# Original article

## Directed shift of vaginal flora after topical application of sucrose gel in a phase Ⅲ clinical trial: a novel treatment for bacterial vaginosis

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***Keywords:*** *bacterial vaginosis; vaginal flora; sucrose; metronidazole; clinical trial*

**Background** Bacterial vaginosis (BV) is one of the most common infectious diseases among sexually active women and is associated with the increased acquisition of a variety of sexually transmitted diseases. This study aimed to compare the efficacy of a non-antibiotic sucrose gel against an antibiotic metronidazole gel for the treatment of BV.

**Methods** A randomized, double-blinded, multi-center, parallel-group, placebo-controlled phase III clinical trial was conducted at eight hospitals in China. A total of 560 subjects with clinically diagnosed BV were randomly assigned into three groups for vaginally receiving sucrose, metronidazole, and placebo gels, respectively, twice daily for five consecutive days. The efficacy of therapeutic cure, defined as an achievement of both microbiologic cure (a Nugent score of 3 or less) and clinical cure (a resolution of the clinical findings from the baseline visit), was evaluated at the 1st and 2nd test-of-cure (TOC) visits at 7–10 and 21–35 days after the start of treatment, respectively.

**Results** Therapeutic cure rates for sucrose, metronidazole, and placebo gel groups were 83.13%, 71.30% and 0.92%, at the 1st TOC, and 61.04%, 66.67% and 7.34%, at the 2nd TOC, respectively. While there was no significant difference between the sucrose and metronidazole gel groups at the 2nd TOC ($P$=0.305), and sucrose gel was more effective than metronidazole gel at the 1st TOC ($P$=0.009).

**Conclusion** These findings suggest that sucrose gel restores normal vaginal flora more rapidly than metronidazole gel and can be used as a novel treatment for BV.

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Bacterial vaginosis (BV), one of the most common infectious diseases among women of childbearing age, accounts for 40%–50% of all cases of vaginitis.[1] BV is associated with the risks of preterm delivery,[2] pelvic inflammatory diseases,[3] and increased acquisition of a variety of sexually transmitted diseases (STD) caused by pathogens like *Neisseria gonorrhoeae* and *Chlamydia trachomatis*,[4] human papillomavirus (HPV),[5] herpes simplex virus type 2 (HSV-2),[6] and human immunodeficiency virus (HIV).[7] BV is characterized by a shift in the vaginal flora from a predominance of protective *lactobacilli* to pathogenic anaerobic bacteria, including *Gardnerella vaginalis*, *Mobiluncus spp*, *Peptostreptococcus*, *Mycoplasma homonis*, and *Protella*.[8] The protective *lactobacilli*, such as *Lactobacillus gasseri*, *L. crispatus*, *L. iners*, and *L. jensenii*, play an important role in maintaining a healthy vaginal ecosystem through different mechanisms. First, they can produce lactic acid to maintain a low vaginal pH (<4.5), which is not only suitable for growth of *lactobacilli*, but also for inhibiting replication of anaerobic bacteria. Second, *lactobacilli* can also secrete other antibacterial substances, including hydrogen peroxide ($H_2O_2$), bacteriocin and biosurfactants.[8] Third, by their micropili, *lactobacilli* can adhere to receptors on vaginal epithelial cells, thereby

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preventing adherence of potential pathogens to the vaginal mucosa.[9]

Antibiotics against anaerobes, such as metronidazole and clindamycin, administered orally or intravaginally, are recommended by the U.S. CDC for treatment of BV.[8] These antibiotics are able to kill or slow the growth of pathogenic anaerobic bacteria, leading to the restoration of the *lactobacilli*-predominant normal vaginal flora, thus achieving an effective therapeutic cure. However, repeated use of antibiotics, such as metronidazole, can induce the emergence of drug-resistant pathogenic anaerobic bacterial variants, which may, in turn, spread among women of childbearing age.[10] Since antibiotic resistance is one of the greatest threats to public health,[11] developing non-antibiotic therapeutics as an alternative for the treatment of BV is essential.

Here we report a randomized, double-blinded, and placebo-controlled clinical study to compare the efficacy of a non-antibiotic sucrose gel against an antibiotic metronidazole gel for the treatment of BV.

## METHODS

### Materials

Metronidazole gel (0.75%), an OTC product, was obtained from Beijing Ziguang Pharmaceutical Limited Inc., China. Both sucrose gel and placebo gel were produced under GMP conditions in the Bioengineering Center of Hebei Medial University. The main ingredient of the placebo gel is xanthan gum, and sucrose gel was prepared by adding 9% sucrose to the placebo gel.

### Study design

A randomized, double-blinded, multi-center, parallel-group, placebo-controlled phase III clinical trial was conducted to evaluate the efficacy of sucrose gel in restoring vaginal microflora, leading to the cure of BV, at eight hospitals in China between September and November of 2008. The study was approved by the Chinese State Food and Drug Administration (SFDA) and the Ethics Committees of the participating hospitals. All enrolled subjects signed a written informed consent. Subjects were screened for the study based on the following inclusion criteria were: (1) having clinically diagnosed BV (at least three of the four Amsel criteria[12] were present[13]: (a) thin, white, homogeneous discharge; (b) presence of "clue cells" ≥ 20% of the total epithelial cells on microscopic examination of the wet mount; (c) pH of vaginal fluid >4.5; and (d) a positive Whiff test). (2) with Nugent score 7 –10; (3) being between the ages of 18 and 50; and (4) having signed a written informed consent; and the exclusion criteria: (1) suffering from other vaginal infections, such as *candidal vulvovaginitis, trichomoniasis, gonorrhea*, HSV infection or condyloma; (2) having a history of systemic or vaginal antibiotic or antifungal therapy within the previous two weeks; (3) with Nugent score <7; (4) being pregnant or breast feeding; (5) having a baseline diagnosis of liver dysfunction, kidney dysfunction, blood diseases, mental diseases, diabetes or other serious diseases; (6) being poor drug compliance; and (7) being allergic to metronidazole.

### Sample and data collection

The BV patients were randomly assigned to three groups for vaginally receiving 5.0 g of sucrose gel, metronidazole gel and placebo gel, respectively, twice a day (once in the morning and once at night before sleeping) for five consecutive days. The patients were called at day 3–4 to record their general conditions, adverse events and other medications used. The therapeutic efficacy was evaluated at the 1st and 2nd test-of-cure (TOC) visits at 7–10 and 21–35 days after the start of treatment, respectively. Swabs were used to collect samples from the junction of the upper one third and lower two thirds of the lateral vaginal wall. The swabs were touched to pH sticks (pH range 3.8–5.4, Shanghai San Ai Si Reagent, Inc.) for measuring pH values. Under microscopic examination of wet prep, the squamous epithelial cells that were covered with bacteria and had "shaggy or furlike" appearance were considered as "clue cells" and the presence of "clue cells" ≥20% of the total epithelial cells on microscopic examination is diagnostic positive.[14] The Whiff test was performed by mixing the vaginal discharge with potassium hydroxide as described by Cohrssen et al.[15] The sample with a characteristic fishy odor was considered as positive. Therapeutic cure was defined as an achievement of both microbiologic cure and clinical cure, according to U.S. FDA guidelines.[16] A microbiologic cure was defined by a low (≤3) Nugent score, which was calculated by determining the presence of bacteria per oil immersion field (OF), including *lactobacillus* morphotypes: 0 = > 30, 1 = 5–30, 2 = 1–4, 3 = < 1, and 4 = 0 per OF; *G. vaginalis* and *bacteroides* morphotypes: 0 = 0, 1 = < 1, 2 = 1–4, 3 = 5–30, and 4 = > 30 per OF; *Mobiluncus spp*: 0 = 0, 1 = < 4, 2 = ≥ 5 per OF.[14] Therefore, a high Nugent score as an indication of BV is associated with a relatively low number of beneficial bacteria, i.e., *lactobacilli*, and a relatively high number of pathogenic bacteria, including *G. vaginalis*, *bacteroides* and *Mobiluncus spp*. morphotypes. A patient with a Nugent score of 4 to 10 is considered to have BV.[14] A clinical cure was further defined as resolution of the clinical findings from the baseline visit, including normal vaginal secretion, negative Whiff test, absence of clue cells, and vaginal fluid pH ≤4.5.[17] All the microbiologic evaluations were performed at the clinical laboratory of the corresponding hospital using a standard procedure by the technicians (or technologists) who were blind to the treatment.

### Statistical analysis

Data were analyzed using SAS 6.12 software. One-way analysis of variance (ANOVA) *F* test and Wilcoxon rank sum test were used for multiple comparisons between treatment groups with samples of unequal sizes. All between-group differences in bacterial scores (Tables 1–3),

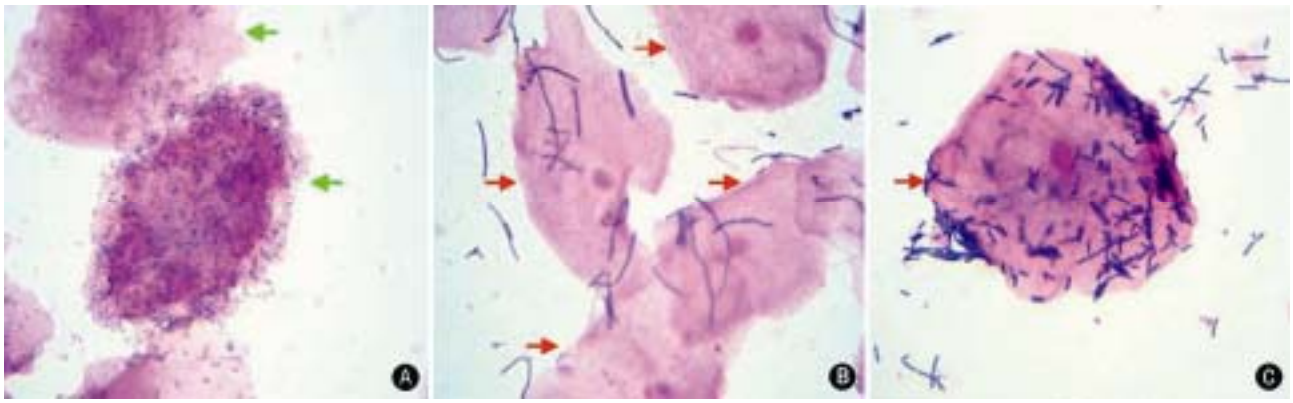

**Figure.** Gram-stained vaginal smear observation of samples from a BV patient before and after sucrose gel treatment (oil immersion original magnification ×1500). **A:** Before treatment. In the observed field, there were two clue cells (pointed by green arrows) which are squamous epithelial cells with shaggy borders coated with *coccobacilli*, but almost no Gram positive *bacilli*. **B:** Five days after treatment. The squamous epithelial cells (pointed by red arrows) became normal and the Gram positive *bacilli* became predominant, although there were still some *coccobacilli* presented. **C:** Thirty days after treatment. Normal epithelial cells (pointed by red arrow) and predominant Gram positive *bacilli* were presented.

**Table 1.** Lactobacillus scores before and after treatment with sucrose gel, metronidazole gel, and placebo gel

| Time point | Group* (*n*) | *Lactobacillus* score (*n* (%))§ 0 | 1 | 2 | 3 | 4 | Mean±SD | *P* values | |
|---|---|---|---|---|---|---|---|---|---|
| Baseline (before treatment) | A (326) | 0 (0.0) | 3 (0.9) | 15 (4.6) | 174 (53.4) | 134 (41.1) | 3.368±0.571 | A vs. B: | 0.499 |
| | B (108) | 0 (0.0) | 0 (0.0) | 2 (1.9) | 57 (52.8) | 49 (45.4) | 3.546±0.500 | B vs. C: | 0.863 |
| | C (109) | 0 (0.0) | 1 (0.9) | 3 (2.8) | 59 (54.1) | 46 (42.2) | 3.417±0.514 | C vs. A: | 0.916 |
| 1st TOC visit (7-10 days after treatment) | A (326) | 256 (78.5) | 37 (11.4) | 23 (7.1) | 8 (2.5) | 2 (0.6) | 0.353±0.800 | A vs. B: | <0.001 |
| | B (108) | 41 (38.0) | 11 (10.2) | 13 (12.0) | 19 (17.6) | 24 (22.2) | 1.759±1.628 | B vs. C: | <0.001 |
| | C (109) | 7 (6.4) | 11 (10.1) | 28 (25.7) | 44 (40.4) | 19 (17.4) | 2.546±1.071 | C vs. A: | <0.001 |
| 2nd TOC visit (21-35 days after treatment) | A (326) | 206 (63.2) | 34 (10.4) | 36 (11.0) | 34 (10.4) | 16 (4.9) | 0.834±1.256 | A vs. B: | 0.550 |
| | B (108) | 76 (70.4) | 6 (5.6) | 12 (11.1) | 10 (9.3) | 4 (3.7) | 0.704±1.202 | B vs. C: | <0.001 |
| | C (109) | 9 (8.3) | 10 (9.3) | 24 (22.2) | 43 (39.8) | 22 (20.4) | 2.713±1.216 | C vs. A: | <0.001 |

*Group A: Sucrose gel; Group B: Metronidazole gel; Group C: Placebo gel. *n*: the number of patients with non-missing data in each group. (*n* (%))§: number and percentage of patients within each endpoint for each treatment group.

clinical cure rate (Table 4), and therapeutic cure rate (Table 5) among BV patients treated with sucrose gel, metronidazole gel and placebo gel, respectively, were compared using a $\chi^2$ test except that the between-group differences in *lactobacilli* scores and *G.vaginalis* and *bacteroides* sores at baseline were compared using Fisher exact test. A *P* value of less than 0.05 was considered to be statistically significant.

## RESULTS

### Enrollment and baseline characteristics of the patients

A total of 560 patients with diagnosed BV were enrolled between September and November of 2008, were randomly assigned into three groups, A (*n*=335), B (*n*=112) and C (*n*=113), and received intravaginally 5.0 g sucrose gel, metronidazole gel and placebo gel, respectively, twice daily (once in the morning and once at night before bedtime). Nine (2.68%), 4 (3.54%), and 4 (3.48%) of the patients in groups A, B and C, respectively, were lost of follow-up visit or withdrawn from study. There were no significant differences in baseline characteristics among the three groups with respect to age, height, weight, blood pressure, pulse, history of vaginal medication, and vaginal flora (based on Nugent scores and Amsel criteria; *P* >0.05).

### Topical application of sucrose gel rapidly restored the *lactobacilli*-predominated vaginal flora based on Nugent score

All the participants had a baseline Nugent score >7 before the treatment, suggesting that their vaginal flora contained a decreased number of the protective *lactobacilli* and increased number of pathogenic bacteria, including *G.vaginalis*, *bacteroides* and *Mobiluncus spp*. morphotypes. Intravaginal use of sucrose and metronidazole gel resulted in restoration of the *lactobacilli*-predominated vaginal flora as shown by the increased number of the *lactobacilli* (Table 1 and Figure) and decreased number of pathogenic bacteria, *G.vaginalis/bacteroides* (Table 2) and *Mobiluncus* (Table 3). Notably, at the first TOC visit (7–10 days after the start of treatment) the *lactobacilli* score of the sucrose gel group is significantly lower than metronidazole gel ($P < 0.001$, Table 1), while the *G. vaginalis/bacteroides* score of the former is higher than that of the latter ($P < 0.001$, Table 2). At the second TOC visit (21–35 days following the start of treatment), the sucrose gel exhibited a *lactobacilli* score similar to the metronidazole gel group (0.834 vs. 0.704, *P*=0.550), which was much lower than that for the placebo gel (1.713, $P < 0.001$, Table 1). These results confirm that augmentation of endogenous *lactobacilli* in the vagina flora is critical for treatment of BV.

**Table 2.** *G. vaginalis/bacteroides* scores before and after treatment with sucrose gel, metronidazole gel, and placebo gel

| Time point | Group* (*n*) | *G. vaginalis/bacteroides* score (*n* (%))§ | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | 0 | 1 | 2 | 3 | 4 | Mean±SD | *P* values | |
| Baseline (before treatment) | A (326) | 0 (0.0 ) | 0 (0.0) | 3 (0.9) | 11 (3.4) | 312 (95.7) | 3.960±0.211 | A vs. B: | 0.802 |
| | B (108) | 0 (0.0) | 0 (0.0) | 1 (0.9) | 2 (1.9) | 105 (97.2) | 4.000±0.000 | B vs. C: | 0.810 |
| | C (109) | 0 (0.0) | 0 (0.0) | 1 (0.9) | 1 (0.9) | 107(98.2) | 4.000±0.000 | C vs. A: | 0.435 |
| 1st TOC visit (7–10 days after treatment) | A (326) | 128 (39.3) | 50 (15.3) | 49 (15.0) | 57 (17.5) | 42 (12.9) | 1.494±1.471 | A vs. B: | <0.001 |
| | B (108) | 86 (79.6) | 13 (12.0) | 6 (5.6) | 0 (0.0) | 3 (2.8) | 0.343±0.822 | B vs. C | <0.001 |
| | C (109) | 1 (0.9) | 0 (0.0) | 5 (4.6) | 11 (10.1) | 92 (84.4) | 3.806±0.502 | C vs. A: | <0.001 |
| 2nd TOC visit (21–35 days after treatment) | A (326) | 134 (41.1) | 27 (8.3) | 22 (6.8) | 48 (4.7) | 95 (29.1) | 1.825±1.737 | A vs. B: | 0.001 |
| | B (108) | 69 (63.9) | 5 (4.6) | 6 (5.6) | 7 (6.5) | 21 (9.4) | 1.130±1.647 | B vs. C: | <0.001 |
| | C (109) | 6 (5.6) | 2 (1.9) | 5 (4.63) | 7 (6.5) | 88 (81.5) | 3.565±1.061 | C vs. A: | <0.001 |

*Group A: Sucrose gel; Group B: Metronidazole gel; Group C: Placebo gel. *n*: number of patients with non-missing data in each group. (*n* (%))§: number and percentage of patients within each endpoint for each treatment group.

**Table 3.** *Mobiluncus* scores before and after treatment with sucrose gel, metronidazole gel and placebo gel

| Time point | Group* (*n*) | Mobiluncus score (*n* (%))§ | | | | | |
|---|---|---|---|---|---|---|---|
| | | 0 | 1 | 2 | Mean±SD | *P* values | |
| Baseline (before treatment) | A (326) | 204 (62.6) | 60 (18.4) | 62 (19.0) | 0.561±0.793 | A vs. B: | 0.11 |
| | B (108) | 68 (63.0) | 12 (11.1) | 28 (25.9) | 0.769±0.923 | B vs. C: | 0.553 |
| | C (109) | 68 (62.4) | 17 (15.6) | 24 (22.0) | 0.602±0.831 | C vs. A: | 0.694 |
| 1st TOC visit (7–10 days after treatment) | A (326) | 316 (96.9) | 9 (2.8) | 1 (0.3) | 0.037±0.204 | A vs. B: | 0.804 |
| | B (108) | 106 (98.2) | 2 (1.9) | 0 (0.0) | 0.019±0.135 | B vs. C: | 0.001 |
| | C (109) | 71 (65.1) | 19 (17.4) | 19 (17.4) | 0.528±0.779 | C vs. A: | <0.001 |
| 2st TOC visit (21–35 days after treatment) | A (326) | 275 (84.4) | 20 (6.1) | 31 (9.5) | 0.252±0.616 | A vs. B: | 0.011 |
| | B (108) | 103 (95.4) | 2 (1.9) | 3 (2.8) | 0.074±0.354 | B vs. C: | <0.001 |
| | C (109) | 63 (58.3) | 17 (15.7) | 28 (25.9) | 0.676±0.683 | C vs. A: | <0.001 |

*Group A: Sucrose gel; Group B: Metronidazole gel; Group C: Placebo gel. *n*: number of patients with non-missing data in each group. (*n* (%))§: number and percentage of patients within each endpoint for each treatment group.

### Intravaginal administration of sucrose gel resulted in clinical cure of BV based on Amsel criteria

The clinical cure was assessed based on the Amsel criteria. As shown in Table 2, topical application of sucrose gel twice a day for 5 days resulted in resolution of the clinical findings from the baseline visit. At the 1st visit (7–10 days after the start of treatment), more than 86% of the patients in both sucrose and metronidazole gel groups had normal vaginal secretion, negative Whiff test, absence of clue cells, and vaginal fluid pH ≤4.5, while only 7%–15% of patients in the placebo group had similar normal vaginal flora ($P$ <0.001). At the 2nd visit (21–35 days following the start of treatment), more than 67% of the patients in both sucrose and metronidazole gel groups had been clinically cured. These results suggest that sucrose gel is equally effective as metronidazole gel in clinically curing BV patients based on Amsel criteria.

### Compared with metronidazole gel, sucrose gel is more effective at the 1st TOC visit and equally effective at the 2nd TOC visit for therapeutic cure of BV

Finally, we compared the rates of therapeutic cure, an achievement of both microbiologic cure and clinical cure. At the first TOC visit (7–10 days following the start of treatment), the therapeutic rates for sucrose, metronidazole, and placebo gel groups were 83.13%, 71.30% and 0.92%, respectively. Like the metronidazole gel group, the sucrose gel group had much greater cure rates than the placebo group ($P$ <0.001, 95% *CI*: 77.77–86.65). Notably, the cure rate of sucrose gel was significantly higher than that of metronidazole gel ($P$=0.009, 95% *CI*: 2.38–21.28). These findings suggest that sucrose gel restores normal vaginal flora more rapidly than metronidazole gel. At the second TOC visit, the therapeutic rates for sucrose, metronidazole, and placebo gel groups were 61.04%, 66.67% and 7.34%, respectively. Similarly, therapeutic rates of both treatment groups are significantly higher than that of the placebo group ($P$ <0.001, 95% *CI*: 46.49–60.91 between sucrose and placebo gel groups), but there is no significance between the sucrose gel and metronidazole gel groups ($P$=0.305, 95% *CI*: –15.97–4.72). This result indicates that sucrose gel is as effective as metronidazole gel at the 2nd TOC visit.

### Sucrose gel is safe for intravaginal application

Any treatment-associated adverse event was self-reported and recorded immediately after it occurred or monitored at each follow-up visit. No serious adverse events were recorded by patients participating in this study. Eighty-nine participants reported 98 mild and moderate adverse events, including, for example, abdominal distension and pain, lower back pain, sagging of lower abdomen, and mycotic infection. The rates of the treatment-associated adverse events for sucrose, metronidazole, and placebo gel groups were 16.62%, 15.60% and 15.18%, respectively. Thus, there is no significant difference between either of the treatment groups and the placebo group ($P$ >0.05), suggesting that, like metronidazole gel, sucrose gel is also safe for intravaginal application.

## DISCUSSION

The objective of this study is to compare and evaluate the efficacy of a non-antibiotic sucrose gel for treatment of

**Table 4.** Number and percentage ($n$ (%))[§] of patients within each clinical endpoint (based on Amsel's criteria) after treatment with sucrose gel, metronidazole gel, and placebo gel

| Endpoint | Evaluated at | Sucrose gel (A: $n$=326)[*] | Metronidazole gel (B: $n$=108) | Placebo gel (C: $n$=109) | *P* values | | |
|---|---|---|---|---|---|---|---|
| | | | | | A vs.B | B vs.C | C vs. A |
| Normal discharge | Baseline | 0 (0) | 0 (0) | 0 (0) | — | — | — |
| | 1st TOC visit | 282 (86.5) | 101 (93.5) | 7 (6.4) | 0.057 | <0.001 | <0.001 |
| | 2nd TOC visit | 218 (66.8) | 81 (75.0) | 14 (12.8) | 0.121 | <0.001 | <0.001 |
| Negative Whiff Test | Baseline | 0 (0) | 0 (0) | 0 (0) | — | — | — |
| | 1st TOC visit | 285 (87.4) | 103 (95.4) | 10 (9.2) | 0.029 | <0.001 | <0.001 |
| | 2nd TOC visit | 232 (71.2) | 87 (80.6) | 20 (18.4) | 0.060 | <0.001 | <0.001 |
| < 20% clue cells | Baseline | 0 (0) | 0 (0) | 0 (0) | — | — | — |
| | 1st TOC visit | 292 (89.6) | 104 (96.3) | 15 (13.7) | 0.047 | <0.001 | <0.001 |
| | 2nd TOC visit | 233 (71.5) | 90 (83.3) | 24 (22.0) | 0.015 | <0.001 | <0.001 |
| pH ≤ 4.5 | Baseline | 0 (0) | 0 (0) | 0 (0) | — | — | — |
| | 1st TOC visit | 284 (87.1) | 93 (86.1) | 8 (7.3) | 0.869 | <0.001 | <0.001 |
| | 2nd TOC visit | 101 (77.8) | 24 (69.0) | 15 (13.8) | 0.087 | <0.001 | <0.001 |

[*]$n$: number of patients with non-missing data in each group. ($n$ (%))[§]: number and percentage of patients within each endpoint for each treatment group. The 1st and 2nd TOC visits were performed 7–10 and 21–35 days after the start of treatment, respectively.

**Table 5.** Therapeutic cure rate ($n$ (%))[§] among BV patients treated with sucrose gel, metronidazole gel, and placebo gel (based on the microbiologic and clinical cure rates)

| Endpoint | Evaluated at | Sucrose gel (A: $n$=326)[*] | Metronidazole gel (B: $n$=108) | Placebo gel (C: $n$=109) | *P* values | | |
|---|---|---|---|---|---|---|---|
| | | | | | A vs. B | B vs. C | C vs. A |
| Microbiologic cure | 1st TOC visit | 276 (84.7) | 78 (72.2) | 1 (0.9) | 0.005 | < 0.001 | < 0.001 |
| | 2nd TOC visit | 204 (62.6) | 80 (74.1) | 8 (7.3) | 0.035 | < 0.001 | < 0.001 |
| Clinical cure | 1st TOC visit | 276 (84.7) | 92 (85.2) | 2 (1.8) | 1.000 | < 0.001 | < 0.001 |
| | 2nd TOC visit | 207 (63.5) | 76 (70.4) | 10 (9.2) | 0.202 | < 0.001 | < 0.001 |
| Therapeutic cure | 1st TOC visit | 271 (83.1) | 77 (71.3) | 1 (0.9) | 0.009 | < 0.001 | < 0.001 |
| | 2nd TOC visit | 199 (61.0) | 72 (66.7) | 8 (7.3) | 0.305 | < 0.001 | < 0.001 |

[*]$n$: number of patients with non-missing data in each group. ($n$ (%))[§]: number and percentage of patients within each endpoint for each treatment group. The 1st and 2nd TOC visits were performed 7–10 and 21–35 days after the start of treatment, respectively.

BV against that of antibiotic metronidazole gel. Our previous studies have shown that some oligosaccharides, such as oligo-fructoses or oligo-galactoses, can selectively promote the growth of some protective vaginal bacteria, such as *lactobacilli*. We therefore tested the gel containing sucrose, a disaccharide of glucose and fructose, in a rhesus macaque BV model and found that it was able to quickly restore the normal vaginal flora (data not shown). Since sucrose, one of the most commonly used excipients in drug products, is defined by the U.S. FDA as a generally safe (GRAS) material for human use, the SFDA approved our IND application of sucrose gel in a phase II clinical trial for treating BV without doing the phase I (safety) clinical trial. A total of 96 BV patients participated in the phase II clinical study and received intravaginally 5 g (group A, $n$=32) or 7.5 g (group B, $n$=32) sucrose gel or placebo gel (group C, $n$=32). The therapeutic cure rates at day 8 post-treatment were 84.38%, 86.67% and 3.13% for groups A, B, and C, respectively. There is significant difference between the placebo group and each of the treatment groups ($P$ <0.001), but no difference between treatment groups A and B ($P$ >0.05), suggesting that both 5 g and 7.5 g sucrose gel dosages are equally effective in treating BV (unpublished data). Therefore, we selected the 5 g sucrose gel for the phase III clinical trial.

A total of 560 patients with BV participated in this randomized, double-blinded, multi-center, parallel-group, placebo-controlled phase III clinical trial. The subjects in the Group A, B, and C received intravaginally 5 g of sucrose, metronidazole, and placebo gels, respectively, twice daily for five consecutive days, and the treatment efficacy was evaluated at the 1st and 2nd TOC visits at 7–10 and 21–35 days after the start of treatment. While the sucrose gel exhibited a therapeutic cure rate similar to the metronidazole gel at the 2nd TOC visit 21–35 days following the start of treatment, the former proved more effective than the latter at the 1st TOC visit (7–10 days after the start of treatment). These results suggest that sucrose gel restores normal vaginal flora more rapidly than metronidazole gel. Based on the Nugent score calculated for the numbers of beneficial and pathogenic bacteria, there is significant difference between the sucrose and metronidazole gel groups at the 1st TOC visit. The number of *lactobacilli* in the sucrose gel group was significantly higher than that of metronidazole gel group, suggesting that treatment of BV with sucrose gel results in a quick increase of *lactobacillus* number in the vaginal flora and that metronidazole gel takes a longer time than sucrose gel to recover *lactobacillus* levels. On the other hand, the numbers of the *G.vaginalis/bacteroides* and *Mobiluncus spp* in the metronidazole gel groups were significantly lower than the sucrose gel group. However, the numbers of these pathogens in both treatment groups were remarkably lower than those found in the placebo group, indicating that metronidazole suppresses the growth of pathogenic bacteria more rapidly than sucrose gel, while the sucrose gel is more effective in promoting the growth of lactobacilli. These findings indicate that sucrose gel and metronidazole gel have different mechanisms of action in treating BV. That is, sucrose gel

mediated a shift of vaginal bacterial flora by promoting the growth of *lactobacilli*, which, in turn, generate lactic acid to lower the vaginal pH and secrete antibacterial substances to inhibit the adhesion and replication of the pathogenic anaerobic bacteria. Dr. ZENG Zhong-ming defined such kind of flora substitution mediated by substances other than antibiotic chemicals as directed shift of flora. By controlling shift of flora, a pathogenic BV flora can be changed into a beneficial *lactobacilli*-dominated flora. Though the sucrose gel itself does not have any bactericidial or antibacterial effect, it is very effective in curing BV by inducing vaginal flora shift from a pathogenic BV flora to a beneficial lactobacillus-dominated flora.

On the other hand, metronidazole gel appears to inhibit the growth of both the pathogenic bacteria and the beneficial *lactobacilli*. Simoes et al[18] demonstrated that high concentration (e.g., ≥5 mg/ml) of metronidazole could completely suppress the *in vitro* growth of *lactobacillus*, while the clinically used intravaginal gel for treating BV contains 0.75% (=7.5 mg/ml) of metronidazole. Nevertheless, the *lactobacillus* is expected to recover faster than the pathogenic bacteria after stop of the metronidazole gel treatment because *lactobacillus* is more resistant to metronidazole than anaerobic bacteria.

In both the sucrose and metronidazole gel treatment groups, the therapeutic cure rates at the 2nd TOC visit (21–35 days following the start of treatment) was significantly lower than those at the 1st TOC visit, suggesting that the treatment effect is not well sustained, either because of relapse or reinfection. Therefore, further clinical study of prolonged treatment with sucrose gel is warranted since it was reported that extending the duration of metronidazole therapy from 7 days to 14 days resulted in a significantly greater cure rate for treating BV.[17]

Nonetheless, sucrose gel carries less risk of inducing pathogenic bacterial-resistant variants than the currently used antibiotics, including metronidazole. Therefore, sucrose gel can be developed as a novel, safe therapeutic alternative for treatment of BV. Because sucrose gel and metronidazole use different mechanisms to treat BV, topical application of sucrose gel in combination with metronidazole (orally or intravaginally) may have synergistic effect in treating BV. Such synergism may result in a more rapid restoration of the normal vaginal flora and more effective inhibition of pathogenic bacteria. Also, because of this synergism, a reduced dose of metronidazole in the combination therapy may gain therapeutic efficacy similar to that of metronidazole alone at the regular dose, which may delay the emergence of drug resistance.

Whether BV is sexually transmitted remains unsettled, but it is well known that BV is associated with an increased risk of acquiring STDs.[4-6] Therefore, topical application of sucrose gel for treatment of BV and restoration of *lactobacilli*-predominant normal vaginal flora is expected to reduce the risk of sexual transmission of certain STD pathogens, such as *Neisseria gonorrhoeae*, *Chlamydia trachomatis*, HPV, and HSV-2. Sucrose gel may be used in combination with the corresponding antibacterial or antiviral agents for treatment of BV and the associated STDs. Furthermore, sexual transmission is the major route for HIV infection worldwide,[19] and development of an effective and safe anti-HIV microbicide for prevention of HIV/AIDS is urgently needed.[20] Previous reports have shown that BV is associated with increased acquisition of HIV infection because BV-induced alterations in cervical immune cell populations and local inflammatory cytokines can enhance local HIV replication.[7,21,22] Therefore, treatment of BV resulting in restoration of *lactobacilli*-predominant normal vaginal flora by sucrose gel is expected to reduce women's risk of acquiring HIV. Topical application of the sucrose gel in combination with a specific anti-HIV agent in a microbicide formulation may achieve double protective effects. These would include normalization of vaginal flora and inhibition of HIV infection, resulting in a higher efficacy of prevention against the sexual transmission of HIV than use of an anti-HIV microbicide alone.

In conclusion, topical use of sucrose gel is a novel alternative treatment for bacterial vaginosis because its mechanism of action is different from traditional antibacterial treatments and because its use reduces the risk of inducing antibiotic resistance. It has been shown in this report to rapidly restore normal vaginal flora by promoting the growth of the protective *lactobacilli*, which, in turn, suppresses the replication of pathogenic bacteria. Therefore, this unique strategy of *directed shift of vaginal flora* is expected to be an attractive alternative option of treating flora-associated diseases because instead of using antibiotics or other antimicrobial chemicals, the microbial diseases, such as bacterial vaginosis, could be cured by use of sucrose, one of the most abundant carbohydrates from natural resource. Sucrose gel may also be used in combination with specific antimicrobial agents for the treatment of BV-associated STDs and the prevention of sexual transmission of HIV and other STD pathogens.

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