Vaccine Development for the Prevention of Urinary Tract Infections

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The effectiveness of antibiotic prophylaxis for recurrent urinary tract infections is being compromised as increasing numbers of uropathogens develop resistance to conventional antibiotics. Because one alternative to antibiotic therapy is immunization of susceptible patients to increase innate resistance, several different vaccines are currently being developed. Four of the vaccines contain a mixture of whole bacteria or an antigenic extract and are administered as a vaginal suppository or oral tablet. A parenteral route is being used in clinical trials of the *Escherichia coli* type 1 fimbrial adhesin and its chaperone protein. The safety of both the mucosal and parenteral vaccines has been demonstrated in phase 1 clinical trials. Phase 2 trials have shown the efficacy of a vaginal mucosal vaccine containing whole bacteria and an oral vaccine prepared from bacterial lysates. Further clinical trials will allow comparisons of the various vaccines and evaluation of their effectiveness relative to prophylactic antibiotic therapy.

Introduction

Recurrent urinary tract infections (UTIs) in women continue to be a significant clinical problem in terms of patient morbidity and health care costs [1]. They are primarily caused by *Escherichia coli* strains, but an increasing number of UTIs are being attributed to *Proteus* species, *Enterococcus* species, *Klebsiella* species, and *Staphylococcus saprophyticus* [2]. Antibiotics usually are effective in treating acute infections and are the primary means of prophylaxis for recurrent UTI patients; however, their value is being lessened by the emergence of increasing numbers of drug-resistant bacteria [3]. Consequently, it is important that alternative prevention strategies be developed, and one approach being actively explored is immunization of susceptible individuals to increase natural immunity against infection.

Strategies in Vaccine Development

The primary objectives in developing a UTI vaccine are efficacy and safety. Ideally, the vaccine will increase patient resistance to the most common uropathogens without causing significant adverse effects. Additional benefits would be obtained if the vaccine could be administered easily at low cost and have broad patient acceptance. Achieving these goals is largely dependent on how a potential UTI vaccine is formulated and its route of administration.

The components of the vaccine should necessarily reflect the antigenic profile of bacteria responsible for the largest number of UTIs, which currently are various strains of *E. coli*, *Klebsiella*, and *Proteus* species [3]. Immunogens prepared from these bacteria can be whole organisms inactivated by chemical or physical treatment, lysates prepared from intact bacteria, or purified cellular structures that are known to be associated with uropathogenicity. The advantage of intact bacteria or crude lysates is that the vaccine will contain a large number of urovirulence factors and potentially afford protection against many different strains of uropathogens. One problem, however, is that bacterial components such as the endotoxin of gram-negative bacteria are likely to be present and cause unacceptable adverse reactions. This drawback can be overcome by using detoxified bacterial lysates or purified virulence factor immunogens. While these preparations are less toxic, the number of bacterial antigens present, and therefore the spectrum of antibodies induced, will decrease and conceivably lessen the ability of the vaccine to protect against a wide range of pathogens.

The route of administration is another important consideration in vaccine development. Our current understanding of UTI etiology is that uropathogens from the intestinal flora sequentially colonize mucosal surfaces of the vagina and urethra prior to establishing an infection on the bladder mucosa. Thus, one objective of an effective immunization program would be to increase mucosal antibody levels against uropathogens in these organs and thereby decrease both initial and prolonged colonization. An effective means of inducing local antibodies is to introduce the immunogen onto the mucosal surface that may become infected or onto a distant mucosal site because of the integrated nature of the mucosal immune system [4]. Alternatively, the immunogen could be administered parenterally; however, this route would be expected to induce lower amounts of specific antibody in mucosal secretions.

Table 1. Summary of vaccines currently in development for prevention of recurrent urinary tract infections in adult women

Vaccine	Immunogen	Administration	Clinical trials
Urovac® (SolcoBasel, Basel, Switzerland and Protein Express, Cincinnati, OH)	Inactivated whole-cell; 10 uropathogens	Vaginal suppository; primary + monthly boosters	Phase 1: patients only; phase 2: vaccine or placebo in patients
Uro-Vaxom® (OM Pharma, Myerin, Switzerland)	Extract from 18 uropathogens	Oral capsule; daily for 3 months	Vaccine or placebo in patients
Urvakol (Institute of Microbiology; Olomouc, Czech Republic)	Inactivated whole-cell; multistrain	Oral tablet; daily for 6 months	Patients only
Urostim (Bulbio; National Center for Infectious and Parasitic Diseases, Sofia, Bulgaria)	Inactivated whole-cells and lysate from four uropathogens	Oral tablet; daily for 3 months	Patients only
FimCH (Medimmune, Gaithersburg, MD)	*Escherichia coli* type 1 fimbrial adhesin and its chaperone	Parenteral	Phase 1: controls only

Various combinations of immunogens and immunization routes have been explored in clinical trials of UTI vaccines. Each of the vaccines currently being tested will be reviewed here and evaluated on the basis of the criteria proposed above for an effective and safe vaccine. A summary of the UTI vaccines now under development is presented in Table 1.

Mucosal Vaccines

Most of the UTI vaccines now being investigated are administered mucosally and differ in the composition of vaccine components and immunization site. One approach has been to administer vaccine onto the vaginal mucosal surface using whole bacteria contained in a suppository. Bacterial antigens taken up and processed by mucosal Langerhans' cells will induce an immune response in regional lymph nodes, after which antibody-producing cells migrate back to the vaginal mucosa. When given as an oral vaccine in tablet or capsule form, antigens present on whole bacteria or in lysates are taken up through the mucosal surface of the small intestine and stimulate antibody responses in the Peyers' patches lining the small intestine. Either route would induce antibacterial antibody in secretions of the immunizing tissue as well as at other mucosal sites such as the bladder. One advantage of vaginal mucosal immunization is that the highest amounts of protective antibodies should be produced in cervicovaginal secretions and inhibit vaginal colonization by uropathogens, thus reducing their ability to establish infections of the urethra and bladder. The mucosally administered vaccines currently under study are Urovac® (SolcoBasel, Basel, Switzerland and Protein Express, Cincinnati, OH); Uro-Vaxom® (OM Pharma, Myerin, Switzerland); Urvakol (Institute of Microbiology, Olomouc, Czech Republic); and Urostim (BulBio; National Center of Infectious and Parasitic Diseases, Sofia, Bulgaria).

Urovac®

One of the earliest reported trials of a UTI vaccine used Urovac®, a vaccine prepared from 10 strains of heat-killed uropathogens isolated from women with acute UTIs. Each vaccine dose contained equal numbers of bacteria from six *E. coli* strains and individual strains of *Proteus mirabilis*, *Proteus morganii*, *Klebsiella pneumoniae*, and *Enterococcus faecalis* at a final concentration of 1 x 10^9 bacteria per dose.

After preliminary animal studies, Urovac® was given intramuscularly to 14 susceptible women who later showed significantly increased urinary secretory immunoglobulin (Ig)A [5]. In a follow-up study, 400 women were randomized to receive parenteral vaccine plus antibiotics as needed or antibiotics alone. The immunized women had fewer reinfections, but 47% had "postvaccinal reactions" including 3.5% with fevers up to 38° C [6]. These adverse reactions were attributed to endotoxin present in the whole-cell preparation.

In order to obviate the adverse reactions of parenteral administration and to induce mucosal immune responses in the urogenital tract, the vaccine has more recently been given as a vaginal suppository containing the same proportions and amounts of bacteria as in the parenteral version. Evidence that administration by this mucosal route is immunostimulating comes from mouse studies where the vaccine was incorporated into a water-oil emulsion and resulted in increased numbers of splenic antibody-forming cells against vaccine components [7]. In monkeys, vaginal immunization with Urovac® resulted in faster resolution of an induced *E. coli* UTI and increased levels of anti-*E. coli* IgG and IgA [8]. Based on these animal studies, phase 1 and 2 clinical trials of the vaccine as a vaginal immunogen were initiated.

The phase 1 clinical trail of Urovac® treated 25 UTI-susceptible women with vaccine given vaginally, and no serious adverse reactions were observed [9]. Subsequently, a phase 2 study entered 91 susceptible women

into a double-blind, randomized, placebo-controlled trial using the vaccine contained in a vaginal suppository [10••]. Immunogen-treated women showed a significant delay in interval to reinfection during the first 8 weeks; mean interval to reinfection was delayed from 8.7 weeks for placebo-treated women to 13 weeks for women receiving vaccine. Again, no serious adverse reactions were observed. In a second phase 2 study focused on extending the period of protection, a group of 36 susceptible women were randomized into three treatment groups of 12 patients each. The first group received three weekly doses of Urovac® in vaginal suppositories followed by three monthly vaginal booster doses of vaccine. The second group received three weekly immunizations plus three monthly placebo suppositories, and the third group was given placebo suppositories at all six timepoints [11]. Women receiving six doses of vaccine showed a significant increase in the time until first reinfection extending through 23 weeks, which was 10 weeks longer than in the previous trial without boosters. From these studies, it appears that Urovac® given as a vaginal suppository is an effective and safe way to decrease UTI susceptibility in patients with recurrent infections. A multicenter, phase 3 trial is currently being planned.

Uro-Vaxom®

The oral route provides an additional method of mucosal immunization and has been used in animal studies and clinical trials of Uro-Vaxom®, a vaccine containing immunostimulating components from 18 uropathogenic *E. coli* strains [12]. Several studies in mice have demonstrated its immunogenicity when administered either orally or parenterally [12–14]. The most recent animal experiments have reported the induction of antibody response to uropathogen antigens not present in the vaccine, primarily *Klebsiella*, *Proteus*, and *Enterococcus* species [15••]. The Uro-Vaxom® immunogen preparation is also immunologically active in humans where experiments in vitro have shown its ability to induce cytokine production in human peripheral blood cells [16].

Several clinical trials of Uro-Vaxom® in UTI-susceptible women have been conducted to determine vaccine efficacy and safety [15••]. Patients with a history of recurent infections were randomized into two groups that received either vaccine or placebo in a double-blind protocol. Each subject took one daily capsule of vaccine or placebo for 3 months and was monitored for recurrence of UTIs caused by *E. coli* or non-*E. coli* organisms over the treatment period and the following 3 months. Adverse effects were reported to be minor or reversible over the treatment and follow-up periods. A statistical meta-analysis of five studies has been performed using the number of UTI recurrences in vaccine- and placebo-treated groups as a measure of efficacy. According to the investigators, results of the analysis indicated a medically relevant difference in the efficacy of Uro-Vaxom® over placebo as a prophylaxis for recurrent UTI.

Urvakol and Urostim

Other oral UTI vaccines have been clinically tested in Europe and include Urvakol in the Czech Republic [17] and Urostim in Bulgaria [18]. Urvakol contains a mixture of inactivated, whole *E. coli*, *P. mirabilis*, *Pseudomonas aeruginosa*, and *E. faecalis* in tablet form. This vaccine has been shown to have immunostimulating activity in animal and patient studies [17]. Urostim is also given as a tablet and contains freeze-dried excipient plus lysates of killed *E. coli*, *P. mirabilis*, *K. pneumoniae*, and *E. faecalis* [18]. Patient studies have suggested an increase in urinary secretory IgA over 12 months of treatment.

Clinical trials conducted for both of these vaccines have not been well structured in terms of consistency in the primary urologic illness of entered patients or the measures of vaccine efficacy during follow-up. Studies with both preparations have included both male and female patients with previous or current episodes of cystitis, pyelonephritis, urethritis, or prostatitis [17,18]. Patients followed treatment protocols for both Urvakol and Urostim in which they took one vaccine tablet each morning for between 6 and 12 months. Recurrences of UTIs in females or prostatitis and urethritis in males were recorded over the study period. Results of the Urvakol studies indicated that 88% of the 34 patients evaluated were either improved or did not experience infections. In reports from the Urostim trials, 46% of patients receiving vaccine, including some with urethritis and prostatitis, were effectively treated. The primary difficulty in statistically analyzing results from clinical studies of these two vaccines is the lack of adequately randomized treatment groups, inclusion of control subjects, and consistent measures of efficacy. The apparently positive results obtained thus far could be strengthened with additional studies using redesigned protocols.

Parenteral Vaccines

Parenteral vaccines for UTI have long been studied and are now undergoing clinical trials. Since P fimbriae are thought to be one of the most important virulence factors for *E. coli* kidney infections in humans, a purified P fimbrial vaccine has been investigated and found to be protective against induced *E. coli* pyelonephritis in nonhuman primates [19]. Type 1 fimbriae, which mediate attachment to vaginal and bladder epithelial cells, are also thought to be important in the induction and pathogenicity of *E. coli* UTIs [20,21,22••]. Consequently, a vaccine that will interfere with the adherence function of type 1 fimbirae to uroepithelial cells is being developed (Medimmune, Gaithersburg, MD). The immunogen consists of the type 1 fimbrial adhesin (FimH) complexed with its chaperone protein (FimC) and combined with an adjuvant [21]. Infection

studies with FimH gene knockout *E. coli* in mice and nonhuman primates have demonstrated that the FimH adhesin is essential for cystitis induction [22••,23] and thus confirmed the hypothesis that it would be a suitable candidate immunogen. A recombinant *E. coli* subunit vaccine consisting of FimH and FimC (FimCH), when given IP to mice, induces specific immunoglobulins capable of blocking attachment of uroviulent *E. coli* to bladder epithelial cells in vitro [22]. Vaccinated monkeys developed anti-FimH antibodies, and three of four animals did not develop cystitis after live *E. coli* were instilled into the bladder [23]. The monkeys did not experience adverse reactions or shifts in fecal bacterial populations.

Based on positive results from these experimental animal studies, human clinical trials have begun with the FimCH vaccine. In a phase 1 trial, 48 adult women without baseline serum antibodies to the adhesin or its chaperone were randomized to receive one of four different doses of vaccine plus adjuvant or adjuvant alone (Personal communication). Subjects were given intramuscular injections at 0, 1, and 4 months of the study and monitored over a 12-month period for increases in anti-FimH antibodies in serum, urine, or vaginal secretions. All vaccine recipients developed serum IgG antibodies to FimH that inhibited binding of uropathogenic *E. coli* to uroepithelial cells, and some immunized women had increases in anti-FimH in urine and vaginal IgG. Adverse reactions to the vaccine within the first 3 to 4 days of injection were considered mild to moderate. Phase 2 studies of vaccine efficacy are in progress.

Conclusions

The encouraging results of clinical trials conducted within the past several years in the United States and Europe point toward successful development of a UTI vaccine that may be generally available in the near future. The Urovac® and Uro-vaxom® mucosal vaccines have progressed the furthest thus far and have shown efficacy against a variety of uropathogens in more than one randomized, placebo-controlled phase 2 trial. Additional advantages of these two vaccines are a low incidence of significant adverse reactions, good patient acceptance, and the ability to be self-administered. The one parenteral vaccine currently in development, FimCH, has proven to be safe in a phase I trial and has the prospect of lessening the incidence of UTIs caused by bacteria with type 1 fimbriae. A drawback to this preparation is the need for parenteral injection, which may preclude it from being administered by the patient herself. Completion of FimCH phase 2 trials will allow a comparison of protection afforded by the three most promising vaccines and provide an indication of which will be successful in completing pivotal clinical trials leading to drug approval.

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