asikoglu, Pharmaceutical Technology Department, Faculty of Pharmacy, Ege University, Izmir, Turkey.

Materials and Methods

Materials

Drug and chemicals were as follows: isoconazole nitrate (Schering AG Berlin, Germany); polyethylene glycol (PEG) 1500 and PEG 6000 (Hoechst AG Werk Gendorf, Burgkirchen); PEG 4000 and Witepsol H15 (Sandoz, Istanbul, Turkey); Cremao (Aarhus Olie fabrik A/S, Bruunsgade, Denmark); Novata BD (Henkel KGaA., Düsseldorf, Germany); erythrosin B, thymol blue, bromcresol green, chlorophenol red, phenol red, alkali blue, disodium hydrogen phosphate and potassium dihydrogen phosphate (Merck, Darmstadt, Germany); agar (Himedia, Atabay, Istanbul, Turkey).

All chemicals used were analytical grade, isoconazole nitrate and suppository bases were pharmaceutical grade, and were used as received.

Methods

Preparation of suppositories and discs. All suppositories containing 25 mg and discs containing 8 mg isoconazole nitrate were prepared by the fusion method (Remington's Pharmaceutical Science 1990) with PEG 1500, PEG 4000, PEG 6000, Cremao, Witepsol H15, Novata BD. Each suppository was torpedo shaped. The discs of different bases were prepared with 8 mg isoconazole nitrate and without drug (Fig. 1). The discs of different bases prepared without drug were used as the control discs. The lipophilic and hydrophilic suppository bases were melted in a water bath at 45 and 65°C respectively, isoconazole nitrate was added and mixed. The masses were cooled to 36–38°C with gentle stirring, poured into steel moulds and allowed to solidify at room temperature (21°C). The suppositories and discs were wrapped individually in aluminium foil. All suppositories and discs were stored in a refrigerator (+4°C) until they were used for experiments. Before use, all suppositories were allowed to stand for 24 h at room temperature.

Weight variation. Twenty suppositories from each base were weighed, and the average weight and percent deviation for each suppository was calculated.

Content uniformity tests. Five suppositories were randomly selected for each base and these were assayed individually. The suppository was melted and dissolved in ethanol/ether (1:1). One millilitre of sample was transferred to the 10-mL volumetric flask and the volume was adjusted to 10 mL with ethanol/ether (1:1). The absorbance was measured on a spectrophotometer (Shimadzu Double Beam Spectrophotometer UV-150-02 JAPAN) at 273 nm against the blank sample. The concentration of isoconazole nitrate in samples was calculated from the standard curve.

Melting time. The suppositories were stored at room temperature for at least 24 h then placed into a glass tube (2·5 cm diam.); 2 mL Sorensen's phosphate buffer (pH:7·8) was added. The tube was placed in a water-bath at 37 ± 0·5°C. The time required for each suppository to completely melt and disintegrate at 37 ± 0·5°C was determined.

Differential scanning calorimetry (DSC). Differential scanning calorimetry (DSC) (DSC92 France) analyses were carried out on samples of 8·1–9·9 mg. The suppositories prepared with the bases contained ~1·5% isoconazole nitrate and the discs prepared with PEG 6000 as an example of the hydrophilic base and Witepsol H15 as an example of the lipophilic suppository base containing ~17% drug were scanned in aluminium pans from 20 to 200°C at a rate of 10°C min^{-1} in air. The phase transition range of prepared suppositories was determined.

Hardness. The suppositories were stored for at least 24 h at 25°C. The hardness of each suppository was determined using a fracture-point testing apparatus (Erweka Apparatebau-GmbH Germany).

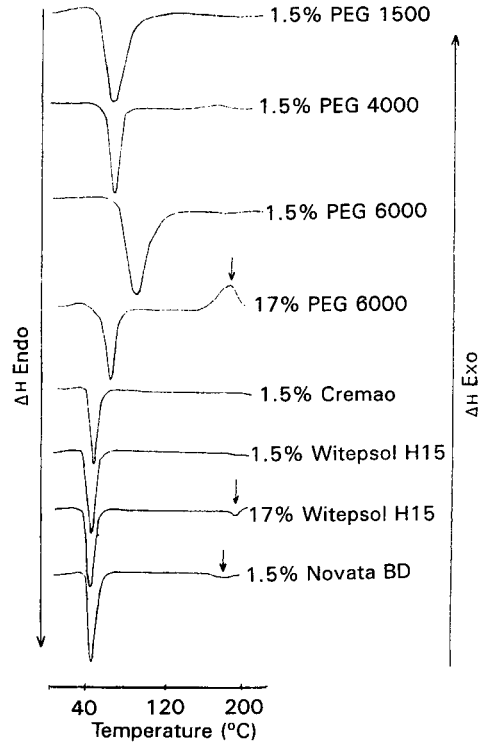

FIG. 1. The DSC scans obtained with isoconazole nitrate-mixed suppository bases, showing the melting endotherms and the degradation exotherm of the prepared suppositories.

Dissolution-controlled drug release. The British Pharmacopoeia dissolution apparatus was used for the determination of release rates in this work. Each suppository was placed in a flask containing 150 mL Sorensen's phosphate buffer solution (pH 7·8). The paddle was rotated at 100 rotations min^{-1} at a constant temperature (37 ± 0·5°C). Five millilitre samples were taken at different time intervals and the volumes were adjusted to 10 mL with ethanol. Five millilitres of phosphate buffer was added to the dissolution medium to compensate for sampling. The absorbances of these solutions were measured at 273 nm on a spectrophotometer against the blank sample and the drug concentrations were calculated from the standard curve.

Anticandidal testing. The anticandidal activity of the drug from different suppository bases was determined against *Candida albicans* (Inst. Pasteur 628) (Baver et al 1966; National Committee for Clinical Laboratory Standards 1984). The suspension of *Candida albicans* was streaked on double plates of Sabouraud dextrose-agar. The discs of different bases (polyethylene glycols, Novata BD, Cremao, and Witepsol H15) prepared with 8 mg drug and without drug, were placed on the inoculated plates and were incubated at 37°C for 3 days. The diameters of inhibition zones were measured and the radii were calculated as the inhibition distance. The discs for each base without isoconazole nitrate were used as the controls.

Physicochemical diffusion procedure. Physicochemical diffusion studies were carried out to show that the drug and not the base inhibits the growth of *Candida albicans*. In the

Table 1. The weight variation, content uniformity, melting time and hardness of suppositories.

Base	Weight variation		Content uniformity (% ± s.d.)	Melting time (min ± s.d.)	Hardness (kg ± s.d.)
	Suppository (g ± s.d.)	Disc (g ± s.d.)			
PEG 6000	1·979±0·013	0·045±0·00012	4·571±0·107	95·4±0·400	4·54±0·040
PEG 4000	2·020±0·009	0·044±0·00019	0·757±0·061	82·2±0·800	4·22±0·049
PEG 1500	2·070±0·074	0·046±0·00029	5·085±0·046	44·6±0·678	2·24±0·060
Witepslol H15	1·627±0·012	0·037±0·00020	1·814±0·055	13·6±0·245	4·32±0·058
Novata BD	1·650±0·021	0·036±0·00010	2·086±0·062	13·8±0·374	4·42±0·049
Cremao	1·593±0·008	0·036±0·00015	4·757±0·130	11·8±0·735	3·28±0·037

physicochemical investigations, 1·5 mL indicator solution was added to 24 mL 2% agar gel at 50°C. Isoconazole nitrate was formed as a disc with different suppository bases as described above. The control discs were prepared with each base without isoconazole nitrate, and were placed in holes made with the lower part of a hard gelatin capsule (size 4) in the agar gels at 37°C. The active substance diffused from suppository bases to the agar and coloured zones appeared. After 21 h, the release rate of isoconazole nitrate was determined by measuring the diameter of the coloured zones as a diffusion distance. It was also observed that the colours of the zones are the result of the active substance reacting with the indicators. Diffusion distances were calculated from the equation:

$$\text{Diffusion distance (cm)} = a - b/2 \quad (1)$$

where a is the diameter of the coloured zone and b is the diameter of the disc.

Indicators. The indicator solutions were as follows: erythrosin B (pH: 0·0–3·6), 0·1 g in 100 mL water; thymol blue (pH: 1·2–2·8), 0·04 g in 100 mL 20% ethanol; bromocresol green (pH: 3·8–5·4), 0·1 g in 100 mL 20% ethanol; chlorophenol red (pH: 4·8–6·4), 0·1 g in 100 mL 20% ethanol; alkali blue (pH: 9·4–14), 0·1 g in 100 mL 96% ethanol; and phenol red (pH: 6·4–8·2), 0·05 g in 2·85 mL 0·05 M NaOH with 5 mL 90% ethanol and water to make up to 100 mL.

Results and Discussion

The results of weight variation, content uniformity, melting time and hardness of suppositories are shown in Table 1. The weight variation and content uniformity of suppositories and discs were found to be within ± 5%.

A narrow melting range is important in maintaining the shape of the suppository and in controlling the melting time of the suppository after application. The melting times of isoconazole nitrate suppositories in the glass tube were in the order PEG 6000 > PEG 4000 > PEG 1500 > Novata BD = Witepsol H15 > Cremao. The melting point of isoconazole nitrate is 182–183°C. In the DSC studies (Fig. 1, Table 2), for the suppositories prepared with PEG 6000, PEG 4000, PEG 1500, Witepsol H15 and Cremao, containing 1·5% isoconazole nitrate, no sharp thermal event corresponding to the melting of isoconazole nitrate crystal domains was observed. Isoconazole nitrate forms a solid solution with the suppository bases. For the suppositories of isoconazole nitrate prepared with Novata BD (1·5%) and PEG 6000

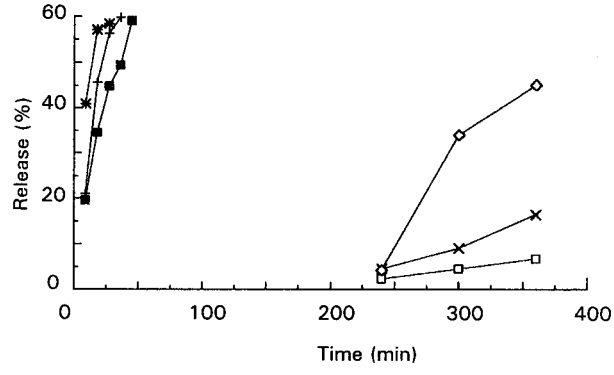

FIG. 2. The dissolution rates of isoconazole nitrate from different bases. ■ PEG 6000, + PEG 400, * PEG 1500, □ Novata BD, × Cremao, ◇ Witepsol H15.

Table 2. Experimental heats of melting obtained for the different suppositories compared with the theoretical melting points of the pour bases.

Sample	Mass (mg)	Phase transition range (°C)	Top of the peak (°C)	Onset temperature (°C)	Enthalpy H ($J\ g^{-1}$)	Theoretical melting point (°C)
PEG 6000 (1·5%)	9·7	66·08–117·72	86·07	72·26	−147·810	55–63
PEG 4000 (1·5%)	9·6	55·35–91.70	71·79	64·35	−169·966	50–58
PEG 1500 (1·5%)	9·9	51·10–97·76	69·96	57·57	−113·788	44–48
Witepsol H15 (1·5%)	8·9	35·34–65·97	48·32	42·08	−129·830	33·5–35·5
Novata BD (1·5%)	8·8	33·14–65·82	47.86	42.03	−124·908	33·5–35·54
		169·31–189·50	182·45	174·24	−2·631	
Cremao (1·5%)	9·6	35·68–68·17	49·47	43·01	−101·536	32–34
PEG 6000 (17%)	9·1	44·61–84·01	68·09	59·16	−101·488	
		158·46–196·77	181·94	160·82	35·114	
Witepsol H15 (17%)	8·6	31·78–61·65	45·64	39·93	−93·038	
		179·02–190·43	184·18	180·23	−1·378	

Table 3. The inhibition and physicochemical diffusion distance of isoconazole nitrate.

Base	Inhibition distance (cm)	Diffusion distance (cm)				Chlorophenol red	Alkali blue	Phenol red
		Erythrosin B	Thymol blue	Bromocresol green				
PEG 6000	1.80	0.955	0.935	1.010	0.945	0.905	0.850	0.900
PEG 4000	1.75	0.920	0.905	0.910	0.845	0.820	0.835	0.890
PEG 1500	1.70	0.875	0.785	0.880	0.785	0.815	0.770	0.855
Witepsol H15	1.75	0.765	0.735	0.830	0.690	0.820	0.660	0.685
Novata BD	1.55	0.750	0.680	0.795	0.685	0.795	0.650	0.665
Cremao	0.80	—	—	—	—	—	—	—

and Witepsol H15 containing ~17% drug, the melting event of isoconazole nitrate was clearly visible on the DSC scans.

The order of suppository hardness was PEG 6000 > Novata BD = Witepsol H15 > PEG 4000 > Cremao > PEG 1500 (Table 1).

Using the British Pharmacopoeia apparatus, the amount of isoconazole nitrate release is shown in Fig. 2. The release of active substance from the polyethylene glycols was rapid due to the high water-solubility of the bases; sustained release was observed for the oily bases.

In the microbiological investigations, the discs were prepared by using different suppository bases. Isoconazole nitrate diffused through the agar and inhibition zones were formed. The inhibiting capability of the active substance on the growth of *Candida albicans* is shown in Table 3. It was observed that the release of the drug from suppository bases is different. Higher release rates were obtained from PEG 6000, PEG 4000, Witepsol H15 and PEG 1500. Lower release rates were obtained from Cremao and Novata BD.

In this study, the control discs prepared with the PEG bases without active substance prevented the growth of *Candida albicans*, but lypophilic suppository bases (without isoconazole nitrate) did not stop or prevent the growth of the fungi. Polyethyleneglycols do not support microbial growth, nor do they become rancid. The antibacterial activity of certain antibiotics, particularly penicillin and bacitracin, is reduced in polyethyleneglycol bases (Price et al 1986) but polyethyleneglycols did not decrease the antibacterial activity of isoconazole nitrate in this study.

The release of isoconazole nitrate increases depending on the size of the polyethylene glycol. Polyethylene glycols have the general chemical formula $HOCH_2(CH_2OCH_2)nCH_2OH$, where n represents the average number of oxyethylene groups. The highest drug release was found for PEG 6000 and the inhibition distances were as follows: PEG 6000, 1·80 cm (n > 84); PEG 4000, 1·75 cm (n = 69–84); PEG, 1·70 cm (n = 30–36).

The diffusion distances were measured in the physicochemical investigation. The coloured borders caused by isoconazole nitrate reacted with indicators added to the agar gel were determined. The drug diffused from suppository bases to the agar and coloured zones appeared. The diameters of the coloured zones were measured and the diffusion distances were calculated. There was only a slight increase in the release of isoconazole nitrate from Cremao and the diffusion distance could not measured. The release of isoconazole nitrate was in the order PEG derivatives > Witepsol H15 > Novata BD.

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