THE EFFECT OF DIFLUNISAL ON THE ELIMINATION OF TRIAMTERENE IN HUMAN VOLUNTEERS

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SUMMARY

A major metabolic pathway for triamterene (a potassium sparing diuretic) is aromatic hydroxylation followed by sulphate conjugation. Diflunisal (a salicylate anti-inflammatory agent) also undergoes sulphate conjugation of its phenolic group as a major pathway. We investigated the possible effect of diflunisal on the elimination of triamterene (competition for phenolic sulphonation) in six healthy volunteers by studying the disposition of single doses of triamterene (100 mg) taken alone and in the presence of steady-state levels of diflunisal. Diflunisal coadministration (500 mg b.i.d.) had no effect on the pharmacokinetics of triamterene itself. However, plasma AUC of *p*-hydroxytriamterene sulphate was greater (4.6 times), and its renal clearance lower (0.24 times), in the presence of diflunisal. There was no change in the formation clearance or protein binding of *p*-hydroxytriamterene sulphate in the presence of diflunisal. The data point to competition for renal excretory pathways rather than sulphonation capacity. This interaction could have clinical relevance since *p*-hydroxytriamterene sulphate is pharmacologically active.

KEY WORDS

triamterene, diflunisal, drug-drug interaction, elimination, metabolism

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INTRODUCTION

Triamterene is a well-established potassium-sparing diuretic which is still widely used. It is extensively metabolised and is eliminated mainly as its metabolites in urine and bile /1/. The phase I metabolite, *p*-hydroxytriamterene, is rapidly and extensively converted to the pharmacologically active phase II sulphate conjugate, *p*-hydroxytriamterene sulphate /2/, which attains much higher concentrations in plasma and urine than does triamterene /3/. Diflunisal, an analgesic and anti-inflammatory agent derived from salicylic acid, is likely to be co-administered with triamterene, particularly in the elderly. Diflunisal is metabolised to two glucuronide conjugates (a phenolic-glucuronide and an acyl-glucuronide) as well as a sulphate conjugate /4/. As sulphonation of a phenolic group is a major metabolic pathway for both drugs, this raised the possibility of a drug-drug interaction at the metabolic level.

Triamterene undergoes renal elimination by being actively secreted into the lumen of the proximal tubule by the organic cation transport system /1/. The sulphate conjugate of *p*-hydroxytriamterene also undergoes tubular secretion by the organic anion transport system in the rat /5/, and probably by the same mechanism in humans /6/. Active tubular secretion is also responsible for the renal excretion of the acyl and phenolic glucuronides of diflunisal /7/. Renal clearances of the glucuronides are markedly reduced (by as much as 70%) during probenecid coadministration, which supports tubular secretion by the organic anion transport pathway as the operative mechanism /7/.

The present study in healthy volunteers was designed to investigate the effects of diflunisal on the elimination of triamterene, by studying the disposition of single doses of triamterene taken alone and in the presence of steady-state levels of diflunisal.

METHODS

Study design

Triamterene and *p*-hydroxytriamterene sulphate were provided by Alphapharm Pty Ltd. Salicylic acid was purchased from BDH Chemicals. Diflunisal and cloflbric acid were purchased from Sigma

Chem Co. All other reagents and solvents were of analytical grade or better, and were obtained from commercial sources.

Three male and three female volunteers (aged 21-54 yr), all within 20% of ideal body weight for height and build (as published in the Geigy Scientific Tables), not receiving any medication, ingested two tablets of Hydrene 25/50® (Alphapharm Pty Ltd.; total dose 100 mg triamterene and 50 mg hydrochlorothiazide) at approximately 8 a.m. after an overnight fast. Blood samples were collected immediately pre-dose and post-dose serially at appropriate times until 144 h via an indwelling forearm venous catheter or by single venepuncture. Urine was collected immediately pre-dose (an untimed collection), and then all urine passed post-dose for 48 h was collected (4x12 h intervals). After a one-week washout period, the volunteers were given diflunisal (Dolobid®; Merck, Sharp & Dohme (Aust) Pty Ltd.) at a starting dose of 1000 mg followed by 500 mg twice daily. One subject had a low body height and weight and was given reduced doses of diflunisal (starting dose 600 mg, followed by 300 mg twice daily). Volunteers continued the dosing regimen for one week to achieve steady state conditions. At this time, whilst continuing taking diflunisal at the same dose, they took a single dose of triamterene/hydrochlorothiazide as described above. Blood and urine collections were conducted in the same manner as before. Aliquots of the urine samples and plasma from all blood samples were stored at -20°C until assayed. The Medical Research Ethics Committee of the University of Queensland approved all experiments.

Analytical methods

Triamterene and *p*-hydroxytriamterene sulphate were assayed simultaneously by a sensitive HPLC method using salicylic acid as the internal standard, with fluorescence detection to take advantage of the intense fluorescence of the analytes. The analytical column was a Waters RCM-100 radial compression module with C_{18} Novapak (4 μm) cartridge insert, which was preceded by a guard column packed with Waters C_{18} Corasil Bondapak. The mobile phase consisted of a solution of glacial acetic acid (10 ml) in water (890 ml) to which was added triethylamine (1 ml). The final pH was adjusted to 3.75 by dropwise addition of sodium hydroxide (1 M). To 900 ml of this solution was added 100 ml acetonitrile, after which the solution was filtered through a Millipore Waters membrane (0.5 μm pore size). The

mobile phase was prepared freshly at the start of each batch of assays and was pumped through the system at 1.8 ml/min. The fluorescence detector (Waters 470) was used with excitation and emission wavelengths of 365 and 440 nm for both triamterene and *p*-hydroxytriamterene sulphate, and 320 and 440 nm respectively for salicylic acid. Elution times were 4, 8.5 and 14 min for *p*-hydroxytriamterene sulphate, salicylic acid and triamterene respectively. Quantification was in terms of peak area ratios.

For plasma, the assay was established over two separate calibration ranges, to permit precise and accurate analyses over a very broad concentration range. The low range covered 0.5 to 5.0 ng/ml for triamterene and 0.1 to 50.0 ng/ml for *p*-hydroxytriamterene sulphate. The high range covered 5.0 to 500 ng/ml for triamterene and 50 to 5000 ng/ml for *p*-hydroxytriamterene sulphate. Sample preparation was non-extractive, and involved the evaporation of internal standard solution (100 µl of a solution of salicylic acid in acetonitrile, 20 µg/ml) followed by addition of a 0.5 ml aliquot of plasma. Acetonitrile (1.5 ml for the high calibration range, 2.5 ml for the low calibration range) was then added, and after thorough vortex agitation the mixture was centrifuged. The supernatant (0.3 ml for the high range, 3.0 ml for the low range) was evaporated to dryness in a vacuum centrifuge, redissolved in mobile phase (300 µl for the high range, 200 µl for the low range) and an aliquot (30 µl for high range, 100 µl for low range) applied to the HPLC. In urine, the calibration ranges were 0.05 to 10.0 µg/ml for triamterene and 0.1 to 20.0 µg/ml for *p*-hydroxytriamterene sulphate. Sample preparation involved mixing a 100 µl aliquot of urine with an equal volume of aqueous sodium hydroxide (0.01 M), followed by salicylic acid (50 µl of an aqueous solution, 400 µg/ml) and mobile phase (850 µl). After vortex mixing, a 100 µl aliquot was applied to the HPLC. Within-batch and between-batch analyses of standards and quality control samples showed that the methods were precise to within 15% of mean values and accurate to within 15% of true values. The minimum quantified concentration was defined as the lowest point on the standard curves with precision and accuracy within 20% (in each case this was the lowest point on the ranges stated above).

Plasma diflunisal concentrations were determined from the blood sample taken prior to dosing with triamterene. The calibration range used was 20 to 500 µg diflunisal/ml plasma. Sample preparation

involved the addition of 1 ml of internal standard solution (20 μg clofibric acid/ml acetonitrile) to a 0.5 ml plasma sample. The mixture was vortex agitated and centrifuged. A sample of supernatant (20 μl) was injected into the HPLC system fitted with a Waters μ-Bondapak C_{18} (30 cm x 3.9 mm) column preceded by a C_{18} Corasil Bondapak guard column. Diflunisal was detected at a wavelength of 226 nm, and eluted at 5.6 min. The mobile phase was 55% methanol and 45% sodium acetate buffer. The flow rate through the system was 1 ml/min.

Pharmacokinetic calculations and statistical analyses

Standard formulas were used for the calculation of all pharmacokinetic parameters. Maximum plasma concentration (C_{max}) and time to C_{max} (t_{max}) were obtained from the measured values. Area under the plasma concentration curve (AUC) was determined by the linear trapezoidal method. Apparent plasma clearance (CL) of triamterene was calculated by dividing the dose by $AUC_{0-\infty}$. Renal clearances (CL_R) were determined by dividing the amount of drug or metabolite excreted in urine over a certain period by its plasma AUC over that period. Formation clearances (CL_f) of plasma triamterene to urinary *p*-hydroxytriamterene sulphate were calculated as the product of fractional recovery in urine multiplied by the apparent plasma clearance of triamterene. The half-lives ($t_{½}$) of triamterene and *p*-hydroxytriamterene sulphate were calculated by linear regression analyses of the terminal data points (5 points for triamterene; 6 points for *p*-hydroxytriamterene sulphate) of the logarithmic plasma concentration-time curves.

Since the variances in the pharmacokinetic data collected were high (i.e. variance [S.D.^{2}] being > mean), differences in pharmacokinetic parameters were analysed by a non-parametric test (Wilcoxon Matched-Pair Signed Ranks Test). A P value of 0.05 was considered to represent a statistically significant difference.

RESULTS

The plasma concentration-time profiles for triamterene taken alone and in the presence of steady-state concentrations of diflunisal are shown in Figure 1. It is apparent from the graph that there were no significant changes in the plasma pharmacokinetics of triamterene in

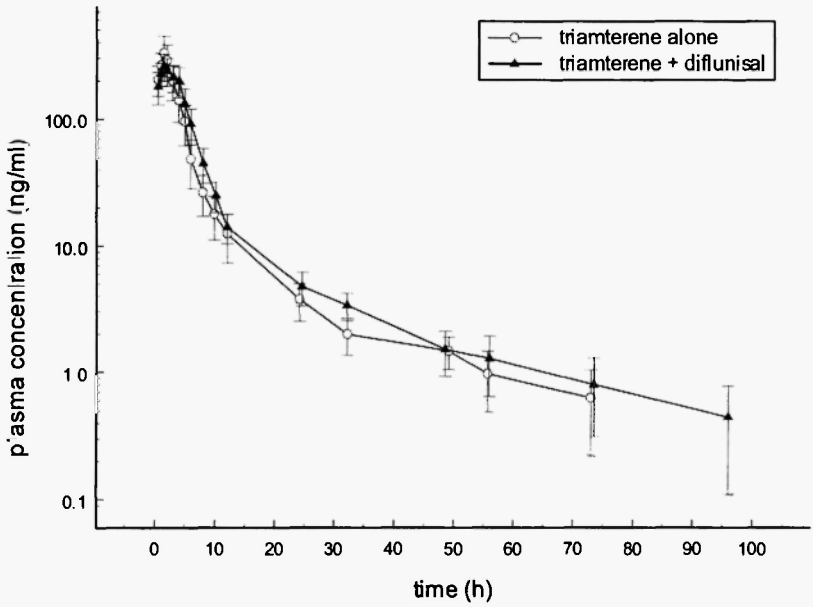

Fig. 1: Mean plasma concentration profile of triamterene after a single dose of triamterene compared with the plasma concentration curve after the same dose of triamterene while on steady-state diflunisal. Error bars represent standard error of the mean.

the presence of diflunisal, and this was confirmed by the absence of statistically significant differences between the two treatment periods for all pharmacokinetic parameters measured except for the slight delay seen in t_{max} (Table 1).

Despite this, however, there were substantial differences in the disposition of *p*-hydroxytriamterene sulphate between the two treatment periods. Plasma concentrations of this metabolite were markedly elevated at all time points in the presence of diflunisal (Figure 2). This resulted in statistically significant differences in several important pharmacokinetic parameters (Table 2). Thus C_{max}, t_{max} and $AUC_{0-\infty}$ all showed significantly greater values in the presence of diflunisal. Formation clearance to *p*-hydroxytriamterene sulphate was lower in the presence of diflunisal, but not significantly so. Renal clearance of this metabolite was significantly reduced.

TABLE 1

Pharmacokinetic parameters of triamterene in the absence and presence of diflunisal

Pharmacokinetic parameter for triamterene (TA)	Triamterene alone	Triamterene + Diflunisal	P value
C_{max} (ng/ml)	360 20 ± 282.81	292.01 ± 158.54	0.463
t_{max} (h)	1.34 ± 0.40	2.04 ± 1.20	0.043*
$AUC_{0-\infty}$ (μg h/l)	1431.51 ± 971.48	1392.44 ± 833.61	0.463
$t_{1/2}$ (h)	17.90 ± 8.71	17.67 ± 9.59	0.753
CL (l/h)	110.43 ± 82.67	74.88 ± 25.02	0.345
Urinary recovery (%)	4.72 ± 2 29	4.27 ± 4.14	0.917
CL_R (l/h)	7.36 ± 2 25	4.99 ± 0.68	0.345
% TA bound to protein	96 ± 0.5	96 ± 0.8	0.753

* Significantly different ($P<0.05$) using Wilcoxon Signed Ranks Test.

TABLE 2

Pharmacokinetic parameters of *p*-hydroxytriamterene sulphate in the presence and absence of diflunisal

Pharmacokinetic parameter for *p*-hydroxytriamterene sulphate (TAS)	Triamterene alone	Triamterene + Diflunisal	P value
C_{max} (ng/ml)	1480.03 ± 954.81	3525.71 ± 1408.37	0.028*
t_{max} (h)	1.26 ± 0.41	3.30 ± 1.81	0.02*
$AUC_{0-\infty}$ (μg.h/l)	6161 ± 2504	28756 ± 12255	0.028*
$t_{½}$ (h)	20.59 ± 11.09	16.29 ± 8.02	0.463
Urinary recovery (%)	21.43 ± 13.83	19.71 ± 11.49	0.600
CL_r (l/h)	25.02 ± 21.57	14.96 ± 8.71	0.249
CL_R (l/h)	5.06 ± 2.61	1.23 ± 1.14	0.028*
% TAS bound to protein	99 ± 0.3	99 ± 0.3	0.035

* Significantly different ($P<0.05$) using Wilcoxon Signed Ranks Test.

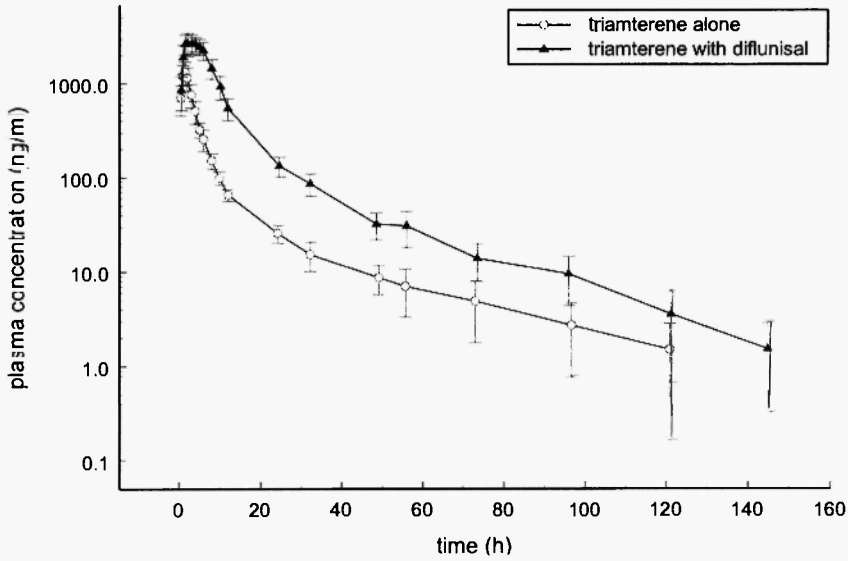

Fig. 2: Mean plasma concentration profile of *p*-hydroxytriamterene sulphate after a single dose of triamterene compared with the plasma concentration curve after the same dose of triamterene while on steady-state diflunisal. Error bars represent standard error of the mean.

Steady-state trough diflunisal concentrations determined for each subject in the blood sample taken prior to dosing with triamterene (89, 110, 111, 129, 99 and 90 μg diflunisal per ml plasma) were in the expected range /7/.

DISCUSSION

This study was designed to investigate the effect of steady-state coadministration of diflunisal on the disposition of triamterene. The underlying hypothesis was that, since the metabolism of both drugs involves the conjugation of phenolic groups with sulphate, one might see a metabolic interaction based on competition for available activated cosubstrate (3′-phosphoadenosine 5′-phosphosulphate) or for a common sulphotransferase isoform.

The steady-state trough diflunisal concentrations were similar for all subjects, including subject 4 who was given lower doses of di-

flunisal (because of substantially lower body weight). The pharmacokinetics of triamterene were unchanged in the presence of diflunisal, compared with those of triamterene taken alone. There was no evidence for a metabolic interaction involving the sulphonation pathway. However the plasma concentrations of the sulphonated metabolite, *p*-hydroxytriamterene sulphate, were much higher in the presence of diflunisal, and the AUC for this metabolite was 4.6 times greater than when triamterene was taken alone. Importantly, there were no significant changes in the protein binding of the metabolite or in its formation clearance during diflunisal coadministration. The higher plasma concentrations of *p*-hydroxytriamterene sulphate were associated with a 76% reduction in the renal clearance of this metabolite in the presence of diflunisal.

Interactions at the level of renal excretion have been reported for many drugs for which this is the major route of elimination /8/. Creatinine clearance, an indicator of glomerular filtration rate (GFR), was not measured in this study. It is highly unlikely that the reduction in CL_R of *p*-hydroxytriamterene sulphate during diflunisal coadministration is due to changes in GFR. In addition, since *p*-hydroxytriamterene sulphate is a hydrophilic compound, it is highly unlikely to undergo passive reabsorption. Taking into consideration the 99% level of protein binding of *p*-hydroxytriamterene sulphate, glomerular filtration should contribute only about 0.07 l/h (1% of GFR) to its renal clearance. The remainder (about 5 l/h) constitutes the great majority of its renal clearance and is most likely due to active tubular secretion. The reduction of CL_R of *p*-hydroxytriamterene sulphate during coadministration of diflunisal can thus be attributed to competition for active tubular secretion. Conducting triamterene-probenecid interaction studies could further test this, since probenecid is the best recognised inhibitor of the organic anion transport system.

Dickinson *et al.* /7/ have shown that the renal clearances of diflunisal acyl and phenolic glucuronides were reduced by 70% during probenecid interaction studies with diflunisal, confirming that these metabolites are excreted via the organic anion transport pump. Therefore the decrease in CL_R of *p*-hydroxytriamterene sulphate seen in the presence of diflunisal in the present study is most probably due to competition between all three organic anion metabolites of diflunisal and *p*-hydroxytriamterene sulphate for the transport pump.

Most of the pharmacokinetic data on triamterene were acquired in the late 1970s and early 1980s using methods of detection which did not allow the quantification of very low concentrations. For this reason several authors have published half-life values calculated from plasma concentration profiles recorded for only 10 to 36 h post-dose /3,9,10/. This has led to various reported half-lives of elimination, depending on which portion of the declining curve was used to calculate the slope. In addition, a few authors were not able to calculate half-life values from their data, as a result of apparent non-linearity of the terminal elimination phase /1,11/. Due to the sensitive method used in this study to quantify triamterene and *p*-hydroxy-triamterene sulphate, a true 'terminal' elimination phase has been defined. The plasma concentration curves of both triamterene and *p*-hydroxytriamterene sulphate declined in a biphasic manner and there were reasonable log-linear terminal elimination phases from which half-lives were calculated of approximately 17 h and 18 h for triamterene and *p*-hydroxytriamterene sulphate respectively. These values are much higher than any of the published data (2-12 h for both compounds) /3,9,10/.

In Australia, triamterene is available only as a combination tablet containing 50 mg triamterene and 25 mg hydrochlorothiazide. This is the formulation that was administered to the six volunteers in this study. Ideally we should have performed the present studies without the potential complicating factor of hydrochlorothiazide coadministration, but we were constrained to the use of drug formulations approved for marketing. Hydrochlorothiazide is eliminated unchanged into the urine also by the organic anion transport system /12/. However due to the relatively low single dose of hydrochlorothiazide (administered in both phases), the results of the present study are not expected to be influenced to any significant extent by its presence.

In the present study we were interested in the effect of diflunisal on triamterene, and obtained no information on the potential reverse interaction. Furthermore the extent to which these observations on single-doses of triamterene can be extrapolated to the continuous dosing situation requires further study. Comment should also be made on possible diflunisal-induced alterations to triamterene bioavailability, which is usually quoted at about 50% after oral administration /3,13/. However, the lack of effect of diflunisal coadministration on plasma triamterene profiles and urinary recoveries of triamterene and

p-hydroxytriamterene sulphate suggests an interaction on this level is unlikely.

In conclusion, a new drug-drug interaction has been identified. The mechanism for this interaction appears not to be metabolic, but rather a competition for renal excretory capacity for anionic conjugated metabolites. These drugs are coadministered in some patients, and the interaction could have some clinical significance given that the documented effect involves a pharmacologically active metabolite.

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