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

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Summary. We investigated whether the glomerular synthesis of prostaglandins modulates the glomerular filtration rate and albuminuria in diabetic nephropathy. The urinary excretion of immunoreactive prostaglandin E_2 (253 pg/min) was significantly elevated in eight Type 1 (insulin-dependent) diabetic women with nephropathy as compared with nine normoalbuminuric Type 1 diabetic women (95 pg/min) and 11 non-diabetic women (132 pg/min), respectively ($p < 0.01$). Glomerular filtration rate (single bolus ^{51}Cr-EDTA technique) and albuminuria (radioimmunoassay) were measured twice within two weeks in the eight Type 1 diabetic women with nephropathy. All eight patients were on a diabetic diet without sodium restriction. The study was performed as a randomized double-blind trial, with the patients receiving either indomethacin (150 mg/day) or placebo for three days prior to the kidney function studies. Indomethacin treatment induced a significant reduction in urinary prostaglandin E_2 excretion (73%, $p < 0.01$), glomerular filtration rate diminished from 120 ± 18 to 106 ± 17 ml/min/1.73 m^2 ($p < 0.05$), albuminuria declined from 148 to 69 μg/min (median and range) ($p < 0.05$) and fractional clearance of albumin diminished 42% ($p < 0.05$). Blood glucose concentrations were comparable during the placebo and indomethacin treatment, 13.4 ± 4 versus 14.2 ± 3 mmol/l, respectively. Our results suggest that glomerular filtration rate in early diabetic nephropathy is dependent on the enhanced glomerular synthesis of vasodilating prostaglandins.

Key words: Albuminuria, diabetic nephropathy, glomerular filtration rate, prostaglandins, Type 1 diabetes.

Diabetic nephropathy is the major cause of the increased morbidity and mortality in Type 1 (insulin-dependent) diabetic patients [1]. The clinical syndrome is characterized by persistent albuminuria and a relentless decline in glomerular filtration rate (GFR) [2, 3]. Quantitative morphometric studies in Type 1 diabetic patients with nephropathy have demonstrated a close correlation between GFR and glomerular capillary filtration surface area [4, 5]. Recently, we have demonstrated that long-term streptozotocin-diabetic rats have a reduced ultrafiltration coefficient (Kf, the product of water permeability of the glomerular capillary wall and the surface area available for filtration) and a compensatory increase in glomerular capillary hydraulic pressure [6]. Furthermore, our results suggest that the prostaglandin system compensates for the reduction in Kf by reducing the arteriolar resistances to increase glomerular capillary pressure and thereby maintaining kidney function [6]. The renin prostaglandin systems are involved in maintaining GFR in chronic renal diseases [7, 8].

Our study was performed to test the hypothesis that increased glomerular synthesis of vasodilating prostaglandins acts to maintain GFR in Type 1 diabetic patients with nephropathy and normal kidney function (GFR > 80 ml/min per 1.73 m^2).

Subjects and methods

Subjects

Eight Type 1 diabetic females with nephropathy were investigated after informed consent (Table 1). The patients were recruited consecutively among female outpatients, and fulfilled the following enrollment criteria: persistent albuminuria, presence of retinopathy, onset of insulin-dependent diabetes before the age of 31 years, duration of diabetes ≥ 10 years, age < 50 years, serum creatinine < 100 μmol/l, normotension and no medication other than insulin. All patients had been insulin-dependent from the time of diagnosis, and all received two daily injections of highly purified porcine insulin. Persistent albuminuria was defined as urinary albumin excretion ≥ 200 μg/min in 2 of 3 consecutive 24-h collections of urine at home. Diabetic nephropathy was diagnosed clinically if there was persistent albuminuria, diabetic retinopathy, diabetes of more than 10 years' duration, and no clinical or laboratory evidence of kidney or renal-tract disease other than diabetic glomerulosclerosis. The study was approved by the local ethical committee.

Table 1. Clinical data of 8 Type 1 diabetic women with nephropathy

Patient	Age (years)	Duration of diabetes (years)	Retinopathy	Body mass index	Insulin dose ($U \cdot kg^{-1} \cdot day^{-1}$)
1	45	19	simplex	20.4	0.62
2	23	11	simplex	21.9	0.64
3	37	23	simplex	21.7	0.77
4	23	18	simplex	26.2	0.67
5	35	28	simplex	22.6	0.55
6	29	14	simplex	20.2	0.69
7	43	36	proliferative	21.6	0.50
8	44	37	proliferative	21.3	0.44
Median	36	21		21.7	0.63
Ranges	(23-45)	(11-37)		(20.2-26.2)	(0.44-0.77)

Methods

The study was performed as a randomized double-blind trial. The investigations were performed twice within 2 weeks, with the patients receiving either indomethacin (50 mg three times a day) or placebo during the last three days preceding the kidney function studies. All patients received their usual diabetic diet (containing 15 to 20% protein) during the study, and sodium restriction was not applied. Measurements were taken in the morning following an overnight fast. The patients had their last injection of insulin at 17.00 hours the day before the study. The patients drank tap water (200 ml/h) during the study. Measurements were performed in the supine position, and the patients were standing only when voiding. Urinary catheters were not used.

Glomerular filtration rate was measured after a single intravenous injection of 100 μCi ^{51}Cr-EDTA (08.20 hours) by determination of plasma radioactivity in venous blood samples taken from the other arm 180, 200, 220, and 240 min after the injection [9]. In our hospital, the small underestimation (10%) of ^{51}Cr-EDTA clearance versus clearance of insulin is corrected for by multiplying the former by 1.10 [10]. The mean intraindividual coefficient of variation of GFR from day to day is 2.8% in our laboratory. Urinary albumin excretion was measured during the 4-h clearance period by radioimmunoassay [11]. This assay has a sensitivity of 0.5 mg/l and an interassay coefficient of variation of 9%. Fractional clearance of albumin was obtained by dividing the clearance of albumin (calculated from UV/P, where U = urine albumin concentration, V = urine flow, and P = plasma albumin concentration) by the simultaneously measured GFR. Urinary excretion of prostaglandin E_2 during the 4-h clearance period, used as an indication of the renal synthesis of prostaglandin E_2, was measured with radioimmunoassay. Urine was extracted with 3% vol ethylacetat at pH 3 [12]. After evaporation of the organic phase, immunoreactive prostaglandin E_2 was determined by a commercial kit (NEK-020) according to the manufacturers' description (New England Nuclear, Boston, Mass, USA). This assay has an interassay coefficient of variation of 7.8%. Urinary sodium excretion was measured during the 4-h clearance period. Plasma indomethacin concentration was measured before and 5 h after the last tablet was taken (08.00 hours) by a gas chromatographical technique [13]. Blood glucose concentration was measured every hour during the clearance by the reflectance meter Reflomat (Boehringer, Mannheim, FRG). Plasma albumin was measured according to Laurell [14]. Stable haemoglobin A_{1c} (HbA_{1c}) was measured at both investigations (normal range 4.1 to 6.1 per cent of total haemoglobin) [15]. Arterial blood pressure was measured every hour on the right arm with a Hawksley random zero device (cuff 25 × 12 cm). Diastolic blood pressure was recorded at the disappearance of the Korotkoff sounds (phase 5). Retinopathy was assessed by direct ophthalmoscopy after pupillary dilation. Beat-to-beat variation in heart rate during hyperventilation was measured as previously described [16].

All patients collected 24 h urine at home during the last 3 days before the kidney function studies were performed. Urinary excretion of albumin and of prostaglandin E_2 was measured as described above. The coefficient of variation for albumin and prostaglandin E_2 collected at home was 24% and 38% respectively.

Three 24-h urine collections at home were performed for determination of albumin, prostaglandin E_2 and sodium excretion in 11 non-diabetic healthy women, aged 35 ± 6 years, and in 9 normoalbuminuric Type 1 diabetic women, aged 36 ± 8 years. The duration of diabetes ranged from 10 to 37 years, median 18 years. Seven patients had simplex retinopathy and two patients lacked retinopathy. All patients were normotensive, with mean arterial blood pressure 132/80 ± 8/4 mmHg, and an average HbA_{1c} of 7.9 ± 0.7 percent. All patients were ketosis-prone and were treated with insulin twice daily. None of the patients were taking other drugs, and the non-diabetic control subjects received no drugs.

Statistical analysis

Wilcoxon's non-parametric test for paired comparison, and the Mann-Whitney non-parametric test for unpaired comparison (prostaglandin E_2) were used. Urinary albumin and prostaglandin E_2 excretion are expressed as median and range, since they are not normally distributed. Coefficient of variation of urinary albumin and urinary prostaglandin E_2 was calculated after logarithmic transformation of the data.

Results

Urinary excretion of prostaglandin E_2 based on 24-h collections at home was significantly elevated in Type 1 diabetic women with nephropathy, 253 (143-697) pg/min, as compared with normoalbuminuric Type 1 diabetic women, 95 (66-225) pg/min and non-diabetic women, 132 (54-263) pg/min ($p < 0.01$). Twenty-four h urinary albumin excretion was 6 (5-18) mg in the normoalbuminuric Type 1 patients and 7 (4-16) mg in the normal subjects. Urinary sodium excretion was 135 ± 41, 112 ± 32 and 168 ± 78 mmol/24 h in control subjects and normo-macroalbuminuric Type 1 diabetic patients respectively (NS).

Indomethacin was not detectable in serum during the placebo periods. Serum indomethacin concentration rose from 0.48 ± 0.29 (07.50 h) to 0.62 ± 0.23 μg/ml (12.50 h) during active treatment. Urinary prostaglandin E_2 excretion diminished with 74 per cent during indomethacin therapy, Table 2 ($p < 0.01$). GFR declined in seven of the eight patients, on average from 120 ± 18 to 106 ± 17 ml/min/1.73 m^2 during indomethacin, Table 2 ($p < 0.05$). Urinary albumin excretion deter-

Table 2. Effects of indomethacin on glomerular filtration rate (GFR), urinary albumin excretion and urinary prostaglandin E_2 excretion in 8 Type 1 diabetic females with nephropathy

Patient	GFR (ml/min per 1.73 m^2)		Urinary albumin excretion (µg/min)		Fractional albumin clearance (10^{-5})		Urinary prostaglandin E_2 excretion (pg/min)		Urinary albumin excretion[a] (µg/min)		Urinary prostaglandin E_2 excretion[a] (pg/min)	
	Placebo	Indomethacin	Placebo	Indomethacin	Placebo	Indomethacin	Placebo	Indomethacin	Placebo	Indomethacin	Placebo	Indomethacin
1	110	101	677	545	17.91	17.42	143	42	608	643	199	26
2	155	122	753	70	15.31	1.70	168	58	972	402	304	34
3	126	135	120	41	2.76	0.95	211	33	219	68	202	30
4	124	103	134	25	3.22	0.69	107	36	200	38	186	74
5	108	90	88	109	2.13	3.27	81	27	209	94	143	52
6	126	111	162	67	3.63	1.81	958	105	200	99	427	282
7	94	83	128	56	4.19	2.26	617	92	210	94	697	97
8	116	103	245	120	5.39	3.37	194	53	448	340	400	127
Median	120[b]	106[b]	148	69	6.83[b]	3.93[b]	181	48	215	97	253	62
Range	18	17	(88-753)	(25-545)	6.16	5.53	(81-958)	(27-105)	(200-972)	(38-643)	(143-697)	(31-264)
p	<0.05		<0.05		<0.05		<0.01		<0.05		<0.01	

[a] Based on three 24-h urine collections
[b] Mean ± SD indicated

mined during the 4-h clearance procedure decreased from 148 to 69 µg/min, Table 2 ($p < 0.05$). The fractional albumin clearance was also significantly reduced, Table 2 ($p < 0.05$). Plasma albumin concentration decreased from 507 ± 38 to 476 ± 36 µmol/l during indomethacin treatment ($p < 0.05$). Blood glucose concentrations and HbA_{1c} were nearly identical during placebo and active treatment, 13.4 ± 4 versus 14.2 ± 3 mmol/l, and 8.3 ± 1 versus 8.2 ± 1 per cent respectively. A slight but insignificant rise in arterial blood pressure occurred during indomethacin treatment, 116/70 ± 9/3 to 121/74 ± 13/5 mmHg. Urinary sodium excretion diminished from 0.16 ± 0.06 to 0.13 ± 0.10 mmol/min during indomethacin treatment (NS). Body weight remained unchanged during the trial, and no side effects were recorded. Five patients had reduced beat-to-beat variation in heart rate (< 15 min^{-1}).

Discussion

We have demonstrated that urinary prostaglandin E_2 excretion is elevated in Type 1 diabetic women with nephropathy compared to matched normoalbuminuric Type 1 diabetic and non-diabetic women. Furthermore, inhibition of cyclooxygenase, the major enzyme in the biosynthesis of all prostaglandins, induces a decline in urinary prostaglandin E_2 excretion, GFR and albuminuria in Type 1 diabetic patients with nephropathy. Urinary prostaglandin E_2 excretion was used as a probe for overall kidney synthesis of vasodilating prostaglandins.

Prostaglandin E_2 and prostacyclin are major vasodilating prostaglandins synthesized in the glomeruli, while thromboxane A_2 represents a vasoconstricting prostanoid [17, 18]. Previous studies have suggested that urinary excretion of prostaglandin E_2 reflects the renal synthesis [17, 18]. However, this suggestion is only valid in females, since prostaglandins are also synthesized in seminal vesicles. Increased production of vasodilating prostaglandins and unchanged synthesis of thromboxane A_2 has been demonstrated in the glomeruli isolated from rats with streptozotocin-induced diabetes mellitus [19, 20].

Previous studies have demonstrated that nonsteroid anti-inflammatory drugs, e.g. indomethacin, reduces GFR in different types of chronic glomerular diseases, including nephrotic syndrome [8, 21, 22, 23]. This effect is immediately reversible when the drug is discontinued [8]. Intravenous infusion of vasodilating prostaglandins improves renal function in chronic glomerular diseases [24]. We have demonstrated that increased renal prostaglandin synthesis maintains GFR in Type 1 diabetic patients with normal kidney function (GFR > 80 ml/min per 1.73 m^2) and nephropathy. It is important to stress that our results were obtained on a normal diabetic diet without sodium restriction. It is well established that sodium restriction enhances the effect of cyclooxygenase inhibition on GFR in patients with chronic glomerular diseases [8, 23]. This exaggerated effect is due to increased activity of the renin-angiotensin system. Inhibition of prostaglandin synthesis has no effect on glomerular function in normal man [25], while conflicting results have been obtained in normoalbuminuric short-term Type 1 diabetic patients [26, 27].

Our study does not elucidate the mechanisms involved in the GFR reduction induced by indomethacin. Determination of renal plasma flow by the classical constant infusion technique requires a complete emptying of the bladder every 20 to 30 min. This crucial requirement cannot be fulfilled in long-term Type 1 diabetic patients with nephropathy and autonomic neuropathy, since diabetic cystopathy is present in approximately 40 per cent of these patients [28]. Serum albumin concentration dropped by 6 per cent during indomethacin. Theoretically, a minimal increase in GFR can be predicted if the other GFR determinants remain unchanged. However, it is well documented that a decrease in serum albumin (oncotic pressure) will induce a reduction in the glomerular capillary permeability surface area product (Kf), thereby opposing the effect [29]. Recently we have demonstrated that indomethacin

infusion in long-term streptozotocin-diabetic rats produced striking effects on glomerular haemodynamics. The afferent arteriolar hydraulic resistance increased substantially while the efferent resistance rose slightly, causing large reductions in single nephron blood flow, glomerular capillary hydraulic pressure and in single nephron glomerular filtration rate [6].

Our findings of diminished albuminuria (55%) can neither be explained by the decrease in GFR (12%) nor by the decline in serum albumin concentration (6%), since the fractional clearance of albumin was also found to decrease significantly during indomethacin treatment. Arisz et al. [8] found an increase in selectivity of proteinuria in indomethacin-treated patients with nephrotic syndrome. This finding suggested either a direct effect of indomethacin on the size and charge selective filter properties of the glomerular capillary wall or a redistribution of renal blood flow to a nephron population with a lower permeability to macromolecules. However, Tiggeler et al. [23] were unable to confirm these results in a large study applying more refined methods for determination of protein selectivity. Lowering of glomerular capillary hydraulic pressure may, as recently demonstrated in indomethacin-treated long-term streptozotocin-diabetic rats, be the crucial factor involved. This suggestion is also supported by the finding of a reduced filtration fraction during indomethacin treatment in patients with nephrotic syndrome [8].

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