Reduction of ACE Activity Is Insufficient to Decrease Microalbuminuria in Normotensive Patients With Type 1 Diabetes

Mats Bojestig, MD, PhD
Bengt E. Karlberg, MD
Torbjörn Lindström, MD, PhD
Fredrik H. Nyström, MD, PhD

OBJECTIVE — To study whether administration of 1.25 and 5.0 mg ramipril daily, compared with placebo treatment, reduces the urinary albumin excretion rate (UAER) in normotensive patients with type 1 diabetes.

RESEARCH DESIGN AND METHODS — Ramipril was administered double blind at two different doses (1.25 \( n = 19 \)) and 5.0 mg \( n = 18 \)), and compared with placebo \( n = 18 \)) after a single-blind placebo period of 1–4 weeks. The patients (total, \( n = 55 \); women, \( n = 14 \)) were followed for 2 years. To document an effect on the renin-angiotensin system, ACE activity and plasma-renin activity (PRA) were measured. In addition, 24-h ambulatory blood pressure (BP) was recorded at baseline and repeated after 1 and 2 years using a Spacelab 90207 ambulatory BP recording device (Spacelab, Redmont, CA).

RESULTS — Both doses of ramipril were sufficient to reduce ACE activity and to increase PRA significantly as compared with placebo \( P < 0.05 \) for both). On the other hand, neither ambulatory nor clinic BP was affected by either dose of ramipril compared with the placebo group. There was no progression of UAER in the placebo group during the 2 years of the study. Analysis of covariance showed no differences in UAER between the three treatment groups at year 1 \( P = 0.94 \) or year 2 \( P = 0.97 \), after adjusting for baseline. Furthermore, there were no statistically significant changes from baseline UAER within any of the three treatment groups.

CONCLUSIONS — Treatment with ramipril did not affect microalbuminuria or clinic or ambulatory BP in this study. On the basis of the present study, we question the clinical use of ACE inhibitors in stably normotensive patients with type 1 diabetes and microalbuminuria in whom a concomitant reduction in BP is not demonstrated.

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ACE inhibitors are often prescribed to normotensive patients with type 1 diabetes and microalbuminuria (1). Numerous reports have proved that treatment with ACE inhibitors reduces the urinary albumin excretion rate (UAER) in this particular category of patients (2–16). However, many of these trials were of rather short duration or contained only a small number of patients (2–8). When the effect of ACE inhibitors on UAER has been compared with that of other antihypertensive drugs, a more profound reduction has often been achieved with ACE inhibition (2,7,8,10). Demonstration of a reduction in UAER, even after correction for changes in blood pressure (BP) (17), and two large studies of the short-acting ACE inhibitor captopril (9,11) also imply that pharmacological inhibition of ACE confers renoprotective effects that are independent of changes in BP in normotensive patients with type 1 diabetes.

We aimed to study whether the reduction of UAER in normotensive patients with type 1 diabetes is dependent on changes of BP or if interference with the renin-angiotensin system is, in itself, sufficient to explain the effects of ACE inhibition on UAER. We thus performed a 2-year study of the long-acting ACE inhibitor ramipril compared with placebo in normotensive patients with type 1 diabetes. Ramipril was administered at two different doses: 1.25 and 5.0 mg; the lower dose was assumed not to reduce BP (18,19). To document an effect on the renin-angiotensin system, ACE activity and plasma-renin activity (PRA) were monitored during the study. Because 24-h ambulatory BP (ABP) correlates better with target organ damage (20) and provides higher reproducibility than clinic BP (21), we recorded ABP as well as regular clinic BP to assess the magnitude of the antihypertensive effect of ramipril.

RESEARCH DESIGN AND METHODS

Subjects

The study was performed at six centers in Sweden. We consecutively recruited 55 normotensive patients with microalbuminuria (clinic diastolic BP <90 mmHg) defined as 20–200 μg/min UAER in two of three urine collections. Two additional urine collections were used to estimate baseline UAER after a single-blind placebo period of 1–4 weeks. Patients treated with any form of antihypertensive medication were not considered for participation. Subjects were then randomized, double-blind, to receive either placebo or ramipril (1.25 or 5.0 mg) once daily in the morning.

From the Department of Medicine and Care, University Hospital of Linköping, Linköping, Sweden. Address correspondence and reprint requests to Fredrik Nyström, MD, PhD, Department of Medicine and Care, University Hospital of Linköping, SE-581 85 Linköping, Sweden. E-mail: fredrik.nyström@lio.se. Received for publication 10 October 2000 and accepted in revised form 8 January 2001.

Abbreviations: ABP, ambulatory blood pressure; ACE, angiotensin I-converting enzyme; BP, blood pressure; GFR, glomerular filtration rate; PRA, plasma-renin activity; UAER, urinary albumin excretion rate. A table elsewhere in this issue shows conventional and Systeme International (SI) units and conversion factors for many substances.
**Methods**

Determinations of ACE activity and PRA were performed at 0, 3, 6, 12, 18, and 24 months of treatment. The methods for analysis of ACE and PRA have been published earlier (22). Glomerular filtration rate (GFR) was determined by iohexol clearance at 0, 6, 12, and 24 months of treatment (23). Collection of urine for analysis of urinary albumin was performed in duplicate every 3 months. Determination of urinary albumin levels was performed by radioimmunoassay. Levels of Hba1c were determined by high-performance liquid chromatography.

Spacelab 90207 ABP recording devices (Spacelab, Redmont, CA) were used to record 24-h ABP at baseline and after 1 and 2 years, as described previously (24). BP was recorded every 20 min during the day (0600–2200) and during the night (2200–0600). Every 3 months, clinic BP was measured twice by a nurse, after 5 and 6 min of rest, in the supine position. The mean value of the two recordings was calculated. The standard cuff size used was 12 cm wide × 32 cm long for arm circumferences <36 cm. If the arm circumference exceeded 36 cm, a 14-cm-wide cuff was used. Phase 1 and V Korotkoff sounds were defined as systolic and diastolic BP, respectively.

**Ethics**

The study was approved by the ethics committee at Linköping University Hospital (Linköping, Sweden), and all subjects gave informed consent.

**Statistics**

The use of parametric or nonparametric statistical methods is given elsewhere in the text. Statistical significance was considered at the 5% level (P = 0.05). It was calculated that a total of 55 subjects was needed to achieve >90% power to detect a 50% reduction in UAER for active treatment compared with no change with placebo treatment.

**RESULTS** — There were no differences between the groups regarding proportions of men and women, age of the subjects, duration of diabetes, or Hba1c levels (Table 1). Hba1c was unaffected by treatment, and the levels remained stable in all three treatment groups throughout the study.

**Effect of treatment on ACE activity, levels of PRA, and BP**

Both doses of ramipril were sufficient to decrease ACE activity and to increase PRA significantly as compared with placebo (Fig. 1A and B). After 2 years of treatment, ACE activity was approximately halved in both groups treated with ramipril, and PRA was almost doubled as compared with placebo. On the other hand, neither ambulatory nor clinic BP was affected by either dose of ramipril compared with placebo (Table 2).

**Effect of treatment on UAER**

There was no progression of UAER in the placebo group during the 2 years of the study. There was no development of hypertension in any of the treatment groups at year 1 (P = 0.77). GFR remained similar during the study. There was no development of hypertension in any of the three treatment groups throughout the study. No regression of proteinuria occurred in any patients during the study. There was no development of normoalbuminuria or development of macroproteinuria occurred in any patients during the study. There was no development of hypertension in any of the treatment groups.

**Adverse events**

In general, there were no differences between treatment groups regarding the proportion of subjects reporting adverse events (P = 0.8). All patients in the placebo group completed the study. Of the patients receiving 1.25 mg ramipril, two withdrew from the study. One withdrew because of arthralgia, which subsided after discontinuation of the drug. The withdrawal of the second patient from the study after 1 year was unrelated to any particular event or side effects. In the group receiving 5.0 mg ramipril, there were also two withdrawals. One was due to cough and one was due to feeling of faintness. The symptoms abated after discontinuation of the drug. The patients who withdrew from the study were used in the outcome calculations.

**CONCLUSIONS** — We found that both doses of ramipril, 1.25 mg or 5.0 mg given once daily, on average halved the ACE activity levels throughout the study, indicating a relevant pharmacological interference with the renin-angiotensin system. Furthermore, PRA was roughly doubled by ramipril, indirectly demonstrating decreased plasma concentrations of angiotensin II. Despite these proofs of pharmacological effects of ramipril, we somewhat surprisingly found no change in either clinic or ambulatory BP with either dose of ramipril. A small BP-reducing effect of ramipril when given to subjects not necessarily hypertensive (average BP, 139/79 mmHg) was found in the Heart Outcomes Prevention Evaluation study, in which 10 mg ramipril (compared with placebo) decreased BP, on average, by 3/2 mmHg (25,26).

**Table 1—Baseline characteristics of patients**

<table>
<thead>
<tr>
<th>Group</th>
<th>n (M/F)</th>
<th>Age (years)</th>
<th>Duration of diabetes (years)</th>
<th>Hba1c (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Placebo</td>
<td>14/4</td>
<td>38 ± 9</td>
<td>21 ± 9</td>
<td>7.4</td>
</tr>
<tr>
<td>Ramipril 1.25 mg</td>
<td>13/6</td>
<td>42 ± 10</td>
<td>28 ± 11</td>
<td>7.6</td>
</tr>
<tr>
<td>Ramipril 5.0 mg</td>
<td>14/4</td>
<td>39 ± 10</td>
<td>22 ± 12</td>
<td>7.2</td>
</tr>
</tbody>
</table>

Data are means ± SD unless otherwise indicated.
compared with placebo was demonstrated (1–17). However, in one small study by Chase et al. (27), therapy with captopril \((n = 7)\) for 2 years did not affect UAER or BP as compared with placebo \((n = 9)\), although the rate of progression of retinopathy was reduced. In contrast to the study presented here, the effects of ACE inhibition on levels of PRA or ACE activity levels was not assessed by Chase et al. (27) nor was ABP performed.

HbA\(_1c\) was similar in the three treatment groups and remained stable during the study. The high-performance liquid chromatography method for HbA\(_1c\) in our study gives values that, in general, are \(~1\%\) lower than, for example, those of the U.K. (28). Thus, for comparison, the patients in our study were not under as good glycemic control as the group allocated to intensive treatment in the Diabetes Control and Complications Trial (29). A reduction of UAER by ACE inhibition was demonstrated earlier in patients with higher (12,14,30) as well as lower HbA\(_1c\) levels (16) than patients in our study. This makes it unlikely that a trial difference

Figure 1—A: Mean values of ACE activity at the different time points during therapy with ramipril 1.25 mg, ramipril 5.0 mg daily, or placebo. \(P < 0.001\) for differences between either 1.25 or 5.0 mg ramipril daily at 24 months of treatment compared with placebo. B: Median values of PRA at the different time points during therapy with ramipril 1.25 mg, ramipril 5.0 mg daily, or placebo. \(P < 0.05\) for differences between either 1.25 or 5.0 mg ramipril daily at 24 months of treatment compared with placebo.
ACE inhibition and microalbuminuria

Data are median (range) from the treatment groups at 0, 1, and 2 years of treatment. There were no statistically significant differences between the groups at any time point.

Table 2—Clinic and ambulatory BP at baseline and after 1 and 2 years of treatment

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Baseline BP (mmHg)</th>
<th>BP after 1 year (mmHg)</th>
<th>BP after 2 years (mmHg)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Clinic BP</td>
<td>Daytime BP</td>
</tr>
<tr>
<td>Placebo</td>
<td>124 ± 13/78 ± 7.2</td>
<td>122 ± 8.9/77 ± 7.1</td>
<td>128 ± 13/90 ± 6.2</td>
</tr>
<tr>
<td>Clinic BP</td>
<td>138 ± 9.7/82 ± 6.0</td>
<td>134 ± 8.3/82 ± 5.3</td>
<td>134 ± 8.1/81 ± 6.4</td>
</tr>
<tr>
<td>Daytime BP</td>
<td>124 ± 8.9/72 ± 7.8</td>
<td>121 ± 8.0/70 ± 6.9</td>
<td>120 ± 7.7/70 ± 5.8</td>
</tr>
<tr>
<td>Nighttime BP</td>
<td>127 ± 12/76 ± 7.2</td>
<td>133 ± 14/74 ± 8.4</td>
<td>132 ± 14/77 ± 8.7</td>
</tr>
<tr>
<td>Ramipril 1.25 mg</td>
<td>136 ± 14/80 ± 8.6</td>
<td>132 ± 13/80 ± 6.2</td>
<td>134 ± 9.4/79 ± 7.5</td>
</tr>
<tr>
<td>Ramipril 5.0 mg</td>
<td>123 ± 13/70 ± 8.0</td>
<td>119 ± 8.2/68 ± 5.8</td>
<td>121 ± 10/67 ± 6.2</td>
</tr>
</tbody>
</table>

Data are means ± SD. There were no statistically significant differences in BP between the groups at any time point.

with regard to glycemic control was the reason for the lack of effect of ramipril on UAER in our study.

We found no differences in UAER between the three groups, or changes from baseline, at any time point during the study. Again, this was true despite a powerful interference with the renin-angiotensin system. We were thus unable to test our hypothesis that 5.0 mg ramipril, which we had assumed would reduce BP in this study, compared with 1.25 mg ramipril, which was confirmed to lack effect on BP, would reduce UAER or cause a decline in GFR. On the other hand, the results of our study do suggest that both 1.25 and 5.0 mg ramipril once daily significantly affect PRA and ACE activity and that this magnitude (doubling of PRA and decreasing ACE activity to approximately half of baseline levels) of interference with the renin-angiotensin system does not decrease UAER in normotensive subjects in whom BP was not reduced. Considered together, our findings imply that a decrease in BP is the main cause of the UAER-reducing effect of ACE inhibition in normotensive subjects with type 1 diabetes and microalbuminuria. This interpretation was strengthened by the statistically significant correlation between the change in systolic BP and the change in UAER. This association suggests that some individuals did experience a decrease in BP and that this was correlated, on an individual level, with a reduction in UAER. On the other hand, it is even possible that some subjects experienced an increase in UAER and BP in parallel, which also serves to cause a positive correlation between changes in clinic BP and UAER.

Thus, it is possible that this, together with reasonably good glycemic control, accounts for stable UAER in the control group.

The lack of an effect of ACE inhibition on UAER in this study was not due to the specific choice of ramipril. In the HOPE study (in the substudy micro-HOPE), it was clearly settled that ramipril reduces UAER as well as microangiopathy in subjects with diabetes (25,26). Furthermore, in the ATLANTIS study, comprising 120 normotensive patients with type 1 diabetes and microalbuminuria, 5.0 mg ramipril decreased BP and UAER. However, this study has hitherto only been published as an abstract (32).

The subjects in the study by Chase et al. (27) (in which no effect on UAER or BP was seen with treatment with captopril) had a mean BP of only 118/78 mmHg at baseline, which was even lower than that of the patients in our study. However, in other trials, patients have been recruited with clininc BP in the same strictly normotensive range as in our study, and still a reduction of UAER and BP was found by inhibition of ACE (9,15).

The main limitation of this study is the size. It is possible, although very unlikely, that it was underpowered to detect relevant changes in UAER. However, we believe that this was compensated for by frequent determination of UAER. Furthermore, we measured clinic as well as ABP, and a significant pharmacological effect of ACE inhibition was demonstrated on ACE activity and PRA levels. Because we did not find even a trend toward a reduction of UAER in this setting of normotensive patients with type 1 diabetes and microalbuminuria, in whom BP was not reduced, we believe a statistical

Table 3—UAER and GFR as estimated by iohexol clearance

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Baseline GFR (ml/min per 1.73 m²)</th>
<th>GFR after 1 year (ml/min per 1.73 m²)</th>
<th>GFR after 2 years (ml/min per 1.73 m²)</th>
<th>Baseline UAER (µg/min)</th>
<th>UAER after 1 year (µg/min)</th>
<th>UAER after 2 years (µg/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Placebo</td>
<td>108 (49–138)</td>
<td>100 (78–145)</td>
<td>102 (60–173)</td>
<td>103 (49–202)</td>
<td>103 (22–338)</td>
<td>96 (48–308)</td>
</tr>
<tr>
<td>n = 16</td>
<td></td>
<td></td>
<td></td>
<td>n = 18</td>
<td>n = 18</td>
<td>n = 18</td>
</tr>
<tr>
<td>Ramipril 1.25 mg</td>
<td>100 (63–144)</td>
<td>102 (67–127)</td>
<td>95 (61–132)</td>
<td>109 (25–550)</td>
<td>110 (35–768)</td>
<td>94 (23–1,112)</td>
</tr>
<tr>
<td>n = 19</td>
<td></td>
<td></td>
<td></td>
<td>n = 17</td>
<td>n = 19</td>
<td>n = 17</td>
</tr>
<tr>
<td>Ramipril 5.0 mg</td>
<td>100 (69–134)</td>
<td>115 (75–139)</td>
<td>104 (57–135)</td>
<td>69 (16–466)</td>
<td>78 (22–482)</td>
<td>81 (10–1,450)</td>
</tr>
<tr>
<td>n = 16</td>
<td></td>
<td></td>
<td></td>
<td>n = 16</td>
<td>n = 17</td>
<td>n = 16</td>
</tr>
</tbody>
</table>

Data are median (range) from the treatment groups at 0, 1, and 2 years of treatment. There were no statistically significant differences between the groups at any time point.
type II error is very unlikely. However, this cannot be entirely excluded.

Treatment with ramipril did not affect microalbuminuria, clinic BP, or ABP in our study. The quite general recommendation to treat normotensive patients with type 1 diabetes and microalbuminuria with ACE inhibitors (1) might need to be reconsidered. On the basis of the present study, we question the clinical use of ACE inhibitors in stably normotensive patients with type 1 diabetes and microalbuminuria who do not demonstrate a concomitant reduction in BP.

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