Effect on blood pressure of combined inhibition of endothelin-converting enzyme and neutral endopeptidase with daglutril in patients with type 2 diabetes who have albuminuria: a randomised, crossover, double-blind, placebo-controlled trial

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Summary

Background Effective reduction of albuminuria and blood pressure in patients with type 2 diabetes who have nephropathy is seldom achieved with available treatments. We tested the effects of treatment of such patients with daglutril, a combined endothelin-converting enzyme and neutral endopeptidase inhibitor.

Methods We did this randomised, crossover trial in two hospitals in Italy. Eligibility criteria were: age 18 years or older, urinary albumin excretion 20–999 µg/min, systolic blood pressure (BP) less than 140 mm Hg, and diastolic BP less than 90 mm Hg. Patients were randomly assigned (1:1) with a computer-generated randomised sequence to receive either daglutril (300 mg/day) then placebo for 8 weeks each or vice versa, with a 4-week washout period. Patients also took losartan throughout. Participants and investigators were masked to treatment allocation. The primary endpoint was 24-h urinary albumin excretion in the intention-to-treat population. Secondary endpoints were median office and ambulatory (24 h, daytime, and night-time) BP, renal haemodynamics and sieving function, and metabolic and laboratory test results. This study is registered with ClinicalTrials.gov, number NCT00160225.

Findings We screened 58 patients, of whom 45 were enrolled (22 assigned to daglutril then placebo, 23 to placebo then daglutril; enrolment from May, 2005, to December, 2006) and 42 (20 *vs* 22) were included in the primary analysis. Daglutril did not significantly affect 24-h urinary albumin excretion compared with placebo (difference in change –7·6 µg/min, IQR –78·7 to 19·0; p=0·559). 34 patients had complete 24-h BP readings; compared with placebo, daglutril significantly reduced 24-h systolic (difference –5·2 mm Hg, SD 9·4; p=0·0013), diastolic (–2·5, 6·2; p=0·015), pulse (–3·0, 6·3; p=0·019), and mean (–3·1, 6·2; p=0·003) BP, as well as all night-time BP readings and daytime systolic, pulse, and mean BP, but not diastolic BP. Compared with placebo, daglutril also significantly reduced office systolic BP (–5·4, 15·4; p=0·028), but not diastolic (–1·8, 9·9; p=0·245), pulse (–3·1, 10·6; p=0·210), or mean (–2·1, 10·4; p=0·205) BP, and increased big endothelin serum concentration. Other secondary outcomes did not differ significantly between treatment periods. Three patients taking placebo and six patients taking daglutril had mild treatment-related adverse events—the most common was facial or peripheral oedema (in four patients taking daglutril).

Interpretation Daglutril improved control of BP in hypertensive patients with type 2 diabetes and nephropathy and had an acceptable safety profile. Combined endothelin-converting enzyme and neutral endopeptidase inhibition could provide a new approach to hypertension in this high-risk population.

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Introduction

Hypertension and diabetes are leading causes of end-stage renal disease and cardiovascular disease worldwide.[1] Inhibitors of the renin–angiotensin system are first-line treatment for hypertensive patients with type 2 diabetes mellitus; angiotensin-converting enzyme inhibitors and angiotensin 2 receptor blockers reduce renal and cardiovascular events in such patients more effectively than do other drugs that reduce blood pressure, even if blood pressure is controlled to the same degree.[1–4] However, the protective effects of inhibition of the renin–angiotensin system are negligible in patients with advanced stages of diabetic renal disease and 7–10% of diabetic patients with overt nephropathy still progress to end-stage renal disease each year.[3,4] Even more patients die from cardiovascular causes before progressing to end-stage renal disease.[5] The excess risk in this population is probably a result of poorly controlled hypertension and residual proteinuria despite treatment with several antihypertensive drugs combined with angiotensin-converting enzyme inhibitors or angiotensin 2 receptor blockers.[4] Unfortunately, intensification of treatment by combining angiotensin 2 receptor blockers with an angiotensin-converting enzyme inhibitor or a direct renin

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inhibitor is associated with an increased risk of acute renal failure or cardiovascular events.[6,7] Addition of an aldosterone receptor antagonist to renin–angiotensin system inhibitors further reduces albuminuria, but can lead to life-threatening hyperkalaemia.[8]

After the seminal discovery by Yanagisawa and coworkers[9] of endothelin 1 (EDN1)—a potent vasoconstrictor—antagonism of this molecule has been proposed as a new approach to hypertension.[10] EDN1 also causes progressive renal damage by induction of cell proliferation and interstitial inflammation.[11,12] The actions of EDN1 can be antagonised by mixed or selective inhibition of its receptors—EDNRA and EDNRB—or by diminishing its production through inhibition of endothelin-converting enzyme, which catalyses the generation of biologically active EDN1 from its precursor, big EDN1.[11,13]

Daglutril is a compound that inhibits both endothelin-converting enzyme and neutral endopeptidase. Inhibition of neutral endopeptidase has several effects, including increasing bioavailability of natriuretic peptides, bradykinin, and substance P, which might partly contribute to the natriuretic, diuretic, vasodilatatory, and anti-proliferative properties of the inhibitor.[13] In diabetic rats, combined inhibition of endothelin-converting enzyme and neutral endopeptidase by daglutril or a similar compound reduces blood pressure and proteinuria, and prevents nephrosclerosis as effectively as the angiotensin-converting enzyme inhibitor captopril.[14,15] Phase 1 studies[16] show that daglutril is safe and well-tolerated in healthy people. However, the risk–benefit profile of treatment with daglutril for patients with diabetes and nephropathy has not been investigated. Therefore, we assessed the effect of daglutril on urinary albumin excretion, blood pressure, and renal function in hypertensive patients with type 2 diabetes mellitus and albuminuria.

Methods

Study design and participants

We did this prospective, randomised, cross-over, placebo-controlled trial in the outpatient clinics of the Diabetology and Nephrology Units of the Azienda Ospedaliera Ospedali Riuniti di Bergamo and of the Diabetology Unit of the Azienda Ospedaliera of Treviglio, all in Italy. Eligibility criteria were: type 2 diabetes mellitus according to WHO criteria, age 18 years or older, 24-h urinary albumin excretion 20–999 µg/min, systolic blood pressure less than 140 mm Hg and diastolic blood pressure less than 90 mm Hg with antihypertensive drugs, and no use of contraceptive methods despite child-bearing potential. We excluded patients with concomitant diseases that might interfere with the study, documented cardiovascular events within the past 6 months, previous adverse reactions to angiotensin 2 receptor blockers, liver aminotransferase concentrations exceeding two times the upper limit of the normal range, serum creatinine concentration of 200 µmol/L or more, mitral or aortic valve stenosis, hypertrophic cardiomyopathy, or decompensated chronic heart failure.

For the **study protocol** see http://clintrials.marionegri.it/index.php/main-trials/main-trials-completed.html

The study was done in accordance with the EU Clinical Trial Directive (2001/20/EC), Good Clinical Practice, and the Declaration of Helsinki. It was approved by the ethics committee of the local health agency of Bergamo, Italy. All patients provided written informed consent.

Randomisation and masking

Enrolment was from May, 2005, to December, 2006. Patients who completed a 4-week run-in treatment period (during which patients were masked to treatment, investigators were not) with losartan (100 mg/day) and placebo and at baseline assessment fulfilled the eligibility criteria were randomly assigned. Patients were allocated centrally by a computer-generated randomisation list prepared by the Clinical Supplies Department of Solvay Pharmaceuticals (Weesp, Netherlands). Patients were randomly assigned (1:1) to 8-week treatment with daglutril (300 mg/day) followed by 8-week placebo, or vice versa. All study participants, investigators, and data assessors were masked to treatment allocation.

Procedures

All patients were taking losartan (100 mg/day) in addition to their randomly assigned treatment. Participants who completed their first 8-week treatment period crossed over to the second 8-week period after a 4-week washout that—in view of the 2–4 h half-life of daglutril—was deemed sufficient to eliminate possible carry-over effects from the first treatment. After the second 8-week treatment period, patients stopped taking the study drug and continued to take losartan for 4 weeks of follow-up. No systematic change in diet or co-medication was introduced during the study. The daglutril dosage was recommended by the manufacturer on the basis of evidence that 300 mg of the daglutril formulation used in this study would provide a bioavailability of the active metabolite that was equivalent to that provided by a 400 mg dose of the previous formulation of daglutril that had had the largest antihypertensive effect in a phase 2 study (unpublished data). Details of the study protocol are available online.

We measured trough blood pressures with a semiautomatic device (Omron HEM-705CP, Tokyo, Japan). Office blood pressure was the mean of three measurements taken 2 min apart in the morning before treatment administration after a 10 min rest, while the participant was sitting. 24-h ambulatory blood pressure was monitored at the start and end of each treatment period by Spacelabs equipment (Redmont, Washington, USA) that was set to obtain measurements at 15 min intervals during daytime (0600–2200) and 30 min intervals during night-time (2200–0600). Pulse pressure was the difference between systolic and diastolic blood pressures. Mean arterial pressure was the diastolic blood

pressure plus a third of pulse pressure. Urinary albumin concentration was measured by nephelometry, and the median of three consecutive 24-h urine collections was recorded. Glomerular filtration rate was measured by plasma clearance of iohexol (Omnipaque 3000; GE Healthcare, Milan, Italy) and renal plasma flow was measured by plasma clearance of para-aminohippuric acid (Iacopo Monico, Mestre, Italy).[17] Filtration fraction was calculated as glomerular filtration rate/renal plasma flow, and renal vascular resistance was calculated as mean blood pressure/renal plasma flow. Albumin and IgG fractional clearances were calculated by adjusting albumin and IgG clearances for the simultaneously measured glomerular filtration rates. Data were locally recorded in case report forms and then entered twice in a central database of the clinical research centre.

At each visit, adverse events were recorded and physical and laboratory parameters were assessed for safety. Seriousness and severity of adverse events and their relation with study drug were assessed according to Good Clinical Practice guidelines. Further details are provided in the study protocol. The primary outcome was 24-h urinary albumin excretion after 8 weeks of treatment. The secondary outcomes were office and ambulatory blood pressure (24-h, daytime, and night-time), renal haemodynamics and other kidney function, metabolic and laboratory test results.

Statistical analysis

We estimated the required sample size assuming use of paired *t* test (two sided) of the difference on the log-scale between placebo and daglutril groups for the primary outcome. Based on data from patients at the clinical research centre, we assumed a baseline, pretreatment urinary albumin excretion rate of 5·20 mg/min (SD 1·12) for post-treatment differences of log-transformed values. With these assumptions, we calculated that we would need 40 patients to detect a 40% mean difference between daglutril and placebo groups with a 5% significance level and a power of 80%. Assuming that 10% of patients would drop out, we aimed to enrol 45 participants.

On the basis of ambulatory blood pressure recordings from patients referred to the clinical research centre, participants were expected to have mean a pretreatment 24-h systolic blood pressure of 135 mm Hg (SD 10). We calculated a priori that a sample size of 40 patients would have an 86% power to detect a 5 mm Hg mean difference (130 *vs* 135 mmHg) in 24-h systolic blood pressure between daglutril and placebo groups with a 5% significance level.

We analysed the primary and secondary outcomes in the modified intention-to-treat population, consisting of all randomly assigned patients who took at least one dose of study drug and who had at least one efficacy measurement after the first dose of study drug, irrespective of protocol violations. We tested the difference between groups with a repeated measures ANOVA. We did a mixed model ANOVA, with treatment and period as fixed factors and participant as a random factor; the dependent variable was absolute change between pretreatment and post-treatment. We then compared changes during the two treatment periods. This model had no baseline covariates because we assumed that baseline variation was accounted for by adjustment for period and participant effects and all effects of carry-over had disappeared by the time of baseline for the second treatment period. Carry-over effects were assessed by visual inspection. We did per-protocol subgroup analyses of patients with microalbuminuria and macroalbuminuria for the primary efficacy variable. We assessed patterns of rhythmical and non-rhythmical fluctuations in ambulatory blood pressure over 24 h by autoregressive modelling.[18] Albuminuria was log-transformed. To assess the effect of missing data, we did a sensitivity analysis, imputing missing values. We used both parametric multiple imputation by chained equations (the ice command in STATA 12) and a non-parametric simple

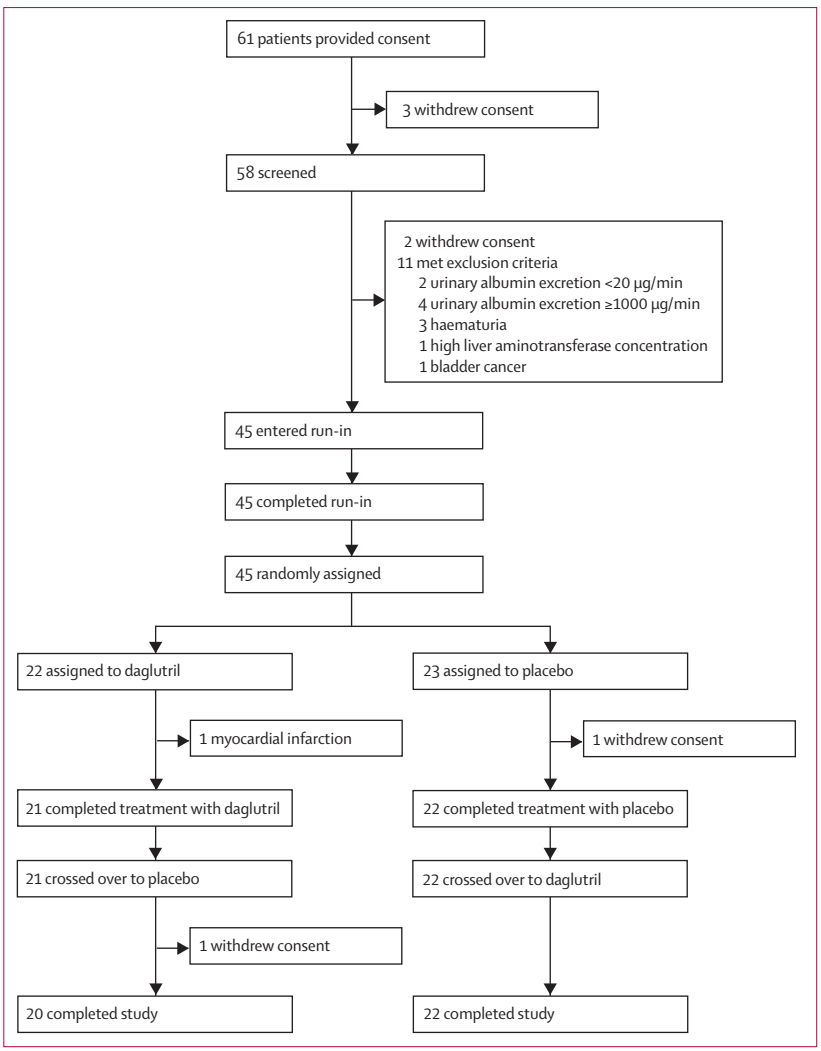

Figure 1: **Trial profile**

For the **Good Clinical Practice guidelines** see http://www.ich.org/products/guidelines/efficacy/article/efficacy-guidelines.html

mean imputation replacing the missing values with the arithmetic average. All statistical tests were done with a two-sided significance level of 5%. Continuous and categorical variables (sex, microalbuminuria or macroalbuminuria stratum, concomitant drugs) were summarised as mean (SD) or median (IQR), or by counts and percentages. We did all statistical analyses with SAS (version 9·1) and STATA (version 12).

This study is registered with ClinicalTrials.gov (number NCT00160225).

	Daglutril to placebo (n=22)	Placebo to daglutril (n=23)
Age (years)	62·0 (7·7)	65·7 (6·4)
Sex		
Men	22 (100%)	20 (87%)
Women	0	3 (13%)
Body-mass index (kg/m²)	30·6 (4·5)	30·8 (6·2)
HbA_{1c}		
%	6·20 (1·43)	5·79 (1·26)
mmol/mol	58·4 (13·5)	53·9 (11·7)
Blood pressure (mm Hg)		
Office		
Systolic	135·9 (9·3)	139·4 (9·2)
Diastolic	77·6 (6·9)	77·4 (6·4)
Pulse	59·5 (11·5)	65·0 (12·4)
Mean	99·3 (7·5)	101·9 (7·2)
24-h		
Systolic	132·6 (9·9)	130·7 (12·1)
Diastolic	74·7 (7·3)	73·5 (8·2)
Pulse	59·0 (9·3)	57·8 (10·1)
Mean	93·6 (7·0)	91·7 (8·4)
Daytime		
Systolic	134·6 (10·5)	132·6 (12·6)
Diastolic	76·5 (7·6)	75·2 (8·3)
Pulse	59·2 (9·4)	58·0 (10·5)
Mean	96·2 (7·6)	94·3 (8·6)
Night-time		
Systolic	126·6 (11·0)	124·0 (12·2)
Diastolic	68·3 (7·8)	66·8 (9·0)
Pulse	58·2 (10·4)	57·2 (9·3)
Mean	87·7 (7·5)	85·9 (9·2)
24-h UAE (µg/min)	136·2 (81·7–219·3)	73·4 (38·9–192·8)
Renal function		
GFR (mL/min per 1·73 m²)	88·9 (20·5)	71·7 (24·6)
RPF (mL/min per 1·73 m²)	387·7 (102·1)	331·6 (107·0)
Filtration fraction (%)	26·7 (17·3)	21·3 (5·0)
RVR (mm Hg/mL per min/1·73 m²)	0·28 (0·07)	0·34 (0·12)
Albumin fractional clearance (×10⁵)	4·70 (3·71)	6·71 (7·09)
IgG fractional clearance (×10⁵)	0·84 (1·2)	0·96 (0·89)
Na excretion (mmol/day)	249·3 (89·1)	227·8 (65·3)
Big END1 concentration (fmol/mL)	0·35 (0·05)	0·37 (0·13)
Pro-atrial natriuretic peptide concentration (pmol/l)	1759·1 (1274·3)	2212·8 (1349·2)
Patients taking BP-lowering drugs		
Losartan	22 (100%)	23 (100%)
Antiadrenergic drugs*	11 (50%)	8 (35%)
Beta-blockers	4 (18%)	8 (35%)
Calcium-channel blockers	7 (32%)	12 (52%)
Diuretics	21 (95%)	18 (78%)
Patients taking losartan alone	1 (5%)	2 (9%)
Patients taking losartan and one other drug	9 (41%)	6 (26%)
Patients taking losartan and two other drugs	10 (45%)	10 (43%)
Patients taking losartan and three other drugs	2 (9%)	3 (13%)
Patients taking losartan and four other drugs	0 (0%)	2 (9%)
Number of BP-lowering drugs per patient	3 (2–3)	3 (2–3)
Patients with BP <130/80 mm Hg	4 (18%)	1 (4%)

Data are mean (SD) or median (IQR) for continuous variables and n (%) for dichotomous variables. Daytime was defined as 0600–2200 h and night-time as 2200–0600 h. HbA_{1c}=glycated haemoglobin. BP=blood pressure. GFR=glomerular filtration rate. RPF=renal plasma flow. RVR=renal vascular resistance. UAE=urinary albumin excretion. *Clonidine (n=11) or doxazosin (n=8).

Table 1: **Baseline characteristics**

Role of the funding source

The sponsor supplied study drug, but had no role in study design, data collection, data analysis, data interpretation, or writing of the report. The database of the study was released by the company to the investigators on October, 2011, after all the data had been collected. APa, APe, GR, and PR had access to the raw data. The corresponding author had full access to all of the data and had final responsibility to submit for publication.

Results

We screened 58 patients, of whom 45 were enrolled, 22 assigned to daglutril then placebo, 23 assigned to placebo then daglutril (figure 1). Three participants withdrew during the study: one because of an acute myocardial infarction followed by a fatal cerebrovascular event that the investigators deemed not related to study drug. 42 participants were included in the primary analysis. Full ambulatory blood pressure recordings were available for 34 participants and renal functional data for 36 participants. Baseline characteristics were much the same between study arms (table 1). At randomisation, most patients were taking losartan plus one or two other drugs that reduce blood pressure (table 1). All participants were taking oral blood-glucose lowering drugs and 14 were also taking insulin.

	Daglutril treatment			Placebo treatment			Daglutril *vs* placebo	
	Before	After	p value*	Before	After	p value*	Absolute difference	p value†
Overall (n=42)	116·0 (51·3 to 211·6)	116·3 (48·3 to 203·3)	0·808	87·8 (45·1 to 190·5)	102·7 (52·7 to 231·0)	0·467	–7·6 (–78·7 to 19·0)	0·559
Patients with microalbuminuria (n=30)	80·9 (43·3 to 134·9)	91·0 (38·3 to 129·3)	0·443	62·6 (40·3 to 105·1)	73·3 (45·7 to 118·8)	0·217	–7·4 (–53·4 to 18·8)	0·762
Patients with macroalbuminuria (n=12)	274·5 (232·7 to 382·5)	249·5 (186·1 to 378·2)	0·135	258·3 (178·1 to 396·7)	253·7 (190·1 to 352·7)	0·423	–73·9 (–119·5 to 47·6)	0·482

Data are median (IQR), in µg/min, unless stated otherwise. Assessed by modified intention-to-treat analysis for overall albuminuria and by per protocol for microalbuminuria or macroalbuminuria at randomisation. *Paired *t* test of after versus before. †Paired *t* test of daglutril versus placebo.

Table 2: **24-h urinary albumin excretion**

	Daglutril treatment			Placebo treatment			Daglutril *vs* placebo	
	Before	After	p value*	Before	After	p value*	Absolute difference	p value†
Office (n=42)								
Systolic	140·6 (14·0)	140·8 (14·7)	0·975	139·5 (13·0)	144·7 (16·7)	0·006	–5·4 (15·4)	0·028
Diastolic	79·0 (7·9)	78·9 (8·5)	0·886	77·6 (7·9)	79·9 (7·5)	0·032	–1·8 (9·9)	0·245
Pulse	59·5 (11·5)	61·9 (14·5)	0·267	63·6 (14·1)	64·9 (15·8)	0·296	–3·1 (10·6)	0·210
Mean	100·6 (8·7)	99·5 (8·5)	0·355	100·2 (8·5)	101·5 (8·6)	0·326	–2·1 (10·4)	0·205
24 h (n=34)								
Systolic	132·9 (10·7)	129·9 (12·0)	0·016	132·4 (11·3)	134·4 (11·5)	0·118	–5·2 (9·4)	0·0013
Diastolic	74·6 (7·6)	73·9 (8·0)	0·247	74·0 (7·4)	76·0 (7·3)	0·041	–2·5 (6·2)	0·015
Pulse	59·1 (9·9)	56·9 (11·1)	0·0034	59·0 (10·7)	59·4 (10·9)	0·372	–3·0 (6·3)	0·019
Mean	93·3 (7·3)	91·7 (8·2)	0·058	92·9 (7·3)	94·4 (7·4)	0·106	–3·1 (6·2)	0·0030
Daytime (n=34)								
Systolic	134·7 (11·0)	132·2 (12·0)	0·052	134·6 (11·8)	136·6 (12·1)	0·142	–4·5 (10·3)	0·0080
Diastolic	76·3 (7·8)	75·8 (8·2)	0·568	75·9 (7·6)	77·8 (7·6)	0·061	–2·1 (7·0)	0·071
Pulse	59·3 (10·2)	57·1 (11·1)	0·0034	59·3 (11·0)	59·7 (11·2)	0·46	–2·9 (6·5)	0·0067
Mean	95·7 (7·5)	94·6 (8·0)	0·214	95·5 (7·7)	97·4 (7·7)	0·076	–2·9 (7·7)	0·022
Night-time (n=34)								
Systolic	126·6 (12·4)	122·2 (15·0)	0·0055	124·4 (11·4)	126·9 (11·7)	0·086	–7·5 (11·8)	0·0003
Diastolic	68·3 (8·5)	65·7 (9·0)	0·0026	66·7 (7·8)	68·6 (8·0)	0·087	–4·3 (6·6)	0·0002
Pulse	58·3 (10·0)	56·5 (11·9)	0·062	57·6 (10·6)	58·3 (10·8)	0·372	–3·2 (8·1)	0·019
Mean	87·8 (8·8)	84·6 (9·9)	0·0026	85·9 (7·7)	88·1 (7·9)	0·065	–5·4 (7·8)	<0·0001

Data are mean (SD), in mm Hg. *Repeated measures ANOVA: after versus before. †Repeated measures ANOVA: daglutril versus placebo.

Table 3: **Blood pressure before and after treatment with daglutril and placebo and differences between treatments**

24-h urinary albumin excretion did not change significantly throughout each treatment period, in the study group as a whole, or in subgroups of participants with microalbuminuria or macroalbuminuria (table 2).

For office recordings, systolic and diastolic blood pressure did not change significantly before and after treatment with daglutril, but increased when patients took placebo (table 3). Systolic blood pressure was significantly different when patients were given daglutril compared with placebo, whereas diastolic, pulse, and mean blood pressures did not differ significantly between the two treatments (table 3, figure 2).

For 24-h recordings of blood pressure, daglutril treatment was associated with a significant decrease in systolic and pulse pressure, but mean or diastolic blood pressure did not change significantly (table 3). Only diastolic blood pressure changed significantly with placebo. Daglutril compared with placebo resulted in a significant reduction in all 24-h blood pressures, with the largest effect for systolic (table 3, figures 2 and 3). According to the autoregressive model (appendix), both diastolic and systolic blood pressure after treatment were significantly different (figure 3). Sensitivity analyses using parametric and non-parametric imputation methods confirmed the robustness of the results (appendix).

According to daytime recordings, pulse blood pressure decreased with daglutril treatment, but did not change significantly with placebo. Compared with placebo, the effect of daglutril was significant for systolic, pulse, and mean blood pressures (table 3, figures 2 and 3). Results of night-time recordings show that systolic, diastolic, and mean blood pressure all significantly decreased with daglutril treatment, whereas pulse pressure did not change significantly. No significant changes occurred

with placebo. The effect of daglutril compared with that of placebo was significant for all night-time blood pressures (table 3, figures 2 and 3).

Glomerular filtration rate, renal plasma flow, filtration fraction, and renal vascular resistance did not change significantly before and after each treatment period, and the effect of daglutril treatment was not significantly different from that of placebo (table 4). IgG fractional clearance significantly increased during daglutril treatment, but did not with placebo. The effect of daglutril on both IgG and albumin fractional clearance was not significantly different from that of placebo. 24-h urinary sodium excretion was stable throughout the study (table 4).

Serum concentration of big EDN1 significantly increased during daglutril treatment but that of pro-atrial natriuretic peptide did not. Neither changed significantly with placebo treatment. Compared with placebo, the effect of daglutril on big EDN1 was significant, but on pro-atrial natriuretic peptide was not (table 4). Per-protocol analyses of primary and secondary efficacy variables confirmed the results obtained in the modified intention-to-treat analyses (data not shown). We did not record any substantial carry-over effect for all outcomes.

Treatment was well tolerated in all participants and no serious treatment-related adverse events were reported. Six patients taking daglutril had non-serious treatment-related events compared with two taking placebo. Three patients had peripheral oedema and one had facial oedema during daglutril treatment. In one participant, peripheral oedema resolved after reduction of lacidipine dose from 8 mg/day to 4 mg/day and increase of hydrochlorothiazide dose from 25 mg/day to 50 mg/day. In another, oedema persisted after the dose of hydrochlorothiazide was increased from 12·5 to 25·0 mg/day. Because it was still present 2 weeks after the end of the study, it was judged a result of underlying renal disease rather than a treatment-related effect. The facial oedema was associated with signs of fluid retention (weight gain, and reduced haematocrit and haemoglobin concentration) and resolved after completion of treatment. A first-degree atrioventricular block was reported in two participants taking placebo and in one taking daglutril. One patient taking daglutril had hypotension. None of the events required down-titration or withdrawal of treatment. Daglutril did not affect haematocrit or haemoglobin concentration, body-mass index, or serum concentrations of liver enzymes, lipids, fasting glucose, or glycated haemoglobin (data not shown).

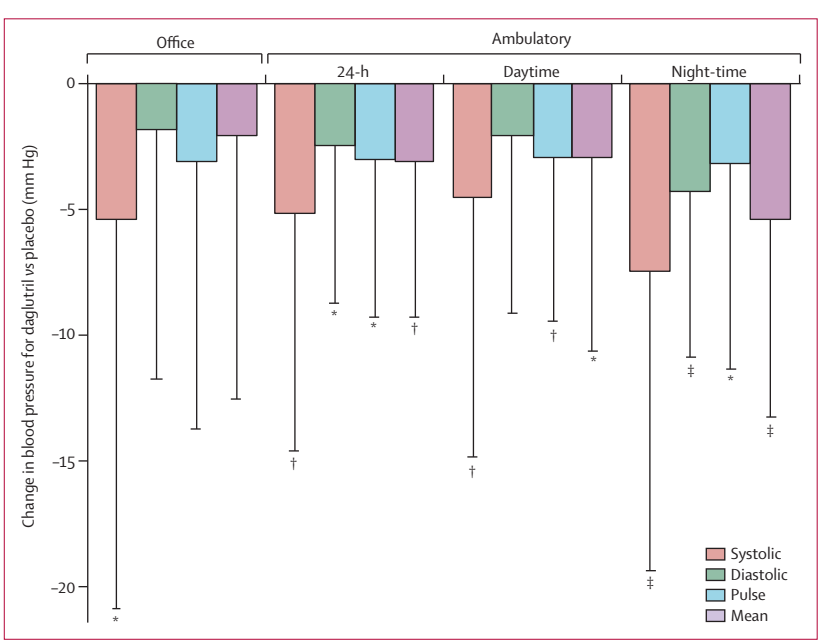

Figure 2: **Mean changes in blood pressures for daglutril versus placebo**
Error bars are SDs. *p<0·05. †p<0·01. ‡p<0·001.

Discussion

8-week treatment with daglutril plus losartan and other antihypertensive drugs did not significantly affect urinary albumin excretion, nor renal haemodynamic measures or sieving function, but it did decrease ambulatory blood pressure in hypertensive patients with type 2 diabetes mellitus and albuminuria. Treatment was safe and well tolerated in all participants.

Because dietary salt intake and concomitant anti-hypertensive treatment were not systematically changed and 24-h urinary sodium excretion was stable during the study, we can reasonably exclude any confounding effect of intensified hypertension treatment or reduced sodium exposure. Moreover, we detected no substantial carry-over effect and the crossover design avoided confounding related to interpatient data heterogeneity. Thus, the reduction of blood pressure associated with daglutril seems to be a genuine treatment effect.

To the best of our knowledge, this study is the first randomised clinical trial reporting the beneficial effects of daglutril on arterial hypertension in patients with type 2 diabetes mellitus (panel). Hypertension affects most patients with diabetes and almost all of those with some renal involvement;[1,3] systolic hypertension is almost always present. When combined with increased pulse pressure, it is almost always a result of increased vascular stiffness—a major risk factor for cardiovascular morbidity and mortality in this population.[19] Systolic hypertension is often resistant to drug treatment,[19] especially in patients with diabetes with renal involvement; in our study, systolic blood pressure averaged 140 mm Hg, despite background treatment with losartan, plus two or more antihypertensive drugs, and also a diuretic in most cases. This blood pressure exceeds the 130 mm Hg target that was recommended when the study was designed, but accords with the most recent guidelines,[20] which recommend less stringent control of blood pressure in patients with diabetes.

Thus, daglutril effectively improved both office and ambulatory systolic hypertension with much smaller effects on diastolic blood pressure. Reduction in systolic blood pressure is normally associated with a concomitant reduction in diastolic blood pressure, which

can result in decreased left ventricular perfusion and a heightened risk of cardiovascular events—the so called J curve.[21] Therefore, availability of a drug that can reduce systolic and pulse pressure with marginal effects on diastolic blood pressure might have major clinical implications. Indeed, a 10 mm Hg reduction in systolic blood pressure has been associated with a 22% reduction in coronary heart disease and 41% reduction in stroke.[22] Whether the effect of daglutril observed in our study is a result of improved vascular stiffness should be investigated. The treatment effect of daglutril on ambulatory blood pressure was larger during night-time, and was achieved on top of full-dose losartan plus two or more additional hypotensive drugs in most patients. This might also have clinical implications, because night-time hypertension is a strong cardiovascular risk factor independent of trough, 24-h, or daytime blood pressure control, especially in patients with diabetes who have renal disease.[23]

We recorded a significant increase in office blood pressure, and a non-significant increase in other blood pressures, during placebo treatment, which might be a result of progression of renal disease with a consequent worsening of hypertension. Daglutril maintained all measures of office blood pressure and decreased ambulatory systolic and pulse blood pressure, an effect that translated into net differences compared with placebo that were larger for ambulatory than for office blood pressure.

The increase in serum concentrations of big EDN1 suggests that the treatment effect was mainly sustained by inhibition of endothelin-converting enzyme. In the vasculature, EDNRA and EDNRB are expressed on vascular smooth-muscle cells and mediate the vasoconstrictory effects of EDN1. ENDRB is also located on vascular endothelial cells, where its activation promotes vasodilation through release of nitric oxide and prostacyclin.[10] In patients with mild-to-moderate hypertension without antihypertensive treatment, the mixed endothelin receptor antagonist bosentan has been reported to significantly reduce office and 24-h systolic and diastolic blood pressure compared with placebo, and to a similar extent as the angiotensin-converting enzyme inhibitor, enalapril.[24] Furthermore, the selective EDNRA antagonist darusentan—when added to at least three other antihypertensive drugs—significantly reduced office and 24-h systolic and diastolic blood pressure in patients with treatment-resistant hypertension, and to a larger extent than had been shown for bosentan.[25] Finally, avosentan—an EDNRA antagonist that is less selective than darusentan—improved albuminuria when given with angiotensin-converting enzyme inhibitors or angiotensin 2 receptor blockers in patients with overt diabetic nephropathy, but had no antihypertensive effect.[26] Theoretically, avoiding inhibition of EDNRB would be preferable, because it also mediates the clearance of circulating EDN1 in people, and in animal studies it has a role in regulation of natriuresis and diuresis. Thus, endothelin-converting enzyme inhibitors are promising new drugs—they will antagonise endothelin without affecting EDNRB-mediated clearance of EDN1.[10]

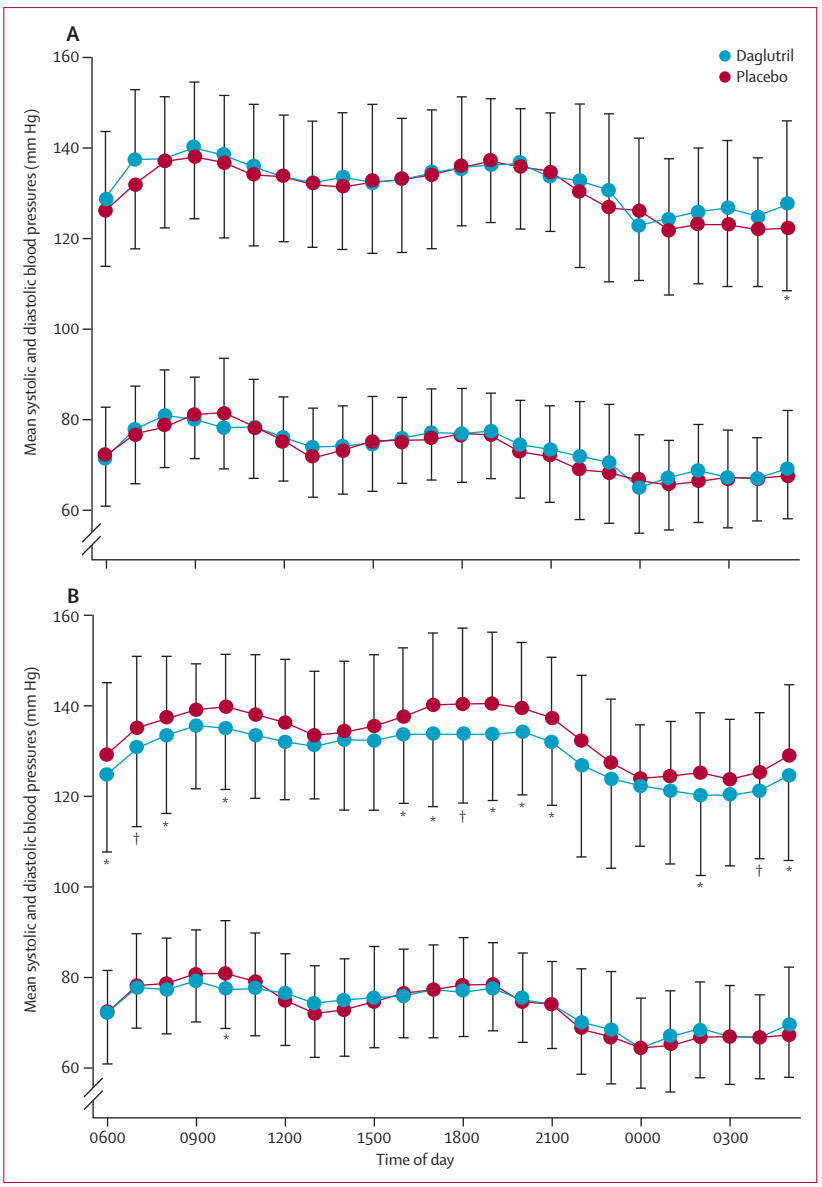

Figure 3: **24-h systolic and diastolic blood pressures**
Note, y axes are broken. Before treatment (A), and after treatment (B). Error bars are SDs. According to the autoregressive model, the difference in systolic and diastolic blood pressures after treatment with placebo versus daglutril are significant (p<0·0001 and p=0·010). *p<0·05. †p<0·01.

Blood pressure reduction during daglutril treatment was not associated with any significant change in 24-h albuminuria, renal haemodynamics, or albumin and IgG fractional clearances compared with placebo. One explanation could be that patients had increased bioavailability of pro-atrial natriuretic peptide secondary to inhibition of neutral endopeptidase, which could have increased glomerular permeability to plasma macromolecules.[27] The consequent increase in albumin

	Daglutril treatment			Placebo treatment			Daglutril *vs* placebo	
	Before	After	p value*	Before	After	p value*	Absolute difference	p value†
Renal function parameters								
GFR (mL/min per 1·73 m²)	84·7 (23·7)	82·9 (24·4)	0·417	81·8 (29·6)	85·5 (26·7)	0·343	−4·8 (23·2)	0·185
RPF (mL/min per 1·73 m²)	370·7 (103·6)	361·1 (126·1)	0·444	357·6 (134·3)	410·1 (177·4)	0·114	−36·9 (181·0)	0·328
Filtration fraction (%)	24·5 (12·4)	23·8 (7·3)	0·563	22·3 (5·7)	23·2 (5·6)	0·555	−5·3 (17·0)	0·138
RVR (mm Hg/mL per min/1·73 m²)	0·30 (0·11)	0·31 (0·10)	0·456	0·32 (0·12)	0·32 (0·18)	0·991	0·0 (0·2)	0·917
Albumin fractional clearance (×10⁵)	6·15 (6·82)	8·41 (9·44)	0·054	6·16 (6·95)	6·52 (6·37)	0·804	2·0 (8·6)	0·144
IgG fractional clearance (×10⁵)	0·96 (1·06)	1·35 (1·50)	0·016	0·82 (0·77)	0·87 (0·62)	0·667	0·4 (1·7)	0·178
Urinary sodium excretion (mmol/day)	231·0 (79·8)	216·2 (62·5)	0·344	234·2 (65·1)	234·7 (99·1)	0·360	0·0 (0·4)	0·814
Explicative variables								
Big END1 concentration (fmol/mL)	0·36 (0·09)	0·42 (0·14)	0·0055	0·36 (0·09)	0·35 (0·05)	0·747	0·06 (0·15)	0·010
Pro-atrial natriuretic peptide concentration (pmol/L)	1901·3 (1112·3)	2089·8 (1337·2)	0·109	1943·1 (1351·1)	1937·1 (1297·0)	0·950	194·5 (1112·7)	0·264

Data are mean (SD). GFR=glomerular filtration rate. RPF=renal plasma flow. RVR=renal vascular resistance. *Repeated measures ANOVA: after versus before. †Repeated measures ANOVA: daglutril versus placebo.

Table 4: **Renal function and explicative variables**

Panel: **Research in context**

Systematic review

We searched PubMed for original reports in English, between Jan 1, 1990, and Dec 31, 2012, with the terms "endothelin converting enzyme (ECE) inhibition", "neutral endopeptidase (NEP) inhibition", "combined ECE/NEP inhibition", "daglutril", "endothelin-1 antagonism", "type 2 diabetic nephropathy", and "clinical trials". We did not identify any clinical studies of the effect of daglutril on 24-h urinary albumin excretion rate, or on office and ambulatory blood pressure and renal function parameters in hypertensive patients with type 2 diabetes mellitus and nephropathy.

Interpretation

This study is, to our knowledge, the first randomised clinical trial to report that the combined oral endothelin-converting enzyme and neutral endopeptidase inhibitor daglutril reduces blood pressure in hypertensive patients with type 2 diabetes mellitus who have microalbuminuria or macroalbuminuria. The risk–benefit profile of daglutril compared favourably with that previously reported for endothelin-1 receptor antagonists, which suggests that combined endothelin-converting enzyme and neutral endopeptidase inhibition might help to improve control of blood pressure in this high-risk population.

ultrafiltration might have offset the reduction in albuminuria expected from decreased kidney perfusion pressure and postglomerular vasodilatation from antagonism of endothelin.[28,29] Natriuretic peptides might also induce preglomerular vasodilatation that maintains glomerular perfusion and filtration despite reduced blood pressure.[30] This hypothesis might explain why glomerular filtration rate and renal plasma flow were not reduced by daglutril treatment.

Our safety data compare favourably to the side-effects reported during treatment with endothelin receptor antagonists.[24,25] Darusentan has been associated with a doubled incidence of fluid overload or oedema compared with placebo. Another study[28] examining the effects of avosentan on progression of overt diabetic nephropathy had to be stopped prematurely because of an excess of fluid overload and congestive heart failure in the avosentan group. Kohan and colleagues[29] reported that oedema occurred in up to 46% of patients receiving increasing doses of the highly selective EDNRA antagonist atrasentan. Notably, no angio-oedema occurred during our study, a finding of clinical relevance, because combined inhibition of angiotensin-converting enzyme and neutral endopeptidase has previously been associated with increased incidence of angio-oedema caused by decreased breakdown of bradykinin, leading to increased nitric oxide concentrations.[31] Additional inhibition of endothelin-converting enzyme—as provided by daglutril—might alleviate this effect by reducing activation of EDNRB, thus decreasing production of nitric oxide.[13]

Further studies should be done to address whether higher doses of daglutril than were used in this study are needed to detect the antiproteinuric effects previously reported in animal studies and whether daglutril's blood-pressure lowering effects apply to patients with non-diabetic nephropathies.[14] The predominance of men in our study could be a result of the excess of men in the average population of patients with type 2 diabetes mellitus who have nephropathy and perhaps environmental factors that result in more men than women consenting to take part in the study. However, the large number of men does not affect the internal validity of the study and should not affect the generalisability of the findings to both sexes; no evidence exists of sex-specific effects of endothelin on hypertension, and previous studies[25] of endothelin receptor

antagonists showed the same antihypertensive effects in both men and women. Our results from autoregressive modelling[18] provide additional evidence that daglutril has an antihypertensive effect—particularly on systolic blood pressure—throughout the whole 24-h observation period, independent of rhythmical (circadian) and non-rhythmical changes in blood pressure. Our sensitivity analyses confirmed the robustness of these results. The study design, measurement of 24-h blood pressure, and the gold-standard procedures used to measure albuminuria and renal haemodynamic and sieving function parameters are major strengths. Results of our per-protocol analyses of efficacy variables were similar to those of the modified intention-to-treat analyses, which confirmed the robustness of our findings. Long-term clinical trials are needed to test whether the blood-pressure lowering effect of daglutril provides consistent nephroprotection and cardioprotection in this high-risk population.

Contributors

GR and PR had the original idea and wrote the study protocol. APa and II treated and monitored all participants. RT and AB helped in patient selection and follow-up. APe did the statistical analyses. AB and FG coordinated and supervised all the laboratory measurements. IMvdM wrote the first draft of the report and PR wrote the final version. All authors contributed to data interpretation, critically revised the draft, and approved the final report.

Conflicts of interest

We declare that we have no conflicts of interest.

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