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Persistence of Isoconazole in Vaginal Secretion after Single Application

Verweildauer von Isoconazol im Vaginalsekret nach einmaliger Applikation

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Summary: After intravaginal application of 2 vaginal tablets each containing 300 mg isoconazole nitrate, isoconazole concentration in vaginal secretion above MBC- and MIC-levels of Candida spec. was maintained for at least 72 hours. 7 days after insertion no isoconazole could be detected in the vagina.

Obviously the single dose therapy of vaginal mycoses with isoconazole nitrate is as effective and reliable as multi-dose-therapies due to the high long lasting isoconazole levels at the site of infection. Doubts that the high cultural cure rates observed 7 days after application in clinical trials may be caused by still available antimycotic in the vagina are not justified.

Zusammenfassung: Nach intravaginaler Applikation von 2 Vaginaltabletten mit jeweils 300 mg Isoconazolnitrat lagen die Isoconazolkonzentrationen im Vaginalsekret mindestens 72 Stunden lang über den MBK- und MHK-Werten für Candida-Keime. 7 Tage nach dem Einführen konnte kein Isoconazol mehr in der Vagina nachgewiesen werden.

Die mehrere Tage anhaltenden hohen Isoconazol-Spiegel am Ort der Infektion sind offensichtlich der Grund dafür, daß sich die Ein-Dosis-Therapie von Vaginalmykosen mit Isoconazolnitrat als ebenso wirksam und verläßlich erwies als Mehr-Dosis-Therapien. Zweifel, daß die hohen kulturellen Heilungsraten 7 Tage nach der Applikation durch noch vorhandenen antimykotischen Wirkstoff in der Vagina vorgetäuscht seien, sind nicht gerechtfertigt.

Introduction

The duration of local treatment of vaginal mycoses with imidazoles showed a striking change during the last 10 years. Initially starting with a 14-days-therapy (1) the duration of treatment was continuously shortend to 10 days then to 7 and 6 days and finally to 3 days (2). Isocona-

zole nitrate was the first imidazole with enabled a once-only-treatment of vaginal moniliasis (3).

From pharmacokinetic studies with ^{3}H-labeled isoconazole nitrate the following hypothesis on the mode of action has been discussed: Intravaginal administration of two tablets with 300 mg isoconazole nitrate creates a local depot which long lasting high concentrations of isoconazole in the vaginal secretion and the vaginal epithelium (4). The present study in young healthy women was undertaken to determine the concentration of isoconazole in the vaginal secretion as a function of the time after a single application of 2 vaginal tablets with isoconazole nitrate by means of a microbiological method.

Subjects and Methods

2 vaginal tablets each containing 300 mg isoconazole nitrate* were inserted intravaginally to 6 healthy non-pregnant female volunteers aged between 29 and 37 years. The application was performed by a gynaecologist at the department of clinical endocrine pharmacology of Schering AG.

Collection of Secretions

Samples of vaginal secretion were collected just before insertion and at intervals up to 10 days afterwards. The samples were collected with sterile sponge swabs** (size: 2 × 2 cm) tied with cotton to wooden applicator sticks. Swabs were weighed before and after sampling for determination of actual weight of the secretion. The sponge swabs were placed in barrels of 5 ml plastic syringes. Immediately after sampling 400 to 800 µl of phosphate buffer (0.06 Mol/l Na_2HPO_4, 0.003 Mol/l KH_2PO_4, pH 7.9 – + 0.1% Triton® WR) was added and fluid rapidly expressed from the swabs by pressure from a syringe plunger. The average amount of secretion was 88 ± 45 mg.

The addition of buffer was necessary because it proved difficult to obtain the secretion eluted from the swabs without dilution with buffer. At a pH 7.9 the solubility of isoconazole nitrate is very low, thus preventing dissolution of undissolved crystals which might falsify the results.

Estimation of Isoconazole Concentration

Isoconazole concentrations in vaginal fluid were measured by agar plate bioassay. An indicator strain of Candida albicans, grown for 48 hours on Sabouraud Dextrose agar, was seeded to a concentration of 10^4 cells/ml in agar containing yeast nitrogen base (Difco Laboratories).

The seeded agar was poured in flat rectangular large (24 × 34 cm) glass vessels. A series of wells, each 4 mm in diameter, were cut and 25 µl of vaginal fluid samples and standard solution of isoconazole nitrate of 4, 8, 16, 31, 63, 125, 250 and 500 µg/ml prepared in phospate buffer from a stock solution of 500 µg/ml in phospate buffer (+ 5% Ethanol, 0.1% Triton® WR) were placed in the wells according to a randomisation scheme devised in advance. The dishes of agar were stored at room temperature for 4 hours (prediffusion-time) and afterwards incubated at 37°C for 48 hours.

The isoconazole concentration in the elution fluid was determined from diameters of inhibition zones by reference to a standard curve of log isoconazole concentration against zone diameter. The concentration in the original (undiluted) vaginal secretion sample was calculated according to equation (1).

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*Gyno-Travogen®, Schering AG Berlin
**Spongostan®, Ferrosan, Denmark

Table 1
Isoconazole concentration in elution medium (A) and in undiluted vaginal secretion (B) in µg/ml

A. Elution medium (results of bioassay)

Time after dose (days)	Subject No. 1	2	3	4	5	6	Q25	median	Q75
0	0	0	0	0	0	0	0	0	0
0.25	3.6	5.6	125	70	82	19	5.1	44.5	93
1	150	110	180	155	135	150	129	150	161
3	35	5.8	100	4.8	3.6	3.8	3.8	5.3	51
5	6.8	0	7.5	0	0	0	0	0	7
7	0	0	0	0	0	0	0	0	0
10	0	0	0	0	0	0	0	0	0

B. Vaginal secretion (calculated from A by means of equation [1])

Time after dose (days)	Subject No. 1	2	3	4	5	6	Q25	median	Q75
0	0	0	0	0	0	0	0	0	0
0.25	28	31	690	388	455	105	30	247	514
1	6300	930	1380	1370	760	1650	888	1375	2812
3	207	35	417	53	15	28	25	44	259
5	28	0	37	0	0	0	0	0	30
7	0	0	0	0	0	0	0	0	0
10	0	0	0	0	0	0	0	0	0

0 detection limit

$$(1)\ C_{VS} = \frac{C_{EL} \cdot (V_{EL} + V_{VS})}{V_{VS}}$$

C_{VS} = isoconazole concentration in vaginal secretion (µg/ml)
C_{EL} = measured isoconazole concentration after elution (µg/ml)
V_{EL} = volume of buffer added into the syringe for elution in ml
V_{VS} = original volume of vaginal secretion in ml (= weight in g × density)

Results

The time course of the isoconazole concentration in vaginal secretion of individual subjects is summarized in Table 1B. Isoconazole concentration in vaginal secretion increased during the first day reaching its maximum value of 1375 µg/ml (median-value) 24 hours after administration.

From the evaluation of the individual time courses a half-life for isoconazole in the vaginal secretion of 10–12 hours can be estimated. In 4 of 6 subjects zero levels were already obtained 5 days after administration. 7 days after administration no isoconazole was available in the vagina in any subject.

Discussion

In the local therapy of vaginal mycoses the availability of the antifungal agent in dissolved form at the site of infection is a necessary prerequisite for an effective therapy. At least minimal inhibitory, better fungicidal concentrations should be maintained for a time interval which is sufficient to reduce the number of fungi to a level which can be controlled by the body itself.

The present study is a pharmacokinetic contribution to the understanding of the mechanism of action of isoconazole in the once-only-treatment of vaginal mycoses. Table 1 shows that isoconazole is available in the vaginal secretion in all test subjects in multiples of the fungicidal concentrations (0.1–10 µg/ml) for at least 3 days.

The isoconazole levels in the vaginal secretion are considerably higher than those reported for miconazole (5). The very high clotrimazole levels in vaginal fluid - 68.1±42.9 mg/ml have been measured 24 hours after insertion of a 500 mg tablet - cannot be compared to our values, because they mainly represent undissolved suspended material (6).

High isoconazole concentration ranging between 1500–2700 µg/ml have been measured also in the vaginal epithelium (4).

Oral administration of ketoconazole led only to low levels of the antimycotic in the vaginal tissue (<1 µg/ml) which decayed rapidly with a half life of 1.4 hours. Ketoconazole concentrations in the vaginal secretions were not presented but thought to be much higher because of ion-trapping (8).

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 (9, 10). 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 (11).

7 days after application isoconazole was completely removed from the vagina in all subjects. Therefore 7 day healing rates - determined as absence of fungal growth in culture - are real healing rates and cannot be explained by the presence of remaining antimycotic in the vagina.

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