ORIGINAL ARTICLE

Urinary concentrations and urine ex-vivo effect of mecillinam and sulphamethizole

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ABSTRACT

Healthy adult volunteers received 1 g of sulphamethizole orally ($n = 10$) and later 400 mg of pivmecillinam (274 mg of mecillinam) ($n = 9$). All urine was collected in defined periods over 24 h, and the drug concentrations in urine were determined. For sulphamethizole, the maximum urine concentration for seven subjects was reached in 0–3 h, and for the remaining three in 3–6 h. For mecillinam, eight of the nine subjects attained a maximum urine concentration in 0–3 h, after which the concentration declined rapidly for six subjects in 3–6 h. Strains of *Escherichia coli* with different MICs for sulphamethizole and mecillinam were exposed to collected urine for 2.5 h and 5 h. The results indicated that a sensitive *E. coli* population should be suppressed by sulphamethizole in urine for two-thirds of the time (with 1 g twice-daily) and by mecillinam in urine throughout the 24-h period (with 400 mg three times a day). There was a slight but significant correlation between the ex-vivo effect ($\Delta \log_{10}$ CFU/mL) and the $\log_{10}$ concentration/MIC ratio after exposure to sulphamethizole for 5 h ($r^2 = 0.27$, $p < 0.0001$), and a significant correlation between the variables with mecillinam ($r^2 = 0.66$, $p < 0.0001$).

Keywords Urinary concentrations, mecillinam, sulphamethizole, ex-vivo

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INTRODUCTION

The sulphonamides have been used since the 1930s in the treatment of urinary tract infection (UTI), but have now been replaced in most of the world by either the combination trimethoprim–sulphamethoxazole (introduced in 1968) or trimethoprim alone. In Denmark, sulphamethizole is still used as empirical treatment for uncomplicated UTI, but mecillinam is currently being discussed as an alternative. The therapeutic effect in UTI depends on the concentration of the antibiotic in urine [1–3] and the distribution of pathogen MIC values. Despite this, the pharmacokinetic profile of sulphamethizole is poorly described—probably because extensive documentation was not required in the 1930s. Studies often declare, without any documentation, that the sulphamethizole concentration in urine is sufficiently high to eradicate almost all bacteria. The excretion of mecillinam is well documented, but only a few studies have established the actual urine concentration after oral administration. Therefore, this study investigated the concentrations in urine of sulphamethizole and mecillinam, and the current National Committee for Clinical Laboratory Standards breakpoints were evaluated. In addition, the inhibitory effect of the excreted urine containing antibiotic on susceptible and resistant strains of *Escherichia coli* was measured.

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MATERIALS AND METHODS

In-vivo study participants

Ten healthy adult volunteers (five women, five men) participated in the study, which was approved by the local ethical committees (Copenhagen and Frederiksberg). Each participant was given oral and written information, and written informed consent was obtained from each subject. No use of antibiotics was allowed during the 2 weeks preceding the study, and all alcohol and caffeine-containing beverages were avoided from 24 h before the study. Participants ranged from 28 to 49 years in age, and their weights ranged from 52 to 85 kg (mean:

68.3 kg). Creatinine clearance of the subjects was determined from a blood sample and a 24-h urine collection; the range of 81–127 mL/min (mean: 104 mL/min) indicated that all had normal kidney function. The study of the two antibiotics was separated by 4 weeks.

Drug administration

Sulphamethizole was administered orally (two 500-mg tablets of Sulfamethizol Ophtha; Unikem, Copenhagen, Denmark). Sulphamethizole is excreted in the urine by an active transport mechanism, and *c.* 10% is acetylated (inactive) [4]. Mecillinam was given orally as 400 mg of pivmecillinam (two 200-mg tablets of Selexid; Løvens Kemiske Fabrik, Ballerup, Denmark), corresponding to a total of 274 mg of mecillinam. About 21% of the mecillinam dose is excreted in the urine (three inactive metabolites and one active metabolite (0.5%)). Most mecillinam is excreted via the bile into the faeces [5]. The subjects fasted from 22.00 hours on the night before drug administration, and at 07.00 hours the standard dose of antibiotic was ingested. One hour later, they were allowed to eat and drink (they all recorded the amount of liquid that they consumed during the entire experiment).

Urine samples

All urine was collected, and samples were pooled in the following intervals after antibiotic ingestion: 0 h (control), 0–3 h, 3–6 h, 6–12 h, and 12–24 h. Immediately after collection, urine samples were placed in a refrigerator. The amount of urine pooled in each time interval was determined by weight, and the pH was measured by a colour-fixed indicator stick (pH Test Strips 4.5–10; Sigma, St Louis, MO, USA). A sample of each pool was placed in a −20 °C freezer for further processing.

Antibiotic concentration determination

Drug concentrations in urine were determined by the bioassay method for mecillinam, and by spectrophotometry for sulphamethizole. For the bioassay, the mecillinam-sensitive *E. coli* strain Leo HA2, grown on Mueller–Hinton BBL-II agar (Becton Dickinson, Erembodegem-Aalst, Belgium), was used. Each sample was measured in duplicate and matched to a standard curve of six four-fold dilutions. The concentrations (prepared in phosphate buffer, pH 5.0) started at 2500 mg/L and ended at 2.44 mg/L (detection limit 0.5 mg/L). The coefficient of variation was 1.7–8.5%.

The spectrophotometric assay (Ultrospec 2000; Pharmacia Biotech, Piscataway, NJ, USA) for sulphamethizole was performed as described previously [6,7], with standard curves of four two-fold dilutions starting at 200 mg/L. This method does not measure the acetylated, inactive fraction. The dilutions were made in the control urine sample pooled from two women and two men (randomly selected). One standard curve based on the mean values of these four curves (coefficient of variation 1.6–7.4%) was used to measure the concentration in all urine samples.

Urine samples that exceeded the standard curve upper limit were diluted before retesting. The detection limit was 2.5 mg/L.

Bacteria and MICs

MICs were determined by the agar dilution method according to National Committee for Clinical Laboratory Standards criteria [8], using Mueller–Hinton BBL II agar. For the sulphamethizole ex-vivo study, the following *E. coli* strains were used: KMA-26883 (strain I), MIC 128 mg/L; UVI-203 (strain II), MIC 512 mg/L; and KMA-28523 (strain III), MIC > 2048 mg/L (*sulII* gene positive). For the mecillinam ex-vivo study, the following *E. coli* strains were used: 21623884-114 (strain IV), MIC 0.5 mg/L; 21773360-98 (strain V), MIC 16 mg/L; and Eco518 (strain VI), MIC 128 mg/L. Data on the distribution of MIC values in the *E. coli* population isolated from UTIs in general practice [9] were used for comparison with the concentrations in urine of the antibiotics.

Ex-vivo study

The bactericidal activity of urine from individuals from each collecting period was tested against the three test strains in a microtitre plate format (Nunc, Neerijse, Belgium). Before the experiment, all urine samples were thawed and filtered through a 0.45-μm filter (Millipore, Glostrup, Denmark). To each 180-μL urine sample, a 20-μL inoculum, adjusted to 10^7 CFU/mL in Mueller–Hinton broth (BBL-II), was added to give a final inoculum of 1×10^6 CFU/mL and a concentration of 10% v/v Mueller–Hinton broth. The microtitre plates were incubated in a shaking incubator at 35 °C with constant shaking (400 rev/min). Samples were taken at 0, 2.5 and 5 h for determination of viable counts on lactose bromothymol blue agar plates (Statens Serum Institut, Copenhagen, Denmark) by the spread plate technique (Eddy Jet; IUL Instruments, Barcelona, Spain). After incubation for 18–24 h, the bacterial concentration (CFU/mL) was determined with a Countermat Flash (IUL Instruments). The detection limit was estimated to be 50 CFU/mL.

Statistics

The paired *t*-test (two-tailed) and Pearson's linear regression analyses were used to compare the ex-vivo effects between the *E. coli* strains; p values < 0.05 were considered significant (GraphPad Prism v. 3.00 for Windows; GraphPad Software, San Diego, CA, USA).

RESULTS

Antibiotic concentrations in urine

All ten participants completed the study with sulphamethizole, but one male subject did not participate in the study with mecillinam because of illness. For both antibiotics, the accumulated volume of excreted urine (mL), the urine concentration (mg/L) and the accumulated excreted amount, of antibiotic (mg) are displayed in Fig. 1. The median and range of the urine concentration, accumulated excreted amount, and the urine pH value are shown in Table 1 for the four

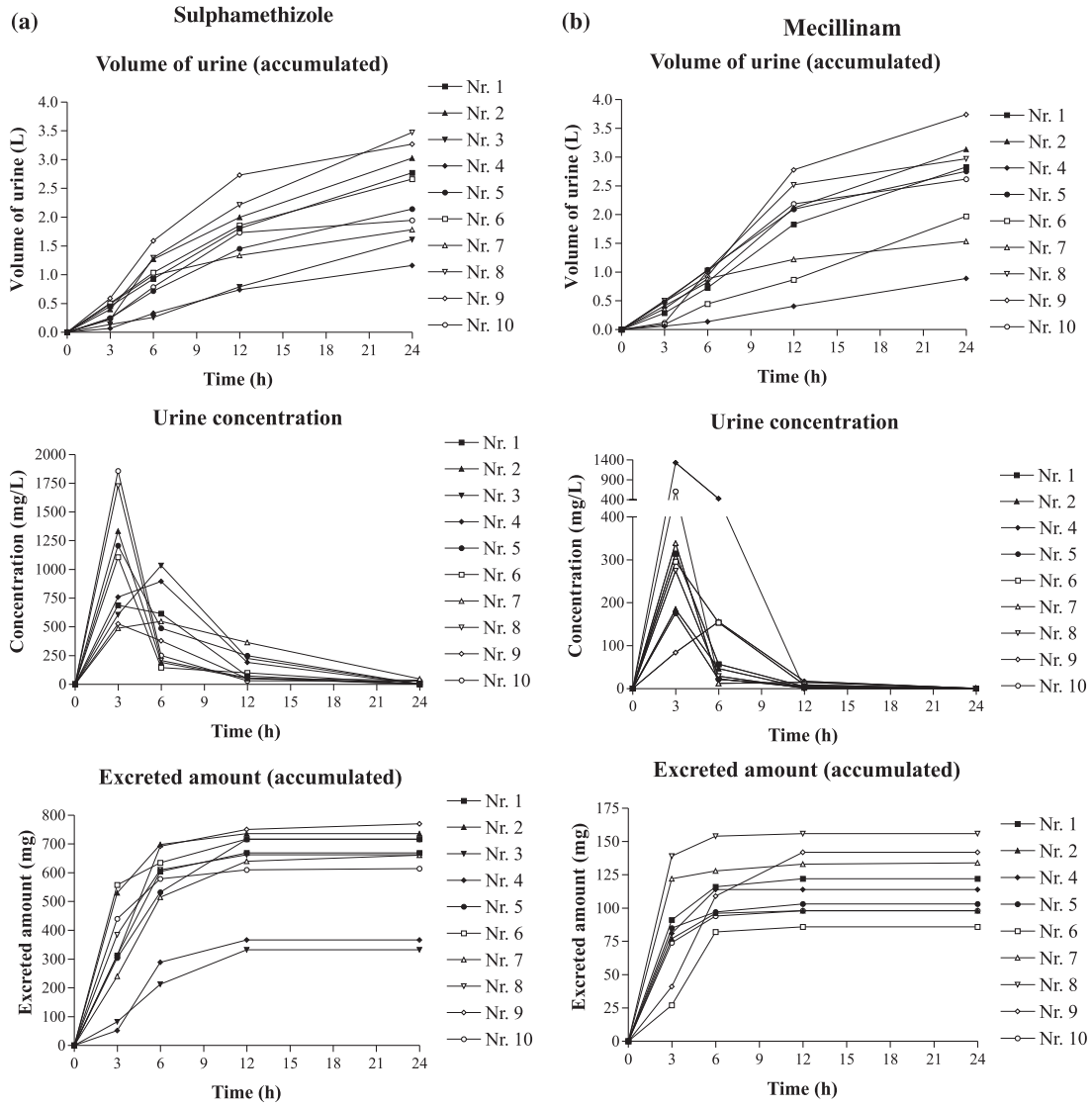

Fig. 1. (a) Urinary sulphamethizole excretion for ten subjects after an oral dose of 1000 mg. The accumulated volume of urine, the urine concentration and the accumulated amount excreted are shown as functions of time. (b) Urinary mecillinam excretion for nine subjects after an oral dose of 274 mg. The accumulated volume of urine, the urine concentration and the accumulated amount excreted are shown as functions of time.

collection intervals. In both experiments, there was a marked difference in the amount of urine excreted, which correlated well with the registered amount of liquid consumed (data not shown).

For sulphamethizole, the maximum urine concentration for seven individuals was reached in 0–3 h (range: 528–1857 mg/L), and for the remaining three subjects in 3–6 h (range: 549–1034 mg/L) (mean $T_{½\ \mathrm{urine}} = 3.36$ h). There appeared to be two patterns of excretion for sulphamethizole: five subjects (2, 5, 6, 8 and 10) reached the highest concentration in 0–3 h, the concentration declining thereafter to a minimum in 3–6 h, whereas the other five subjects attained a lower maximum concentration that lasted for a longer period of time. This observation was not related to the subject's age or gender. For seven subjects, the concentration of sulphamethizole in the 12–24-h samples was below the detection limit of 2.5 mg/L. Two subjects excreted *c.* 50% less sulphamethizole than the rest, which seemed to be connected to the low urine production of these two subjects. In general *c.* 30% of the administered sulphamethizole dose (1 g) was excreted in 0–3 h, 60% in 3–6 h,

Table 1. Human urinary excretion data for mecillinam and sulphamethizole after one oral standard dose

	Median (range)					
	Mecillinam (n = 9)			Sulphamethizole (n = 10)		
Collection interval (h)	Urine concentration (mg/L) (range)	Accumulated excreted (mg) (range)	Urine pH value (range)	Urine concentration (mg/L) (range)	Accumulated excreted (mg) (range)	Urine pH value (range)
Control (0 sample)	0	0	5.5 (5.0–6.0)	0	0	5.3 (5.0–6.0)
0–3	296 (84–1324)	81 (27–139)	6.0 (5.0–8.0)	934 (489–1857)	313 (52–558)	5.5 (5.0–7.5)
3–6	47 (12–432)	109 (82–154)	7.0 (5.0–7.5)	434 (145–1034)	592 (213–698)	6.5 (5.0–7.0)
6–12	5 (0[a]–17)	114 (86–156)	6.5 (4.5–7.0)	87 (33–364)	666 (333–751)	6.3 (5.5–7.0)
12–24	0 (0[a]–1)	114 (86–156)	6.0 (4.5–7.0)	0 (0[b]–47)	666 (333–770)	6.0 (5.0–6.5)

[a]Below the detection limit of 0.5 mg/L, values set to 0.
[b]Below the detection limit of 2.5 mg/L, values set to 0.

and 66% in 6–12 h. The pH of the sulphamethizole urine samples increased slightly between the 0–3-h and 3–6-h periods, and then declined.

For mecillinam, eight of the nine subjects attained a maximum urine concentration in 0–3 h (range: 176–1324 mg/L), after which the concentration declined rapidly in six subjects during the 3–6-h period to 12–57 mg/L (mean $T_{½\ \mathrm{urine}} = 1.79$ h). The low urine production observed in 3–6 h for subjects 4 and 6 resulted in higher urine concentrations during this period. Subject 9 achieved a maximum urine concentration of 176 mg/L after 3–6 h. In the 12–24-h period, the mecillinam concentration in the urine declined to a level below the detection limit of 0.5 mg/L. From Table 1 it can be deduced that *c.* 30% of the mecillinam dose (274 mg of mecillinam) was excreted in 0–3 h, 40% in 3–6 h, and 42% in 6–12 h. The pH of the mecillinam urine samples increased slightly in the 0–3-h and the 3–6-h periods, after which it decreased.

Relationship between urine concentrations and MICs

The relationship between the urine concentrations of the two antibiotics and the MIC distribution for *E. coli* strains from UTIs in general practice is displayed in Fig. 2. The urine concentration of sulphamethizole exceeded the MIC for 50% of the strains for *c.* 12 h, but the concentration never reached the MIC_{90} level of > 2048 mg/L. The data on mecillinam revealed that the MIC_{50} value of 0.5 mg/L was exceeded for most subjects for 24 h, and for 90% of the strains for *c.* 21 h.

Ex-vivo study

The effect of the urine samples containing sulphamethizole and mecillinam on each set of three *E. coli* strains with increasing MIC values after exposure for 2.5 h and 5 h is shown in Fig. 3. Overall, the reduction in viability was higher with mecillinam than with sulphamethizole, and the reduction in CFUs was more pronounced after 5 h than after 2.5 h. The effect generally decreased from the first collection interval (0–3 h) to the fourth collection interval (12–24 h), corresponding to the decreasing antibiotic concentration over time.

After incubation for 2.5 h with the urine samples containing sulphamethizole, a minimal decrease in CFU/mL was observed in the 0–3-h sample, with a significant difference in the effect on strain I compared to strain III. For the remaining collection intervals, the viable counts of all strains after incubation for 2.5 h were almost identical to the control. The results after incubation for 5 h showed a significant difference between strains I and II, and between strains II and III, in all collection intervals. In the third and fourth collection intervals, strain III started to grow like the control. A significant difference between strains I and II was observed only in the 6–12-h collection interval.

Strains IV and VI showed a significant difference in the CFU reduction for all mecillinam urine samples after exposure for 2.5 h. There was a significant difference between strains IV and V in the 6–12-h and 12–24-h collections. Significant differences after exposure for 5 h were observed between all strains and in all collection intervals, apart from strains V and VI in the first and fourth

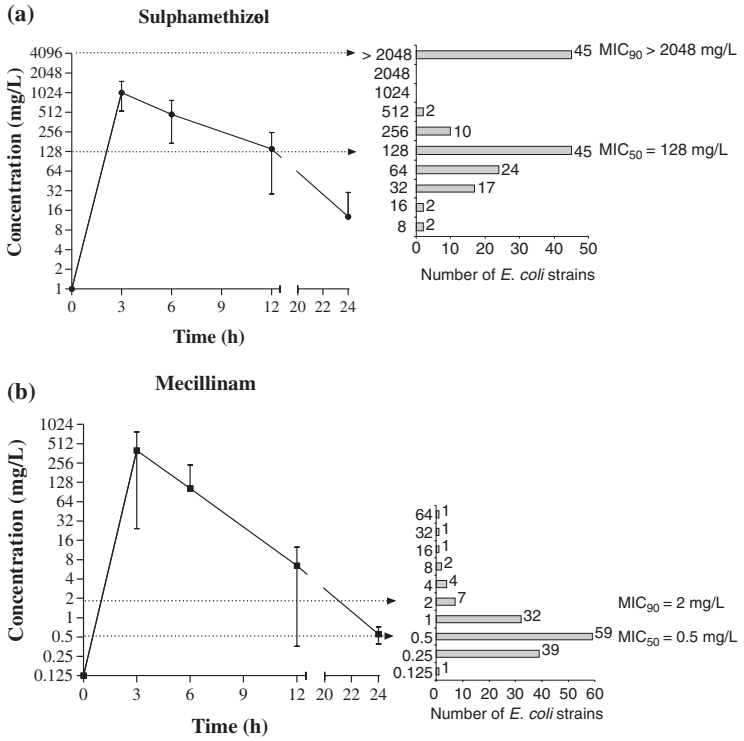

Fig. 2. (a) Mean of the ten subjects' sulphamethizole concentration curves related to the distribution of sulphamethizole MICs for *E. coli* strains ($n = 147$) from urinary tract infections in general practice. Arrows indicate the MIC_{50} and MIC_{90} values. Error bars indicate the standard deviation. (b) Mean of the nine subjects' mecillinam concentration curves related to the distribution of mecillinam MIC values in *E. coli* strains ($n = 147$) from urinary tract infections in general practice. Arrows indicate the MIC_{50} and MIC_{90} values. Error bars indicate the standard deviation.

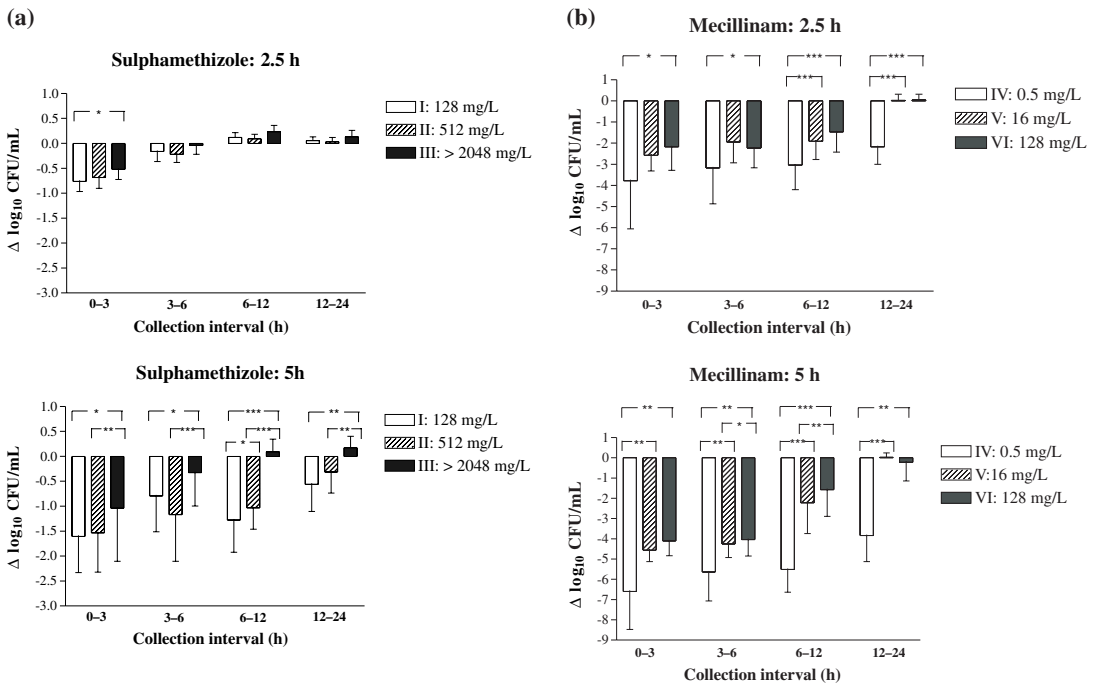

Fig. 3. (a) The mean effect of sulphamethizole urine samples ($n = 10$) on three *E. coli* strains after 2.5 h and 5 h. The $\Delta \log_{10}$ CFU/mL is calculated as the difference between the CFU/mL of the sample and the CFU/mL of the control at the same time. p values are indicated on the figure: *p = 0.01–0.05, **p = 0.001–0.01, ***p = < 0.001. Error bars indicate the standard deviation. (b) The mean effect of mecillinam urine samples ($n = 9$) on three *E. coli* strains after 2.5 h and 5 h. The $\Delta \log_{10}$ CFU/mL is calculated as the difference between the CFU/mL of the sample and the CFU/mL of the control at the same time. p values are indicated on the figure: *p = 0.01–0.05, **p = 0.001–0.01, ***p = < 0.001. Error bars indicate the standard deviation.

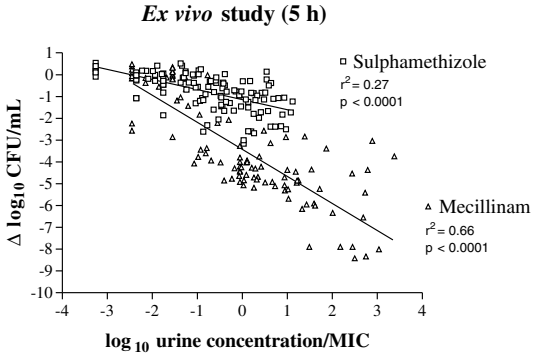

Fig. 4. Correlation between $\log_{10}$ antibiotic concentration in urine/MIC and Δ $\log_{10}$ CFU/mL after exposure for 5 h to sulphamethizole or mecillinam.

intervals. At both time points, growth was inhibited in all samples, except in the 12–24-h sample, where strains V and VI grew almost like the control.

Correlation between ex-vivo effect and MIC

Fig. 4 shows the correlation between the ex-vivo effect (Δ $\log_{10}$ CFU/mL) and the $\log_{10}$ concentration/MIC ratio for all combinations of strains and collection intervals for sulphamethizole and mecillinam after exposure for 5 h. The concentrations of antibiotic were adjusted, since the urine samples were diluted by 10% after addition of the inoculum. The strain with a sulphamethizole MIC > 2048 mg/L was set to 4096 mg/L in the calculations.

For sulphamethizole, there was a slight but significant correlation between the two variables ($r^2 = 0.27$, $p < 0.0001$), and a significant correlation was seen with mecillinam ($r^2 = 0.66$, $p < 0.0001$). Some strains were affected even though the antibiotic concentration was below the MIC. The corresponding relationships after exposure for 2.5 h were $r^2 = 0.06$, $p < 0.01$ for sulphamethizole and $r^2 = 0.42$, $p < 0.0001$ for mecillinam (data not shown).

DISCUSSION

Despite use since the 1930s, there are only limited data available on the concentrations in urine of antibiotics belonging to the sulphamethizole group [10], and it is usually stated that the concentrations are high (*c.* 200–400 mg/L) [11,12]. While the excretion of mecillinam has been studied more thoroughly since its introduction in the 1970s, fewer data are available on its concentration in urine.

In this study, 6 h after an oral dose of 400 mg of pivmecillinam had been given to normal subjects, *c.* 40% of the dose was excreted in the urine as mecillinam, after which the excretion declined, as reported elsewhere [5,13–15]. Other studies have reported excretion to be 25–30% [16,17], which could be explained by the rapid degradation of mecillinam in samples. The actual concentration of mecillinam in urine after oral administration of 400 mg of pivmecillinam has been reported to vary from 92 to 365 mg/L [17] and from 37 to 787 mg/L (mean: 187 mg/L) [18] in the first 6-h pooled urine sample. A study in which 400 mg of pivmecillinam was administered four times daily showed a mean mecillinam concentration in urine over 24 h of *c.* 300 mg/L [19]. The present study revealed the same degree of variability in the level of urine concentrations after one dose as did the above mentioned studies, but also shows that the peak concentration occurs in the first 3 h after ingestion.

In a South African study, the maximum 6-h sulphamethizole urine level was measured, and a mean of 374 mg/L was found (range 64–768 mg/L), after an oral dose of 160 mg of sulphamethizole [7]. In a Danish experiment, 500 mg of sulphamethizole was given orally four times daily, yielding urine concentrations of 200–12 000 mg/L [20]. It is unclear whether, in these studies, all excreted urine was collected or merely samples at indicated times. Therefore, it is difficult to compare the results with the current data, since all urine was collected in the present study to give an average concentration at the collection interval in question. To our knowledge, there are no other studies reporting the urine concentrations of sulphamethizole in humans.

The MIC breakpoint should, in theory, be determined by the pharmacokinetic profile of the antibiotic at the site of infection and the distribution of MIC values in pathogens. The current recommended National Committee for Clinical Laboratory Standards urinary breakpoint for sulphonamides is $R \geq 512$ mg/L, and for mecillinam it is $R \geq 32$ mg/L [21]. The results of the current study indicate that, with administration of 1 g of sulphamethizole twice-daily, the antibiotic concentration in the urine would exceed the MICs of sensitive *E. coli* for *c.* 66% of the time. It is also possible that the urine concentration could exceed

the MIC for the resistant population over a very short period, 0–3 h after administration. In comparison, the current recommended regimen for treating uncomplicated UTI with pivmecillinam is 400 mg three times daily for 3 days. The observations in this study indicate that a sensitive *E. coli* population should be suppressed by mecillinam in the urine throughout the 24-h period.

Clinical data have shown that treatment of uncomplicated UTI with 1 g of sulphamethizole twice-daily for 1 week resulted in cure rates of 58–74% for resistant strains and 71–97% for sensitive strains [22,23]. A dose of 1 g of sulphamethizole three times daily for 14 days resulted in a total cure rate of 92%, although the cure rate was only 64% when resistant strains were involved [24]. Whether the reported strains were actually resistant, and to what degree, was not reported. Despite this, there seemed to be an overall lower cure rate when the pathogen was found to be resistant *in vitro*.

A range of clinical trials on mecillinam has been performed with different dosing regimens and lengths of treatment. Treatment of uncomplicated UTI with 400 mg of pivmecillinam three or four times daily for 7–10 days resulted in cure rates of 87–90% [25–27]. Failures were often observed with strains that were susceptible *in vitro*, and strains resistant *in vitro* could be cured *in vivo*, so laboratory tests did not necessarily predict the clinical outcome. In general, most investigators do not take account of the fact that 11% of untreated women with uncomplicated UTI will have sterile urine after 1 week, and 47% will recover after 2 weeks [28]. This might explain why some infections, despite in-vitro resistance, can be treated *in vivo*. Recent studies have shown that 3-day courses consisting of pivmecillinam with a dose of 200 mg three times daily, or only twice-daily for 5 days, cured 91–100% of patients with uncomplicated UTI [29–31]. The beneficial effects reported with the lower dose simply reflect the fact that halving the dose only decreases the time above the MIC by one $T_{½}$, which, according to the present data, is *c.* 2 h. A comparison of standard mecillinam (400 mg three times daily for 3 days) and sulphamethizole (1 g twice-daily for 6 days) in the treatment of uncomplicated UTI has been performed, and showed no significant difference between the cure rates (90–96%), although no information was given about the susceptibility of the strains in this study [32].

A relationship between the bactericidal or bacteriostatic activities of urine samples containing excreted antibiotic and the MIC values for pathogens has been demonstrated for several antibiotics. Madsen *et al.* found that excreted amounts of different sulphonamides inhibited the growth of a single strain [33], while back-titration of urine samples containing mecillinam inhibits bacteria with different MIC values [25]. A clinical study with oxytetracycline demonstrated that activity in the urine separated the cures from the failures [1]. The association between the bactericidal titre in urine and MICs for the tested strains has been established with quinolones [34,35]. On the other hand, no correlation could be demonstrated between the time required to kill 90% of the bacteria, the concentration of co-trimoxazole, and the organism's susceptibility [36]. This is consistent with the findings obtained in the present study, and it is possible that it might be difficult to obtain such a correlation with bacteriostatic antibiotics because the effect is much less than with bactericidal antibiotics.

In conclusion, these observations indicate that sensitive *E. coli* populations should be suppressed by a sufficient sulphamethizole urine concentration for two-thirds of the time with a dose of 1 g twice-daily, and by a satisfactory mecillinam urine concentration throughout the 24-h period with a dose of 400 mg three times daily. A correlation between the ex-vivo effect ($\Delta \log_{10}$ CFU/mL) and the $\log_{10}$ concentration/MIC ratio was observed for both antibiotics, although it was more pronounced for mecillinam. It seems that there is a lack of clinical studies focusing on the relationship between the predictability of in-vitro susceptibility tests and clinical outcome in UTI. Some would argue that it would not be ethically correct to continue treatment of a resistant pathogen, but that is, in fact, what is done when empirical treatment is practised.

REFERENCES

1. Stamey TA, Fair WR, Timothy MM, Millar MA, Mihara G, Lowery YC. Serum versus urinary antimicrobial concentrations in cure of urinary-tract infections. *N Engl J Med* 1974; **291**: 1159–1163.
2. Anderson JD. The relevance of urine and serum antibacterial activity to the treatment of urinary tract infections. *J Antimicrob Chemother* 1976; **2**: 226–228.

3. Klastersky J, Daneau D, Swings G, Weerts D. Antibacterial activity in serum and urine as a therapeutic guide in bacterial infections. *J Infect Dis* 1974; **129**: 187–193.
4. Bergan T. Valg av sulfonamider basert på deres kjemiske og farmakokinetiske egenskaper. *Norsk Farm Tidsskrift* 1973; **81**: 387–395.
5. Roholt K. Pharmacokinetic studies with mecillinam and pivmecillinam. *J Antimicrob Chemother* 1977; **3**(suppl B): 71–81.
6. Bratton AC, Marshall EK. A new coupling component for sulfanilamide determination. *J Biol Chem* 1939; **122**: 537–550.
7. Jacobs MR, Robinson RG, Koornhof HJ. Antibacterial activity of nitroxoline and sulfamethizole alone and in combination in urinary tract infections. *South Afr Med J* 1978; **54**: 959–962.
8. National Committee for Clinical Laboratory Standards. *Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically*, 5th edn. Approved standard M7-A5. Villanova, Pa: NCCLS, 2000.
9. Kerrn MB, Klemmensen T, Frimodt-Møller N, Espersen F. Susceptibility of Danish *Escherichia coli* strains isolated from urinary tract infections and bacteraemia, and distribution of *sul* genes conferring sulphonamide resistance. *J Antimicrob Chemother* 2002; **50**: 513–516.
10. Weinstein L, Madoff MA, Samet CM. The sulfonamides. *N Engl J Med* 1960; **263**: 793–800.
11. Welling PG, Craig WA, Amidon GL, Kunin CM. Pharmacokinetics of trimethoprim and sulfamethoxazole in normal subjects and in patients with renal failure. *J Infect Dis* 1973; **128**(suppl): S556–S566.
12. Bach MC, Gold O, Finland M. Absorption and urinary execretion of trimethoprim, sulfamethoxazole, and trimethoprim–sulfamethoxazole: results with single doses in normal young adults and preliminary observations during therapy with trimethoprim–sulfamethoxazole. *J Infect Dis* 1973; **128**(suppl): S584–S599.
13. Parson RL, Hossack GM, Paddock GM. Plasma profile and urinary excretion of mecillinam after pivmecillinam. In: Connots TA, Williams JD, Geddes AM, Hellmann K eds. *Chemotherapy*. New York: Plenum Press, 1976; 173–181.
14. Roholt K, Nielsen B, Kristensen E. Pharmacokinetic studies with mecillinam and pivmecillinam. *Chemotherapy* 1975; **21**: 146–166.
15. Heikkila A, Pyykko K, Erkkola R, Iisalo E. The pharmacokinetics of mecillinam and pivmecillinam in pregnant and non-pregnant women. *Br J Clin Pharmacol* 1992; **33**: 629–633.
16. Neu HC, Srinivasan S, Francke EL, Ortiz Neu C, Christenson JG. Pharmacokinetics of amdinocillin and pivamdinocillin in normal volunteers. *Am J Med* 1983; **75**: 60–64.
17. Williams JD, Andrews J, Mitchard M, Kendall MJ. Bacteriology and pharmacokinetics of the new amidino penicillin mecillinam. *J Antimicrob Chemother* 1976; **2**: 61–69.
18. Wise R, Pippard M, Reeves DS. A laboratory and clinical investigation of mecillinam and its ester pivmecillinam in the treatment of urinary tract infections. *J Antimicrob Chemother* 1977; **3**(suppl B): 113–120.
19. Anderson JD, Adams MA. Urinary excretion of mecillinam by volunteers receiving film-coated tablets of pivmecillinam hydrochloride. *Chemotherapy* 1979; **25**: 1–4.
20. Johansen KS, Grosen G, Holm Nielsen E *et al*. Patients with symptoms of acute urinary infections treated with piromidic acid or sulfamethizole in general practice. A double-blind clinical controlled multicentre study. *Ugeskr Laeger* 1981; **143**: 2491–2494.
21. National Committee for Clinical Laboratory Standards. *Performance standards for antimicrobial susceptibility testing; MIC interpretive standards (μg/mL) for Enterobacteriaceae*. Approved standard M100-S12. Villanova, Pa: NCCLS, 2001.
22. Mabeck CE, Vejlsgaard R. Treatment of urinary tract infections in general practice with sulfamethizole, trimethoprim or co-trimazine (sulphadiazine–trimethoprim). *J Antimicrob Chemother* 1980; **6**: 701–708.
23. Andersen B, Bonderup OK, Tilma A. Urinary tract infection in general practice treated with sulfamethizole in a single dose or for 6 days. *Ugeskr Laeger* 1986; **148**: 511–513.
24. Bergan T, Skjerven O. Double blind comparison of short and medium term sulfonamides, sulfamethizole and sulfamethoxazole, in uncomplicated acute urinary tract infections. *Scand J Infect Dis* 1979; **11**: 219–223.
25. Clarke PD, Geddes AM, McGhie D, Wall JC. Pivmecillinam in urinary tract infections: a correlation of urinary bactericidal activity with clinical efficacy. *J Antimicrob Chemother* 1977; **3**: 169–173.
26. Bresky B. Controlled randomized study comparing amoxycillin and pivmecillinam in adult out-patients presenting with symptoms of acute urinary tract infection. *J Antimicrob Chemother* 1977; **3**(suppl B): 121–127.
27. Guttmann D. A comparison of pivmecillinam and cotrimoxazole in the treatment of simple cystitis in general practice. *J Antimicrob Chemother* 1977; **3**(suppl B): 137–140.
28. Mabeck CE. Treatment of uncomplicated urinary tract infection in non-pregnant women. *Postgrad Med J* 1972; **48**: 69–75.
29. Menday AP. Comparison of pivmecillinam and cephalexin in acute uncomplicated urinary tract infection. *Int J Antimicrob Agents* 2000; **13**: 183–187.
30. Pitkajarvi T, Pyykonen ML, Kannisto K, Piippo T, Viita P. Pivmecillinam treatment in acute cystitis. Three versus seven days study. *Arzneimittelforschung* 1990; **40**: 1156–1158.
31. Skinner JL, Venables TL, Sheldon MG. A general practice evaluation of pivmecillinam given twice daily as a treatment for acute urinary tract infection. *Pharmatherapeutica* 1984; **4**: 25–31.
32. Bitsch M, Hansen PH, Pagh J. Treatment of acute urinary infections. Comparison between pivmecillinam for 3 days and sulfamethizole therapy for 6 days. *Ugeskr Laeger* 1985; **147**: 1392–1395.
33. Madsen ST. Antibacterial effect of urine after single oral doses of sulfonamide. *Chemotherapy* 1968; **13**: 16–22.
34. Naber KG, Theuretzbacher U, Kinzig M, Savov O, Sorgel F. Urinary excretion and bactericidal activities of a single oral dose of 400 milligrams of fleroxacin versus a single oral dose of 800 milligrams of pefloxacin in healthy volunteers. *Antimicrob Agents Chemother* 1998; **42**: 1659–1665.
35. Well M, Naber KG, Kinzig-Schippers M, Sorgel F. Urinary bactericidal activity and pharmacokinetics of enoxacin versus norfloxacin and ciprofloxacin in healthy volunteers after a single oral dose. *Int J Antimicrob Agents* 1998; **10**: 31–38.
36. Broughall JM, Bywater MJ, Holt HA, Reeves DS. Bactericidal or bacteristatic effects of two sulphonamide plus trimethoprim preparations in human urine. *Infection* 1979; **7**: 113–118.