Early ACE-I Intervention in Microalbuminuric Patients with Type 1 Diabetes: Effects on Albumin Excretion, 24 H Ambulatory Blood Pressure, and Renal Function

PL. Poulsen, E. Ebbehøj, R. Nosadini (1), P. Fiorettò (1), G. Deferrari (1), G. Crepaldi (1), C.E. Mogensen

SUMMARY - Objectives: To study the effects of ACE-i in type 1 diabetic patients with early microalbuminuria with regard to: i) UAE, ii) 24 h AMBP, including the effect on diurnal BP variation, and iii) renal hemodynamics.

Material and Methods: 58 patients with urinary albumin excretion (UAE) between 20-70 µg/min were treated for two years with either the ACE inhibitor (ACE-i) lisinopril (20 mg od) or placebo in two randomised placebo controlled double blind studies. In a subgroup of patients (n = 22) we performed 24 h ambulatory blood pressure measurements (AMBP) and renal function tests (constant infusion technique).

Results: i) Changes in UAE over the two years were significantly different (p < 0.01) in the two groups with final UAE in the lisinopril group of 19.1 ± 2.5 µg/min (=2.5 (geometric mean x±tolerance factor) and 44.1 ± 2.8 in the placebo group. In the lisinopril group 20 patients (60.6%) reversed to normoalbuminuria compared to 6 patients (24%) in the placebo group (p < 0.02). ii) Clinical BP measurements revealed no differences between groups, but by AMBP significant reductions were detectable in the lisinopril group, primarily in night AMBP (systolic/diastolic: – 6.9 ± 6.8/– 6.0 ± 5.3 mmHg, p < 0.01) as opposed to increases in the placebo group (3.1 ± 9.3/1.9 ± 7.3 mmHg). iii) Changes in UAE and changes in filtration fraction (FF) were positively correlated in the intervention group (r = 0.3, p < 0.01), i.e. the patients who showed the greatest fall in UAE were the ones with the greatest fall in FF.

Conclusions: ACE-i treatment in patients with low-grade microalbuminuria reduces 24 h AMBP without attenuating diurnal blood pressure variation, reduces UAE significantly, with changes in UAE being strongly associated with changes in FF. Furthermore, compared to placebo, ACE-i reverses micro- to normoalbuminuria in a significant fraction of patients.

RÉSUMÉ - Intervention précoce par IEC chez des diabétiques de type 1 microalbuminuriques : effets sur l’excrétion d’albumine, la pression artérielle ambulatoire sur 24 h et la fonction rénale.

Objectifs : Étudier les effets d’un IEC chez des diabétiques de type 1 avec microalbuminurie précoce sur : 1) UAE, 2) 24 h AMBP, notamment les effets sur la variation tensionnelle diurne, et 3) l’hémodynamique rénale.

Matériel et Méthodes : 58 patients avec microalbuminurie (UAE) entre 20-70 µg/min ont été traités pendant deux ans soit par l’IEC lisinopril (20 mg) soit par placebo lors de deux études en double insu randomisées contre placebo. Dans un sous-groupe de patients (n = 22) nous avons enregistré la pression artérielle ambulatoire de 24 h (AMBP) et la fonction rénale (technique d’infusion constante).

Résultats : 1) Les changements de UAE sur les deux années ont significativement différé entre les deux groupes (p < 0.01) avec une UAE finale dans le groupe lisinopril de 19,1 µg/min (<2.5 (moyenne géométrique x±facteur de tolérance) et de 44,1 µg/min <2.8 dans le groupe placebo. Dans le groupe lisinopril 20 patients (60,6 %) ont normalisé leur albuminurie contre 6 patients (24 %) dans le groupe placebo (p < 0.02). 2) Les mesures cliniques de pression artérielle n’ont révélé aucune différence entre les groupes, mais les valeurs d’AMBP étaient significativement réduites dans le groupe lisinopril, principalement la nuit (systolique/ diastolique : – 6,9 ± 6,6/– 6,0 ± 5,3 mmHg, p < 0.01) et au contraire augmentées dans le groupe placebo (3,1 ± 9,3/1,9 ± 7,3 mmHg). 3) Les modifications d’UAE et de fraction de filtration (FF) étaient positivement corrélées dans le groupe traité (r = 0,9, p < 0,01), ainsi les patients qui ont présenté la plus forte chute d’UAE étaient ceux présentant la plus forte chute de FF.

Conclusions : Le traitement par IEC chez les patients avec microalbuminurie débutante réduit la pression artérielle ambulatoire sans atténuer les variations diurnes, réduit significativement l’UAE, dont les changements sont fortement liés aux modifications de FF. En outre, par rapport au placebo, l’IEC normalise l’albuminurie chez un nombre significatif de patients.

Mots-clés : néphropathie diabétique, diabète de type 1, albuminurie, pression artérielle ambulatoire, microalbuminurie, inhibiteur de l’enzyme de conversion.
Microalbuminuria is currently the best marker and the most practical method for detection of early diabetic nephropathy [1]. Antihypertensive treatment in particular with ACE inhibitors (ACE-i) in microalbuminuric patients with type 1 diabetes reduces albuminuria, impedes the progression to overt nephropathy [2-6], and importantly, also preserves GFR [7] and apparently prevents progression of glomerulopathy [8]. However, the implementation of sensitive research methods has disclosed a long list of pathophysiological abnormalities in patients with microalbuminuria compared to patients with normoalbuminuria: Blood pressure is elevated [9-12] with an attenuated circadian rhythm [13], and autonomic abnormalities have been documented [14, 15]. Importantly, ultrastructural findings [16, 17] indicate the presence of glomerular and arteriolar abnormalities in type 1 diabetic patients with incipient nephropathy. Thus, early intervention has attracted interest. The EUCLID study [6] confirmed the effect of ACE-i on microalbuminuric patients but found no significant difference in albumin excretion rate between normoalbuminuric (<20 µg/min) placebo and lisinopril treated patients and could not demonstrate a significant reduction in incidence of microalbuminuria in lisinopril treated normoalbuminuric patients. Hence, the clinical question of when to intervene in type 1 diabetic patients still stands. In addition, it is still debated whether the effects of ACE-i are to some extent BP independent. In this context, it is important to realize the relative insensitivity of clinic BP measurements in comparison with 24 h AMBP [11, 18]. In extension of this, new data seem to indicate that high night BP and reduced circadian BP variation may be associated with increased cardiovascular risk [19, 20].

The aims of this study were to assess the effects of ACE-i in type 1 diabetic patients with moderate microalbuminuria (20-70 µg/min) with regard to: 1) UAE, 2) 24 h AMBP, in particular the effect on diurnal BP variation, and 3) Renal haemodynamics.

### SUBJECTS AND METHODS

In two randomised placebo controlled double blind studies the effects of treatment with either lisinopril or placebo were evaluated in microalbuminuric type 1 diabetic patients [21, 22]. We here present a post hoc analysis of 58 patients with urinary albumin excretion (UAE) between 20-70 µg/min treated for two years. In the subgroup of patients examined in Aarhus (n = 22) we performed 24 h ambulatory blood pressure measurements (AMBP) and renal function tests (constant infusion technique). Inclusion criteria were: Type 1 diabetes with no other chronic illness, and no medical treatment apart from insulin. Mean age was 36.5 ± 11.3 y (range 18-65 y), and mean duration of diabetes was 17.9 ± 9 (range 3-41 y) Blood pressure was < 160/90. None of the patients were on a protein restricted diet. UAE was measured at 6 monthly intervals by RIA and expressed as the geometric mean of three overnight collections made within one week. In one patient urinary collections failed at year two, instead UAE after 1.5 y is used in the calculations. HbA1c was determined by HPLC (non-diabetic range 4.4-6.4%). Renal haemodynamic measurements were measured by constant infusion technique [23] at 6 monthly intervals. In one patient renal function could not be performed due to problems with i.v. access. 125I-iothalamate (bolus 0.52 MBq, infusion 0.0074 MBq/min) was used as a marker for glomerular filtration rate (GFR) and effective renal plasma flow (RPF) was measured by 131I-hippuran (bolus 0.37 MBq, infusion 0.0072 MBq/min). Results are given corrected to 1.73 m² body surface. To promote stable urination, subjects were asked to drink 250 ml of tap water from 07.00 hours and throughout the study period. Six clearance periods each of 20 min were obtained in 93% of examinations, otherwise five periods were obtained. In the calculations the first period was omitted in order to allow stabilization of renal function. Patients were investigated after an overnight fast without receiving their usual morning insulin. AMBP was measured at baseline and at 12 and 24 months by an oscillometric technique (Spacelabs 90202 [24]). Readings were obtained at 20-minute intervals during the day and once an hour from 24.00 to 06.00. Patients’ reported times for rising and going to bed were implemented in the calculation of day and night blood pressure [25]. 93.1% of recordings included 22 hours or more. 94.7% of reported sleeping time was recorded and in no patients more than two night hours were missed. Three auscultatory BP measurements were made by a random zero sphygmomanometer (Hawksley, Lancing, U.K.), the average of these three measurements is termed the clinic BP. Patients gave their informed consent, and the study was approved by the local ethics committee.

### STATISTICAL ANALYSIS

Before analysis UAE were logtransformed to approximate normal distribution. Group results are given as mean ± SD, except for UAE, which is presented as geometric mean x± tolerance factor.

Differences between the two groups were tested by Student’s t test (continuous parameters) and Chi-square test (discrete variables). Multiple linear regression analysis was implemented in the description of associations between changes in UAE, AMBP, filtration fraction and HbA1c. All statistical tests were 2 sided and carried out at the 5% level of statistical significance.
Table I. Clinical characteristics (mean ± SD. except when indicated).

<table>
<thead>
<tr>
<th></th>
<th>Placebo [subgroup examined in Aarhus]</th>
<th>Lisinopril [subgroup examined in Aarhus]</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>n (males/females)</td>
<td>25 (18/7) [10 (9/1)]</td>
<td>33 (22/11) [12 (8/4)]</td>
<td>ns</td>
</tr>
<tr>
<td>Age (y.)</td>
<td>38.5 ± 10.4 [35.8 ± 11.3]</td>
<td>34.9 ± 11.8 [30.1 ± 10.1]</td>
<td>ns</td>
</tr>
<tr>
<td>Duration (y.)</td>
<td>19.5 ± 10.0 [19.4 ± 8.2]</td>
<td>16.6 ± 8.2 [16.7 ± 5.1]</td>
<td>ns</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>24.3 ± 2.7 [24.2 ± 2.7]</td>
<td>24.0 ± 2.6 [24.0 ± 2.6]</td>
<td>ns</td>
</tr>
<tr>
<td>HbA1c (%)</td>
<td>8.7 ± 1.3 [9.0 ± 1.0]</td>
<td>8.7 ± 1.6 [9.4 ± 1.7]</td>
<td>ns</td>
</tr>
</tbody>
</table>

Mean and SD. ns: Not Significant.

RESULTS

UAE

As indicated in Table I placebo and lisinopril treated patients were similar regarding age, duration of disease, BMI, and glycemic control and the subgroup examined in Aarhus was comparable to the total group. UAE was similar in placebo and lisinopril patients at baseline (36.2 ± 1.4 and 35.4 ± 1.5 µg/min, respectively). Over the two years study period UAE increased significantly in the placebo group to 44.1 ± 2.8 µg/min (p < 0.01), a relative increase of 1.2 × ± 2.6. In the lisinopril group UAE was reduced to 19.1 ± 2.5 µg/min (a relative decrease of 0.5 × ± 2.6). The changes in UAE in the two groups were significantly different (p < 0.01). The UAE course for the patients examined in Aarhus was similar although not statistically significant (relative increase of 1.2 × ± 3.1 in the placebo group compared to a relative decrease of 0.7 × ± 2.6 in the lisinopril group). 20 lisinopril treated patients (60.6%) reversed to normoalbuminuria (final UAE < 20 µg/min in at least two out of three collections) compared to 6 patients (24%) in the placebo group (p < 0.02).

Table II. Renal haemodynamics and AMBP in subgroup examined in Aarhus (placebo = 10 patients, lisinopril = 12 patients).

<table>
<thead>
<tr>
<th></th>
<th>Baseline</th>
<th>Year two</th>
<th>∆ (Year two - Baseline)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Placebo</td>
<td>Lisinopril</td>
<td>p</td>
<td>Placebo</td>
</tr>
<tr>
<td>GFR (ml/min/1.73 m²)</td>
<td>128 ± 18.0</td>
<td>137 ± 22.6</td>
<td>ns</td>
<td>131 ± 15.4</td>
</tr>
<tr>
<td>RPF (ml/min/1.73 m²)</td>
<td>547 ± 72.1</td>
<td>592 ± 124.0</td>
<td>ns</td>
<td>558 ± 132.0</td>
</tr>
<tr>
<td>FF (%)</td>
<td>23.6 ± 3</td>
<td>23.7 ± 4</td>
<td>ns</td>
<td>23.9 ± 2</td>
</tr>
<tr>
<td>24 h AMBP Sys</td>
<td>130 ± 10.2</td>
<td>126 ± 7.2</td>
<td>ns</td>
<td>131 ± 11.6</td>
</tr>
<tr>
<td>(mmHg)</td>
<td>Dia 76 ± 5.3</td>
<td>74 ± 8.2</td>
<td>ns</td>
<td>76 ± 5.8</td>
</tr>
<tr>
<td>Day AMBP Sys</td>
<td>135 ± 11.1</td>
<td>131 ± 8.5</td>
<td>ns</td>
<td>135 ± 11.6</td>
</tr>
<tr>
<td>(mmHg)</td>
<td>Dia 80 ± 5.3</td>
<td>78 ± 7.8</td>
<td>ns</td>
<td>80 ± 4.7</td>
</tr>
<tr>
<td>Night AMBP Sys</td>
<td>118 ± 8.9</td>
<td>115 ± 6.2</td>
<td>ns</td>
<td>121 ± 12.9</td>
</tr>
<tr>
<td>(mmHg)</td>
<td>Dia 64 ± 7.0</td>
<td>66 ± 7.8</td>
<td>ns</td>
<td>66 ± 8.8</td>
</tr>
<tr>
<td>Clinic BP Sys</td>
<td>129 ± 12.1</td>
<td>124 ± 13.4</td>
<td>ns</td>
<td>127 ± 8.7</td>
</tr>
<tr>
<td>(mmHg)</td>
<td>Dia 79 ± 6.1</td>
<td>83 ± 12.5</td>
<td>ns</td>
<td>77 ± 7.5</td>
</tr>
</tbody>
</table>

Data are presented as mean ± SD. In one patient (lisinopril group) renal function could not be assessed due to technical problems. *p < 0.05 compared to baseline values.
24 h AMBP

Data regarding 24 h ambulatory blood pressure measurements (AMBP) for the patients examined in Aarhus is given in Table II. Initial values BP values were similar in the two groups. In the placebo group, day-time AMBP was almost unchanged over the two years, whereas small increases in night-time systolic and diastolic AMBP was observed indicating a slight attenuation of circadian BP variation (diastolic night/day ratio increased 2.5% over 2 years). In contrast, significant reductions both in day-time and night-time AMBP of approximately 5-6 mmHg were observed in the lisinopril group. Day and night AMBP, as well as night/day ratios are depicted in Fig. 1. The changes in both systolic and diastolic night AMBP were significantly different for the two groups (p < 0.01), and the development in circadian BP variation (diastolic night/day ratio) approached statistical significance (p = 0.08). Clinical BP measurement could not detect any significant differences between the two groups (Table II).

Renal haemodynamics

GFR and effective renal plasma flow (RPF) were similar at baseline, remained stable over the two years with no significant differences between the two groups (Table II). Filtration fraction (FF) was reduced in the lisinopril group and increased in the placebo treated patients, the difference was not statistically significant.

In a multiple regression analysis including all patients with changes in UAE (log values) as dependent variable, changes in systolic night BP and changes in FF were significant explanatory variables (r = 0.60, p < 0.02). Disparate associations between these variables were observed in the placebo and lisinopril treated patients: In the placebo group changes in UAE were significantly associated with changes in night AMBP (r = 0.77, p < 0.01, Fig. 2a) but not with changes in FF (r = 0.03, p = 0.94). In contrast, in the lisinopril group the association between changes in UAE and FF was highly significant (r = 0.91, p<0.01 Fig. 2b) as opposed to the association with night AMBP as (r = -0.1, p = 0.76). There were no treatment differences in HbA1c, and changes in HbA1c did not affect the above mentioned associations.

DISCUSSION

We selected patients with moderate microalbuminuria (UAE 20-70 µgr/min), in which the disease process presumably still is in an early phase and pathophysiological abnormalities e.g. blood pressure changes potentially reversible. This segment of patients, without any symptoms and with perfectly normal GFR, illustrates the clinical question of when to initiate a presumably lifelong treatment in young people. Two years treatment with lisinopril was associated with significantly lower overnight UAE and a significant higher fraction of patients reversing to normalalbuminuria, although spontaneous normalization was also observed, as in other studies [12]. We have recently described that in the same patients, exercise UAE was significantly reduced during the two years of ACE-i treatment [22]. Despite the small numbers, 24 h AMBP demonstrated significant reductions in blood pressure in the lisinopril treated group as opposed to the placebo group who demonstrated small increases in BP. These statistically highly significant differences were undetectable with clinic BP (mean of three random zero measurements). This emphasizes the necessity of a critical appraisal of the sensitivity of clinical BP measurement when evaluating the role of
BP reduction in diabetic nephropathy. Furthermore, 24 h AMBP allows an exploration of circadian BP variation: During the study period the BP increase in placebo treated patients was most pronounced for the night-time blood pressure and consequently night/day ratio increased, i.e. an attenuation in circadian BP variation was observed in the placebo group as opposed to the lisinopril group. The comparison between development in night/day ratio for placebo and lisinopril treated patients approached statistical significance (p = 0.08). The clinical relevance of attenuated circadian BP variation is still uncertain, but new data both in older patients with systolic hypertension and in normotensive type 2 diabetic patients seems to indicate increased cardiovascular risk with a higher night/day ratio independent of the 24-hour BP [19, 20]. A retrospective study in patients with diabetic nephropathy has described a faster decline of creatinine clearance in “non-dippers” compared to “dippers” [26], but conclusions are hampered by the lack of baseline or follow-up AMBP, and the fact that renal function was already reduced in “non-dippers” at the time of AMBP measurement.

In the two groups disparate associations regarding changes in UAE were apparent: in the placebo treated group there was a close association between changes in night BP and changes in overnight UAE whereas there were no signs of association with changes in filtration fraction. Quite the reverse was observed in the ACE-i treated patients where there was a strong positive correlation between Δ UAE and Δ filtration fraction, but no association with changes in BP. One possible interpretation is that ACE-i treatment ameliorates the association between BP and UAE observed in untreated patients and reduces UAE in proportion to reduction in transglomerular pressure [27]. However, there are several caveats: the relation between filtration fraction and transglomerular pressure is complex [28] and obviously the presence or absence of associations in a limited number of patients is not conclusive. In the ACE-i treated patients there was a small reduction in filtration fraction whereas the placebo treated patients had small increases but the difference was not statistically significant. This could be a type two error as several studies have found reduction in FF during ACE-i treatment in diabetic patients [18, 29-31].

In conclusion, treatment with lisinopril in type 1 diabetic patients with low-grade microalbuminuria reduces 24 h AMBP without attenuating diurnal blood pressure variation, reduces UAE significantly, with changes in UAE being strongly associated with changes in filtration fraction. Furthermore, lisinopril reverses micro- to normoalbuminuria in a significant fraction of patients compared to placebo.

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