Diflunisal and its conjugates in patients with renal failure

R. G. DICKINSON, R. K. VERBEECK¹, A. R. KING, A. C. RESTIFO & S. M. POND

Department of Medicine of The University of Queensland and the Princess Alexandra Hospital, Brisbane, Australia and

¹School of Pharmacy, Catholic University of Louvain, Brussels, Belgium

Six patients with renal failure were given a single oral dose (250 mg) of diflunisal. In contrast to the acyl glucuronide, the phenolic glucuronide and sulphate conjugates showed the capacity to accumulate in plasma, suggesting that systemic instability of the acyl glucuronide contributes, via hydrolysis, to plasma concentrations of diflunisal itself. Although earlier studies in renal failure patients have almost certainly underestimated diflunisal clearance (by overestimation of plasma diflunisal concentrations through unrecognized acidic hydrolysis of diflunisal sulphate during analysis), the present results suggest that the reported decrease in clearance was not attributable only to this analytical artifact.

Keywords diflunisal renal failure glucuronide sulphate protein binding

Introduction

Diflunisal, a substituted salicylate with analgesic and antiinflammatory properties, is eliminated from the body almost entirely by biotransformation. The major metabolites are the acyl glucuronide, the phenolic glucuronide and the sulphate conjugates. Their urinary recoveries account for 40–50%, 30–40% and 10–20%, respectively, of single doses to healthy volunteers (Loewen *et al.*, 1988). Whereas the glucuronides have long been known as major metabolites of the drug (Tocco *et al.*, 1975), the sulphate conjugate was identified relatively recently (Loewen *et al.*, 1986).

Verbeeck *et al.* (1979) reported that diflunisal elimination from plasma was markedly impaired in renal failure patients given single doses of the drug. Its terminal half-life increased and its plasma clearance decreased with increasing impairment of renal function. It was suggested that the effects of a saturable biotransformation process were being accentuated by systemic accumulation of diflunisal glucuronides, normally excreted in urine. Alternative explanations were offered by others. Levy (1979) proposed that retention of the glucuronides would promote their biliary excretion and the enterohepatic recirculation of diflunisal. Faed (1980) proposed that retention of the glucuronides would promote the extent of systemic deconjugation of the potentially labile acyl glucuronide. In 1988, we reported that biliary excretion of diflunisal glucuronides accounted for *circa* 9% of the total diflunisal species recovered in urine and bile of patients with T-tube drainage given a single 250 ml oral dose of the drug (Verbeeck *et al.*, 1988). This work provided support for a contribution from the enterohepatic recirculation pathway proposed by Levy (1979). In studies using rats, we have recently

demonstrated the systemic instability of diflunisal acyl glucuronide after i.v. administration of the purified conjugate (Watt *et al.*, 1991).

In 1989, Eriksson *et al.* reported that renal failure, but not old age or arthritis, markedly retarded diflunisal elimination. However, both the studies of Verbeeck *et al.* (1979) and Eriksson *et al.* (1989) were carried out in unawareness of the presence of the sulphate conjugate as a major plasma and urinary metabolite of diflunisal (Loewen *et al.*, 1986; Verbeeck *et al.*, 1990). Because diflunisal sulphate can readily hydrolyse to diflunisal under certain acidic analytical conditions (Dickinson & King, 1989) not unlike those employed in the two renal failure studies mentioned above, it seemed likely that a portion of the diflunisal measured in plasma in those studies could have originated from hydrolysis of diflunisal sulphate during analysis. This would have reduced clearance artifactually.

The present study was carried out to further understanding of the disposition of diflunisal in renal failure, by taking cognizance of recent knowledge of the reactivities of, and using recently-developed specific assays for, the individual acyl glucuronide, phenolic glucuronide and sulphate conjugates of diflunisal.

Methods

Materials

Diflunisal pure substance and [carboxyl-¹⁴C] diflunisal (52.63 $\mu\text{Ci mg}^{-1}$) were gifts from Merck, Sharp & Dohme, Sydney, NSW. Authentic samples of the acyl glucuronide,

Table 1 Some personal details and pharmacokinetic data from renal failure subjects given a single 250 mg oral dose of diflunisal

Subject	Sex	Age (years)	Weight (kg)	Plasma albumin (g l ⁻¹)	Creatinine clearance (ml min ⁻¹)	t _{1/2} [†] (h)	CL _{po} (ml min ⁻¹)	Unbound fraction in plasma	CLu _{po} (ml min ⁻¹)	Urinary recovery* (%)
1	M	56	66	45	10.1	18.7 (16.8)	7.2 (6.6)	0.0015	4800	33.4
2	F	61	89	24	19.7	31.6 (34.8)	10.3 (6.4)	0.0041	2510	19.7
3	F	56	65	41	18.9	29.8 (32.0)	3.1 (2.5)	0.0015	2070	59.8
4	F	28	59	35	7.8	37.1 (96.0)	4.3 (1.6)	0.0022	1950	19.5
5	F	51	66	44	33.4	17.1 (17.6)	4.7 (4.5)	0.0012	3930	49.8
6	F	61	65	43	20.5	18.6 (18.6)	4.8 (4.8)	0.0015	3180	70.4
Mean ± s.e. mean					18.4 ± 3.7	25.5 ± 3.4 (36.0 ± 12.4)	5.7 ± 1.1 (4.4 ± 0.8)	0.0020 ± 0.0004	3070 ± 460	42.1 ± 8.7

[†]Calculated from 36 h

*Total 0–120 h recovery by summation of 12 and 24 h recoveries (see **Methods**); and measured as the sum of diflunisal and its glucuronide and sulphate conjugates after hydrolysis.

Values in parenthesis refer to calculations based on the sum of diflunisal and its sulphate conjugate in plasma.

phenolic glucuronide and sulphate conjugates of diflunisal were obtained as described previously (Loewen *et al.*, 1986). β -Glucuronidase (from *Helix pomatia*, type H-2) was purchased from Sigma, St Louis, MO. Solvents were high performance liquid chromatography (h.p.l.c.) grade and were obtained from Mallinckrodt, Melbourne, VIC. Reagents were of analytical grade purity.

Subjects studied and study protocol

The study was carried out in six adults with varying degrees of renal failure (Table 1). All gave informed consent, and the protocol was approved by the Ethics Committees of The University of Queensland and Princess Alexandra Hospital, Brisbane. All of the subjects were taking multiple medications, but none was on haemodialysis. Routine therapy was not interrupted because of the study. Each patient received a single 250 mg oral dose of diflunisal (Dolobid®, Merck, Sharp & Dohme) in the morning after an overnight fast. Blood samples were taken predose (12 ml) and at 0.5, 1, 1.5, 2, 3, 4, 6, 8, 12, 24, 36, 48, 60, 72, 96 and 120 h (3 ml each) after diflunisal administration, centrifuged immediately thereafter and the plasma stored at -20° C. Urine was collected (under continuous medical supervision) predose and over the intervals 0–12, 12–24, 24–48, 48–72, 72–96, 96–120 h after drug intake and stored at -20° C.

Plasma protein binding of diflunisal

At the first thawing, 1 ml samples of predose, 2, 24 and 48 h plasma samples from the renal failure patients and fresh plasma samples from two young healthy drug-free volunteers were spiked with *circa* 0.25 μ Ci (*circa* 5 μ g) of freshly-purified [¹⁴C]-diflunisal and dialysed against 1 ml of 67 mM sodium phosphate buffer pH 7.4 at 37° C. The methods for diflunisal purification and equilibrium

dialysis were similar to those previously reported (Loewen *et al.*, 1988).

Analysis of diflunisal and its conjugates

Diflunisal and its acyl glucuronide, phenolic glucuronide and sulphate conjugates in plasma were analysed as described previously (Dickinson & King, 1989), using 50 μ l aliquots of the freshly-thawed plasma samples. Isomers of diflunisal acyl glucuronide were measured using the same molar extinction at 226 nm as diflunisal acyl glucuronide itself. In urine samples (500 μ l), total diflunisal content was measured after sequential hydrolysis with β -glucuronidase (1000 units, 37° C for 4 h), alkali (60 μ l of 2 M sodium hydroxide solution, 80° C for 1 h) and acid (120 μ l of 2 M hydrochloric acid solution, 80° C for 1 h).

Pharmacokinetic analysis

The plasma half-life (*t*_{1/2}) of diflunisal was determined from the slope of the terminal portion (from 36 h) of the log plasma drug concentration-time profile. The area under the plasma drug concentration-time curve (AUC) from 0–*t* h was determined by trapezoidal rule integration and the total AUC to infinity by addition of the area under the extrapolated terminal linear phase. The oral clearance of diflunisal (CL_{po}) was determined as the quotient of dose and AUC. The oral clearance of the unbound fraction of diflunisal (CLu_{po}) was calculated as the quotient of CL_{po} and unbound fraction.

Results

Plasma profiles of diflunisal and its conjugates in the renal failure patients are shown in Figure 1. The drug

Figure 1 Plasma profiles of diflunisal (●) and its acyl glucuronide (△), phenolic glucuronide (□) and sulphate (○) conjugates after administration of an oral dose of 250 mg of diflunisal to patients with renal failure. The acyl glucuronide concentrations refer to the sum of the glucuronide itself plus its isomers formed by intramolecular acyl migration.

was absorbed rapidly, achieving peak plasma concentrations of $25\text{--}55\ \mu\text{g ml}^{-1}$ (mean $43 \pm \text{s.e. mean } 4.4\ \mu\text{g ml}^{-1}$) at 1–2 h. Except in subject 6, the phenolic glucuronide and sulphate conjugates were readily measurable in plasma. In contrast, only low or unmeasurable concentrations of the acyl glucuronide, accompanied by its isomers formed by intramolecular acyl migration (Dickinson & King, 1989), were found. The profiles for plasma acyl glucuronide in Figure 1 refer to the sum of the glucuronide and its isomers; measurable concentrations were found only in subjects 1–3.

Retention of the conjugates in plasma correlated broadly with their decreased urinary excretion (Table 1). Thus for subjects 2 and 4, in whom terminal plasma concentrations of the phenolic glucuronide and sulphate conjugates were comparable with those of diflunisal itself, total urinary recovery of the drug over 5 days was low (circa 20% of dose). For subject 6, in whom none of the conjugates was measurable in plasma, urinary recovery

was 70%. Unfortunately, concentrations of the individual conjugates in urine were usually too low for their direct analysis, because the assay procedure does not permit extraction and concentration steps which would hydrolyse diflunisal sulphate. Consequently, only the total diflunisal content in the urines was determined after applying steps which hydrolyse the three conjugates as well as the isomers of the acyl glucuronide.

Pharmacokinetic parameters calculated assuming a terminal linear phase for plasma diflunisal after 36 h are shown in Table 1. Terminal $t_{1/2}$ varied between 17 and 37 h and CL_{po} was between 3.1 and $7.2\ \text{ml min}^{-1}$ except in subject 2 ($10.3\ \text{ml min}^{-1}$), who had a low plasma albumin concentration ($24\ \text{g l}^{-1}$) and a high unbound fraction of diflunisal in plasma (0.0041). Normalising for unbound fraction gave $\text{CL}_{\text{u po}}$ values of $1.9\text{--}4.8\ \text{l min}^{-1}$ (Table 1). The unbound fraction was obtained for the predose, 2, 24 and 48 h plasma samples. There was no convincing evidence for concentration-dependent protein

binding of diflunisal, and because the values within each subject were similar, the unbound fraction for each patient was taken as the mean of the measured values. In plasma from two normal individuals, the unbound fraction was 0.0009 at a diflunisal concentration of *circa* $5 \mu\text{g ml}^{-1}$. This fraction agrees with earlier studies in normal subjects which showed concentration-independent binding of diflunisal over the range $11\text{--}88 \mu\text{g ml}^{-1}$ (Loewen *et al.*, 1988).

Discussion

This study presents for the first time the plasma concentrations of diflunisal and its individual conjugates after the administration of an oral dose of the drug to patients with renal failure. Earlier studies (Eriksson *et al.*, 1989; Verbeeck *et al.*, 1979) had been carried out in ignorance of the sulphate conjugate, which is now known to account for 10–20% of the metabolism of single diflunisal doses (Loewen *et al.*, 1988), and which has recently been shown to hydrolyse to diflunisal under certain acidic analytical conditions (Dickinson & King, 1989). Reproduction of the plasma diflunisal assays reported in the earlier studies gave, in our hands, *circa* 40–80% (Verbeeck *et al.*, 1979) and 10–20% (Wahlin-Boll *et al.*, 1981) hydrolysis of diflunisal sulphate (at *circa* $8 \mu\text{g}$ diflunisal equivalents ml^{-1}) after 1 min contact between diflunisal sulphate and the acidic aqueous/organic media. Thus plasma diflunisal concentrations seem certain to have been overestimated, and clearance underestimated, in those studies.

Table 1 shows the effects (values in parentheses) on pharmacokinetic parameters caused by measuring diflunisal as the sum of diflunisal and diflunisal sulphate. Whilst this leads to increases in AUC and consequent decreases (mean 22%) in CL_{po} , the changes in $t_{1/2}$, (with the exception of subject 4), are quite small, because the terminal plasma profiles for diflunisal and its sulphate conjugate are essentially parallel. However, the same may not be true in patients with more severe renal failure (perhaps exemplified by subject 4). The six subjects of the present study had creatinine clearances of $7.8\text{--}33.4 \text{ ml min}^{-1}$ (Table 1) and mean values for CL_{po} and $t_{1/2}$ of 5.7 ml min^{-1} and 25.5 h, respectively (or 4.4 ml min^{-1} and 36 h, respectively, if diflunisal measurements included its sulphate conjugate). These values are broadly comparable with those for the renal failure subjects reported by Verbeeck *et al.* (1979) and Eriksson *et al.* (1989). More importantly, CLu_{po} ($1.95\text{--}4.80 \text{ l min}^{-1}$, Table 1) was only 15–37% of the mean value of 12.9 l min^{-1} found for healthy volunteers given a single 250 mg dose of diflunisal (Verbeeck *et al.*, 1990). Thus, the data obtained in this study do not support the contention that the previously reported reduced plasma clearance of diflunisal in renal failure was attributable *solely* to analytical artifacts.

Whereas diflunisal sulphate undergoes hydrolysis under acidic conditions *in vitro*, it has been shown, like diflunisal phenolic glucuronide, to be stable *in vitro* at physiological pH (Dickinson & King, 1989). Furthermore, we have recently documented that both diflunisal phenolic glucuronide (Watt *et al.*, 1991) and diflunisal

sulphate (Dickinson *et al.*, 1991) are systemically stable in rats after i.v. administration of the purified conjugates. In contrast, diflunisal acyl glucuronide has been shown to be a labile conjugate at physiological pH *in vitro*, capable of undergoing three related reactions—hydrolysis (to reform diflunisal), rearrangement (isomerisation via intramolecular acyl migration) and intermolecular transacylation/covalent bonding reactions (Dickinson & King, 1989; Hansen-Møller *et al.*, 1988; Watt & Dickinson, 1990b). With the exception of hydrolysis catalysed by enzymes, these three pathways reflect the intrinsic chemical reactivity of acyl glucuronides. In rats given the purified conjugate i.v., hydrolysis was the predominant pathway of diflunisal acyl glucuronide reactivity (Watt *et al.*, 1991). Liberated diflunisal was then available for re-conjugation, and further cycling of reformed diflunisal acyl glucuronide. The net result of such systemic cycling should be steady accumulation of stable diflunisal sulphate and stable diflunisal phenolic glucuronide, and this has been verified in rats with surgical blockage of excretion pathways (Watt & Dickinson, 1990a; Watt *et al.*, 1991). Similar results have been obtained in the present human study, with the phenolic glucuronide and sulphate conjugates, but not the acyl glucuronide, showing the *capacity* to accumulate in plasma of renal failure patients (Figure 1). Age-matched controls with normal renal function were not studied in the present investigation. However, in young healthy volunteers given single 250 or 500 mg doses of diflunisal, none of the diflunisal conjugates achieved measurable concentrations in plasma (Verbeeck *et al.*, 1990).

Given that the acyl glucuronide is a major metabolite of diflunisal, accounting for 40–50% of single doses to normal subjects (Loewen *et al.*, 1988), its hydrolysis during systemic retention in renal failure should make an appreciable contribution to sustaining the concentrations of circulating diflunisal. Lin *et al.* (1986) suggested that systemic hydrolysis of diflunisal acyl glucuronide was unimportant in reduced clearance of diflunisal in rats with experimental renal failure. However, aspects of that study have been questioned, including the suitability of rats as models of the preferred excretion pathways for diflunisal conjugates in man (Watt & Dickinson, 1990a), and the potential for unrecognized hydrolysis of diflunisal sulphate during analysis (Lin, 1990; Verbeeck & Dickinson, 1990) i.e. the same artifact besetting the earlier studies of diflunisal disposition in human renal failure (Eriksson *et al.*, 1989; Verbeeck *et al.*, 1979). A practically unavoidable deficiency of the present and previous studies is the use of renal failure patients taking other drugs. Thus potential effects of such co-medication on diflunisal disposition (e.g. effects on its metabolism or renal excretion of its conjugates) have not been considered. Nonetheless, the results of the present investigation support a contribution from systemic instability of diflunisal acyl glucuronide to the decreased clearance of diflunisal in human renal insufficiency, as originally proposed by Faed (1980).

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References

- Dickinson, R. G. & King, A. R. (1989). Reactivity considerations in the analysis of glucuronide and sulfate conjugates of diflunisal. *Ther. Drug Monitoring*, **11**, 712–720.
- Dickinson, R. G., King, A. R. & Hansen-Møller, J. (1991). The sulphate conjugate of diflunisal: its synthesis and systemic stability in the rat. *Xenobiotica* (in press).
- Eriksson, L.-O., Wahlin-Boll, E., Odar-Cederlöf, I., Lindholm, L. & Melander, A. (1989). Influence of renal failure, rheumatoid arthritis and old age on the pharmacokinetics of diflunisal. *Eur. J. clin. Pharmacol.*, **36**, 165–174.
- Faed, E. M. (1980). Decreased clearance of diflunisal in renal insufficiency—an alternative explanation. *Br. J. clin. Pharmacol.*, **10**, 185–186.
- Hansen-Møller, J., Dalgaard, L. & Hansen, S. H. (1987). Reversed-phase high-performance liquid chromatographic assay for the simultaneous determination of diflunisal and its glucuronides in serum and urine. Rearrangement of the 1-*O*-acylglucuronide. *J. Chromatogr.*, **420**, 99–109.
- Levy, G. (1979). Decreased body clearance of diflunisal in renal insufficiency—an alternative explanation. *Br. J. clin. Pharmacol.*, **8**, 601.
- Lin, J. H. (1990). The role of sulfate conjugation in the elimination kinetics of diflunisal in the rat: Response. *Drug Metab. Dispos.*, **18**, 268–269.
- Lin, J. H., Ulm, E. H. & Duggan, D. E. (1986). Possible mechanisms for reduced plasma clearance of diflunisal in rat experimental renal failure. *J. Pharmac. exp. Ther.*, **238**, 978–984.
- Loewen, G. R., Herman, R. J., Ross, S. G. & Verbeeck, R. K. (1988). Effect of dose on the glucuronidation and sulphation kinetics of diflunisal in man: single dose studies. *Br. J. clin. Pharmacol.*, **26**, 31–39.
- Loewen, G. R., McKay, G. & Verbeeck, R. K. (1986). Isolation and identification of a new major metabolite of diflunisal in man. The sulfate conjugate. *Drug Metab. Dispos.*, **14**, 127–131.
- Tocco, D. J., Breault, G. O., Zacchei, A. G., Steelman, S. L. & Perrier, C. V. (1975). Physiological disposition and metabolism of 5-(2',4'-difluorophenyl)salicylic acid, a new salicylate. *Drug Metab. Dispos.*, **6**, 453–466.
- Verbeeck, R. K. & Dickinson, R. G. (1990). The role of sulfate conjugation in the elimination kinetics of diflunisal in the rat. *Drug Metab. Dispos.*, **18**, 267–268.
- Verbeeck, R. K., Dickinson, R. G. & Pond, S. M. (1988). Biliary excretion of diflunisal conjugates in patients with T-tube drainage. *Eur. J. clin. Pharmacol.*, **34**, 423–426.
- Verbeeck, R. K., Loewen, G. R., Macdonald, J. I. & Herman, R. J. (1990). The effect of multiple dosage on the kinetics of glucuronidation and sulphation of diflunisal in man. *Br. J. clin. Pharmacol.*, **29**, 381–389.
- Verbeeck, R., Tjandramaga, T. B., Mullie, A., Verbesselt, R., Verberckmoes, R. & De Schepper, P. J. (1979). Bio-transformation of diflunisal and renal excretion of its glucuronides in renal insufficiency. *Br. J. clin. Pharmacol.*, **7**, 273–282.
- Wahlin-Boll, E., Brantmark, B., Hanson, A., Melander, A. & Nilsson, C. (1981). High-pressure liquid chromatographic determination of acetylsalicylic acid, salicylic acid, diflunisal, indomethacin, indoprofen and indobufen. *Eur. J. clin. Pharmacol.*, **20**, 375–378.
- Watt, J. A. & Dickinson, R. G. (1990a). Effects of blockage of urine and/or bile flow on diflunisal conjugation and disposition in rats. *Xenobiotica*, **20**, 835–845.
- Watt, J. A. & Dickinson, R. G. (1990b). Reactivity of diflunisal acyl glucuronide in human and rat plasma and albumin solutions. *Biochem. Pharmacol.*, **39**, 1067–1075.
- Watt, J. A., King, A. R. & Dickinson, R. G. (1991). Contrasting systemic stabilities of the acyl and phenolic glucuronides of diflunisal in the rat. *Xenobiotica*, (in press).

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