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Effects of acetazolamide on kidney function in Type 1 (insulin-dependent) diabetic patients with diabetic nephropathy

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Summary. We investigated the effects of 3 days treatment with acetazolamide 250 mg three times daily on kidney function in 8 Type 1 (insulin-dependent) diabetic patients with nephropathy, and in 7 healthy subjects in a double-blind placebo controlled cross-over study. Glomerular filtration rate and extracellular fluid volume were measured with the single injection ^{51}Cr-EDTA technique and fluid flow rate from the proximal tubules was determined by measurement of the renal lithium clearance. A 24% decline in glomerular filtration rate was observed in both groups during acetazolamide treatment (control subjects: 108 ± 11 vs 82 ± 9 ml/min, $p < 0.02$, diabetic patients: 71 ± 19 vs 54 ± 14 ml/min, $p < 0.01$). The renal lithium clearance (ml/min) remained about the same (control subjects: 22 ± 6 vs 27 ± 8, NS, diabetic patients: 14 ± 5 vs 15 ± 4, NS). Absolute proximal tubular reabsorption of water (ml/min) was reduced by about one-third (control subjects: 85 ± 11 vs 56 ± 7, $p < 0.02$, diabetic patients: 55 ± 17 vs 37 ± 6, $p < 0.02$), and fractional proximal reabsorption of water and sodium (%) declined (control subjects: 79 ± 5 vs 67 ± 8, $p < 0.02$, diabetic patients: 79 ± 5 vs 72 ± 6, $p < 0.02$). Renal sodium clearance and distal fractional reabsorption of sodium was unchanged. Extracellular fluid volume declined by 10% in both groups ($p < 0.02$). Albuminuria and fractional albumin clearance decreased significantly in the nephropathic patients ($p < 0.02$). Our study suggests that the effects of acetazolamide on kidney function are similar in healthy subjects and patients with diabetic nephropathy.

Key words: Acetazolamide, albuminuria, diabetic nephropathy, glomerular filtration rate, lithium clearance, normal subjects, proximal tubules, sodium excretion.

Proliferative retinopathy and secondary glaucoma are much more common in Type 1 (insulin-dependent) diabetic patients with diabetic nephropathy than in those without. Carbonic anhydrase inhibitors are widely used to treat glaucoma because they reduce the rate of formation of aqueous humour. Recently we observed large reductions in glomerular filtration rate (GFR) during long-term treatment with carbonic anhydrase inhibitors in 3 Type 1 diabetic patients with nephropathy and glaucoma [1]. Controlled studies on the renal effects of acetazolamide in man are not available. Intravenous injection of acetazolamide induces a slight (10–20%) decline in GFR in normal man [2, 3]. Micropuncture studies in rats have shown reduced proximal tubular reabsorption of fluid and electrolytes, suggesting activation of the tubuloglomerular feedback mechanism as the main cause of the reduced GFR during intravenous administration of acetazolamide [4, 5].

To evaluate the effects of acetazolamide on GFR and on proximal tubular reabsorption of fluid and sodium in diabetic nephropathy, we performed a double-blind placebo controlled cross-over study in Type 1 diabetic patients with nephropathy as compared to healthy control subjects.

Subjects and methods

Subjects

Nine consecutive non-uraemic Type 1 diabetic patients with diabetic nephropathy were investigated (Table 1). Seven patients received antihypertensive treatment, including captopril (3 patients) and furosemide (7 patients), which remained unchanged throughout the whole investigation. All patients received at least two daily injections of insulin. Diabetic nephropathy was diagnosed clinically according to previously described criteria [6]. Seven healthy subjects served as a control group. All subjects took their usual diet.

The subjects agreed to participate in the study after being given oral and written information about the procedure according to the Helsinki Declaration, and the study was approved by the local ethical committee.

One of the diabetic patients completed only one part of the study. She was admitted to Hvidøre Hospital the night before the second study because of severe orthostatic hypotension, peripheral paraesthesia and poor metabolic control. The code was broken, revealing that she had received placebo in the first study and acetazolamide in the second. Thus, she was excluded from the study. Another patient had forgotten to take his lithium tablets before one of the clearance studies and was not included in the analysis of the tubular effects of acetazolamide.

Methods

The effects of 3 days treatment with acetazolamide 250 mg three times daily were studied in a double-blind placebo controlled cross-

Table 1. Clinical data in seven control subjects and eight Type 1 (insulin-dependent) diabetic patients with diabetic nephropathy

	Control subjects	Diabetic patients
Age (years)	33 ± 6	42 ± 10
Sex	3 F/4 M	1 F/7 M
Body mass index (kg/m^2)	21.6 ± 2.3	23.3 ± 1.4
Systolic blood pressure (mm Hg)	110 (98–123)	133 (120–146)
Diastolic blood pressure (mm Hg)	68 (62–79)	78 (72–90)
Duration of diabetes (years)	-	23 ± 7
Urinary albumin excretion (mg/24 h)	9 (4–12)	1025 (112–2556)
Haemoglobin A_{1C} (%)	5.0 (4.4–5.7)	9.0 (5.4–11.4)
Retinopathy (simplex/proliferative)	-	3/5
Insulin dosage ($units \cdot kg^{-1} \cdot day^{-1}$)	-	0.54 ± 0.11

Values are shown as mean ± standard deviation or as median with range in parentheses

Table 2. Renal variables in seven control subjects and eight Type 1 diabetic patients with diabetic nephropathy with and without treatment with acetazolamide 250 mg three times daily

	Control subjects		Diabetic patients	
	Placebo	Acetazolamide	Placebo	Acetazolamide
Glomerular filtration rate (ml/min)	108 ± 11	82 ± 9[b]	71 ± 19	54 ± 14[a]
Lithium clearance (ml/min)	22 ± 6	27 ± 8	14 ± 5	15 ± 4
Urine flow rate (ml/min)	2.8 ± 0.7	2.6 ± 1.3	3.6 ± 1.6	2.4 ± 0.8[c]
Fractional excretion of sodium (%)	1.2 ± 0.5	1.2 ± 0.5	2.9 ± 2.2	1.8 ± 1.3
Renal sodium clearance (ml/min)	1.3 ± 0.4	1.1 ± 0.3	1.9 ± 1.2	1.0 ± 0.6
Renal potassium clearance (ml/min)	15 ± 6	24 ± 6[b]	21 ± 10	19 ± 6

Mean ± standard deviation indicated. [a] $p < 0.01$, [b] $p < 0.02$, [c] $p < 0.05$ vs placebo period

over design. The study periods were separated by two weeks. The subjects were instructed to perform 24-h urine collections during each of the 3 days treatment periods.

The subjects took 600 mg (16.2 mmol) of lithium carbonate (DAK Laboratories, Copenhagen, Denmark) orally the evening before the clearance study. From midnight until the end of the study, no methylxanthine containing beverages and no smoking were allowed.

The clearance studies were performed on the morning of the fourth day of treatment. At the start of the clearance studies, the subjects took the last dose of acetazolamide or placebo. During the clearance studies all subjects were in the supine position, except when voiding. The subjects drank about 150 ml of tap water per h.

GFR and extracellular fluid volume were determined from the total ^{51}Cr-EDTA plasma clearance as previously described [6]. In our hands this method has a coefficient of variation of 2.8% for GFR [6]. The coefficient of variation for extracellular fluid volume in 11 subjects with diabetic nephropathy was 7.5%.

Urine and serum concentrations of lithium were determined by atomic absorption spectrophotometry (Perkin Elmer 2380, Norwalk, Conn, USA) as described by Amdisen [7]. Serum lithium concentrations were determined at the beginning and at the end of the 4-h clearance period. Urine and serum concentrations of sodium and potassium were determined by flame emission spectrophotometry. Urinary albumin concentration was measured by radioimmunoassay [8] and urinary glucose concentration by the hexokinase method [9]. Blood glucose concentration (Hypocount B, Hypoguard, Woodbridge, UK) was measured at least hourly, and supplementary fast acting insulin (Insulin Actrapid, Novo, Bagsværd, Denmark) was given intravenously if blood glucose concentration exceeded 15 mmol/l.

Blood pressure was measured with Hawksley Random Zero sphygmomanometer (Hawksley and Sons, Lancing, UK). Diastolic pressure was read at the disappearance of sounds.

The renal clearance of lithium (C_{Li}) was calculated as the ratio between the urinary excretion rate of lithium and the interpolated mean serum lithium concentration during the 4-h clearance period [10]. The coefficient of variation for renal lithium clearance was 14.8%. The renal sodium clearance was similarly calculated as the ratio between the urinary excretion rate of sodium und serum sodium concentration. The absolute reabsorption rate of water in the proximal tubules was determined as (GFR-C_{Li}), and the absolute proximal reabsorption rate of sodium as the product of serum sodium concentration and absolute proximal reabsorption of water. The fractional reabsorption of sodium and water in the proximal tubules was determined as 1 - (C_{Li}/GFR). The absolute distal reabsorption rate of water was calculated as C_{Li} - urine flow and the fractional distal water reabsorption as 1-urine flow/C_{Li}. The absolute distal reabsorption rate of sodium was calculated as C_{Li} minus sodium clearance multiplied by plasma sodium concentration, and fractional distal sodium reabsorption was determined as the difference between C_{Li} and renal sodium clearance divided by C_{Li}.

If osmotically active substances, such as glucose, are present in the endproximal tubular fluid, a dissociation between distal delivery of sodium and distal delivery of water is induced [11]. It is currently unknown if lithium clearance in this situation measures distal delivery of sodium or distal delivery of water. An estimate of the size of the uncertainty induced by the presence of glucosuria on the interpretation of C_{Li} and derived variables was obtained by assuming that the glucosuria represented the amount of glucose leaving the proximal tubules. Since the proximal tubular fluid is isomotic with plasma, it can be calculated from an estimate of plasma osmolarity (2 · plasma sodium concentration + 2 · plasma potassium concentration + mean blood glucose concentration), how much water was osmotically obliged by the glucosuria. This amount of water represents the uncertainty induced by glucosuria.

Haemoglobin A_{1c} was measured as previously described [12]. Serum acetazolamide concentrations were measured by high performance liquid chromatography; 0.5 ml serum was extracted with ethylacetate:dichloromethane, reconstituted in methanol, and eluted on a LiChrosorp RP 18 column (10 µm, 25 cm) using acetonitril with phosphoric acid in water as mobile phase. Acetazolamide was detected with an ultraviolet detector at 264 nm using an internal standard. Plasma bicarbonate concentrations were measured using conventional laboratory technique.

Statistical analysis

The Wilcoxon test for paired differences with Pratts modification was used for comparisons within each of the two groups investigated [13]. A p-value of < 0.05 was considered significant. Data are reported as mean ± standard deviation or as median with range in parenthesis.

Table 3. Calculated segmental renal tubular reabsorption in seven control subjects and eight Type 1 diabetic patients with diabetic nephropathy with and without treatment with acetazolamide 250 mg three times daily

	Control subjects		Diabetic patients	
	Placebo	Acetazolamide	Placebo	Acetazolamide
Absolute proximal reabsorption rate of water (ml/min)	85±11	56±7[a]	55±17	37±6[a]
Absolute proximal reabsorption rate of sodium (mmol/min)	11.8±1.5	7.7±1.1[a]	7.5±2.3	5.0±1.6[a]
Fractional proximal sodium (or water) reabsorption (%)	79±5	67±8[a]	79±5	72±6[a]
Absolute distal reabsorption rate of sodium (mmol/min)	2.9±0.8	3.7±1.2[b]	1.7±0.6	1.8±0.5
Absolute distal reabsorption rate of water (ml/min)	20±6	24±7[b]	11±4	12±4
Fractional distalsodium reabsorption (%)	94±3	95±2	87±9	94±4
Fractional distal water reabsorption (%)	87±3	91±3[b]	74±12	84±5[a]

Mean ± standard deviation indicated. [a] $p < 0.02$, [b] $p < 0.05$ vs placebo period

Table 4. Blood glucose concentration, urinary albumin excretion rate, extracellular fluid volume, mean arterial blood pressure and venous plasma bicarbonate concentration in seven control subjects and eight Type 1 diabetic patients with diabetic nephropathy with and without treatment with acetazolamide 250 mg three times daily

	Control subjects		Diabetic patients	
	Placebo	Acetazolamide	Placebo	Acetazolamide
Mean blood glucose concentration during investigation (mmol/l)	4.3±0.5	4.4±0.5	13.2±6.0	15.6±4.4
Urinary albumin excretion rate during investigation (μg/min)[d]	6 (3-31)	6 (1-21)	705 (44-1493)	20[a] (11-1300)
Extracellular fluid volume (l)	14.4±1.9	13.1±2.2[b]	15.1±1.6	13.6±2.1[a]
Mean arterial blood pressure (mm Hg)[d]	83 (74-93)	81 (75-94)	95 (89-109)	93[c] (75-99)
Plasma bicarbonate concentration (mmol/l)	22±2	16±2[c]	24±3	18±3[c]

Mean ± standard deviation indicated. [a] $p < 0.01$, [b] $p < 0.02$, [c] $p < 0.05$ vs placebo period, [d] median and range indicated

Table 5. Time course of 24-h sodium excretion (mmol/24 h) during treatment with acetazolamide 250 mg three times daily for three days in 7 healthy control subjects and 8 patients with diabetic nephropathy

	Placebo period (mean of 3 days)	Acetazolamide treatment		
		Day 1	Day 2	Day 3
Healthy subjects	146±39	343± 58[a]	155±51	175± 89
Diabetic patients	153±33	238±120[b]	181±61	194±136

Mean ± standard deviation indicated. [a] $p < 0.02$, [b] $p < 0.05$ vs placebo period

Table 6. Estimates of the influence of glucosuria on the interpretation of indices of tubular function in patients with diabetic nephropathy

	Placebo	Acetazolamide
Glucosuria (μmol/min)	52 (0-256)	10 (0-170)
Estimated isosmotic volume represented by the glucosuria (ml/min)	0.18 (0-0.86)	0.03 (0-0.59)
Possible percentage error induced by glucosuria on the estimate of		
Lithium clearance	1 (0-8)	0 (0-5)
Absolute proximal reabsorption rate of water or sodium	0 (0-3)	0 (0-3)
Absolute distal reabsorption rate of sodium	1 (0-10)	0 (0-5)
Absolute distal reabsorption rate of water	2 (0-12)	0 (0-7)

Median and range indicated

Results

In both control subjects and in patients with diabetic nephropathy acetazolamide induced a 24% decline in GFR, and a 33% reduction in absolute proximal reabsorption rate of sodium and water ($p < 0.02$) (Tables 2 and 3). The output of water from the descending straight part of the proximal tubules into the descending limb of Henle's loop as estimated by lithium clearance did not change significantly (Table 2). Distal fractional sodium reabsorption was unchanged, while an increase in fractional distal water reabsorption took place in both groups ($p < 0.05$) (Table 3).

During acetazolamide treatment urinary albumin excretion rate during the clearance period was unaffected in the control subjects, while it declined in all patients (Table 4). Fractional albumin clearance also fell significantly in the patients from a median value of 365×10^{-6} to 189×10^{-6} ($p < 0.02$). Extracellular fluid volume declined significantly by 10% in both groups. Mean arterial blood pressure did not change in the control subjects, while in the diabetic patients it fell significantly ($p < 0.05$).

Plasma bicarbonate concentration declined during acetazolamide treatment in both control subjects and

diabetic patients. None of the patients developed ketonuria.

As compared to the natriuresis during the placebo period, the natriuresis was significantly enhanced only on the first day of acetazolamide treatment in both control subjects and patients (Table 5).

Mean serum acetazolamide concentrations at 08.00 hours and 13.00 hours was 58 ± 41 and 59 ± 13 μmol/l during active treatment and zero during placebo in the control subjects, and 42 ± 24 and 60 ± 26 μmol/l during active treatment and below detection limit during placebo in the diabetic patients.

The uncertainty on the estimates of tubular function induced by glucose induced osmotic diuresis is shown in Table 6.

The mean supplementary dose of insulin necessary during the clearance study on the acetazolamide day was 7.9 U, range 0–29 U, and on the placebo day 3.0, range 0–10 U.

Discussion

We have shown that treatment for three days with acetazolamide induces a 24% reduction in GFR and a 33% reduction in absolute proximal reabsorption of sodium and water in healthy subjects and in patients suffering from diabetic nephropathy. The renal functional disturbances induced by acetazolamide seem fully reversible on discontinuation of the drug.

In the human studies on the acute effects of intravenous administration of carbonic anhydrase inhibitors, the average decline in GFR ranged from 12% to 20% [2, 3, 14, 15]. One study found no decline after oral administration of acetazolamide [16]. In the present study, the natriuretic and diuretic effect was only observed on the first day of acetazolamide treatment. One previous study on the long term effects of carbonic anhydrase inhibition on GFR in humans included only 3 subjects [17], in all of whom GFR declined. In a large study 2–4 weeks of carbonic anhydrase inhibition did not change serum creatinine significantly [18], but the limited value of serum creatinine or even creatinine clearance as a marker of GFR has been repeatedly documented [19].

Experimental studies in animals have shown that carbonic anhydrase inhibitors reduce proximal tubular reabsorption of water and sodium [4, 5, 20–22]. Acute administration of acetazolamide has been reported to increase lithium clearance and reduce proximal tubular reabsorption of sodium and water in man [16]. The acute decline in GFR is probably mediated by the tubulo-glomerular feedback mechanism [4, 5, 20, 22].

Mean arterial blood pressure was unaffected by acetazolamide in the healthy subjects, but declined significantly in the patients with diabetic nephropathy. The drop in blood pressure may have contributed to the fall in GFR, as defective autoregulation of GFR has previously been demonstrated in diabetic nephropathy [23].

The overall renal response to acetazolamide was similar in the patients and control subjects, even in patients treated with furosemide, which in acute animal experiments blocks the tubulo-glomerular feedback [24]. Also in patients treated with captopril, the response was similar, although an animal study concluded that angiotensin II mediated the fall in GFR induced by benzolamide [20]. Further studies are needed to evaluate the influence of these changes in a setting of chronic treatment in man. Irrespective of the possible mechanisms, the fluid flow rate from the end of the proximal tubules is a closely regulated parameter, even in early diabetic renal failure.

Patients with diabetic nephropathy and low GFR may be especially sensitive to the volume depletion induced by acetazolamide. The patient who did not complete the present study had a control GFR of 28 ml/min. The patient previously reported presenting with a similar clinical picture of hypotension and dehydration also had a low GFR of 23 ml/min [1].

Urinary albumin excretion rate was unaffected by acetazolamide in the healthy subjects. In the patients with diabetic nephropathy, both urinary albumin excretion rate and fractional albumin clearance declined. This finding may reflect a reduction in transglomerular hydraulic pressure gradient. Proximal intratubular hydrostatic pressure was probably unchanged, since lithium clearance was unchanged. This led us to suggest that acetazolamide induces a reduction in intraglomerular capillary hydraulic pressure.

The validity of the renal lithium clearance as a quantitative estimate of the output of fluid from the proximal tubules has been established in animals [25, 26]. It has been suggested that lithium is reabsorbed also in Henle's loop [27]; but even if this occurs, the reabsorption must be considered quantitatively insignificant [28]. High rates of glucosuria induce a dissociation between the output of water and of sodium from the proximal tubules. Theoretically, this makes the interpretation of lithium clearance difficult [11]. The errors induced by the rates of glucosuria observed in the present study are minimal and primarily affect the estimates of distal tubular function (Table 6). Furthermore, the changes observed in tubular function were qualitatively and quantitatively similar in the healthy control group. Uraemia may also induce significant osmotic diuresis. However, none of the patients completing the study had GFR below 30 ml/min during any part of the study.

Major changes in lithium clearance can be induced by alterations in sodium intake [29]. Sodium clearance and urinary sodium excretion during the clearance studies were similar on the placebo and the acetazolamide study days. Therefore, the present findings are not affected by changes in these parameters. Since sodium excretion was normalised after three days of

acetazolamide treatment, we suggest that the changes observed are similar to the changes induced by long-term treatment.

The increased dosage of insulin given to the diabetic patients during active treatment did not affect the observed changes in proximal tubular function, since insulin stimulates distal tubular reabsorption of sodium in animals and man without affecting proximal reabsorption of sodium and water [30, 31].

Acetazolamide should be used with caution when treating glaucoma in patients with diminished renal function, since it may produce a clinically relevant reduction in renal function. We recommend that renal function be monitored closely during initiation of treatment with acetazolamide in diabetic patients.

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