BACTERIAL PATHOGENICITY

Effect of glucose and pH on uropathogenic and non-uropathogenic *Escherichia coli*: studies with urine from diabetic and non-diabetic individuals

SUZANNE E. GEERLINGS*, ELLEN C. BROUWER†, WIM GAASTRA‡, JAN VERHOEF† and ANDY I. M. HOEPELMAN*†

*Department of Medicine, Division of Infectious Diseases and AIDS, University Hospital, Utrecht, †Eijkman-Winkler Laboratory for Medical Microbiology, University Hospital Utrecht and ‡Department of Bacteriology, Institute of Infectious Diseases and Immunology, Veterinary Faculty, Utrecht University, the Netherlands

It is generally assumed that one of the reasons why diabetics are more susceptible to urinary tract infections than non-diabetics is their 'sweet urine'. However, very little information is available on this subject. Therefore, the growth rates of different *Escherichia coli* strains were studied in human urine with and without added glucose and with and without a constant pH, and compared with their growth rates in Mueller-Hinton broth (MHB). Eight isolates were used (three from blood cultures from urosepsis patients, two urinary isolates, two faecal isolates and one laboratory strain K12). All isolates grew better in MHB than in urine, but with the exception of the laboratory strain, they had the same growth rate in urine. No significant difference was found between the growth rate in urine from diabetics without glucosuria and that in urine from non-diabetics. The addition of glucose (up to a concentration of 1000 mg/dl) to urine and MHB enhanced the growth rate of all isolates. However, very high concentrations of glucose (up to 10 000 mg/dl) in urine and MHB caused a decrease in bacterial growth rate when the urinary pH was not kept constant. The stationary phase was reached later and the final bacterial yield was greater when the urine was made less acidic. As the uropathogenic strains did not grow better in urine than the other isolates, it may be concluded that better growth in urine is not one of the causes of the greater virulence of these strains.

Introduction

Diabetic individuals are more susceptible to urinary tract infections than non-diabetic subjects [1, 2]. The following factors have been suggested as possible causes: (i) neutrophil function is impaired; (ii) glucose in urine ('sweet urine') supports bacterial growth better; and (iii) adherence to uro-epithelial cells is increased [3].

In a recent study from this laboratory [4], no difference in granulocyte function was found between diabetic females with bacteriuria, diabetic females without bacteriuria, and controls, which makes the first explanation unlikely. The third possibility is currently being investigated, because animal experiments seem to support it. As very little information

about the second possible cause of increased susceptibility is available, this has been investigated and the results are presented in the present paper.

It has been established that uropathogenic bacteria grow well in human urine, whereas non-uropathogenic bacteria do not [5], and that the rapid growth of some uropathogenic *Escherichia coli* in urine may permit colonisation even in the absence of specific adherence organelles [6]. It has also been suggested that not only pH and osmolality, but also glucosuria enhance bacterial growth [7]. In 1961, O'Sullivan *et al.* [8] compared the growth of *E. coli* (from a patient with asymptomatic bacteriuria) in urine with and without the addition of glucose 2% (200 mg/dl, moderate glucosuria). They found a four-fold increase in bacterial yield 2 h after the addition of glucose. In an earlier study, Jackson and Griebble [9] had shown that lower concentrations of glucose (0.1%; 10 mg/dl) had no effect on bacterial yield, although the growth rate was increased. In a recent study [4], 50% of

Received 24 April 1998; revised version received 7 Sept. 1998; accepted 15 Sept. 1998.

Corresponding author: Dr S. E. Geerlings.

diabetic patients with a positive urine culture were found to have glucosuria in the range 300–1000 mg/dl.

Osmolality and pH may also affect host defences. Gargan *et al.* [10] showed that raising urinary pH (from pH 6 to 8) and reducing urinary osmolality (to 485 and 200 mosm) increased the ability of phagocytes to eliminate infecting organisms.

In the present investigation, bacterial growth was studied in human urine at glucose concentrations such as those found in diabetic urine. The following questions were also addressed: (i) are there differences in bacterial growth between uropathogenic and non-uropathogenic strains?; (ii) is there a difference in support of bacterial growth between urine from diabetics and that from non-diabetics?; (iii) what are the effects of glucose and pH on uropathogenic and non-uropathogenic strains?

Materials and methods

Bacterial strains

Eight *E. coli* strains were used: three blood culture isolates (*E. coli* 89-91 from a diabetic patient with urosepsis, *E. coli* 90-606 from a non-diabetic patient with urosepsis and *E. coli* 97-121 from a non-diabetic patient with urosepsis); two isolates from urine (from a patient with prostatitis and from a patient before transurethral resection of the prostate); two isolates from faeces and the laboratory strain K12 (for serotyping and genotyping of bacterial strains; see Table 1). The strains were frozen in skimmed milk at -70°C and subcultured on sheep blood agar plates. The day before the experiment, two colonies of each strain were inoculated into Mueller-Hinton broth (MHB; Difco) 5 ml and incubated overnight at 37°C . The OD_{660} was measured and the bacteria were centrifuged at 3000 g at room temperature for 10 min and resuspended in 1 ml of PBS. (The initial inoculum from these suspensions was determined by preparing 10-fold serial dilutions in phosphate-buffered saline (PBS) pH 7.4; 0.03-ml volumes from each dilution were spread on sheep blood agar plates which were then incubated overnight. The final bacterial count was 1×10^{10} cfu/ml.) At the beginning of the experiment

30 μl of bacterial suspension in 12-ml tubes containing 3 ml of urine resulted in an OD_{660} of 0.1 (which corresponded with a viable count of 1×10^8 cfu/ml urine as initial inoculum).

Urine

Freshly voided midstream urine obtained from three diabetic and two non-diabetic females (investigators), all of whom showed no glucosuria, were not taking antibiotics, and had no history of recurrent urinary tract infections was used directly after micturition. The urine was filtered (Acrodisc 32 0.45- μm pore size; Gelman Sciences, Ann Arbor, MI, USA) before use.

Glucose

Five glucose concentrations were used. These were the same concentrations as used in the clinic to differentiate between minimal 50 mg/dl (+), moderate 100 mg/dl (++), high 300 mg/dl (+++), 1000 mg/dl (++++) and extremely high 10 000 mg/dl glucosuria (Combur-Test Boehringer Mannheim, Almere, The Netherlands). High glucosuria was found in a recent study of diabetic females in this hospital [4]. Growth of the various strains in urine with different glucose concentrations was studied and compared with growth in MHB without glucose. The bacterial growth rate was also measured in MHB after the addition of glucose in the same concentrations as in the glucosuria tests.

pH

Bacterial growth rate was measured twice at a glucose concentration of 10 000 mg/dl. The pH was kept at a constant value (5.5) for the first test, by adding 0.1 N NaOH after 5 h; then in a second trial the pH was not kept constant. Bacterial growth rate was measured in MHB with a pH of 5.0 and of 7.0.

Bacterial growth rate

Bacterial growth rate was measured with the Dr Lange photometer (Berlin, Germany) (660 nm), for a period of 6–7 h, with hourly reading points. The final bacterial count was the number of bacteria counted during the stationary phase of bacterial growth.

Table 1. Serotypes and genotypes of bacterial strains studied

<i>E. coli</i>	O:K:H -serotyping	Type 1 fimbriae	Subunit A P-fimbriae	G-Adhesin P-fimbriae	CNF	Aerobactin
Strain 89-91	O6K ⁻	p	p	p	p	a
Strain 90-606	OntH ⁻	p	a	a	a	p
Strain 97-121	O9K(A)30H ⁻	p	a	a	a	p
Uropathogen	O2K1Hnt	p	p	p	p	a
Uropathogen	O21KntH ⁻	a	a	a	a	p
Faecal isolate	OntHnt	p	a	a	a	p
Faecal isolate	OntHnt	p	a	a	a	a

p, present; a, absent; nt, non-typable; CNF, cytotoxic necrotising factor.

Statistics

Student's *t* test was used to test differences in OD₆₆₀ between two growth curves at the same time point.

Results

Growth differences between uropathogenic and non-uropathogenic strains

All *E. coli* strains grew better in MHB (pH 7.4) than in normal urine (pH 6.5) (Fig. 1: urosepsis strains; data from urinary, faecal and laboratory isolates are not shown). To exclude the effect of pH differences, the growth rate of *E. coli* was measured in urine and MHB with the same pH (7.4). No significant differences were found between growth in urine with a pH of 6.5 and with a pH of 7.4. Lowering the pH of MHB to 5.0 also had no effect on the growth rate (data not shown).

All non-laboratory *E. coli* strains grew at the same rate in urine and no differences were found between uropathogenic and non-uropathogenic strains. Only the laboratory strain *E. coli* K12 grew at a lower rate in urine than the other strains.

Comparison of growth rate in urine from diabetic and non-diabetics individuals

The growth rate of all *E. coli* strains was measured in urine from diabetic females (without glucosuria) and from non-diabetic females (also without glucosuria) with the same pH and a comparable osmolality (osmolality values between 500 and 900 mosm/kg in the three experiments). Although differences were found between growth in urine from diabetic and from

Fig. 1. Growth of blood culture isolates *E. coli* 89-91, 90-606 and 97-121 in MHB (●) (pH 7.4) and in urine from a healthy non-diabetic female (■) (pH 6.5). Results are the means and SEM of the three isolates. Experiments were performed five times.

non-diabetic subjects in the individual experiments, no overall significant differences were seen.

Effects of the addition of glucose

Moderate (100 mg/dl) or high (300 or 1000 mg/dl) amounts of glucose, such as are found in diabetic urines in the outpatient clinic of this hospital, enhanced the growth rate of all strains tested in both MHB (data not shown) and urine from healthy volunteers. After 6 h, these differences were statistically significant ($p < 0.01$) (Fig. 2). The differences in bacterial growth rate after the addition of low (50 mg/dl) or extremely high (10 000 mg/dl) amounts of glucose compared to that in normal urine without glucose were not significant. However, a non-significant decrease in growth rate was found in urine with a glucose concentration of 10 000 mg/dl compared with urine with a glucose concentration of 1000 mg/dl. In some strains (*E. coli* 89-91, one *E. coli* isolate from faeces, *E. coli* K12), extremely high glucose concentrations (10 000 mg/dl) inhibited growth when compared with that in urine without glucose.

Effect of changing pH

E. coli 89-91 reached the stationary phase later in urine with a constant pH of 5.5 (glucose concentration 10 000 mg/dl) than in urine with a more acidic pH of 5.0. The difference between the bacterial growth rates of the two urines was apparent after 5 h. The final bacterial population was greater in urine with a constant pH (Fig. 3). However, with the *t* test for each time point, these differences were not statistically

Fig. 2. Growth of blood culture isolates *E. coli* 89-91, 90-606 and 97-121 in urine from a healthy non-diabetic female without added glucose (■) (pH 6.5) and with added glucose at concentrations of 100 mg/dl (▼), 300 mg/dl (◆), 1000 mg/dl (★) and 10 000 mg/dl (●). Results are the mean and SEM of the three isolates. Experiments were performed five times.

Fig. 3. Bacterial growth rate of blood culture isolate *E. coli* 89-91 measured twice at a glucose concentration of 10 000 mg/dl. First the pH was kept at a constant value (5.5) by adding 0.1 N NaOH after 5 h (▲); then the pH was not kept constant and after 5 h had decreased to 5.0 (■).

significant ($p = 0.064$). The urinary osmolality before and after the addition of glucose (10 000 mg/dl) changed from 383 mosm/kg to 940 mosm/kg. Presumably this change in osmolality would not affect growth [7].

Discussion

It is generally assumed that glucosuria is one of the most important reasons for the high prevalence of urinary tract infections in diabetics. In this study, the effects of glucose and pH on the growth rates of different *E. coli* strains in human urine were investigated. Moreover, the bacterial growth rate in urine samples from diabetic females without glucosuria was compared with that in samples from non-diabetic controls. The different *E. coli* strains used in this study grew better in MHB than in urine (Fig. 1). This was not due to a difference in pH, as the bacterial growth rate was better in MHB (pH 7.4 or pH 5.0) than in urine with the same pH.

No differences were observed between the bacterial growth rates of uropathogenic and non-uropathogenic strains. Only the laboratory strain *E. coli* K12 grew at a lower rate in human urine than the other strains. Therefore, the conclusion may be drawn that the difference in bacterial growth rate in urine is not one of the causes of the higher virulence of the urosepsis strains.

The glucose content of normal urine ranges from 0.0 to 6.0 mg/dl [11]. The present study found that concentrations between 100 and 1000 mg/dl (i.e.,

moderate to severe glucosuria), stimulated bacterial growth the most. Moreover, a decrease in bacterial growth rate in urine and MHB was found at levels of 10 000 mg/dl glucose. Therefore, it may be concluded that moderate and severe glucosuria enhance bacterial growth and may provide one of the explanations for the increased susceptibility of diabetics to urinary tract infections. No significant differences in growth rate in normal and diabetic, non-glucosuric urine were found. This means that there is no other factor than glucose in diabetic urine that enhances bacterial growth.

The results of the present study are in agreement with those of Weiser *et al.* [12] who studied the effects of urinary glucose concentration on the growth rate of *E. coli*. They found that glucose concentrations between 2.8 and 10 mg/dl had no effect on the bacterial growth rate, although at a urinary glucose concentration of 100 mg/dl the final bacterial yield was twice as large as the yield at 2.8 mg/dl (2.8 mg/dl, 5×10^7 ; 100 mg/dl, 1×10^8). These authors also found that a further increase in glucose concentration did not cause an increase in the final bacterial population. Growth inhibition was found at extremely high glucose concentrations (10 000 mg/dl). This might be either a pH-related or an osmolality effect. Asscher *et al.* [7] showed that growth rate was impaired at a pH < 5.5 or > 7.6. They also showed that variations in osmolality in the physiological range (300–1200 mosm/kg) did not affect the bacterial growth rate. Considering this and the results described above, the growth inhibition found at extremely high glucose concentrations in this study is probably caused by the decreasing pH (to 5.0).

The studies described took place in the laboratory, whereas the human urinary tract is a dynamic system where fresh urine is constantly being formed and normal micturition occurs once every 4–6 h. This urine flow is also important in the defence against infection. Furthermore, the urinary pH and osmolality change over time. For these reasons, the low urinary pH, as a result of bacterial metabolism, probably does not play an important role as an inhibitor of further bacterial growth *in vivo* and, in the clinical situation, very high glucose concentrations probably enhance bacterial growth. This may explain the greater susceptibility of diabetics to urinary tract infection.

This study was supported by grants from the Dutch Diabetes Fund. Two blood culture isolates (*E. coli* 89-91 and *E. coli* 90-606) were kindly provided by Thecla Hekker of the Vrije Universiteit, Amsterdam, the Netherlands.

References

1. Hansen RØ. Bacteriuria in diabetic and non-diabetic out-patients. *Acta Medica Scandinavica* 1964; **176**: 721–730.
2. Patterson JE, Andriole VT. Bacterial urinary tract infections in diabetes. *Infect Dis Clin North Am* 1995; **9**: 25–51.
3. Hoepelman IM. Urinary tract infection in patients with diabetes

- mellitus. *Int J Antimicrobial Agents* 1994; **4**: 113–116.
4. Balasoiu D, van Kessel KC, van Kats-Renaud HJ, Collet TJ, Hoepelman AI. Granulocyte function in women with diabetes and asymptomatic bacteriuria. *Diabetes Care* 1997; **20**: 392–395.
 5. Stamey TA, Mihara G. Observations on the growth of urethral and vaginal bacteria in sterile urine. *J Urol* 1980; **124**: 461–463.
 6. Gordon DM, Riley MA. A theoretical and experimental analysis of bacterial growth in the bladder. *Mol Microbiol* 1992; **6**: 555–562.
 7. Asscher AW, Sussman M, Waters WE, Harvard Davis R, Chick S. Urine as a medium for bacterial growth. *Lancet* 1966; **2**: 1037–1041.
 8. O'Sullivan DJ, FitzGerald MG, Meynell MJ, Malins JM. Urinary tract infection. A comparative study in the diabetic and general populations. *BMJ* 1961; **1**: 786–788.
 9. Jackson GG, Gribble HG. Urinary tract infection. Pathogenesis of renal infection. *Arch Intern Med* 1957; **100**: 692–700.
 10. Gargan RA, Hamilton-Miller JMT, Brumfitt W. Effect of pH and osmolality on in vitro phagocytosis and killing by neutrophils in urine. *Infect Immun* 1993; **61**: 8–12.
 11. Fine J. Glucose content of normal urine. *BMJ* 1965; **1**: 1209–1214.
 12. Weiser R, Asscher AW, Sussman M. Glycosuria and the growth of urinary pathogens. *Invest Urol* 1969; **6**: 650–656.