

Safety, Tolerability and Pharmacokinetics of Intravaginal Pentamycin

B. Frey Tirri^a J. Bitzer^a B. Geudelin^c J. Drewe^b

Departments of ^aObstetrics and Gynecology, and ^bClinical Pharmacology, University Hospital Basel, and ^cMediante GmbH, Basel, Switzerland

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.

Copyright © 2010 S. Karger AG, Basel

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.

Table 1. Inclusion and exclusion criteria

Inclusion criteria	Exclusion criteria
<p><i>Part A of the study</i></p> <p>Age range: 18–45 years. Body mass index: 19–25. Use of a reliable contraception method. Intact vaginal mucosa. Healthy status, as determined by medical history, physical examination, and 12-lead electrocardiogram at screening. Normal clinical laboratory tests at screening. Negative tests for hepatitis B (surface antigen), hepatitis C and human immunodeficiency virus at screening. Ability to understand the protocol and give informed consent.</p>	<p>Pregnant or lactating women. History of genital problems that might interfere with the interpretation of results; acute gynecological symptoms at the time of screening or inclusion; significant abnormalities of the vaginal epithelium at the screening colposcopy. Diseases involving other organs or systemic disorders; presence of an inflammatory process or an acute infection at screening or inclusion; abnormal liver or renal function. Unwillingness to suspend the use of other vaginal products, such as douches, tampons, spermicides or herbal preparations during the study period. History of relevant drug hypersensitivity. Smoking; history of drug abuse or alcoholism, or consumption of more than 21 units of alcohol a week. Use of prescription drugs or over-the-counter medications within 2 weeks of first dosing; use of investigational drugs within 1 month of first dosing. Inability to communicate reliably with the investigator or to cooperate properly during the study.</p>
<p><i>Part B of the study</i></p> <p>Age ≥18 years. Symptoms of vaginitis, including itching, burning, leukorrhea and xanthorrhoea, and dyspareunia. Confirmed diagnosis of bacterial vaginosis, candidiasis, or trichomoniasis. Negative Papanicolaou smear documented less than 1 year before screening. Use of a reliable contraception method. Ability to understand the protocol and give informed consent.</p>	<p>Pregnant or lactating women. Abnormal liver or renal function; recent serious medical conditions that made the patients unsuitable for study participation. Presence of a concomitant infection that needed an additional antimicrobial agent. Unwillingness to suspend the use of other vaginal products, such as douches, tampons, spermicides or herbal preparations, during the study period. History of relevant drug hypersensitivity. Smoking; history of drug abuse or alcoholism, or consumption of more than 21 units of alcohol a week. Use of investigational drugs within 1 month prior to screening. Inability to communicate reliably with the investigator or to cooperate properly during the study.</p>

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

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

Group 1	Group 2	Group 3	Group 4	Group 5
<i>AEs considered as not related or unlikely related to study drug</i>				
Active treatment (n = 3/group)				
1 Metrorrhagia (T)	1 Abdominal pain (1 day) 1 Aphthous stomatitis (1 day) 1 Metrorrhagia (T)	1 Diarrhea (T) 1 Malaise (4 days) 1 Viral infection (1 day) 1 Vaginal burning sensation (3 days) 1 Vulvovaginal pruritus (T)	1 Flatulence (T) 1 Laryngitis (5 days) 1 Nasopharyngitis (4 days)	1 Dizziness (T) 1 Headache (T) 1 Perianal erythema/eczema (13 days) 1 Pityriasis (23 days)
Vehicle (n = 1/group)				
Tendinitis (2 days)		Headache (3 days) Malaise (3 days) Nausea (1 day)	Back pain (1 day) Conjunctival irritation (2 days) Pelvic pain (1 day)	
<i>AEs considered as possibly or probably related to study drug</i>				
Active treatment (n = 3/group)				
3 Vaginal discharge (T-2 days) 1 Vulvar erythema (T)	3 Vaginal discharge (T-4 days)	3 Vaginal discharge (1–4 days) 2 Abdominal pain (1–5 days) 1 Abdominal discomfort (7 days) 1 Cystitis (4 days) ¹ 1 Micturition urgency (4 days) ¹ 1 Vaginal hemorrhage (4 days) 1 Vaginal pain (unchanged at follow-up) 1 Vulvar edema (NR) 1 Vulvovaginal pruritus (T)	3 Vaginal discharge (5–13 days) 1 Vaginal burning sensation (2 days) 1 Vaginal pain (1 day) 1 Vulvovaginal pruritus (7 days)	3 Vaginal discharge (5–6 days)
Vehicle (n = 1/group)				
Vaginal burning sensation (1 day) Vaginal discharge (4 days)	Vaginal discharge (3 days) Vulvovaginal pruritus (2 days)	Abdominal pain (2 days) Vaginal discharge (3 days)	Pelvic pain (T) Vaginal discharge (11 days)	Vaginal discharge (3 days)
T = Transient AE, resolving on the day of its occurrence; NR = not recorded.				
¹ This AE occurred 26 days after treatment end.				

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 trimethoprim and sulfamethoxazole and symptoms resolved completely.

All subjects who received the active treatment or the vehicle reported the appearance of mild or moderate vag-

inal 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

Table 3. Blood pressure recording for part A of the study

Treatment	Mean blood pressure on day 1, mm Hg				Mean blood pressure on day 7, mm Hg			
	supine		standing		supine		standing	
	SBP	DBP	SBP	DBP	SBP	DBP	SBP	DBP
Active treatment								
Group 1	111	70	116	69	110	69	104	75
Group 2	106	70	109	73	117	76	101	67
Group 3	112	70	111	77	110	67	103	76
Group 4	112	68	116	76	110	67	109	71
Group 5	115	71	118	79	106	68	113	79
Vehicle	107	67	120	72	108	68	114	72

SBP = Systolic blood pressure; DBP = diastolic blood pressure. Active treatment: n = 3/group; vehicle: n = 5, 1/group.

tablets. Persistent vaginal discharge was reported at the follow-up visit by 5 women: 4 had received the active treatment (one in group 1, one in group 3 and 2 in group 5) and one had received the vehicle (group 5). One case of vaginal pain in a woman who received the active treatment in group 3 (table 2) was also reported as unchanged at the follow-up visit. This AE started 2 days before the end of the treatment period. Two women who received the active treatment in group 5 experienced perianal erythema/eczema and pythiasis versicolor, respectively, during the study period (table 2). None of these AEs were considered to be related to study drug and were treated with specific topical agents. The other AEs resolved spontaneously by the end of the study.

There were no substantial changes in blood pressure from the time of treatment start to the end of the treatment period across the dose groups and between the subjects treated with the active compound and those who received the vehicle (table 3). On ECG recordings, sinus bradycardia (sinus rate <60 beats/min) was noted in 5 cases at the screening visit and was confirmed at the end of the treatment period only in one case. This woman was the one who reported a mild and short-lasting episode of dizziness during the application of the active compound in group 5 (table 2) and such event was not considered as treatment-related. In another woman, bradycardia was not present at the screening visit but was detected on day 7. This subject had received the vehicle. The laboratory parameters did not show significant changes attributable to the type of treatment received or to the dose of active ingredient applied during the treatment period. The plas-

ma levels of pentamycin were below the limit of quantification in all samples.

Part B of the Study

All enrolled women reported symptoms of vaginitis, including itching, burning, leukorrhea and xanthorrhea, and dyspareunia, as required by the protocol. The cause of vaginitis was investigated by using saline wet mount and the potassium hydroxide test. Four women were diagnosed with bacterial vaginosis and 8 with mixed infections (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 the azole 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 vaginoses 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-

tions [1, 3], which are pathogen-specific and require laboratory tests to ascertain the nature of the causative agent, intravaginal pentamycin offers the advantage of being effective in more than one form of vaginitis [20, 22]. 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 observa-

tion 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.

Acknowledgements

This study was sponsored by Lumavita AG (formerly Shogoo Pharmaceutical AG), Basel, Switzerland. The investigators of the study (Dr. B. Frey Tirri, Prof. J. Bitzer and Prof. J. Drewe) have no additional potential conflicts of interest to disclose. Dr. B. Geudelin received consultancy fees from the sponsor. The sponsor did not have a direct role in the collection, analysis and interpretation of the data or in the writing of the manuscript. Medical writing support was provided by Avail GmbH, Basel, Switzerland.

References

- 1 Owen MK, Clenney TL: Management of vaginitis. *Am Fam Physician* 2004;70:2125–2132.
- 2 Egan ME, Lipsky MS: Diagnosis of vaginitis. *Am Fam Physician* 2000;62:1095–1104.
- 3 Center for Disease Control and Prevention: Sexually transmitted diseases treatment guidelines, 2006. *MMWR* 2006;55(RR-11):1–94.
- 4 Klebanoff MA, Schwebke JR, Zhang J, Nansel TR, Yu KF, Andrews WW: Vulvovaginal symptoms in women with bacterial vaginosis. *Obstet Gynecol* 2004;104:267–272.
- 5 Weir E: Bacterial vaginosis: more questions than answers. *Can Med Assoc J* 2004;171:448–501.
- 6 Soper DE: Taking the guesswork out of diagnosing and managing vaginitis. *Contemporary OB/GYN* 2005;50:32–39.

- 7 Landers DV, Wiesenfel HC, Heine RP, Krohn MA, Hillier SL: Predictive value of the clinical diagnosis of lower genital tract infection in women. *Am J Obstet Gynecol* 2004;190:1004–1010.
- 8 Mikamo H, Kawazoe K, Izumi K, Ito K, Katoh N, Watanabe K, Ueno K, Tamaya T: Bacteriological epidemiology and treatment of bacterial vaginosis. *Chemotherapy* 1996;42:78–84.
- 9 Mikamo H, Kawazoe K, Sato Y, Hayasaki Y, Tamaya T: Comparative study on the effectiveness of antifungal agents in different regimens against vaginal candidiasis. *Chemotherapy* 1998;44:364–368.
- 10 Hill LVH, Embil JA: Vaginitis: current microbiologic and clinical concepts. *Can Med Assoc J* 1986;134:321–331.
- 11 Ferris DG, Nyirjesy P, Sobel JD, Soper D, Pavletic A, Litaker MS: Over-the-counter antifungal drug misuse associated with patient-diagnosed vulvovaginal candidiasis. *Obstet Gynecol* 2002;99:419–425.
- 12 Rosoloso D, Vanáčová S, Tomková E, Rázga J, Hrdy I, Tachezý J, Kulda J: Mechanisms of in vitro development of resistance to metronidazole in *Trichomonas vaginalis*. *Microbiology* 2002;148:2467–2477.
- 13 Crowell AL, Sanders-Lewis KA, Secor WE: In vitro metronidazole and tinidazole activities against metronidazole-resistant strains of *Trichomonas vaginalis*. *Antimicrob Chemother* 2003;47:1407–1409.
- 14 Kanno M, Sobel JD: Late recurrence of resistant *Trichomonas vaginalis* vaginitis: relapse or re-infection? *Sex Transm Infect* 2003;79:260–261.
- 15 Cleveland A: Vaginitis: finding the cause prevents treatment failure. *Cleve Clin J Med* 2000;67:634–642, 645–646.
- 16 Umezawa S, Tanaka Y, Ooka M, Shiotsu S: A new antifungal antibiotic, pentamycin. *J Antibiot (Tokyo)* 1958;11:26–29.
- 17 Zygmunt WA: Intracellular loss of potassium in *Candida albicans* after exposure to polyene antifungal antibiotics. *Appl Microbiol* 1966;14:953–956.
- 18 Cusumano V, Zamboni C, Leonardi MS: The antimycotic activity of pentamycin (in Italian). *G Ital Chemioter* 1977;24:109–111.
- 19 Dubini F: Attività della pentamicina nelle candidosi vaginali. *Riv Ost Gin Perin* 1987;1:45–53.
- 20 Braga A: Preliminary clinical trials of pentamycin (in Italian). *Clin Ter* 1980;92:137–142.
- 21 Sweetman S (ed): Martindale: The Complete Drug Reference. London, Pharmaceutical Press. <http://www.medicinescomplete.com>; accessed on 16 November 2009.
- 22 Balmer JA: Treatment of vaginal infections with intravaginal pentamycin in clinical practice. *Internet J Gynecol Obstet* 2009;11. http://www.ispub.com/journal/the_internet_journal_of_gynecology_and_obstetrics/volume_11_number_1_6/article/treatment_of_vaginal_infections_with_intravaginal_pentamycin_in_clinical_practice.html; accessed on 29 April 2009.
- 23 Polosini I, Scarpignato C: Rifamixin, a peculiar rifamycin derivative: established and potential clinical use outside the gastrointestinal tract. *Chemotherapy* 2005;51(suppl 1):122–130.
- 24 Sitheequ MAM, Panagoda GJ, Yau J, Amarakoon AMT, Udagama URN, Samaranyake LP: Antifungal activity of black tea polyphenols (catechins and theaflavins) against *Candida* species. *Chemotherapy* 2009;55:189–196.