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Direct-gradient high-performance liquid chromatographic analysis and preliminary pharmacokinetics of flumequine and flumequine acyl glucuronide in humans: effect of probenecid

T. B. Vree

Department of Clinical Pharmacy and Institute of Anaesthesiology, Sint Radboud Hospital, University of Nijmegen, Geert Grooteplein Zuid 8, 6525 GA Nijmegen (Netherlands)

E. W. J. van Ewijk-Beneken Kolmer

Department of Clinical Pharmacy, Sint Radboud Hospital, University of Nijmegen, Geert Grooteplein Zuid 8, 6525 GA Nijmegen (Netherlands)

J. F. M. Nouws

Farma Research, Snelliusstraat 6, 6535 NV Nijmegen (Netherlands)

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ABSTRACT

A gradient high-performance liquid chromatographic analysis for the direct measurement of flumequine, with its acyl glucuronide, in plasma and urine of humans has been developed. In order to prevent hydrolysis and isomerization of flumequine acyl glucuronide, the samples were acidified by the oral intake of four 1.2-g amounts of ammonium chloride per day. In contrast to the acyl glucuronides of non-steroidal anti-inflammatory drugs, flumequine and its acyl glucuronide were stable in urine of pH 5.0–8.0. Flumequine acyl glucuronide is unstable at pH 1.5. In acidic urine (pH 5–6), almost no flumequine is excreted unchanged (1%): it is excreted chiefly as acyl glucuronide (84.2%). Probenecid co-medication reduces the renal excretion rate of flumequine acyl glucuronide from 662 to 447 μg/min ($p = 0.00080$), but not the percentage of glucuronidation.

INTRODUCTION

Flumequine (racemic 9-fluoro-6,7-dihydro-5-methyl-1-oxo-1*H*,5*H*-benzo[*ij*]quinolizine-2-carboxylic acid) may be considered as a hybrid of nalidixic acid and the racemic quinolone ofloxacin (Fig. 1). It is used as an antimicrobial drug in veterinary medicine [1–7]. Nalidixic acid is considered to be the first clinically applied quinolone derivative. Because of its role in antimicrobial therapy, the group of the quinolones is at present a centre of attention.

The kinetics of quinolones are investigated with high-performance liquid chromatographic (HPLC) methodology, but this is of limited use for the pre-quinolone nalidixic acid [8–13]. Flu-

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

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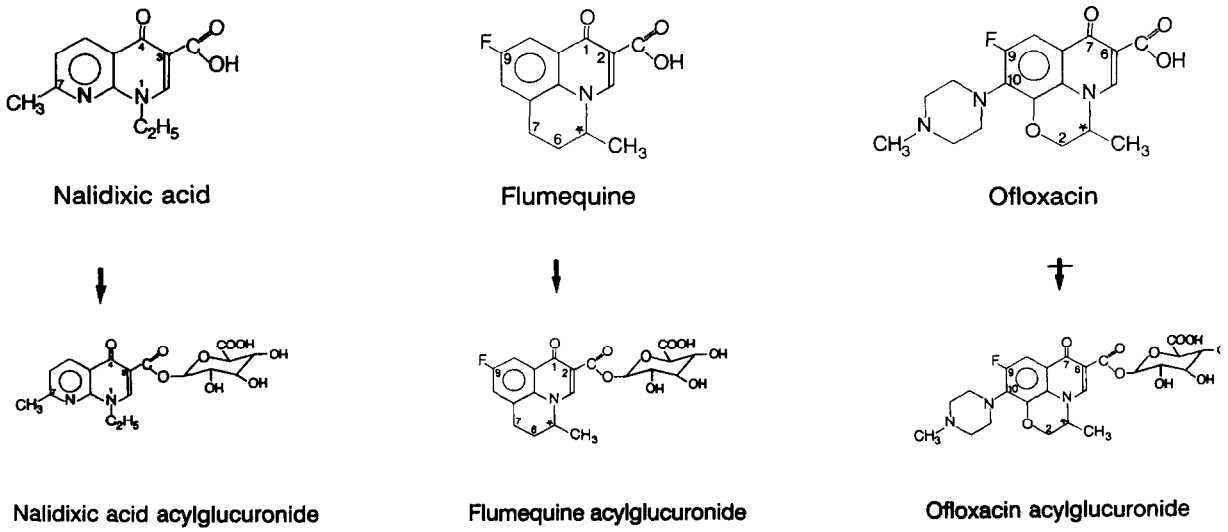

Fig. 1. Structures of flumequine, nalidixic acid, ofloxacin and their corresponding glucuronide conjugates.

mequine is analysed by either a microbiological assay [7] or by HPLC, the glucuronide being measured after enzymic hydrolysis [2,3,14–17].

When [^{14}C]flumequine was administered orally to humans, 75% of the radiolabel was excreted in the urine and 10% in the faeces [18]. It was reported that flumequine was oxidized at the 7 position to 7-hydroxyflumequine and conjugated with glucuronic acid to form an acyl glucuronide [16,17,19]; these reactions may depend on the species [1,3,18]. The glucuronide conjugates of flumequine and the quinolones are acyl glucuronides, which analogously to acyl glucuronides of non-steroidal anti-inflammatory drugs (NSAIDs), may be unstable at alkaline urine pH values [20–22].

Moreover, flumequine acyl glucuronide may be formed, like nalidixic acid acyl glucuronide and probenecid acyl glucuronide, in the renal tubules because the latter two glucuronides were not detectable in plasma [23,24]. When this is the case, competition between probenecid and flumequine for glucuronidation may be expected.

The aims of this investigation were: (a) to develop a direct HPLC procedure for racemic flumequine and its possible glucuronide conjugate; (b) to investigate the pharmacokinetics of flumequine in a pilot experiment in a human volunteer; and (c) to investigate in a pilot experiment a possible inhibitory effect of probenecid on the glucuronidation of flumequine.

EXPERIMENTAL

Drugs

(±)-Flumequine and (±)-7-hydroxyflumequine were obtained from Riker/3M (Zoeterwoude, Netherlands). Probenecid (Benemid) was obtained from the hospital pharmacy and Merck, Sharp and Dohme (Haarlem, Netherlands). Ammonium chloride (Ammonchlor) was obtained from Südmedica (Munich, Germany). Flumequine acyl glucuronide was detected in human urine.

Chemicals

The following chemicals were used: HPLC-grade acetonitrile (Betron, Rotterdam, Netherlands), orthophosphoric acid, 99% (Merck, Darmstadt, Germany), diethylamine and dimethylformamide (Merck-Schuchardt, Darmstadt, Germany), all analytical-grade.

Gradient HPLC analysis

The HPLC system consisted of a Spectra Physics SP 8775 autosampler (Spectra Physics, Eind-

hoven, Netherlands), an SP 8800 ternary HPLC pump, a Kratos Spectroflow 783 UV detector (Separations, Hendrik Ido Ambacht, Netherlands) and an SP 4290 integrator. The analytical column (25 cm × 4.6 mm I.D.) was packed with Cp-Spher-C8, particle size 8 μm (Cat. No. 28502, Chrompack, Middelburg, Netherlands), with a guard column (7.5 cm × 2.1 mm I.D.) packed with 10-μm pellicular reversed-phase (Chrompack, Cat. No. 028640). A 20-μl injection loop was used.

The mobile phase was a mixture of dimethylformamide (solvent A), acetonitrile (solvent B) and orthophosphoric acid (solvent C; 6 g of H_3PO_4 and 2 ml of diethylamine in 1000 ml of distilled water). At $t = 0$ the mixture consisted of 15% A, 15% B and 70% C, and this was changed linearly in 7.5 min to 22.5% A, 60% B, and 17.5% C. At $t = 7.5$ min, the system was changed to its initial state in 1.5 min and was left for 1 min to equilibrate (10 min total time). The flow-rate was 2.0 ml/min. Flumequine and its acyl glucuronide were detected at 320 nm. The assay was carried out at room temperature. The retention times and capacity factors are given in Table I.

Sample preparation

To 0.1 ml of plasma, 0.1 ml of acetonitrile was added. The solution was mixed thoroughly and centrifuged for 5 min at 2600 *g*.

To 100 μl of urine, 0.9 ml of 0.2 *M* KH_2PO_4 buffer (pH 5.0) was added, and the solution was mixed.

TABLE I

RETENTION TIMES AND CAPACITY FACTORS OF FLUMEQUINE AND FLUMEQUINE ACYL GLUCURONIDE

Compound	t_R (min)	k'
t_0	1.1	
Flumequine acyl glucuronide	3.31	2.0
Flumequine	6.21	4.6

Calibration curves

Nine urine samples from one subject (experiment 1) containing the full range of different concentrations of flumequine acyl glucuronide were selected by peak area, because the peak area of flumequine acyl glucuronide correlated well with the increase of the concentration of flumequine after deconjugation. The samples were deconjugated by system D (see *Deconjugation* below). The increase in the concentration of flumequine represented the concentration of the conjugate. A calibration curve was constructed with the help of the following formula:

$$[\text{Fgluc}] = d[\text{F}] \times M_{\text{Fgluc}}/M_{\text{F}}$$

where $d[\text{F}]$ is the difference in concentration of flumequine before and after deconjugation and M is relative molecular mass ($r = 0.9997$; Tables II and III). Calibration curves for flumequine were made by adding known amounts of flumequine to blank human plasma and urine (correlation coefficients of greater than 0.999).

Stability

The stability of flumequine acyl glucuronide in urine was tested as follows. Four samples of 2 ml of urine were brought to pH 1.5, 5.0, 6.0 and 8.0 and incubated at 37°C for 6 h. At regular time intervals, a 100-μl sample was taken and diluted with 0.9 ml of 0.2 *M* KH_2PO_4 buffer (pH 5.0), and 20 μl were injected onto the column.

The stability of flumequine acyl glucuronide in plasma was tested as follows. A sample of 2 ml of plasma (pH 7.4) (1.9 ml of plasma spiked with 100 μl of urine containing flumequine acyl glucuronide) was incubated at 37°C for 6 h. At regular time intervals a 100-μl sample was taken and the reaction was stopped by deproteinizing with 100 μl of acetonitrile. The reaction was repeated at room temperature (21°C) and at 4°C.

Limits of quantitation

Quantitation limits for flumequine and flumequine acyl glucuronide were 50 ng/ml in plasma and 1 μg/ml in urine, at a signal-to-noise (S/N) ratio of 3.

TABLE II

CALIBRATION CURVE OF FLUMEQUINE ACYL GLUCURONIDE IN PLASMA

The calibration curve of flumequine acyl glucuronide in plasma is: peak area = 2285 (concentration) − 143 ($0.32 < x < 3.60$ μg/ml) with $r = 0.9969$. The calibration curve of flumequine in plasma is: peak area = 2944 (concentration) − 2069 ($0.0 < x < 46.70$ μg/ml) with $r = 0.9994$.

Plasma sample	Before deglucuronidation		After deglucuronidation[a]		
	Area Fgluc (IG)[b]	[F] (μg/ml)	[F] (μg/ml)	δ[F] (μg/ml)	[Fgluc] (μg/ml)
Blank	–	–	–	–	–
3	1003	0.27	0.64	0.37	0.62
6	5599	0.24	1.92	1.68	2.81
8	13 638	–	3.59	3.59	6.02
9	7677	–	1.91	1.91	3.19
16	3317	–	0.84	1.41	245
22	1254	–	0.32	0.32	0.54

[a] After deglucuronidation no Fgluc was detectable.
[b] IG = integration units.

Deconjugation

Deglucuronidation was carried out with 100 μl of urine, 100 μl of β-glucuronidase and 800 μl of 0.2 *M* $(Na_2H–KH_2)PO_4$ buffer at 37°C for 2 h.

Four different glucuronidase enzymes (A–D) were used: (A) 100 000 U/ml β-glucuronidase type B1 (bovine liver, Sigma, St. Louis, MO, USA, Cat. No. G-0251) and phosphate buffer (pH 5.0); (B) 107 200 U/ml β-glucuronidase type H2 (*Helix pomatia*, Sigma, Cat. No. G-0876) and

TABLE III

CALIBRATION CURVE OF FLUMEQUINE ACYL GLUCURONIDE IN URINE

The calibration curve of flumequine acyl glucuronide in urine is: peak area = 579.3 (concentration) + 2059 ($24.8 < x < 466$ μg/ml) with $r = 0.9997$. The calibration curve of flumequine in urine is: peak area = 810 (concentration) − 731 ($11.9 < x < 95.2$ μg/ml) with $r = 0.9990$.

Urine sample	Before deglucuronidation		After deglucuronidation[a]		
	Area Fgluc (IG)[b]	[F] (μg/ml)	[F] (μg/ml)	δ[F] (μg/ml)	[Fgluc] (μg/ml)
Blank	–	–	–	–	–
4	192 091	1.68	194.96	193.28	323.48
5	278 161	2.53	281.20	278.67	466.40
6	226 658	2.73	224.53	221.80	371.22
7	90 378	1.17	86.52	85.35	142.85
8	150 396	2.95	149.39	146.39	245.01
9	67 674	1.02	63.76	62.74	105.01
11	33 699	0.83	33.70	32.87	55.01
15	14 989	–	14.79	14.79	24.75

[a] After deglucuronidation no Fgluc was detectable.
[b] IG = integration units.

phosphate buffer (pH 5.0); (C) 100 000 U/ml β-glucuronidase type LII (lyophilized powder from limpets *Patella vulgata*, Sigma, Cat. No. G-8132) and phosphate buffer (pH 3.8); (D) 20 000 U/ml β-glucuronidase type VIIA (*Escherichia coli*, Sigma, Cat. No. G-7646) and phosphate buffer (pH 6.8).

Sample deglucuronidation for peak identification

To 100 μl of plasma, 10 μl of a β-glucuronidase solution (system D, *E. coli*) were added. The sample was mixed thoroughly, 100 μl of acetonitrile were added, and the solution was centrifuged at 2600 *g*.

To 100 μl of urine, 100 μl of the β-glucuronidase solution (system D) and 300 μl of phosphate buffer (pH 6.8) were added. The solution was mixed and centrifuged.

Subject

One Caucasian male with normal liver and kidney function (82 kg, 45 years) volunteered for this study. The study had the approval of the Sint Radboud Hospital Ethics Committee. A single oral dose of 256 mg of flumequine was administered in a gelatine capsule after an overnight fast.

One month later, 1 g of probenecid (Benemid) was administered after an overnight fast, followed 1 h later by 254 mg of flumequine.

One month later, 1 g of probenecid (Benemid) was administered after an overnight fast, followed by two 500-mg doses at intervals of 10 h. Flumequine (256 mg) was administered 1 h after the first probenecid dose. In all experiments the urine was kept acidic by the administration four times a day of 1.2 g of ammonium chloride (Ammonchlor, Südmedica).

Sampling procedures

At regular intervals, 2-ml blood samples were collected by means of fingertip puncture with Monolet lancets (Monoject, St. Louis, MO, USA) in 2-ml Eppendorf tubes containing a few crystals of solid heparin. After centrifugation, plasma samples were stored at −20°C pending analysis.

Urine was collected upon spontaneous voiding. The total time of sample collection was 72 h (seven times the expected $T_{1/2}$). The urine volume and pH were measured immediately after collection. Of each urine void, five samples of 5 ml were stored at −20°C pending analysis.

Probenecid analysis

Probenecid and probenecid acyl glucuronide were measured by direct HPLC analysis as described earlier [23].

Renal clearance

The apparent renal clearance values (Cl_r) of flumequine and its acyl glucuronide were calculated from the amount (mg) excreted divided by the plasma AUC_∞ (area under the curve) of drug or metabolite, and from the average renal excretion rate in each urine sample divided by the extrapolated plasma concentration at the midpoint of the measured time interval.

Pharmacokinetic analysis

Regression lines, standard deviations and Student's *t*-test were calculated according to standard statistical procedures [26]. The AUC and AUMC were calculated with the trapezoidal rule with extrapolation to infinity. Cl = dose × AUC^{-1} (F = 1); MRT = AUMC × AUC^{-1} (assuming the $MRT_{absorption} \ll MRT_{elimination}$). The intrinsic mean residence time (MRT_{int}) of flumequine acyl glucuronide was calculated as $MRT_{int} = MRT_{metabolite} - MRT_{parent}$ according to Veng-Pedersen [25]. V_{ss} = dose × AUMC × AUC^{-2}.

Statistics

The Student's *t*-test was performed according to standard procedures [26].

RESULTS

HPLC analysis

Fig. 2 shows HPLC chromatograms of flumequine and its acyl glucuronide in a plasma and in a urine sample. No 7-hydroxyflumequine could be detected (retention time 4 min) in either plasma or urine.

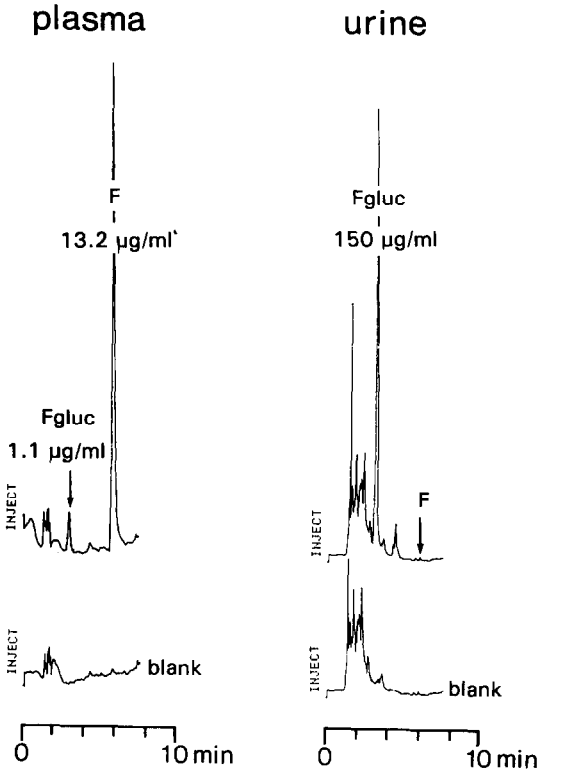

Fig. 2. Chromatograms of a plasma sample and a urine sample from a volunteer after oral administration of 256 mg of flumequine. Peaks: F = flumequine; Fgluc = flumequine acyl glucuronide.

Flumequine acyl glucuronide was moderately stable in plasma at a pH 7.4 and 37°C ($T_{1/2}$ 24 h), and stable when the temperature was kept lower at 4 and 21°C, as shown in Fig. 3. Fig. 4 shows the stability of the acyl glucuronide in urine at various pH values: it was stable at pH 5.0, 6.0 and 8.0, but unstable at pH 1.5.

Glucuronidase hydrolysis of the acyl glucuronides for all four glucuronidase systems tested was almost instantaneous just after mixing of the components. System D, with type VIIA *E. coli*, was preferred because of the cleaner chromatograms. The intra-day and inter-day coefficient of variation (C.V.) of flumequine and flumequine acyl glucuronide are shown in Table IV.

Pharmacokinetics of flumequine

Fig. 5 shows the plasma concentration–time curves and the renal excretion rate–time profiles

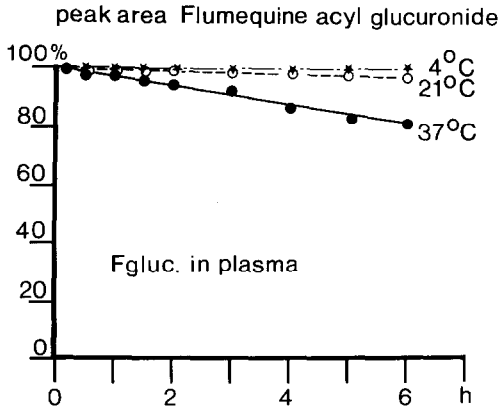

Fig. 3. Stability of flumequine acyl glucuronide in plasma at pH 7.4 and at different temperatures. Starting concentration, 10 μg/ml.

of (±)-flumequine (F), with its acyl glucuronide (Fgluc), in the volunteer after an oral dose of 256 mg of flumequine. Two phases can be distinguished in the plasma elimination curve, characterized by half-lifes of 3.5 and 8.0 h, respectively. The acyl glucuronide metabolite showed a half-life of 3.5 h, which is similar to the initial half-life of flumequine.

The renal excretion rate time profiles of the parent drug and its metabolites show a half-life of 3.5 and 8.0 h. Approximately 84% of the administered dose is excreted in the urine, predominantly as the acyl glucuronide. Only a small fraction of the dose (1%) is excreted unconjugated. Table V summarizes some pharmacokinetic pa-

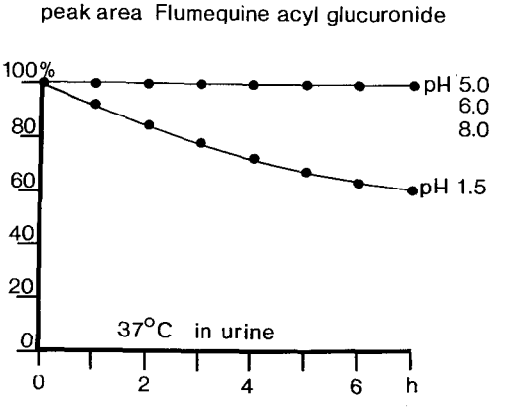

Fig. 4. Stability of flumequine acyl glucuronide in urine at different pH values at 37°C. Starting concentration, 371 μg/ml.

TABLE IV

INTRA- AND INTER-DAY COEFFICIENTS OF VARIATION OF FLUMEQUINE AND FLUMEQUINE ACYL GLUCURONIDE IN PLASMA AND URINE

Concentration (μg/ml)	Coefficient of variation (n = 4) (%)	
	Intra-day	Inter-day
Plasma		
Flumequine		
11.42	3.4	9.2
5.71	2.6	6.6
1.14	1.0	6.0
Flumequine acyl glucuronide		
3.8	1.3	2.2
1.7	1.4	5.0
0.9	0.54	1.2
Urine		
Flumequine acyl glucuronide		
114	2.3	1.2
52.9	1.8	1.4
28.6	2.5	3.5

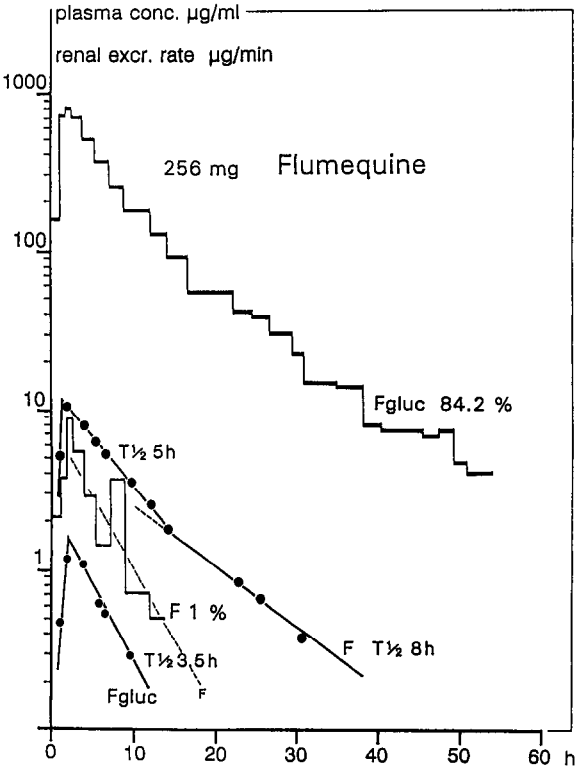

Fig. 5. Plasma concentration–time curves and renal excretion rate–time profiles of flumequine (F) and its glucuronide (Fgluc) in a volunteer after an oral dose of 256 mg of flumequine. The dashed line (F) is obtained after line feathering and represents the first phase of the elimination of flumequine. This phase has the same $T_{1/2}$ of 3.5 h as that of flumequine acyl glucuronide in plasma.

rameters of flumequine and its metabolite in the volunteer.

Effect of probenecid

Fig. 6 shows the pharmacokinetic profile of flumequine with its conjugate under probenecid co-medication. Inhibition of renal clearance and renal glucuronidation did not occur during the period (15 h) of constant and maximal renal excretion of probenecid acyl glucuronide. Probenecid eliminates the first phase in the elimination of flumequine, which was characterized by the $T_{1/2}$ of 3.5 h. Table IV shows the pharmacokinetic parameters of flumequine with and without probenecid co-medication. The renal excretion patterns of flumequine and flumequine acyl glucuronide were lower than without probenecid. Probenecid reduces the total body clearance of flumequine acyl glucuronide from 926 to 558 ml/min, and the renal clearance from 642 ± 95 to 447 ± 93 ml/min (p = 0.00080). The recovery of flumequine acyl glucuronide was unchanged.

The second experiment with probenecid co-medication resulted in the continuous presence of probenecid acyl glucuronide in the kidney for 40 h, and in a constant renal excretion rate of 600–700 μg/min. In this period, flumequine was metabolized and excreted. Probenecid had no effect on the total amount or percentage of the drug glucuronidated (Table V). The renal clearance of flumequine was similar to that obtained after one dose of probenecid (p = 0.45960) and smaller than that obtained without probenecid (p = 0.00070).

DISCUSSION

HPLC analysis

The aims of the study were to develop a direct

TABLE V

SOME PHARMACOKINETIC PARAMETERS OF FLUMEQUINE WITH AND WITHOUT PROBENECID CO-MEDICATION

The urine pH was in the range 5.0–5.5. C_{max} = maximum plasma concentration; T_{max} = time to reach C_{max}; $T_{1/2}$ = half-life; MRT = mean residence time; AUC_{∞} = area under the plasma concentration–time curve extrapolated to infinity; AUMC = area under the AUC moment curve; Cl = body clearance; Cl_r = renal clearance; V_{ss} = steady state volume of distribution; F = relative bioavailability.

Parameter	Without probenecid	With 1 g of probenecid	With 1 + 0.5 + 0.5 g of probenecid
Dose (mg)	256	254	256
Body weight (kg)	82	82	82
Flumequine			
C_{max} (μg ml^{-1})	10.6	13.2	10.7
T_{max} (h)	1.6	1.0	1.0
$T_{1/2}$ (h)	3.5 + 8.0	8.0	8.0
MRT (h)	10.7	10.0	11.6
AUC_{∞} (mg l^{-1} h)	99.3	113.3	105.2
AUMC (mg l^{-1} h^2)	1064	1134	1220
Cl (ml min^{-1})	43.0	37.4	40.4
Cl_r (ml min^{-1})	0.42	–	–
V_{ss} (l)	27.6	22.4	28.0
Flumequine acyl glucuronide			
F	1.0	1.0	1.0
C_{max} (μg ml^{-1})	1.2	1.4	1.4
T_{max} (h)	1.6	3.0	4.0
$T_{1/2}$ (h)	3.5	4.0	5.0
MRT (h)	6.0	7.3	8.6
MRT_{int} (h)	4.7	2.7	3.0
AUC_{∞} (mg l^{-1} h)	7.70	12.68	14.10
AUMC (mg l^{-1} h^2)	46.6	92.1	121
Cl (ml min^{-1})	926	558	504
Cl_r (ml min^{-1})	778	477	428
V_{ss} (l)	337	243	258
Percentage of the dose excreted			
Flumequine	1.0	–	–
Flumeqine acyl glucuronide	84.2	90.9	85.1
Renal clearance (ml min^{-1})			
Flumequine	0.58 ± 0.37	–	–
Flumequine acyl glucuronide	662. ± 95	447. ± 93	442. ± 74

HPLC method for the measurement of flumequine and its acyl glucuronide, to demonstrate that the method was able to measure plasma and urine samples of a volunteer, and to obtain preliminary pharmacokinetic data for the parent drug and its metabolite. However, meaningful kinetics will be obtained when the enantiomers of flumequine and its acyl glucuronide are quantitated, like for ofloxacin [27–29].

Stability

Flumequine acyl glucuronide is an acyl glucuronide, which may form unstable glucuronides, like those of the NSAIDs [20–24]. The instability

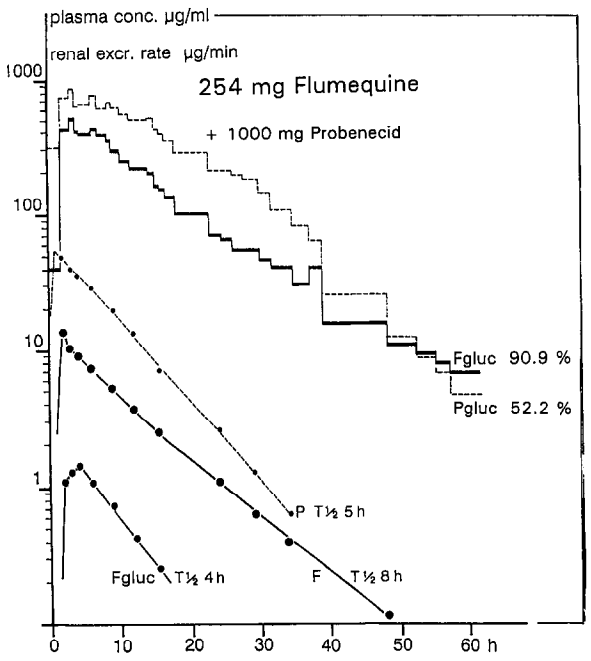

Fig. 6. Plasma concentration–time curves and renal excretion rate–time profiles of flumequine (F) and flumequine acyl glucuronide (Fgluc) in a volunteer after an oral dose of 1 g of probenecid followed 1 h later by 254 mg of flumequine. The plasma concentration of probenecid (P) and the renal excretion rate of probenecid acyl glucuronide (Pgluc) are shown (dashed lines).

of these glucuronides can be recognized easily when urine containing these glucuronides is kept at room temperature and at slightly alkaline pH. Alkaline acyl glucuronide hydrolysis results in an increase of free aglycone or in glucuronide isomers that are not substrates for β-glucuronidase. Flumequine acyl glucuronide was found to be stable over the pH range 5.0–8.0, but not at pH 1.5. If isomerization occurs, hydrolysis is also present and the presence of flumequine would have been noticed. If the gradient had been too fast for isomerization to be detected, then hydrolysis to flumequine would have indicated that the acyl glucuronide was unstable. In this respect, it clearly behaves different from the NSAIDs and from its structural analogue nalidixic acid. Apparently the extra ring system, making a fused three-ring system, increases the stability of the acyl glucuronide.

Metabolism

Flumequine may be metabolized by phase I hydroxylation to 7-hydroflumequine and by phase II conjugation to its acyl glucuronide [15–19]. This study shows that the glucuronide conjugate is the main metabolite in humans and that less than 1% of flumequine itself is excreted unchanged under acidic urine conditions. No 7-hydroxyflumequine was present in measurable concentrations (< 1 µg/ml, S/N = 3). This observation differs from those reported by Schuppan *et al.* [19] and Decolin *et al.* [15]. In those reports, 7-hydroxyflumequine was detected as the main metabolite, but no flumequine acyl glucuronide.

The plasma concentration–time curve of flumequine shows two phases, characterized by half-lives of 3.5 and 8.0 h. The renal excretion rate–time profile shows a gradual change in elimination phase with half-lives of 3.5 and 8.0 h. These half-lives are longer than those of nalidixic acid (1.5 and 3.5 h) [13]. The second phase in the elimination may be the result of an enterohepatic loop of flumequine acyl glucuronide, which may be excreted by the bile and hydrolysed in the intestines. Hydrolysis of this glucuronide in the intestines must therefore be slower than that of nalidixic acid acyl glucuronide (final $T_{1/2}$ = 3.5 h).

The plasma concentration–time curve of flumequine acyl glucuronide shows one phase ($T_{1/2}$ = 3.5 h). A second phase may be observed when the dose is higher (400 mg) or with lower limits of quantitation. The acyl glucuronide is moderately stable and present in blood of pH 7.4, and can be excreted by the kidney by glomerular filtration and active tubular secretion. When probenecid is co-administered, this short $T_{1/2}$ of 3.5 h of flumequine acyl glucuronide is similar (4 h).

The extra ring in flumequine compared with nalidixic acid has no influence on the conjugation to form the acyl glucuronide. Oxidation of flumequine at the 7-position is minimal, whereas nalidixic acid is 50% oxidized at its 7-methyl group. The 7-hydroxyflumequine metabolite has been reported as present in animals, but in very low concentrations and yields [1–3,18,19]. The structural analogue ofloxacin is glucuronidated to a negligle extent (1–2%) [29].

Renal excretion

The renal excretion of flumequine itself is negligible (1.0%) and its renal clearance is low (0.5 ml/min). Between 80 and 90% of a dose of flumequine is glucuronidated.

Renal glucuronidation?

When the renal clearance of the acyl glucuronide could not be measured because they were not present in the plasma, it may be high, owing to glomerular filtration and active tubular secretion, or the glucuronides are synthesized in the renal tubule, analogously to probenecid [24], nalidixic acid [13,30] and the N_1-glucuronides of the methoxysulphonamides sulphadimethoxine [31,32], sulpha-6-monomethoxine [33], sulphamethomidine [34] and sulphaphenazole [35].

Probenecid reduces the glucuronidation of nalidixic acid from 53 to 15% of the dose [36]. This can be understood when both compounds are conjugated by the same glucuronyltransferase, with probenecid having the higher affinity. Probenecid reduces the renal clearance of flumequine acyl glucuronide from 778 to 477 ml/min but, in the pilot experiment, it had effect no clear-cut effect on the glucuronidation of flumequine. The remaining renal clearance of flumequine acyl glucuronide was well above the glomerular filtration rate of 125 ml/min. This implies that renal glucuronidation is still possible, and that the renal clearance values obtained are apparently too high. It is most likely that flumequine is glucuronidated predominantly in the liver and partly by the kidney, whereas nalidixic acid and probenecid are predominantly renally glucuronidated. In calves, probenecid reduces the plasma elimination of flumequine and increases the $T_{1/2}$ twofold ($T_{1/2}$ = 145–>240 min) [7].

CONCLUSION

This HPLC method enables the analysis of flumequine and flumequine acyl glucuronide in human plasma and urine. Preliminary pharmacokinetic data demonstrate that the compound is mainly conjugated to its acyl glucuronide. The acyl glucuronide is relatively stable in plasma and is present in low concentrations, demonstrating extremely high renal clearance. The high renal clearance of the acyl glucuronide may, in part, be the result of renal glucuronidation. Probenecid had no effect on the acyl glucuronidation of flumequine while it was itself glucuronidated at maximal capacity in the kidney tubules. It reduced the renal clearance of flumequine acyl glucuronide.

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