Safety, Tolerability and Pharmacokinetics of Intravaginal Pentamycin

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Key Words
Antimicrobial agents · Pentamycin · Pharmacokinetics · Polyene antifungal antibiotic · Vaginal infections

Abstract
Background/Aims: Intravaginal pentamycin is a polyene macrolide with a broad spectrum of antimicrobial activity and is effective in various forms of infectious vaginitis. We evaluated the safety, tolerability and pharmacokinetics of escalating doses of this product. Methods: Nineteen healthy volunteers were randomized to receive double blind one of five doses of intravaginal pentamycin (3, 10, 30, 60 or 100 mg) or the corresponding dose of pentamycin vehicle daily for 6 days. Patients with symptomatic vaginitis received a single dose of 60 (n = 6) or 100 mg (n = 6) of intravaginal pentamycin. Safety and tolerability parameters were monitored throughout the study. Plasma concentrations of pentamycin were measured daily in the healthy volunteers and on the day of drug application in the patients. Results: The most frequently reported adverse events were mild or moderate vaginal discharge and mild symptoms of vaginal irritation (mainly pruritus or burning sensation), which also occurred in women who applied the vehicle. No patient with symptomatic vaginitis reported treatment-related adverse events. The plasma levels of pentamycin were below the quantification limit in all samples. Conclusion: Intravaginal pentamycin does not cause adverse reactions compared with vehicle and is not absorbed through the intact or the inflamed vagina.

Introduction
The most common vaginal disorders include vaginal candidiasis, vaginal trichomoniasis and bacterial vaginosis [1–5]. Forms of infectious vaginitis sustained by mixed micro-organisms are also observed frequently [6]. Vaginal infections can be successfully treated by using one or more pathogen-specific antimicrobials [1, 3, 7–9], but, before vaginitis is treated, the cause must be ascertained by using appropriate laboratory tests [1, 2, 6, 7, 10]. Because some strains of Candida albicans resistant to topical azole antifungals [3, 11] and strains of Trichomonas vaginalis resistant to treatment with metronidazole and tinidazole [12–14] have been recently isolated, it is also necessary to verify the susceptibility of micro-organisms to the recommended antimicrobials in certain cases [3]. Therefore, many physicians find it difficult to diagnose accurately and manage these disorders in clinical practice [15]. The availability of a drug effective in more than one form of infectious vaginitis would reduce the need for a confirmatory diagnosis and the risk of treatment failure.
Pentamycin is a polyene macrolide [16, 17] with a broad spectrum of antimicrobial activity [16, 18–20]. The 3-mg dose strength of intravaginal pentamycin (Prurix®, 3-mg vaginal tablet) is registered in Switzerland for the treatment of various forms of infectious vaginitis (vaginal candidiasis, mixed infections and trichomoniasis). The currently approved treatment regimen is 3–6 mg daily for 5–10 days. A higher-dosed vaginal tablet of pentamycin (10-mg dose strength) may be effectively used for shorter treatment periods than the 3-mg dosage strength and is currently under clinical development. The purpose of this study was to evaluate the safety, tolerability and systemic absorption of escalating doses of intravaginal pentamycin in healthy female volunteers and in volunteers with symptomatic vaginitis.

Patients and Methods

Ethics
The study was conducted in full compliance with the principles of the Declaration of Helsinki III and in accordance with the international guidelines of Good Clinical Practice. The study protocol was approved by an independent Ethics Committee (Ethische Kommission beider Basel, Basel, Switzerland). Written informed consent to participate in the study was obtained from all subjects prior to study start.

Part A of the Study
Part A was a double-blind, randomized, vehicle-controlled, escalating dose study in 19 female healthy volunteers (mean age: 28.5 years; age range: 21–36 years). The inclusion and exclusion criteria are listed in table 1. The screening visit was performed between 24 h and 20 days before inclusion (day 1) and consisted of a medical history interview, collection of demographic characteristics, physical examination, gynecological examination with colposcopy, measurement of pulse rate and blood pressure after 3 min of rest in the supine position and after 1 min of standing, 12-lead electrocardiogram (ECG), collection of blood and urine samples for laboratory tests, pregnancy test (for women in reproductive age), and review of the inclusion and exclusion criteria. On day 1, the enrolled women were allocated to five dose groups as it follows:

- Group 1: 3 mg of pentamycin or only vehicle (1 vaginal tablet) daily for 6 days;
- Group 2: 10 mg of pentamycin or only vehicle (1 vaginal tablet) daily for 6 days;
- Group 3: 30 mg of pentamycin or only vehicle (1 vaginal tablet) daily for 6 days;
- Group 4: 60 mg of pentamycin or only vehicle (2 vaginal tablets, containing each 30 mg of pentamycin or only vehicle, in single application) daily for 6 days;
- Group 5: 100 mg of pentamycin or only vehicle (1 vaginal tablet) daily for 6 days.

Each dose was administered to 4 subjects. Within each group, subjects were assigned 3:1 to treatment with the active compound or to the vehicle alone under blind conditions, according to a randomization code. Subjects allocated to Groups 2–5 received treatment only if women treated with the study drug in group 1 had not reported significant adverse events (AEs). One of the 19 enrolled women received more than one treatment (groups 1 and 4). This procedure was allowed by protocol and there were at least 2 weeks of wash-out between completion of the first treatment and initiation of the second one. Drug application was avoided during menses. Male partners were instructed to use condoms during the treatment period and up to 48 h following the last application of the vaginal tablets.

Subjects returned daily to the clinic for drug supply, recording of AEs and evaluation of the plasma concentrations of pentamycin. Additional visits were conducted on day 3 (3rd day of treatment), on day 7 (24 h after the last dose), and 8 ± 2 days after the last treatment day (follow-up visit). A physical examination was performed at each of these visits. Pulse rate and blood pressure were recorded on the day of treatment start and on the last day of treatment. On both occasion, the pulse rate and blood pressure were measured after 3 min of rest in the supine position and after 1 min of standing. The complete gynecological examination, ECG recording and laboratory assessment were repeated on day 7. Blood sampling for the evaluation of pentamycin plasma concentrations was performed on day 1 before the first dose and then at 30 min, 1, 2, 4, 6, 12 and 24 h after the first dose. On days 2–5, blood sampling was performed only pre-dose. On day 6 (last application), blood sampling was performed as on day 1. The last blood sampling was performed 24 h after the last dose (day 7).

All vaginal tablets were prepared by an independent organization (Fisher Clinical Services GmbH, Allschwil, Switzerland) and had similar aspect, shape and color. The randomization list was provided by the study sponsor (Lumavita AG, formerly Shogoo Pharmaceutical AG, Basel, Switzerland). The investigators received code-breaking envelopes, containing the identity of the treatment dispensed to each subject. Breaking the code was allowed only in case of a clinical emergency and upon receiving prior approval from the sponsor. Any code break had to be justified in the case report form. At the end of the study, the unused code-breaking envelopes were collected by the monitor for verification.

Part B of the Study
Part B was an open-label study in 12 female volunteers (mean age: 32.7 years; age range: 19–59 years) with symptomatic vaginitis. The inclusion and exclusion criteria are listed in table 1. The screening visit was performed between 24 and 48 h before inclusion and treatment (day 1). It consisted of a medical history interview, collection of demographic characteristics, physical examination, gynecological examination with colposcopy, recording of pulse rate and blood pressure, collection of blood and urine samples for laboratory tests, pregnancy test (for women in reproductive age), and review of the inclusion and exclusion criteria. The cause of vaginitis was investigated by using saline wet mount and the potassium hydroxide test. On day 1, 6 women were asked to apply two vaginal tablets, each containing 30 mg of pentamycin (total dose: 60 mg in a single application). In absence of intolerances, the remaining 6 women were asked to apply one vaginal tablet, containing 100 mg of pentamycin. Blood sampling for determination of pentamycin plasma concentrations was performed pre-dose and then at 1, 3 and 8 h post-dose. The occurrence of AEs was registered during the entire post-dose period of observation.

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Dosing was not performed during menses, and male partners were instructed to use condoms for 48 h following the application of the vaginal tablets.

Assessment of AEs, Laboratory Parameters and Plasma Levels of Pentamycin

All systemic and local AEs were recorded in the case report forms. For each AE, the investigator had to obtain adequate information concerning the intensity, causal relationship with treatment and outcome. AEs that met the criteria for classification as serious AEs were immediately reported to the sponsor.

For the laboratory parameters, the normal range values were the standard reference values of the central laboratory of the University Hospital of Basel (Switzerland) where the analyses were performed. Plasma levels of pentamycin were measured by an independent laboratory (Swiss BioAnalytics AG, Birsfelden, Switzerland) using high-performance liquid chromatography for separation of plasma proteins and mass spectrometry for detection of pentamycin concentrations. The lower limit of quantification of the assay was 5 ng/ml.

Results

Part A of the Study

All enrolled subjects completed part A of the study and were evaluated for safety and tolerability. No major differences in the occurrence of AEs could be detected across the dose groups and between the subjects treated with the active compound and those who received the vehicle (table 2). There was no report of serious AEs, and all reported AEs were events of mild or moderate severity in most cases, with the sole exception of one case of severe abdominal pain in group 2 (table 2), which was not considered to be related to study drug. Mild symptoms of vaginal irritation (mainly pruritus or burning sensation) were reported in women who applied 30 or 60 mg of pentamycin daily (group 3 and 4) and also in women who applied the vehicle alone (group 1 and 2; table 2). The AEs
listed as metrorrhagia according to the MedDRA Medical Dictionary (table 2) were actually cases of intermediate bleeding that predominantly occurred in women with a history of irregular menses and intermediate bleeding and were not considered to be treatment related. In group 3, one woman who received the active compound experienced several AEs. Two of these events were not considered to be treatment-related (viral infection and vaginal burning sensation; table 2). The other events included vaginal hemorrhage, vaginal discharge, vulvovaginal pruritus, and vaginal pain and were all considered as possibly or probably related to study drug (table 2). About 4 weeks after the last application of intravaginal pentamycin, this subject experienced micturition urgency and cystitis, which were also considered by the investigator as possibly related to study drug (table 2). The woman was treated for 5 days with a combination of trimethoprine and sulfamethoxazole and symptoms resolved completely.

All subjects who received the active treatment or the vehicle reported the appearance of mild or moderate vaginal discharge from the 2nd or 3rd day of study drug application (table 2). The discharge was usually white-grey and not disturbing, except for underwear staining. This AE was suspected to be due to an excipient of the vaginal tablet, and a sample of the discharge from women in group 5 was taken for analysis. Five batches of film-coated tablets, containing the active ingredient, and the residues of drug product after application (vaginal discharge) were compared by high-performance liquid chromatography followed by mass spectrometry and ultraviolet detection. This analysis excluded the presence of the active ingredient in the vaginal discharge.

The gynecological examination performed within 24 h after the last application of study drug confirmed the presence of vaginal discharge in most cases at the speculum exam but did not reveal alterations of the vaginal mucosa. Parts of incompletely dissolved vaginal tablets were found in 7 women who had received the active treatment (one in group 2, one in group 3, 3 in group 4, and 2 in group 5). According to the investigator, this finding is commonly observed with locally applied vaginal discharge.

### Table 2. AEs, duration, and causality assessment for part A of the study

<table>
<thead>
<tr>
<th>Group 1</th>
<th>Group 2</th>
<th>Group 3</th>
<th>Group 4</th>
<th>Group 5</th>
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<tr>
<td><strong>AEs considered as not related or unlikely related to study drug</strong></td>
<td></td>
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<tr>
<td>Active treatment (n = 3/group)</td>
<td>Vehicle (n = 1/group)</td>
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<tr>
<td>1 Metrorrhagia (T)</td>
<td>Tendinitis (2 days)</td>
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<td>1 Abdominal pain (1 day)</td>
<td>Headache (3 days)</td>
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<td>1 Aphthous stomatitis (1 day)</td>
<td>Malaise (3 days)</td>
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<tr>
<td>1 Metrorrhagia (T)</td>
<td>Nausea (1 day)</td>
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<td><strong>AEs considered as possibly or probably related to study drug</strong></td>
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<td>Active treatment (n = 3/group)</td>
<td>Vehicle (n = 1/group)</td>
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<tr>
<td>3 Vaginal discharge (T-2 days)</td>
<td>Vaginal burning sensation (1 day)</td>
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<tr>
<td>1 Vulvar erythema (T)</td>
<td>Vaginal discharge (3 days)</td>
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<tr>
<td>3 Vaginal discharge (T-4 days)</td>
<td>Abdominal pain (2 days)</td>
<td>Pelvic pain (T)</td>
<td>Vaginal discharge (3 days)</td>
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<tr>
<td>3 Vaginal discharge (1–4 days)</td>
<td>Pelvic pain (T)</td>
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<tr>
<td>2 Abdominal pain (1–5 days)</td>
<td>Vaginal discharge (3 days)</td>
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<tr>
<td>1 Abdominal discomfort (7 days)</td>
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<tr>
<td>1 Cystitis (4 days)¹</td>
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<tr>
<td>1 Micturition urgency (4 days)¹</td>
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<tr>
<td>1 Vaginal hemorrhage (4 days)</td>
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<tr>
<td>1 Vaginal pain (unchanged at follow-up)</td>
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<tr>
<td>1 Vulvar edema (NR)</td>
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<tr>
<td>1 Vulvovaginal pruritus (T)</td>
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T = Transient AE, resolving on the day of its occurrence; NR = not recorded.

¹ This AE occurred 26 days after treatment end.
tions (bacterial vaginosis and candidiasis). No case of trichomoniasis was identified. In 6 cases, the vaginal discharge specimen was sent to the local Institute of Microbiology, although microbiology testing was not required by protocol. The isolation of *C. albicans* and/or *Gardnerella vaginalis* confirmed the diagnosis of candidiasis, mixed infections or bacterial vaginosis in these women.

Five of 6 patients in the 60-mg dose group reported the following AEs: vaginal burning sensation (2 women), vaginal discharge (2 women), pruritus (one woman), vaginal pain (one woman) and cystitis (one woman). Only one of the patients who received the highest dose of pentamycin reported an AE (cystitis). All events were mild or moderate in severity and were considered as not related or unlikely related to study drug. Except for one case of vaginal discharge (60-mg dose group) and one case of cystitis (100-mg dose group) the events resolved promptly after appropriate treatment (started 8 h following the application of study drug). The plasma levels of pentamycin were below the limit of quantification in all samples.

**Discussion**

Pentamycin exhibits a broad spectrum of antimicrobial activity in vitro and in vivo [16, 18–20]. In particular, its in vitro activity against various *Candida* species is similar to that of theazole antifungal agent miconazole and its activity against trichomonads is similar to that of metronidazole. Pentamycin is also active against several pathogenic bacterial strains, including *G. vaginalis* and other strains involved in bacterial vaginosis and mixed infections, but it is inactive against the lactobacilli that colonize the normal vagina [19]. Because of the high molecular weight (molar mass = 670.85 g/mol) and bipolar molecular structure [21], topically applied pentamycin has a low potential of penetrating through the intact mucosa into the circulation. Compared to other antimicrobials recommended for the treatment of vaginal infec-
or drug candidates is also superior to other potential therapeutic options because of a broader spectrum of antimicrobial activity, it is also superior to other potential therapeutic options [23] or drug candidates [24]. The favorable tolerability profile of this drug is supported by the absence of reports of adverse reactions in the population of over 40,000 patients who have been treated with intravaginal pentamycin in clinical practice since the first registration in Switzerland of the 3-mg dose strength (data on file at Lumavita AG, Basel, Switzerland).

As part of the clinical development program for a higher-dosed vaginal tablet of pentamycin (10-mg dose strength), this study evaluated the safety, tolerability and systemic absorption of escalating doses of intravaginal pentamycin. We found that the administration of increasing doses of intravaginal pentamycin up to a maximal dose of 100 mg daily for 6 days in healthy women did not result in a dose-dependent increase in the risk of systemic or local AEs as compared to the vehicle alone. Most reported AEs were of mild or moderate intensity. There was no serious AE and no discontinuation due to AEs. Irrespective of the dose group and treatment received (either the active compound or the vehicle), all women reported the appearance of a white-grey vaginal discharge from the 2nd or 3rd day of study drug application. The vaginal discharge did not contain the active ingredient and was probably due to an excipient of the vaginal tablets. The gynecological examination performed after treatment completion did not reveal any abnormality of the vaginal mucosa. In a woman who received the active treatment, cystitis was diagnosed approximately 4 weeks after the last dosing. The time elapsed between treatment completion and the occurrence of symptoms of cystitis would suggest a doubtful causal relationship between the event and the application of intravaginal pentamycin. Moreover, the subject had reported a viral infection not considered as treatment-related during the treatment period and this AE may have represented a confounding factor. Blood pressure, ECG parameters and laboratory parameters did not change significantly in relation to the treatment received during the study period or in relation to the dose of the active compound. The intravaginal application of pentamycin did not result in a detectable systemic absorption of the active compound into the circulation because the plasma levels of pentamycin were invariably below the detection limit of 5 ng/ml, irrespective of the dose of pentamycin administered. The total observation period was 120 treatment days (90 for the active compound and 30 for the placebo) and the highest dose applied was 10× the assumed therapeutic dose of 10 mg daily that should be given for 3 days.

The AEs reported by patients with symptomatic vaginitis who applied a single dose of either 60 or 100 mg of intravaginal pentamycin in the part B of this study were all mild or moderate local events. These AEs were not considered to be treatment related and were probably due to the underlying disease. Notably, the administration of a dose of intravaginal pentamycin that is 10× the assumed therapeutic dose of 10 mg daily in these patients with inflamed vagina was not associated with the presence of detectable levels of the active ingredients in the circulation.

In conclusion, our study demonstrates that the intravaginal administration of pentamycin at a dose as high as 100 mg daily for up to 6 days does not cause an increase in the occurrence of local or systemic adverse reactions in comparison with the vehicle alone and is not associated with systemic absorption of the active ingredient through the intact or the inflamed vagina.

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