

Vaginal Mucosal Vaccine for Recurrent Urinary Tract Infections in Women: Results of a Phase 2 Clinical Trial

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Purpose: We assessed the clinical efficacy of vaginal mucosal immunization with a multivalent bacterial vaccine in women with recurrent urinary tract infections.

Materials and Methods: A total of 75 patients in a double-blind study were randomly assigned to receive placebo only, primary immunization without boosters, or primary immunization plus boosters using vaginal suppositories containing placebo or vaccine. Vaccine suppositories contained 10 strains of heat-killed uropathogenic bacteria and placebo suppositories had no vaccine organisms. All women were monitored for 6 months to record the number of infections and adverse events.

Results: Analysis of data on urinary tract infections caused by any bacteria showed the greatest difference in infection rates between patients in the vaccine plus boosters protocol compared to those receiving placebo only ($p = 0.100$). When only *E. coli* urinary tract infections were considered in the analysis, urinary tract infection recurrence rates were significantly less in women given booster immunizations compared to placebo ($p = 0.0015$). Furthermore, women who received vaccine with boosters and who were sexually active, less than 52 years old, or had not undergone hysterectomy had *E. coli* urinary tract infections at a much lower rate than women given placebo only ($p = 0.0002, 0.002$ and 0.003 , respectively). No significant adverse events were associated with vaccine treatment.

Conclusions: This study demonstrated the efficacy of vaginal mucosal immunization with a multivalent vaccine in reducing recurrence of *E. coli* urinary tract infections. The results suggest that the vaccine may provide the most benefit to sexually active women in the 20 to 50-year-old age group.

Key Words: clinical trial, urinary tract infections, vaccines

Uropathogenic *Escherichia coli* cause 80% of community acquired UTIs and approximately 50% of UTIs in hospitalized patients and diabetics.¹ Various *Proteus*, *Enterococcus* and *Klebsiella* species, as well as *Staphylococcus saprophyticus* strains, are causative organisms in an increasing number of infections.² While antibiotics are effective in treating acute infections and are the primary means of prophylaxis in patients with recurrent infections, increased drug resistance is reducing their usefulness.³ As an alternative to prophylactic antibiotics, several vaccines have been developed and have undergone clinical testing.⁴

Our efforts to develop an effective immunization regimen for women with recurrent UTIs have used what we believe to be the most effective immunogen and route of administration. The vaccine tested previously⁵⁻⁷ and in the current study contains multiple uropathogens administered as a mucosal immunogen. This approach has the advantage of inducing primarily immunoglobulin G and immunoglobulin A in the urogenital tract, thereby reducing potential colonization of the vagina and bladder with uropathogens. Mucosal vaccines also elicit minimal adverse effects. In contrast, parenteral immunization regimens induce primarily systemic antibodies and can stimulate severe local inflammatory responses.

In our previous phase 2 clinical trial, patients received 3 weekly immunizations with a vaccine containing inactivated uropathogens in a vaginal suppository.⁶ The number of re-infections was significantly less in women treated with vaccine until 8 weeks after the last immunization, suggesting that vaccine efficacy had decreased over time. In the current study we examined whether the infection-free period could be extended by additional vaccine doses and demonstrated that booster immunizations can increase the vaccine's effectiveness for up to 6 months.

METHODS

Vaccine Composition

Vaginal suppositories were prepared using Urovac™, a vaccine containing heat-killed bacteria from 10 human uropathogenic strains.⁸ Virulence factors present in the 6 *E. coli* strains were determined using polymerase chain reaction

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primers specific for uropathogenicity genes.⁹ The *E. coli* strains belong to the B2 phylogenetic group,¹⁰ and possess type 1 and p fimbriae, hemolysin, S fimbrial adhesin, cytotoxic necrotizing factor 1, several siderophores and protectins, and the CFT073 pathogenicity associated island marker. The vaccine also contained 1 strain each of *Proteus mirabilis*, *Morganella morganii*, *Klebsiella pneumoniae* and *Enterococcus faecalis*. The 10 bacterial strains were combined in equal numbers (1×10^8 each) in a polyethylene glycol base to form suppositories containing a total of 1×10^9 bacteria each. Placebo suppositories were prepared with polyethylene glycol base only.

Study Design and Participants

This study protocol was approved by the University of Wisconsin Health Sciences Institutional Review Board. A total of 75 women with 3 or more UTIs in the previous year were entered into a randomized, double-blind, placebo controlled clinical trial. All patients were interviewed to document oral or vaginal estrogen use, use of birth control pills, whether they were sexually active, and to obtain history of childhood UTIs. All patients underwent excretory urogram or renal ultrasound and cystoscopy. Specific exclusion criteria were anatomical abnormalities, neurogenic bladder, interstitial cystitis, kidney stones, indwelling catheter, or urinary diversion. Patients discontinued prophylactic antibiotics 1 week before entering the study.

Sample size was determined by power calculations using a relative risk of at least 0.36 determined from infection rates of placebo treated patients in previous phase 2 studies⁶ using the re-infection risk ratio¹¹ tested by the log rank statistic.¹² A total of 25 patients per group with 160 days of followup provided at least 80% power. Randomization was accomplished before starting the study by randomizing treatments in blocks of 6 with 2 of each treatment assignment per block. Women were sequentially enrolled in the study and assigned to a treatment group: placebo only, primary immunization without boosters, or primary immunization with boosters. Each patient received 3 initial suppositories at weekly intervals followed by 3 additional suppositories at monthly intervals. The placebo group received placebo in all 6 suppositories. Primary immunization consisted of 3 vaccine containing suppositories followed by 3 placebo suppositories. A third treatment group received primary immunization plus 3 boosters with vaccine suppositories. Samples of urine and vaginal secretions were collected before entry and at 2, 6, 10, 14, 18 and 22 weeks after the first suppository. Anti-*E. coli* antibodies were quantified by an enzyme linked immunosorbent assay technique.¹³ All UTIs and any adverse effects were reported to the study coordinator. Most infections were verified by urine culture and all were treated with full doses of conventional antibiotics.

Statistical Analysis

The distribution of patients with different characteristics in each of the 3 treatment groups was evaluated using Fisher's 2-tailed exact test or Kruskal-Wallis 1-way ANOVA, as appropriate for a specific data set. The proportions of patients in each treatment group remaining infection-free over time were compared using the log rank test. Changes in antibody levels were evaluated by repeated measures ANOVA for

unbalanced data. All analyses were performed using SAS® statistical software.¹²

RESULTS

Patient Characteristics

The table summarizes characteristics of study participants in each treatment group. Patient age ranged from late teens to early 70s. The average number of UTIs in the previous year ranged from 6 to 7 and several women reported up to 20 infections. Less than 20% of the patient population had a history of childhood UTIs, which was similar to the proportion in each treatment group. Approximately a third of all patients had a hysterectomy. More than half of all patients were receiving estrogen as replacement therapy or in birth control pills. Women on birth control pills made up 25% of the study population. A large proportion of women overall were sexually active (79%), as were the subjects in each treatment group.

The distribution of patients with each characteristic across treatment groups was statistically analyzed to determine whether there were any disparities that might influence subsequent analyses of vaccine efficacy. There were no significant differences between treatment groups in mean ages or mean number of UTIs in the previous year (Kruskal-Wallis ANOVA). The number of women with a childhood history of UTI, estrogen use, use of birth control pills, or who were sexual active was not significantly different between treatments (Fisher's exact test).

Vaccine Efficacy

The primary measures of vaccine efficacy were the rates at which patients experienced their first UTI after the start of

<i>Characteristics of patients in study</i>			
	Placebo	Primary Immunization Without Boosters	Primary Immunization+ Boosters
No. pts	25	24	26
Age:			
Mean	54.3	45.0	45.2
Range	26-74	21-70	19-70
UTIs in previous 12 mos:			
Mean	6.2	7.0	7.2
Range	3-12	3-20	3-20
No. childhood UTI history:			
Yes	5	7	2
No	20	17	24
No. hysterectomy:			
Yes	10	5	10
No	15	19	15
No. estrogen use*:			
Yes	10	12	19
No	15	12	7
No. birth control pill use:			
Yes	3	6	10
No	22	18	16
No. sexually active:			
Yes	17	20	22
No	8	4	4

All patients received 3 initial suppositories at weekly intervals followed by 3 additional suppositories at monthly intervals. Women in the placebo group had placebo in each suppository. Vaccine treatment consisted of 3 initial suppositories containing vaccine followed by placebo or vaccine in all 6 treatments.

* Number of patients on estrogen replacement therapy or using birth control pills.

treatment and the proportion of patients without any reported infections at the end of the study. Figure 1 presents Kaplan-Meier curves of patients in each treatment group remaining free of infections caused by any bacteria. The greatest difference in re-infection rates was between patients given vaccine plus boosters compared to those receiving placebo only ($p = 0.100$). Primary immunization without boosters did not differ from placebo treatment ($p = 0.265$). The proportions of women remaining infection-free in the vaccine plus boosters, vaccine without boosters and placebo only groups were 46.0%, 25.0% and 16.7%, respectively.

We further analyzed the data to determine efficacy against UTIs caused by *E. coli*. There were a total of 26 *E. coli* UTIs and 22 infections caused by other bacteria. The rates at which patients in the 3 treatment groups acquired *E. coli* infections are presented in fig. 2. The most statistically significant difference in infection rates was between patients who had primary and booster doses of vaccine compared to those on placebo ($p = 0.0015$). Patients who were given the initial 3 vaccine doses, but no boosters, also acquired their first UTI more slowly than patients receiving placebo only ($p = 0.038$). The proportions of women remaining infection-free in the vaccine plus boosters, vaccine without boosters and placebo only groups were 72.5%, 57.0% and 30.0%, respectively.

The data on *E. coli* infections in women receiving vaccine plus boosters or placebo only were stratified by patient characteristics and reanalyzed to determine efficacy in these subgroups. Nearly 80% of the women in this study trial were sexually active, and *E. coli* UTIs developed in those receiving the initial vaccine doses plus boosters at a significantly slower ($p = 0.0002$) rate than similar women on placebo (fig. 3). In addition, more than 70% (16 of 22) of sexually active, vaccine treated patients had no *E. coli* UTIs during the study, whereas less than 20% (3 of 17) of those on placebo remained infection-free. Statistically significant lower rates of re-infection were observed for vaccine treated women younger than 52 years, without a childhood history of recurrent UTIs, 6 or more UTIs in the previous year, without a hysterectomy, using

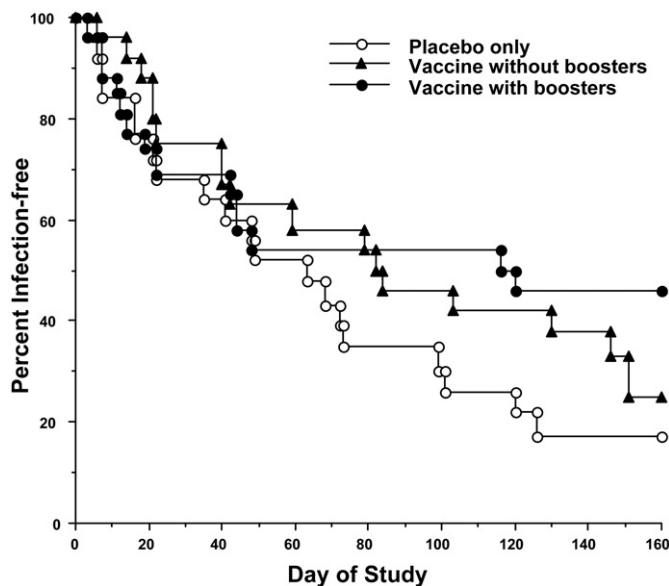


FIG. 1. Percent of women in different treatment groups remaining free of urinary tract infections caused by any bacterial strain.

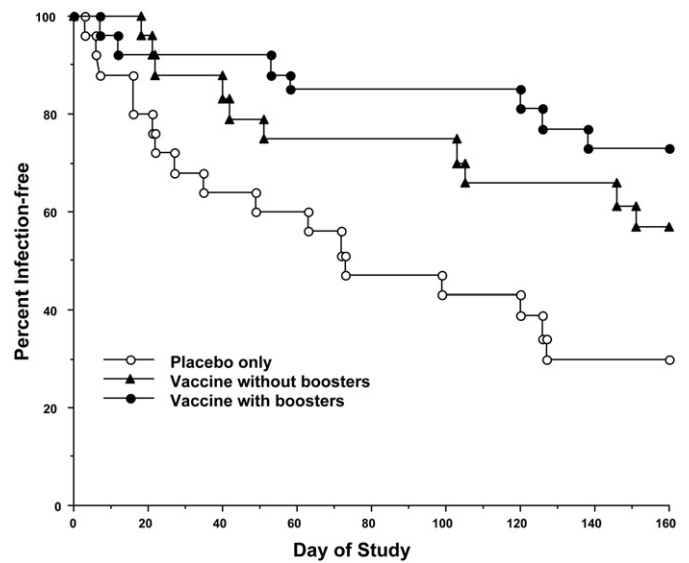


FIG. 2. Percent of women in different treatment groups remaining free of urinary tract infections caused by *E. coli*.

estrogen and using birth control pills ($p = 0.002, 0.003, 0.009, 0.001, 0.002$ and 0.0001 , respectively). For patients younger than 52 years, there were 14 patients in each of the vaccine groups and 9 in the placebo group. The proportions of patients in these subgroups remaining infection-free during the study period ranged from 70.1% to 88.8%. However, it should be noted that while the most significant p value observed was from comparing vaccine with boosters to placebo treatment in women using birth control pills, the placebo group had only 3 subjects.

Antibody Responses

Samples of urine and vaginal secretions were collected from all patients 1 week before their first treatment and throughout the study. Concentrations of urinary and vaginal secre-

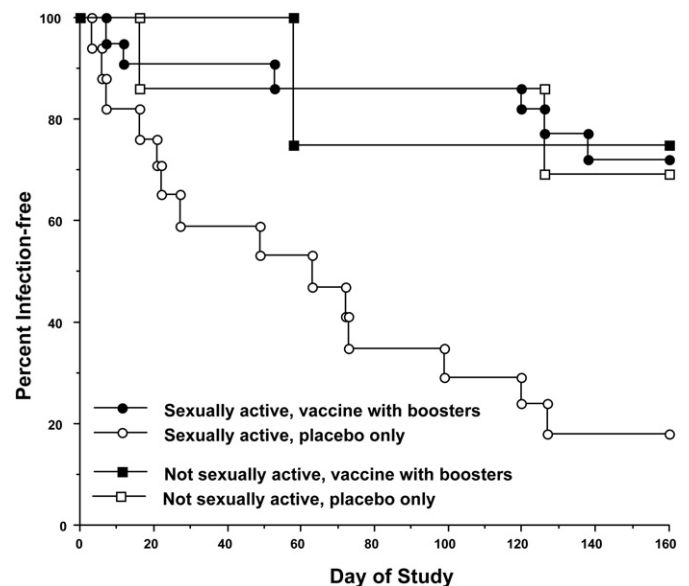


FIG. 3. Percent of women in vaccine with boosters or placebo treatment group who were sexually active and remained free of urinary tract infections caused by *E. coli*.

tory immunoglobulin A anti-*E. coli* antibodies during the study ranged from 15.4 to 36.6 $\mu\text{g}/\text{mg}$ creatinine and 2.0 to 7.5 ng/ml, respectively. Urinary and vaginal anti-*E. coli* secretory immunoglobulin A and immunoglobulin G levels ranged from 10.1 to 47.1 $\mu\text{g}/\text{mg}$ creatinine and 1.9 to 6.8 ng/ml, respectively. The mean urinary and vaginal antibody levels for the 3 treatment groups were statistically analyzed to determine correlations between vaccine treatment and antibody response. While some individual patients on vaccine regimens showed increases in antibody levels over time, there were no statistically significant increases between treatment groups over time or between treatment groups at specific time points.

Adverse Events

Patients in each treatment group experienced some type of adverse event. Women receiving vaccine suppositories reported a total of 6 instances of a burning sensation shortly after treatment. Three women in the placebo group had similar symptoms. A total of 4 patients from the vaccine groups experienced single occurrences of low grade fever, nausea, vaginal bleeding, or vaginal rash. Four women receiving placebo suppositories had, on 1 occasion, low grade fever, headache, bladder pain, or a body rash. There were no statistically significant differences in the frequency of adverse effects among the 3 treatment groups. Two patients reported more severe adverse events. One woman in the placebo only group had pyelonephritis and 1 patient in the vaccine without boosters group had a gallstone removed. Neither of these 2 events was considered vaccine related.

DISCUSSION

The principal objective of this clinical trial was to determine whether primary and booster immunizations with multiple bacterial immunogens in a vaginal suppository could reduce UTIs during a 6-month period in women with a history of recurrent infections. Vaccine efficacy was primarily evaluated by comparing rates at which patients in different treatment groups were re-infected following the initiation of vaccine or placebo treatment. When UTIs were caused by any bacterial strain, there was a trend toward statistical differences between patients who received vaccine plus boosters and those who had only placebo. These results were further analyzed to determine efficacy against *E. coli* UTIs. Women who had 3 initial doses of vaccine plus boosters had a highly significant decrease in the rate of *E. coli* re-infection compared to placebo treatment. Thus, the vaccine appears to be successful in reducing the incidence of *E. coli* UTIs in susceptible women.

Further data analysis examined vaccine efficacy in subgroups of patients based on age, UTI history, estrogen use and sexual activity. When comparing *E. coli* re-infection rates in women receiving vaccine plus boosters to rates in the placebo only group, women younger than 52 years acquired UTIs less rapidly than women older than that age. The vaccine was also found to be effective in women who were sexually active, had not had a hysterectomy, or had 6 or more UTIs in the previous year. Thus, the current results suggest that the vaccine tested here may provide the most benefit to sexually active women who are in the 20 to 50-year-old age range and have frequent UTIs. It is not clear why the older women showed relatively less improvement

after vaccine treatment. One explanation may be the immune senescence that occurs with aging,¹⁴ potentially causing decreased immunological responses to bacterial antigens. Those patients who had hysterectomies also had a poorer clinical response to the vaccine. In this case, it is plausible that loss of immunologically active tissue in the cervix may have diminished induction of protective immune responses in the urogenital tract.¹⁵

As in our previous clinical trials of this vaccine, levels of anti-*E. coli* antibodies were assessed in urine and vaginal secretion samples. No statistically significant differences in mean antibody concentrations at several time points were observed between women in the placebo and vaccine or vaccine plus booster arms of the trial. While there were increased urinary anti-*E. coli* antibodies over time in some vaccine treated patients, mean differences between treatment groups were not significant. There is sufficient evidence from previous experimental animal studies of vaginal mucosal immunization with whole bacteria to anticipate that these women would have similar increases over time.^{16,17} We believe that 1 explanation for the apparent lack of, or weak, urogenital antibody responses is the variability associated with testing single urine or vaginal secretion samples taken at weekly or monthly intervals. Future clinical studies can address this issue by obtaining urine samples collected during a longer period, such as 24 hours, and at more closely spaced intervals.

A potential limitation of this study is that we did not have information on sexual activity beyond whether a patient was sexually active. Therefore, we could not use intercourse frequency, a UTI risk factor, as a covariate in statistical analyses. A possible bias in the study could be introduced if women receiving placebo only had a higher intercourse frequency than those in either of the vaccine groups. In this case women in the placebo group could acquire UTIs at a relatively higher rate and increase the probability of demonstrating a positive vaccine effect. We do not believe that is likely because the randomization procedure evenly allocated patients with all other characteristics into each of the 3 arms of the study.

CONCLUSIONS

The efficacy demonstrated by this vaccine in 2 independent phase 2 clinical trials indicates that it may provide an alternative to long-term antibiotic prophylaxis for recurrent UTIs in susceptible women. We believe that this success may be attributed to vaccine composition and method of delivery. The broad spectrum of antigens present on bacterial virulence factors preserved in the whole-cell vaccine can potentially induce antibodies reactive against similar antigens found on *E. coli* and other UTI inducing strains. Another advantage of this vaccine is its use as a mucosal immunogen, giving it a greater potential to induce protective antibodies on the mucosal surfaces of the vagina and bladder than a parenteral vaccine. A further benefit of mucosal application using vaginal suppositories is that a small number of minor adverse effects have occurred in 2 separate clinical trials. These encouraging results provide the foundation for larger, multicenter studies in which efficacy can be further verified and better insight can be gained into which populations of susceptible women will benefit from vaccine treatment.

Abbreviations and Acronyms

UTI = urinary tract infection

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