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Effect of pentoxifylline on proteinuria, markers of tubular injury and oxidative stress in non-diabetic patients with chronic kidney disease — placebo controlled, randomized, cross-over study

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Background: Inhibition of the renin-angiotensin-aldosterone system (RAAS) with angiotensin converting enzyme inhibitors (ACEI) and/or angiotensin II subtype 1 receptor antagonists (ARB) is a common strategy used in the management of patients with chronic kidney disease (CKD). However, there is no universal therapy that can stop progression of CKD. Pentoxifylline (PTE) is a non-specific phosphodiesterase inhibitor with anti-inflammatory properties. It has been reported to have promising effects in CKD treatment. Methods: In a placebo-controlled, randomized, cross-over study we evaluated the influence of PTE (1200 mg/day) added to RAAS blockade on proteinuria, surrogate markers of tubular injury and oxidative stress-dependent products in 22 non-diabetic patients with proteinuria (0.4–4.3 g per 24 h) with normal or declined kidney function [eGFR 37–178 mL/min]. In an eight-week run-in period, therapy using ACEI and/or ARB was adjusted to achieve a blood pressure below 130/80 mm Hg. Next, patients were randomly assigned to one of two treatment sequences: PTE/washout/placebo or placebo/washout/PTE. Clinical evaluation and laboratory tests were performed at the randomization point and after each period of the study. Results: The PTE therapy reduced proteinuria (by 26%) as compared to placebo. There were no differences in α_1-microglobulin, urine excretion of *N*-acetyl-β-D-glucosaminidase (NAG), hsCRP, the urinary excretion of 15-F_{2t}-isoprostane, blood pressure (BP), eGFR and serum creatinine between the PTE and placebo groups. Conclusion: Pentoxifylline may decrease proteinuria in non-diabetic patients with CKD.

Keywords: pentoxifylline, oxidative stress, kidney, chronic kidney disease, proteinuria, tubular injury

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INTRODUCTION

The incidence and prevalence of chronic kidney disease (CKD) is increasing worldwide. Pharmacological inhibition of the renin-angiotensin-aldosterone system (RAAS) constitutes a cornerstone strategy in the management of patients with chronic nephropathies with proteinuria (Tylicki *et al.,* 2005). Angiotensin converting enzyme inhibitors (ACEI) and angiotensin II subtype 1 receptor antagonists (ARB) have been shown to decrease proteinuria, reduce local renal inflammatory processes and slow down the progression of renal insufficiency (Renke *et al.,* 2004; Renke *et al.,* 2005; Rutkowski *et al.,* 2004; Tylicki *et al.,* 2007a; 2007b). Despite recent progress, there is still no optimal therapy that stops progression of CKD. Therefore, it is necessary to search for alternative therapeutic strategies which can further improve renal outcome.

Considering the prognostic impact of proteinuria reduction on long-term renal outcome, in the present study we evaluated the effects of pentoxifylline (PTE) addition to background nephroprotective therapy consisting of ACEI and/or ARB. PTE, a methyl-xanthine derivative, is a non-selective phosphodiesterase inhibitor with anti-inflammatory and immunomodulatory effects. PTE is also widely used to treat peripheral vascular disorders because of its potent hemorrheological properties (Frampton & Brogden, 1995). Moreover, PTE potently inhibits cell proliferation and extracellular matrix accumulation, factors that play important roles in CKD progression. The PTE's benefit when administered in conjunction with RAAS blockade in patients with CKD is not clear. Our study evaluated the effects of this treatment on proteinuria, inflammation, oxidative stress, renal function and surrogate markers of tubular injury.

MATERIAL AND METHODS

Individuals. Patients were selected from the cohort that attended our renal outpatient department. The inclusion criteria were as follows: age 18–65 years, chronic non-diabetic proteinuric nephropathy without dyslipidemia, normal or slightly impaired stable renal function expressed as estimated glomerular filtration rate (eGFR)

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Abbreviations: ACEI, angiotensin converting enzyme inhibitors; ARB, angiotensin II subtype 1 receptor antagonists; BP, blood pressure; CKD, chronic kidney disease; CVD, cardiovascular diseases; DPE, 24-h urinary protein excretion; eGFR, estiamted glomerular filtration rate; PTE, pentoxifylline; RAAS, renin-angiotensin-aldosterone system.

Table 1. Patient characteristics at baseline

Parameter	
Gender: female/male (n)	7/15
Mean age years (±S.E.M.)	38.6±10.3
Mean systolic blood pressure mm Hg (±S.E.M.)	123.8±12.6
Mean diastolic blood pressure mm Hg	75.3 (70.6–81.0)
Urinary protein excretion g/24 h	1.2 (0.4–4.3)
Serum creatinine mg/dL	1.0 (0.9–1.3)
eGFR mL/min (±S.E.M.)	121.8±50.2
hsCRP mg/L	2.36 (0.29–10.4)
BMI kg/m^2	27.7 (19.3–36.1)
Histopathological diagnosis: (n)	14
Mesangial glomerulonephritis	4
Mesangiocapillary glomerulonephritis	1
Membranous glomerulonephritis	2
Focal segmental glomerulosclerosis (FSGS)	2
IgA nephropathy	5
Unknown non-diabetic proteinuric chronic kidney diseases	8
Background hypotensive therapy: (n)	
ACEI and ARB	14
ACEI	7
ARB	1

above 30 mL/min, stable proteinuria above 300 mg/24 h, and no steroids or other immunosuppressive treatment for a minimum of six months before the study. Stable renal function and proteinuria were defined as a variability of serum creatinine and proteinuria less than 20% during 6 months before the start of the study. Exclusion criteria were as follows: fertile women who were not taking oral contraceptives, pregnant or lactating women, and a history of adverse reactions to PTE.

General protocol. The study was a prospective, placebo-controlled, randomized, two-period cross-over trial in which the renal effects of adding PTE to a background nephroprotective therapy with ACEI and/or ARB were evaluated. Subjects entered an eight-week run-in period during which background nephroprotective therapy using pharmacological blockade of RAAS was adjusted to keep target blood pressure (BP) below 130/80 mm Hg (Table 1). At the end of the run-in period, patients were randomly assigned to one of two treatment sequences: 8-week PTE (1200 mg/day)/8-week washout — background therapy/8-week placebo (sequence 1) or 8-week placebo/8-week washout — background therapy/8-week PTE (1200 mg/day) (sequence 2) (Fig. 1). The allocation was performed according to a computer-generated randomization list by a person that was independent of the research team. The patients received 1200 mg of PTE, in tablet form (Polfilin 400, Polpharma), once a day. The dosages of ACEI, ARB and diuretics, once established in the run-in period, were left unchanged throughout the study. At the randomization point, and after the end of each treatment period, office through BP, serum creatinine, potassium, hsCRP, proteinuria (measured as 24-h

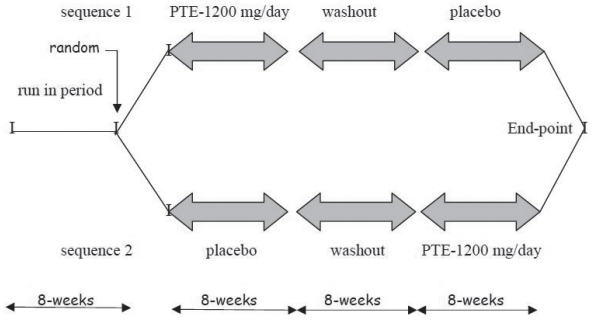

Figure 1. Study scheme

urinary protein excretion (DPE)), sodium excretion (Na ex), and urea excretion were measured. Further, surrogate markers of tubular injury were analyzed, namely urine excretion of *N*-acetyl-β-D-glucosaminidase (NAG), α-$_1$microglobulin (α1m) and 15-F_{2t}-isoprostane. 15-F_{2t}-isoprostane is accepted as a reliable and sensitive marker of oxidative stress in human pathologies (Fam & Morrow, 2003). The study was approved by the local ethical committee and the investigated patients all gave informed consent.

Procedures and laboratory analyses. The office through BP was measured with a Speidel+Keller sphyngomanometer in a sitting position after 10 min of rest and expressed as a mean value of two consecutive measurements taken 2 min apart. DPE, Na ex and urea excretion were evaluated on the basis of 24-h urine collection. All patients were equipped with a graded container and were informed how to collect 24-h urine. They collected two 24-h urines — of those the mean value of DPE was calculated for data evaluation. Patients were asked not to perform heavy physical activity on the urine collection days and were recommended not to change their usual daily protein and sodium intake during the study period. The excretion of urea was used to calculate the protein intake according to the Maroni equation: protein intake normalized to weight (g/kg per day) = 6.25×([urea-N-excretion urine 24 h (g/day)] + [0.0031 × body weight (kg)])/body weight (kg) (Maroni *et al.,* 1985). eGFR was calculated according to the Cockcroft-Gault formula (Cockcroft & Gault, 1976). NAG and α1m were analyzed in the second morning spot urine sample. NAG was determined by the spectrophotometric method according to Maruhn (1976). Incubation medium had a final volume of 0.4 mL, containing 5 nmol/L *p*-nitrophenyl-2-acetamido-β-D-glucopyranoside as a substrate in 50 mmol/L citrate buffer (pH 4.14). The reaction was started by the addition of 0.2 mL of undialysed urine, carried out for 15 min at 37 °C, and then terminated with 1 mL of glycine buffer, pH 10.5. Absorbance was measured at 405 nm against a sample terminated at time zero. The calculation of the NAG level was made from the molar absorption coefficient of the product of the reaction, *p*-nitrophenol, which is 18.5 cm^2/μmol. From preliminary experiments it was clear that the dialysis did not affect NAG levels in the urine. Immunoturbidimetric test (Tina-quant α_1-microglobulin, Roche, Basel, Switzerland) was used for the quantification of α1m in urine. The detection limit of the method was 2 mg/L. Urinary NAG, and α1m were reported per mass of urine creatinine to correct for the variation in urine concentration. High sensitivity C-reac-

Table 2. Changes of parameters after PTE and placebo

	PTE — Baseline (Δ)	Placebo — Baseline (Δ)	*P*
Proteinuria (DPE) g/24 h	–0.41 ± 0.48	0.01 ± 0.62	0.11
α1m excretion mg/g creatinine	0.61 (–8.4–9.6)	0.41 (–5.7–6.5)	0.96
NAG excretion IU/ g creatinine	1.0 ± 1.96	1.16 ± 4.9	0.91
hsCRP mg/L	–1.66 ± 1.77	–0.89 ± 4.69	0.63
Urine excretion of iPF2α ng/mg creatinine	0.08 (–0.01–0.18)	–0.04 (–0.12–0.03)	0.8

tive protein (hsCRP) was measured with a commercial ELISA kit (DRG, EIA-3954) and reported as mg/L. A commercial ELISA kit (Cayman Chemical Co.) was then used to measure the urinary excretion of 15-F_{2t}-isoprostane in the treated patients. Potassium, sodium, urea, protein and creatinine levels were measured in fresh blood samples drawn after fasting overnight for at least 12 h. These parameters were measured using standard laboratory techniques. Body mass index (BMI) was calculated as weight (kilograms) divided by height (meters) squared. Adverse effects were recorded at each visit in response to questionnaires or as observed by the investigators.

Statistics. The primary end point of this study was a change in DPE in measurements available for each patient after treatment with PTE and placebo. The sample size of 16 patients adequately allowed a power of 80% to detect a difference in variables equal to within one standard deviation, that is a standardized effect size of 1.0 at a significance level of 0.05 (two-tailed). Secondary end points included urine NAG, α1m, and 15-F_{2t}-isoprostane excretions. Normality and homogeneity of the variances were verified by means of the Shapiro-Wilk test and Levene test, respectively. Because of their skewed distribution, diastolic BP, DPE, NAG excretion, 15-F_{2t}-isoprostane, hsCRP, serum creatinine and daily protein intake were logarithmically transformed before statistical analysis, and expressed as geometric means and 95% confidence intervals. Other results are presented as means ± S.E.M. Differences in the variables' changes between treatment with PTE and placebo were assessed using Student's *t*-test (Table 2). The differences in the variables measured more than twice (Table 3) were assessed using ANOVA. *P* less than 0.05 (2-tailed) was considered statistically significant. Data were evaluated using Statistica (version 7.1; StatSoft Inc, Tulsa, OK) software package.

RESULTS

Of the 22 patients who entered the study, 14 (64%) completed the protocol. Five of the patients dropped out because of the withdrawal of informed consent due to a side effect of therapy (gastrointestinal symptoms — 23%). The other patients resigned from participation in the study for personal reasons. Clinical characteristics of the patients are listed in Table 1.

24-h urinary protein excretion (DPE)

The PTE therapy reduced proteinuria (by 26%) as compared to placebo, but the result was not significant ($P = 0.11$) (Table 2). The exact change of DPE in single patients before and after PTE is shown separately (Fig. 2).

Urinary NAG and α1m excretion

There were no significant changes in urinary NAG ($P = 0.91$) and α1m excretion level ($P = 0.96$) using PTE as compared to placebo (Table 2).

15-F_{2t}-isoprostane excretions and hsCRP

There were no significant changes in15-F_{2t}-isoprostane excretions and hsCRP during the study (Table 2).

Blood pressure, renal function, sodium and protein intake

The control of BP was adequate in all study periods; all patients reached the target office trough BP below 130/80 mm Hg. There were no differences in the office through systolic and diastolic BP between the treatment periods. Renal function assessed by means of serum creatinine and eGFR remained stable during the study periods. There were no differences in sodium and protein intake between treatment periods (Table 3).

Safety

Interestingly, the PTE therapy was not well tolerated in this study. Adverse effects were reported in five patients (22.7%) who suffered from gastrointestinal symptoms — nausea, dyspepsia and diarrhea.

Table 3. Changes of parameters during study

Parameter	Randomization point	After PTE	After Placebo	*P*
Na urinary excretion mmol/24 h	295 ± 30.2	247 ± 34.5	268 ± 35.5	0.64
Daily protein intake g/24 h	1.1 ± 0.3	1.1 ± 0.3	1.0 ± 0.3	0.45
Serum creatinine mg/dL	1.0 (0.9–1.3)	1.1 (0.9–1.4)	1.1 (0.9–1.5)	0.86
Systolic BP mm Hg	123.8 ± 12.6	122.9 ± 11.2	123.8 ± 10	0.55
Diastolic BP mm Hg	75.3 (70.6–81.0)	74.3 (70.2–79.0)	77.6 (73.8–82.1)	0.64

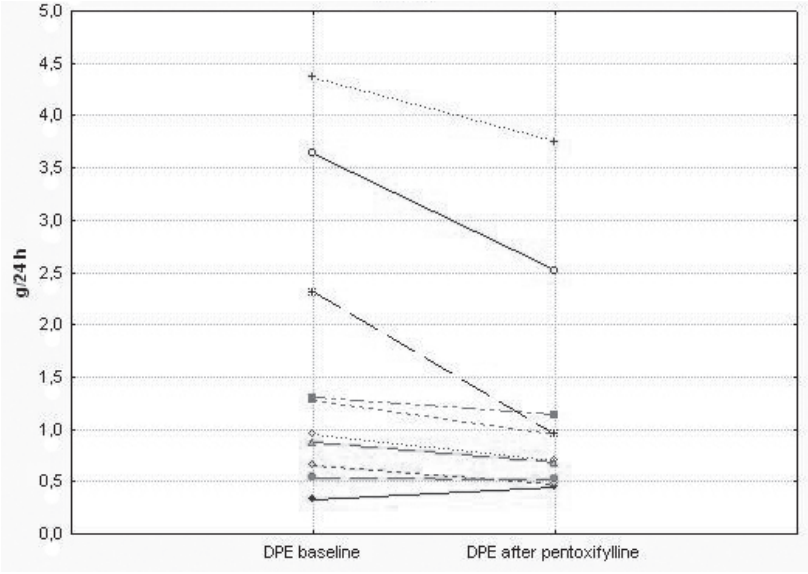

Figure 2. Daily protein excretion (DPE) before and after the therapy with pentoxifylline.

DISCUSSION

To the best of our knowledge the present study was the first to evaluate tubulointerstitial effects of pentoxifylline in proteinuric non-diabetic CKD patients. PTE has potential value as an antiproliferative and antifibrogenic agent, an effect documented in animal research (Chen *et al.,* 1999a; 1999b; Lin *et al.,* 2005) and in patients with diabetic kidney disease (Navarro *et al.,* 2003). Considering that tubular epithelial cell injury may initiate the fibrotic process in kidneys and that the extent of tubulointerstitial damage is a crucial predictor of renal outcome, tubular cells have become a site of particular interest. To evaluate the tubulointerstitial effects of the described interventions, the tubular involvement markers NAG and α1m were analyzed (Bazzi *et al.,* 2002). An increased excretion of NAG is thought to be a specific marker of tubular injury in many renal pathologies including non-diabetic CKD (Bazzi *et al.,* 2002). Increased urinary excretion of α1m, a low-molecular weight protein physiologically filtered and reabsorbed by tubular cells, may indicate a reduced capacity of reabsorption by tubular cells, and thus can act as a marker of established tubular damage, with greater urinary concentrations suggesting greater severity of damage (Holdt-Lehmann *et al.,* 2000). Our results show that treatment with PTE had no influence on these markers of tubular injury.

The effects of PTE (1200 mg/day) on proteinuria were also analyzed. Proteinuria is considered a marker of long-term renal outcome. In the present study, the administration of PTE decreased the proteinuria levels in non-diabetic CKD patients, but this was not significant ($P=0.11$). Only a few randomized controlled trials directly addressing the effect of PTE on renal function and proteinuria have been reported. Most of those studies were of small size or short duration, used a variety of doses, and many did not include a placebo arm. Some of these studies suggest that PTE reduces proteinuria (Ducloux *et al.,* 2001; Galindo-Rodriguez *et al.,* 2003; Lin *et al.,* 2008) and the rate of GFR decline (Perkins *et al.,* 2009). These positive effects were summarized in published meta-analyses (McCormick *et al.,* 2008) and review articles (Lin *et al.,* 2004; Lin *et al.,* 2005; Renke *et al.,* 2008; Vilayur & Harris, 2009). The pleiotropic effects of PTE have important clinical implications, as it displays anti-tumor necrosis factor alpha (TNF-α) (Mandell, 1995) and anti-interferon gamma (IFN-γ) (Benbernou *et al.,* 1995; Bienvenu *et al.,* 1995) action, as well as antioxidant (Freitas & Filipe, 1995) and antiapoptotic effects (Belloc *et al.,* 1995). Patients with CKD are at increased risk for cardiovascular disease (CVD), and recent reviews suggest that inflammation and oxidative stress could be the primary mediators of CVD in CKD patients (Arici & Walls, 2001). Moreover, inflammation plays a central role in the progression of CKD (Tonelli *et al.,* 2005; Zoja *et al.,* 2006). In our study we used hsCRP, a protein found in the blood, as a marker of inflammatory process. Interestingly, patients with elevated basal levels of CRP are at an increased risk of diabetes, hypertension and cardiovascular disease (Pradhan *et al.,* 2001; Dehghan *et al.,* 2007). In our study this parameter had a tendency to decrease with PTE treatment (70%), but the result was not statistically significant ($P=0.63$). The facts that most of the patients had serum hsCRP levels within the normal range at the beginning of the study and the small number of participants are probably the main reasons why our results differ from those of other studies. The urinary excretion of 15-F_{2t}-isoprostane, a reliable and sensitive marker of oxidative stress, was also measured. Urinary excretion of 15-F_{2t}-isoprostane was not found to change with treatment ($P=0.8$). Interestingly, the PTE therapy was not well tolerated in this study, a finding in contrast to the perception that PTE has few side effects in CKD patients (Ward & Clissold, 1987; McCormick *et al.,* 2008). Adverse effects, namely gastrointestinal symptoms, were reported in 5 patients (23%) during the study period. This finding is perhaps attributable to accumulation of PTE metabolites, a known mechanism of toxicity in patients with chronic renal failure (Paap *et al.,* 1996). In the present study, the PTE doses were unchanged in patients with moderate renal dysfunction (Navarro *et al.,* 2003).

A potential limitation of the study is the relatively small sample size, which was unsufficiently powered to detect a significant difference equal to the S.D. value between treatment periods. Further, 24-h urine collections used to assess proteinuria may be associated with significant collection errors, largely because of improper timing and missed samples, leading to overcollection and undercollection.

In conclusion, the study results suggest that treatment with PTE (1200 mg/day) for 8 weeks in nondialysed patients with CKD induced the reduction of DPE (by 26%), without affecting markers of tubular injury and

oxidative stress. However, the potential nephroprotective properties of PTE need to be addressed further in future controlled long term studies.

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