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Influence of urological irrigation fluids on urothelial bacterial adherence

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Summary. The influence of various urological irrigation solutions on bacterial growth and adherence to urothelium was investigated in in vitro and guinea pig models. The irrigation solutions glycine 1.5%, glycine 1.5% and ethanol 1%, glycerol 3%, mannose 6%, sorbitol 2.7% and mannitol 0.54% all inhibited bacterial growth compared with normal saline. In guinea pigs, the influence on bacterial adherence of four irrigation solutions (glycine 1.5%, glycine 1.5% and ethanol 1%, mannose 6%, povidone-iodine) was investigated using two different strains of *E. coli*. After cauterizing one side of the bladder and inoculation with 2.7×10^8 colony forming units under high or low pressure, the bladder was irrigated with the irrigation solutions. There was a stronger adherence of *E. coli* O6 (with type I pili) than of *E. coli* ATCC 25922 (without type I pili) to bladder urothelium, particularly to the injured side. There was no significant difference between the high- and low-pressure groups. None of the various irrigation solutions was clearly superior. As mannose 6% effectively inhibited type I pili and also had some antibacterial activity it may reduce urinary tract infection if used as irrigation solution.

Key words: Adherence – Bladder irrigation – Transurethral surgery – Urothelium

Urinary tract infection following transurethral surgery occurs in up to 30% of patients [5]. The bacteria originate from the external meatus, urethra, prostate, bladder tumors, contaminated irrigation fluids or from the inevitable eye-lens contact during resection. Once the bacteria have gained access to the bladder, adherence to the urothelium is the first step to infection [14]. The influence of the irrigation fluids used during transurethral resection on bacterial adherence to the bladder mucosa is unknown. We therefore investigated the influence of various well-known irrigation solutions, as well as some newer ones, on bacterial adherence in in vitro and in vivo studies.

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Materials and methods

Irrigation solutions

The following irrigation solutions were investigated: glycine 1.5%, glycine 1.5% and ethanol 1%, glycerol 3%, and mannose 6%. Additionally, in the in vitro experiments the mixture of sorbitol 2.7% and mannitol 0.54% was studied. As a control normal saline and povidone-iodine (Betadine, Napp; diluted 1:10) was used for the in vitro and in vivo studies, respectively.

Preparation of bacterial solution

Two different strains of *Escherichia coli* were used: *E. coli* ATCC 25922, a commercially available and widely used standard strain, and *E. coli* O6, and O serotype often found in urinary tract infections and also pathogenic in rats [3]. The latter strain was isolated from a patient with an urinary tract infection. The bacteria were grown overnight in Mueller Hinton broth (BBL Microbiology Systems, Cockeysville, Mass.) and 1 ml of this solution was injected undiluted into the bladder, giving a mean inoculum of 2.7×10^8 colony forming units per millilitre (CFU/ml).

In vitro studies

The influence of the irrigation fluids (glycine 1.5%, glycine 1.5% and ethanol 1%, glycerol 3%, mannose 6%, sorbitol 2.7%, and mannitol 0.54%) on bacterial type I pili and on bacterial growth was studied in an in vitro model.

Inhibition of type I pili

Among other factors type I pili play an important role in the mechanism of bacterial adherence to the urothelium [11]. The presence of type I pili was determined by an in vitro agglutination

Table 1. pH values of the irrigation solutions investigated and their ability to block bacterial agglutination

Irrigation solution	pH	Blocking effect
Glycine 1.5%	6.00	No
Glycine 1.5%/ethanol 1%	6.01	No
Glycerol 3%	7.12	No
Mannose 6%	4.74	Yes
Sorbitol 2.7%/mannitol 0.54%	6.02	No

test of guinea pig red blood cells [13]. Briefly, 1 ml guinea pig blood was centrifuged at 5000 rpm for 10 min. The red cells were then resuspended in normal saline and centrifuged again. This procedure was repeated three times. Fifty microliters of the packed red cells were then resuspended in 2 ml normal saline, giving a 5% solution. One hundred microliters of this solution were placed on a microscope slide and an equal volume of bacterial solution added. The results were read after 3–5 min with a scanning microscope. In the presence of type I pili an agglutination of red cells was noted.

To test whether these type I pili are inhibited by the irrigation fluids under investigation, one hundred microliters of the fluid were added to the red cells before adding bacteria. The experiments were performed in duplicate. The results were read after 3–5 min. If the type I pili are inhibited no agglutination will occur.

Antibacterial activity of irrigation solution

To investigate whether the various irrigation solutions have any antibacterial activity or, on the contrary, enhance bacterial growth, 3.4×10^5 CFU *E. coli* ATCC 25922 were dissolved in 10 ml of each of the irrigation fluids. This solution was incubated at 37°C. After 15, 30, 45, 60, and 120 min, 0.1 ml of this solution was streaked out on MacConkey agar (Difco Laboratories, Detroit, Mich.). The plates were incubated overnight and the colonies counted the next day with a scanning microscope. The percentage of the original inoculum was calculated over time and "killing curves" were created.

Additionally, the pH of the solutions was determined using a pH meter (Orion Research Model 801A, Hawthorn, N.Y.).

In vivo studies

White Hartley guinea pigs (Charmaney Farms, Madison, Wis.) with a mean weight of 491 g were used for the experiments. The animals were anesthetized with ketamine hydrochloride (60 mg/100 g body weight) and xylazine (6 mg/100 g body weight) intramuscularly. The shaved skin was prepared with merthiolate (Eli Lilly, Indianapolis, Ind.). A lower midline laparotomy was performed, and the bladder carefully exposed and opened at the dome with a knife blade. To mimic surgery involving electrocautery, the left side of the bladder was cauterized with a small electrode for 3 s. A small 1.09 mm polyethylene tube (Clay Adams, Parsippany, N.J.) was introduced into the bladder and secured with a purse-string suture. After clamping the bladder neck to prevent leakage, 1 ml of *E. coli* solution containing 2.7×10^8 CFU was injected into the bladder via the tubing. To mimic high-pressure conditions an intravesical pressure of 60 cmH_2O was maintained. For this, the polyethylene tubing was connected via a three-way connector to a container filled with 60 cm of water at room temperature. After 30 min the clamp was removed, and bladder irrigation was started. An adjustable pump connected to the three-way was started. During irrigation the intravesical pressure was continuously monitored. The irrigation fluid was drained via the urethra. The bladder was irrigated at low pressure (less than 20 cmH_2O) for 15 min with a mean volume of 60 ml (4 ml/min). After 15 min the irrigation was stopped. The bladder was carefully removed to avoid additional damage to the urothelium, and the animal was killed. The tissue which was macroscopically affected by the purse-string suture was removed. After rinsing in three consecutive baths of sterile saline the bladder was cut in half and the cauterized left and non-cauterized right side were examined separately.

A total of 137 animals were studied in 30 subgroups with three to six animals in each. Each of the irrigation solutions (povidone-iodine 1:10, mannose 6%, glycine 1.5%, glycine 1.5% and ethanol 1%, glycerol 3%), was tested with the two different *E. coli* strains (ATCC 25922 and O6). The group with *E. coli* ATCC 25922 was examined with and without cauterizing the bladder and under high- and low-pressure conditions. The group with *E. coli* O6 was studied with and without cauterizing the bladder under high-pressure conditions only. Therefore, six subgroups for each of the five irrigation solutions were created, comprising a total of 30 (see also Table 2).

Bacterial counts

To express the bacterial counts in the bladder, the two sides of the bladder were placed in separate preweighed plastic tubes containing 2 ml sterile saline, and the weight of the tissue was determined. The tissue was homogenized in a polytron homogenizer (Kinematica, Lucerne, Switzerland). Serial dilutions of 1:5 were made of the homogenate and 0.1 ml of each dilution was streaked out on MacConkey agar. The plates were incubated overnight at 37°C, and the number of CFU counted using a scanning microscope. Only bright red colonies were accepted as *E. coli.*

Statistics

For the killing curves analysis of variance (ANOVA) was used for data analysis. For the in vivo experiments three way analysis of covariance (ANCOVA) with fixed effects was used. The statistical calculations were performed on a personal computer using the statistics program Statistica (Stattsoft Co., USA). A P value of <0.05 was considered significant.

Results

In vitro studies

The pH values of the various irrigation solutions are listed in Table 1. The *E. coli* ATCC 25922 did not cause agglutination of red cells. Conversely, with *E. coli* O6 a strong agglutination of red cells was noted, indicating presence of type I pili. This agglutination could be completely blocked with mannose 6%. However, none of the other irrigation solutions studied inhibited the red cell clotting.

The killing curves of the various solutions studied are shown in Fig. 1. Compared with normal saline a clear reduction in the number of bacteria was noted. After 120 min the most pronounced reduction was noted with mannose 6%, where only 48% of the initial inoculum was present. This was followed by sorbitol/mannitol (58%), glycerol (71%), glycine/ethanol (72%) and glycine (88%). However, the killing curves were not significantly different, probably due to the small numbers of experiments.

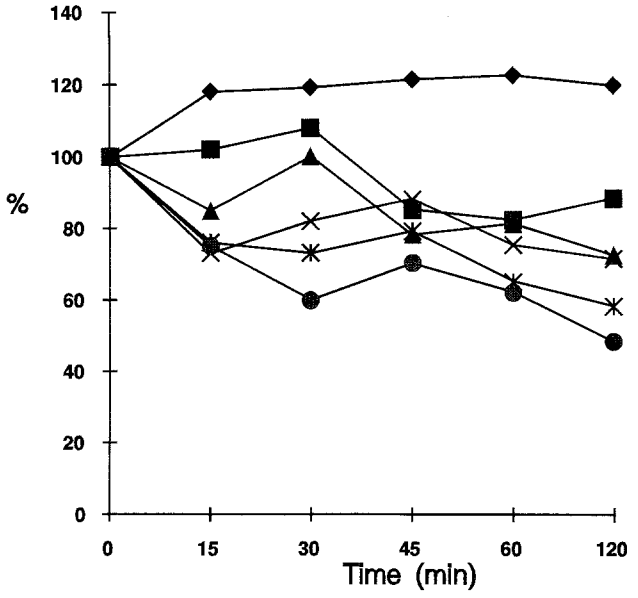

Fig. 1. "Killing curves" of *E. coli* ATCC 25922 in various irrigation solutions. %, percentage of original inoculum
—◆— Saline; —■— glycine; —▲— glycine/ethanol; —✕— glycerol; —✳— sorbitol/mannitol; —●— mannose

In vivo studies

With the inoculum of 2.7×10^8 CFU all bladder samples became heavily infected. Bacterial adherence to urothelium of *E. coli* serotype O6 was clearly stronger than that of *E. coli* ATCC 25922 (Table 2). The difference was statistically highly significant ($P = 0.00015$).

For the *E. coli* O6 the bacterial count on the cauterized side of the bladder was consistently higher than on the non-cauterized side. However, as for the *E. coli* ATCC 25922, the difference between the cauterized and the non-cauterized sides was statistically not significant ($P = 0.9994$). In the *E. coli* O6 group, the lowest and highest colony counts were found for glycine and glycerol, respectively, and with povidone-iodine no considerable reduction in bacteria as compared with the other irrigation solutions was noted. In the *E. coli* ATCC 25922 group the lowest colony counts were found when povidone-iodine was used as an irrigation solution (Table 2). The results of the statistical evaluation (P value) among the various irrigation fluids investigated are listed in Table 3.

Discussion

Transurethral resection of the prostate (TURP) is one of the most common urological operations performed, and urinary tract infection following TURP is still a source of considerable morbidity and cost [5]. Huge amounts of irrigation solutions are used each year, and although irrigation fluids have been blamed as a possible source of infection following TURP [7] little is known as to their influence on the development of postoperative infection. Also, information concerning electrocautery urothelial damage and urinary tract infection is sparse.

Urinary catheters are routinely placed following TURP. Catheters adsorb components from the urine and form a biofilm on the surface [9]. This may enhance the binding of uropathogens to the catheter and, therefore, promote infection.

Bacterial adherence to the mucosa is considered the first step of infection [14]. However, the exact mechanism of bacterial adherence to the bladder mucosa is not

Table 2. Bacterial adherence to bladder urothelium (colony forming units per milligram tissue, median)

	E. coli O6		*E. coli* ATCC 25922			
	High pressure/ cautery	High pressure/ no cautery	High pressure/ cautery	Low pressure/ cautery	High pressure/ no cautery	Low pressure/ no cautery
Povidone-iodine	38960	20390	495	264	948	689
Mannose	39123	15768	10589	4696	1877	10175
Glycine	12311	10455	582	3006	602	535
Glycine/ethanol	24152	20829	8484	1221	3309	788
Glycerol	59484	34576	2455	18642	2128	1834

Table 3. Levels of significance (P values) among the irrigation solutions investigated (three-way ANCOVA)

	Povidone-iodine	Mannose	Glycine	Glycine/ethanol	Glycerol
Povidone-iodine	–	0.9262	0.3242	0.4393	0.0196
Mannose	0.9262	–	0.3680	0.4933	0.0167
Glycine	0.3242	0.3680	–	0.8204	0.0037
Glycine/ethanol	0.4393	0.4933	0.8204	–	0.0054
Glycerol	0.0196	0.0167	0.0037	0.0054	–

completely understood [15]. On the one hand bacteria must possess the ability to adhere to a mucous surface. Among adhesins, type I fimbriae are the most common, occurring in 80% of *E. coli* strains [4]. On the other hand the natural defense mechanism of the bladder must be altered to facilitate adherence [10]. The bladder defense mechanism consists of direct removal of bacteria by washing out with urine, and of a glycosaminoglycan (GAG) layer, histologically overlying the urothelium at the luminal surface of the bladder and thus preventing bacterial adherence to the bladder surface [8].

It has long been recognized that mannose, a simple sugar, can inhibit bacterial binding to different target cells [6]. Consistent with this finding type I pili were blocked by mannose 6% in vitro. Conversely, none of the other irrigation solutions prevented red cell agglutination. This suggests that mannose could possibly reduce the risk of postoperative urinary tract infection with type I pili *E. coli* better than the other solutions.

Irrigation solutions for TURP are selected mainly on the basis of their composition with regard to possible hemolysis and absorption during the operation. However, they also have been proven to have limited antibacterial activity. The most pronounced antibacterial effect was found for mannose, the least for glycine alone. This may be due to the differences in pH [17]. The combination of glycine and ethanol 1% was studied since early detection of its excessive absorption during TURP may be possible with a breathalyzer test. However, due to the very low concentration of alcohol, no enhancement of the antibacterial activity of glycine was noted.

Our study confirms the importance of bacterial adhesins and of disturbed urothelial integrity as prerequisites for the development of urinary tract infections.

The *E. coli* serotype O6 with type I pili clearly adhered more strongly to the bladder mucosa than the *E. coli* ATCC 25922 without type I pili. The difference was statistically highly significant. Interestingly, with *E. coli* O6 more bacteria adhered to the injured side than to the non-injured of the bladder. However, the difference was statistically not significant, possibly due to the relatively small numbers and the variation between experiments done on different days [12]. Stronger adhesion to the injured side has also been found by other investigators. In the study by Bagley et al. [1], after injury to the bladder and subsequent intravesical bacterial administration more than 90% of the bacteria were recovered from the portion of the bladder containing the injured site.

Conversely, with *E. coli* ATCC 25922 no such difference was found. This suggests increased adherence to damaged urothelium if *E. coli* containing type I pili is the infecting organism. The already higher virulence of *E. coli* with type I pili appears to be further enhanced by urothelial damage due to electrocautery.

In our study, we did not find a difference between animals with high and low intravesical pressure. This may be because we investigated only *E. coli* ATCC 25922 under both high and low intravesical pressures. Conversely, Iversen and Madsen [2] found in a rat study that higher bacterial adherence of *E. coli* occurred in animals irrigated with high intravesical pressure. They investigated the irrigation fluids glycine 1.5%, sorbitol 2.7% and mannitol 0.54%, and sterile water and found no differences in bacterial adherence among the solutions studied. Also the temperature of the solution was of no influence. Conversely, others found adherence to be temperature dependent [17]. In our study, the influence of the temperature on bacterial adherence was not examined, the irrigation solution being consistently kept at room temperature. Povidone-iodine clearly reduced the number of bacteria in the bladder when *E. coli* ATCC 25922 was the infecting organism, but when the serotype O6 was used no antibacterial activity was noted. This suggests that bacteria are protected against the antibacterial effect of local antiseptics once they adhere to the bladder mucosa. An antiadherent effect with instillations of an organic iodine complex with a pH adjusted to 6.8 prior to the bacterial challenge has been reported [16]. The opacity of povidone-iodine, however, prevents it from being used as an irrigation solution during transurethral resection. As mannose 6% effectively inhibits type I pili and thus diminishes bacterial adherence it may reduce postoperative urinary tract infections if the preoperative urine is sterile.

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