Estrogen Increases Menopausal Host Susceptibility to Experimental Ascending Urinary-Tract Infection

Edward M. Curran,1,2 Audrey Hart–Van Tassell,2 Barbara M. Judy,1,2 Bogdan Nowicki,3,4 Valerie Montgomery-Rice,1,2 D. Mark Estes,1,3 and Stella Nowicki5

Departments of 1Pediatrics and 1Biochemistry and Molecular Biology and 3Sealy Center for Vaccine Development, University of Texas Medical Branch, Galveston; Departments of 2Obstetrics and Gynecology and 4Microbiology, Meharry Medical College, Nashville, Tennessee

(See the editorial commentary by Stamm, on pages 623–4.)

The protective effect of estrogen replacement on ascending urinary-tract infection (UTI) is controversial. We designed a study using an experimental model of UTI in which surgically menopausal mice were supplemented with estrogen and the susceptibility to UTI was evaluated after experimental Escherichia coli infection. The mean rate of E. coli infection in the group not treated with estrogen was 2 × 10^4 cfu/g of renal tissue, compared with 9 × 10^4 cfu/g (P < .001) in the estrogen-treated group. Surprisingly, despite the hypothesis that estrogen would protect mice from infection, estrogen treatment significantly increased the susceptibility of the mice to ascending UTI.

Urinary-tract infection (UTI) accounts for 8 million patient visits in the United States annually and requires billions of dollars for management. One of the most significant clinical issues of UTI is recurrence, which affects 25%–50% of patients [1]. The underlying mechanisms of recurrent UTI are only partially understood. Among the clinical groups at increased risk for recurrence are postmenopausal women, who exhibit a drastic cessation of estrogen production. Controlled clinical trials with estrogen replacement in postmenopausal women with recurrent UTI significantly decreased reported rates of recurrent UTI [1, 2] and established estrogen-replacement guidelines for the prevention of UTI. Nevertheless, some clinical studies suggested that hormone replacement is associated with an increased risk of UTI [3].

There is evidence that the female urethra plays an important role in combating UTI, in addition to the powerful antimicrobial defenses of the bladder [4]. Interestingly, the vaginal and urethral epithelial linings share a common embryonic origin and are estrogen responsive, undergoing changes with menstrual cycle and age. Hormonally induced cyclic changes in exfoliation of the urethral and vaginal epithelium may be important in regulating the normal microbial flora, including intruding pathogenic microbes. Exfoliation of infected bladder epithelial cells is thought to be one of the established defense mechanisms against infection [5].

Several bacterial colonization factors seem to be crucial for the initiation and etiology of disease pathogenesis in UTIs. Urinary-tract pathogenic Escherichia coli express adhesive proteins on the cell surface called pili, fimbriae, or adhesins, including P pili, type 1 pili, and Dr adhesins, which mediate the adherence of the bacterium to uroepithelial cells [5–7]. E. coli P fimbriae are associated with acute pyelonephritis [7]. The initial binding of P fimbriae triggers ceramide release and the subsequent activation of intracellular signaling pathways [8]. P fimbriae require Toll-like receptor (TLR)–4 to act in a lipopolysaccharide (LPS)–independent manner as a coreceptor for intracellular signaling [9]. A strong proinflammatory response mediated by E. coli P fimbriae is important for the acute infection process. E. coli type 1 fimbriae are associated with cystitis and mediate the invasion of bladder epithelial cells, which activates the production of interleukin-6 in the same cells via a classical LPS-dependent pathway requiring CD14/TLR4 signaling [9]. E. coli Dr fimbriae are associated with an increased risk of recurrent UTI and cause experimental chronic pyelonephritis. E. coli Dr adhesins interact with uroepithelium by recognizing CD55 (also called decay-accelerating factor), type IV collagen, and carcinoembryonic antigen, and they invade the epithelium using lipid rafts and microtubule networks [10]. The fimbrial antigen can persist in infected tissues for several months [11].

Because recurrent/chronic UTI is a significant clinical problem, we developed an experimental ascending, nonobstructive UTI model in C3H/HeJ mice in which Dr fimbriated E. coli
Figure 1. Enhancement of infection rate in experimental urinary-tract infection (UTI) by estrogen (E2) replacement. Ovariectomized C3H/HeJ mice (Toll-like receptor–4 nonresponders) were either implanted with a 17β-estradiol pellet or sham implanted and then inoculated 1 week later with 50 mL of cfu/mL of clinical isolates of Escherichia coli \(81/H11003\) expressing either type 1, P, or Dr fimbriae. After 4 days of incubation, the mice were killed, and the infection rate was determined by homogenization of bladder and kidney tissues, followed by plating of homogenates on MacConkey’s agar plates. The infection rate for sham-implanted control mice (black symbols) vs. E2-treated mice (white symbols) is expressed as colony-forming units per gram of bladder (A) or kidney (B) tissues. Horizontal bars indicate the median infection rate for each group. The statistical significance (*) of differences in the medians between groups was determined using the Mann-Whitney rank-sum test. The statistical significance of differences in colony-forming units between treatment groups was evaluated using a non-parametric Mann-Whitney rank-sum test. Calculations were done with the GraphPad Prism statistical software package (version 4; GraphPad Software).

Results. Ovariectomy results in estrogen levels that are at or below the lowest levels observed during diestrus in mice, mimicking a postmenopausal state in which estrogen levels can persist for >1 year [11]. In the present study, we used this established model to investigate the controversial impact of estrogen replacement on UTI in mice with surgically established menopause. We treated mouse strains more susceptible (C3H/HeJ) or more resistant (C3H/HeN) to UTI with a high dose of 17β-estradiol and then infected them with \(E. \ coli\) strains expressing either Dr fimbriae associated with chronic/recurrent UTI, P fimbriae causing acute pyelonephritis, or type 1 fimbriae causing cystitis. We measured the severity of infection by evaluating the proportion of mice infected and by quantitation of colony-forming units per gram of bladder or renal tissue, and we show that, instead of the expected protection against UTI, estrogen treatment increased the severity of UTI in the mouse model.

Materials and methods. C3H/HeJ (LPS nonresponders) or C3H/HeN (LPS responders) mice were purchased from Jackson Laboratories. For ovariectomy, mice were anesthetized with iso-flurane, and bilateral incisions were made in the abdominal cavity. Each ovary was removed, the wound was closed, and the mice were allowed to recover for 10–14 days. Mice were divided into 2 groups: 1 group received a 0.25 mg, 21-day-release 17β-estradiol pellet (Innovative Research) by incision with a 12-gauge trocar needle under isoflurane anesthesia; the other group was subjected to the same surgical procedure, but no pellet was implanted. 17β-estradiol treatment was continued for 7 days. This dose of 17β-estradiol pellet results in serum estrogen levels of ∼800 pg/mL in mice [12]. The mice were inoculated with 50 μL of a 1 × 10⁷ cfu/mL \(E. \ coli\) suspension delivered by transurethral catheterization, monitored for 4 days, and killed by cervical dislocation, and bladder and kidney tissues were collected and homogenized for determination of bacterial infection rates [11]. \(E. \ coli\) used for infection were strains 3166 (type 1), 7446 (P), and 11128 (Dr) isolated from the urine of patients with UTI [11]. In total, >100 mice were infected with the 3 different \(E. \ coli\) strains. To determine the level of bacterial infection, bladder and kidney tissues from each mouse were weighed, homogenized, and serially diluted, and 50 μL of the suspension plated on Luria broth and MacConkey’s agar plates. The plates were incubated overnight at 37°C, bacterial colonies were counted, and the number of colony-forming units per gram was calculated. To reduce the potential effects of exogenous estrogens, mice were fed a casein-based diet that contained few or no phytoestrogens.

The statistical significance of differences in colony-forming units between treatment groups was evaluated using a non-parametric Mann-Whitney rank-sum test. Calculation were done with the GraphPad Prism statistical software package (version 4; GraphPad Software).
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One week later, 50 μL of 1 × 10⁶ cfu/mL of Escherichia coli obtained from clinical isolates expressing Dr fimbriae were inoculated by transurethral injection into the bladders of the mice. Increased infection was seen after estrogen replacement in mice infected with Dr⁺ E. coli but not with E. coli mutants that do not express Dr adhesin. Ovariectomized C3H/HeJ (TLR4 nonresponder) mice were either implanted with a 17β-estradiol pellet or sham implanted. One week later 50 μL of 1 × 10⁶ cfu/mL Dr fimbriae–deficient (−) of E. coli were inoculated by transurethral injection into the bladders of the mice. The infection rate for sham-implanted control mice (black symbols) vs. E2-treated mice (white symbols) is expressed as colony-forming units per gram of bladder for kidney tissues. The statistical significance (P < .05) of differences in the medians between groups was determined using the Mann-Whitney rank-sum test.

Figure 2. Impact of estrogen (E2) replacement on infection rate in lipopolysaccharide (LPS) responder (A) and LPS nonresponder (B) mice. Ovariectomized C3H/HeN (Toll-like receptor–[TLR]–4 responder) mice were either implanted with a 17β-estradiol pellet or sham implanted. One week later 50 μL of 1 × 10⁶ cfu/mL of Escherichia coli obtained from clinical isolates expressing Dr fimbriae were inoculated by transurethral injection into the bladders of the mice. Increased infection was seen after estrogen replacement in mice infected with Dr⁺ E. coli but not with E. coli mutants that do not express Dr adhesin. Ovariectomized C3H/HeJ (TLR4 nonresponder) mice were either implanted with a 17β-estradiol pellet or sham implanted. One week later 50 μL of 1 × 10⁶ cfu/mL Dr fimbriae–deficient (−) of E. coli were inoculated by transurethral injection into the bladders of the mice. The infection rate for sham-implanted control mice (black symbols) vs. E2-treated mice (white symbols) is expressed as colony-forming units per gram of bladder for kidney tissues. The statistical significance (P < .05) of differences in the medians between groups was determined using the Mann-Whitney rank-sum test.

In contrast to the bladder, a significantly higher bacterial infection rate was observed in the kidneys of estrogen-treated mice for E. coli expressing type 1 fimbriae (median, 1.6 × 10⁴ cfu in control mice vs. 2.9 × 10⁷ cfu in estrogen-treated mice; P = .0076), P fimbriae (median, 2.8 × 10⁴ cfu in control mice vs. 1.0 × 10⁷ cfu in estrogen-treated mice; P = .0006) or Dr fimbriae (median, 2.0 × 10⁴ cfu in control mice vs. 3.2 × 10⁴ cfu in estrogen-treated mice; P = .0147) (n = 9–10 mice/treatment group and E. coli strain) (figure 1B). This suggests that estrogen treatment increased the susceptibility of mice to renal infection.

We then asked whether the estrogen effect would be manifested in mice with normal TLR4 signaling in LPS responder mice (C3H/HeN). Because all 3 E. coli strains showed an increased rate of infection in E2-treated C3H/HeN mice, we selected the E. coli Dr⁺ strain for subsequent experiments, given that this strain was used to establish the chronic pyelonephritis model. As expected, the bacterial infection rates were much lower in C3H/HeN than in C3H/HeJ mice. Estrogen-treated C3H/HeN mice exhibited a higher bacterial infection rate (6/7 mice infected) than controls (2/7 mice infected), although this did not reach statistical significance in the Mann-Whitney rank-sum test (median, 0 in control mice vs. 10 cfu/g in estrogen-treated mice; P = .0530) when E. coli expressing Dr fimbriae were inoculated (figure 2A). However, the biological trend (P = .0530) toward a higher infection rate in estrogen-treated C3H/HeN TLR4 responsive mice suggests that estrogen plays a role in increased host susceptibility to experimental UTI even when TLR4 signaling is not affected.

To determine whether estrogen treatment would have a similar impact on bacteria lacking Dr adhesin (and therefore the capacity to colonize and invade urogenital tissues), we used the mutant strain E. coli Dr14, which does not express Dr fimbriae, and the control clinical E. coli strain IH11128 [11]; we then
tested the infection rate in response to estrogen. In contrast to intact E. coli, which caused more severe infection with estrogen treatment, there were no significant differences in numbers of colony-forming units in kidney in estrogen-treated mice, compared with nontreated mice infected with E. coli Dr 14, which lacks the capacity to attach and invade (figure 2B). These data suggest that the effect of estrogen on mice susceptibility to infection appeared to be at least partially dependent on the expression of Dr adhesin.

Discussion. We have demonstrated that high-dose estrogen treatment of C3H/HeJ (LPS nonresponder) mice resulted in higher bacterial infection rates in the kidney for 3 clinical isolates of uropathogenic E. coli expressing type 1, P, and Dr fimbriae. High-dose estrogen treatment also appeared to alter infection rates in C3H/HeN (LPS responder) mice, suggesting that the effect of estrogen treatment was not an artifact due to the lack of LPS signaling. The effect of estrogen treatment appeared to be dependent on the presence of adhesins, because infection with Dr-deficient E. coli was not significantly affected in estrogen-treated mice. The idea that estrogen can alter the attachment of uropathogenic E. coli is supported by older studies in humans in which the use of oral contraceptives was associated with an increased attachment potential of E. coli to exfoliated uroepithelial cells [13]. Furthermore, in vitro treatment of exfoliated uroepithelial cells with estrogen and progesterins resulted in a significantly greater attachment in treated cells.

The mechanisms of UTI involve the ability of E. coli to colonize different regions of the urinary tract depending on the expression of attachment factors and the receptors specific for those urogenital-tract regions. Our present results suggest that the effect of estrogen on UTIs appears to be both organ and E. coli adhesin specific and, thus, appears to resemble some aspects of the pathogenesis of UTI in humans. All E. coli strains showed an increased bacterial infection rate in the kidney. The severe decrease in estrogen levels at the time of menopause may be associated with an increased incidence of recurrent UTIs that can be partially reversed by the administration of local (topical vaginal creams) and/or systemic (hormone replacement therapy) estrogen [2]. Interestingly, in an earlier study, postmenopausal women (>3600) receiving long-term estrogen therapy (>1 year) had a 2-fold increased risk for UTI, compared with >19,000 control patients [3]. One possible explanation is that differences between the studies may be related to the “dose” or route of delivered estrogen. A common medical issue in postmenopausal women is atrophic vaginitis, which often presents with symptoms mimicking cystitis and is a complaint relieved by estrogen replacement. Therefore, atrophic vaginitis could be a confounding factor affecting the results of clinical studies and accounting for the discrepancy in reported findings. Our experimental data illustrate the necessity for further understanding the role played by steroid hormones in the physiology of the urinary tract and the immune defense against pathogens of the urogenital tract, and animal models will be important in this regard.

The present results illustrate the potential for 17β-estradiol modulation of disease severity during the course of UTIs and challenge the existing paradigm that hormone replacement therapy protects against recurrent UTI. Estrogen replacement can restore the vaginal environment and therefore reduce menopausal complaints of atrophic vaginitis and the risk for UTI. However, there remains a significant proportion of the patient population in which estrogen replacement therapy does not help, and these patients require further evaluation for possible estrogen protection, compared with the increased risk for UTI caused by E. coli strains.

References