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The renal clearance of cefuroxime and ceftazidime and the effect of probenecid on their tubular excretion

C. A. VERHAGEN*, H. MATTIE & E. VAN STRIJEN
Leiden University Hospital, Department of Infectious Diseases, P.O. Box 9600, 2300 RC Leiden, The Netherlands

1 The renal tubular excretion of cefuroxime and ceftazidime in relation to the co-administration of probenecid was investigated in eight and two healthy subjects, respectively.
2 Cefuroxime or ceftazidime were administered by i.v. infusion and 1 g probenecid was administered orally after steady state plasma concentrations of the cephalosporin were reached.
3 In a second session the same antibiotic was administered at increasing infusion rates such that three different levels of plasma drug concentration were achieved.
4 The renal clearance of antibiotic was calculated based upon unbound plasma concentration, and tubular clearance was estimated by subtracting inulin clearance from the renal clearance of the antibiotic.
5 Non-linear regression analysis was used to estimate parameters describing the saturability of tubular excretion and the effect of probenecid inhibition, i.e. EC_{50} and $R_{tub,max}$, could be established for cefuroxime: EC_{50} was 248 (s.d. 130) mg l^{-1} and $R_{tub,max}$ was 1.852 (s.d. 0.577) mg h^{-1}. Tubular excretion of ceftazidime was practically zero. The EC_{50} of probenecid for inhibition of the tubular excretion of cefuroxime was 0.80 (s.d. 0.31) mg l^{-1}.
6 The results indicate that in the therapeutic plasma concentration range of cefuroxime its renal clearance is not saturated. Probenecid at therapeutic doses will block tubular excretion of cefuroxime almost completely.

Keywords cefuroxime ceftazidime probenecid clearance

Introduction

The renal clearance of most β-lactam antibiotics is partly by glomerular filtration and partly by tubular excretion. In previous studies large differences in maximum tubular excretion rate as well as in the affinity of the drug for the transport mechanism were found between β-lactam antibiotics [1, 2]. Probenecid competitively inhibits tubular excretion of β-lactam antibiotics [3], and for benzylpenicillin it was shown that the degree of this inhibition is dose-dependent [2]. In these previous studies [1, 2] tubular clearance of the antibiotic was calculated by subtracting the glomerular filtration rate, estimated from creatinine clearance, from the total renal clearance of the drug. Disadvantages of this method are that creatinine is also partly secreted by the tubular cells [4], and that cephalosporins may interfere with the assay of creatinine [5].

The present study was undertaken to elucidate further the determinants of tubular excretion, i.e. maximum excretion rate and affinity for the transport mechanism, since available information does not indicate whether the large variation between β-lactam antibiotics with respect to tubular excretion is due to variation in maximum capacity or affinity, or both. Since competition between the antibiotic and probenecid is related to their respective affinity, further assessment of the relationship between tubular excretion and the effect of probenecid should allow conclusions with respect to the determinants of tubular excretion. Cefuroxime was chosen as a model drug because its plasma protein binding is low, thereby excluding competition for binding as a possible source of error, and tubular excretion was expected to be small [6]. Ceftazidime was used for comparison because it

*Present address: Nijmegen University Hospital, Department of Medical Oncology, P.O. Box 9101, 6500 HB Nijmegen, The Netherlands
Correspondence: Dr H. Mattie, Department of Infectious Diseases, Bldg 1, C5-P, P.O. Box 9600, 2300 RC Leiden, The Netherlands

does not appear to be excreted actively [7]. To obtain an accurate measurement of glomerular filtration rate inulin clearance was determined.

Methods

Drugs

Vials containing 1.5 g cefuroxime and 2.0 g ceftazidime from single batches were obtained from Glaxo, Nieuwegein, The Netherlands. A solution of 10% w/v inulin was prepared freshly by the Hospital Pharmacy. Probenecid tablets (500 mg) were obtained from the Hospital Pharmacy, all from one batch.

Subjects

The study was approved by the Review Committee on Human Research of Leiden University Hospital. Eight male volunteers (age range 20–27 years) were recruited and gave written informed consent to the study. They were healthy as determined by medical history, physical examination, ECG, urinalysis, blood chemistry and haematological tests. The subjects were asked not to take any other drugs from 1 week before the first day of the study to the last day of the study. No tobacco, caffeine or alcohol-containing beverages were allowed during the study.

Procedures

Cefuroxime Six subjects took part in two sessions. In the first session the influence of probenecid on the tubular transport of cefuroxime was studied; in the second session the relation between the plasma concentration of cefuroxime and its tubular excretion rate was established. Before each session intravenous catheters were introduced into the antecubital veins of both forearms. One catheter was used for drug infusion and hydration, the other for blood sampling. Dextrose 5% w/v was administered i.v. until a urine flow of at least 500 ml h^{-1} was achieved, thus minimizing tubular drug reabsorption. Sufficient diuresis was ensured throughout the experiment by infusion of 5% w/v dextrose at a rate of 500 ml h^{-1}. Stable plasma concentrations of the antibiotics and of inulin were established by giving bolus i.v. injections followed by continuous infusions using a Harvard pump 22 (Harvard apparatus; Edenbridge, Kent, UK). The dosage of cefuroxime was based on mean kinetic data reported by Brogden *et al.* [8]. Before drug administration and at 30 min intervals throughout the experiment 5 ml blood samples were taken from the second cannula. The samples were centrifuged and the plasma was separated and stored at −15° C. Subjects emptied their bladders at 30 min intervals, immediately after each blood sample was drawn. The volume of urine was recorded and the samples were stored at −15° C pending assay within 7 days. In the first session cefuroxime was administered as an i.v. injection of 750 mg followed by a continuous i.v. infusion of 420 mg h^{-1}. Inulin was administered as an i.v. bolus of 40 mg kg^{-1} followed by a continuous i.v. infusion of 2400 mg h^{-1}. Probenecid (1 g) was administered orally 30 min after the start of the administration of the antibiotic. Blood and urine samples were collected every 30 min for 6.5 h.

The second session took place at least 1 week after the first. During this session three steady plasma concentrations of cefuroxime were established. Thus, after prehydration, cefuroxime was administered as an i.v. bolus of 375 mg followed by a continuous infusion of 210 mg h^{-1}; after 2.5 h the bolus injection of 375 mg was repeated, followed by a continuous infusion of 420 mg h^{-1}; after 5 h a bolus injection of 750 mg was given, followed by a continuous infusion of 840 mg h^{-1}. Blood and urine samples were collected every 30 min for 7.5 h. Inulin was administered as in the first session. To avoid carry-over effects data collected within 1 h of a change in dose were not included in the calculations.

Ceftazidime Two subjects took part in this study. The procedures for prehydration and fluid loading during the experiment, inulin administration and blood and urine sampling were as described for cefuroxime. In the first session a dose of 1 g ceftazidime was administered as an i.v. bolus, followed by a continuous infusion of 400 mg h^{-1}. After 30 min 1 g probenecid was administered orally. Blood and urine samples were collected every 30 min for 4.5 h. The second session was at least 1 week after the first. Ceftazidime was administered as an i.v. bolus of 500 mg followed by a continuous infusion of 200 mg h^{-1}. After 2.5 h the bolus injection of 500 mg was repeated followed by a continuous infusion of 400 mg h^{-1}. After 5 h a bolus injection of 1 g was given followed by a continuous infusion of 800 mg h^{-1}. Probenecid was administered orally in a dose of 1 g 5.5 h after the start of the experiment. Blood and urine samples were collected every 30 min for 9.5 h. To avoid carry-over effects data collected within 1 h of a change in dose were not included in the calculations.

Analytical methods

Cefuroxime, ceftazidime and probenecid were assayed by h.p.l.c. as described by Van Gulpen *et al.* [9]. For cefuroxime the level of detection was 0.1 mg l^{-1}. At the actual concentrations measured the coefficient of variation was 1.3%. For ceftazidime these values were 0.5 mg l^{-1} and 4%, respectively, and for probenecid 0.5 mg l^{-1} and 3%, respectively.

To assay probenecid glucuronide β-glucuronidase was added to plasma or urine at a concentration of 1 in 20 and incubated for 24 h at 37° C. The total probenecid concentration was measured as described above and the glucuronide concentration was estimated as the difference between total concentration and concentration before hydrolysis.

Inulin was assayed by the method of Heyrovsky [10]. The level of detection in urine was 300 mg l^{-1} and in plasma 50 mg l^{-1}. The coefficient of variation was 1.5%. Protein binding was determined by equilibrium dialysis of plasma against buffered saline in a Dianorm® apparatus (Diachema AG, Zurich, Switzerland) [11]. The coefficients of variation of this procedure for the determination of the bound fraction were 1.6% for probenecid and 15% for cefuroxime.

Pharmacokinetic analysis

Tubular excretion rate (R_{tub}) was calculated from:

$$R_{tub} = R_R - CL(in) \cdot Cu \quad (1)$$

where R_R is the total renal drug excretion rate, CL(in) is inulin clearance, and Cu is the unbound plasma drug concentration. Tubular excretion was assumed to depend on the concentrations of antibiotics and probenecid as described by equation (2):

$$R_{tub} = R_{tub,max} \frac{Cu_{cef}}{Cu_{cef} + EC_{50,cef} + Cu_{prob} \frac{EC_{50,cef}}{EC_{50,prob}}} \quad (2)$$

where $R_{tub,max}$ is the maximum tubular excretion rate of the cephalosporin, Cu_{cef} is unbound plasma concentration of the cephalosporin, $EC_{50,cef}$ is the cephalosporin concentration at which 50% of $R_{tub,max}$ is obtained in the absence of probenecid, Cu_{prob} is the unbound plasma concentration of probenecid and $EC_{50,prob}$ is the probenecid concentration at which 50% of the transport capacity is occupied by probenecid. Application of non-linear regression (NONLIN; SYSTAT 5.0: Systat Inc., Evanston, Ill, USA) allowed separate estimates of the respective constants using all of the data. The algorithm used a quasi-Newton non-linear least squares estimator. Analysis of covariance (MGLH; Systat 5.0) was performed to assess urine flow as a determinant of passive drug reabsorption and to assess the effect of probenecid on the clearance of ceftazidime.

Table 1 Parameters describing the tubular excretion of cefuroxime and its inhibition by probenecid in five healthy volunteers. Concentrations refer to unbound drug in plasma. $R_{tub,max}$ is the maximum tubular excretion rate of cefuroxime, $EC_{50,cef}$ is the cefuroxime concentration at which 50% of $R_{tub,max}$ is obtained in the absence of probenecid and $EC_{50,prob}$ is the probenecid concentration at which 50% of the transport capacity is occupied by probenecid; CL(in) is inulin clearance, CL_{tub} is tubular clearance of cefuroxime and $CL_{tub,max}$ is the maximal tubular clearance of cefuroxime

Subject	*Urine flow* ($l\,h^{-1}$)	$EC_{50,cef}$ ($mg\,l^{-1}$)	$R_{tub,max}$ ($mg\,h^{-1}$)	$EC_{50,prob}$ ($mg\,l^{-1}$)
1	1.00	380	2220	0.39
2	1.04	391	2600	0.61
3	1.03	186	1839	1.08
4	0.90	180	1424	1.10
5	1.26	105	1178	0.82
Mean	1.04	248	1852	0.80
s.d.	0.13	129	577	0.32

	CL(in) ($l\,h^{-1}$)	CL_{tub} ($l\,h^{-1}$)	$CL_{tub,max}$ ($l\,h^{-1}$)
1	8.97	5.40	5.84
2	7.56	5.77	6.65
3	8.11	8.31	9.89
4	9.05	6.53	7.91
5	10.57	6.30	11.22
Mean	8.85	6.46	8.30
s.d.	1.15	1.12	2.23

Results

Cefuroxime

The mean urinary flow maintained during the sessions was about $1\,l\,h^{-1}$ (Table 1). The mean extent of plasma protein binding of cefuroxime was 17.2% (s.d. 4.2%) and that of probenecid was 92.6% (s.d. 1.1%). Non-linear regression analysis of complete data according to equation [2] provided estimates of the pharmacokinetic parameters in four of the six subjects (Table 1), while in one other subject the data from the second session without probenecid were used to calculate $EC_{50,cef}$ and $R_{tub,max}$ which were then used to calculate the parameters of the interaction with probenecid using the data from the first session. A three-dimensional plot of tubular drug excretion against unbound concentrations of cefuroxime and probenecid in a representative subject is shown in Figure 1. The curved plane was constructed using equation 2 with the estimated parameters for that individual (subject 3, Table 1). The parameter estimates for the individual subjects showed a large error. Moreover, a strong covariation between the estimates of EC_{50} and $R_{tub,max}$ for cefuroxime existed, but not between these parameters and the EC_{50} of probenecid. Because of this covariation the maximum tubular clearance ($CL_{tub,max}$) was calculated as the quotient of $R_{tub,max}$ and EC_{50}, which is the limit of CL_{tub} when the plasma concentration goes to zero. These values were less variable than the values of EC_{50} and $R_{tub,max}$ (Table 1).

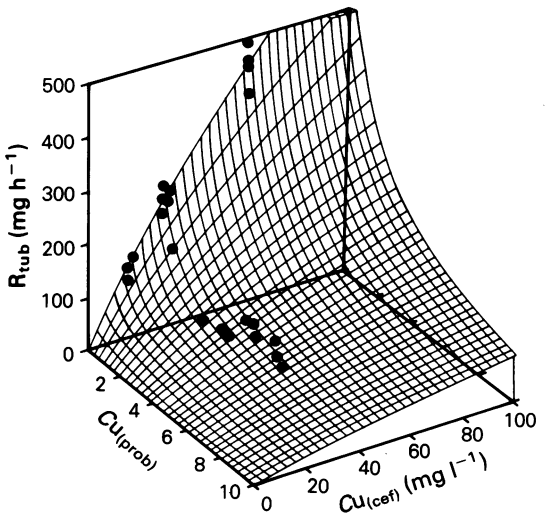

Figure 1 Tubular excretion of cefuroxime as a function of unbound plasma concentrations of cefuroxime (Cu_{cef}) and probenecid (Cu_{prob}). The curved plane was derived from the equation:

$$R_{tub} = R_{tub,max}\, Cu_{cef}/(Cu_{cef} + EC_{50,cef} + Cu_{prob} \cdot EC_{50,cef}/EC_{50,prob})$$

with the parameters given in Table 1 for subject 3. Data points refer to all calculated values of R_{tub} in two experiments.

Non-linear regression analysis using the urinary concentration of probenecid instead of its unbound plasma concentration gave correlation coefficients that were generally lower.

For the sessions without probenecid analysis of covariance of the data from all five subjects showed a highly significant dependence of the calculated tubular clearance of cefuroxime on the reciprocal value of urinary flow ($P < 0.001$).

For probenecid itself a negative tubular clearance was calculated, the mean value for the five subjects being -8.3 (s.d. 0.7) $l\ h^{-1}$.

Ceftazidime

Ceftazidime was not bound to plasma protein. The estimated values of clearance by tubular excretion of ceftazidime showed a large variation about zero for both subjects, indicating no net tubular excretion. Therefore, it was not possible to use non-linear regression analysis of the calculated tubular clearance of ceftazidime. Nevertheless, analysis of covariance of these values as the dependent variable and unbound plasma concentrations of ceftazidime and probenecid as the independent variables showed a significant negative effect of probenecid in both cases ($P < 0.05$). The clearance of probenecid glucuronide in the two subjects was similar ($56\ l\ h^{-1}$ and $48\ l\ h^{-1}$).

Discussion

The results indicate that the tubular excretion of cefuroxime is saturable and that this excretion is inhibited by probenecid. The latter finding confirms earlier observations [8, 12]. For ceftazidime no tubular excretion could be shown, as suggested previously by Balant *et al.* [7] and Bergan [12].

The analysis of the interaction of the two cephalosporins with probenecid indicates that the method used may also be applicable for studying the interaction of other drugs with probenecid at sites of tubular transport. The calculated values of EC_{50} and $R_{tub,max}$ far exceeded the range of the actual observations; the highest concentrations of cefuroxime observed in individual subjects were between 16 and 40% of the EC_{50}. The observed values of tubular clearance were about 80% of the estimated maximum value of tubular clearance ($CL_{tub,max}$), giving further indication that the excretory process was far from saturated. However, to approach the EC_{50} more closely much higher dosages would have been necessary, dosages which far exceed those used clinically, and this was not considered ethically justifiable. Nevertheless, the highest observed concentrations were much higher than those seen in patients treated with those antibiotics. Since the pharmacokinetic model gives an accurate description of the quantitative relation between the concentration in plasma and the excretory mechanism, our results allow conclusions with regard to therapeutic concentration ranges. The calculated values of EC_{50} refer to concentrations in the renal artery and, therefore, they do not represent the value of this parameter at the site of tubular transport. However, it is not possible to estimate such values in man. The value of the EC_{50} of cefuroxime was similar to that found for cephradine in a previous study (1), *viz*, 266 $mg\ l^{-1}$, and clearly higher than those for benzylpenicillin (93 $mg\ l^{-1}$) and cloxacillin (7.7 $mg\ l^{-1}$). The value of 1852 $mg\ h^{-1}$ for the $R_{tub,max}$ of cefuroxime was less than that for cephradine (4537 $mg\ h^{-1}$), although there was substantial overlap of individual values. Mean values for benzylpenicillin and cloxacillin were 5535 and 1017 $mg\ h^{-1}$, respectively. These values indicate that in the therapeutic concentration range differences in tubular excretion rate between the antibiotics are mainly determined by differences in EC_{50}. The high affinity of cloxacillin for the transport mechanism in combination with the limited capacity will lead to significant decrease in tubular clearance at higher therapeutic concentrations. This has been confirmed in a clinical study [13].

Even at relatively high concentrations the tubular excretion of ceftazidime is negligible. This indicates that the absence of tubular excretion is due to a very low affinity for the transport system, since a limited maximal capacity would easily have been detected by our analysis. Our results differ from those of Lüthy *et al.* [14] and Mouton *et al.* [15]. In the first of these studies ceftazidime clearance was about 60% of creatinine clearance, and no effect of probenecid was found, and in the second study it was 90%. This suggests the presence of significant tubular reabsorption. In our study the total renal clearance of ceftazidime was similar to inulin clearance, indicating that there is neither net tubular excretion nor tubular reabsorption. However, the significant decrease of total renal clearance in the presence of probenecid indicates that some tubular excretion occurs, which may be offset by passive reabsorption. Since urinary flow in our study was very high, passive reabsorption would be expected to be less than in the studies cited above [14, 15].

If this explanation is correct with respect to ceftazidime, the possibility of passive reabsorption should also be considered in the case of cefuroxime. This would explain the negative correlation between tubular excretion of cefuroxime and the reciprocal of the urinary flow. The estimate of the unbound EC_{50} of probenecid was 0.80 $mg\ l^{-1}$, corresponding to a total plasma concentration of 108 $mg\ l^{-1}$. Actual plasma concentrations of probenecid were much higher than the EC_{50}. Therefore, therapeutic doses of probenecid may be expected to block the tubular excretion of β-lactam antibiotics almost completely. In a previous study [2] a value of 52 $mg\ l^{-1}$ was found for the EC_{50} (total plasma concentrations) of probenecid at steady plasma concentrations when administered together with benzylpenicillin in one subject. However, in view of the limited observations on the interaction between benzylpenicillin and probenecid this difference may be spurious.

The calculated tubular clearance of probenecid was negative, indicating net tubular reabsorption, either by active tubular reabsorption or by passive reabsorption.

In conclusion, there are considerable differences in the tubular excretion of different β-lactam antibiotics. Tubular excretion is saturable but, except in the case of cloxacillin, this does not seem to occur to considerable extent within the therapeutic concentration range. Inhibition of tubular excretion by probenecid appears to be competitive.

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