

Long-Term Treatment With the Dual Antithromboxane Agent Picotamide Decreases Microalbuminuria in Normotensive Type 2 Diabetic Patients

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Picotamide both inhibits thromboxane synthetase and acts as a thromboxane antagonist at the receptor level. We investigated the long-term effect of picotamide on urinary albumin excretion (UAE) at rest and induced by exercise in 30 type 2 diabetic patients who were normotensive and had microalbuminuria while at rest. The subjects of our study had a mean age of 52.5 ± 1.6 years, BMI of $28.5 \pm 0.7 \text{ kg/m}^2$, diabetes duration of 9.1 ± 1.8 years, and HbA_{1c} of $7.0 \pm 0.8\%$. The study was a randomized double-blind placebo-controlled trial. The patients were randomly allocated to receive for 1 year either picotamide, 300 mg, 3 tablets/day, or placebo, 3 tablets/day. The patients were asked to visit our outpatient clinic after 1, 3, 6, 9, and 12 months of treatment. At all times, blood pressure, microalbuminuria at rest, blood glucose, serum creatinine, serum picotamide, and creatinine clearance were measured; at baseline and after 6 and 12 months, all patients underwent submaximal physical exercise. After 6 months of picotamide, baseline and exercise-induced microalbuminuria were significantly decreased (up to one-third) as compared with the baseline and placebo level, with no further drops at month 12 of picotamide treatment. On placebo treatment, UAE at rest and after exercise was slightly increased compared with baseline values. The effects of picotamide occurred without significant side effects or changes in either blood pressure levels or glycometabolic control. Our study is the first long-term intervention trial in type 2 diabetes showing that an antithromboxane agent is able to decrease microalbuminuria, which in this disease is a dual marker of macro- and microangiopathy. Our findings suggest an important role for thromboxane in the pathophysiology of microalbuminuria in diabetes; moreover, we hypothesize that antithromboxane agents may have a place in the treatment/prevention of both macro- and microvascular complications in type 2 diabetic patients. *Diabetes* 47:423–430, 1998

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ECG, electrocardiogram; UAE, urinary albumin excretion.

Platelets from patients with diabetes and from diabetic rats have been reported to synthesize more thromboxane than do normal platelets in response to a variety of agonists that induce deacylation of arachidonate from membrane phospholipids (1). Moreover, platelets obtained from patients with type 2 diabetes synthesize significantly higher amounts of thromboxane B₂ than those from patients with type 1 diabetes or matched control subjects (2,3). In both type 1 and type 2 diabetes, microalbuminuria (urinary albumin excretion [UAE] between 20 and 200 $\mu\text{g}/\text{min}$) predicts the onset of overt diabetic nephropathy, which may lead to chronic renal failure (4–6). Type 1 and type 2 diabetic subjects with microalbuminuria also have an increased mortality rate from cardiovascular diseases (6–8). Moreover, UAE has been shown to be associated with electrocardiogram (ECG) changes in type 2 diabetic patients (8). Microalbuminuria has also been found in both hypertensive (9) and normotensive (10) nondiabetic subjects, and microalbuminuria has been associated with a higher prevalence of coronary heart disease. Exercise can induce abnormal increases in UAE in diabetic patients with microalbuminuria at rest (11). It has been suggested that in subjects with microalbuminuria at rest, UAE after exercise might be studied to monitor the progression of microalbuminuria and might enable the effects of various drug treatment to be evaluated (12).

Some have suggested that platelet activation contributes to the pathogenesis of both the macrovascular and microvascular complications of diabetes (1,13,14). Evidence linking platelet activation with increased UAE comes from a study showing that an experimental specific inhibitor of thromboxane synthetase (UK-38,485) significantly reduced microalbuminuria in type 1 diabetic patients at rest compared with patients given a placebo (15). Dipyridamole, a phosphodiesterase inhibitor, has also been reported, either alone or associated with aspirin, to decrease UAE in type 1 diabetic patients with either overt (16,17) or incipient (18) diabetic nephropathy. Another antiplatelet agent, indomethacin, a cyclooxygenase inhibitor, has been shown to have beneficial effects on kidney function in type 1 diabetes (19). No significant information on the effects of antiplatelet agents on UAE in type 2 diabetes is available to date.

Picotamide (Novartis, Origgio, Italy), a bisamidic 4-OH isophthalic acid derivative recently introduced in clinical practice, has shown platelet inhibitory effects both in vitro and

in humans (20). Recent findings indicate that this drug both inhibits thromboxane synthetase and acts as a thromboxane antagonist at the receptor level in vitro and in vivo (21). We have recently shown that both picotamide and ACE inhibitors (11) are able, after short-term administration, to significantly decrease exercise-induced microalbuminuria in normotensive type 2 diabetic patients (22). On the other hand, it has been clearly demonstrated that after long-term administration, ACE inhibitors may reduce proteinuria and slow the progression of diabetic nephropathy (23). Our study investigated the long-term effect of picotamide on UAE at rest and induced by exercise in type 2 diabetic patients who were normotensive and had microalbuminuria while at rest.

RESEARCH DESIGN AND METHODS

Patients. The subjects of our study were 26 male and 4 female type 2 diabetic patients (age 52.5 ± 1.6 years, BMI 28.5 ± 0.7 kg/m²; Table 1). We used the following inclusion criteria: 40–65 years of age, known duration of diabetes >12 months, glycosylated hemoglobin (HbA_{1c}) <10%, stable BMI < 35 kg/m², supine blood pressure <140/90 mmHg, serum creatinine < 106 μmol/l, 24-h UAE between 20 and 200 μg/min in samples assessed weekly during the 3 months before the first evaluation and in the 15-day placebo run-in period, and no cardiovascular, hepatic, or systemic diseases before the beginning of the study. The patients were not taking any drugs other than those for the treatment of their diabetes. Owing to the high intraindividual variability of UAE, only patients who had variations within 15% of their average rate, measured by weekly assays in the month before the first evaluation, were included in this study (24). The patients were treated with diet and oral hypoglycemic agents (5–15 mg/day glyburide or glipizide or 80–160 mg/day gliclazide). For 3 months before and throughout the duration of the study, the patients followed an isocaloric diet (-0.13 mJ \times kg⁻¹ \times day⁻¹; 50% carbohydrates, 35% lipids, 15% proteins) with no restriction on sodium intake. Adherence to dietary recommendations was checked at each visit. Main exclusion criteria were presence of renal or hepatic diseases, ECG abnormalities at rest or induced by exercise, peptic ulcer diseases or previous hemorrhagic episodes, and treatment with either other antiplatelet drugs or ACE inhibitors.

Study design. The study was a randomized, double-blind, placebo-controlled trial. Randomization was carried out with a block size of four. Blinded treatment allocation was obtained by central randomization. The study drugs consisted of picotamide tablets (300 mg) and placebo tablets that were identical in appearance. The initial evaluation included a medical-history taking and a complete physical examination. If the patients met the inclusion criteria at the first evaluation (time T - 1), they were allocated to a 15-day run-in period during which they only assumed placebo, 3 tablets/day. If after this study period the patients were still meeting our inclusion criteria, they were randomly allocated to receive long-term medication (picotamide 300 mg, 3 tablets/day, i.e., 1 tab every 8 h) or placebo (3 tablets/day, i.e., 1 tab every 8 h) (time T0).

Follow-up evaluations. The patients were asked to visit our outpatient clinic after 1 (time T + 1), 3 (time T + 3), 6 (time T + 6), 9 (time T + 9), and 12 (time T + 12) months of treatment. At all times, the following baseline examinations were performed: supine blood pressure measurements, BMI, microalbuminuria at rest (mean of three 24-h urine samples collected the week before), blood glucose, HbA_{1c}, 24-h urine glucose, serum creatinine, creatinine clearance, and ECG. At times T - 1, T0, T + 6, and T + 12, routine hematochemical tests were also performed. Moreover, at times T - 1, T0, T + 6, and T + 12, all patients underwent a submaximal physical exercise for evaluation of exercise-induced albuminuria. Compliance with treatment was determined by pill count and blood monitoring

(plasma was obtained at each visit for measurement of picotamide trough levels). At T + 12, three urinary samples were collected for urinary thromboxane measurement in 10 patients (5 on picotamide and 5 on placebo).

Exercise-induced albuminuria. The diabetic patients performed four physical exercise tests during the study. For each test, all subjects came to the outpatient department at 0800 with a 24-h urine sample collected the previous day for UAE measurements. During the 24-h period of urine collection, the subjects were asked to follow the isocaloric diet detailed above and to avoid heavy or unusual physical exercises. A variation in UAE < 15% of the subject's average rate was considered a reasonable parameter to validate that subjects followed the dietary and exercise instructions. Subjects underwent a blood sampling for routine hematochemical tests and then remained recumbent for 1 h and drank 500 ml of water. At 0900, each subject performed a submaximal exercise test (90% of maximal theoretical heart rate) on a cycle ergometer according to a protocol previously described (11). The average duration of the exercise period was 10 min. Blood pressure, heart rate, and ECG were monitored constantly during the exercise and recovery period. When heart rate and blood pressure had returned to basal values (after 10 to 20 min), the subjects provided a sample of urine for assay of albumin concentration. Each subject again remained recumbent for 1 h and drank 500 ml of water, then provided another urine sample for UAE measurement. Blood glucose concentrations were monitored at rest, at the end of exercise, and at 30, 60, and 90 min after the end of exercise. Subjects were asked to collect their urine until 0800 the next day for albumin assay. The same investigator followed up with the same subjects.

Sample-size calculation and statistical analysis. The study sample size was calculated taking into account our previous data regarding the effect of short-term picotamide administration on exercise-induced UAE (22). For an assumed standardized difference in exercise-induced UAE between picotamide and placebo of 1.3, with an $\alpha = 0.05$ and $\beta = 0.90$, the sample would have to consist of a total of 30 patients. The study was stopped when the last of the 30 patients finished the trial, and only at that time were randomization codes broken by the same investigators who followed-up the trial. No interim analyses have been performed, and both the manufacturer of the tested drug and the investigators were masked to data until the end of the trial. The data, always expressed as means \pm SD, were analyzed by nonparametric methods to avoid assumptions about the distributions of the measured variables. Microalbuminuria at rest is expressed at all times as the mean of three samples. Postexercise UAE is always expressed as the maximal value obtained after exercise, regardless of the timing of the peak (either immediately or 1 h after exercise). ANOVA tests for repeated measurements were used to assess the interaction between time and treatments. Spearman's rank-correlation test was used to calculate the relationship between the different parameters. For all statistical tests, two-tailed *P* values <0.05 were considered to indicate statistical significance.

Assays. Albuminuria was assayed by an immunonephelometric method (nephelometer BNA 100; Behring, Scoppito, Italy; intra-assay CV 4.3%; inter-assay CV 4.4%; sensitivity limit of the assay 1.76 mg/l); HbA_{1c} was assayed by a chromatographic method (Bio-Rad, Milan, Italy; normal range 3–6%). Plasma glucose was determined by the glucose oxidase method (Beckman Glucose Analyzer II) and serum creatinine with a method based on the Jaffe reaction. Immunoreactive thromboxane B2 was extracted from 20 ml aliquots of each urine sample on SEP-PACK C18 cartridges, eluted with ethyl-acetate, and assayed by radioimmunoassay as previously described (3). Plasma picotamide was assayed by a validated high-performance liquid chromatographic method (25).

RESULTS

Clinical characteristics of the subjects. Table 1 shows the clinical data of the subjects studied. The two groups were well matched concerning the main biochemical and clinical vari-

TABLE 1
Baseline clinical characteristics of the two groups of patients randomly assigned to receive either picotamide (900 mg/day) or placebo (3 tablets/day)

Treatment allocation	Sex (M/W)	Age (years)	Diabetes duration (years)	BMI (kg/m ²)	HbA _{1c} (%)	Serum glucose (mmol/l)	sBP/dBP (mmHg)	Creatinine clearance (ml/s)	Serum creatinine (μmol/l)	UAE at rest (μg/min)
Picotamide	13/2	56 \pm 2	9.5 \pm 2.0	27 \pm 2	6.9 \pm 1.1	9.1 \pm 1.9	133/85 \pm 17/6	2 \pm 0.6	79.5 \pm 8.8	60 \pm 39
Placebo	13/2	57 \pm 3	8.8 \pm 1.6	28 \pm 4	7.0 \pm 0.6	10.7 \pm 1.8	132/84 \pm 18/8	1.8 \pm 0.4	79.5 \pm 17.6	72 \pm 45

Data are means \pm SD. sBP, systolic blood pressure; dBP, diastolic blood pressure.

TABLE 2

Serum creatinine, creatinine clearance, blood glucose, and glycosylated hemoglobin (HbA_{1c}) in the two study groups at baseline and during the various stages of treatment

Time (months)	Serum creatinine (μmol/l)		Creatinine clearance (ml/s)		Blood glucose (mmol/l)		HbA _{1c} (%)	
	Picotamide	Placebo	Picotamide	Placebo	Picotamide	Placebo	Picotamide	Placebo
Baseline	81.3 ± 8.8	79.5 ± 17.6	2 ± 0.6	1.8 ± 0.4	9.1 ± 1.9	10.7 ± 1.8	6.9 ± 1	7.0 ± 0.6
1	88.4 ± 8.8	70.7 ± 8.8	1.9 ± 0.4	2.3 ± 0.7	9.2 ± 2.8	10.6 ± 1.3	6.8 ± 1	6.8 ± 0.6
3	88.4 ± 8.8	70.7 ± 8.8	2 ± 0.4	2.5 ± 0.5	10.3 ± 2.6	10.7 ± 2.3	7.1 ± 0.8	6.9 ± 0.8
6	88.4 ± 8.8	70.7 ± 8.8	1.9 ± 0.5	2.6 ± 0.4	9.3 ± 2	10.9 ± 2.3	7.2 ± 1	6.9 ± 0.8
9	79.5 ± 8.8	70.7 ± 8.8	1.9 ± 0.5	2.2 ± 1	10.4 ± 4.5	10.6 ± 1.4	6.9 ± 1	6.7 ± 0.6
12	79.5 ± 8.8	70.7 ± 8.8	1.8 ± 0.5	2.1 ± 0.5	10.3 ± 2.2	11.6 ± 3.1	6.9 ± 1	7.0 ± 0.6

Data are means ± SD.

ables considered. Both groups had the same higher prevalence of men compared with women (13 vs. 2). The known duration of disease was similar in the two groups. The degree of glycometabolic control was fairly good and similar in both groups. Moreover, no significant changes in either HbA_{1c} or blood glucose (Table 2) were observed during the duration of the study. In none of the patients was either type or dose of antidiabetic treatment changed during the follow-up period. The exercise tests did not cause significant changes in blood glucose levels throughout the study in either picotamide- or placebo-treated subjects. All the patients had normal renal function at baseline, and no differences were noticed in either serum creatinine or creatinine clearance between groups. Moreover, these parameters did not significantly change throughout the study during either picotamide or placebo administration (Table 2).

Side effects and compliance. An overall number of 33 patients were enrolled in the study. Three patients spontaneously withdrew from the study during the first 3 months of follow-up due to lack of compliance. Two of these patients were in the placebo group, and the other was in the picotamide group. No significant side effects were recorded in any of the patients, with the exception of reported facial flushing in a patient treated with placebo. The compliance of the subjects was assessed through measurement of picotamide in the blood. Serum picotamide was always lower than 50 ng/ml in the placebo group. In the drug-treated group, significantly higher blood picotamide levels compared with baseline lev-

els were observed at all follow-up appointments. A wide range in serum drug level was observed in the treated subjects, probably due to differences in gastrointestinal absorption; however, all treated subjects had, throughout the study, serum picotamide values higher than 150 ng/ml, the threshold level chosen for assessing good compliance to the treatment (25). In two patients in the placebo group, significant ECG abnormalities were recorded during the exercise performed at the end of the study (T + 12). In detail, in one patient an episode of ventricular tachycardia in the recovery period and in the other patient a 1.8-mm depression of the ST segment at maximal exercise were observed.

No significant differences in baseline blood pressure were observed in the two groups of diabetic patients entering picotamide or placebo treatment. All subjects were normotensive (blood pressure <140/90 mmHg), and no significant differences among the two groups were observed in blood pressure at the end of the first two exercise tests. In all patients, systolic and diastolic blood pressure at rest and after exercise did not change significantly during either picotamide or placebo treatment (Table 3). Consistently, at the end of the study, no significant differences in baseline and exercise-induced blood pressure levels were found among placebo- and picotamide-treated groups.

Microalbuminuria at rest. At baseline evaluation (T₀), no significant differences in UAE at rest between patients entering the placebo- and picotamide-treated groups were observed (72 ± 45 vs. 60 ± 39 μg/min).

TABLE 3

Systolic and diastolic blood pressure and heart rate at rest and at the end of exercise in the two study groups at baseline and during treatment

Time (months)	sBP/dBP at rest (mmHg)		Heart rate at rest (beats/min)		sBP/dBP after exercise (mmHg)		Heart rate after exercise (beats/min)	
	Picotamide	Placebo	Picotamide	Placebo	Picotamide	Placebo	Picotamide	Placebo
Baseline	133/85 ± 17/5	132/84 ± 18/8	75 ± 14	83 ± 8	200/109 ± 15/16	195/108 ± 29/16	132 ± 12	126 ± 11
1	129/80 ± 13/5	134/84 ± 16/11	77 ± 11	76 ± 6	—	—	—	—
3	126/84 ± 11/5	137/88 ± 20/9	75 ± 12	82 ± 9	194/106 ± 21/12	197/105 ± 30/16	126 ± 13	130 ± 8
6	128/85 ± 16/6	132/83 ± 12/6	74 ± 11	77 ± 11	194/106 ± 30/10	195/102 ± 24/11	124 ± 12	130 ± 10
9	133/81 ± 17/8	130/85 ± 13/8	72 ± 9	78 ± 9	—	—	—	—
12	137/88 ± 19/7	136/89 ± 16/7	75 ± 11	76 ± 6	202/113 ± 29/13	—	—	—

Data are means ± SD. dBP, diastolic blood pressure; sBP, systolic blood pressure.

In the picotamide-treated group, UAE remained stable during the first 3 months of the study. After 6 months of picotamide administration, a significantly decreased level of microalbuminuria at rest compared with baseline was observed. Consistently, the 9- and 12-month levels of microalbuminuria remained suppressed compared with the baseline levels, without, however, a statistically significant further drop compared with the 6-month level of microalbuminuria (Fig. 1). At the end of the study, UAE in the group of picotamide-treated patients was cut to about 50% of the baseline level ($32 \pm 24 \mu\text{g}/\text{min}$). Inspection of the individual values shows that in 14 of 15 subjects, UAE values at the end of the study were reduced compared with baseline; in only 1 patient was UAE at the end of the study increased compared with baseline (37 vs. 27 $\mu\text{g}/\text{min}$). Moreover, in five patients, UAE was reduced under the threshold level of microalbuminuria.

In the placebo group, UAE remained stable throughout the whole duration of the study. At the end of the study, UAE was slightly, but not significantly, increased compared with the baseline level (86 ± 53 vs. $72 \pm 45 \mu\text{g}/\text{min}$; Fig. 1). Individual UAE values in 5 of 15 patients at the end of the study were within $\pm 10\%$ of baseline values (3 patients within $+10\%$, 2 within -10%). Of the other 10 patients, only 1 had reduced microalbuminuria at the end compared with the beginning of the study, and 1 patient became macroalbuminuric at the end of the study.

The comparison of picotamide- and placebo-treated groups shows a significantly lower value of UAE at rest in the picotamide-treated patients from month 6 to the end of the study (Fig. 1).

Exercise-induced microalbuminuria. At baseline evaluation (T0), no significant differences in peak UAE after exercise between patients entering the placebo- and picotamide-treated groups were observed (206 ± 105 vs. $246 \pm 162 \mu\text{g}/\text{min}$).

At the first testing (1 month of picotamide treatment), a clear—though not statistically significant—reduction in peak exercise-induced microalbuminuria was already observed. From the second testing (month 6 of treatment), this decrease became highly significant (to one-third of baseline levels), without further drops at month 12 of picotamide treatment ($84 \pm 54 \mu\text{g}/\text{min}$; Fig. 2). In all 15 patients, picotamide caused a reduction in UAE after exercise.

In the placebo group, UAE after exercise remained stable throughout the duration of the study. At the end of this study, it was slightly, but not significantly, increased compared with baseline values (268 ± 221 vs. $206 \pm 105 \mu\text{g}/\text{min}$; Fig. 2). Individual values at the end of the study show that in 6 of 15 patients, a slight (10–25%) decrease in peak exercise-induced UAE was observed compared with baseline. Conversely, in the remaining nine patients, peak UAE after exercise was increased (up to 1000%) at the end of the study compared with baseline.

The comparison of picotamide- and placebo-treated groups shows that from month 6 to the end of this study, a significantly lower value of UAE after exercise was found in picotamide-treated patients (Fig. 2). Neither picotamide nor placebo caused any changes in the duration of exercise period compared with baseline.

Urinary thromboxane levels. The urinary excretion rate of thromboxane B_2 averaged $27.3 \pm 8 \text{ ng}/24 \text{ h}$ in the five patients

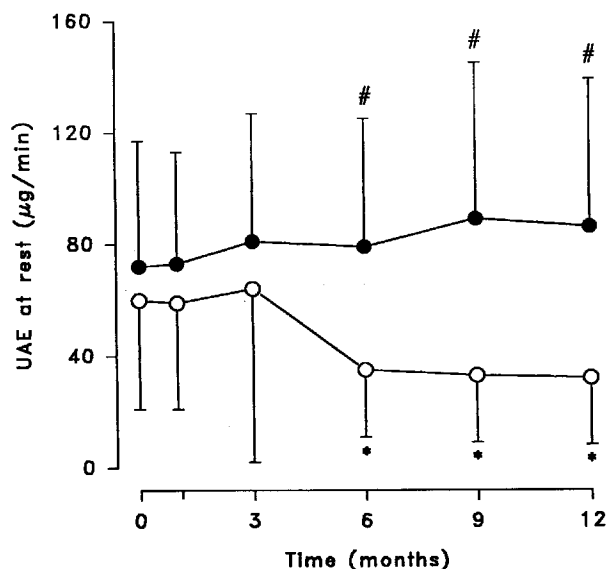


FIG. 1. Means \pm SD UAE at rest ($\mu\text{g}/\text{min}$) in 30 type 2 diabetic patients at baseline and after 1, 3, 6, 9, and 12 months of treatment with either picotamide (\circ ; $n = 15$; $300 \text{ mg} \times 3/\text{day}$) or placebo (\bullet ; $n = 15$; 3 tablets/day). * $P < 0.05$ vs. baseline; # $P < 0.05$ vs. picotamide.

treated with placebo. A slightly, but not statistically significant, lower urinary level of thromboxane B_2 in the five type 2 diabetic patients treated with picotamide ($16.9 \pm 6 \text{ ng}/24 \text{ h}$) was found. At the end of the study, urinary thromboxane B_2 was positively correlated with UAE after exercise ($r_s = 0.57$; $P < 0.05$) and with UAE at rest ($r_s = 0.49$; $P = 0.059$) and inversely correlated with serum picotamide level ($r_s = 0.73$; $P < 0.05$).

DISCUSSION

Our data show that long-term administration (1 year) of picotamide, a new clinically available dual antithromboxane agent, significantly decreases microalbuminuria at rest and after exercise in a group of normotensive patients with mild type 2 diabetes and microalbuminuria at rest, compared with placebo. This effect of picotamide occurs without changes in either blood pressure levels or glycometabolic control. Moreover, in a subgroup of 10 patients treated with either picotamide or placebo, we found a linear relationship between the urinary thromboxane excretion rate and the exercise-induced microalbuminuria at the end of the study.

There is a well-established association of diabetes with the development of atherosclerosis and its thromboembolic complications (26) and with the occurrence of disorders of the renal and retinal microvasculature (27). Abnormal platelet activation might contribute to the pathogenesis of both types of complications (3). The results of a recent study on the rate of thromboxane biosynthesis in type 2 diabetic patients in vivo by measuring its major urinary metabolite (29), 11-dehydro-thromboxane B_2 , suggest that enhanced thromboxane formation in type 2 diabetes is not a consequence but rather a causal factor of macrovascular disease (28). Platelet hyperactivity has also been hypothesized to play a role in the pathogenesis of diabetic nephropathy, although the mechanism underlying this action remains unknown (30,31).

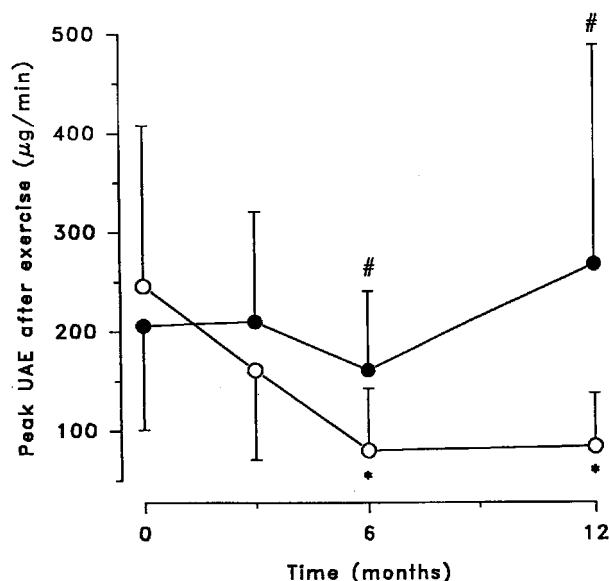


FIG. 2. Means \pm SD peak UAE after exercise ($\mu\text{g}/\text{min}$) in 30 type 2 diabetic patients at baseline and after 3, 6, and 12 months of treatment with either picotamide (\circ ; $n = 15$; 300 mg \times 3/day) or placebo (\bullet ; $n = 15$; 3 tablets/day). * $P < 0.05$ vs. baseline; # $P < 0.05$ vs. picotamide.

Microalbuminuria is a well-established marker of early diabetic nephropathy (4,5,32). In addition, it was clearly demonstrated that both type 1 and type 2 diabetic patients with microalbuminuria have a significantly higher probability of developing clinically overt nephropathy than those without microalbuminuria (6,33). Several studies have also shown that microalbuminuria predicts cardiovascular mortality in type 1 (34) and type 2 (35) diabetic subjects and nondiabetic elderly subjects (36).

The mechanism linking microalbuminuria to both macrovascular and microvascular diseases of diabetes is at present unclear. It may be speculated that the vascular permeability that permits glomerular leakage of albumin (37) may also be found in other vessels, thereby permitting the escape of lipoproteins into arterial walls. On the other hand, microalbuminuria has been associated with the accumulation of extracellular matrix in glomeruli and large-vessel walls (38). Moreover, it has been associated with changes in extracellular matrix (39,40) and proliferation of mesangial and myomedial cells (41). Similar changes in the extracellular matrix of vessel walls have been found in atherosclerosis (42). Finally, it was recently shown that endothelial dysfunction, estimated by plasma concentration of von Willebrand factor, is strongly related to the development of microalbuminuria and the occurrence of cardiovascular events in type 2 diabetes (43).

Microalbuminuria after exercise has been proposed both as an early marker for identifying normoalbuminuric diabetic patients susceptible to the later development of microalbuminuria at rest (44,45) and as a sensitive tool for evaluating the effects of various drug treatments on UAE (11,12,22). It has been hypothesized that the increase in systemic blood pressure during exercise, and consequently the higher intraglomerular filtration pressure in patients who may already have either structural or functional micro- or macrovascular changes in the glomerulus, may induce

increased excretion of albumin as compared with normal subjects (44). Normalization of albuminuria induced by exercise in type 1 diabetic patients has been seen after continuous subcutaneous administration of insulin with portable pumps (46). Moreover, strict metabolic control has also been shown to significantly decrease UAE at rest in type 1 diabetes (47). In fact, in the Diabetes Control and Complications Trial, long-term (6.5 years) intensified insulin therapy reduced the occurrence of microalbuminuria by 39% and that of macroalbuminuria by 54% in type 1 diabetic patients. In the only available large-scale intervention study in type 2 diabetes, the University Group Diabetes Programme, the therapeutic allocations to intensified glycometabolic control on UAE were ended because of a tendency to increase rather than decrease the incidence of major cardiovascular events (48).

A decrease in UAE at rest and after exercise has been reported in hypertensive diabetic and nondiabetic patients with microalbuminuria at rest who are given different antihypertensive treatments, such as β -blockers (50), clonidine (51), and calcium-channel blockers (52,53). Long-term treatment with ACE inhibitors has recently been shown to induce a significant decrease in UAE at rest in normotensive type 1 diabetic patients with microalbuminuria (54). Moreover, Lewis et al. (23) have demonstrated that treatment with the ACE inhibitor captopril is associated with a 50% reduction, compared with placebo, in the risk of the combined end points of death, dialysis, and transplantation in type 1 diabetic patients with diabetic nephropathy not dependent on the small effect of captopril on blood pressure. We also have shown that short-term inhibition of ACE significantly decreases exercise-induced microalbuminuria without substantially affecting blood pressure in normotensive type 1 and type 2 diabetic patients. It can be hypothesized that the primary effect of ACE inhibition on UAE in normotensive diabetic patients is a decrease in intraglomerular capillary pressure independent of variations in systemic blood pressure (10,54,55). Information is scanty about the effects in patients with type 2 diabetes of hypoglycemic and antihypertensive treatments on microalbuminuria, which in these patients is an important marker of both micro- and macroangiopathy.

Among patients at high risk of occlusive vascular disease, such as diabetic patients, some years of antiplatelet therapy offer worthwhile protection against myocardial infarction, stroke, and death (56). In patients with diabetes, the potential effect of antiplatelet treatment in the prevention of diabetic nephropathy is not known. However, this issue has recently been addressed by a number of researchers. Donadio et al. (17) found that 7 of 28 (25%) patients with diabetes and overt nephropathy who were treated for 4 years with aspirin and dipyridamole had a reduction in proteinuria at rest and no decrease in glomerular filtration rate. Dipyridamole alone has also been shown to decrease baseline UAE in patients with type 1 diabetes (18), and indomethacin, a cyclooxygenase inhibitor, has shown the same effect in patients with type 1 diabetes and incipient nephropathy (19). A previous study demonstrated that long-term administration of an experimental thromboxane synthetase inhibitor did cause a 73% decrease in UAE in resting patients with type 1 diabetes and microalbuminuria (15). However, in this study, the control group showed a 24% decrease in albuminuria, and two of the control subjects had a greater decrease of UAE than did the treated patients

Picotamide (*N,N'*-bis[3-picolyl]-4-methoxyisophtalamide) has potent platelet inhibitory effects *in vitro* and in humans (20). *In vitro* experiments have indicated that this drug both inhibits thromboxane synthetase and acts as a thromboxane antagonist at the receptor level (21). In fact, picotamide has been shown to competitively inhibit human platelet aggregation induced by the stable endoperoxide analog U46619 and by natural thromboxane. Picotamide has also been shown to be equally effective in inhibiting thromboxane synthetase and thromboxane receptor binding and to exert both effects simultaneously at active concentrations (21). This is important because while the inhibition of thromboxane synthetase enhances the endogenous synthesis of prostacyclin, thromboxane receptor antagonism prevents accumulated endoperoxides from activating platelets and smooth muscle cells (57,58). Picotamide has already been shown in the clinical setting to be able to influence the progression of several types of atherosclerotic lesions and also, in acute studies, UAE in patients with diabetes. In fact, in patients with peripheral atherosclerosis, picotamide significantly increased the walking distance and the ankle-arm pressure index (59). Moreover, in a double-blind, randomized, multicenter, long-term study, picotamide significantly reduced the rate of vascular events in diabetic (60) and non-diabetic (61) patients with peripheral occlusive arteriopathy. Finally, in a prospective, randomized, double-blind study, long-term picotamide treatment has been shown to slow the evolution of early carotid atherosclerotic lesions compared with placebo in diabetic subjects, inhibiting progression and plaque growth and reducing the appearance of new lesions (62). The overall incidence of severe adverse reactions with picotamide in these studies has been low and similar to that observed with placebo (63). On the other hand, we have recently reported that short-term picotamide administration (10 days) decreases exercise-induced microalbuminuria in type 2 diabetes (22). Moreover, in a long-term uncontrolled open pilot study, four patients with type 2 diabetes and microalbuminuria at rest were treated for 9 months with picotamide (900 mg/day). At the end of the study, the picotamide-treated patients demonstrated a significant decrease compared with baseline in microalbuminuria at rest (at baseline 41.7 ± 12.7 vs. 11.8 ± 3 $\mu\text{g}/\text{min}$) and after exercise (peak at baseline 103 ± 36 vs. 65.8 ± 11 $\mu\text{g}/\text{min}$ after treatment; 64). These preliminary findings suggested that long-term picotamide treatment was potentially able to decrease UAE both at rest and after exercise in patients with type 2 diabetes. However, a long-term controlled trial was needed to strengthen these findings and to quantify the potential beneficial effects of picotamide in a population study.

The data of the present study show that picotamide significantly decreases UAE at rest and after exercise compared with placebo in normotensive type 2 diabetic patients with microalbuminuria at rest. Importantly, the two study groups were well matched concerning the duration of disease, the baseline glycometabolic and blood pressure control, and the degree of renal involvement as assessed by UAE, serum creatinine, and creatinine clearance. In the 1 year of follow-up, the diabetic patients treated with placebo, taken as a whole, showed a tendency toward progression of both at-rest and exercise-induced microalbuminuria (+20 and +30%, respectively) without any tendency toward an increase in either blood pressure or HbA_{1c} levels. In two-thirds of the patients,

a progression in UAE at rest and after exercise was observed: in one of these patients, UAE levels progressed to macroalbuminuria. The higher relative increase at the end of the study in UAE after exercise compared with at rest in patients on placebo suggests that the first marker may be more sensitive than the latter in revealing the progression of vascular damage. The 1-year intervention with picotamide in the type 2 diabetic subjects determined a significant reduction in UAE at rest and after exercise compared with baseline levels (-50 and -70%, respectively) and with the levels in placebo-treated patients (-70 and -80%, respectively) without changes either in blood pressure or in glycometabolic control. The exercise-induced data confirm on a long-term basis those obtained in the previously performed short-term study, which showed a decrease to -70% of exercise-induced microalbuminuria compared with placebo (22). Interestingly, just as seems to be the case for ACE inhibitors (54), these acute effects of picotamide on exercise-induced microalbuminuria appear to be able to predict the long-term efficacy of picotamide on microalbuminuria at rest, not only from the qualitative but also from the quantitative (-70% compared with placebo) point of view. Moreover, it should be noticed that the blunting effect on microalbuminuria at rest obtained with picotamide is at least comparable to, if not greater than, that observed in long-term trials with either ACE inhibitors (54) or strict metabolic control (47) in diabetic patients.

The mechanism via which picotamide exerts its effects on microalbuminuria is alternative to intensified glycometabolic control and antihypertensive regimens and can be hypothesized to be thromboxane inhibition. This is suggested by the linear inverse correlation between serum levels of picotamide and urinary excretion of thromboxane at the end of the study and by the positive correlation between urinary thromboxane levels and microalbuminuria, both exercise-induced (statistically significant) and at rest (approaching statistical significance). These findings are in agreement with previous work suggesting involvement of increased platelet thromboxane production in the pathogenesis of macro- (28) and microvascular (15) complications of type 2 diabetes. That exercise-induced UAE is more sensitive than at-rest UAE to the picotamide-mediated thromboxane reduction suggests that the hemodynamic effects of thromboxane, *i.e.*, increase in intraglomerular capillary pressure (22), may be of significant importance in the pathogenesis of exercise-induced microalbuminuria (22,65). Alternatively, it can be hypothesized that picotamide affects glomerular permeability either directly or via thromboxane inhibition. Interestingly, a possible further explanation of the mechanism of picotamide-mediated decrease in microalbuminuria has been suggested by recent findings showing that picotamide may block *in vitro* the myogenic action of platelet-derived growth factor on smooth muscle cells proliferation at concentrations similar to those obtained after *in vivo* administration (66). This effect on platelet-derived growth factor suggests that the picotamide-mediated decreasing action on microalbuminuria may be a part and/or a marker of a systemic anti-atherosclerotic effect of the drug. Hyperinsulinemia and insulin resistance are distinctive features of patients with type 2 diabetes (67), and they were reported to be associated with microalbuminuria in these patients (68). Our data cannot rule out that the effect of picotamide on microalbuminuria may be mediated via a decrease in insulin resistance.

However, this mechanism seems unlikely because no improvement in glycometabolic control or change in the need for antidiabetic treatment was observed in the picotamide-treated patients.

Our study is the first long-term intervention trial in type 2 diabetes showing that a dual antithromboxane agent consistently decreases microalbuminuria, which in this disease can be considered a dual marker of macro- and microangiopathy. These findings are important for the understanding of the pathophysiology of microalbuminuria, showing that thromboxane may play a major role in its determinism. The relevance of our positive results to the clinical practice is increased by the fact that antiplatelet agents in general, and antithromboxane drugs such as picotamide in particular, are widely recognized to be indicated in type 2 diabetic patients, a high-risk group for coronary artery disease (56,60). Finally, it is intriguing to speculate that the effect of antithromboxane agents on microalbuminuria may be a positive marker of their efficacy in the prevention of clinically relevant atherosclerotic lesions.

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