The effect of probenecid on the renal tubular excretion of benzylpenicillin

D. OVERBOSCH*, C. VAN GULPEN, J. HERMANS¹ & H. MATTIE

Department of Infectious Diseases, University Hospital Leiden, P. O. Box 9600, 2300 RC Leiden and

¹Department of Medical Statistics, State University, Leiden, The Netherlands

1 The aim of this study was to establish whether the renal tubular excretion of benzylpenicillin is saturable and whether the effect of probenecid on the tubular excretion of benzylpenicillin is dose-dependent.

2 Each of four volunteers underwent three experiments. In each experiment benzylpenicillin was administered by continuous infusion, such that three different consecutive concentration levels were reached. In the first experiment no probenecid was given; in the second and third experiments, probenecid was administered by continuous infusion at a low and higher rate, respectively.

3 Plasma and urinary concentrations of benzylpenicillin were determined at 30 min intervals by high performance liquid chromatography.

4 By fitting the equation $R_{\text{tub}} = R_{\text{tub,max}} \cdot C_p / (EC_{50} + C_p)$ to the values of the tubular excretion rate found for benzylpenicillin (R_{tub}) vs the free plasma concentration (C_p), the values of $R_{\text{tub,max}}$ and EC_{50} could be calculated: $3350 (\pm 606) \text{ mg h}^{-1}$ for $R_{\text{tub,max}}$ and $48.0 (\pm 17.8) \text{ mg l}^{-1}$ for EC_{50} (in the absence of probenecid).

5 The EC_{50} for benzylpenicillin increased significantly with increasing doses of probenecid.

6 The dose of probenecid at which 50% of the excretory system is occupied by probenecid in the absence of benzylpenicillin (ED_{50}) ranged from 13.2 to 108.5 mg h^{-1} .

7 The EC_{50} of probenecid in one subject could actually be measured: 52.3 mg l^{-1} .

8 Extrapolating these results to the clinical situation, the commonly used daily dose of 2 g of probenecid is likely to be close to the maximal effective dose for inhibition of the tubular excretion of benzylpenicillin.

Keywords probenecid benzylpenicillin tubular excretion

Introduction

Benzylpenicillin, which is eliminated mainly by tubular excretion and glomerular filtration, has a plasma half-life of about 30 min (Plaut *et al.*, 1969). In an attempt to prolong this plasma half-life probenecid was developed, a substance inhibiting the transport of organic acids across epithelial membranes such as that found in the renal tubule (Beyer *et al.*, 1944, 1950; Martindale, 1982). A quantitative extensive study of the

effects of probenecid on the tubular excretion of benzylpenicillin in man has not yet been carried out, although Beyer *et al.* (1951) showed that the inhibition by probenecid of the tubular excretion of benzylpenicillin in dogs is clearly dose-related. If the inhibition is attributable to competition for the transport system, as Beyer *et al.* (1951) suggested, then the effect of probenecid on the renal tubule should depend on the concentrations

*Present address: Rode Kruis Ziekenhuis, Sportlaan 600, 2566 MJ The Hague, The Netherlands

of both probenecid and penicillin. Probenecid has a high degree of plasma-binding of 83–95%; 77–88% of the dose is excreted in urine, mostly in the form of metabolites since only 4–13% is excreted as unchanged drug. The plasma half-life of probenecid is 6–12 h, depending upon dose, urinary pH and urine flow (Dayton *et al.*, 1963, 1971; Cunningham *et al.*, 1981). To study the interaction between probenecid and benzylpenicillin in relation to concentration it is therefore necessary to maintain fairly constant plasma concentrations. The aim of the study was to quantify the inhibition of tubular excretion of benzylpenicillin by probenecid.

Methods

Drugs

Benzylpenicillin, obtained as a sodium salt (Gist-brocades, Delft, the Netherlands, batch no. 79D29) was administered intravenously in all experiments. A solution of 100 mg probenecid ml^{-1} (kindly donated in the form of a sodium salt by Merck, Sharp and Dohme Research Laboratories, Westpoint, PA, USA) was used for the experiments with probenecid.

Volunteers

Four healthy volunteers (two males and two females) between 20 and 27 years of age, entered the study. They received both verbal and written information on the nature and design of the study before they consented to participate, according to the declaration of Helsinki/Tokyo/Venice (KNMG, 1984). The study was approved by the Hospital Review Committee. A history was taken to rule out gross liver and kidney diseases, peptic ulcer, gout, nephrolithiasis and convulsions. Appropriate laboratory tests were done with the same purpose in mind. The volunteers were asked to refrain from drinking coffee, tea and alcoholic beverages at least 12 h prior to the start of the study.

Experimental design

In order to study the saturable tubular excretion of benzylpenicillin three experiments were carried out with each volunteer. In all three experiments benzylpenicillin was infused such that three different more or less constant concentration levels were maintained per experiment. In order to study the effect of probenecid on the tubular excretion of benzylpenicillin, benzylpenicillin was infused as the only drug in

the first experiment; in the second and third experiments probenecid was added by a separate continuous infusion such that a low and a higher plasma probenecid concentration, respectively, would be attained.

The three experiments were carried out at intervals of at least 48 h. In all experiments, a loading dose of benzylpenicillin was administered via a continuous infusion within 10 min, followed by a lower dose given in order to maintain a constant plasma concentration of benzylpenicillin for 2 h. During this period blood samples were taken and urine was collected at approximately 30 min intervals. To ensure a constant plasma concentration at a higher level a larger loading dose of benzylpenicillin was administered and the continuous infusion rate adjusted. Blood samples and urine were collected as during the first period. Finally, a third loading dose was administered, followed again by a higher infusion rate, aiming at a still higher plasma concentration. Since higher infusion rates of benzylpenicillin, especially during the loading dose, were sometimes accompanied by side-effects, such as nausea, the loading doses and infusion rates were individualized. During each experiment the fluid intake, both oral and parenteral, was high in order to ensure an urinary flow of more than 500 ml h^{-1} to minimize tubular reabsorption.

In the second and third experiments probenecid was also administered: an initial loading dose of probenecid was infused intravenously within 10 min, 30 min prior to the start of the benzylpenicillin infusion, and the plasma probenecid concentrations were maintained at a lower and a higher level, respectively, by means of a continuous infusion.

Assessment of benzylpenicillin and probenecid concentrations in blood and urine

Blood samples were collected in heparinized tubes; plasma was separated and benzylpenicillin assays were carried out immediately after collection. Urine was also assayed immediately after collection.

Benzylpenicillin concentrations were measured by high performance liquid chromatography with u.v. detection, as described elsewhere in detail (Van Gulpen *et al.*, 1986). For one volunteer (no. 4) the plasma concentrations of probenecid could also be measured by high performance liquid chromatography. The limits of detection for benzylpenicillin and probenecid were 0.5 and 0.25 mg l^{-1} , respectively, and the coefficients of variation ranged from 2 to 5% for day-to-day reproducibility (Van Gulpen *et al.*, 1986).

Determination of the creatinine concentrations

Serum creatinine concentrations were determined with a Technicon-SMAC® automated analyser (Technicon Instruments Corp., Tarrytown, New York, USA) (Chaon *et al.*, 1961). Urine creatinine concentrations were determined with a Beckman-Astra® automated analyser (Beckman, Berkeley, California, USA) (Heinegard & Tiderstrom, 1973).

Pharmacokinetic calculations

The total renal clearance (CL_R) of benzylpenicillin is determined by glomerular filtration, tubular excretion and tubular reabsorption. Since reabsorption was kept to a minimum as a result of the high urinary flow, tubular reabsorption was considered negligible in our calculations. The relation between the renal clearance components may be written as follows:

$$CL_{uR} = CL_{uG} + CL_{uT} \quad (1)$$

in which CL_{uG} is the glomerular clearance of the free drug and CL_{uT} its tubular clearance. The glomerular clearance of free (i.e. unbound) benzylpenicillin was considered equal to the creatinine clearance. Equation (1) can then be rewritten to find the tubular excretion of free benzylpenicillin:

$$R_{tub} = R_R - CL_{cr} \times Cu \quad (2)$$

in which Cu is the plasma concentration of free benzylpenicillin. To calculate free concentrations from total concentrations a plasma-binding percentage of 53% was used. This value had been previously established in our laboratory, with a standard deviation of 7.7%, and without concentration dependency in the range of concentrations encountered (Bins & Mattie, 1987). In order to establish the relationship between R_{tub} and Cu the values of R_{tub} were plotted against those of the free concentration of benzylpenicillin and fitted by non-linear regression analyses (Metzler *et al.*, 1974) according to the following equation:

$$R_{tub} = R_{tub,max} \times \frac{Cu}{EC_{50} + Cu} \quad (3)$$

in which $R_{tub,max}$ is the maximum tubular excretion rate and EC_{50} is the concentration of free benzylpenicillin at which the tubular excretion rate becomes 50% of $R_{tub,max}$. Therefore inhibition of the tubular excretion of benzylpenicillin by probenecid was assumed to be competitive. Curve fitting was performed using the data from all three experiments with each volunteer with the same value for $R_{tub,max}$ for the

three experiments and different values of EC_{50} for each dosage level of probenecid. The change in EC_{50} for benzylpenicillin caused by probenecid can be described as follows:

$$EC_{50} = EC_{50,0} + \frac{D_{prob} \times EC_{50,0}}{ED_{50}} \quad (4)$$

Hence equation (3) may be written as:

$$R_{tub} = R_{tub,max} \times \frac{Cu}{(EC_{50,0} + D_{prob} \times EC_{50,0}/ED_{50}) + Cu} \quad (5)$$

in which $EC_{50,0}$ is the EC_{50} for benzylpenicillin in the absence of probenecid; D_{prob} is the dose of probenecid, expressed as an infusion rate, and ED_{50} is the dose of probenecid that effectively doubles the EC_{50} of benzylpenicillin (Wilson *et al.*, 1975). The values of $EC_{50,0}$ for benzylpenicillin are obtained by linear regression analysis of the values of EC_{50} calculated from equation (3) with respect to the corresponding infusion rates of probenecid (D_{prob}). The intercept represents $EC_{50,0}$ for benzylpenicillin which together with the slope of the regression line yields the value of ED_{50} .

Results

Four subjects participated in our study and each subject underwent three experiments. Due to differences between subjects in tolerance for the high rates of infusion of benzylpenicillin, the infusion rates and thus the resulting plasma concentrations show considerable variation. However, for each infusion rate in each experiment three plasma samples were obtained; the mean benzylpenicillin concentration determined for these samples is given in Table 1 together with the benzylpenicillin excretion rate, creatinine clearance and infusion rates for probenecid and benzylpenicillin.

The calculated values of the tubular excretion of benzylpenicillin (R_{tub}) and the free plasma concentration (Cu) were fitted to equation (3) separately for each experiment, thus providing the best estimates of the maximum tubular excretion rate for benzylpenicillin ($R_{tub,max}$) for each subject and the EC_{50} for benzylpenicillin in each experiment. The values of $R_{tub,max}$ and EC_{50} in relation to the probenecid dose are given in Table 2. The curves fitted to equation (3) for each subject are shown in Figure 1. It is evident that the measured concentrations were far from the concentrations that yield maximal excretion rates, but such concentrations cannot be maintained safely in human subjects. The mean maximum excretion rate for benzylpenicillin, as

Table 1 Mean values for four volunteers receiving continuous infusions of benzylpenicillin and probenecid

Subject	$k_{o,prob}$	$k_{o,pen}$	Cu	CLu _R	R _{tub}	CL _{cr}
1	0.0	600	19.0	1035.4	977.1	118.1
		1800	31.3	783.6	1247.9	105.9
		3600	127.8	460.0	2580.7	122.1
	31.3	600	10.4	1165.5	643.7	134.2
		1200	22.6	1243.0	1499.1	137.5
		2400	63.5	754.1	2347.7	137.5
	200.3	200	5.8	305.0	79.8	74.5
		600	20.1	357.5	287.8	117.1
		1200	53.6	288.2	555.2	115.5
	0.0	600	14.5	974.0	746.7	119.9
		1800	75.5	600.3	2031.4	131.9
		3600	132.2	523.5	3042.8	114.6
2	31.3	600	11.8	509.1	298.7	78.5
		1200	30.2	573.9	867.4	94.6
		2250	73.6	464.1	1620.7	93.7
	125.0	200	6.8	318.6	80.2	105.8
		600	22.1	377.5	338.2	121.9
		1200	51.4	318.0	612.9	118.8
	0.0	600	12.3	820.0	502.2	141.0
		1800	49.9	677.6	1654.3	125.7
		3600	154.0	371.3	2284.9	123.5
	31.3	600	10.3	774.8	390.4	141.2
		1200	25.9	572.2	679.0	132.4
		2400	67.0	455.8	1198.4	157.8
3	93.8	750	24.5	556.8	600.2	125.2
		1500	53.5	480.1	1116.3	132.6
		2250	98.2	430.1	1658.1	148.3
	0.0	858	6.1	1506.4	495.3	140.2
		2091	34.5	877.0	1523.7	138.0
		4570	100.9	490.7	2045.6	152.4
	21.4	486	6.9	875.0	300.2	150.1
		975	17.2	712.1	578.2	152.2
		2116	34.7	622.5	971.8	156.3
	140.1	1424	29.4	540.9	711.5	135.9
		2241	55.0	408.9	895.5	136.8
		2984	95.2	362.7	1229.4	147.3

$k_{o,prob}$ = infusion rate (mg h^{-1}) for probenecid. $k_{o,pen}$ = infusion rate (mg h^{-1}) for benzylpenicillin. Cu = free plasma concentration (mg l^{-1}) of benzylpenicillin. CLu_R = renal clearance of free benzylpenicillin (ml min^{-1}). R_{tub} = tubular excretion of benzylpenicillin (mg h^{-1}). CL_{cr} = creatinine clearance (ml min^{-1}).

derived from equation (3), was $3350 (\pm 606) \text{ mg h}^{-1}$. The mean EC₅₀ in the absence of probenecid was $48.0 (\pm 17.8) \text{ mg l}^{-1}$. The values of EC₅₀ are plotted against the corresponding infusion rate for probenecid for all four subjects in Figure 2. The individual values of ED₅₀ for probenecid were calculated from the intercept of the regression lines (EC_{50,0}) and the slope (Table 2). The mean value for ED₅₀ was 47.1 mg h^{-1} with a range from 13.2 to 108.5 mg h^{-1} . Actual probenecid concentrations could only be measured in subject no. 4. The mean concentration of probenecid was 6.7 mg l^{-1} and 35.1 mg l^{-1} for the low and high infusion rates, respectively.

These values could be used to calculate an EC₅₀ for probenecid, which represents the concentration of probenecid that effectively doubles the EC₅₀ of benzylpenicillin. The value of this EC₅₀ for probenecid was 52.3 mg l^{-1} . The corresponding EC_{50,0} for benzylpenicillin, obtained by regression analysis, was 67.9 mg l^{-1} .

Discussion

In the present study several aspects of the interaction between probenecid and benzylpenicillin were studied.

Table 2 Maximum tubular excretion, EC_{50} for benzylpenicillin and ED_{50} for probenecid in four volunteers

Subject	$k_{o,prob}$	$R_{tub,max}$	EC_{50}	ED_{50}
1	0	3779 (594)	60.2 (21.6)	41.3
	31.3		38.8 (13.3)	
	200.0		297.1 (129.9)	
2	0	4131 (815)	60.5 (30.4)	108.5
	31.3		116.5 (39.2)	
	125.0		285.7 (117.2)	
3	0	3065 (211)	48.7 (9.1)	25.4
	31.3		96.8 (13.5)	
	93.8		90.5 (12.8)	
4	0	2493 (122)	22.6 (3.5)	13.2
	21.4		54.8 (5.8)	
	140.1		91.7 (9.6)	

$k_{o,prob}$ = infusion rate ($mg\ h^{-1}$) for probenecid.

$R_{tub,max}$ = maximal tubular excretion rate for benzylpenicillin ($mg\ h^{-1}$).

EC_{50} = concentration of benzylpenicillin at which half of $R_{tub,max}$ is reached ($mg\ l^{-1}$).

ED_{50} = dose of probenecid, expressed as infusion rate ($mg\ h^{-1}$) at which 50% of the tubular transport mechanism is occupied by probenecid in the absence of benzylpenicillin.

In parentheses: s.e. mean.

The tubular excretion of benzylpenicillin is saturable with a mean maximum excretion ($R_{tub,max}$) of $3.4\ g\ h^{-1}$; the mean concentration at which 50% of the excreting capacity of the tubular system is saturated ($EC_{50,0}$) is $48\ mg\ l^{-1}$. These findings confirm the results of earlier studies from our group, although the actual values of $R_{tub,max}$ are somewhat lower than those reported (Bins & Mattie, 1988). There is also some discrepancy between the values found for the EC_{50} for benzylpenicillin (Bins & Mattie, 1988): $48\ vs\ 73\ mg\ l^{-1}$. The reason for this is not clear, but the values are at least on the same order of magnitude. Our maximal excretion rates are higher than the $1.8\ g\ h^{-1}$ reported by Bryner *et al.* (1948). However, these authors used a kinetic model with a fixed maximum excretion rate that was arbitrarily chosen rather than calculated.

It was assumed that the tubular excretion of benzylpenicillin is inhibited by probenecid due to competition. The relation between the measured tubular excretion and the free plasma concentration of benzylpenicillin should therefore fit equation (3). With increasing doses of probenecid the EC_{50} for benzylpenicillin should then increase without affecting maximum tubular excretion. Our results actually did fit equation (3) although the plasma concentrations were far below those at which saturation of the tubular excretory system would be expected. Because of

this curve fitting for the separate experiments would lead to considerable variation between the three values of $R_{tub,max}$ for each individual volunteer. Because the calculated values of EC_{50} and $R_{tub,max}$ are interdependent, this procedure would not allow meaningful conclusions regarding EC_{50} . Concentration levels closer to the saturation level could not be achieved because these are not considered safe (Lerner *et al.*, 1967; Conway *et al.*, 1968; Seamans *et al.*, 1968).

Our aim was to study the dose-related effect of probenecid on the tubular excretion of benzylpenicillin. Linear regression analysis of EC_{50} and the dose of probenecid showed that the effect of the dose on the EC_{50} for benzylpenicillin varied greatly between the four subjects. However, in three of four subjects linear regression was highly significant. Consequently, we may conclude that the effect of probenecid on the tubular excretion of benzylpenicillin is dose-related. The ED_{50} calculated from the EC_{50} for benzylpenicillin and the slope of the regression line, represents the affinity of the tubular excretory system for probenecid. As may be seen in Figure 2, the ED_{50} varied greatly between subjects, ranging from 13.2 to $108.5\ mg\ h^{-1}$. However, apparent differences in the efficacy of probenecid, as expressed by a dose-derived parameter like the ED_{50} , do not necessarily mean that a concentration-derived parameter like the

Figure 1 Plasma concentrations of free benzylpenicillin and tubular excretion rates found for four volunteers. Curves were fitted non-linearly to equation (3) (see Methods) ($\circ$ no probenecid, $\square$ low dose probenecid, $\triangle$ high-dose probenecid).

Figure 2 Values of the EC₅₀ for benzylpenicillin plotted against the dose of probenecid as determined for four volunteers. (— $\bullet$ — subject no. 1; --- $\square$ — subject no. 2; ··· $\diamond$ ··· subject no. 3; — $\triangle$ — subject no. 4).

EC₅₀ for probenecid would show the same variation. The effect of probenecid depends most likely on the actual plasma concentrations at the

target site, i.e. the tubular system; therefore the variation in ED₅₀ is more likely to be due to differences in plasma clearance of the drug between subjects. We were able to measure actual probenecid concentrations in one subject (no. 4) so that an EC₅₀ for probenecid could be calculated. However, due to the above-mentioned possible variations in the plasma clearance of probenecid, no solid quantitative conclusions can be drawn from these measurements. Unfortunately, the solution for intravenous administration of probenecid is no longer available and therefore we felt it appropriate to present our findings at this time.

Our findings may have consequences for the optimal dose regimen of probenecid. In our experiments the dose of probenecid was expressed as an infusion rate. Because the absorption of probenecid after oral administration is rapid and complete (Dayton *et al.*, 1963; Dayton & Perel, 1971; Tjandramaga *et al.*, 1972), it seems justified to extrapolate the doses given in our experiments to a 24 h oral dose. An infusion rate

of 83 mg h^{-1} corresponds then to the usual daily dose of 2 g of probenecid. Using equation (5) it can be calculated that the latter dose produces about 90% inhibition of the tubular excretion of benzylpenicillin when the average total plasma concentration of benzylpenicillin is 25 mg l^{-1} . To illustrate this effect we plotted the estimated effect of probenecid on the tubular clearance of benzylpenicillin for the four volunteers in Figure 3a. This figure shows that doses of more than 2 g of probenecid per day do not proportionally increase inhibition of the tubular excretion of benzylpenicillin. At much higher plasma concentrations of benzylpenicillin, however, tubular clearance is already decreased due to saturation of the tubular excretory system. Probenecid at the usual dose then elicits only a small additive inhibitory effect on the benzylpenicillin clearance. This is illustrated in figure 3b. In clinical practice total plasma concentrations of benzylpenicillin vary from 25 to 50 mg l^{-1} , rather

than 200 mg l^{-1} , so that a daily dose of 2 g seems appropriate.

Probenecid was developed initially because at that time benzylpenicillin was extremely expensive. Nowadays it is a cheap antibiotic and the desired clinical effect may simply be attained by increasing the dosage, thus avoiding the need for probenecid. Other β -lactam antibiotics however are also widely used and they are often very costly. Probenecid could again play a role in reducing the costs of treatment (Editorial, 1984). For these drugs, however, the EC_{50} values may be quite different (Overbosch *et al.*, 1985; Bins & Mattie, 1988), and therefore the optimal doses of probenecid may well differ from the 2 g found for benzylpenicillin. This optimal dose has to be established for each drug.

Figure 3 Calculated relation between tubular clearance of benzylpenicillin and dose of probenecid for four volunteers when the total plasma concentration of benzylpenicillin is 25 mg l^{-1} (a) and 200 mg l^{-1} (b) (— subject no. 1; ---- subject no. 2; subject no. 3; -.- subject no. 4).

References

- Beyer, K. H., Painter, R. H. & Weibelhaus, V. D. (1950). Enzymatic factors in tubular secretion of phenol red. *Am. J. Physiol.*, **161**, 259.
- Beyer, K. H., Russo, H. F., Tillson, E. K., Miller, A. K., Verwey, W. F. & Gass, S. R. (1951). 'Benemid', p-(di-n-propylsulfamyl)-benzoic acid: Its renal affinity and its elimination. *Am. J. Physiol.*, **166**, 625-640.
- Beyer, K. H., Woodward, R., Peters, L., Verwey, W. F. & Mattis, P. A. (1944). The prolongation of penicillin retention in the body by means of para-aminohippuric acid. *Science*, **100**, 107-108.
- Bins, J. W. & Mattie, H. (1988). Saturation of the tubular excretion of beta-lactam antibiotics. *Br. J. clin. Pharmacol.*, **24**, 41-50.
- Bryner, S., Clark, W. H., Randall, A. & Rantz, L. A. (1948). Renal tubular excretory capacity for penicillin in health and in subacute bacterial endocarditis. *Am. J. Med.*, **5**, 202-209.
- Chaon, A. L., Grady, H. T. & Staneley, M. A. (1961). Determination of creatinine by means of automatic chemical analysis. *Am. J. clin. Pathol.*, **35**, 83-88.
- Conway, N., Beck, E. & Somerville, J. (1968). Penicillin encephalopathy. *Postgrad. med. J.*, **44**, 891-897.

- Cunningham, R. F., Israili, Z. H. & Dayton, P. G. (1981). Clinical pharmacokinetics of probenecid. *Clin. Pharmacokin.*, **6**, 135–151.
- Dayton, P. G. & Perel, J. M. (1971). The metabolism of probenecid in man. *Ann. N. Y. Acad. Sci.*, **179**, 399–402.
- Dayton, P. G., Perel, J. M., Snell, M. M., Yü, T. F. & Gutman, A. B. (1963). The physiological disposition of probenecid including renal clearance in man; studies by an improved method for its estimation in biological material. *J. Pharmac. exp. Ther.*, **140**, 278–286.
- Editorial (1984). Plugging the penicillin leak with probenecid. *Lancet*, **ii**, 499.
- Heinegard, D. & Tiderstrom, G. (1973). Determination of serum creatinine by a direct calorimetric method. *Clin. Chim. Acta*, **43**, 305–310.
- KNMG, Koninklijke Nederlandsche Maatschappij tot Bevordering der Geneeskunst. (1984). *Gedragsregels voor artsen*, pp 66–69. 2nd ed. Utrecht, The Netherlands.
- Lerner, P. I., Smith, H. & Weinstein, L. (1967). Penicillin neurotoxicity. *Ann. N. Y. Acad. Sci.*, **145**, 310–318.
- Martindale, (1982). *Extra Pharmacopoeia*. pp 419–420. 28 ed., London: Pharmaceutical Press.
- Metzler, C. M., Elfring, G. L. & McEwen, A. J. (1974). A package of computer programs for pharmacokinetic modelling. *Biometrics*, **30**, 562–570.
- Overbosch, D., Van Gulpen, C. & Mattie, H. (1985). Renal clearance of temocillin in volunteers. *Drugs*, **29** (Suppl. 5), 128–134.
- Plaut, M. E., O'Connell, C. J., Pabico, R. C. & Davidson, D. (1969). Penicillin handling in normal and azotemic patients. *J. lab. clin. Med.*, **74**, 12–18.
- Seamans, K. B., Gloor, P., Dobell, A. R. C. & Wyant, J. D. (1968). Penicillin-induced seizures during cardio-pulmonary bypass. *New Engl. J. Med.*, **278**, 861–868.
- Tjandramaga, T. B., Cucinell, S. A., Israili, Z. H., Perel, J. M., Dayton, P. G., Yü, T. F. & Gutman, A. B. (1972). Observations on the disposition of probenecid in patients receiving allopurinol. *Pharmacology*, **8**, 259–272.
- Van Gulpen, C., Brokerhof, A. W., Van der Kaay, M., Tjaden, U. R. & Mattie, H. (1986). Determination of benzylpenicillin and probenecid in human body fluids by high-performance liquid chromatography. *J. Chromatogr.*, **381**, 365–372.
- Wilson, A., Schild, H. O., & Modell, W. (1975). Drug-receptor interactions. In *Applied pharmacology*, 11th ed. pp. 8–15. London: Churchill Livingstone.

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