A Double-Blind, Randomized, Placebo-Controlled Clinical Trial on Benfotiamine Treatment in Patients With Diabetic Nephropathy

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OBJECTIVE — To investigate the effect of benfotiamine on urinary albumin excretion (UAE) and the tubular damage marker kidney injury molecule-1 (KIM-1) in patients with type 2 diabetes and nephropathy.

RESEARCH DESIGN AND METHODS — Patients with type 2 diabetes and UAE equivalent to 15–300 mg/24 h, despite ACE inhibitors (ACE-Is) or angiotensin receptor blockers (ARBs), were randomly assigned to 12 weeks of benfotiamine (900 mg/day) ($n = 39$) or placebo ($n = 43$).

RESULTS — Compared with placebo, benfotiamine treatment resulted in significant improvement of thiamine status ($P < 0.001$). Benfotiamine treatment did not significantly decrease 24-h UAE or 24-h KIM-1 excretion.

CONCLUSIONS — In patients with type 2 diabetes and nephropathy, high-dose benfotiamine treatment for 12 weeks in addition to ACE-Is or ARBs did not reduce UAE or KIM-1 excretion, despite improvement of thiamine status.

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The pathophysiology of diabetic nephropathy includes albuminuria as a consequence of glomerular endothelial damage and further progression due to tubulointerstitial inflammation and fibrosis (1,2). Despite protective treatment with ACE inhibitors (ACE-Is) and angiotensin receptor blockers (ARBs), many patients progress to end-stage renal disease (3).

Thiamine and benfotiamine have been proposed as protective agents for diabetes complications (4,5). Benfotiamine is a lipophilic thiamine derivative with high bioavailability (6). In animal studies, both compounds had beneficial effects on microvascular complications, including diabetic nephropathy (5,7).

We investigated whether benfotiamine results in reduction in urinary albumin excretion (UAE) or tubulointerstitial damage markers in patients with type 2 diabetes and increased UAE despite ACE-Is or ARBs.

RESEARCH DESIGN AND METHODS

— Participants were recruited at the Isala Clinics (Zwolle, the Netherlands). Inclusion criteria were type 2 diabetes, age 40–75 years, active diabetic nephropathy (UAE 15–300 mg/24 h or equivalent albumin-to-creatinine ratio [males 1.25–25, females 1.75–35 mg/mmol] in two of three samples within 2–6 weeks) despite ACE-Is and/or ARBs in unchanged dose for at least 3 months, glycated hemoglobin (A1C) <8.5%, and an estimated glomerular filtration rate of >30 ml/min. Exclusion criteria were participation in another study, renal impairment by causes other than diabetes, elevated liver enzymes, hyper- or hypothyroidism, blood pressure >160/90 mmHg, neoplasm, severe general diseases, drug abuse, pregnancy, lactation, active menses, hypersensitivity to benfotiamine, use of vitamin B–containing supplements, changes in concomitant medication during the previous 3 months, and use of nonsteroidal anti-inflammatory drugs >3 times per week. In total, 2,711 patients were screened. Eligible patients were included after written informed consent was received. The trial was approved by the medical ethics committee.

Patients were randomized to oral benfotiamine 300 mg three times daily or placebo for 12 weeks. Study medication was prepared by Wörwag Pharma (Böblingen, Germany). Participants were evaluated at baseline and after 6 and 12 weeks. Each visit, 24-h urine, spot morning urine, and blood samples were collected. Noncompliance was considered if <80% of the study medication had been taken.

Thiamine concentration was measured in whole blood and plasma by high-performance liquid chromatography (8). Erythrocyte transketolase activity was measured in erythrocytes (9). Urinary albumin was measured by immunonephelometry (Behring Nephelometer; Mannheim, Germany), threshold 1.8–2.3 mg/l, intra- and interassay coefficients of variation (CV) 2.2 and 2.6%, respectively. Urinary kidney injury molecule-1 (KIM-1) was measured by ELISA, threshold 0.12 ng/ml, intra- and interassay CV 7.9 and 14.4%, respectively (10). Neutro-

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Table 1—*Baseline characteristics and changes in thiamine status parameters, primary outcome measures, secondary outcome measures, and clinical characteristics over time*

	Benfotiamine (n = 39)			Placebo (n = 43)			
	Baseline	6 weeks	12 weeks	Baseline	6 weeks	12 weeks	P
Males	30			33			0.98
Age (years)	65.3 ± 5.9			64.6 ± 6.1			0.63
BMI (kg/m^2)	32.1 ± 5.1			31.9 ± 5.9			0.93
Duration of diabetes (years)	12 (9–18)			10 (7–18)			0.41
Insulin treatment	31 (79)			29 (67)			0.22
Oral hypoglycaemic agents	19 (49)			29 (67)			0.05
Plasma thiamine (nmol/l)	31.8 ± 7.7			31.6 ± 9.8			0.92
Thiamine status							
Whole blood thiamine (nmol/l)	126 ± 23	290 ± 31	300 ± 0	122 ± 23	124 ± 25	138 ± 30	<0.001
TK activity (mU/mgHb)	0.41 ± 0.10	0.51 ± 0.12	0.53 ± 0.15	0.38 ± 0.11	0.39 ± 0.08	0.41 ± 0.10	<0.001
Primary outcome parameters							
UAE (mg/24 h)	90 (38–267)	75 (49–280)	72 (38–199)	97 (48–177)	99 (43–200)	96 (45–200)	0.36
U-KIM-1 (μg/24 h)	1.67 (0.95–2.47)	1.51 (0.86–2.59)	1.68 (1.06–2.40)	1.56 (1.06–1.83)	1.56 (1.06–1.83)	1.39 (1.02–2.01)	0.12
Secondary outcome parameters							
24-h UACR (mg/mmol)	10.3 (3.7–23.4)	6.1 (3.0–17.7)	4.9 (2.5–18.4)	7.6 (4.3–13.3)	7.4 (2.8–11.0)	7.1 (4.0–12.5)	0.37
Spot-urine UACR (mg/mmol)	9.3 (2.4–16.8)	5.8 (3.7–17.9)	7.1 (3.6–17.8)	6.2 (3.4–10.5)	8.2 (3.9–14.2)	8.1 (4.6–15.9)	0.58
U-KIM-1/creatinine (ng/mmol)	103 (63–158)	95 (66–170)	96 (77–148)	99 (79–141)	89 (58–130)	81 (66–150)	0.37
U-α1m (mg/24 h)	9.4 (4.3–24.4)	11.9 (4.4–20.2)	11.2 (4.1–18.8)	8.2 (4.3–20.3)	9.0 (5.7–21.1)	10.2 (2.5–19.7)	0.33
U-α1m/creatinine (mg/mmol)	0.6 (0.3–1.4)	0.7 (0.3–1.3)	0.6 (0.3–1.2)	0.6 (0.3–1.3)	0.6 (0.3–1.4)	0.7 (0.2–1.1)	0.47
U-Ngal (mg/24 h)	131 (67–227)	118 (77–229)	115 (73–284)	122 (53–224)	112 (52–218)	99 (52–222)	0.17
U-Ngal/creatinine (mg/mmol)	6.7 (4.3–13.9)	6.2 (3.4–15.9)	5.1 (3.2–12.9)	7.7 (4.2–18.9)	6.4 (3.2–15.1)	8.5 (3.3–13.1)	0.18
Clinical characteristics							
SBP (mmHg)	140 ± 16	139 ± 14	143 ± 17	137 ± 20	140 ± 20	140 ± 17	0.60
DBP (mmHg)	76 ± 8	77 ± 10	76 ± 9	76 ± 10	76 ± 9	76 ± 10	0.68
A1C (%)	7.3 ± 0.9	7.1 ± 0.9	7.3 ± 1.0	7.4 ± 0.9	7.2 ± 0.9	7.2 ± 0.9	0.33
Plasma creatinine (μmol/l)	84 ± 19	89 ± 19	88 ± 20	87 ± 23	89 ± 25	87 ± 21	0.04
Creatinine clearance (ml/min)	135 ± 51	129 ± 53	133 ± 45	130 ± 58	139 ± 58	131 ± 64	0.57
Cystatin C (mg/l)	1.01 ± 0.21	1.06 ± 0.22	1.09 ± 0.23	1.03 ± 0.23	1.10 ± 0.26	1.11 ± 0.23	0.53
LDL cholesterol (mmol/l)	1.9 ± 0.7	1.9 ± 0.8	2.1 ± 0.8	1.8 ± 0.9	1.8 ± 0.9	1.9 ± 0.9	0.55
HDL cholesterol (mmol/l)	1.2 ± 0.3	1.1 ± 0.3	1.2 ± 0.3	1.1 ± 0.3	1.1 ± 0.3	1.1 ± 0.3	0.25
Triglycerides (mmol/l)	1.8 (1.4–2.6)	1.9 (1.4–2.8)	1.7 (1.2–2.6)	2.1 (1.4–3.4)	2.2 (1.4–2.9)	2.0 (1.2–2.9)	0.06

Data are means ± SD, n (%), or median (interquartile range). Comparison of baseline characteristics was performed by unpaired Student t test (for normally distributed variables) or Mann-Whitney U test (for non-normally distributed variables). χ^2 test was used to compare noncontinuous variables. Changes in thiamine status parameters, primary outcome measures, secondary outcome measures, and clinical characteristics over time were analyzed by ANOVA for repeated measures, with log-transformation of variables with skewed distribution prior to analysis. DBP, diastolic blood pressure; SBP, systolic blood pressure; TK, transketolase; U-α1m, urinary excretion of α1-microglobulin; U-KIM-1, urinary excretion of KIM-1; U-Ngal, urinary excretion of neutrophil gelatinase–associated lipocalin; UACR, urinary albumin-to-creatinine ratio.

phil gelatinase–associated lipocalin and α1-microglobulin were measured by ELISA and cystatin C by immunoassay (Gentian; Moss, Norway). Other laboratory measurements were performed according to standard procedures.

Statistical analyses

Variables with normal distribution are presented as means ± SD. Variables with skewed distribution were log-transformed before analysis and are presented as median (interquartile range). Changes were analyzed by ANOVA for repeated measurements. *P* values for change over time are presented. Results were considered statistically significant with $P < 0.05$.

To test whether benfotiamine reduces 24-h UAE or 24-h KIM-1 excretion, 38 evaluable patients per group were required to detect an effect of size 0.65 (power 80%, $\alpha = 0.05$, one-sided test). To compensate for drop-out, we enrolled 43 patients per group. One-sided *P* values were calculated for primary outcome measures and two-sided *P* values for other outcomes. Statistics were done by SPSS, version 16.0 (Chicago, IL). Intention-to-treat analysis and per-protocol analyses were planned. In cases of drop-out, data were not replaced.

RESULTS — Baseline characteristics and results at 6 and 12 weeks are shown in Table 1. In the benfotiamine group, two patients did not complete the study because of newly diagnosed malignancy and two others withdrew informed consent (dizziness and urticaria). In patients receiving benfotiamine, parameters of thiamine status improved significantly. Benfotiamine treatment had no significant effect on primary or secondary outcome parameters. Change in UAE between baseline and 12 weeks was −18 mg/24 h in the benfotiamine group and −1 mg/24 h in the placebo group. For individual differences, respective changes were −9 (−53 to 34)mg/24 h and −7 mg/24 h(−56 to 65). With respect to clinical characteristics, benfotiamine resulted in a borderline significant increase in plasma creatinine, but this was not accompanied by changes in creatinine clearance or cystatin C.

During the study, two patients were noncompliant (one per group) and two protocol violations occurred in the placebo group (ACE-I stopped), resulting in 38 patients in the benfotiamine and 40 in the placebo group for per-protocol analyses, of which results (data not shown) were not materially different from presented analyses.

CONCLUSIONS — We found that 12-week treatment with high-dose benfotiamine did not result in decreases in 24-h–UAE or 24-h–KIM-1 excretion despite significant improvement of thiamine status.

Our findings differ from those of a pilot study of 40 patients with type 2 diabetes in which 12 weeks of 300 mg/day of thiamine resulted in a significant decrease in UAE by 17.7 mg/24 h (11). In that study, baseline UAE was 44 mg/24 h (33–121) in the thiamine and 51 mg/24 h (32–122) in the placebo group, which is approximately 2 times lower than in our study, despite 100% of ACE-I and ARB treatment in our study versus <50% in the pilot study (12). Thus, thiamine derivatives may provide protective effects in earlier diabetic nephropathy stages, which is in line with an animal study in which development of albuminuria after induction of diabetes was inhibited by thiamine and benfotiamine (5). Furthermore, we investigated Caucasian patients, and the other study was of Pakistani patients. Thus, differences in diet, baseline prevalence of thiamine deficiency, or genetic susceptibility may also play a role. In our study, baseline plasma thiamine concentrations of 31.8 ± 7.7 nmol/l in the benfotiamine group and 31.6 ± 9.8 nmol/l in the placebo group were higher than the 16.3 ± 11.5 nmol/l reported for patients with type 2 diabetes in the U.K., but deficient compared to the 64.1 ± 12.0 nmol/l reported for healthy control subjects in that study (13).

It is important to realize that thiamine and benfotiamine are supposed to antagonize detrimental effects of hyperglycemia (7). Yet, in two large intervention studies, it took years of lowering A1C before a difference in UAE was found between strict metabolic control and standard therapy (14,15). Our study may therefore have been too short to demonstrate the effect of benfotiamine.

In conclusion, longer-term intervention studies and/or intervention studies in earlier stages of diabetic nephropathy are necessary to discern whether benfotiamine has an effect on the development of diabetic nephropathy.

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A.A. researched data and wrote the manuscript. A.K., W.V.O., and R.J.S. researched data. U.A. and G.S.M. contributed to the discussion. N.K., H.J.G.B., R.O.B.G., G.J.N., and S.J.L.B. contributed to the discussion and reviewed/edited the manuscript.

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