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# BIOTRANSFORMATION OF DIFLUNISAL AND RENAL EXCRETION OF ITS GLUCURONIDES IN RENAL INSUFFICIENCY

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**1** A single oral dose of 500 mg diflunisal was administered to control subjects and patients with varying degrees of renal insufficiency to estimate the disposition kinetics of this drug.

**2** Diflunisal and the sum of its ester and ether glucuronides conjugates were measured fluorimetrically.

**3** In normals terminal plasma half-lives ($T_{\frac{1}{2}}\beta$) of diflunisal and its glucuronides were very similar: 10.8 h and 11.8 h respectively. The finding that plasma half-life was shortened with declining diflunisal plasma levels suggests capacity-limited elimination.

**4** In subjects with normal renal function $78.6 \pm 2.7\%$ of the administered dose was recovered in 72 h urine, mainly as the glucuronide conjugates.

**5** With increasing degree of renal function impairment $T_{\frac{1}{2}}\beta$ of diflunisal was progressively prolonged up to ten times normal probably due to slowed biotransformation. This was associated with increasing retention of the conjugated metabolites in plasma due to marked reduction of the urinary excretion of the glucuronide conjugates.

**6** The apparent volume of distribution of diflunisal was very small in normals ($7.3 \pm 0.4$ l) and was significantly increased in patients with renal insufficiency (up to $16.2 \pm 2.2$ l).

**7** Diflunisal elimination studies performed during haemodialysis did not reveal any significant change in diflunisal plasma half-time. *In vivo* ultrafiltration studies during haemodialysis have shown that diflunisal is 98–99% plasma protein bound in uraemic patients.

**8** The present study indicates that although diflunisal is primarily eliminated by biotransformation, $T_{\frac{1}{2}}\beta$ is prolonged in renal insufficiency and dose adjustment will accordingly be required in patients with renal function impairment.

## Introduction

Diflunisal or 5–(2′, 4′–difluorophenyl) salicylic acid (MK 647, MSD Laboratories) is a new salicylate derivative which in patient studies has shown analgesic and uricosuric properties (Van Winzum & Rodda, 1977; Tempero, Franklin, Reger & Kappas, 1976; Tempero, Cirillo & Steelman, 1977). Preliminary pharmacokinetic studies (Tocco, Breault, Zacchei, Steelman & Perrier, 1975; Steelman, Breault, Tocco, Besselaar, Tempero, Lutterbeck, Perrier, Gribnau & Hinselmann, 1975) have indicated that diflunisal is well-absorbed after oral administration and reaches peak plasma concentrations within 2 to 3 h following ingestion. Most of the drug found in plasma is unchanged diflunisal which is 99% bound to plasma proteins. Elimination of diflunisal in man primarily involves biotransformation to the ester and ether glucuronide conjugates that are predominantly excreted in the urine. Only a minor fraction ($\pm 3\%$) of the administered dose is excreted unchanged through the kidneys (Figure 1). Accumulation of the glucuronides due to lowered urinary excretion can therefore be anticipated in the presence of renal function impairment. The present study was undertaken to evaluate the effect of decreased renal function on the disposition characteristics of diflunisal in subjects with varying degrees of renal insufficiency. The influence of haemodialysis on the plasma elimination half-time of the parent drug and its metabolites was examined in renal patients undergoing regular haemodialysis treatment.

## Methods

### *Study subjects and clinical protocol*

Five healthy male volunteers with normal renal function, creatinine clearance ($Cl_{cr}$) > 100 ml/min,

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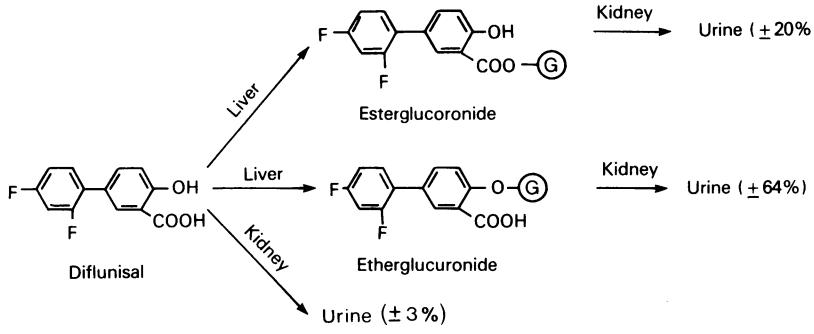


**Figure 1** Schematic diagram showing the diflunisal elimination in subjects with normal renal function (Tocco, Breault, Zacchei, Steelman & Perrier, 1975).

and seventeen consenting patients with varying degrees of renal function impairment participated in this investigation. The clinical characteristics of the participating subjects are described in Table 1. The study was conducted in accordance with the Declaration of Helsinki on biomedical research involving human subjects.

Nine of the patients had moderate renal insufficiency with $Cl_{cr}$ of 10 to 50 ml/min. Three patients had preterminal renal insufficiency with $Cl_{cr}$ of 2 to 10 ml/min and the remaining five patients had terminal renal insufficiency with $Cl_{cr}$ of less than 2 ml/min, requiring regular haemodialysis treatment.

All subjects received a single oral dose of 500 mg (two tablets) diflunisal in the morning after an overnight fast. Food was permitted 2 h following drug intake. Heparinized blood samples (10 ml) were collected before and at 1, 2, 4, 6, 8, 10, 12, 24, 30, 36 and 48 h after diflunisal intake, and plasma was separated by centrifugation. In patients with renal insufficiency, additional samples were drawn up to 72 h after drug administration. None of the subjects received any other medication during the study. Maintenance therapy with aluminium hydroxide, however, was interrupted only the morning of diflunisal intake in patients with end-stage renal failure.

In three of the patients with terminal renal insufficiency, hourly blood collections were obtained during a 4 to 6 h haemodialysis treatment (twin-coil artificial kidney, cuprophane membrane), which was initiated 48 h after diflunisal ingestion. Both at the start and at the end of haemodialysis, arterial plasma and ultrafiltrate samples from the coil kidney were collected as previously described (Tjandramaga, Thomas, Verbeeck, Verbesselt, Verberckmoes & De Schepper, 1976) to estimate the extent of the *in vivo* binding of diflunisal and its glucuronides to plasma proteins.

Urine was collected before drug intake and during the following time intervals after the dose: 0–6, 6–12, 12–18, 18–24, 24–36, 36–48 and 48–72 h. The plasma, ultrafiltrate and urine samples were stored at −20°C until analyzed.

### *Determination of diflunisal and total diflunisal glucuronides concentrations*

Diflunisal concentrations in plasma, urine and ultrafiltrate were measured by the fluorimetric method described by Tocco *et al.* (1975) involving the following procedure:

a. For the unchanged drug (i.e. unmetabolized diflunisal) in plasma and ultrafiltrate, 0.1–0.2 ml aliquot of the biological fluid was adjusted to 1.0 ml volume with distilled water and subsequently made acidic with an equal volume of 70% $HClO_4$, then extracted with chloroform. A 10 ml aliquot of the organic layer was re-extracted in 0.1 M phosphate buffer (pH 8) and the fluorescence of the aqueous phase was measured in a Farrand spectrophotofluorimeter at activation and emission wavelengths of 260 and 425 nm respectively.

b. Total drug (unchanged diflunisal plus diflunisal glucuronides) in plasma, ultrafiltrate and urine was determined following a hydrolytic procedure of the conjugates with 70% $HClO_4$ in a boiling water bath for 30 min. After cooling to room temperature, the samples were extracted with chloroform and processed according to the fluorimetric procedure described above.

From the values obtained with and without preliminary hydrolysis, the concentrations of total diflunisal glucuronides could be derived. Since previous studies by Tocco *et al.* (1975) have shown that only very small amounts of unchanged diflunisal are excreted in the urine of normal volunteers (approximately 3% of the administered dose), no attempt was made in the present study to measure the small quantities of unchanged diflunisal present in urine.

**Table 1** Clinical characteristics of subjects participating in the study

| *Subject* | *Sex* | *Age (years)* | *Weight (kg)* | *BUN (mg%)* | *Serum creatinine (mg%)* | *Creatinine clearance (ml/min)* | *Serum albumin (g%)* | *Clinical condition* | *Medication** |
|---|---|---|---|---|---|---|---|---|---|
| 1. Normal subjects | | | | | | | | | |
| 1. D.C. | M | 24 | 62 | 36 | 1.00 | 125 | 4.4 | Normal | none |
| 2. D.M. | M | 24 | 64 | 33 | 1.00 | 107 | 3.9 | Normal | none |
| 3. L.J. | M | 25 | 71 | 21 | 0.85 | 113 | 4.2 | Normal | none |
| 4. V.D.F. | M | 23 | 71 | 19 | 0.89 | 129 | 4.3 | Normal | none |
| 5. V.R. | M | 26 | 70 | 23 | 0.81 | 149 | 4.7 | Normal | none |
| 2. Moderate renal insufficiency | | | | | | | | | |
| 6. B.K. | M | 69 | 62 | 179 | 5.50 | 16 | 3.0 | Actute renal failure – aortic thrombosis | none |
| 7. C.A. | F | 64 | 60 | 136 | 3.50 | 29 | 2.6 | Chronic glomerulonephritis | clonidine, ampicillin, chlorthalidone |
| 8. M.V. | F | 86 | 75 | 58 | 1.20 | 40 | 3.6 | Geriatric, arthrosis | isosorbide dinitrate, triamterene-frusemide |
| 9. T.A. | F | 75 | 59 | 27 | 0.83 | 25 | 3.4 | Geriatric, femoral fracture | dipyridamole, diazepam |
| 10. T.J. | M | 32 | 74 | 41 | 1.80 | 45 | 2.9 | Acute renal failure-post shock due to trauma | none |
| 11. V.A. | M | 39 | 68 | 88 | 2.70 | 34 | 3.7 | Chronic glomerulonephritis | none |
| 12. V.C. | M | 72 | 65 | 34 | 0.97 | 44 | 2.7 | Geriatric, prostatic carcinoma | none |
| 13. V.D.M. | F | 23 | 52 | 239 | 3.00 | 25 | 3.4 | Acute renal failure – postabortion | none |
| 14. V.R.G. | M | 61 | 75 | 98 | 2.80 | 40 | 4.0 | Chronic pyelonephritis | none |
| 3. Preterminal renal insufficiency | | | | | | | | | |
| 15. V.B.C. | F | 46 | 51 | 159 | 7.60 | 6 | 3.9 | Analgesic nephropathy | clonidine, propranolol |
| 16. V.E. | F | 46 | 51 | 159 | 7.60 | 6 | 3.9 | Analgesic nephropathy | clonidine, propranolol |
| 17. V.L. | M | 61 | 52 | 106 | 4.50 | 9 | 3.1 | Chronic pyelonephritis | none |
| 4. Terminal renal insufficiency | | | | | | | | | |
| 18. B.M. | F | 68 | 46 | 180 | 15.00 | 0.5 | 3.9 | Analgesic nephropathy | propranolol, aluminium hydroxide |
| 19. V.E.M. | F | 55 | 48 | 300 | 10.20 | 1.0 | 3.5 | Chronic pyelonephritis | methyldopa, ampicillin, aluminium hydroxide |
| 20. B.J. | M | 52 | 69 | 177 | 7.70 | 1.0 | 2.7 | Diabetic nephropathy | insulin, aluminium hydroxide |
| 21. C.A1. | M | 53 | 51 | 220 | 17.00 | 0.5 | 3.2 | Chronic glomerulonephritis | dicloxacillin, ampicillin, aluminium hydroxide |
| 22. V.F. | M | 49 | 68 | 400 | 20.00 | 1.0 | 3.5 | Chronic glomerulonephritis | propranolol, aluminium hydroxide |

*Except for insulin, medication was interrupted the day of diflunisal administration. Maintenance therapy with aluminium hydroxide was interrupted only the morning of diflunisal intake.

*Reagents*

All reagents used were analytical grade.

*Pharmacokinetic analysis*

The terminal plasma half-life, $T_{\frac{1}{2}}\beta$, of the drug was estimated using the equation $T_{\frac{1}{2}}\beta = \ln 2/\beta$. The rate constant for drug elimination from the body ($\beta$) was calculated from the slope of the terminal linear decline of the semilogarithmic plot of the plasma concentrations: slope $= -\beta/2.303$. Each regression line was based on at least five points of plasma concentrations from the 12–48 h data in normals and from the 12–72 h data in patients with renal insufficiency. The apparent volume of distribution, $V_d$(area), was calculated using the equation $V_d(\text{area}) = FD/AUC_{0-\infty}.\beta$ in which $AUC_{0-\infty}$ is the total area under the diflunisal plasma concentration-time curve. D is the administered dose and F is the fraction of the dose entering the systemic circulation as unchanged drug (Gibaldi & Perrier, 1975). Except for the group of patients with terminal renal insufficiency where bioavailability of diflunisal was decreased by 40% (F = 0.6) due to interaction with aluminium hydroxide (Verbeeck, Tjandramaga, Mullie & De Schepper, 1979), our calculations in the other subject groups were based on the assumption that the orally administered doses of diflunisal were totally absorbed (F = 1). Total body clearance of diflunisal ($Cl_b$) was derived using the equation $Cl_b = \beta \times V_d$ (area). Results are presented as mean ± s.e. mean in the text and tables. Where appropriate, statistical analysis was performed using Student's *t*-test. A *P*-value of 0.05 or less was considered to be statistically significant.

## Results

The pharmacokinetic parameters derived from the plasma concentration and urinary excretion data of diflunisal and its glucuronides in the four groups of subjects are summarized in Table 2.

*Diflunisal and diflunisal glucuronides in plasma*

The time course of the mean plasma concentrations of diflunisal and its glucuronides following administration of a single oral dose of 500 mg to normal subjects and to patients with varying degrees of renal function impairment is shown in in the semilogarithmic plots of Figure 2. A rapid increase in the plasma concentrations of unchanged diflunisal was observed during the first 2 h following drug administration to reach peak values averaging 60 to 80 μg/ml in both normal subjects and patients with moderate and preterminal renal insufficiency. The difference in peak plasma concentrations between these three groups of subjects was statistically not significant. The terminal renal insufficiency group, however, had a significantly lower average peak plasma concentration of about 40 μg/ml at 2 to 4 h after diflunisal intake.

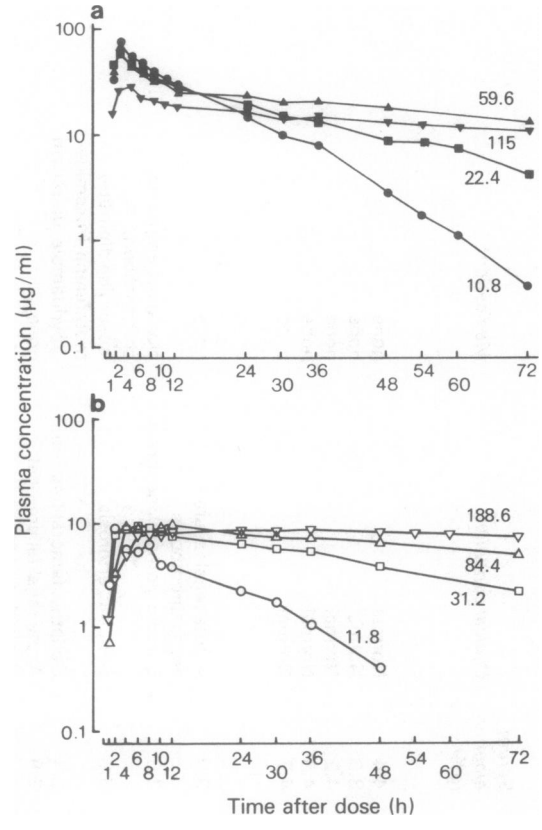


**Figure 2** Semilogarithmic plots of the mean concentrations of a) unchanged diflunisal (closed symbols) and b) its glucuronides (open symbols) in plasma from normal subjects (●,○) and patients with varying degrees of renal function impairment (moderate renal insufficiency ■, □; preterminal renal insufficiency ▲, △; terminal renal insufficiency ▼, ▽) after single oral administration of 500 mg diflunisal. The numbers indicate $T_{\frac{1}{2}}\beta$ in (h).

Diflunisal glucuronides appeared rapidly in plasma of normal subjects to reach maximum values of approximately 9 μg/ml within 2 h after drug administration. In patients with renal insufficiency, however, similar peak plasma concentrations of the glucuronide conjugates were reached at a later time, about 6 to 12 h after drug intake.

In normal subjects ($Cl_{cr} > 100$ ml/min) the mean (± s.e. mean) terminal plasma half-life of unchanged drug ($T_{\frac{1}{2}}\beta$) was 10.8 ± 0.8 h as compared to 22.4 ± 2.5 h in patients with moderate renal insufficiency. With

**Table 2** Pharmacokinetic data of diflunisal obtained from four groups of subjects with varying degrees of renal function

| | *Subject* | *Creatinine clearance (ml/min)* | *Peak plasma concentration (μg/ml)* | *Time to peak plasma concentration (h)* | $T_{\frac{1}{2}}\beta$ *(h)* | $\beta$ *($h^{-1}$)* | $V_d$*(area) (l)* | $Cl_{body}$ *(ml/min)* | *Recovery in 72 h urine*[a] *(% of dose)* |
|---|---|---|---|---|---|---|---|---|---|
| 1. Normal | 1. D.C. | 125 | 75.0 | 2 | 11.8 | 0.059 | 6.2 | 6.1 | 79.8 |
| | 2. D.M. | 107 | 92.5 | 2 | 11.7 | 0.059 | 8.0 | 7.9 | 70.2 |
| | 3. L.J. | 113 | 58.0 | 2 | 8.4 | 0.083 | 6.8 | 9.3 | 75.2 |
| | 4. V.D.F. | 129 | 72.0 | 2 | 9.8 | 0.070 | 8.2 | 9.6 | 84.1 |
| | 5. V.R. | 149 | 86.0 | 2 | 12.5 | 0.056 | 7.3 | 6.8 | 83.1 |
| | Mean | 124.6 | 76.7 | 2 | 10.8 | 0.065 | 7.53 | 7.9 | 78.6 |
| | ±s.e. mean | ±7.3 | ±6.0 | ±0 | ±0.8 | ±0.005 | ±0.4 | ±0.7 | ±2.7 |
| 2. Moderate renal insufficiency | 6. B.K. | 16 | 49.2 | 2 | 31.8 | 0.022 | 17.3 | 6.4 | 34.0 |
| | 7. C.A. | 29 | 54.3 | 2 | 31.2 | 0.022 | 20.5 | 7.5 | 44.3 |
| | 8. M.V. | 40 | 126.0 | 1 | 21.2 | 0.033 | 8.6 | 4.7 | 56.9 |
| | 9. T.A. | 25 | 68.0 | 1 | 15.8 | 0.044 | 7.8 | 5.7 | 46.4 |
| | 10. T.J. | 45 | 55.8 | 2 | 14.7 | 0.047 | 15.8 | 12.4 | 48.6 |
| | 11. V.A. | 34 | 85.2 | 2 | 20.8 | 0.033 | 7.4 | 4.1 | 57.8 |
| | 12. V.C. | 44 | 39.0 | 6 | 15.9 | 0.044 | 15.4 | 11.3 | 83.0 |
| | 13. V.D.M. | 25 | 72.5 | 2 | 17.3 | 0.040 | 8.1 | 5.4 | 58.8 |
| | 14. V.R.G. | 40 | 56.7 | 2 | 32.9 | 0.021 | 13.7 | 4.8 | 48.2 |
| | Mean | 33.1* | 67.4 | 2.2 | 22.4* | 0.034* | 12.7* | 6.9 | 53.1* |
| | ±s.e. mean | ±3,3 | ±8.6 | ±0.5 | ±2.5 | ±0.004 | ±1.6 | ±1.0 | ±4.6 |
| 3. Preterminal Renal Insufficiency | 15. V.B.C. | 6 | 68 | 2 | 54.0 | 0.013 | 14.7 | 3.2 | 8.3 |
| | 16. V.E. | 2 | 63 | 2 | 65.3 | 0.011 | 15.3 | 2.8 | 1.0 |
| | 17. V.L. | 9 | 59 | 2 | 59.4 | 0.012 | 12.9 | 2.6 | 19.1 |
| | Mean | 5.7* | 63.3 | 2 | 59.6* | 0.012* | 14.3* | 2.9* | 9.5* |
| | ± s.e. mean | ±2.0 | ±2.6 | ±0 | ±3.3 | ±0.001 | ±0.27 | ±0.2 | ±5.2 |
| 4. Terminal Renal insufficiency | 18. B.M. | 0.5 | 66.8 | 1 | 98.1 | 0.007 | 10.4 | 1.2 | 0.5 |
| | 19. V.E.M. | 1.0 | 42.0 | 2 | 136.9 | 0.005 | 15.1 | 1.3 | 4.5 |
| | 20. B.J. | 1.0 | 34.5 | 4 | 133.7 | 0.005 | 14.8 | 1.2 | 3.9 |
| | 21. C.A1. | 0.5 | 13.3 | 12 | 137.5 | 0.005 | 23.8 | 2.0 | — |
| | 22. V.F. | 1.0 | 32.5 | 4 | 68.3 | 0.010 | 16.9 | 2.8 | 1.8 |
| | Mean | 0.8* | 37.8* | 4.6 | 114.9* | 0.006* | 16.2* | 1.7* | 2.7* |
| | ±s.e. mean | ±0.1 | ±8.7 | ±1.9 | ±13.8 | ±0.001 | ±2.2 | ±0.3 | ±0.9 |

[a]recovery in urine represents the sum of parent drug + glucuronides
*value significantly different ($P<0.05$) from the corresponding value in control subjects for the estimation of $V_d$ (area) and $Cl_b$ in the terminal renal insufficiency group an F-value of 0.6 has been assumed

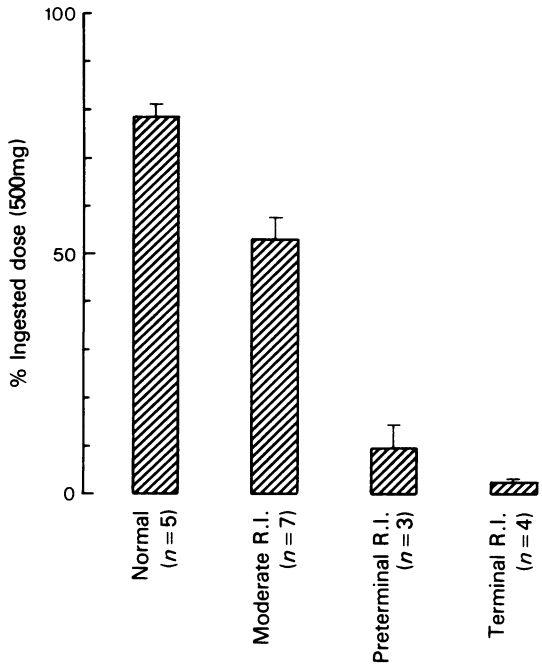


**Figure 3** The 72 h cumulative urinary excretion of diflunisal (almost exclusively as glucuronides) in the four groups of subjects with increasing degree of renal function impairment. R.I. renal insufficiency.

further decrease in renal function in patients with preterminal renal insufficiency ($Cl_{cr}$: 2 to 10 ml/min), the plasma half-life was $59.6 \pm 3.3$ h, while in patients with terminal renal insufficieny ($Cl_{cr} < 2$ ml/min) $T_{\frac{1}{2}}\beta$ was apparently $114.9 \pm 13.8$ h. In all four patient groups half-life was based on data points from 12 h following drug administration onwards.

The apparent terminal half-life ($T_{\frac{1}{2}}\beta$) of diflunisal glucuronides was on the average 11.8 h for the normal group, 31.2 h for the patient group with moderate renal insufficiency, 84.4 h for the preterminal, and 188.6 h for the terminal renal insufficiency group. These values were obtained from the mean plasma concentration-time data of the glucuronides for each patient group.

Comparison of the apparent volume of distribution in normal subjects ($7.5 \pm 0.4$ l) and in patients with renal function impairment ($12.7 \pm 1.6$ l, $14.3 \pm 0.7$ l, and $16.2 \pm 2.2$ l in moderate, preterminal and terminal renal insufficiency respectively) demonstrated a statistically significant volume increase when patients had creatinine clearances measuring less than 50 ml/min.

Total body clearance of unchanged diflunisal is very small, only 7.9 ml/min in normals and even less in patients with moderate, preterminal and terminal renal insufficiency (6.9 ml/min, 2.9 ml/min and 1.7 ml/min respectively).

*Urinary excretion data*

The 72 h urinary excretion of diflunisal in both normals and patients with increasing degrees of renal function impairment is shown in Figure 3. The amount of total diflunisal excreted by normals (predominantly as glucuronides) during 72 h was on the average $78.6 \pm 2.7\%$ of the ingested dose. The corresponding values in patients were progressively lowered in the presence of increasing degree of renal function impairment: $53.1 \pm 4.6\%$ of the dose was recovered in the patient group with moderate renal insufficiency, $9.5 \pm 5.2\%$ in preterminal renal insufficiency and only $2.7 \pm 0.9\%$ in terminal renal insufficiency.

*Effect of haemodialysis*

In three of the five patients with terminal renal insufficiency (C.A1., V.F., B.J.), the concentrations of both unchanged diflunisal and diflunisal glucuronides in arterial plasma were determined during a 4 to 5 h haemodialysis procedure. The plasma concentration data from one representative study (patient V.F.) are illustrated in Figure 4. The slopes of the decay curves of both diflunisal and its glucuronides do not appear to be markedly affected by the haemodialysis procedure indicating that the effect of dialysis on the plasma concentrations and removeal of both unchanged diflunisal and diflunisal glucuronides was insignificant.

The concentrations of diflunisal and its glucuronides obtained from the arterial plasma samples and its ultrafiltrates at the start and at the end of the haemodialysis in the three patients, are listed in Table 3. Since electrophoretic examination of the ultrafiltrates from the coil kidney did not reveal the presence of any proteins, the fractions recovered in the ultrafiltrate could be taken to represent the free dialyzable fraction of the drug. This free fraction was approximately 1.8% for intact diflunisal and 10% for the diflunisal glucuronides.

*Side-effects*

No side-effects related to diflunisal intake was observed in any of the participating subjects.

## Discussion

The present study shows that subjects with normal renal function and patients with renal function impairment rapidly absorb orally administered diflunisal (500 mg) to obtain comparable peak plasma concentrations (60 to 80 μg/ml) within 2 h after drug administration (Figure 2). The lower average peak

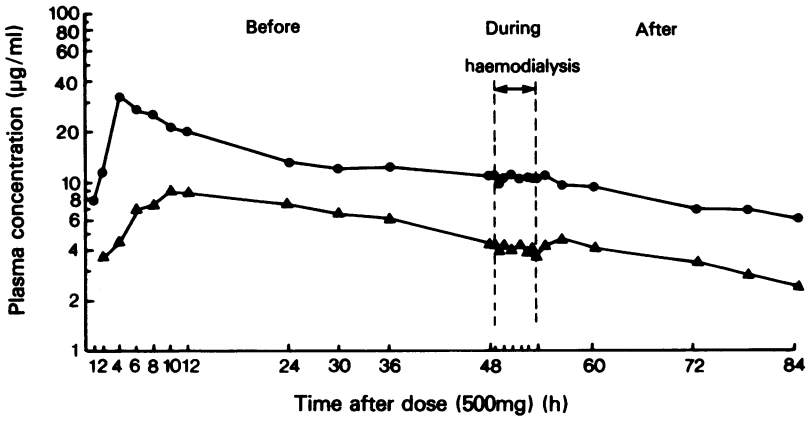


**Figure 4** Plasma concentration-time course of diflunisal (●) and its glucuronides (▲) in a patient with terminal renal insufficiency (V.F., $Cl_{cr}$ = 1 ml/min) undergoing haemodialysis approximately 48 h after a single oral dose of 500 mg diflunisal.

plasma concentration of about 40 µg/ml in patients with terminal renal insufficiency was due to lower bioavailability of the drug as a result of an interaction with chronic aluminium hydroxide administration (Verbeeck *et al.*, 1979). The terminal sections of the plasma concentration-time curves of diflunisal and its glucuronides exhibit parallel slopes (Figure 2). This can only occur when the metabolite is more rapidly eliminated than the parent drug, so that elimination of the metabolite is determined by its formation rate from the parent drug. In these circumstances, the real elimination half-life of the metabolite cannot be calculated on the basis of the plasma concentration-time curve data (Van Rossum, Van Ginneken, Henderson, Ketelaars & Vree, 1977). The present results thus indicate that the glucuronidation of diflunisal is the rate determining step for the elimination of the glucuronides. In normals the mean terminal plasma half-life of diflunisal is 10.8 h (measured from 12 to 72 h after drug ingestion). This is in good agreement with previously published values of 10 h (Tocco *et al.*, 1975) and 11 h (Steelman *et al.*, 1975). The apparent plasma half-life of the glucuronides is about 12 h which reasonably corresponds with the $T_{\frac{1}{2}}\beta$ value of the parent drug.

However, upon further analysis of the plasma concentration-time data in normals according to the two segments of decay curves, from 10 to 36 h and from 36 to 72 h, it is noticeable that the elimination half-lives of both unchanged diflunisal and of its glucuronides become smaller with decreasing plasma concentrations. Between 10 h and 36 h after drug intake the half-lives of unchanged diflunisal and its glucuronides are respectively 12.3 h and 16.2 h, while between 36 h and 72 h the half-lives have significantly decreased to 8.3 h and 8.6 h.

These findings can only be explained by assuming that the biotransformation of diflunisal to its glucuronide conjugates involves some saturable process. Tocco *et al.*, (1975) earlier suggested dose-dependent kinetics for diflunisal based on their findings that the areas under the curve for [$^{14}$C]-diflunisal in plasma of healthy volunteers were approximately 18 times higher following the 500 mg dose than the 50 mg dose. Moreover, these authors reported a shorter elimination half-life of 5.6 h in normal subjects after a 50 mg oral dose as compared to the value found in the present study: 10.8 h after 500 mg oral diflunisal administration to healthy volunteers. These cited observations support our interpretation for the present findings which indicate that elimination of diflunisal for plasma is capacity-limited. In addition, pharmacokinetic studies with sodium salicylate, a compound possessing a chemical structure very close to that of diflunisal, have clearly demonstrated that man has a limited capacity for salicyl phenolic glucuronide formation even within the therapeutic dose range (Levy & Procknal, 1968; Levy, Tsuchiya & Amsel, 1972), whereas salicyl acyl glucuronide formation is known to involve an apparent first-order process (Levy *et al.*, 1972). Based on the above considerations, it is thus very likely that formation of 5–(2′, 4′–difluorophenyl) salicyl phenolic glucuronide (=ether glucuronide), which is quantitatively the most important metabolic pathway for diflunisal, is capacity-limited.

As previously mentioned, the elimination of diflunisal is almost exclusively dependent on glucuronidation of the parent compound to an ester and ether glucuronide (Figure 1) which subsequently are excreted in the urine. A previous study using [$^{14}$C]-diflunisal in normal volunteers has revealed only

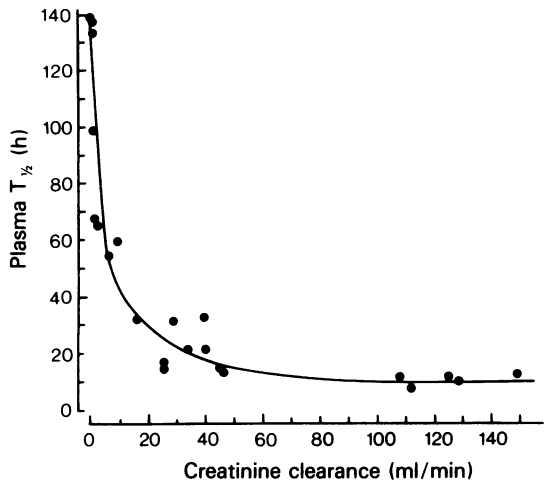


**Figure 5** Relationship between the terminal plasma half-lives of diflunisal and the endogenous creatinine clearance in normal subjects and patients with varying degrees of renal insufficiency.

minor quantities of unchanged drug in urine, less than 4% of a 500 mg dose within 96 h (Tocco, *et al.*, 1975). Therefore, a decrease in renal excretion of diflunisal glucuronides with a resultant delay in the elimination rate of these metabolites from plasma is to be anticipated and has been found in the present study in patients with renal function impairment (Figure 2). Conversely, one would not expect to find a significant prolongation of the elimination half-life of diflunisal if biotransformation of the drug to its glucuronides is not altered in renal insufficiency. The present data, however, demonstrate not only markedly prolonged terminal elimination half-life of the glucuronides according to the degree of renal insufficiency but also similarly prolonged $T_{\frac{1}{2}}\beta$ values of the unchanged drug. No experimental evidence for altered glucuronidation rates of drugs in renal patients has thus far been reported in the literature (Reidenberg, 1977). The elimination half-life of three drugs that are predominantly eliminated in man by glucuronidation such as chloramphenicol (Kunin, Glazko & Finland, 1959), acetaminophen (Lowenthal, Øie, Van Stone, Briggs & Levy, 1976) and lorazepam (Verbeeck, Tjandramaga, Verberckmoes & De Schepper, 1976), has previously been shown to be independent of renal function indicating unaltered glucuronidation of these drugs in the presence of renal insufficiency. Although the exact mechanism for the observed prolonged $T_{\frac{1}{2}}\beta$ values of diflunisal in association with the decreased renal excretion of its glucuronides in renal insufficiency is not known, it is apparent that some saturable process in the biotransformation of diflunisal, already present in subjects with normal kidney function, is accentuated by accumulation of the glucuronides in plasma. Such an accumulation of the conjugates both in plasma and at the site of enzymatic biotransformation in the liver should thus have resulted in a clearer manifestation of the limited capacity of the ether glucuronide formation from diflunisal.

It is generally recognized that the extent to which decreased renal function influences drug elimination is a function of the percentage of circulating drug cleared unchanged through the kidney. This general statement, however, is not applicable in the case of diflunisal as shown by the present investigation. According to the classification of Dettli (1974), diflunisal is a type B compound so that plasma half-life should essentially be independent of creatinine clearance. The relationship of diflunisal elimination half-life ($T_{\frac{1}{2}}\beta$) to the endogenous creatinine clearance in normal subjects and patients with renal function impairment, however, has a similar pattern as the common curve obtained for a drug which is cleared virtually unchanged through the kidney (Figure 5).

Diflunisal elimination studies performed in three patients with terminal renal insufficiency before, during and after haemodialysis did not reveal any noticeable change in plasma half-life of diflunisal, indicating that the drug is only slightly dialyzable (Figure 4). The plasma concentration-time curve of the glucuronides significantly increased up to several hours after terminating the haemodialysis treatment. This may result from a slow equilibration between the plasma and tissue compartment indicating also that there should have been some removal of diflunisal glucuronides from the central compartment (plasma) during haemodialysis (Gibson & Nelson, 1977). Estimates of the *in vivo* plasma protein binding of the drug in patients undergoing plasma ultrafiltration studies at the start and at the end of haemodialysis revealed values between 1.3 to 2.4% for the fraction of diflunisal recovered in the ultrafiltrates (Table 2). For the glucuronides, the dialyzable or unbound fraction is somewhat higher ($\pm$ 10%). It should be pointed out, however, that the small amounts of diflunisal and its glucuronides present in the ultrafiltrate samples are at the lower limit of detection by the fluorimetric analysis method used. Additional *in vitro* binding studies using [$^{4}$C]-diflunisal have demonstrated a protein binding of 99.56% (=0.44% unbound) in uraemic plasma and 99.88% (=0.12% unbound) in normal plasma (Verbeeck *et al.*, 1978). Such an increase in unbound fraction of diflunisal in uraemic plasma can explain the significantly larger apparent volumes of distribution observed in the present study in patients with renal insufficiency.

With regard to dose adjustment recommendations in renal patients, it is generally proposed that drugs which are extensively metabolized (to pharmacologically inactive metabolites) can be given

in normal doses to uraemic patients (Dettli, 1974; Bennett, Singer & Coggins, 1974). Several such drugs e.g. digitoxin, rifampicin, phenytoin and antipyrine have normal or even shorter plasma half-lives in uraemic subjects as compared to controls (Reidenberg, 1977).

Diflunisal appears to be an exception to this general rule and dose adjustment is certainly required to avoid accumulation of this drug to excessive levels during repeated dosing to patients with renal function impairment. Since treatment with commonly used analgesic drugs such as phenacetin, aspirin and acetaminophen may be associated with some nephrotoxic effects (Anderson, Gambertoglio & Schrier, 1976; Cheigh, 1977) the use of diflunisal as an alternative analgesic in renal patients may be indicated. However, in view of the variably and markedly prolonged (6 to 14 times ( $T_{\frac{1}{2}}\beta$ values in patients with very severe degree of renal insufficiency ($Cl_{cr}$: less than 10 ml/min), the use of this drug in such patients is not recommended. Unaltered plasma diflunisal $T_{\frac{1}{2}}\beta$ values and the small $T_{\frac{1}{2}}\beta$ changes associated with lesser degree of renal function impairment, however, suggest that prescribed doses of diflunisal need not be adjusted in patients having renal function impairment to as low as half its normal value, whereas in patients with creatinine clearance measurements of less than 50 ml/min down to approximately 15 ml/min diflunisal maintenance dosage should be reduced and/or the dosing interval lengthened in accordance with the 2 to 3 times prolonged $T_{\frac{1}{2}}\beta$ values.

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