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The Release of Isoconazole Nitrate from Different Suppository Bases: In-vivo Release of Drug Labelled with ^{99m}Tc in Rabbits

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Abstract

The influence of the suppository bases on the in-vivo release of ^{99m}Tc-labelled isoconazole nitrate was investigated. The single-dose vaginal suppository formulations for local treatment of vaginitis were prepared by a fusion method using polyethylene glycols, Witepsol H15, Novata BD and Cremao.

Prepared vaginal suppositories containing solid-labelled substance were applied to the vagina of rabbits and at 0, 2 and 24 h after administration, the amounts of radioactivity in the vagina were detected by the SPECT Gamma Camera and the release rates of the drug were calculated. The percent released was found to be in the following order; polyethylene glycol (PEG) 6000 > PEG 4000 > Witepsol > PEG 1500 > Novata BD > Cremao. The results obtained in both in-vitro and in-vivo studies indicated that the vaginal suppository of isoconazole nitrate prepared with polyethylene glycols could confidently be used in therapy.

Suppositories, administered either vaginally or rectally, are utilized as a dosage form for various drugs. The vaginal route is mainly used for the achievement of local effects, e.g. in the case of *Trichomonas* and *Candida* infections. Some drugs are, however, vaginally administered to achieve systemic effects (Blaey & Tukker 1988). Passive drug absorption via the vaginal mucosa as with other mucosal tissues, is influenced by absorption site, pH and the solubility and partition characteristics of the drug. Following intravaginal administration, some drug absorption from the intact vaginal mucosa is likely, even when the drug is employed for a local effect. Although extensive drug absorption can occur from the vagina, only limited reports of research on in-vitro and in-vivo aspects of vaginal absorption have appeared in the literature (Remington's Pharmaceutical Sciences 1990).

Since there are very few means of obtaining in-vivo release information this will usually have to be interpreted from in-vitro release, which introduces the problem of in-vitro/in-vivo correlation. The present accumulation of knowledge does not permit the choice of an in-vitro method with a high predictive power for in-vivo performance. There are two main classes of vehicles in use, the glyceride-type fatty bases and the water-soluble bases. Although the ideal suppository base has not been found, the large variety of bases which is available enables a well-considered choice for drugs that need to be formulated as suppositories. Choosing the optimum base requires much practical experience and it can at present only partly be guided by scientifically sound data (Blaey & Tukker 1988). The release of a drug from a suppository is greatly dependent on the suppository formulation (Suleiman & Najib 1990). Some studies have been made to develop rectal and vaginal suppositories (Ondracek et al 1988).

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Materials and Methods

The following chemicals were used as received: isoconazole nitrate obtained from Schering (Schering AG Berlin, Germany); polyethylene glycols whose average molecular weights were 1500, 4000, 6000 Da; lipophilic suppository bases, Novata BD, Cremao and Witepsol H15; PEG 6000 and PEG 1500 (Hoechst AG Werk Gendorf, Burgkirchen, Germany); PEG 4000 and Witepsol H15 (Sandoz, Istanbul, Turkey); Novata BD (Henkel KGaA., Düsseldorf, Germany); stannous chloride (Merck, Darmstadt, Germany). ITLC/SG Silica gel impregnated glass fiber sheets, (Gelman Instrument Company, Ann Arbor, Michigan, USA) were used for instant thin-layer chromatography. The membrane filter (Type HA, 0·45μ pore size, Millipore, Bedford, MA, USA) was used as a filter.

Labelling of isoconazole nitrate with ^{99m}Tc

Isoconazole nitrate (10 mg) was dissolved in 1 mL ethanol. One millilitre of water and 0·3 mL stannous chloride solution were added and mixed well. The mixture was filtered from the Millipore filter (0·45μ pore size) into a sterile bottle and 10 mCi ^{99m}Tc ($^{99m}TcO_4$-) solution was added, diluted with ethanol to 3mL, and left for incubation at room temperature (21°C) for 10 min.

For the preparation of stannous chloride solution, 10 mg stannous chloride ($SnCl_2 \cdot 2H_2O$) was dissolved in 10 mL water, purged with nitrogen gas to remove dissolved oxygen.

The radiochemical purity was confirmed with instant thin-layer chromatography ministrips developed with 0·9% NaCl. The labelled compound stayed at the origin and pertechnetate moved with the solvent front. The percentage of labelling was calculated by dividing the number of counts at the origin by the total radioactivity on the strips.

The TLC technique was used for the estimation of radiochemical purity. The TLC techniques, most commonly used in hospital radiopharmacy, are so-called miniaturized procedures for ^{99m}Tc radiopharmaceuticals. These techniques were developed from the chromatographic systems originally reported by Billinghurst (1973) and many reports have described systems for the routine analysis of ^{99m}Tc radiopharmaceuticals (Zimmer & Pavel 1977). The stationary phases used in these miniaturized procedures are Instant Thin-Layer Chromatography (ITLC, Gelman) and 31 ET paper (Whatman) and a regimen which is based entirely on ITLC and which has been found useful for many commonly used ^{99m}Tc radiopharmaceuticals.

The influence of temperature on the radiochemical purity

The influence of the temperature on the radiochemical purity of ^{99m}Tc-labelled compound used during the preparation of suppositories was investigated. Radiochemical purity of the solution of ^{99m}Tc-isoconazole nitrate used in the labelled suppositories was calculated. Two millilitres of this solution was dried with N_2 at 60°C and was dissolved in 2 mL ethanol. Radiochemical purity of these solutions was determined as described above and compared with the initial values (Millar 1989).

Preparation of labelled suppositories

All suppositories, containing 7 mg isoconazole nitrate and 2 mCi ^{99m}Tc each, were prepared by the fusion method, using different hydrophilic and lipophilic bases. A stock solution was prepared by dissolving 100 mg isoconazole nitrate in 10 mL ethanol. A solution of 3 mL stannous chloride (1 mg mL^{-1}) was added. The mixture was stirred and filtered (Type HA, 0·45 μm pore size). Part (0·91 mL) of the filtrate was taken for each suppository. An appropriate amount of pertechnetate was added. A sufficient amount of ethanol was added to make a solution containing approximately 2 mCi ^{99m} Tc, which was incubated for 10 min. One milliliter of the ^{99m}Tc-isoconazole nitrate solution was taken for each suppository and the mixture was heated to 60°C drying with nitrogen, then the required amount of each suppository base was calculated and mixed. The suppositories were prepared using the dried ^{99m}Tc-isoconazole nitrate with different bases obtained by the fusion method. The labelled isoconazole nitrate was mixed in the melted suppository bases (the lipophilic and hydrophilic bases at 45 and 65°C, respectively). The resultant base mixture was poured into the moulds and allowed to solidify at room temperature. The prepared cylindrical suppositories for in-vivo studies containing 7 mg drug were 1·67 cm long with a diameter of 0·65 cm. The suppositories weighed approximately 0·5 g.

In-vivo application of suppositories in rabbits

Twenty four female rabbits, 2·5-3 kg, were kept in a temperature-controlled room (25°C) with free access to water and a regular diet and were randomly divided into six groups of four animals each.

The single-dose suppositories of ^{99m}Tc-labelled isoconazole nitrate were administered intravaginally. The vaginal suppositories containing 2 mCi ^{99m}Tc-labelled isoconazole nitrate were evaluated using the gamma camera. The animals were placed in a supine position under the detector of the camera. Scintigraphic images were obtained and the initial value of the radioactivity and the value of radioactivity after 2 and 24 h at the same geometry were monitored using a SPECT Gamma Camera fitted with a low-energy, parallel-hole collimator. The initial count rate (C_i) in the vagina was accepted as 100% of the administered dose. The other count rates (after 2 h, 24 h) were compared with the initial value. Activity in the vagina was derived from the number of counts of this organ. An ROI (region of interest) was drawn around the vagina in the images and the counts from a background ROI drawn in a low-count density region were subtracted from the vagina counts after correction for the relative number of pixels in each ROI. The amount of isoconazole nitrate released from the vagina was determined from mass balance calculations. The percentage of release (R) in the vagina at each time was obtained by the equation:

$$R_1 = C_i - C_t/C_i \times 100 \quad (1)$$

where C_i is the geometric mean of counts immediately after administration and C_t is the geometric mean of vagina counts at time t after administration.

Results and Discussion

The in-vivo release of ^{99m}Tc-labelled isoconazole nitrate from different suppository bases is summarized in Table 1.

For the evaluation of local effectiveness of drugs, the residence time and the concentration of the drug on the application site is important. The duration of local treatment of vaginal mycoses with imidazoles showed a striking change. Initially starting with a 14-day therapy, the duration of treatment was continuously shortened to 10 days then to 7 and 6 days and finally to 3 days (Tauber et al 1984). Once-only therapy of vaginal mycoses with isoconazole nitrate obviously leads to long-lasting fungicidal concentration of the antimycotic at the site of infection–the vaginal epithelium and the vaginal secretion–which explains the high healing rates. Probably high concentrations of imidazoles over a limited time period are more effective than low concentrations over a longer time period as previously reported for nystatin (Rumler & Hein 1982). The release of isoconazole nitrate from PEG bases was rapid and it appears that the PEGs may make a more

Table 1. Isoconazole nitrate release rates.

Base	Time (h)	Release (% ± s.d.)
PEG 6000	2	28·83 ± 6·33
	24	94·50 ± 1·42
PEG 4000	2	25·56 ± 3·97
	24	94·12 ± 0·55
PEG 1500	2	12·15 ± 4·33
	24	91·71 ± 1·83
Witepsol H15	2	17·33 ± 0·55
	24	93·20 ± 0·52
Novata BD	2	11·01 ± 1·95
	24	91·22 ± 0·89
Cremao	2	14·50 ± 1·95
	24	89·51 ± 0·70

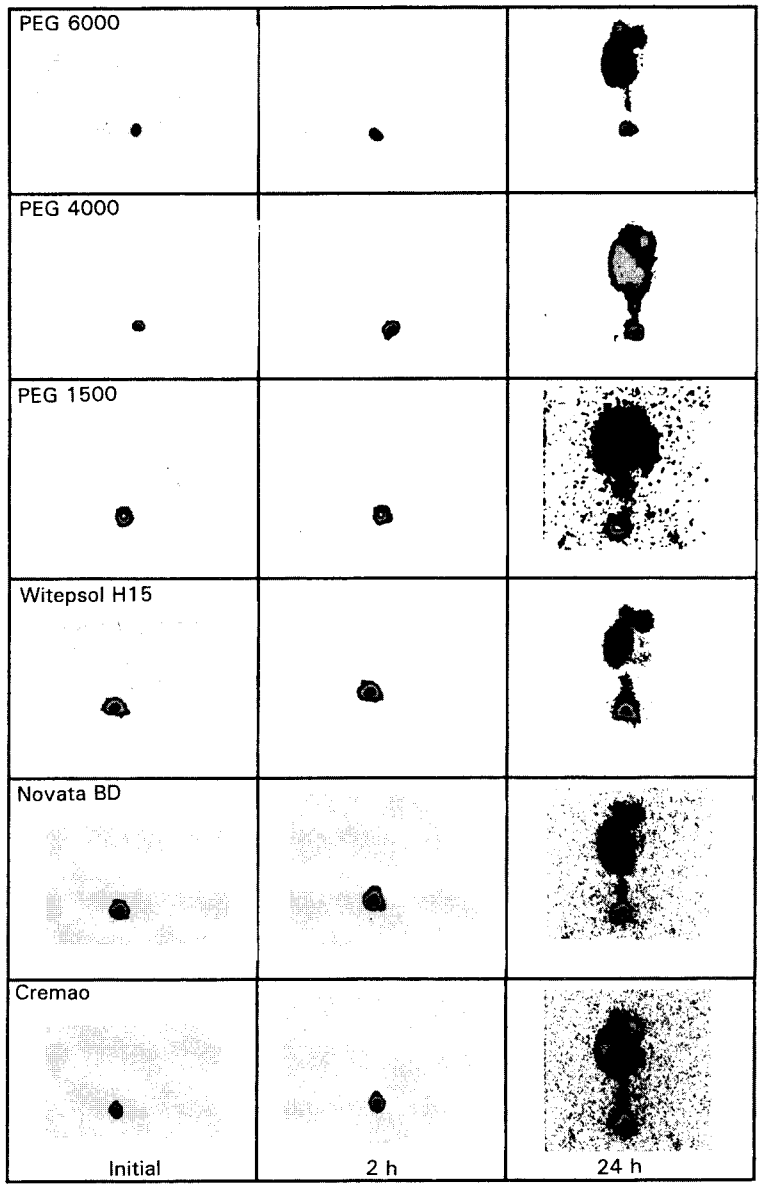

FIG. 1. The scintigraphic images of the release of ^{99m}Tc-labelled isoconazole nitrate from different bases within 0, 2 and 24 h.

satisfactory base for the immediate treatment of *Candida* infections. The percent released was found at 24 h to be in the following order: PEG 6000 > PEG 4000 > Witepsol H15 > PEG 1500 > Novata BD > Cremao. These results were in agreement with those obtained from in-vitro studies.

The scintigraphic images of rabbits obtained at 0, 2 and 24 h post-administration are presented for all six bases in Fig. 1. The release of radioactivity from the initial location of the suppository could not be detected visually on the 2-h image, but was clearly observed on the 24-h image, which showed absorption of radioactivity from the vagina and location in some organs. The highest release of isoconazole nitrate was found in PEG derivatives. The lowest release was found from the suppositories prepared with Cremao, which remained locally in the vagina, but, in the in-vitro studies with the suppositories prepared with Cremao, the antifungal effect was less than that of the other bases against *Candida albicans*.

The influence of temperature on the radiochemical purity was investigated. The results showed that the radiochemical purity was greater then 99% and unaffected by temperature under the study conditions (at 60°C and with N_2).

Our results showed that ^{99m}Tc-labelled isoconazole nitrate produced good quality radioscintigraphic images within 2–24 h following administration. The method is non-invasive, safe, simple and without any side-effects. Its cost, convenience, availability, and physical characteristics make ^{99m}Tc the most desirable radionuclide for imaging. This study demonstrated the feasibility of monitoring the release characteristics of suppositories by scintigraphic methods. This method can be used with other drugs and with different bases in future studies.

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