Br J clin Pharmac 1995; **39**: 692–695

Probenecid inhibits the renal clearance of frusemide and its acyl glucuronide

TOM B. VREE[1,2], MAGDALENA VAN DEN BIGGELAAR-MARTEA[1] & CORRIEN P. W. G. M. VERWEY-VAN WISSEN[1]

[1]Department of Clinical Pharmacy, [2]Institute of Anaesthesiology, Academic Hospital Nijmegen Sint Radboud, Geert Grooteplein Zuid 8, 6525 GA Nijmegen, Netherlands

The effect of oral probenecid (1 g) on the pharmacokinetics of frusemide (80 mg p.o.) and its acyl glucuronide was studied in nine healthy subjects. Probenecid significantly increased the $t_{1/2,z}$ of frusemide from 2.01 ± 0.68 to 3.40 ± 1.48 h (P = 0.0015) and significantly decreased oral clearance from 164 ± 67.0 to 58.3 ± 28.1 ml min^{-1} (P = 0.0001). No effect of probenecid on the plasma protein binding of frusemide was detected. Probenecid significantly increased the t_{max} of the metabolite frusemide acyl glucuronide from 1.4 to 2.6 h, but had no effect on the t_{lag}, C_{max}, $t_{1/2,z}$ and plasma protein binding. The urinary recoveries of unchanged frusemide (39.2 ± 10.2 *vs* 34.4 ± 8.6%, P = 0.28) and its acyl glucuronide (12.1 ± 2.7 *vs* 11.8 ± 3.7%, $P > 0.8$) were not altered by probenecid. However, probenecid decreased the renal clearance of both frusemide (128 ± 49 *vs* 44.0 ± 18.6 ml min^{-1}, P = 0.0002) and the acyl glucuronide (552 ± 298 *vs* 158 ± 94.0 ml min^{-1}, $P < 0.0001$). The non-renal clearance of frusemide (36.7 ± 21.0 *vs* 15.2 ± 13.4 ml min^{-1}, P = 0.0068) was also decreased. The clinical relevance of the study relates to the possible conjugation of frusemide in the kidney and the role of the conjugate in the pharmacodynamic effect.

Keywords frusemide pharmacokinetics acyl glucuronide probenecid renal clearance interaction

Introduction

Frusemide is metabolised to an acyl glucuronide (1-*O*-glucuronide) in man [1–3]. Acyl glucuronides are unstable at slightly alkaline pH, undergoing hydrolysis and isomerization via acyl migration [4]. The presence of isoglucuronides (2-, 3-, and 4-*O*-glucuronide) in urine is expected because of isomerization in blood at pH 7.4 [5–7]. To prevent acyl glucuronide degradation in urine *in vivo*, the urine must be kept acidified to pH values of about 5.0 [4, 6–8].

Pharmacodynamic and pharmacokinetic modelling have correlated frusemide kinetics with the kinetics of chloride, sodium and the urine production but have neglected the possible effect of the acyl glucuronide [5, 9, 10].

Several studies have reported that probenecid increases the plasma concentration of frusemide, suggesting inhibiton of its tubular secretion [11–14]. In addition, if glucuronidation of frusemide occurs in the kidney during cellular transport [15, 16], as previously suggested for indomethacin [17, 18], nalidixic acid [19] and probenecid [20], probenecid might inhibit this process.

The aims of this investigation were to study in healthy volunteers a) the effect of probenecid on the glucuronidation of frusemide, and b) the effect of probenecid on the renal excretion of parent drug and its acyl glucuronide. The clinical relevance of the study relates to the possible conjugation of frusemide in the kidney and the role of the conjugate in the pharmacodynamic effect.

Correspondence: Dr T. B. Vree, Department of Clinical Pharmacy, Academic Hospital Nijmegen Sint Radboud, Geert Grooteplein Zuid 8, 6525 GA Nijmegen, Netherlands

Methods

Protocol

Nine subjects (six males, three females 35 ± 6 (s.d.) years, 78 ± 8 (s.d.) kg) participated in the study. In a cross-over design they took 80 mg frusemide orally (Lasix®) after an overnight fast and again 2 weeks later after an overnight fast and 1 h after the oral administration of 1 g probenecid (Benemid®, MSD, Haarlem Netherlands). The study was approved by the hospital ethics committee and the subjects gave their informed consent.

Fingertip blood samples (2 ml), obtained with Monolet® lancets (Monoject, St Louis, USA), were collected in heparinised Eppendorf® vials (2 ml) at various times up to 12 h. After centrifuging, plasma was stored at –20° C pending analysis.

Urine was collected up to 12 h. Urinary pH was kept acid (pH 5.0–5.5) by the oral administration of 1 g ammonium chloride four times daily (Ammonchlor®, Südmedica, Munich, Germany). Four urine samples of 5 ml from each void were stored immediately at –20° C pending analysis.

Each urine void (of ± 300 ml) was followed by ingestion of 100 ml water.

Drug analysis

Frusemide and its acyl glucuronide were assayed by h.p.l.c. as described by Vree *et al.* [21].

The limits of quantitation of frusemide and its acyl glucuronide in plasma were 0.007 μg ml^{-1}, and 0.01 μg ml^{-1} respectively. The limits of quantitation in urine were 0.10 μg ml^{-1} and 0.15 μg ml^{-1} respectively. The intra- and interday coefficients of the assays were <5% [21].

Plasma samples (100 μl) were deproteinized with 100 μl acetonitrile, centrifuged at 3000 *g*, 20 μl of the supernatant was injected immediately onto the column. Frusemide acyl glucuronide is only stable at pH 7.4 for 30 min.

Urine samples were diluted 1:1 with water, and 20 μl was injected into the column.

Plasma binding

The plasma binding of frusemide and and its acyl glucuronide was measured in *ex vivo* samples using the Amicon Micropartition system MPS-1 (Grace BV, Amico Division, Capelle aan de IJssel, Netherlands). The average binding (± s.d.) was calculated from two plasma samples from each volunteer obtained 1–2 h after drug administration. No non-specific drug binding to the filters was observed.

Data analysis

Curve fitting was carried out using the MediWare® computer program [22]. Values of C_{max}, t_{max} and t_{lag} were noted directly from the data. AUC(0, 12h) values were calculated using the linear trapezoidal rule. Oral clearance (CL_o) and mean residence time (MRT) of frusemide were calculated by standard methods [22]. The intrinsic mean residence time (MRT_i) was defined as $MRT_i = MRT_{metabolite}\text{-}MRT_{parent}$. Total renal clearances (CL_R) were calculated by dividing the total urinary recovery by the corresponding AUC(0, 12h). Non-renal clearance (CL_{NR}) was defined as $CL_{NR} = CL_O – CL_R$.

Results

Plasma concentrations and urinary excretion rates of frusemide and its acyl glucuronide in a representative subject with and without probenecid treatment are shown in Figure 1. Table 1 summarises mean pharmacokinetic parameters.

The $t_{1/2,z}$ values of frusemide and its acyl glucuronide were 2.01 ± 0.68 h and 2.69 ± 1.48 h respectively ($P = 0.21$).

Probenecid increased the t_{max} values of frusemide and its acyl glucuronide and the $t_{1/2,z}$ of frusemide; it did not change the $t_{1/2,z}$ of the glucuronide significantly, nor the MRT values of both compounds. The oral clearance of frusemide, the renal clearance and the non-renal clearance of frusemide were decreased by probenecid to approximately 35% of their baseline value, but their urinary recoveries were unaltered. As a result of decreased oral and renal clearance the AUC of frusemide as well as its glucuronide increased three-fold. Probenecid did not influence the plasma binding of either frusemide or its glucuronide.

Discussion

Probenecid decreased the oral clearance and renal clearance of frusemide, consistant with previous reports [11, 12, 14]. Also the calculated non-renal clearance of frusemide was decreased, which may indicate that active secretion into bile, or the rate of hepatic glucuronidation is decreased. All three clearance parameters were reduced to approximately 35% of their baseline value.

Probenecid had no effect on the t_{lag}, C_{max}, and $t_{1/2,z}$ of frusemide acyl glucuronide but decreased the renal clearance of the metabolite to approximately 30% of its baseline value. Therefore tubular secretion of the metabolite is partly inhibited by probenecid, leaving a renal clearance which is still higher than the glomerular filtration rate. The extremely high apparent renal clearance of frusemide acyl glucuronide, which is four times higher than that of the parent drug, suggests that the kidney contributes to the overall glucuronidation of frusemide. The effect of probenecid on the renal clearance of both parent drug and acyl glucuronide is of the same magnitude: it reduces the renal clearance to approximately 35% of its baseline value.

Renal excretion is the main route of elimination of frusemide (40%), with metabolic conjugation accounting for 12%. Thus 50% of the oral dose is unaccounted for.

Impaired kidney function will reduce the renal se-

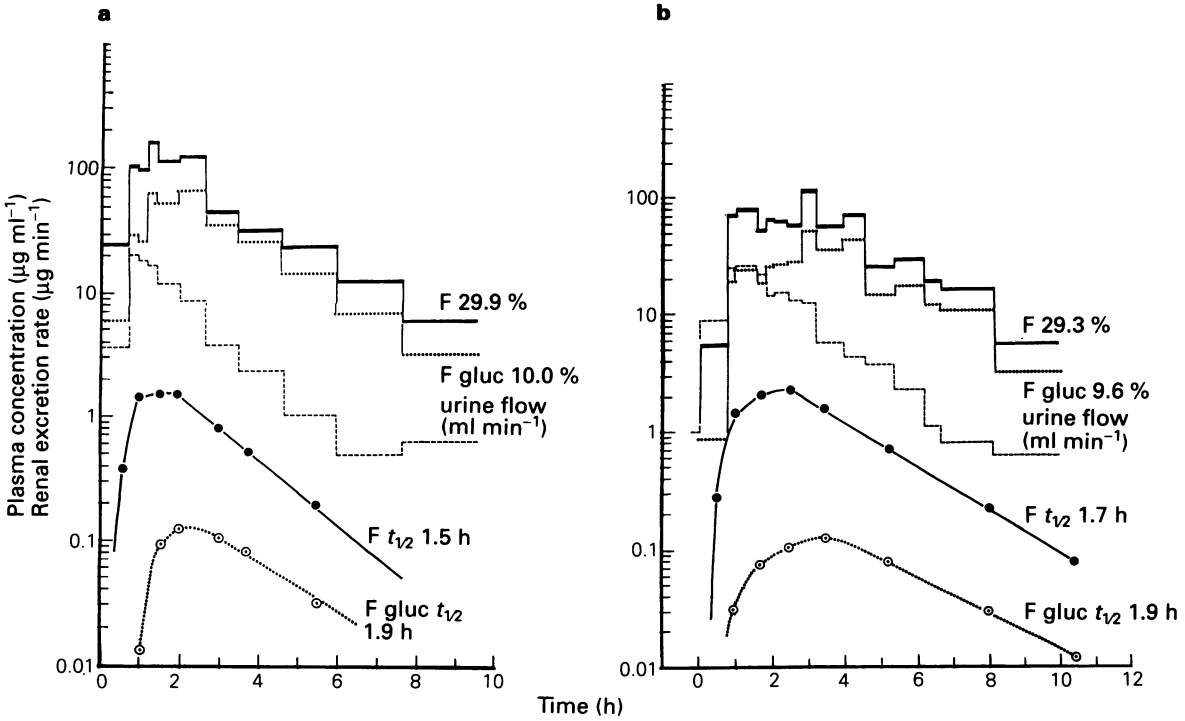

Figure 1 Plasma concentrations and urinary excretion rates of frusemide (F), frusemide acyl glucuronide (Fgluc) in a representative subject after an oral dose of 80 mg frusemide without (a) and with (b) probenecid (1 g).

Table 1 Pharmacokinetic parameters of frusemide (80 mg) with and without probenecid (1g)

Parameter	*Mean + s.d. without*	*with probenecid*	*95% confidence interval of difference*	P
Frusemide				
t_{lag} (h)	0.26 ± 0.21	0.51 ± 0.58	−0.76–0.27	0.07
t_{max} (h)	0.79 (0.6–1.37)	1.93 (0.16–2.46)	−1.30– −0.07	0.0273
C_{max} (μg ml^{-1})	1.79 ± 0.65	2.77 ± 1.71	−2.30–0.36	0.0284
$t_{½,z}$ (h)	2.01 ± 0.68	3.40 ± 1.48	−2.70– −0.14	0.0015
MRT (h)	2.67 ± 0.46	4.41 ± 1.14	−2.64– −0.84	0.0258
AUC(0,12h) (mg l^{-1} h)	4.46 ± 1.13	11.95 ± 4.58	−10.4– −4.34	<0.0001
CL_O (ml min^{-1})	164.0 ± 67.0	58.3 ± 28.1	63.9–149.0	0.0001
CL_R (ml min^{-1})	128.0 ± 49.1	44.0 ± 18.6	53.6–83.7	0.0002
CL_{NR} (ml min^{-1})	36.7 ± 21.0	15.2 ± 13.4	8.66–34.4	0.0068
Urinary recovery (% μmol dose)	39.3 ± 10.2	34.4 ± 8.6	0.39–9.48	0.28
Plasma binding (%)	97.8 ± 1.9	98.8 ± 0.7	−3.30–8.25	0.30
Frusemide acylglucuronide				
t_{lag} (h)	0.30 ± 0.17	0.53 ± 0.60	−0.77–0.31	0.10
t_{max} (h)	1.43 (0.78–2.59)	2.59 (0.50–2.59)	−2.42– −0.06	0.0391
C_{max} (μg ml^{-1})	0.09 ± 0.06	0.18 ± 0.12	−0.15–0.03	0.07
$t_{½,z}$ (h)	2.69 ± 1.48	2.82 ± 0.91	−1.25–1.07	0.77
MRT (h)	3.82 ± 1.32	5.75 ± 1.98	−3.08– −0.79	0.0196
MRT_i (h)	1.54 ± 1.31	2.17 ± 0.96	−1.77–0.71	0.52
AUC(0,12h) (mg l^{-1} h)	0.43 ± 0.35	1.33 ± 0.85	−1.51– −0.29	0.0097
CL_R (ml min^{-1})	552.0 ± 298	158.0± 94.0	197.0–591	<0.0001
Urinary recovery (% μmol dose)	12.1 ± 2.7	11.8 ± 3.7	−18.6–2.51	>0.8
Plasma binding (%)	95.5 ± 1.8	94.2 ± 5.7	−10.2–40.1	0.58

Values are expressed as mean ± s.d except t_{max} (median and range (n = 9).

cretion of both parent drug and conjugate. Reduction of kidney function by probenecid did not affect the percentage of the dose recovered as frusemide glucuronide; however, it slowed the elimination processes. Impaired kidney function (for example in older patients) will therefore not alter the overall yield (%) of glucuronidation. It is not yet certain whether frusemide alone, or its acyl glucuronide or both are active diuretics. To solve this question both parent drug and conjugate will need to be included in pharmacodynamic-pharmacokinetic modelling of frusemide.

References

1 Beerman B, Dalén E, Lindström B, Rosén A. (1975). On the fate of furosemide in man. *Eur J clin Pharmac* 1975; **9**: 51–61.

2 Benet LZ. Pharmacokinetics/pharmacodynamics of furosemide in man, a review. *J Pharmacokin Biopharm* 1979; **7**: 1–27.

3 Hammarlund-Udenaes M, Benet LZ. Furosemide pharmacokinetics and pharmacodynamics in health and disease, an update. *J Pharmacokin Biopharm* 1989; **17**: 1–46.

4 Faed EM. Properties of acyl glucuronides: implications for studies of the pharmacokinetics and metabolism of acidic drugs. *Drug Metab Rev* 1984; **15**: 1213–1249.

5 Benet LZ, Smith DE, Lin ET, Vincenti F, Gambertoglio JG. Furosemide assays and disposition in healthy volunteers and renal transplant patients. *Fed Proc* 1983; **42**: 1695–1698.

6 Vree TB, Beneken Kolmer EWJ. Direct measurement of probenecid and its glucuronide conjugate by means of high performance liquid chromatography in plasma and urine of humans. *Pharm Weekbl [Sci]*, 1992; **14**: 83–87.

7 Vree TB, Biggelaar-Martea M van den, Verwey-van Wissen CPWGM, Vree ML, Guelen PJM. Pharmacokinetics of naproxen, its metabolite *O*-desmethylnaproxen and their acyl glucuronides in humans. Effect of cimetidine. *Br J clin Pharmac* 1993; **35**: 467–472.

8 Rachmel A, Hazelton GA, Yergey AL, Liberato DJ. Furosemide 1-O-acyl glucuronide. *In vitro* biosynthesis and pH-dependent isomerization to β-glucuronidase-resistant forms. *Drug Metab Dispos* 1985; **13**: 705–710.

9 Andreasen F, Mikkelsen E. Distribution, elimination and effect of furosemide in normal subjects and in patients with heart failure. *Eur J clin Pharmac* 1977; **12**: 15–22.

10 Vree TB, Kleijn E van der, Gusdorf ChF, Zum Vörde Sive Vörding JGM. Biologische beschikbaarheid van twee furosemide preparaten (Biovailability of two furosemide preparations). *Pharm Weekbl* 1983; **118**: 121–123.

11 Brater DC. Effects of probenecid on furosemide response. *Clin Pharmac Ther* 1978; **24**: 548–554.

12 Chennavasin P, Seiwell R, Brater DC, Liang WMM. Pharmacodynamic analysis of the furosemide-probenecid interaction in man. *Kidney Int* 1979; **16**: 187–195.

13 Ponto LLB, Schoenwald RD. Furosemide. A pharmacokinetic/pharmacodynamic review. *Clin Pharmacokin* 1990; **18**: 381–408.

14 Smith DE, Gee WL, Brater DC, Lin ET, Benet LZ. Preliminary evaluation of furosemide-probenecid interaction in humans. *J pharm Sci* 1980; **69**: 571–575.

15 Smith DE, Lin ET, Benet LZ. Absorption and disposition of furosemide in healthy volunteers measured with a metabolite specific assay. *Drug Metab Dispos* 1980; **8**: 337–342.

16 Smith DE, Benet LZ. Biotransformation of furosemide in kidney transplant patients. *Eur J clin Pharmac* 1983; **24**: 787–790.

17 Chennavasin P, Seiwell R, Brater DC. Pharmacokinetic-dynamic analysis of the indomethacin-furosemide interaction in man. *J Pharmac Exp Ther* 1980; **215**: 77–81.

18 Vree TB, Biggelaar-Martea M van den, Vewey-van Wissen CPWGM, Ewijk-Beneken Kolmer EWJ van. Probenecid inhibits the glucuronidation of indomethacin and O-desmethylindomethacin in humans. *Pharm World Sci* 1994; **16**: 22–26.

19 Vree TB, Biggelaar-Martea M van den, Beneken Kolmer EWJ, Hekster YA. Probenecid inhibits the renal clearance and renal glucuronidation of nalidixic acid. *Pharm Weekbl [Sci]* 1993; **15**: 165–170.

20 Vree TB, Beneken Kolmer EWJ, Wuis EW, Hekster YA. Capacity limited renal glucuronidation of probenecid by humans. A pilot Vmax finding study. *Pharm Weekbl [Sci]* 1992; **14**: 325–331.

21 Vree TB, Biggelaar-Martea M van den, Verwey-van Wissen CPWGM. Determination of furosemide with its acyl glucuronide in human plasma and urine by means of direct gradient high performance liquid chromatographic analysis with fluorescence detection. Preliminary pharmacokinetics and effect of probenecid. *J Chromatogr* 1994; **655**: 53–62.

22 Proost JH, Meijer DKF. MW/Pharm, an integrated software package for drug dosage regimen calculation and therapeutic drug monitoring. *Comput Biol Med* 1992; **22**: 155–163.

(Received 5 October 1994, accepted 8 February 1995)

