

Lack of Impact of Low-Dose Acetylsalicylic Acid on Kidney Function in Type 1 Diabetic Patients With Microalbuminuria

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OBJECTIVE — High-dose treatment with cyclooxygenase inhibitors reduces urinary albumin excretion rate (AER) in type 1 diabetic patients with microalbuminuria and macroalbuminuria. This effect may lead to an incorrect classification of albuminuria (normo-, micro-, and macroalbuminuria) and jeopardize the monitoring of antiproteinuric treatment (e.g., ACE inhibition). Whether similar difficulties exist using low-dose acetylsalicylic acid (ASA), now widely recommended for primary and secondary prevention of cardiovascular events in type 1 diabetic patients with micro- and macroalbuminuria, remains to be elucidated.

RESEARCH DESIGN AND METHODS — We performed a randomized double-blind crossover trial in 17 type 1 diabetic patients with microalbuminuria (urinary AER 30–300 mg/24 h). Patients were given ASA (150 mg/daily) for 4 weeks followed by placebo for 4 weeks with at least a 2-week washout period in random order. At the end of each treatment period, AER (enzyme-linked immunosorbent assay), glomerular filtration rate (GFR) (plasma clearance of $^{51}\text{Cr-EDTA}$), blood pressure (BP) (Hawksley), and HbA_{1c} (by high-performance liquid chromatography) were measured. Patients were advised to follow a normal diabetes diet without sodium restriction and received their usual antihypertensive treatment during the investigation.

RESULTS — During the study (ASA vs. placebo), urinary AER (geometric mean 64 [95% CI 39–105] vs. 59 [40–87] mg/24 h), GFR (mean 106 [93–118] vs. 104 [90–117] $\text{ml} \cdot \text{min}^{-1} \cdot 1.73 \text{ m}^{-2}$), systolic BP (mean 130 [119–141] vs. 130 [119–142] mmHg), diastolic BP (mean 71 [65–78] vs. 71 [64–78] mmHg), and HbA_{1c} (mean 8.4% [8.0–9.0] vs. 8.5% [8.1–9.0]) remained unchanged.

CONCLUSIONS — Treatment with 150 mg ASA daily does not have any impact on AER or GFR in type 1 diabetic patients with microalbuminuria. Consequently, primary and secondary prevention of cardiovascular events with low-dose ASA does not interfere with the classification of AER or monitoring of antiproteinuric treatment in such patients.

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In patients with type 1 diabetes, microalbuminuria has been shown to be a predictor of both micro- and macrovascular complications (1–4). Since low-dose acetylsalicylic acid (ASA) has been shown to offer substantial primary (5,6) and secondary pro-

tection against macrovascular morbidity and mortality in diabetic patients at high risk for cardiovascular events (7), ASA should now be considered a primary prevention strategy in high-risk patients with type 1 or type 2 diabetes if no contraindications exist (8).

Elevated renal synthesis of vasodilating prostaglandins is present in type 1 diabetic patients with microalbuminuria (9), and treatment with high-dose cyclooxygenase inhibitors reduces urinary albumin excretion rate (AER) in type 1 diabetic patients with microalbuminuria (150 mg indometacin daily) (9) or diabetic nephropathy (990 mg ASA + 225 mg dipyridamole daily or 150 mg indometacin) (10,11). This effect occurs shortly after initiation of treatment and may lead to an incorrect classification of the level of albuminuria (normo-, micro-, and macroalbuminuria) and jeopardize the monitoring effect of antiproteinuric treatment (e.g., ACE inhibition). Whether similar difficulties exist using low-dose ASA remains to be elucidated.

The primary aim of the present study was to investigate the effect of short-term low-dose ASA on urinary AER as well as the effect on glomerular filtration rate (GFR) and endothelial function in type 1 diabetic patients with microalbuminuria.

RESEARCH DESIGN AND METHODS

Patients

Seventeen consecutive type 1 diabetic patients with persistent microalbuminuria (urinary AER between 30 and 300 mg/24 h in at least two of three sterile urine samples) were enrolled. All patients had been insulin dependent from the time of diagnosis and all were receiving at least two daily injections of insulin. Patients were excluded if they had systolic blood pressure (BP) >200 mmHg, were users of cyclooxygenase inhibitors, had acute gastritis or peptic ulcer disease, or if they were pregnant. During the investigation, patients received their usual antihypertensive treatment ($n = 15$; ACE inhibitors [$n = 14$] and/or non-ACE inhibitors [$n = 11$]). The study protocol was in accordance with the Helsinki Declaration. All subjects included in the study were Caucasians, and all gave informed consent to participate. The study was approved by the local ethics committee.

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Abbreviations: AER, albumin excretion rate; ASA, acetylsalicylic acid; BP, blood pressure; GFR, glomerular filtration rate; TER_{alb} , transcapillary escape rate of albumin; θ_{alb} , fractional clearance of albumin.

A table elsewhere in this issue shows conventional and Système International (SI) units and conversion factors for many substances.

Table 1—Clinical characteristics of 17 type 1 diabetic patients with persistent microalbuminuria

Sex (F/M)	12/5
Age (years)	43 ± 9
Duration of diabetes (years)	28 ± 8
Antihypertensive treatment (ACEI/non-ACEI/none)	14/1/2
Rethinopathy (none/simplex/proliferative)	3/7/7

Data are *n* or means ± SD. ACEI, ACE inhibitor.

Crossover design

All patients entered a randomized double-blind crossover trial. With concealed randomization, patients were allocated to take one tablet of ASA (150 mg) or one matching placebo tablet per day (in the morning). After 4 weeks of treatment, there was at least a 2-week washout period before patients crossed over to the opposite treatment for another 4 weeks.

Methods

Patients were investigated at the end of each treatment period of the double-blind crossover phase. All of the investigations were carried out with the patients in the supine position between 8:00 A.M. and 1:00 P.M. The investigations were started in the morning after an overnight fast. Patients had breakfast and their morning insulin after the start of the GFR investigation. They drank 150–200 ml tap water per hour during the study period.

Transcapillary escape rate of albumin

At 8:30 A.M. after 30 min of rest, an intravenous injection of 40 kBq ¹²⁵I-labeled human serum albumin was given (code MIAK; Institute of Atomic Energy, Kjeller, Norway), and eight venous blood samples were drawn 10, 15, 20, 30, 40, 50, 55, and 60 min after the injection. Transcapillary escape rate of albumin (TER_{alb}) was determined as the rate constant of the mono-exponential decrease in plasma radioactivity over the first 60 min after injection of tracer albumin, as calculated by the least square method and as described in detail by Parving et al. (12). Thus, TER_{alb} is defined as the fraction of intravascular mass of albumin leaving the vascular bed per hour (percent per hour).

GFR

GFR was measured after a single intravenous injection of 3.7 MBq ⁵¹Cr-EDTA immediately after termination of the TER_{alb} examination, by determining the

radioactivity in venous blood samples taken 180, 200, 220, and 240 min after the injection (13). Extra renal loss was corrected for by subtracting 3.7 ml/min¹ (14). The small underestimation (10%) of ⁵¹Cr-EDTA renal clearance versus renal clearance of insulin was corrected for by multiplying the EDTA clearance by 1.10 (14). The results were standardized for 1.73 m² body surface area. The mean day-to-day coefficient of variation in the GFR is 4% in our laboratory.

BP

Office BP was measured twice on the right arm, after at least 30 min of rest in the supine position with a Hawksley random zero sphygmomanometer and an appropriate cuff size (25 × 12 cm [upper arm circumference <35 cm] and 30 × 15 cm [upper arm circumference >35 cm]). Diastolic BP was recorded at the disappearance of Korotkoff sounds (phase V). The individual BP level was determined as the mean of at least two measurements performed during the GFR determination.

Urinary albumin excretion and fractional clearance of albumin

On the basis of the three consecutive 24-h urine collections completed immediately before each visit, urinary albumin excretion was measured by an enzyme-linked immunosorbent assay (coefficient of variation was 2.1%) (15). Fractional clearance of albumin (θ_{alb}) was obtained by dividing

the clearance of albumin (calculated as $[U \times V]/P$, where *U* is urine albumin concentration [mg/l], *V* [l/24 h] is urine flow, and *P* [g/l] is plasma albumin concentration) with the measured GFR to correct albumin excretion for changes in plasma albumin concentration and in GFR.

HbA_{1c} and blood glucose

From venous blood samples, HbA_{1c} was measured by high-performance liquid chromatography (Variant; Bio-Rad Laboratories, Hercules, CA). The normal range of HbA_{1c} in our laboratory is 4.1–6.4%. The venous blood glucose concentration was measured three times during the clearance period by a One Touch II (LifeScan, Milpitas, CA).

Statistical analysis

Results are expressed as means ± SD or 95% CI as appropriate. Urinary excretion of albumin and θ_{alb} are logarithmically transformed before statistical analysis because of their positively skewed distribution and are given as geometric mean (95% CI). Changes in variables between visits are expressed as means (95% CI). All comparisons of normally or log-normally distributed parameters were done with a paired Student's *t* test. Data were tested for a period effect and a treatment-period interaction with a two-sample *t* test (16). Before the present study, we calculated the SD (log₁₀ scale: 0.1771) of the mean difference in urinary AER in three consecutive 24-h urine samples collected twice within 3 months in 36 type 1 diabetic patients with diabetic nephropathy. On the basis of these data, a sample-size calculation revealed a necessary minimum of 16 patients to detect a 25% difference in change in urinary AER (α = 0.05 and β = 0.8). All calculations were made using Statistical Package for Social Science program for Windows (SPSS, Chicago). A *P* value of <0.05 was considered significant (two-tailed).

Table 2—Urinary AER, fractional clearance of albumin, and GFR during a randomized double-blind crossover trial with 4 weeks of treatment with ASA and 4 weeks of treatment with placebo in 17 type 1 diabetic patients with microalbuminuria

Variable	Placebo	ASA (150 mg)	Difference	<i>P</i>
Urinary AER (mg/24 h)	59 (40 to 87)*	64 (39 to 105)*	10 (−28 to 68)†	0.65
Fractional clearance of albumin (×10 ^{−6})	155 (103 to 234)*	198 (127 to 308)*	20 (−24 to 91)†	0.41
GFR (ml · min ^{−1} · 1.73 m ^{−2})	104 (90 to 117)	106 (93 to 118)	2 (−2 to 6)	0.33

Data are means (95% CI), *geometric means (95% CI), or †relative change (%).

Table 3—Transcapillary escape rate of albumin, systolic BP, diastolic BP, and HbA_{1c} during a randomized double-blind crossover trial with 4 weeks of treatment with ASA (150 mg/day) and 4 weeks of treatment with placebo in 17 type 1 diabetic patients with microalbuminuria

Variable	Placebo	ASA (150 mg)	Difference	P
Transcapillary escape rate of albumin (%/h)	6.5 (5.1 to 8.0)	6.2 (5.4 to 7.0)	-0.3 (-1.8 to 1.1)	0.64
Systolic BP (mmHg)	130 (119 to 142)	130 (119 to 141)	-0.6 (-6.5 to 5.3)	0.83
Diastolic BP (mmHg)	71 (64 to 78)	71 (65 to 78)	-0.1 (-4.5 to 4.3)	0.96
HbA _{1c} (%)	8.5 (8.1 to 9.0)	8.4 (8.0 to 9.0)	-0.1 (-0.4 to 0.2)	0.41

Data are means (95% CI).

RESULTS

Randomized double-blind crossover phase

All randomized patients completed the study. Table 1 shows the clinical characteristics of the patients. Table 2 shows data for urinary AER, θ_{alb} , and GFR after each of the two treatment periods. Table 3 shows the data of TER_{alb}, systolic and diastolic BPs, and HbA_{1c} after each of the two treatment periods.

No change in medication except shift from ASA and placebo tablets and vice versa was recorded in any patient during the investigation. On average, the washout period was 19.4 days (95% CI 17.0–21.7). Nine patients were smokers.

During the first treatment period, eight patients received ASA and nine received placebo. Neither a period effect (ASA/placebo vs. placebo/ASA; average differences [\log_{10} scale] -0.018 ± 0.35 vs. -0.092 ± 0.38 , $P = 0.68$) nor a treatment-period interaction (ASA/placebo vs. placebo/ASA; average levels [\log_{10} scale] 1.69 ± 0.24 vs. 1.87 ± 0.39 , $P = 0.28$) upon urinary AER was found.

Compliance

Using simple tablet counting, adherence was high, with >99% of tablets taken during period one and >97% taken during period two (NS). Blood ASA was not measured.

Side effects

No significant differences in side effects were observed between treatment with 150 mg ASA and placebo. Dyspepsia was equally reported during treatment with low-dose ASA and placebo (n [%]: 3 [18] and 3 [18], NS).

CONCLUSIONS— Our randomized double-blind crossover trial has demonstrated that short-term treatment with 150 mg ASA daily does not have any clinical

impact on urinary AER, GFR, and TER_{alb} in type 1 diabetic patients with microalbuminuria receiving antihypertensive treatment. Consequently, primary and secondary prevention of cardiovascular events with low-dose ASA does not interfere with the classification of albuminuria or monitoring of antiproteinuric treatment in these patients.

Low-dose ASA (40–325 mg/day) has been shown to offer substantial primary (5,6) and secondary protection against macrovascular morbidity and mortality in diabetic patients at high risk for cardiovascular events (7). Previous studies support the concept that the inactivation of the constitutive platelet cyclooxygenase-1 by ASA, and the consequent reduced formation of platelet thromboxane, largely account for the anti-thrombotic effects of ASA (17,18).

Elevated renal synthesis of vasodilating prostaglandins has been demonstrated in type 1 diabetic patients with microalbuminuria (9), and inhibition with high-dose indometacin (150 mg/day), another inhibitor of cyclooxygenase-1, induced a reduction in renal prostaglandin synthesis, urinary AER, and θ_{alb} , while GFR remained stable (9). Furthermore, treatment with high-dose ASA (990 mg ASA + 225 mg dipyridamole daily) reduced urinary AER in type 1 diabetic patients with diabetic nephropathy (10). These effects occur shortly after start of treatment. On the contrary, our study showed that urinary AER, θ_{alb} , and GFR remained unchanged during short-term treatment with low-dose (150 mg/day) ASA. The tubular protein reabsorption capacity is normal in type 1 diabetic patients with microalbuminuria (19), suggesting that microalbuminuria is of glomerular origin. The transglomerular passage of albumin is determined by the size- and charge-selective properties of the glomerular capillary wall and the transglomerular hydraulic pressure (20). We

have no direct information on these variables; however, our data indirectly suggest that low-dose ASA does not affect the intrarenal synthesis of prostaglandins.

A possible pathophysiological link between elevated urinary AER and cardiovascular disease has been hypothesized by Deckert et al. (21), who suggested that albuminuria reflects widespread vascular damage due to generalized vascular (endothelial) dysfunction (the Steno hypothesis). This concept has been supported by the finding of increased TER_{alb} in type 1 diabetic patients with microalbuminuria (22). The TER_{alb} is determined by the permeability surface area product of the microvasculature and the transcapillary hydraulic pressure gradient (23). The permeability is governed by the size- and charge-selective properties of the capillary barrier. Our study has confirmed that the TER_{alb} is elevated in type 1 diabetic patients with microalbuminuria (22). Since systemic BP remained unchanged during the present study, it seems reasonable to conclude that no major changes in size or charge selectivity were observed during low-dose ASA therapy.

In conclusion, treatment with low-dose ASA (150 mg once a day) does not have any clinical impact on urinary AER, GFR, or the TER_{alb} in type 1 diabetic patients with microalbuminuria. Consequently, primary and secondary prevention of cardiovascular events with low-dose ASA does not interfere with the correct classification of albuminuria or monitoring of antiproteinuric treatment.

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