Int Urogynecol J (1993) 4:194–198

International Urogynecology Journal

Original Article

Influence of the Spermicidal Compound Nonoxynol-9 on the Adhesion of *E. coli* to Human Epithelial Cells

J. A. McGroarty[1] D. Faguy[2], A. W. Bruce[3] and G. Reid[3,4]

[1]Department of Biological Sciences, Glasgow Caledonian University, Glasgow, Scotland; [2]Department of Microbiology and Immunology, Queens University, Kingston, Ontario; [3]Division of Urology, Department of Surgery, University of Toronto, Ontario and [4]Department of Microbiology and Immunology, University of Western Ontario, London, Ontario, Canada

Abstract: Two type I piliated strains of *Escherichia coli* were cultured in medium supplemented with physiologically used concentrations of the spermicidal compound nonoxynol-9. Their ability to adhere to HeLa cells in tissue culture was found to increase significantly ($p<0.05$). For strain 12313, there was a 2.3- and 1.9-fold increase when cultured in 5% and 12.5% (w/v) nonoxynol-9 respectively, and adhesion of strain 12269 increased by 1.7 times after growth in 12.5% (w/v) nonoxynol-9. The increased adhesion was accompanied by loss of mannose-sensitive yeast agglutination and hemagglutination. The absence of type I piliation was confirmed by electron miscroscopy and was accompanied by a decrease in cell surface hydrophobicity. The ability of *E. coli* to grow in high concentrations of nonoxynol-9, combined with increased adhesion to human epithelial cells in vitro, may contribute to the increased incidence of *E. coli* urinary tract infection seen in women using diaphragms and spermicidal preparations for contraception.

Keywords: Adhesion; *E. coli*; Epithelium; Nonoxynol-9; Pili; Spermicide

Introduction

The use of a diaphragm in conjunction with spermicide has been associated with an increased risk of urinary tract infection. Women using this form of contraception have been found, in a number of studies, to be 1.5 [1], 2.0 [2], 2.5 [2] and 4.1 [3] times more likely to develop a urinary tract infection. It has been postulated that this may be due to intermittent obstruction of the urethra caused by the rim of the diaphragm, which has been shown to impede urine flow [4]. This could prevent the washout of uropathogenic organisms, or cause mechanical damage to the urethra, making the tissues more susceptible to infection. However, in 1985 Fihn et al. [2] suggested that diaphragm use might predispose to urinary tract infection through alteration of the vaginal flora, as they and others [5–8] recovered aerobic Gram-negative rods more frequently from the vaginas of diaphragm users than from non-users. In addition, diaphragm-spermicide use could be correlated with a vaginal pH of >5.0, which was interpreted as possibly being due to depletion of lactobacilli from the vaginal flora [2,8].

Lactobacilli are believed to play a protective role in the urogenital tract. It has been suggested that they prevent the overgrowth of pathogenic bacteria by elaboration of antimicrobial substances such as lactic acid, hydrogen peroxide and bacteriocin-like compounds, and also by competitively excluding the adhesion of pathogens [9]. McGroarty et al. [10] have shown that the majority of vaginal lactobacillus isolates are sensitive to concentrations of nonoxynol-9 as low as 0.1%–1.0% (w/v) in vitro, and uropathogenic *E. coli* and enterococci are capable of growth in the presence of up to 25% (w/v) nonoxynol-9. As nonoxynol-9 is generally used at a concentration of 5% (w/v) in cream, and 12.5% (w/v) in spermicidal foam, it is feasible that the presence of nonoxynol-9 in the vagina could upset the delicate ecological balance of the bacterial flora. By

Correspondence and offprint requests to: Dr G. Reid, Office of Research Services, SLB 328, University of Western Ontario, London, Ontario, Canada M6A 5B8.

inhibiting the protective lactobacilli, it may favor colonization by pathogenic microorganisms. The aim of the present study was to determine the effect of nonoxynol-9 on the adhesion of *E. coli* to human epithelial cells in vitro.

Materials and Methods

Preparation of Bacteria

E. coli strains 12313 and 12269, previously isolated from the urine of female patients (non-diaphragm/spermicide users) with urinary tract infection, were used throughout. Both strains were type I piliated and non P-piliated. The bacteria were subcultured once, before use in all experiments, in either brain, heart infusion broth (Difco) supplemented with 2% (w/v) yeast extract, termed BYE, or BYE supplemented with 1%, 5% or 12.5% (w/v) nonoxynol-9 at 37°C. Stationary phase cultures were used for all experiments. Isolates were stored at −70°C in BYE supplemented with 20% (w/v) glycerol. Bacteria were harvested by centrifugation and washed three times in calcium- and magnesium-free phosphate-buffered saline (PBS) (pH 7.1). Prior to inclusion in the adhesion assay the bacteria were resuspended to a concentration of 10^8 cells/ml in Dulbecco's PBS (PBS plus 0.01% (w/v) $MgCl_2.6H_2O$ and 0.01% (w/v) $CaCl_2$ anh.).

Growth of E. coli Cultured in BYE Supplemented With Nonoxynol-9

The growth of both *E. coli* strains was monitored spectrophotometrically over 24 hours, and the corresponding viable count was determined at intervals by plating serial tenfold dilutions onto BYE agar.

Preparation of HeLa Cells

HeLa cells (ATCC CCL2) were cultured in monolayers on glass coverslips in multiwell tissue culture trays in Eagle's minimal essential medium, containing glutamine (Gibco) supplemented with 2.2 g/l $NaHCO_3$, 10% (v/v) fetal bovine serum, 100 units penicillin/ml and 100 mg streptomycin/ml. Confluent monolayers formed after 3–4 days' incubation at 37° in an atmosphere of 10% CO_2.

Adhesion Assay

The adhesion of *E. coli*, cultured in BYE medium with and without nonoxynol-9, to HeLa cells was determined using the method of Samaranayake and MacFarlane [11]. Briefly, washed HeLa cell monolayers were incubated with *E. coli* suspended in Dulbecco's PBS at 37°C in an orbital shaker at 60 rpm for 60 minutes. The monolayers were washed in PBS, fixed in 10% (v/v) formal saline and Gram stained. The number of *E. coli* adherent to 50 HeLa cells was counted by light microscopy, and each condition was assessed three times in duplicate.

Cell Surface Hydrophobicity

Qualitative measurements of cell surface hydrophobicity were obtained for both *E. coli* strains, cultured in either BYE or BYE supplemented with nonoxynol-9, using the adhesion to hydrocarbon method of Rosenberg et al. [12]. Briefly, the bacteria were washed three times in PBS and resuspended to give an absorbance of approximately 0.6 in PUM buffer, pH 7.1 at 400 nm. Hexadecane (200 μl) was layered onto 3 ml of *E. coli* suspension, vortexed for 2 minutes and allowed to separate for 15 minutes. The decrease in absorbance of the aqueous layer, after the addition of hexadecane, was expressed as a proportion of the original culture absorbance. Each condition was assayed three times in triplicate.

Hemagglutination/Yeast Agglutination Assay

The assays were performed using standard techniques [13,14], on suspensions of washed bacteria, cultured in BYE or BYE supplemented with nonoxynol-9. The *E. coli* were resuspended to a concentration of 10^{10}/ml in PBS and tested for their ability to agglutinate 2% (v/v) washed human and horse erythrocytes and glutaraldehyde-fixed *Candida albicans* suspensions in the presence and absence of D-mannose.

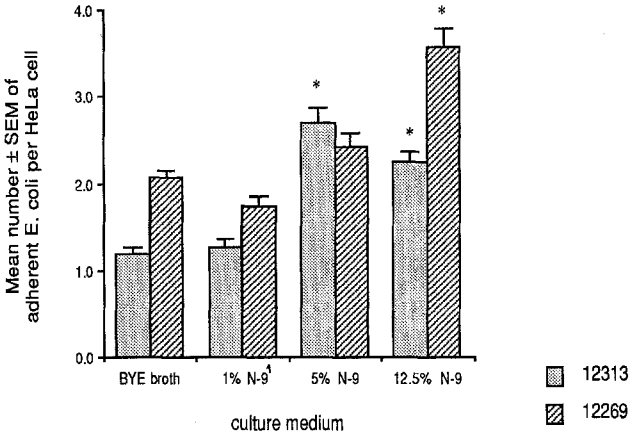
Electron Microscopy

The *E. coli* strains were examined for the presence of pili using negative staining. Briefly, a drop of washed suspension of *E. coli* (10^9 cells/ml) in distilled water was placed onto formvar-coated grids, followed by a drop of 1% (w/v) phosphotungstic acid, pH 7.0. The grid was filter-point dried and examined using a transmission electron microscope.

Results

Growth Rate of E. coli

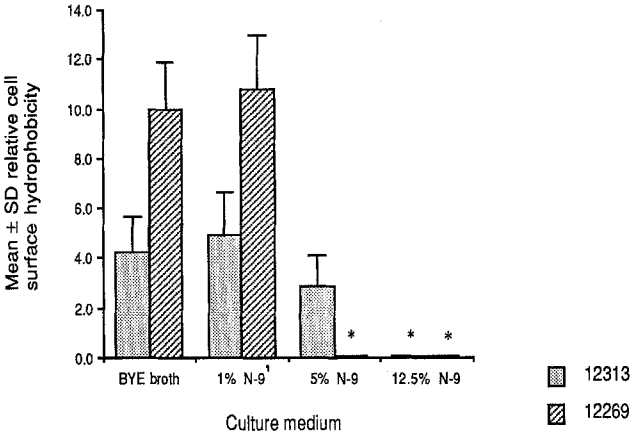
The inclusion of nonoxynol-9 in the growth medium did not affect the growth rate of either *E. coli* strain. Growth rates were similar for both strains, which reached the stationary phase by 18 hours.



* - P< .05 versus control by student t-test

1 - nonoxynol-9

Fig. 1. Adhesion of *E. coli* strains 12313 and 12269, cultured in BYE broth alone or supplemented with nonoxynol-9, to HeLa cells.



* - P< .05 versus control by student t-test

1 - nonoxynol-9

Fig. 2. Cell surface hydrophobicity of *E. coli* strains 12313 and 12269, cultured in BYE broth alone or supplemented with nonoxynol-9.

Adhesion of E. coli to HeLa Cells

E. coli strain 21313 showed increased adhesion when cultured in BYE medium containing 5% and 12.5% (w/v) nonoxynol-9 (Fig. 1). Adhesion increased by 2.3 and 1.9 times respectively ($p<0.05$, Student's *t*-test). Analysis of the adhesion data showed that the increased adhesion was not simply due to bacterial aggregation on the surface of the HeLa cells. There was a significant increase ($p<0.05$, Student's *t*-test) in the proportion of HeLa cells with one or more adherent *E. coli* cultured in these concentrations of nonoxynol-9. The proportion of cells with one or more adherent *E. coli* 12313 increased from 53% (control) to 64% (5% w/v nonoxynol-9) and 67% (12.5% (w/v) nonoxynol-9). The adhesion of strain 12269 increased by 1.7-fold after growth in 12.5% (w/v) nonoxynol-9 (Fig. 1). When cultured in BYE alone, 60% of the HeLa cells had one or more adherent *E. coli* 12269. This increased to 78% when cultured in 12.5% (w/v) nonoxynol-9 ($p<0.001$, versus control by Student's *t*-test).

Cell Surface Hydrophobicity

There was a marked difference between the cell surface hydrophobicity of the two strains tested (Fig. 2). Strain 12313 showed 4.25% reduction in absorbance, and strain 12269 a 9.96% reduction. The hydrophobicity of both strains decreased after growth in 5% and 12.5% (w/v) nonoxynol-9.

Hemagglutination/Yeast Agglutination

Both strains of *E. coli* demonstrated mannose-sensitive hemagglutination of horse erythrocytes, when cultured in BYE broth. No hemagglutination was observed when the *E. coli* were cultured in medium supplemented with 5% and 12.5% (w/v) nonoxynol-9. This indicated a functional loss of type I piliation. No hemagglutination of human erithrocytes occurred, and neither of the organisms expressed mannose-resistant hemagglutinins under any growth condition tested. Since erythrocytes would be sensitive to lysis by any nonoxynol-9 residue in the *E. coli* cultures, the yeast agglutination assay was performed and gave identical results to the horse erythrocytes.

Electron Microscopy

Electron microscopic examination of the *E. coli* strains showed them to be heavily piliated when grown in BYE or BYE supplemented with 1% (w/v) nonoxynol-9 (Fig. 3). Pili were absent from the cells grown in 5% and 12.5% (w/v) nonoxynol-9-supplemented cultures (Fig. 4).

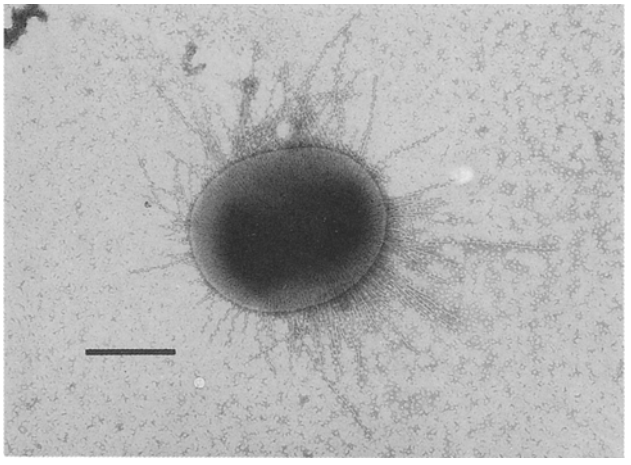


Fig. 3. *E. coli* strain 12313 cultured in BYE broth, stained with 1% (w/v) PTA, viewed end-on. Bar = 1 μm.

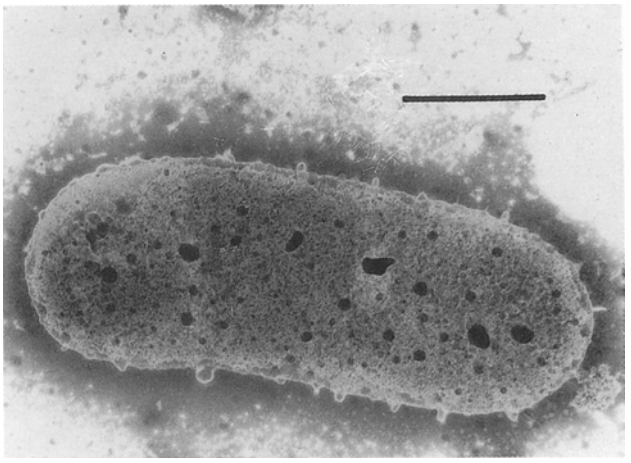


Fig. 4. *E. coli* strain 12313 cultured in BYE supplemented with 5% (w/v) nonoxynol-9, stained with 1% (w/v) PTA. Bar = 1 μm.

Discussion

Nonoxynol-9 is inhibitory to many clinical isolates of lactobacilli, specifically hydrogen peroxide-producing strains [15], and it has been suggested that by killing or inhibiting the growth of these organisms, nonoxynol-9 may cause the vagina to become more susceptible to colonization by uropathogens. However, in addition to this, nonoxynol-9 could affect the virulence of uropathogens. We have explored this possibility by examining the growth and adhesion of *E. coli*, the most commonly isolated uropathogen from diaphragm/spermicide users.

The adhesion of strain 12313 increased by approximately twofold when grown in 5% and 12.5% (w/v) nonoxynol-9, and for strain 12269 when cultured in 12.5 (w/v) nonoxynol-9. Although the increases seen were modest, the assay were performed over 60 minutes only, and the starting concentration of the *E. coli* suspension was chosen so that approximately 50% of the HeLa cells would have one or more adherent bacteria. The results may have clinical significance; women who use diaphragms for contraception are twice as likely to harbour *E. coli* as part of the vaginal flora [5]. This may, in part, be due to the increased adhesiveness of these organisms. Analysis of the adhesion data indicated that the increases were not simply due to aggregation of the bacteria on the surface of the HeLa cells. In another study, nonoxynol-9 was found to affect the adhesion of *C. albicans* in a similar fashion [16].

Type I pili are filamentous, proteinaceous bacterial appendages produced by the majority of uropathogenic *E. coli* strains. They are the mediators of mannose-sensitive hemagglutination [13]. In the urinary tract, piliated organisms have been shown to adhere to epithelial cells more efficiently than non-piliated organisms [17]. Pili production is affected by culture conditions: most broth-grown organisms are piliated, whereas agar-grown organisms do not tend to express pili. *E. coli* cultured in BYE supplemented with nonoxynol-9 lost the ability to agglutinate horse erythrocytes, and electron microscopy confirmed the absence of pili, either attached to the *E. coli* or in suspension. The cell surface hydrophobicity of the nonoxynol-9 grown cells decreased significantly, which correlates with the finding that non-piliated *E. coli* are less hydrophobic than piliated cells [18]. However, contrary to expectation, the nonoxynol-9-grown non-piliated *E. coli* showed increased adhesion to epithelial cells. The suspension of *E. coli* in nonoxynol-9 has previously been shown not to affect adhesion [10]. This suggests that the absence of pili in nonoxynol-9-grown *E. coli* was more effective at promoting adhesion than type I pili. Nickerson et al. [19] have described 'detergent shock proteins' induced in several genera of Gram-negative bacteria in response to growth in sodium dodecylsulphate. Nonoxynol-9 possibly acts in a similar way; preliminary data suggest that novel outer membrane proteins may contribute to the increased adhesiveness of nonoxynol-9-grown *E. coli* (unpublished observation).

In conclusion, nonoxynol-9 does not inhibit the growth of uropathogenic *E. coli*. On the contrary, the presence of physiologically used concentrations in growth medium suppresses the formation of type I pili, which is accompanied by decreased cell surface hydrophobicity and a twofold increase in adhesion to epithelial cells. We suggest that the increased adhesion of *E. coli* may play a role in the pathogenesis of *E. coli* urinary tract infections in women using diaphragms in conjunction with spermicides for contraception.

Acknowledgements. Funding for this project was obtained from MRC (Canada) and in part from the Ontario Ministry of Health and University Research Incentive Fund. We thank Gary Lee for his enthusiastic technical assistance.

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EDITORIAL COMMENT: Understanding why barrier contraception with spermicides induces urinary tract infections in women has been an ongoing puzzle. This well designed study evaluates the role of type I pili in urinary tract infections associated with spermicide and diaphragm use. In the presence of spermicide *E. coli* lose their type I pili, but there is an increase in bacterial adhesiveness, which may be the mechanism responsible for the increase in urinary tract infections in these women. The demonstration of this phenomenon will also help our understanding of bacterial pathogenicity.

Review of Current Literature

Treatment of Bacteriuria in Pregnancy

Tan JS[1] and File TM Jr[2]

[1]Department of Medicine, 74 Arch St, Suite 105, Akron City Hospital, Akron, Ohio; [2]Department of Medicine, Infectious Disease Section, Northeastern Ohio Universities College of Medicine, Rootstown, Ohio, USA
Drugs 1992;44:972–980

Bacteriuria during pregnancy increases the chance of acute pyelonephritis. Although 5.9% of normal pregnant women have bacteriuria, 60% of diabetics and those with a history of previous urinary tract infection have bacteriuria. Between 23% and 40% of these bacteriuric women develop acute pyelonephritis later in pregnancy. Treatment of bacteriuria in pregnancy reduces the risk of subsequent symptomatic disease. Diagnosis of asymptomatic bacteriuria is best made by midstream clean-catch urine culture with a colony count of 100000 cfu/ml on repeat samples. Many patients (60% or more) were treated successfully with single-dose therapy with amoxicillin 3 gm, nitrofurantoin 200 mg, cephalexin 2 g, or even sulfisoxazole 2 g. The culture is repeated 1 week after initial therapy. Positive results are followed by treatment for 7–10 days with either the same or a different antibiotic. Continued positive cultures require prophylaxis, either continuously or after intercourse. If the culture is negative after the initial therapy, then cultures are repeated monthly. Medications considered unsafe in pregnancy because of fetal effects include trimethoprim (folate antagonist), tetracyclines (tooth and bone growth inhibition) and quinolones (arthropathy). Preferred drugs for prophylaxis include nitrofurantoin and cephalexin.

Comment

This is a review article rather than a scientific study, but it is of importance since it makes a number of statements and recommendations regarding bacteriuria in pregnancy. The diagnosis is made by midstream clean-catch urine for culture, and does not require a catheterized specimen. The clean-catch culture should show more than 100000 cfu/ml on two occasions. Treatment may be successful with single-dose therapy, and increases compliance greatly. Failures of therapy require a longer course, and often prophylaxis for the duration of the pregnancy. Generally trimethoprim and quinolones should be avoided in pregnancy. Symtomatic infections should be treated with antibiotic therapy at diagnosis, and intravenous therapy in the case of pyelonephritis.

Renal Colic in Pregnancy

Stothers L and Lee LM

Division of Urology, Department of Surgery, University of British Columbia, Vancouver, BC, Canada
J Urol 1992;148:1382–1387

Eighty patients had a discharge diagnosis of renal colic for an incidence of 0.12% in pregnancy. Stones were confirmed in 57 patients, but all had normal renal function. Stones were passed spontaneously during the pregnancy in 40 of the patients, 8 passed the stones spontaneously postpartum, 1 had the stone removed during pregnancy, and 8 had intervention in the postpartum period. Although hematuria is almost universal in stone disease, it was not noted in 25% of the confirmed stone patients until the urinalysis had been repeated two or three times. Ultrasound is useful in detecting hydronephrosis in pregnancy, but limited IVP is required for the diagnosis of most stones. The least amount of radiation with the maximum diagnostic benefit is achieved with a preliminary plain abdominal film followed by a 30-s film and a 20-min film. Ninety-nine per cent of the stones occurred in the second or third trimester. Thirty-seven procedures were done in 23 patients during pregnancy because of persistent pain, sepsis, progressive hydronephrosis, solitary kidney or high-grade obstruction. Most of these patients had placement of an ureteral stent, and all of these patients had spontaneous vaginal delivery.

Comment

Stone disease in pregnancy is not an uncommon event, although pregnancy does not increase the incidence. Most patients are initially mistreated for urinary tract infection. This can be prevented in most cases by taking a good history and following this with a physical examination and microscopic assessment of the urine. Urinary tract infection rarely produces unilateral pain unless there is pyelonephritis, and in these cases there should be temparature elevation, the patient looks toxic rather than thrashing around in agony, and the urine will usually show many pus cells and bacteria, rather than red cells. Conservative management includes hydration, providing pain relief, and straining the urine. The complicated situations include sepsis in addition to the stone disease, persistent pain, progressive hydronephrosis, high-grade obstruction and solitary kidney. Evaluation may include ultrasound to rule out hydronephrosis, and a modified IVP.