INTERACTION OF BEMETIZIDE AND INDOMETHACIN IN THE KIDNEY

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1 The effect of a single oral dose of 25 mg bemetizide on renal function without and with concomitant administration of the prostaglandin synthesis inhibitor indomethacin was investigated in ten healthy volunteers during sustained water diuresis.

2 Bemetizide induced a significant increase in urinary sodium and chloride excretion from 196 ± 30 and $163 \pm 28 \mu\text{mol/min}$ to 690 ± 54 and $537 \pm 51 \mu\text{mol/min}$ ($P < 0.01$). This effect occurred in the absence of changes in glomerular filtration rate, urinary excretion of phosphate or the delivery of chloride beyond the proximal nephron to the distal tubules (distal delivery) $[(C_{H_2O} + C_{Cl})/\text{GFR} \cdot 100]$, but was associated with a significant decrease in distal fractional chloride absorption (DFA_{Cl}) $[C_{H_2O}/(C_{H_2O} + C_{Cl})]$ from 0.84 ± 0.02 to 0.63 ± 0.02 ($P < 0.01$). Bemetizide also increased urinary excretion of prostaglandin (PG) E_2 .

3 Concomitant indomethacin administration significantly suppressed urinary excretion of PGE_2 and markedly decreased urinary excretion of sodium and chloride during control and following bemetizide administration. Indomethacin had no effect on glomerular filtration rate, urinary excretion of phosphate, distal delivery or the urinary excretion of bemetizide but significantly increased DFA_{Cl} both during control and after bemetizide administration.

4 Our results show that bemetizide as a thiazide-diuretic acts in the diluting segments of the nephron. Indomethacin administration induces retention of sodium and chloride and blunts the renal effects of bemetizide via increased absorption in the diluting segments. The interaction of both drugs most likely represents a pharmacodynamic interaction.

Keywords bemetizide indomethacin interaction renal function

Introduction

Considerable evidence supports the concept that renal prostaglandins (PG) may be modulators of renal blood flow and renal water and electrolyte excretion (Dunn & Hood, 1977; Düsing & Kramer, 1978). Administration of nonsteroidal anti-inflammatory drugs such as aspirin or indomethacin may promote salt retention in experimental animals and humans (Attallah & Lee, 1973; Frölich *et al.*, 1976; Düsing *et al.*, 1976, 1977, 1982; Leyssac *et al.*, 1975). This effect occurs in the absence of changes in glomerular filtration rate (GFR) or proximal tubular function (Higashihara *et al.*, 1979; Foman & Kauker, 1978; Düsing *et al.*, 1982). Moreover, *in vitro* experiments in isolated tubules (Iino & Imai, 1978; Stokes, 1979; Stokes & Kokko, 1977) and *in vivo* papillary and cortical micro-puncture studies in the rat (Higashihara *et al.*, 1979) and clearance studies in healthy humans (Düsing *et al.*, 1982) have shown that NaCl retention following administration of nonsteroidal anti-inflammatory drugs is associated

with increased NaCl absorption in the thick ascending limb of Henle and/or the cortical and outer medullary collecting tubule.

In addition to a possible role of prostaglandins in transtubular NaCl movement it has also been suggested that they may, at least in part, mediate the natriuretic and chloruretic action of some diuretics. Evidence for a possible interaction of diuretics with nonsteroidal anti-inflammatory drugs was first presented by Elliot (1962) and Tweedale & Ogilvie (1973), who demonstrated that the renal effects of spironolactone are markedly blunted during concomitant aspirin treatment. Some investigators have also found an increase in urinary prostaglandin excretion during frusemide treatment (Abe *et al.*, 1977; Brater *et al.*, 1980; Düsing *et al.*, 1982; Kramer *et al.*, 1980; Patak *et al.*, 1979; Williamson *et al.*, 1975) and that its natriuretic effect may be blunted during concomitant inhibition of prostaglandin synthesis (Chennavasin *et al.*, 1980;

Frölich *et al.*, 1976; Kramer *et al.*, 1980; Patak *et al.*, 1975). Hydrochlorothiazide also increases urinary PGE₂ excretion and this diuretic's renal effects may also be blunted during concomitant indomethacin treatment (Kramer *et al.*, 1980). The benzothiadiazine-derivative bemetizide [3-(α -methylbenzyl)-6-chloro-7-sulphamyl-3,14-dihydro-1,2, 4-benzothiadiazine-1,1-dioxide] is functionally classified as a thiazide-diuretic (Deetjen, 1980; Jacobi & Fontaine, 1966 a,b; Noack, 1980). Data on its tubular site of action in man and a possible interaction with nonsteroidal anti-inflammatory drugs are not available to date.

The present study was therefore designed to investigate the tubular site of action of bemetizide in healthy human volunteers using clearance methods and to determine the mechanism of a possible interaction with indomethacin.

Methods

The study was performed in 10 healthy volunteers (five male and five female medical students), aged 22–27 years, while they were on a constant intake of 150 mmol/day of sodium and 80 mmol/day of potassium. The purpose of the study and the nature of all procedures were explained in detail, and informed consent was obtained from all volunteers. After overnight fasting, all subjects received 20 ml/kg body weight of water between 07.00 and 09.00 h. Starting at 08.00 h, the volunteers were given 5% dextrose in water intravenously at a rate of 0.2 ml min⁻¹ kg⁻¹ body weight. Urine was collected during five consecutive 45 min clearance periods beginning at 08.45 h. The first clearance period served as a control period (C). At 09.30 h all volunteers received a single oral dose of 25 mg bemetizide which was followed by four experimental clearance periods I–IV. Urine was collected by voluntary voiding and venous blood was drawn through an indwelling needle at the mid-point of each clearance period. In all volunteers, the same experimental protocol was repeated after a 10 day interval. In this second study, the volunteers received 100 mg of indomethacin p.o. at 22.00 h on the previous day and another 100 mg at 07.00 h on the day of the study.

Serum and urinary sodium and potassium concentrations were determined by internal-standard flame photometry; serum and urinary chloride concentrations were measured with a Corning chloride meter. Urinary phosphate concentration was determined photometrically. Serum and urinary osmolalities were measured with a Knauer osmometer. Creatinine concentrations in plasma and urine were determined enzymatically (Wahlefeld *et al.*, 1974). Urinary excretion of immuno-reactive PGE₂ was determined by radioimmunoassay according to previously described methods (Jaffe *et al.*, 1973). In brief, 2 ml urine were

acidified with 0.1 N HCl (pH 3.5) and extracted twice with ethyl acetate. The organic phase was removed and evaporated to dryness under a nitrogen stream. The extract was then further purified by silicic acid column chromatography. The PGE fraction was quantified by radioimmunoassay using a highly specific PGE₂ antibody (Institut Pasteur, Paris/France). The specificity and sensitivity of this antiserum have been reported previously (Dray *et al.*, 1975). [³H]-PGE₂ (New England Nuclear, Boston, Mass. /USA) was purified by column chromatography before use as recovery trace and in the assay. Unlabeled PGE₂ was kindly provided by the Upjohn Co., Kalamazoo, Mich. /USA. All assays were performed in duplicate. Results were corrected for recoveries and expressed as pmol/min. Urinary excretion of bemetizide was measured according to methods previously described (Brodie *et al.*, 1978).

GFR was estimated as clearance of endogenous creatinine. Clearance of free water (C_{H₂O}) was calculated by the formula $C_{H_2O} = V - U_{osm} \bar{V} / S_{osm}$, where V = urine volume, U_{osm} = urinary osmolality and S_{osm} = serum osmolality; the clearance of chloride (C_{Cl}) was calculated by the formula $C_{Cl} = U_{Cl} V / S_{Cl}$, where U_{Cl} = urinary chloride concentration and S_{Cl} = serum chloride concentration. Delivery of chloride beyond the proximal nephron to the distal tubules (distal delivery) and distal fractional chloride absorption (DFA_{Cl}) were estimated by clearance methods and calculated as $[(C_{H_2O} + C_{Cl} / GFR \cdot 100)]$ and $[C_{H_2O} / C_{H_2O} + C_{Cl}]$, respectively (Danovitch & Bricker, 1976).

Statistical analysis was performed using the Wilcoxon matched-pairs signed rank test (Milton, 1964). Results are presented as means $\pm$ s.e. mean.

Results

Effects of bemetizide on renal function

In both protocols, urinary osmolality had fallen to below 80 mosm/kg H₂O in all volunteers before the control clearance period. The effect of a single oral dose of 25 mg bemetizide on renal function during sustained water diuresis is summarized in Table 1. Due to the maximal water diuresis already achieved during the control period, bemetizide had no effect on urine volume. Both, $U_{Na} V$ and $U_{Cl} V$ remained unchanged during the first clearance period after drug administration and progressively increased from the second to the fourth clearance period. No change in $U_K V$ could be noted following bemetizide administration. The effects of bemetizide occurred in the absence of changes in GFR, urinary excretion of inorganic phosphate ($U_{Phos} V$) and distal delivery, but were associated with a significant decrease in distal fractional chloride absorption (Figure 1).

Table 1 Urine volume (V), urinary excretion of sodium ($U_{Na}V$), potassium (U_KV), chloride ($U_{Cl}V$) and inorganic phosphate ($U_{Phos}V$), creatinine clearance (C_{Cr}), distal delivery (DD) and distal fractional absorption of chloride (DFA_{Cl}) during control (C) and during four consecutive 45 min clearance periods following an oral dose of 25 mg bemetizide in 10 healthy volunteers during sustained water diuresis.

	C	I	II	III	IV
V (ml/min)	15.46 ± 1.21	15.43 ± 0.88	15.49 ± 1.02	15.41 ± 1.22	16.55 ± 1.21
$U_{Na}V$ (μmol/min)	196 ± 30	218 ± 16	441* ± 64	631* ± 62	690* ± 54
U_KV (μmol/min)	65 ± 11	50 ± 8	61 ± 10	72 ± 9	77 ± 10
$U_{Cl}V$ (μmol/min)	163 ± 28	141 ± 21	375* ± 64	533* ± 54	537* ± 51
$U_{Phos}V$ (μmol/min)	20.3 ± 1.7	17.7 ± 1.0	18.6 ± 1.5	16.7 ± 2.4	19.5 ± 2.4
C_{Cr} (ml/min)	126 ± 5	124 ± 8	123 ± 6	125 ± 4	125 ± 4
DD (ml min ⁻¹ 100 ml ⁻¹ GFR)	9.68 ± 0.85	9.72 ± 0.84	10.69 ± 0.95	11.86 ± 0.86	11.35 ± 0.91
DFA_{Cl}	0.84 ± 0.02	0.88 ± 0.02	0.74* ± 0.03	0.61* ± 0.02	0.63* ± 0.02

* $P < 0.01$ as compared to control values (C)

Figure 1 Distal fractional chloride absorption [$C_{H_2O}/(C_{H_2O} + C_{Cl})$] plotted as a function of distal delivery [$(C_{H_2O} + C_{Cl})/GFR \cdot 100$] in 10 healthy volunteers during control (●) and during the fourth clearance period following a single oral dose of 25 mg bemetizide (○).

Effects of indomethacin during control periods and during concomitant bemetizide administration

During the control period indomethacin pretreatment significantly decreased $U_{Na}V$ and $U_{Cl}V$ by an average of 34 and 55%, respectively. The NaCl retention was not accompanied by changes in urine volume, GFR, distal delivery or $U_{Phos}V$, but was associated with a marked increase in the distal fractional absorption of chloride ($P < 0.01$) (Table 2).

The effect of bemetizide on urinary NaCl excretion was markedly blunted during concomitant indomethacin treatment. During the fourth clearance period, $U_{Na}V$ and $U_{Cl}V$ were decreased by an average of 47 and 44%, respectively, as compared to treatment with bemetizide alone. This blunted natriuretic and chloruretic effect of bemetizide was not associated with changes in urine volume, GFR, $U_{Phos}V$ or distal delivery. In contrast, during all clearance periods following bemetizide, distal fractional chloride absorption was significantly higher during concomitant indomethacin treatment as compared to bemetizide administration alone (Table 2).

Urinary PGE_2 excretion

$U_{PGE_2}V$ during control and during the four experimental clearance periods without and with concomitant indomethacin administration is illustrated in

Table 2 Urine volume (V), urinary excretion of sodium ($U_{Na} V$), potassium ($U_K V$), chloride ($U_{Cl} V$) and inorganic phosphate ($U_{Phos} V$), creatinine clearance (C_{Cr}), distal delivery (DD) and distal fractional absorption of chloride (DFA_{Cl}) during control (C) and during four consecutive 45 min clearance periods following an oral dose of 25 mg bemetizide during concomitant indomethacin administration in 10 healthy volunteers during sustained water diuresis.

	C	I	II	III	IV
V (ml/min)	15.75 $\pm$ 1.21	15.03 $\pm$ 0.72	14.48 $\pm$ 0.76	14.63 $\pm$ 0.94	14.13 $\pm$ 0.72
$U_{Na} V$ (μ mol/min)	129* $\pm$ 14	166* $\pm$ 29	204* $\pm$ 32	342* $\pm$ 51	363* $\pm$ 51
$U_K V$ (μ mol/min)	64 $\pm$ 9	55 $\pm$ 10	52 $\pm$ 8	70 $\pm$ 9	76 $\pm$ 12
$U_{Cl} V$ (μ mol/min)	73* $\pm$ 13	85* $\pm$ 21	121* $\pm$ 36	261* $\pm$ 52	301* $\pm$ 56
$U_{Phos} V$ (μ mol/min)	20.9 $\pm$ 2.2	18.9 $\pm$ 1.6	18.9 $\pm$ 1.6	17.6 $\pm$ 1.2	19.3 $\pm$ 2.1
C_{Cr} (ml/min)	122 $\pm$ 5	121 $\pm$ 5	124 $\pm$ 4	123 $\pm$ 4	123 $\pm$ 4
DD (ml min ⁻¹ 100 ml ⁻¹ GFR)	9.20 $\pm$ 0.85	8.92 $\pm$ 0.48	8.87 $\pm$ 0.46	9.64 $\pm$ 1.34	9.45 $\pm$ 0.81
DFA_{Cl}	0.95* $\pm$ 0.01	0.93* $\pm$ 0.02	0.89* $\pm$ 0.04	0.78* $\pm$ 0.04	0.76* $\pm$ 0.04

* $P < 0.01$ as compared to the experiments without indomethacin (results in Table 1).

Figure 2 $U_{PGE_2} V$ during control (C) and four clearance periods following a single oral dose of 25 mg bemetizide without (▨) and with concomitant indomethacin treatment (□).

Figure 2. During control, $U_{PGE_2} V$ averaged 2.24 ± 0.36 pmol/min and significantly increased to 5.10 ± 0.98 pmol/min ($P < 0.01$) during the fourth clearance period following bemetizide administration. Indomethacin significantly decreased $U_{PGE_2} V$ both during the control period (0.71 ± 0.16 pmol/min; $P < 0.01$) and after bemetizide administration ($P < 0.01$).

Excretion of bemetizide in the urine

Significant urinary excretion of bemetizide was observed already during the second clearance period following bemetizide administration and averaged 0.63 ± 0.19 nmol/min. The excretion rate increased to 2.52 ± 0.99 and 4.34 ± 0.50 nmol/min during the third and fourth clearance periods. Concomitant indomethacin administration had no effect on urinary excretion of the diuretic and averaged 1.05 ± 0.33 , 3.32 ± 0.42 and 4.37 ± 0.80 nmol/min respectively, during clearance periods II-IV.

Discussion

Clearance methods have repeatedly been employed to localize the site of action of diuretics (Seldin *et al.*, 1966; Seldin & Rector, 1973). The assessment of proximal and distal tubular function by the indirect clearance techniques used in the present study rests

on several assumptions which have been described in detail elsewhere (Danovitch & Bricker, 1976). Of critical importance is the requirement that the formation of solute-free water accurately represents distal tubular NaCl absorption. Therefore, in the present study, experiments were performed during maximal water diuresis to suppress the release of endogenous antidiuretic hormone. However, since water movement in the distal nephron may occur even in the absence of antidiuretic hormone (Jamison *et al.*, 1971), it is obvious that the clearance terms used in the present study can only be approximations of proximal and distal tubular reabsorptive capacity. In the present study, GFR was estimated by the clearance of endogenous creatinine, proximal tubular function assessed by urine volume, distal delivery, $U_{\text{Phos}} V$ and distal nephron function by the calculation of distal fractional chloride absorption.

Our results show that bemetizide in the dose administered induces a moderate 3.5-fold increase in $U_{\text{Na}} V$ and a 3.3-fold increase in $U_{\text{Cl}} V$. Since GFR and all three parameters of proximal tubular function were unchanged, the natriuretic and chloruretic effects of bemetizide are entirely due to an inhibitory action on tubular NaCl absorption in the diluting segments of the nephron. This is in agreement with the results of microperfusion experiments which also characterized the ascending limb of Henle as the site of action of bemetizide (Deetjen, 1980). Thus, bemetizide's mode of action closely resembles that of other benzothiadiazine-derivatives such as chlorothiazide (Clapp & Robinson, 1968; Earley *et al.*, 1961; Heinemann *et al.*, 1959; Laragh *et al.*, 1958). The complete lack of a proximal tubular effect of bemetizide demonstrates that this diuretic, in contrast to chlorothiazide and frusemide, does not possess carbonic anhydrase inhibitory activity (Goldberg *et al.*, 1973).

Under the conditions chosen in the present study, bemetizide had no effect on $U_{\text{K}} V$. In addition to a diuretic-induced secondary aldosteronism it has been suggested that the rise in distal tubular flow rate is responsible for renal potassium losses during diuretic therapy (Good & Wright, 1979). In the present study,

due to the maximal water diuresis already established before bemetizide administration, the 'diuretic' did not increase urine volume and presumably distal tubular flow rate with the consequence of unchanged $U_{\text{K}} V$.

Furthermore, our results confirm earlier studies which demonstrated that pharmacological inhibition of prostaglandin synthesis may induce NaCl retention (Attallah & Lee, 1973; Düsing *et al.*, 1976, 1977, 1982; Frölich *et al.*, 1976; Leyssac *et al.*, 1975). Also in agreement with various other studies is the localization of the effect of indomethacin in the diluting segments of the distal tubule (Düsing *et al.*, 1982; Iino & Imai, 1978; Higashihara *et al.*, 1979; Stokes, 1979; Stokes & Kokko, 1977).

In the present study, the natriuretic and chloruretic effects of bemetizide were markedly blunted during concomitant administration of indomethacin. One possible mechanism of this effect is a pharmacokinetic drug interaction. However, time course and total amount of bemetizide excretion were similar in both protocols without and with concomitant indomethacin treatment. In contrast, our finding of a markedly decreased natriuretic and chloruretic response to bemetizide in the presence of unchanged urinary excretion of the diuretic and supposedly similar local concentrations in the distal nephron during concomitant indomethacin treatment favours a pharmacodynamic interaction of both drugs. This is in agreement with the results of a study investigating the mechanism of the indomethacin-frusemide interaction in man (Chennavasin *et al.*, 1980). In this study, indomethacin markedly altered the dose-response relationship of frusemide also indicating a pharmacodynamic interaction of these two drugs. The exact mechanism of the pharmacodynamic interaction of bemetizide and indomethacin, however, remains unclear.

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