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Effect of D-Mannose and D-Glucose on *Escherichia coli* Bacteriuria in Rats

E. K. Michaels*[1], J. S. Chmiel[2], B. J. Plotkin[1] and A. J. Schaeffer[1]

Departments of Urology[1] and Community Health and Preventive Medicine, Cancer Center Biometry Section[2], Northwestern University Medical School, Chicago, Illinois, USA

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Summary. The effect of D-mannose and D-glucose on bacteriuria due to *Escherichia coli* with mannose-sensitive adhesins was investigated in adult male Sprague-Dawley rats undergoing diuresis. Inocula of 10^5, 10^7, or 10^8 bacteria in 0.1 ml of normal saline or 2.5% or 10% D-mannose or D-glucose were injected intravesically and urine was cultured 1, 3, 5, 7 and 9 days later. The levels of bacteriuria on days 1 and 5 were significantly lower in rats inoculated with 10^5 *E. coli* and 10% D-mannose than in controls ($p < 0.05$ and 0.01 respectively) and the percentages of rats with < 100 bacteria/ml were higher on days 1 and 3 ($p = 0.05$ and 0.02 respectively). Bacteriuria was significantly lower in rats inoculated with 10^7 bacteria and 10% D-mannose than in controls on days 5 and 7 ($p < 0.01$ for each day) and the percentage of rats with < 100 bacteria/ml was higher on day 7 ($p = 0.01$). D-glucose reduced bacteriuria significantly only with a concentration of 10% after instillation of 10^5 *E. coli* ($p < 0.05$, day 1). The results indicate that D-mannose and D-glucose can significantly reduce bacteriuria within 1 day and that their efficacy is dependent upon the concentration of both saccharide and bacteria.

Key words: *Escherichia coli,* D-mannose, Urinary tract infections, Rats, Adherence.

Introduction

Successful invasion of the urinary tract by *E. coli* may involve binding of the bacterium to the uroepithelium [6, 17, 19–21]. This interaction between *E. coli* and uroepithelial cells appears to be mediated by the surface components on both cell types. Competitive inhibition assays which have been used to probe the biochemical nature of the components involved in the mediation of adherence have shown that *E. coli* adherence to epithelial cells can be blocked by the addition of D-mannose but not D-glucose to the bacterial-epithelial cell mixture [10, 11]. These results support the hypothesis that *E. coli* can recognize and bind to D-mannose or D-mannose-like receptors on the epithelial cell surface.

The effect of the mannose derivative, methyl-α-D-mannose on experimental ascending urinary tract infections in female mice was investigated by Aronson [2]. Bacteriuria was monitored by collecting voided urine specimens. Injection of *E. coli* with mannose-sensitive adhesins in the presence of the methylated saccharide resulted in a significant reduction in the number of bacteriuric mice on the fifth day post inoculation. However, specimens collected within 5 days were judged unreliable since both controls and test animals were usually found to be bacteriuric. The purpose of this study was to examine the effect of D-mannose and D-glucose on bacteriuria in rats after intravesical inoculation with *E. coli* possessing mannose-sensitive adhesins. To prevent contamination associated with voided specimens, we obtained urine by percutaneous aspiration through a ventral bladder hernia which does not alter the response of rats to intravesical innoculation with *E. coli* [8]. The results suggest that D-mannose and to a lesser extent D-glucose can significantly reduce bacteriuria within one day of inoculation and that their effect is dependent upon the concentration of the saccharide and the size of the inoculum.

Materials and Methods

Bacteria

The *E. coli* strain (serogroup 018) used in all experiments was isolated from bladder urine. The strain has fimbriae, exhibits D-mannose-sensitive agglutination of guinea pig erythrocytes and readily adheres to human uroepithelial cells in vitro; adherence is completely inhibited in the presence of D-mannose and not affected by D-glucose [16]. *E. coli* were grown in brian heart infusion broth

* *Present address:* Loyola Medical Center, Department of Urology, Maywood, Illinois, USA

(BHI, Difco, Detroit, Michigan, USA) for 72 h, harvested by centrifugation, and washed once in phosphate buffered saline (PBS, Difco; pH 7.2). To minimize bacterial variation between experiments, the *E. coli* were suspended in BHI plus dimethyl sulfoxide (DMSO, Fisher Scientific, Chicago, Illinois, USA) at a final concentration of 5% (vol/vol) and frozen immediately in an acetone dry ice bath [16]. The frozen aliquots of bacteria were stored at −20 °C in screw-capped tubes. When preparing bacteria for an experiment, an aliquot was thawed at 37 °C, washed once in PBS, inoculated into yeast nitrogen base containing 0.5% glucose (YNB, Difco; pH 7) and incubated overnight at 37 °C. The bacteria were harvested, washed once in PBS, and inoculated to an optical density of 0.03 at 540 nm (Coleman Spectrophotometer Model 44) into YNB. Log phase bacteria (optical density = 0.15) were harvested, washed once in PBS, resuspended and serially diluted in 0.85% sodium chloride to from 10^6 to 10^9 colony forming units (CFU)/ml.

Adherence Assay

Log phase bacteria (optical density = 0.15) were harvested and suspended in minimal essential medium (MEM, GIBCO, Lawrence, Mass, USA; ph 5.4) containing 2.5% D-mannose or 2.5% D-glucose (Sigma) or in MEM alone, to a concentration of 10^8 CFU/ml. Bladder epithelial cells were obtained by gently scraping the mucosal surface of bladders which had been removed from rats drinking tap water or 5% glucose water. The cells were suspended in MEM, washed once and resuspended to a concentration of 10^5 cells per ml, as determined by hemocytometer count. Cell viability was greater than 95% as determined by trypan dye exclusion. For the assay, equal volumes of the bacterial and epithelial cell suspensions were mixed and incubated for 30 min at 37 °C. Non-adherent bacteria were separated from the bacteria-cell complex by vacuum filtration using 5.0 μm polycarbonate filters (Nuclepore). The number of bacteria per cell was determined by indirect fluorescent microscopy.

Indirect Immunofluorescence

Impression smears of each filter were incubated with serogroup 018 antisera (Difco) for 30 min at 37 °C. After repeated washing with normal saline to remove any unbound antibody, a drop of 0.25 mg/ml of FITC conjugated *Staphylococcus aureus.* Protein A (Pharmacia Fine Chemicals) was placed on the smear and the slide incubated for an additional 30 min at 37 °C. Unbound FITC-Protein A was removed by repeated washing. The stained smears were examined for the number of fluorescing bacteria per cell for a minimum of 50 cells. All assays were done in duplicate. Smears of epithelial cells only were also stained as a control for non-specific fluorescence.

Experimental Animals

Male albino Sprague-Dawley rats (Harlan/Sprague-Dawley; Madison, Wisconsin) weighing 200–300 g were allowed unlimited access to Purina Rat Chow. To permit accurate percutaneous aspiration of urine, a ventral bladder hernia was created by surgically repositioning the bladder dome in a subcutaneous suprapubic location [8]. The base of the bladder remained in its normal position. Animals were allowed 2 weeks recuperation before inoculation with bacteria. Previous studies have shown that this procedure does not alter the incidence of bacteriuria in control or polyuric rats after intravesical inoculation of *E. coli* [8].

Bacterial Inoculation

The model of Freedman which is based on the injection of *E. coli* into the bladder of rats undergoing diuresis was used [7]. Six days prior to inoculation, the animals were allowed unlimited access to 5% glucose water. Animals were excluded from the study if their intake on any day was less than 100 ml/day, the amount required to induce a level of polyuria sufficient to maintain bacteriuria. Animals were anaesthetized by ether inhalation. The skin overlying the bladder dome was prepared with Betadine and alcohol. Urine was aspirated for baseline culture through a 27 gauge needle, reducing the residual bladder volume to approximately 0.2 ml. Inocula of 10^5, 10^7 or 10^8 *E. coli* suspended in 0.1 ml of 0.85% sodium chloride, 2.5% or 10% D-mannose or 2.5% or 10% D-glucose were injected. For each experiment a saline group was included as a control to insure the virulence and viability of the bacteria used. Suprapubic aspirates of urine were obtained, 1, 3, 5, 7, and 9 days after inoculation. Cultures were performed by plating 0.1 ml of urine on blood and Mac Conkey agar plates (Difco) which were incubated at 37 °C for 48 h. Positive urine cultures were periodically serotyped.

Statistical Analysis

Urine cultures in individual animals were recorded as CFU/ml of urine. The data were grouped into categories (five intervals of equal length on a logarithmetic scale, and one open ended interval) and coded as 0 (less than 10 CFU/ml), 1 (10 up to 10^2 CFU), etc. thru 5 (10^5 CFU or more). For each day post-inoculation these grouped data were used to compute the mean value (± standard error) for each experimental group. Differences among these means were examined statistically, for each day, using one-way analysis of variance methods [18], with two-sided significance levels for pairwise comparisons determined using the Tukey-Kramer modification (for unequal sample sizes) of Tukey's method for multiple comparisons [5]. For each active test group the percentage of animals having fewer than 100 CFU/ml was compared to the respective control group percentage using a Chi-square test corrected for continuity [18]. For simplicity in these latter analyses, significance levels are reported without adjustment for multiple comparisons.

Results

Effect of D-mannose and D-glucose on E. coli

Exposure of suspensions of *E. coli* in filter sterilized urine containing 10% D-mannose or 10% D-glucose at 37 °C for 24 h did not effect viability; instead there were insignificant increases in the numbers of organisms.

Effect of D-mannose and D-glucose on Adherence of E. coli in Vitro

The uropathogenic *E. coli* adhered to bladder epithelial cells obtained from both diuretic and non-diuretic rats to a similar extent (3.8 ± 1.2 (mean of three experiments ± standard deviation) and 4.2 ± 2.0 bacteria per cell, respectively). Adherence was totally inhibited by 2.5% D-mannose and was not affected by D-glucose.

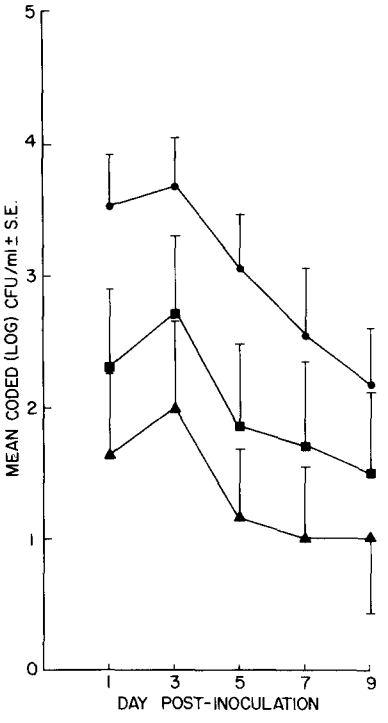

Fig. 1. Effect of D-mannose on bacteriuria. Colony forming units per ml after intravesical inoculation of 10^5 *E. coli* suspended in 0.85% sodium chloride (•), or 2.5% (■) or 10% (▲) D-mannose. N = 25, 14, and 12 animals respectively

Effect of D-mannose of Bacteriuria

Figure 1 shows the mean number (coded log scale) of CFU/ml (± standard error) of urine following inoculation with 10^5 *E. coli*. In control animals, the mean level of bacteriuria was at least 10^3 CFU/ml for days 1, 3 and 5 and then decreased gradually. A consistent dose dependent reduction in the level of bacteriuria was observed for animals in the 2.5% and 10% D-mannose groups compared to the control group. The mean level of bacteriuria for animals in the 10% D-mannose group was significantly lower than the level observed in controls on day 1 and 5 ($p < 0.05$ and 0.01 respectively). The percentages of animals in each group having less than 100, at least 100 but less than 10,000, or 10,000 or more CFU/ml on each day after inoculation are shown in Fig. 2. A gradual increase in the number of control animals with less than 100 CFU/ml was observed on each day post inoculation. With the exception of day 1, instillation of 2.5% D-mannose produced a consistent increase in the number of animals with less than 100 CFU/ml on each day post inoculation. Ten percent D-mannose was more effective and produced a statistically significant increase in the number of animals with less than 100 CFU/ml when compared to controls on the first and third day after inoculation (p = 0.05 and 0.02 respectively).

The effect of 10% D-mannose on bacteriuria after inoculation with 10^7 *E. coli* is shown in Fig. 3. In control animals, the mean level of bacteriuria increased from 10^4

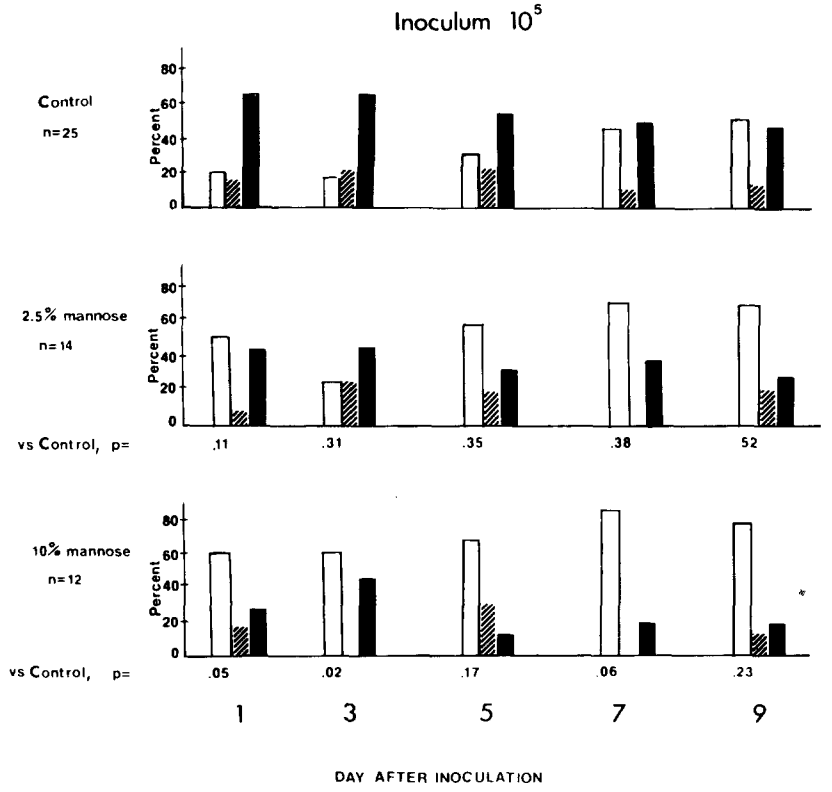

Fig. 2. Effect of D-mannose on bacteriuria. Percent of animals with <100 (□), 100 to <10,000 (▨) or at least 10,000 (■) colony forming units per ml urine after intravesical inoculation of 10^5 *E. coli* suspended in 0.85% sodium chloride or 2.5% or 10% D-mannose

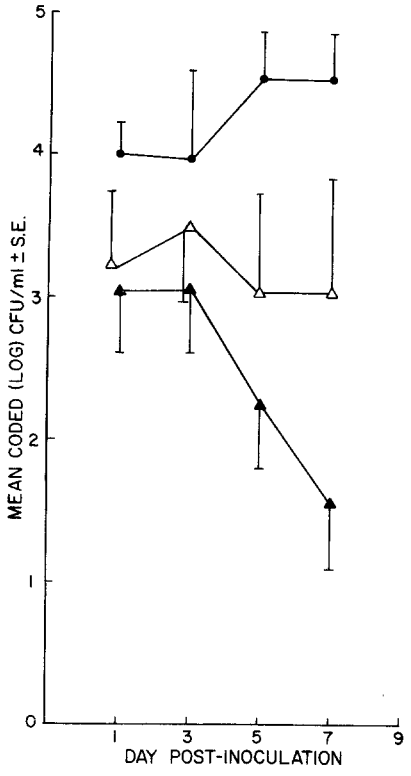

Fig. 3. Effect of D-mannose or D-glucose on bacteriuria. Colony forming units per ml after intravesical inoculation of 10^7 *E. coli* suspended in 0.85% sodium chloride (•), 10% D-glucose (△) or 10% D-mannose (▲). N = 8, 23, and 10 animals respectively

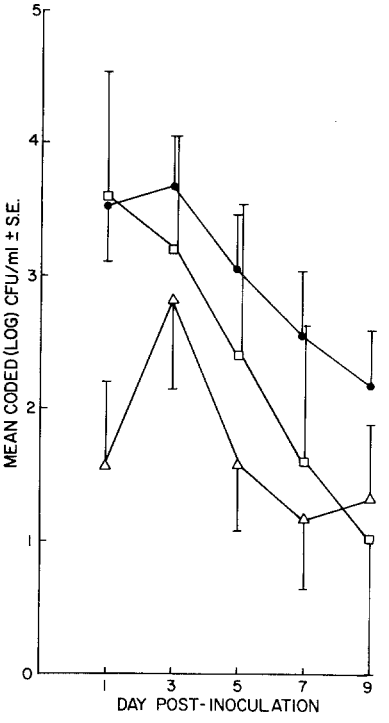

Fig. 4. Effect of D-glucose on bacteriuria. Colony forming units per ml after intravesical inoculation of 10^5 *E. coli* suspended in 0.85% sodium chloride (•) or 2.5% (□) or 10% (△) D-glucose. N = 25, 15 and 12 animals respectively

to approximately 5 x 10^4 CFU/ml. In comparison to control animals, the mean level of bacteriuria in the 10% D-mannose group was lower by a factor of 10 on the first and third days post inoculation, and the mean level was statistically significantly reduced by a factor greater than 100 on days 5 and 7 ($p < 0.01$ for each day). All of the control animals had at least 10^3 CFU/ml with at least 75% of the animals, at any time, having $\geqslant 10^4$ CFU/ml (data not shown). After instillation of 10% mannose, the percentage of animals with $\geqslant 10^4$ CFU/ml decreased from 58% (day 1) to 26% (day 7). Concomitantly, the number of animals with < 100 CFU/ml increased from 22% (day 1) to 62% (day 7) ($p = 0.01$ for day 7 versus control).

The effect of 2.5% and 10% D-mannose on bacteriuria after inoculating 10^8 *E. coli* was a statistically significant reduction in the level of bacteriuria for animals in the 2.5% D-mannose test group as compared to controls ($p < 0.05$) only on day 1. For the remaining observations the effect of D-mannose was not consistently dependent upon its concentration or time post inoculation.

Effect of D-glucose on Bacteriuria

D-glucose is a six carbon carbohydrate which differs from D-mannose only in the position of the hydroxyl group at the second carbon. The effect of 2.5% and 10% D-glucose on an inocula of 10^5 *E. coli* is shown in Fig. 4. Comparison with controls revealed a significant reduction in bacteriuria only for animals in the 10% D-glucose group on day 1 ($p < 0.05$). This effect was not evident on day 1 after instillation of 2.5% D-glucose. For most of the other comparisons including those after instillation of 10^7 *E. coli* in 10% D-glucose (Fig. 3), the trend was toward a reduction in the level of bacteriuria compared to that in the control animals but the differences were not statistically significant.

Discussion

Although voiding appears to be a very efficient mechanism for the clearance of bacteria from the urinary tract, the ability of even a few potential pathogens to overcome this defense mechanism by adhering to the uroepithelium may be an important step in the development of urinary tract infections [4, 9, 12–14]. In vitro experiments with buccal, vaginal, and bladder epithelial cells suggest that adherence of some strains of *E. coli* is mediated by a mannose-specific lectin-like substance on the surface of the bacterium which binds to a mannose-like residue site on the epithelial cell [3, 11, 16]. The results of this study indicate that D-mannose can cause a significant reduction in the level and

prevalence of bacteriuria in diuretic rats and that the efficacy of D-mannose is dependent upon the concentration of both saccharide and bacteria.

A significant reduction in the level and prevalence of bacteriuria was observed when rats were instilled with 10^5 *E. coli* and 10% D-mannose. The reduction in the level of bacteriuria was either not as great or not demonstrably evident when lower concentrations of D-mannose or larger inocula were used. However, in all instances where D-mannose was instilled an increase was observed in the percentage of rats with <100 CFU/ml. In addition to exerting an in vivo effect on *E. colli,* D-mannose also inhibited the in vitro adherence of *E. coli* to rat bladder transitional epithelial cells. These results are consistent with other in vitro studies which have shown that the inhibitory effect of D-mannose was dose related and linear [16]. When the concentration of D-mannose added to a bacterial suspension was varied its effect on adherence was altered. At a concentration of 2.5%, D-mannose completely inhibited *E. coli* adherence to vaginal epithelial cells, whereas when concentrations of between 1.0 and 0.1% were added, only partial inhibition of adherence occurred. In addition, the inhibitory effect of a fixed concentration of D-mannose decreased if bacterial adherence ability increased. Taken together, these data suggest that D-mannose inhibits *E. coli* adherence by either competing with the bacteria for the epithelial cell receptor or by binding the bacterial adhesin thus preventing it from interacting with the receptor.

The significant reduction in bacteriuria observed at 1 day post inoculation when 10% D-mannose was combined with 10^5 *E. coli* suggests that the inhibitory effect is immediate. When 10% D-mannose was added to 10^7 *E. coli* a significant reduction in bacteriuria was delayed until the 5th day post inoculation. Since it is unlikely that at 5 days post-instillation D-mannose would still be present at a concentration sufficient to influence adherence, the inhibitory effect of D-mannose may occur when the bacteria are inoculated. D-mannose may reduce bacteriuria by binding bacteria and thus cause a reduction in the number of bacteria able to interact with the bladder mucosa. When high concentrations of bacteria are used the system could be overloaded. Thus the initial effect of D-mannose would be masked by large numbers of bacteria with which the D-mannose has not been able to interact. However, by reducing the effective inoculum size, D-mannose could enhance the efficacy of other natural bladder defense mechanisms [9, 14].

Instillation of D-glucose resulted in a reduction in the mean level of bacteriuria which in most cases was at least 50% of that observed with D-mannose. Although this effect was statistically significant in only one instance (one day post instillation of 10% D-glucose, compared to controls) it contrasts with the lack of an in vitro inhibitory effect with rat epithelial cells in this study and in other in vitro and in vivo studies where mouse and human epithelial cells were used [2, 11, 16]. However, if adherence is an important factor in the pathogenesis of *E. coli* urinary tract infections, then the inhibitory effect of D-glucose could be explained by the observation of Saier [15] which indicates that D-glucose may repress the fimbrial operon via cAMP-mediated catabolite repression. Since fimbriae are mediators of *E. coli* adherence the supression of their expression would result in a reduction of *E. coli* adherence to the bladder mucosa.

Previous data indicate that D-mannose does not nonspecifically prevent adherence or infection when bacterial attachment to uroepithelium is independent of mannose-sensitive adhesins [2, Schaeffer, unpublished data]. D-mannose and D-glucose could also nonspecifically reduce bacteriuria by causing an increase in the urine osmolality. Urine osmolality in excess of 800 to 1,000 mOsm per liter can be lethal to gram negative bacteria [9]. However, in our experiments the relatively high concentration of 10% D-mannose or D-glucose (550 mOsm per liter) was offset by the low urine osmolality (100–200 mOsm per liter) characteristically observed in the polyuric rat [1]. Furthermore, in vitro studies showed no bactericidal effect on *E. coli* incubated with 10% D-mannose or D-glucose at 37 °C for 24 h.

The results of this study indicate that D-mannose and to a lesser extent D-glucose can reduce bacteriuria in polyuric rats. The mechanism by which these two saccharides act is still uncertain. However, there is an indication that their mode of action may be via inhibition of *E. coli* adherence to the bladder mucosa. Further studies are needed to confirm this hypothesis.

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Dr. A. J. Schaeffer
Department of Urology
Northwestern University Medical School
303 E. Chicago Avenue.
Chicago, IL 60611
USA