ESTIMATION OF BILIARY EXCRETION OF UREIDOPENICILLINS IN HEALTHY VOLUNTEERS USING MARKER DILUTION TECHNIQUE

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- 1 Aspiration of duodenal fluid and estimation of recovery by a marker dilution technique was adapted to measure the biliary excretion of two chemically related ureidopenicillins, mezlocillin and azlocillin, in healthy volunteers.
- 2 The drug was infused intravenously at a constant rate over a period of 6 h during which duodenal fluid was aspirated through the distal opening of a triple-lumen polyvinyl tube.
- 3 Drug levels in plasma, urine and duodenal fluid were determined by h.p.l.c.
- 4 Biliary excretion rate was calculated by multiplying the concentration in the duodenal fluid by the dilution factor resulting from the dilution of marker substance infused through the proximal opening of the triple-lumen tube.
- 5 Since the results obtained by the marker dilution technique are comparable to data obtained by studies in patients with t-tube drainage following biliary surgery, it can be concluded that the method is useful to measure the biliary excretion of drugs in man.

Introduction

When estimating pharmacokinetic parameters in man it is relatively easy to determine the renal clearance of drugs. It is, however, relatively difficult to establish the proportion of non-renal clearance which is due to biliary excretion of unchanged drug as opposed to that due to metabolic degradation. Studies in patients with t-tube drainage may have disadvantages: the biliary recovery may not be guaranteed and hepatic excretion of drugs into the bile may be influenced by the underlying disease. Estimation of biliary excretion of drugs by studying the faecal recovery may be influenced by enterohepatic recirculation and bacterial degradation of the drug may alter the chemical structure.

We became interested in studying the biliary excretion of drugs in man when we investigated the fate of two newly developed ureidopenicillins, mezlocillin and azlocillin (Figure 1). These two penicillins have similar physico-chemical properties but studies in patients with t-tube drainage showed that their biliary excretion was very different (Gundert-Remy *et al.*, 1978, 1982; Gundert-Remy & Weber, 1982). We have therefore devised a method for examining the biliary excretion rate of azlocillin and mezlocillin in normal subjects. In 1972 Grundy & Metzger described the measurement of hepatic secretion of biliary lipids in man by a marker dilution technique. The method was used in modified form to

Figure 1 Formula of azlocillin and mezlocillin.

determine biliary output of cholesterol in patients with gallstones (Shaffer & Small, 1977). We have adapted this method in order to study the biliary excretion of the two ureidopenicillins in healthy volunteers.

Methods

Volunteers

The study was carried out in 13 healthy male volunteers, aged 21–28 years after written informed consent was obtained. The subjects had a normal physical examination and baseline laboratory investi-

gations. In addition there was no history of asthma, hay fever or allergy and skin scratch test were performed to rule out possible penicillin allergy (de Weck, 1973).

Subjects were asked to abstain from food and drinks as well as cigarettes for 10 h before the beginning of the study and during the study.

Study procedure

The fasting subjects were intubated with a triple-lumen polyvinyl tube. Under fluoroscopy the proximal opening was positioned in the upper part of duodenum. The proximal collecting port was near the ampulla of Vater and the distal collection port was situated about 10 cm distal from the ampulla of Vater in the distal part of duodenum. During the study the position of the tube could be monitored by measuring the pH in the fluid aspirated through the proximal collection port. Amino acid solution (2.73 g l⁻¹-valine, 2.73 g l⁻¹-phenyl-alanine, 5.46 g g l⁻¹-methionine/1000 ml) diluted in 10% glucose was infused at a rate of 100 ml/h into the duodenum through the proximal opening using a peristaltic pump (Infusomat, Fa. Braun, Melsungen, FRG) in order to contract the gallbladder (Ashkin *et al.*, 1978) and provide a source of calories. A nonabsorbable sulphobromothalein solution (0.5 g Bromthalein®, Fa. Merck, Darmstadt, FRG, diluted with water to get 50.0 ml) was infused at a rate of 1.2 ml/h (Perfusor, Fa. Braun, Melsungen, FRG) into the line of the amino acid solution.

Duodenal fluid was continuously aspirated by a reverse pump (Perfusor, Fa. Braun, Melsungen, FRG) from both the proximal and distal collection ports at a rate of 10 ml/h. Since the quantity collected did not represent more than 5% of the duodenal output the enterohepatic circulation of lipids was not significantly influenced (Dowling *et al.*, 1970).

The drug under investigation was administered intravenously at a constant infusion rate of 3 ml/h via an intravenous catheter over 6 h.

Blood was withdrawn at intervals from the contralateral arm into heparinized plastic tubes (NH₄-heparinat, 0.5%). Immediately after centrifugation the plasma was frozen and stored at -20°C and the determination of the drug was done within 24 h.

Urine was collected at hourly intervals up to 5 h and then, whenever possible, at 30 min intervals. Volume and pH of the urine samples were recorded. Aliquots were stored and frozen at -20°C and determination of the drug was performed within 24 h. Samples of duodenal juice were collected over 1 h up to 5 h and then at 30 min intervals. In order to separate detritus the samples were centrifuged (5 min, 3000 g) and the supernatant was stored at -20°C. It was ascertained before that not any drug was precipitated in the detritus by the procedure of centrifugation. Determina-

tion of the drug as well as determination of sulphobromothalein were performed within 24 h.

Drugs

Mezlocillin 4 g was diluted with 24 ml of water for injection. In order to examine the final concentration samples of the solution were withdrawn and stored frozen at -20°C. The concentration of the drug was determined within 24 h.

Azlocillin was handled in the same manner.

Analytical methods

Determination of sulphobromothalein Sulphobromothalein was determined according to a standard method (Laboratoriumsmethoden, Fa. Merck, 1974).

Determinations of ureidopenicillins Mezlocillin and azlocillin concentrations were determined using the h.p.l.c. method previously described (Gundert-Remy & De Vries, 1979).

Calculations

The total, renal and biliary clearance were calculated at steady state.

The total clearance was estimated according to

$$Cl_{tot} = \text{infusion rate of drug}/C$$

where Cl_{tot} = total body clearance
 C = plasma concentration at steady state.

Steady state was defined as the time period during infusion in which the concentration did not change more than 10%. Infusion rate of the drug was calculated by multiplying the concentration of the drug in the solution by the infusion rate.

The renal clearance was calculated according to

$$Cl_{ren} = \Delta U / \Delta t / C$$

where Cl_{ren} = renal clearance
 $\Delta U / \Delta t$ = fractional amount excreted in the urine
 = urinary excretion rate
 C = plasma concentration at steady state.

The biliary clearance was estimated according to

$$Cl_{bil} = \Delta B / \Delta t / C$$

where Cl_{bil} = biliary clearance
 $\Delta B / \Delta t$ = fractional amount excreted in the bile
 = biliary excretion rate
 C = plasma concentration at steady state.

Biliary excretion rate was calculated according to

$$\Delta B / \Delta t = C_{MINF} / C_{MASP} \times Q_{INF} \times C_{DASP}$$

where C_{MINF} = concentration of the marker substance in the intraduodenal infusion

- C_{MASP} = concentration of the marker substance in the duodenal fluid aspirated through the distal collection port
- Q_{INF} = volume of the intraduodenal infusion per unit of time
- C_{DASP} = concentration of drug in the duodenal fluid aspirated through the distal collection port.

The metabolic clearance was estimated according to

$$Cl_{\text{met}} = Cl_{\text{tot}} - Cl_{\text{ren}} - Cl_{\text{bil}}$$

where Cl_{met} = metabolic clearance.

Intestinal reabsorption

In order to examine that intestinal reabsorption of drug occurred after delivery into the duodenum via bile a separate study was performed in three volunteers. A dose of 5 g of each ureidopenicillin was administered directly into the duodenum by intraduodenal tube on two separate occasions. Urine was collected for 8 h and the concentration of the ureidopenicillins was determined in the urine. No drug was detectable. The lower limit of detection of the h.p.l.c. method is 1 $\mu\text{g/ml}$, the volume of urine did not exceed 1000 ml. Hence, the lower limit of detection of trace amounts of drug in the urine would be 1 mg. On the other hand, hepatic extraction of both drugs is about 0.2 ($Cl_{\text{nonren}}/Q = E$ where E = hepatic extraction, Cl_{nonren} = nonrenal clearance and Q = liver blood flow which is assumed to be 1500 ml/min) and renal excretion of both drugs is about 50% of the dose. The amount of 1 mg in the urine would therefore represent the amount absorbed of 2.5 mg, i.e. 0.05% of the dose. We therefore conclude that intraduodenal absorption of the ureidopenicillins is negligible and can be ignored in our calculations.

Results

Mezlocillin

In two of the seven volunteers who received mezlocillin biliary excretion of the drug could not be estimated. In one of the two volunteers it was not possible in the h.p.l.c. to separate beyond doubt mezlocillin from interfering peaks. In the other no steady state of biliary mezlocillin excretion was reached. The data of the two subjects were therefore discarded.

In the remaining five volunteers steady state plasma concentrations were reached within 2.8 ± 1.1 h (mean $\pm$ s.d.) after the start of infusion (Figure 2a). With infusion of a dose of 510 ± 24 mg/h steady state

Figure 2 Plasma concentration (▲), urinary (□) and biliary (▽) excretion rate (a) of mezlocillin (intravenous infusion over 6 h, infusion rate 508.8 ± 22.5 mg/h, (b) of azlocillin (intravenous infusion over 6 h, infusion rate 498 ± 22.6 mg/h). Mean and s.e. mean of the five subjects are given.

concentrations were between 17.5 $\mu\text{g/ml}$ and 48.0 $\mu\text{g/ml}$ with a mean value of 30.5 ± 13.9 $\mu\text{g/ml}$. Total clearance for each subject was estimated as the mean of at least four clearance periods of 30 min under steady state conditions. Intraindividual variability was small (Table 1) the coefficients of variation being between 0 to 5.7%. Hence one could assume that steady state conditions were reached.

Table 1 Mezlocillin steady state concentrations and clearances at steady state

Volunteer	Steady state ² concentration ($\mu\text{g/ml}$)	Cl_{tot} ² (ml/min)	Cl_{ren} ² (ml/min)	Cl_{bil} ² (ml/min)	Cl_{met} (ml/min)
1	48.0 $\pm$ 3.0	189.6 $\pm$ 7.9	93.0 $\pm$ 9.1	95.7 $\pm$ 24.1	0.6
2	43.2 $\pm$ 3.3	187.5 $\pm$ 14.3	108.9 $\pm$ 32.7	45.8 $\pm$ 19.1	16.7
3	17.8 $\pm$ 0	477.0 $\pm$ 0	215.2 $\pm$ 15.7	122.8 $\pm$ 21.9	142.9
4	21.0 $\pm$ 1.8	397.9 $\pm$ 34.1	284.6 $\pm$ 34.2	100.2 $\pm$ 31.6	12.9
5	24.0 $\pm$ 0.7	347.9 $\pm$ 10.1	198.0 $\pm$ 33.6	83.1 $\pm$ 13.9	75.0
Mean $\pm$ s.d.	30.5 $\pm$ 13.9	320.0 $\pm$ 128.6	179.9 $\pm$ 79.2	89.5 $\pm$ 28.3	49.6 $\pm$ 59.5

¹ mean and s.d. of the five volunteers. The individual average was taken for the calculation.

² mean and s.d. of different periods at steady state.

Marked interindividual variability of the total clearance was observed ranging from 187.5 to 477.5 ml/min with a mean value of 320.0 ± 128.6 ml/min (Table 1).

Urinary excretion rates reached steady conditions between 2 and 5 h, mean time being 3.3 ± 1.1 h. Intraindividual variability of urinary excretion rate could be estimated since at least two periods were under steady state conditions. Coefficient of variation ranged from 9.8% to 30.0% and intraindividual variability was more pronounced than the intra-individual variability of total clearance.

Interindividual variability of urinary excretion rate was the same as the interindividual variability of total clearance (Table 2). At steady state urinary elimination accounted for a mean of 55.3% of the administered dose of mezlocillin which corresponds to the excretion rate of 281.6 ± 48.1 mg/h (Table 2). Renal clearance was 179.9 ± 79.2 ml/min (Table 1).

Mezlocillin was first detected in duodenal aspirate obtained during the first and second hour after the infusion of the drug was started. This finding may be explained by the lag time which elapsed between the excretion of mezlocillin at the canalicular membrane of the liver cell and the sampling of mezlocillin in the syringe where the duodenal fluid was aspirated. Steady state conditions were reached between 3 and 5 h after infusion was started. In all subjects biliary excretion rate did not fall immediately after the infu-

sion of the drug was stopped, but steady state excretion lasted for another 0.5–2 h, the reason being the same as for the delayed detection of mezlocillin after beginning the infusion. Mean steady state excretion rates were between 118.7 mg/h and 275.7 mg/h with coefficients of variation ranging from 16.8% to 41.7% (Table 2). Mean value of excretion rate of the five subjects was 154.3 ± 68.0 mg/h. Biliary clearance amounted to 89.5 ± 28.3 ml/min.

The rate of metabolic degradation, the difference between infusion rate and the sum of renal and biliary excretion rate, varied from 1.8 to 152.6 mg/h (Table 2) and exhibited the greatest interindividual variability. Metabolic clearance amounted to 49.6 ± 59.5 ml/min (Table 1).

Azlocillin

Six subjects received azlocillin. Only the data of five subjects who received a mean dose of 498 ± 22.6 mg/h could be evaluated since in one of the volunteers plasma steady state was not reached because of inconsistent infusion of the drug. In the remaining five subjects steady state concentration was reached within 4.4 ± 0.2 h after the infusion was started (Figure 2b). Steady state concentrations were between 23.2 $\mu\text{g/ml}$ and 60.0 $\mu\text{g/ml}$ with a mean of 37.3 ± 16.1 $\mu\text{g/ml}$ (mean $\pm$ s.d.) (Table 3). Intraindividual variability of the concentration at steady

Table 2 Mezlocillin excretion rates under steady state conditions

Volunteer	Infusion rate (mg/h)	Urinary excretion ² rate (mg/h)	Biliary excretion ² rate (mg/h)	Metabolic rate (mg/h)
1	546	268.5 $\pm$ 16.2	275.7 $\pm$ 69.5	1.8
2	486	281.3 $\pm$ 84.3	118.7 $\pm$ 20.2	86.0
3	510	226.2 $\pm$ 16.5	131.2 $\pm$ 23.5	152.6
4	501	358.6 $\pm$ 43.1	126.2 $\pm$ 39.8	16.2
5	501	273.2 $\pm$ 46.4	119.8 $\pm$ 20.1	108.0
Mean $\pm$ s.d. ¹	508.8 $\pm$ 22.5	281.6 $\pm$ 48.1	154.3 $\pm$ 68.0	72.9 $\pm$ 63.3

¹ mean and s.d. of the five volunteers. The individual average was taken for the calculation.

² mean and s.d. of different periods at steady state.

Table 3 Azlocillin steady state concentrations and clearances at steady state

Volunteer	Steady state ² concentration ($\mu\text{g/ml}$)	Cl_{tot} ² (ml/min)	Cl_{ren} ² (ml/min)	Cl_{bil} ² (ml/min)	Cl_{met} (ml/min)
6	60.0 $\pm$ 1.7	139.6 $\pm$ 1.2	121.1 $\pm$ 10.3	1.4 $\pm$ 0.3	16.8
7	26.4 $\pm$ 0.7	342.3 $\pm$ 18.0	122.4 $\pm$ 17.9	6.3 $\pm$ 0.05	213.6
8	23.2 $\pm$ 3.0	374.8 $\pm$ 30.2	84.9 $\pm$ 0.8	25.9 $\pm$ 10.1	315.8
9	27.7 $\pm$ 0.6	304.0 $\pm$ 38.2	150.2 $\pm$ 11.1	3.2 $\pm$ 0.5	150.6
10	49.1 $\pm$ 3.2	173.5 $\pm$ 8.7	83.9 $\pm$ 14.0	6.0 $\pm$ 0.8	80.0
Mean $\pm$ s.d. ¹	37.3 $\pm$ 16.3	266.8 $\pm$ 104.4	112.5 $\pm$ 28.2	8.9 $\pm$ 9.8	155.4 $\pm$ 116.2

¹ mean and s.d. of the five volunteers. The individual average was taken for the calculation.² mean and s.d. of different periods at steady state.

state was small, the coefficient of variation ranging from 2.2% to 13.9%. Therefore, steady state conditions were assumed to be present for the last 1.5 h of the infusion. Marked interindividual variability was observed, the coefficient of variation of the mean being 43.7% (Table 3). Total clearance was calculated to be 266.8 ± 104.4 ml/min with marked inter- and small intraindividual variability (Table 3). Steady state condition of urinary excretion rate was reached at 4.8 ± 0.8 h after infusion was started. Intraindividual variability of urinary excretion rate ranged from 0.9% to 16.7% when estimated by coefficient of variation whereas interindividual variability was marked (Table 4). Urinary excretion at steady state which averaged 242.4 mg/h accounted for 48.7% of the administered dose.

Renal clearance was 112.5 ± 28.2 ml/min ranging from 83.9 ml/min to 150.2 ml/min (Table 3).

Azlocillin could first be detected in duodenal aspirate in the sample obtained during the second collection period between the first and the second hour after the commencement of the infusion. Steady state conditions were reached within 5 to 6 h. Steady state excretion lasted for another 1–2 h after infusion was stopped. Mean biliary excretion rates under steady state conditions were between 4.9 mg/h and 34.7 mg/h with large intra- and interindividual variability (Table 4).

Biliary clearance was calculated to vary between 1.4 ml/min and 25.9 ml/min (Table 3).

The rate of metabolic degradation was calculated as the difference between infusion rate and urinary and biliary excretion rate and was between 60.4 mg/h and 422.2 mg/h (Table 4). In contrast to mezlocillin it was the biliary excretion rate, not the metabolic excretion rate which exhibited the greatest intra-individual variability. Metabolic clearance was estimated to be 155.4 ± 116.2 ml/min (Table 3).

The amount of mezlocillin and azlocillin excreted in the bile under steady state conditions is shown in Table 5.

Table 5 Biliary excreted amount of mezlocillin and azlocillin under steady state conditions¹

Volunteer	Mezlocillin (% of dose)	Volunteer	Azlocillin (% of dose)
1	50.5	6	1.2
2	24.4	7	1.8
3	25.7	8	6.9
4	25.2	9	1.1
5	23.9	10	6.2
Mean $\pm$ s.d.	29.9 $\pm$ 11.5		3.4 $\pm$ 2.8

¹ Biliary excreted amount = infusion rate/biliary excretion rate.**Table 4** Azlocillin excretion rates under steady state conditions

Volunteer	Infusion rate (mg/h)	Urinary excretion ² rate (mg/h)	Biliary excretion ² rate (mg/h)	Metabolic rate (mg/h)
6	501	434.5 $\pm$ 36.8	5.0 $\pm$ 1.2	60.4
7	525	187.8 $\pm$ 27.5	9.6 $\pm$ 0.1	327.6
8	501	113.5 $\pm$ 10.0	34.7 $\pm$ 14.2	422.2
9	462	228.3 $\pm$ 16.8	4.9 $\pm$ 0.7	228.8
10	501	247.8 $\pm$ 41.5	30.9 $\pm$ 4.3	222.3
Mean $\pm$ s.d. ¹	498 $\pm$ 22.6	242.4 $\pm$ 119.1	17.0 $\pm$ 14.6	252.2 $\pm$ 134.9

¹ mean and s.d. of the five volunteers. The individual average was taken for the calculation.² mean and s.d. of different periods at steady state.

Discussion

Until now marker dilution techniques have been used to determine biliary excretion of lipids. The use of this method as a tool for the investigation of biliary excretion of drugs has not been established. When we compare the results obtained in the present study with data obtained in patients with t-tube drainage (Gundert-Remy *et al.*, 1978, 1982; Gundert-Remy & Weber, 1982) it is obvious that the results are extremely similar (Table 6). This confirms our belief that this marker dilution method is an accurate way of measuring biliary excretion of drugs.

For the experiments with mezlocillin and azlocillin duodenal reabsorption could be neglected since neither of the ureidopenicillins is absorbed after intraduodenal administration. However, when reabsorption of a drug is known to occur the extent of reabsorption may be estimated experimentally. This can be achieved by infusion of a constant rate of drug through the opening which in this experiment was the proximal collecting port and comparing the amount infused with the amount recovered from the distal collecting port.

There is no way to separate secretion of drugs across the duodenum from biliary excretion by the duodenal perfusion method. However, for azlocillin and mezlocillin passive duodenal secretion can be ruled out since the pKa-value of both drugs is 3.5 (Gundert-Remy & Weber, 1982).

Relative renal excretion of both drugs were very similar seeing that 55.3% and 48.7% of the dose of mezlocillin and azlocillin respectively were excreted via kidneys. Under the assumption that non-renal clearance mainly represents hepatic clearance, hepatic uptake of both substances is quite similar. The non-renal elimination of mezlocillin was 44.7% and azlocillin 51.3%. Nevertheless hepatic handling of the drugs is different since biliary excretion of

Table 7 Duodenal volumes during perfusion of the duodenum with marker substance

Volunteer	Duodenal volume ^{1,2} (l/h)
1	0.409 ± 0.063
2	0.211 ± 0.083
3	0.309 ± 0.052
4	0.333 ± 0.055
5	0.492 ± 0.680
6	0.385 ± 0.319
7	0.254 ± 0.094
8	0.226 ± 0.044
9	0.154 ± 0.030
10	0.331 ± 0.166
11 ³	0.459 ± 0.095
12 ⁴	0.520 ± 0.187
Mean ± s.d. ⁵	0.340 ± 0.116

¹without subtracting the volume of intra-duodenal infusion (101.2 ml/h)

²mean ± s.d. of different periods

³data of the volunteer having received mezlocillin in whom h.p.l.c. peak of the drug could not be separated

⁴data of the volunteer having received azlocillin in whom intravenous infusion was in-constant

⁵mean ± s.d. of the 12 volunteers. The individual average was taken for the calculation.

unchanged mezlocillin accounts for 66% of the hepatic elimination whereas the hepatic elimination of azlocillin is mainly due to metabolic degradation.

In summary, the method of duodenal perfusion gives reasonable results when used to study biliary excretion of drugs. If in addition urinary excretion rates are available the rate of metabolic elimination can be estimated by indirect means. Since the procedure can be applied in healthy volunteers as well as

Table 6 Comparison of the results obtained by duodenal perfusion with data of patients with t-tube drainage

	Amount biliary excreted (% of dose)	Dose (g)	Application
<i>Mezlocillin</i>			
Patients ¹ without liver impairment (<i>n</i> = 3)	22.1 ± 7.7	2.0	i.v. bolus
Volunteers (<i>n</i> = 5)	29.9 ± 11.5	3.1 ± 0.15	i.v. infusion over 6 h
<i>Azlocillin</i>			
Patients ² without liver impairment (<i>n</i> = 3)	5.3 ± 2.9	2.0	i.v. bolus
Volunteers (<i>n</i> = 5)	3.4 ± 2.8	3.0 ± 0.14	i.v. infusion over 6 h

¹Gundert-Remy *et al.* (1978, 1982).

²Gundert-Remy & Weber (1982).

in patients the influence of disease on biliary as well as on metabolic elimination rates can be investigated. However, the method is restricted to measurements of excretion rates and cannot be used to measure biliary flow rate or biliary concentrations of drugs. Marker dilution enables the amount of drug present in the duodenal contents to be estimated (Table 7).

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