CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER:

21-806

MICROBIOLOGY REVIEW

MICROBIOLOGY REVIEW
DIVISION OF SPECIAL PATHOGEN AND IMMUNOLOGIC DRUG PRODUCTS (HFD-590)

NDA #: 21-806

REVIEWER	: Kalavati Suvarna
CORRESPONDENCE DATE	: 07-19-04
CDER RECEIPT DATE	: 07-19-04
REVIEW ASSIGN DATE	: 08-16-04
REVIEW COMPLETE DATE	: 04-25-05

SPONSOR: TEVA Pharmaceuticals
1090 Horsham Road, PO Box 1090,
North Wales, PA 19454-1090.

SUBMISSION REVIEWED: N-000

DRUG CATEGORY: Anti-bacterial

INDICATION: Treatment of bacterial vaginosis

DOSAGE FORM: 0.75% Vaginal gel

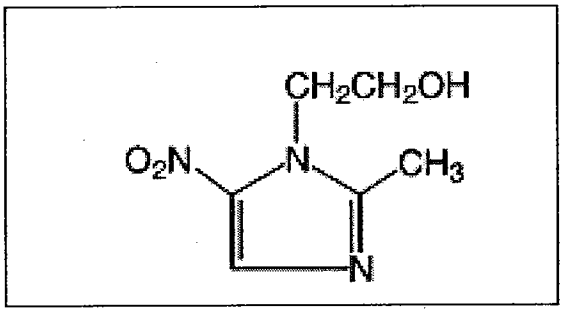
PRODUCT NAMES:

a. **PROPRIETARY:** Metronidazole vaginal gel

b. **NONPROPRIETARY:** Metronidazole

c. **CHEMICAL:** 1H-Imidazole-1-ethynol, 2-methyl-5-nitro-2-methyl-5-nitroimidazole-1-ethanol.

STRUCTURAL FORMULA:



Molecular weight: 171.15
Empirical Formula: $C_6H_9N_3O_3$

SUPPORTING DOCUMENTS: None

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1. EXECUTIVE SUMMARY:

The subject of this NDA is Metronidazole vaginal gel, administered intravaginally once or twice a day for 5 days for the treatment of bacterial vaginosis (BV). The applicant is seeking approval under section 505(b)(2) of the Federal Food, Drug and Cometic Act as the drug product contains the same active ingredient (0.75% metronidazole) as the approved reference drug, MetroGel-Vaginal®.

The support for the preclinical microbiology aspects of the application is based on the approved label for MetroGel-Vaginal®. *In vitro,* metronidazole is active against most strains of *Gardnerella vaginalis, Bacteroides* sp., *Mobiluncus* sp., and *Peptostreptococcus* sp., which are associated with BV. Metronidazole has minimal activity against vaginal *Lactobacillus* isolates *in vitro.* In a study evaluating the effect of metronidazole on vaginal lactobacilli colonization, an improvement in lactobacilli colonization was observed at EOT and at 3 weeks after discontinuation of therapy.

In the clinical study TCR-03, the efficacy of Metronidazole vaginal gel was greater (63%) than MetroGel-Vaginal® (46%) in the treatment of bacterial vaginosis. The study only provided information on the nugent scores using vaginal swabs at baseline and post-treatment. No information was available on the species of bacteria at baseline and post-treatment in patients enrolled in the clinical study. Therefore, the activity of metronidazole against various organisms associated with BV could not be analyzed. However, the cure by nugent criteria suggests that there was an increase in *Lactobacillus* morphotypes and decrease in the *Gardnerella, Bacteroides* and *Mobiluncus* morphotypes in 74% patients treated with metronidazole vaginal gel compared to 61% treated with MetroGel-Vaginal®, at 16 to 25 days after discontinuation of therapy.

2. INTRODUCTION AND BACKGROUND:

The subject of this NDA is Metronidazole vaginal gel, administered intravaginally once or twice a day for 5 days for the treatment of bacterial vaginosis (BV). Metronidazole (0.75%), the active ingredient of Metronidazole vaginal gel, has been approved as a prescription product (MetroGel-Vaginal®) for treatment of BV. Besides metronidazole, clindamycin is approved for the treatment of BV.

Bacterial vaginosis is a common vaginal condition seen in reproductive age women. Patients with BV show an increase in vaginal pH, and milky discharge with fishy odor. The etiology of bacterial vaginosis is thought to be due to replacement of the *Lactobacillus* sp. in the vagina with high concentrations of anaerobic bacteria (for example, *Prevotella* sp., *Mobiluncus* sp., *Gardnerella vaginalis,* and *Mycoplasma hominis*).

In healthy female volunteers administered a single 5 gram intravaginal dose of metronidazole vaginal gel, the mean maximum serum metronidazole concentration was 0.28 µg/ml and the area under the concentration versus time curve (AUC) for metronidazole was 6 µg.hr/ml. The drug concentration in the vaginal tissue was not determined.

3. PRECLINICAL MICROBIOLOGY:

The sponsor is relying on the approved label for MetroGel-Vaginal®, to support the preclinical microbiology section of the label.

3.1. Mechanism of action:

No new studies describing the mechanism of action of metronidazole against bacteria associated with BV were included in this submission.

3.2. Activity *in vitro*:

In vitro, metronidazole is active against most strains of *Gardnerella vaginalis*, *Bacteroides* sp, *Mobiluncus* sp, and *Peptostreptococcus* sp., that are associated with BV (See MetroGel-Vaginal®, approved label).

Activity of metronidazole against *Lactobacillus*

The activity of metronidazole against vaginal isolates of *Lactobacillus* species (*L. casei*, n = 4; *L. acidophilus*, n = 3; *L. jensenii*, n = 1) from healthy subjects was examined *in vitro* (Simoes *et al.*, 2001, Infect Dis Obstet Gynecol., 9: 41-45). The broth microdilution method described in the [redacted] M100-S9 was used to measure *in vitro* susceptibility to metronidazole. No other drug was used as a comparator. At concentrations of 1000 – 4000 µg/ml, metronidazole partially inhibited the growth of *Lactobacillus* (Figure 1). Concentrations of ≥5000 µg/ml showed complete inhibition of growth of lactobacilli up to 12 hours with slight growth between 12 and 20 hours. The percent inhibition of growth of all isolates exposed to metronidazole concentrations of >5000 µg/ml ranged from 86 to 92% at 24 hours (Table 1).

Figure 1: Median *Lactobacillus* growth in metronidazole concentrations ranging from 1000 to 7000 µg/ml.

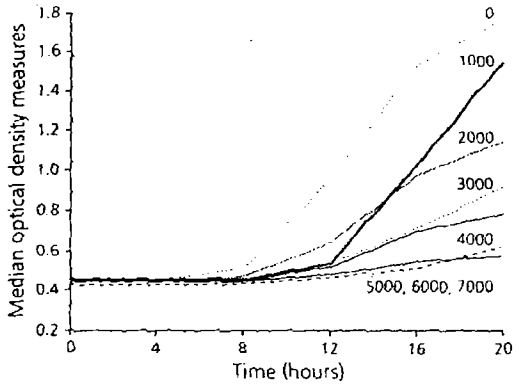


Table 1: Percentage of growth inhibition of lactobacilli by high concentrations of metronidazole (after 24 hours).

	Metronidazole concentration (µg/ml)						
Clinical isolate	*7000*	*6000*	*5000*	*4000*	*3000*	*2000*	*1000*
L. acidophilus (29)	91.1	87.0	88.6	66.7	76.9	58.8	29.0
L. acidophilus (117)	86.1	86.0	70.3	58.8	35.4	19.8	15.4
L. acidophilus (160)	95.1	93.1	84.2	66.5	61.0	37.6	13.2
L. casei (30)	98.9	98.9	96.9	96.6	90.0	90.7	84.7
L. casei (102)	99.8	98.5	94.8	76.2	73.6	48.5	20.8
L. casei (66)	85.6	86.7	73.0	72.2	57.0	57.1	23.2
L. casei (130)	89.5	96.7	93.9	86.8	84.4	65.5	30.0
L. jensenii (135)	92.9	84.6	82.7	75.2	64.8	54.8	21.7

3.3. Activity *in vivo*:

No studies were conducted to evaluate the activity of metronidazole against *Lactobacillus* sp., or the organisms associated with BV *in vivo*.

3.4. Drug Resistance:

Studies examining the development of resistance by BV pathogens to metronidazole were not conducted.

3.5. Cross Resistance:

Studies to examine the development of cross resistance between metronidazole and other antibacterial drugs were not conducted.

3.6. Drug combination:

Studies examining the activity of metronidazole in combination with other antibacterial agents against BV pathogens were not conducted.

3.7. Activity of metabolites:

The 2-hydroxymethyl metabolite of metronidazole has been observed in plasma. However, no studies examining the activity of the 2-hydroxymethyl metabolite against BV pathogens were included in this submission.

4. CLINICAL MICROBIOLOGY:

4.1. Study TCR-03:

This was a multicenter, randomized, double-blind, parallel group study conducted to evaluate the safety and efficacy of metronidazole vaginal gel in 579 non-pregnant women (≥ 18 years of age) with bacterial vaginosis. Subjects exhibiting signs and symptoms of bacterial vaginosis i.e., characteristic homogenous off-white discharge without inflammation, vaginal pH ≥ 4.7, ≥ 20% clue cells in wet mount slides, and positive KOH whiff test were enrolled. Subjects with a gram stain nugent score < 4 [which is based on the morphotype score of the type of organisms (*Lactobacilli*, *Gardnerella/Bacteroides*, and *Mobiluncus* species) observed under oil immersion field in a smear made using a vaginal swab, see Table 2] were excluded. Subjects with cervical neoplasia or vulvovaginal or genital infections such as vulvovaginal candidiasis, trichomonal vaginitis, gonorrhoea, chlamydia or genital herpes were also excluded. Patients were randomized (1:1) to receive metronidazole vaginal gel or MetroGel-Vaginal® intravaginally, once daily for 5 days.

Table 2: Nugent scoring system

Nugent Scoring System for Gram's Stained Vaginal Smears			
SCORE*	Lactobacillus morphotypes	*Gardnerella/Bacteroides* spp. morphotypes	Curved Gram-variable rods
0	**4+	0	0
1	3+	1+	1+ or 2+
2	2+	2+	3+ or 4+
3	1+	3+	
4	0	4+	

* Morphotypes are scored as the average number seen per oil immersion field (minimum of 10-20 fields should be examined). Each morphotype is then given a score from the left hand column. The TOTAL SCORE is calculated by adding up the individual morphotype scores = Lactobacillus + Gardnerella/Bacteroides + Curved Gram-negative rods.

** QUANTIFICATION SCALE: 0 = no morphotypes seen; 1+ = <1 morphotype per field; 2+ = 1 to 4 morphotypes; 3+ = 5 to 30 morphotypes; 4+ = >30 morphotypes per field.

Mobiluncus species represented by curved gram-variable rods

The primary efficacy endpoint was therapeutic cure (clinical cure and a gram stain nugent score ≤ 3) at 21 to 30 days after initiation of therapy (16 to 25 days after discontinuation of therapy). Clinical cure was defined as the resolution of clinical signs and symptoms with normal vaginal discharge (negative whiff test, negative for clue cells in wet mount, and a normal vaginal pH of < 4.7). The secondary efficacy endpoints were (a) analysis of therapeutic cure rates at 7 to 14 days after initiation of therapy (2 to 9 days after discontinuation of therapy), and (b) adverse events by treatment group.

The subjects were evaluated for clinical and microbiologic outcomes using vaginal specimens at baseline, visit 2 (2 to 9 days after discontinuation of therapy), and at the test of cure visit 3 (16 to 25 days after discontinuation of therapy). Microbiologic measurements using vaginal specimens included (1) wet mount and gram stain examination, (2) 10% KOH whiff test, and (3) vaginal pH determination.

Of the 579 patients enrolled in the study, 314 patients (metronidazole vaginal, n = 155; MetroGel – Vaginal®, n = 159) were included in the evaluable per protocol population (defined as patients who met all inclusion/exclusion criteria, received at least 3 consecutive days of therapy and no more than 6 days of therapy, and had no study violations). Patients who were defined as failures at 2 to 9 days after discontinuation of therapy were included in the evaluable per protocol population by using the last observation carried forward (LOCF) method. Of the 314 patients, gram stain nugent score at Visit 2 (2 to 9 days after discontinuation of therapy), and visit 3 (16 to 25 days after discontinuation of therapy) were not obtained for 22, and 60 patients, respectively. For the purpose of this review, these patients were excluded from analysis. Therapeutic cure was observed in 63% patients treated with metronidazole vaginal gel compared to 46% patients treated with MetroGel-Vaginal®, at 16 to 25 days after discontinuation of therapy (Table 3). As expected, the therapeutic cure rates at 2 to 9 days were slightly higher than that at 16 to 25 days, after discontinuation of therapy. Using data on patients that received an antifungal drug for the treatment of vaginal yeast infection, it appears that 10% of patients in the metronidazole vaginal gel arm and 17% in the MetroGel-Vaginal® arm developed a yeast infection. Previous human experience has shown that 6-10% patients treated with metronidazole develop a yeast infection. The reason for higher percentage of patients developing yeast infection in the MetroGel-Vaginal® arm compared to metronidazole vaginal gel arm is unclear. Based on the nugent score, an increase in *Lactobacillus* morphotypes and decrease in the *Gardnerella*, *Bacteroides*, and *Mobiluncus* morphotypes occurred in 74% patients treated with metronidazole vaginal gel compared to 61% with MetroGel-Vaginal®. However, the activity of metronidazole against the different bacterial species associated with BV could not be analyzed as the bacteria at baseline and post-treatment were not speciated and only nugent scores were determined.

Table 3: Efficacy of Metronidazole vaginal gel 0.75% versus Metro Gel-Vaginal® in the evaluable patients who had a nugent score and clinical outcome at the 2 visits (study TCR-03).

Outcome	**Metronidazole vaginal gel 0.75% N = 155**	**MetroGel-Vaginal® N = 159**
Visit 2 (2-9 days after discontinuation of therapy)		
Therapeutic cure	98/145 (68%)	76/147 (52%)
Cure by Nugent criteria	108/145 (74%)	93/147 (63%)
Visit 3 (16-25 days after discontinuation of therapy)		
Therapeutic cure	80/128 (63%)	58/126 (46%)
Cure by Nugent criteria	95/128 (74%)	77/126 (61%)
Development of vaginitis due to yeast	16/155 (10%)	27/159 (17%)

4.2. Interpretive criteria:

No interpretive criteria have been established for pathogens associated with bacterial vaginosis.

4.3. Effect of metronidazole on vaginal colonization by *Lactobacillus*:

The effect of metronidazole on vaginal colonization by *Lactobacillus* was examined in patients with BV (Agnew and Hillier, 1995, Sex. Trans Dis, 22: 269-273). Patients were treated with

0.75% metronidazole vaginal gel (twice daily for 5 days) or 2% clindamycin vaginal cream (once daily for 7 days). The vaginal swabs from patients at baseline, 1 week (end of therapy), and ≥1 month after initiation of therapy (3 weeks after discontinuation of therapy) were examined by gram stain and culture. The lactobacilli were identified by gram stain and colony morphology in Columbia 5% sheep blood agar medium or Rogosa agar. Cultures in Columbia 5% sheep blood agar medium were incubated in 5-7% CO_2 at 37°C for 48-72 hours. Cultures in Rogosa agar were incubated at 37°C in an anaerobic chamber for 5 days. No attempts were made to identify the species of *Lactobacillus* or quantify the lactobacilli. However, the ability of the isolate to produce hydrogen peroxide (H_2O_2) was determined using a tetra-methylbenzidene agar medium.

The number of patients with lactobacilli at baseline, end of therapy (EOT), and ≥1 month after initiation of therapy are shown in Table 4. An increase was observed in the number of patients with lactobacilli at EOT and ≥1 month after initiation of therapy with metronidazole compared to baseline (Table 4). In the case of clindamycin treated patients, a decrease was observed in the number of patients with lactobacilli at EOT but improvement in colonization was observed at ≥1 month after initiation of therapy compared to baseline. The colonization of lactobacilli at ≥1 month after initiation of therapy was observed in 94% and 87% patients treated with metronidazole and clindamycin, respectively. The results suggest that metronidazole does not inhibit lactobacilli colonization at EOT or ≥1 month after initiation of therapy.

Production of H_2O_2 was measured for some of the *Lactobacillus* isolates. Although the authors have shown the results for H_2O_2 producers and nonproducers separately, please note that the patients with mixed lactobacilli were included in these 2 groups (Table 4, see footnote). It would have been useful to analyze the number of patients with mixed lactobacilli (i.e., both H_2O_2 producers and non-producers) separately.

Table 4: Effect of treatment for vaginitis and cervitis on vaginal colonization by lactobacilli (LB).

Treatment	**Number of patients (n)**	**LB**	**Baseline (% patients with lactobacilli)**	**EOT (% patients with lactobacilli)**	**≥ 1month after initial therapy (% patients with lactobacilli)**
Clindamycin vaginal	28	All	50	25	87
		H_2O_2 +ve	29	11	57
		H_2O_2 -ve	25	21	17
Metronidazole vaginal	18	All	67	83	94
		H_2O_2 +ve	22	61	59
		H_2O_2 -ve	56	67	71

LB = *Lactobacillus*; EOT = end of therapy; All = H_2O_2 +ve and –ve lactobacilli

H_2O_2 +ve = patients having hydrogen peroxide producing lactobacilli exclusively or mixed with lactobacilli that do not produce hydrogen peroxide;

H_2O_2 -ve = patients that have lactobacilli which do not produce hydrogen peroxide exclusively or mixed with lactobacilli that produce hydrogen peroxide.

5. CONCLUSIONS:

The sponsor is seeking approval of Metronidazole vaginal gel for the treatment of bacterial vaginosis based the approved label for MetroGel-Vaginal® and 1 well-controlled clinical study in patients with BV.

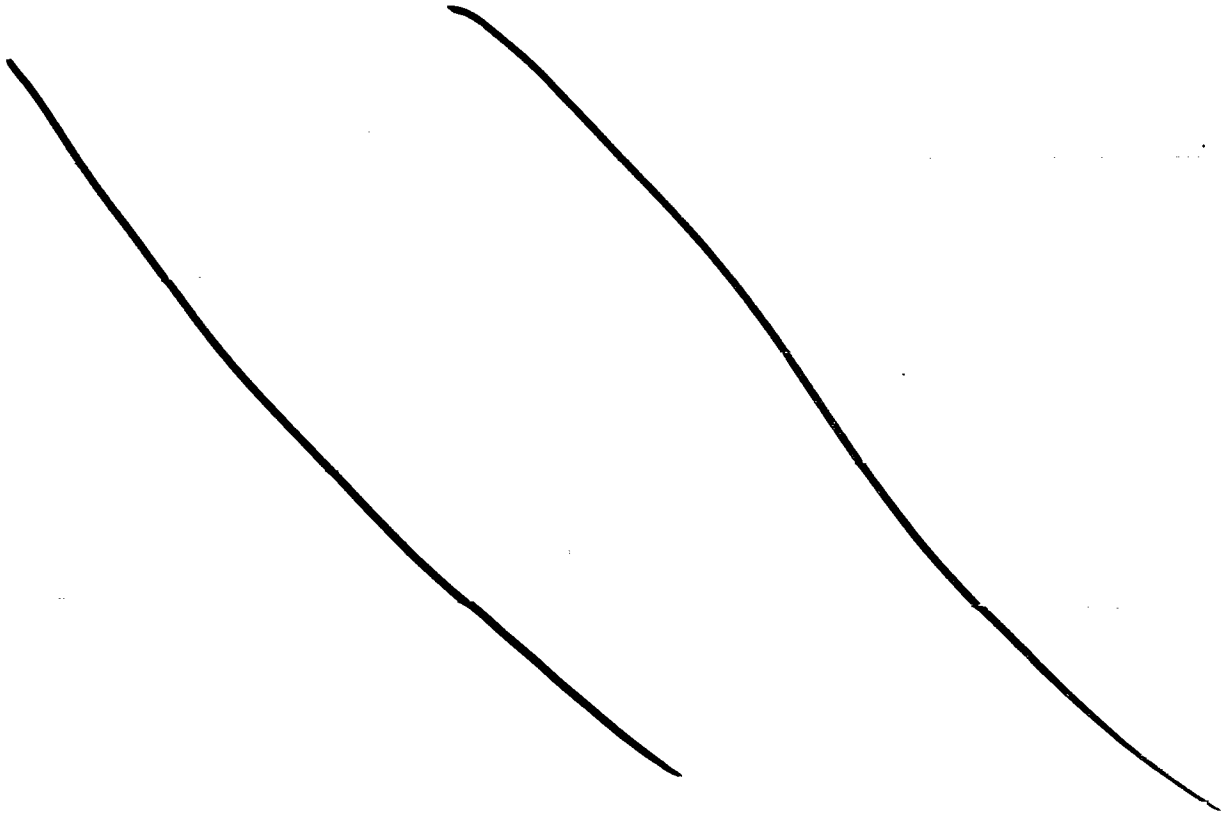
In vitro, metronidazole is active against most strains of *Gardnerella vaginalis*, *Bacteroides* sp., *Mobiluncus* sp., and *Peptostreptococcus* sp., which are associated with BV. At concentrations of 1000 – 4000 µg/ml, metronidazole partially inhibited the growth of vaginal *Lactobacillus* isolates. At higher concentrations (>5000 µg/ml) of metronidazole, 86-92% inhibition of growth was observed at 24 hours. However, in a study that evaluated the effect of metronidazole on vaginal lactobacilli colonization, an improvement in lactobacilli colonization was observed at EOT and at 3 weeks after discontinuation of therapy.

In the clinical study TCR-03, the efficacy of Metronidazole vaginal gel was greater (64%) than MetroGel-Vaginal® (46%) in the treatment of bacterial vaginosis. The study only provided information on the nugent scores using vaginal swabs at baseline and post-treatment. No information was available on the species of bacteria at baseline and post-treatment in patients enrolled in the clinical studies. Therefore, the activity of metronidazole against the bacterial species associated with BV could not be analyzed in the clinical study. The cure by nugent score suggests that there was an increase in *Lactobacillus* morphotypes and decrease in the *Gardnerella, Bacteroides* and *Mobiluncus* morphotypes in 74% patients at 16 to 25 days after discontinuation of therapy with metronidazole vaginal gel compared to 61% in patients treated with MetroGel-Vaginal®. Approximately 10% of patients in the metronidazole vaginal gel arm developed a vaginal yeast infection compared to 17% in the MetroGel-Vaginal® arm.

6. THE LABEL:

6.1. Sponsor's version of the label:

Microbiology



6.2. Comments:

There are no changes to the microbiology section of the label.

7. RECOMMENDATIONS:

The NDA submission should be approved with respect to Microbiology for the treatment of bacterial vaginosis. There are no changes to the Microbiology section of the label.

Kalavati Suvarna
Microbiologist, HFD-590

CONCURRENCES:

HFD-590/Deputy Dir _____Signature _____Date
HFD-590/Micro TL _____Signature _____Date
CC:
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/s/

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