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Antiproteinuric efficacy of verapamil in comparison to trandolapril in non-diabetic renal disease

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Nephrol Dial Transplant 1999: in press.

Introduction

Meta-analyses on antiproteinuric efficacy of different antihypertensive agents show that ACE inhibitors are superior to all other classes of antihypertensive drugs in diabetic and non-diabetic nephropathy at similar blood pressure reducing levels [1-3]. It has been demonstrated that this greater reduction of proteinuria by ACE inhibition is associated with better preservation of long-term renal function [4-7]. The antiproteinuric efficacy of calcium channel antagonists is overall significantly less than that of ACE inhibitors [1-3]. However, dihydropyridine and non-dihydropyridine calcium channel antagonists seem to differ with respect to their antiproteinuric efficacy [8-9]. Dihydropyridine calcium channel antagonists do not reduce proteinuria in long-term studies and may have adverse effects on GFR in diabetic nephropathy despite efficient blood pressure reduction [2]. In contrast, the non-dihydropyridine calcium channel antagonists verapamil and diltiazem reduce proteinuria to a similar extent as ACE inhibitors**,** but to a greater extent as other antihypertensive drugs [10-13]. Combination of verapamil or diltiazem with ACE inhibitors even induced a greater reduction of proteinuria in comparison to each agent alone [11,13].

All available data on non-dihydropyridine calcium channel antagonists are derived from a limited number of open label studies which are carried out in hypertensive non-insulin dependent diabetic patients with overt nephropathy [10-13]. To date, it is unknown whether verapamil reduces proteinuria to a similar extent as ACE inhibitors in patients with non-diabetic renal disease. We therefore performed a double-blind, random cross-over and placebo-controlled study on the antiproteinuric efficacy of the non-dihydropyridine calcium channel antagonist verapamil, the ACE inhibitor trandolapril, as well as the fixed combination of both agents in patients with non-diabetic proteinuria.

Methods

Patients and protocol

Patients were selected from the population who attended our outpatient renal department. Entry criteria for this study were biopsy proven non-diabetic renal disease, diastolic blood pressure below 110 mmHg, creatinine clearance more than 40 ml/min, proteinuria more than 2.0 g/day, and no need for concomitant medication. Patients with diabetes mellitus, edema or renovascular hypertension were not allowed to participate in the study. Antihypertensive, diuretic and immunosuppressive drugs were withdrawn for at least 4 weeks before enrollment. A 50 mmol/day sodium restricted diet as well as stable protein intake was prescribed. All patients gave their informed consent for participation in this protocol, which was approved by the local Medical Ethical Committee.

The study was designed as a double-blind, random crossover, placebo-controlled trial existing of 6 consecutive periods of 6 weeks duration (Figure 1). Patients received verapamil SR 360 mg o.i.d., trandolapril 4 mg o.i.d., and vera/tran

(verapamil SR 180 mg and trandolapril 2 mg o.i.d.) in double dummy capsules. Patients took their medication at each study day between 7.00-10.00 a.m. Patient compliance was estimated by capsule count at the end of each period by which patients should have taken at least 70% of placebo capsules and 90% of drug capsules. At the end of each study period patients collected 24-hr urine during three days. 24-Hour systolic and diastolic blood pressure, and pulse rate, were recorded at 30 minute intervals with an automated device (Spacelab®) during the last 24 hour urine collection. Renal function measurement was carried out at our outpatient renal function laboratory. At 8.00 a.m. a bolus injection of renal function tracers ^{125}I-iothalamate and ^{131}I-hippuran was followed by a constant infusion of these tracers in an anticubital vein. After a 2-hr equilibration period, two clearance periods were performed from 10:00 to 12:00 hr. and 12:00 to 14:00 hr. During the clearance periods blood was drawn to bracket each urine collection. Throughout the study day patients remained in sitting position, except when voiding. Patients were allowed to take their usual breakfast at 8:30 hr and to drink at least 250 ml/hr beverages during the study day to establish a sufficient diuresis. Before the intake of study medication at 8.00 a.m., blood was drawn for determination of chemistry, ACE, PRA, and angiotensin II.

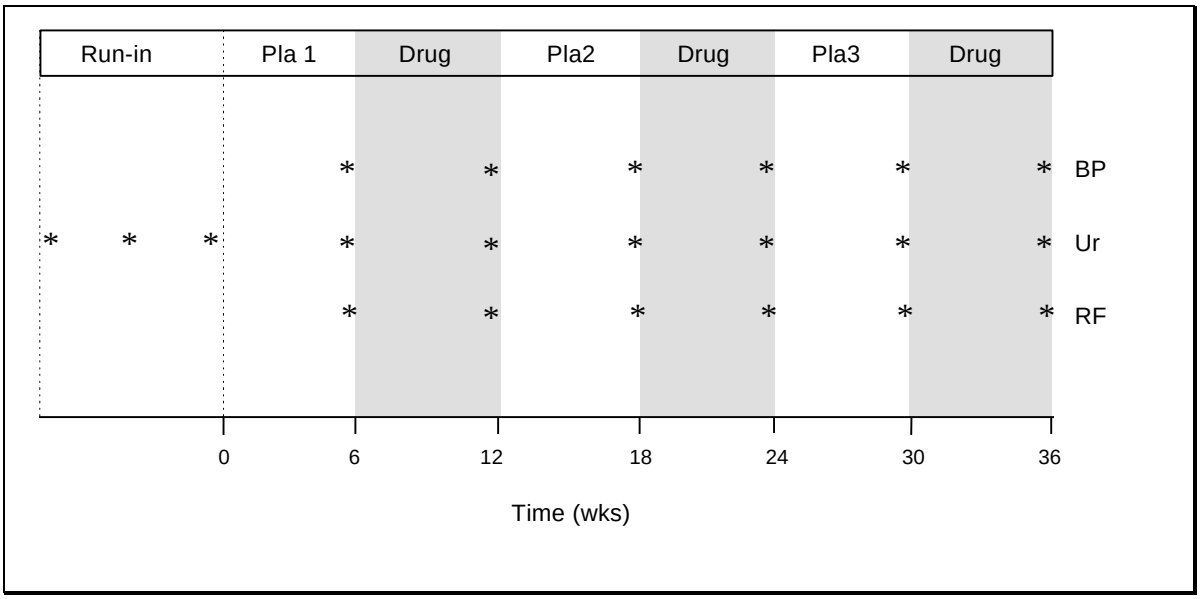

Figure 1. Study design. Drug = treatment phase with in random order trandolapril 4 mg o.i.d., verapamil SR 360 mg o.i.d., and vera/tran (trandolapril 2 mg/verapamil SR 180 mg o.i.d.); BP = 24 hour blood pressure recording; Ur = 24 hour urine collections at three consecutive days; RF = ^{125}I-iothalamate and ^{131}I-hippuran clearances.

Clinical and laboratory procedures

Serum and urinary chemistry were determined with an automated multi-analyzer (SMA-C-II®, Technicon Instruments Inc.,Tarry Town, NY, USA). Urinary protein excretion was determined by the pyrogalloll red-molybdate method [14]. Proteinuria was calculated as the mean of three 24-hr urine collections. Mean arterial pressure was calculated as the sum of one-third of the systolic blood pressure and two-thirds of the diastolic blood pressure for each measurement during 24 hours. GFR and ERPF were estimated as the mean renal clearance of ^{125}I-iothalamate and ^{131}I-hippuran during two clearance periods according to a previously described method [15]. Filtration fraction was calculated as the ratio of GFR and ERPF. Serum ACE was measured using a HPLC assisted assay [16]. PRA was assessed by the quantification of generated angiotensin I as measured by radioimmunoassay (intra-assay variation < 7.8%; inter-assay variation < 8.2%; lower detection range of 0.15 ng/ml/hr). Blood for determination of angiotensin II was collected in prechilled tubes which contained a mixture of EDTA, enalaprilat, ofenantroline, ethanol and neomycin-B-sulfate to prevent in-vitro generation or degradation of angiotensin II. Immediately after centrifugation at 4°C, plasma was stored at -20°C until analysis. Angiotensin II was determined by radioimmunoassay (intra-assay variation < 4.0%; inter-assay variation < 9.3%; lower detection range of 3.8 ng/l).

Table 1. *Baseline characteristics.*

Pat	Sex	Age *yr*	Diagn	SBP *mmHg*	DBP *mmHg*	Cl_{cr} *ml/min*	$U_{protein}$ *g/day*	$S_{albumin}$ *g/l*	S_{chol} *mmol/l*
1	m	49	FSGS	139	96	85	7.0	35	4.4
2	f	37	MGP	136	85	87	5.0	34	4.6
3	m	23	FSGS	137	77	52	7.5	41	6.8
4	m	44	FSGS	122	75	112	4.6	45	6.3
5	m	40	MGP	124	74	107	6.6	28	9.6
6	m	33	IgA	131	80	121	4.6	42	6.9
7	f	20	MPGN	116	61	120	9.6	27	7.7
8	f	48	MGP	132	86	80	6.1	37	6.4
9	m	60	FSGS	197	108	76	3.8	41	6.4
10	f	36	FSGS	161	109	91	13.2	34	8.3
11	m	54	FSGS	158	95	57	8.7	37	6.7

Abbreviations are: Pat = patient no.; Diagn = renal diagnosis; SBP = 24-hr systolic blood pressure; DBP = 24-hr diastolic blood pressure; Cl_{cr} = creatinine clearance; $U_{protein}$ = proteinuria; $S_{albumin}$ = serum albumin; S_{chol} = serum cholesterol; FSGS = focal segmental glomerulosclerosis; MGP = membranous glomerulopathy; IgA = IgA nephropathy; MPGN = membranoproliferative glomerulonephritis.

Analysis

A meta-analysis of Gansevoort et al. [1] on the antiproteinuric capacity of antihypertensive drugs in comparative studies demonstrated that ACE inhibition induced an antiproteinuric response of 40%, whereas non-dihydropyridines induced an antiproteinuric response of 21%. Power analysis based on a 20% or greater difference in the antiproteinuric response between verapamil and trandolapril revealed that at least 9 patients were to be included in the present study to achieve a β-error of 0.15 and an α-error of 0.05.

Data are expressed as a Wilcoxon-based estimated median with 95% confidence interval [17], unless otherwise indicated. Parameters are expressed as absolute value or as percentage change from the preceding placebo value. A Friedmann two way non-parametric ANOVA for paired observations was performed, followed by Duncan's correction for multiple comparisons, to test for differences between the placebo periods in consecutive order as well as for differences between active treatment efficacy [18-19]. Wilcoxon's rank sum test for paired observations was performed to test for carry-over effects of each active treatment by comparison of two placebo periods which preceded and followed active treatment. In addition, differences between active treatment and preceding placebo were tested by Wilcoxon's rank sum test for paired observations. Correlation's between parameters were tested using Spearman's rank sum test. Statistical significance was assumed at a p-level less than 0.05.

Results

Thirteen patients were included in this study. Eleven patients completed the protocol, whereas 2 patients dropped out: one patient needed diuretics because of progressive edema during the first placebo phase and another patient underwent surgery because of acute appendicitis during the first treatment phase with verapamil. Both reasons for discontinuation were not related to the study medication. Baseline characteristics of the patients at the end of the first placebo treatment are presented in table 1. All patients but one were of Caucasian origin. Four of 11 patients (no. 1, 9, 10, and 11) demonstrated hypertension with a mean 24-hr blood pressure higher than 140/90. Renal function was normal to moderately impaired as reflected by a creatinine clearance ranging from 52 to 121 ml/min. Patients had proteinuria ranging from 3.8 to 13.2 g/day without signs of edema. Two patients (no. 5 and 7) demonstrated a serum cholesterol of more than 7.0 mmol/l in combination with a serum albumin below 30 g/l at the start of the study.

The primary parameters MAP, FF and proteinuria did not significantly change during the placebo periods in consecutive order. This excludes presence of time related changes due to changes in intrinsic renal disease. In addition, no significant carry-over effects of any of the active treatments could be demonstrated. Patient compliance met the criteria in all study periods, whereas no significant differences in

patient compliance were observed between active treatment periods. Dietary conditions monitored by 24-hr urinary sodium and urea excretion as well as parameters of biochemistry were not significantly different during the entire study. The effects of each treatment compared to its preceding placebo period are shown in table 2.

Verapamil

Verapamil did not change 24-hr MAP compared to its preceding placebo period. Pulse rate, GFR, ERPF, and FF did neither change significantly. Verapamil slightly reduced proteinuria compared to its preceding placebo period from 6.3 (5.0-7.0) to 5.9 (4.5-6.4) g/day ($p<0.05$). Hypertensive patients tended to have a greater mean reduction of MAP (120 to 112 versus 95 to 96 mmHg; $p=0.16$) and proteinuria (6.3 to 5.4 versus 5.8 to 5.5 g/day; $p=0.16$) than normotensive patients, whereas change in FF did not differ between both groups (Figure 2). Neither ACE, PRA or angiotensin II levels changed. Three patients showed peripheral edema during treatment with verapamil, which spontaneously resolved in the following placebo period.

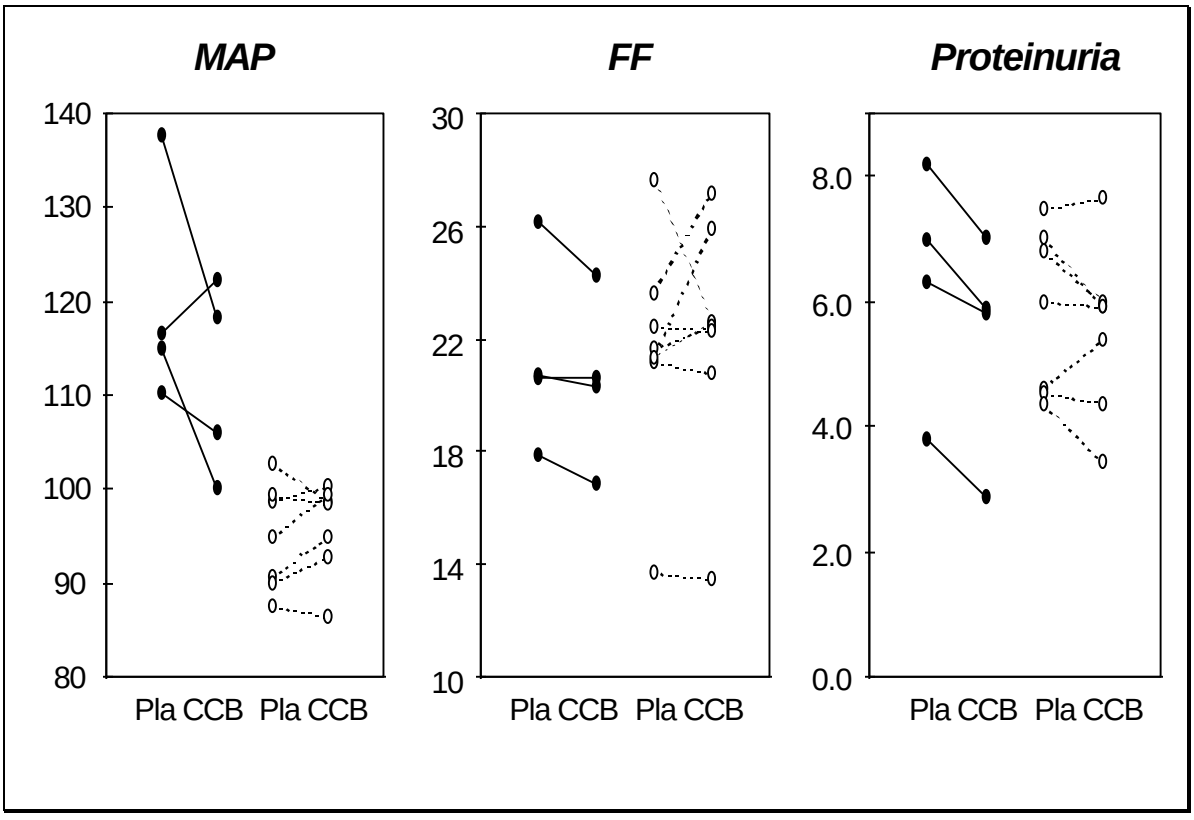

Figure 2. Individual data on mean arterial pressure (MAP), filtration fraction (FF) and proteinuria during placebo (Pla) and verapamil (CCB). Closed circles represent hypertensive patients and open circles represent normotensive patients.

Table 2. *Effects of trandolapril, verapamil, and vera/tran compared to preceding placebo values (n=11).*

	Placebo	Trandolapril	Placebo	Verapamil	Placebo	Vera/tran
MAP, mmHg	99 [95-113]	89 [86-99]*	99 [94-114]	99 [94-108]	99 [97-115]	90 [88-101]*
Pulse rate, bpm	70 [65-78]	69 [65-76]	77 [64-77]	69 [62-76]	75 [66-82]	74 [66-75]
GFR, ml/min/1.73m^2	85 [63-95]	85 [62-99]	80 [60-93]	88 [61-95]	80 [63-97]	84 [62-97]
ERPF, ml/min/1.73m^2	364 [280-448]	436 [323-530]*	378 [271-463]	392 [275-470]	349 [270-479]	413 [316-529]*
FF,%	22.7 [20.3-23.9]	18.3 [17.1-21.0]*	21.3 [19.0-24.1]	22.3 [18.9-24.1]	22.2 [19.8-24.5]	20.1 [17.0-21.4]*
$U_{protein}$, g/day	6.2 [4.9-7.7]	3.7 [2.6-4.6]*	6.3 [5.0-7.0]	5.9 [4.5-6.4]*	6.3 [4.3-8.4]	3.2 [2.6-5.4]*
ACE, U/l	23 [2-75]	15 [10-25]*	22 [4-73]	19 [2-69]	21 [4-66]	15 [10-25]*
PRA, ng/l/hr	1.1 [0.8-1.7]	3.6 [1.3-13.8]*	1.1 [0.8-1.8]	1.0 [0.7-2.0]	1.3 [0.9-1.8]	2.3 [1.0-6.9]*
Ang II, ng/l	86 [60-108]	103 [67-178]	69 [54-114]	98 [64-113]	91 [70-120]	95 [61-148]
$S_{albumin}$, g/l	38 [33-41]	40 [35-42]	38 [33-41]	37 [33-40]	38 [33-41]	40 [34-42]
$S_{cholesterol}$ mmol/l	6.5 [5.6-7.8]	6.2. [5.4-7.2]	6.4 [5.8-7.3]	6.4 [5.5-7.5]	6.7 [6.0-7.9]	6.1 [5.6-7.3]
$U_{Na}E$, mmol/day	108 [81-120]	129 [97-145]	100 [78-148]	114 [93-153]	112 [80-133]	123 [101-137]
$U_{urea}E$, mmol/day	281 [261-370]	358 [303-396]	329 [267-377]	302 [277-357]	321 [294-373]	313 [278-362]

Data are expressed as median and 95% CI. Abbreviations are: MAP = mean arterial pressure; GFR = glomerular filtration rate; ERPF = effective renal plasma flow; FF = filtration fraction; $U_{protein}$ = proteinuria; ACE = angiotensin converting enzyme activity; PRA = plasma renin activity; Ang II = angiotensin II; $S_{albumin}$ = serum albumin; $S_{cholesterol}$ = serum cholesterol; $U_{Na}E$ = urinary sodium excretion; $U_{urea}E$ = urinary urea excretion. *p<0.05 versus preceding placebo value.

Trandolapril

Twenty-four hour MAP was significantly reduced by trandolapril. The pulse rate and GFR remained unchanged, whereas ERPF increased ($p<0.05$) and filtration fraction decreased ($p<0.05$). Trandolapril reduced proteinuria compared to its preceding placebo period from 6.2 (4.9-7.7) to 3.7 (2.6-4.6) g/day ($p<0.05$). The responses of MAP, FF and proteinuria to trandolapril were comparable in normotensive and hypertensive patients. The relative changes of MAP, filtration fraction, and proteinuria during trandolapril were significantly greater than those during verapamil (Figure 3). ACE activity significantly fell, whereas PRA significantly increased and angiotensin II levels remained unchanged. Two patients experienced cough during trandolapril which spontaneously resolved in the following placebo period.

Vera/tran

Vera/tran significantly reduced 24-hour MAP. The pulse rate and GFR did not change significantly, whereas ERPF increased ($p<0.05$) and FF decreased ($p<0.05$). Vera/tran reduced proteinuria compared to its preceding placebo period from 6.3 (4.3-8.4) to 3.2 (2.6-5.4) g/day ($p<0.05$). The responses of MAP, FF and proteinuria to vera/tran were comparable in normotensive and hypertensive patients. The relative changes in MAP, ERPF, FF, and proteinuria during vera/tran were comparable to those during trandolapril, whereas the relative changes in MAP, FF and proteinuria during vera/tran were significantly greater than those during verapamil (Figure 3). ACE activity was significantly reduced, whereas PRA was significantly increased. Angiotensin II levels showed no significant change. One patient experienced cough during vera/tran which spontaneously resolved in the following placebo period.

Correlation's

Baseline MAP was significantly related with relative changes in blood pressure during verapamil ($r=-0.70$; $p<0.02$), whereas such a relation just did not reach statistical significance during trandolapril ($r=-0.60$; $p<0.10$) or vera/tran ($r=-0.59$; $p<0.10$). Baseline MAP only tended to be significantly related to the relative changes of proteinuria during verapamil ($r=-0.55$; $p<0.10$). Relative changes in MAP or FF were not related to relative changes in proteinuria during any of the treatments. Daily sodium excretion was not significantly related to relative changes in MAP, FF or proteinuria during any of the active treatments.

Discussion

In the present study we show that the antiproteinuric and antihypertensive response of the non-dihydropyridine calcium channel antagonist verapamil is less compared to the ACE inhibitor trandolapril in patients with non-diabetic proteinuria.

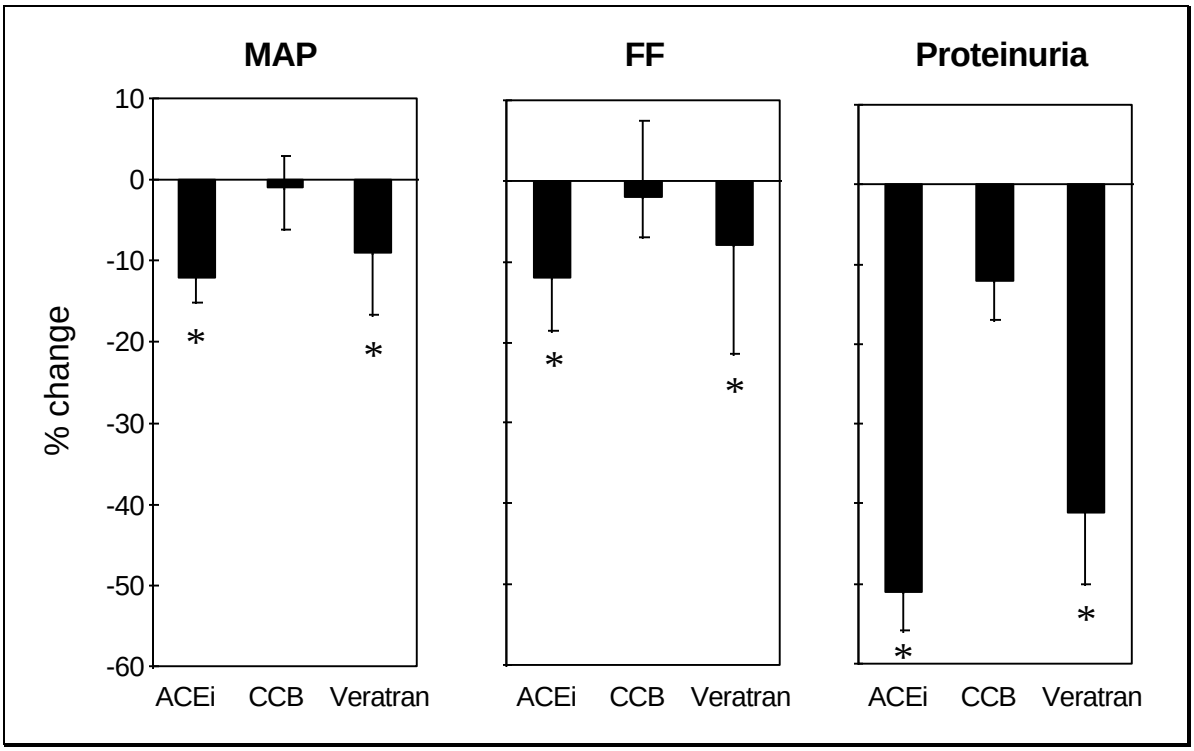

Figure 3. Changes of mean arterial pressure (MAP), filtration fraction (FF) and proteinuria during trandolapril (ACEi), verapamil (CCB) and Vera/tran. Data are expressed as median and 95% confidence interval. * $p<0.05$ versus verapamil.

Treatment with a fixed half dose of trandolapril and verapamil in combination showed similar effects on blood pressure, renal hemodynamics, and proteinuria compared to treatment with trandolapril alone at full dose.

Meta-analyses revealed that ACE inhibitors are superior to all other antihypertensive drugs including calcium channel antagonists with respect to their antiproteinuric response at similar blood pressure reducing levels [1-3]. It has previously been observed that blood pressure reduction mediates the initial 20% reduction of proteinuria by ACE inhibitors which is comparable to the complete antiproteinuric effect of other antihypertensive agents [20]. The additional 30% reduction of proteinuria by ACE inhibition seems to be mediated by a reduction of intraglomerular pressure which is independent from systemic blood pressure reduction and/or non-hemodynamical intrarenal effects of ACE inhibitors [21-22]. Non-dihydropyridine calcium channel antagonists such as verapamil and diltiazem show an antiproteinuric capacity comparable to that of ACE inhibitors, but greater than other antihypertensive drugs, in moderate to severe hypertensive non-insulin dependent diabetic patients with overt nephropathy [10-13]. It has also been observed that non-dihydropyridine calcium channel antagonists slowed progressive

loss of renal function to a similar extent as ACE inhibitors, but to a greater extent than β-blockers in NIDDM [23-24]. This suggest that ACE inhibitors and non-dihydropyridine calcium channel antagonists induce beneficial renal effects beyond their blood pressure lowering effect in diabetic renal disease. The present study firstly elucidated the antiproteinuric capacity of verapamil in comparison to ACE inhibitors in non-diabetic renal disease. Surprisingly, verapamil revealed a smaller antiproteinuric response compared to the ACE inhibitor trandolapril.

Can we explain that verapamil and trandolapril did not reveal a comparable antiproteinuric efficacy? The most important issue seems to be the difference in blood pressure reduction between both treatments in the present study, whereas all previous comparative studies showed equal reductions of blood pressure [10-13]. This difference in blood pressure reduction is the consequence of the absence of blood pressure reduction during treatment with verapamil. Several factors such as study design, study conditions, drug dosage, and patient characteristics may have contributed to this the lack of blood pressure effect during verapamil. The cross-over study design may have induced carry-over and time dependent effects on study parameters. Although the exact time-dependence of the antiproteinuric effect of verapamil is unknown, its antihypertensive effect is maximal after 4 weeks treatment [25]. ACE inhibitors reach their maximal hemodynamic and antiproteinuric effect after 4 weeks treatment, whereas 4 weeks withdrawal of ACE inhibition induces a complete reversal of its hemodynamic and antiproteinuric response [22]. We therefore assumed 6 weeks to be an appropriate study period duration to achieve a maximal efficacy for verapamil and trandolapril. Furthermore, each active treatment is followed by 6 weeks placebo which allows an effective wash-out period of 12 weeks duration for each active treatment. Since blood pressure and other parameters did not significantly change during three placebo periods in consecutive order and carry-over effects could be excluded, it is unlikely that our study design induced a difference in response of blood pressure or other parameters during verapamil or trandolapril. Another factor which has to be taken into consideration is the application of dietary salt restriction in the present study. The hemodynamic and antiproteinuric responses of ACE inhibition are enhanced by dietary salt restriction [26]. However, controversial data exist on the influence of dietary salt restriction on the blood pressure response during calcium channel antagonism. On the one hand, dietary salt restriction attenuated the blood pressure lowering response to verapamil in patients with salt-sensitive essential hypertension [27]. On the other hand, changes in dietary salt intake did not change the blood pressure lowering response of verapamil in hypertensive patients with chronic renal failure [28-30]. Since we could not observe a significant relation between the intake of sodium and the relative change of blood pressure during verapamil, it is unlikely that dietary salt restriction contributed to the lack of blood pressure lowering during verapamil. Drug dosage is another factor which may have a role in the lack of blood pressure reduction during verapamil. The used dosage of verapamil in the present study induces a maximal reduction of blood pressure as well as a reduction of blood pressure comparable to that of ACE inhibitors in hypertensive patients [9,11,23,25]. In contrast, verapamil at

a dose of 160 mg did not acutely reduce blood pressure in normotensive patients [31]. Since no data are available which reveal that higher dosages of verapamil induce a long-term reduction of blood pressure in normotensive patients, it is unlikely that a higher dosage of verapamil would have induced a greater reduction of blood pressure or renal hemodynamic vasodilatation in our population. Lastly, patient characteristics may have contributed to the difference in blood pressure and antiproteinuric response between verapamil and trandolapril. Our population showed a large heterogeneity with respect to blood pressure and renal function. It has been demonstrated that the antiproteinuric response of ACE inhibitors is partly dependent from changes in blood pressure or GFR, but not from baseline patient characteristics [1]. The antiproteinuric response of calcium channel blockers is greatly dependent from change in blood pressure, initial GFR, and type of calcium channel antagonist [1]. Furthermore, calcium channel blockers do not induce systemic or renal hemodynamic changes in normotensive conditions [32-33]. The fact that the majority of our patients are normotensive may thus explain the overall lack of blood pressure, renal hemodynamic and antiproteinuric response during verapamil. Hypertensive patients indeed tended to show greater reductions of blood pressure and proteinuria during verapamil. Higher baseline blood pressure was also related with greater reductions of blood pressure during verapamil. No significant relations could be observed between relative changes of proteinuria and relative changes in blood pressure or GFR, or baseline GFR during any of the treatments. Although our analysis is affected by the small number of included patients, these observations suggest that the antiproteinuric effect of the non-dihydropyridine calcium channel antagonist verapamil is greatly dependent of blood pressure reduction in non-diabetic renal disease, in contrast to the antiproteinuric response of ACE inhibitors. In support of this, an unpublished study revealed that diltiazem induced a smaller reduction of proteinuria compared to ACE inhibition in hypertensive patients with non-diabetic renal disease, despite equivalent blood pressure reductions [34]. It has also been demonstrated that treatment with antihypertensive drugs other than ACE inhibitors, including calcium channel blockers, induces a smaller reduction of blood pressure and proteinuria in non-diabetic than in diabetic patients [1]. It therefore may be that differences between non-diabetic and diabetic renal disease also contributed to the disappointing antiproteinuric efficacy of verapamil in the present study.

Combination treatment of verapamil and ACE inhibitors in half their usual doses reduced proteinuria significantly more than treatment with one of each agent at full dose in hypertensive diabetics with micro-albuminuria and overt nephropathy [9,11,13]. In this respect it is again important to stress that blood pressure reduction during combination and single treatments was comparable in those studies. In the present study, half of the dose of trandolapril and verapamil resulted in a similar response of blood pressure, renal hemodynamics and proteinuria as single treatment with full dose of trandolapril. It has been previously shown that the antiproteinuric effect of ACE inhibition is dose related [35]. Halving the dose of trandolapril should therefore result in less reduction of proteinuria. Since this is not

the case in our study, it could well be that the combination of verapamil and trandolapril exerts additional antiproteinuric effects in non-diabetic patients. Nevertheless, trandolapril already induces a maximal blood pressure lowering effect at a dose of 2 mg [36]. In case the dose response profile of reduction in proteinuria mimics that of blood pressure lowering, a maximal antiproteinuric response may already be induced with half the dose of trandolapril. This would exclude an additional antiproteinuric effect of combination treatment. It is clear that our study does not allow a firm conclusion on the potential additional antiproteinuric effect of treatment with trandolapril and verapamil in combination.

In conclusion, calcium channel blockade with verapamil is not as effective as ACE inhibition with trandolapril in reducing proteinuria and blood pressure in patients with non-diabetic renal disease. In contrast to the antiproteinuric response of trandolapril, the antiproteinuric response of verapamil seems to be greatly dependent from effective blood pressure reduction. The fixed combination of verapamil and ACE inhibition at half doses has similar effects as ACE inhibition at full dose, which suggests, but not prove, that the combination provide additional antiproteinuric efficacy in non-diabetic renal disease.

Acknowledgments

This study was supported by Knoll BV (Amsterdam, The Netherlands) for which we are indebted to L. Sahelijo, M.D., E. Geervliet, Pharm.D. and J. Bots. We are grateful to Dr. W.J. Sluiter for statistical advice. We furthermore acknowledge the practical assistance at our outpatient renal function ward of Mrs. C. Nieuwenhout, Mrs. E. Konneman, Mrs. M. van Kammen and Mrs. A. Drent-Bremer.

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