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Original Paper

The Enhanced Renin-Angiotensin-Aldosteron System Pharmacological Blockade - Which is the Best?

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Key Words
Mineralocorticoid receptor antagonist • Angiotensin II receptor antagonist • Renin inhibitor • Proteinuria • Chronic kidney disease • Renin angiotensin aldosteron system

ABSTRACT
Background/Aims: Pharmacological inhibition of renin-angiotensin-aldosteron system (RAAS) may reduce proteinuria and the rate of chronic kidney disease progression. The aim was to compare the effects on albuminuria of the therapy with either: i) telmisartan 80 mg and aliskiren 300 mg, ii) telmisartan 80 mg and eplerenone 50 mg, iii) telmisartan 160 mg as monotherapy. ***Design and patients:*** Randomized, double-center, double-blind, cross-over, three treatments-three periods of 8 weeks each study. 18 patients with non-diabetic proteinuric CKD stage 1-3 completed the protocol. ***Results:*** There was significant difference in albuminuria between studied therapies (ANOVA; $p<0.01$). The combination therapy with telmisartan plus aliskiren decreased albuminuria more effectively than the treatment with telmisartan plus eplerenone and monotherapy with telmisartan 160 mg OD [376 mg/g creatinine (286-686) vs. 707 (502-1204) vs. 525 (318-763); post-hoc $p<0.01$ and $p<0.05$, respectively]. ***Conclusions:*** The study demonstrated that the combination therapy with angiotensin receptor blocker (ARB) and renin inhibitor was more effective in albuminuria lowering than the concomitant usage of ARB and mineralocorticoid receptor antagonist as well as than ARB in doses two-fold higher than usually used in treatment of hypertension in patients with non-diabetic CKD and that this higher antiproteinuric efficacy was independent on changes in blood pressure.

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Introduction

Angiotensin II and aldosterone are the key players in the development and progression of chronic kidney diseases (CKD), either directly by promoting tissue fibrosis or indirectly through their action on glomerular hemodynamic and enhancing proteinuria [1, 2]. Therefore, pharmacological inhibition of the renin-angiotensin-aldosteron system (RAAS) may have a beneficial impact on renal outcome [2, 3]. Various studies have shown that treatment with angiotensin-converting enzyme inhibitors (ACEI) or angiotensin II receptor blockers (ARB) reduce both proteinuria and the rate of decline in the glomerular filtration rate (GFR) in non-diabetic CKD [4-6]. Mineralocorticoid receptor antagonists and renin inhibitors have been postulated to exert antiproteinuric activity as well [7, 8]. Despite recent progress, however, there is still no optimal therapy which can stop progression of CKD. It has been shown that constant treatment with an ACEI or ARB leads to the return of angiotensin II and aldosterone to their pre-treatment level [9]. Therefore, it is necessary to search for therapeutic strategy which can enhance RAAS blockade and further improve renal outcome. Of particular need is to find the optimal combination of different RAAS blocking drugs and establish the optimal dosing of these agents [10]. To gain insight into this issue, we performed the study to compare the influence of (i) the combination therapy with ARB, telmisartan plus renin inhibitor, aliskiren, (ii) the combination therapy with ARB, telmisartan plus mineralocorticoid receptor antagonist, eplerenone and (iii) monotherapy with ARB, telmisartan in doses two-fold higher than usually used on albuminuria regarded as an independent risk factor for renal disease progression and a surrogate marker of kidney injury extent.

Material and Methods

Individuals

Patients were selected from the cohort that attended renal outpatients' departments in Medical University of Gdansk and Collegium Medicum in Bydgoszcz of Nicolaus Copernicus University in Torun. The inclusion criteria were established as follows: age 18-65 years, non-diabetic proteinuric chronic kidney disease stage 1-3, stable proteinuria above 500 mg/ 24 hours in last 6 months (no variations above 500 mg/24 hours), hypertension treated with at least one agent or hypertension no treated so far with blood pressure above 140/90 mmHg, no steroids or other immunosuppressive treatment for a minimum of six months before the study. Patients with unstable coronary heart disease or decompensated congestive heart failure in the previous 6 months, with an episode of malignant hypertension or stroke in the history, diabetics and estimated glomerular filtration rate of less than 30 ml per minute per 1.73 m^2 of body-surface area were excluded.

General protocol

This was a double center, prospective, randomized, double-blind, cross-over study comparing the renal effects of therapy with either combination of telmisartan 80 mg and eplerenone 50 mg OD (A), or the combination of telmisartan 80 mg and aliskiren 300 mg OD (B), or telmisartan 160 mg OD (C) alone, in three randomized periods of 8 weeks each. At the beginning, subjects who met the inclusion criteria entered the 8 weeks run-in period during which any hypotensive agents previously used were stopped and blood pressure (BP) was controlled by the background therapy with combination of telmisartan 80 mg and ACEI, perindopril 10 mg OD (P1 period). At the end of the run-in period, the subjects were randomly allocated to one of six treatment sequences: ABC, ACB, BAC, BCA, CAB, CBA (Figure 1). Allocation was performed according to a computer generated randomization list. For the ethical reasons there was no washout between the run-in period and three treatment therapies. There was also no washout between the treatments in each sequence. At the end, the same 8-week background therapy as in the run-in period was administered as a control period for stability of the background proteinuria (P5 period). Preparing, labeling and blinding of the study medications was performed by Department of Pharmaceutical Technology, Medical University of Gdańsk. Patients were instructed to take the study medication once daily in the morning. The doses of the study medications were not changed. Patients were recommended not to change their usual daily protein

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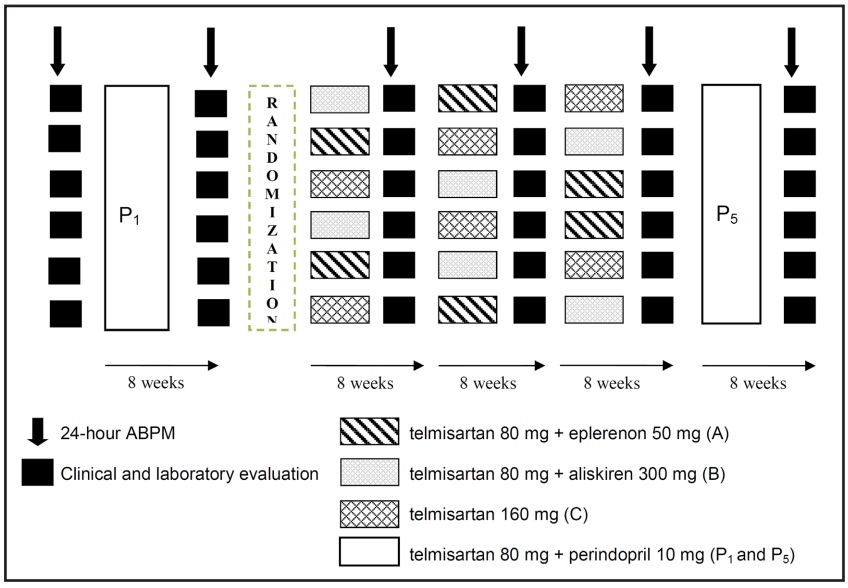
Fig. 1. Study protocol.



and sodium intake during the study period. At the end of each of 3 treatment periods (A,B,C) and after both background therapies (P1 and P5) albuminuria, ambulatory BP, serum concentration of creatinine were determinated and creatinine clearance was calculated. Patients discontinued the trial in case of withdrawal of consent, incompliance with therapy, hyperkalaemia (>6.0 mmol/l), worsening of renal function defined by an increase from baseline serum creatinine greater than 30% and confirmed in two occasions, any other severe adverse events associated with treatment, e.g. cough or angioedema on ACEIs therapy. The study was approved by the local ethical committee, and the investigated patients all gave their informed consent. The study was registered at www.clinicaltrial.gov (identifier: NCT 01541267).

Procedures and laboratory analyses

Ambulatory BP was measured continuously for 24-h using the Mobil-o-graph (version 12) monitoring system. BP was measured every 15 minutes during the day (7.00 a.m. to 10.00 p.m.) and every 30 minutes during the night (10.00 p.m. to 7.00 a.m.). Results of ambulatory BP measurements were analysed for 24h SBP, 24h DBP.

Albumin urine excretion was measured in the first morning spot urine sample and expressed as the ratio of albumin-to-creatinine (UACR) to correct for variations in urinary concentration due to hydration. Two urine sample were collected on separate days - of those the mean value of UACR was calculated for data evaluation. The concentration of albumin was measured by enzyme-linked immunosorbent assay (ELISA) using an *Albumin* (Immunodiagnostic AG, Bensheim, Germany) kit in accordance with manufacturer's recommendations. The intra-assay and inter-assay coefficients of variations for this assay were 5.0% and 8.0%, respectively. Creatinine level was measured in the same urine samples using a modified kinetic Jaffe method.

Serum creatinine was measured by the standard laboratory techniques. Sodium (Na) and urea excretion were evaluated on the basis of 24-hour urine collection. The excretion of urea was used to calculate the protein intake according to Maroni equation : protein intake normalized to weight(g/kg/day)=6.25x[(urea-N-excretion urine 24h(g/day)+(0.0031xbody weight(kg))]/body weight (kg) [11]. Creatinine clearance was calculated according to Cockroft-Gault equation [12].

Statistics

The primary end point of the study was a difference in albuminuria between three studied therapies. A sample size of 18 patients adequately allowed a power of 80% to detect a difference in means across the levels of repeated measures factor equal to within patient standard deviation, that is a standardised effect size of 1.0 at a significance level of 0.05 (two-tailed). Normality and homogeneity of the variances were verified by means of the Shapiro-Wilk test and Levene test, respectively. In the per-protocol design, the variable differences were assessed by analysis of variance (ANOVA) for repeated measurements with

Table 1: Patients` characteristic at baseline

Parameter	
n (Gender: female/male *n)*	18 (4/14)
Age *years*	39.3 ± 2.7
Mean systolic blood pressure *mm Hg*	116.8 ± 2,4
Mean diastolic blood pressure *mm Hg*	73.8 ± 1.8
24 h proteinuria *g*	1.62 (0.98 – 2.26)
Serum creatinine *mg/dl*	1.1 ± 0.11
Creatinine clearence [Cockroft-Gault formula] *ml/min*	94.0 ± 8.1
24-hour urinary sodium *mmol/24 h*	169 ± 21
Serum potasium *mmol/l*	4.47 ± 0.1
Body mass index kg/m^2	26.4 ± 0.79
Diagnosis : *n*	
Mesangial glomerulonephritis	2
Mesangiocapillary glomerulonephritis	2
Membranous glomerulonephritis	2
Focal segmental glomerulonephritis	3
Minimal change nephropathy	1
IgA nephropathy	6
Unknown non-diabetic proteinuric chronic kidney diseases	2
Background hypotensive therapy: *n*	
ACEi and ARB	8 (44.5%)
ACEi (alone)	4 (22.5%)
ARB (alone)	2 (11%)
No hypotensive therapy	4 (22%)

ACEi - angiotensin-converting enzyme inhibitors; ARB - angiotensin II receptor blockers; *Note:* To convert serum creatinine in mg/dL to μmol/L, multiply by 88.4; eGFR in mL/min/1.73 m2 to mL/s/1.73 m2, multiply by 0.01667; Data are expressed as mean ± SEM or geometric mean (95% confidence interval)

Benferroni corrections for paired comparisons. Head to head comparisons between study therapies and background treatment with telmisartan plus perindopril, as secondary analyses, were performed using t-Student test. A P <0.05 (two-tailed) was considered statistically significant. Data were evaluated using a STATISTICA (version 9.0 Stat Soft Inc.) software package. The results were expressed as means ± SEM.

To prevent or limit the risk of "carryover" effect, we planned each treatment period for 8 weeks. Previous studies demonstrated that the effects of RAAS blocking agents on albuminuria and glomerular permselectivity are fully reversible within 4 weeks [13]. Thus, prolonging each treatment period to 8 weeks allowed us to rule out any residual effect of previous treatment at the end of the eighth week, when albuminuria was measured. To prevent or limit the possibility of a "period effect", we introduced a degree of balance into the study design, with a scheme of randomisation allowing every treatment sequence to be represented in every period with the same frequency. Overall, we had six different therapy sequences with the three treatment periods (Figure 1). Equal numbers of patients (n=3) per sequence were randomised. Since no patients were prematurely withdrawn, this balance was fully respected at the study end. To test the presence of "period effect", the difference between albuminuria at the end of P1 and P5 periods was checked.

Results

Of the 18 patients who entered the study, 18 (100%) completed the protocol. Baseline clinical characteristic of patients is listed in Table 1. Before data analysis, the "period effect" (the difference in UACR after P1 and P5 period) was tested and found not to be significant.

24 hour ambulatory BP

There were no differences in systolic and diastolic blood pressure between the treatments (Table 2) BP was stable during whole study time without hyper- and hypotonic episodes.

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Table 2: 24 h systemic blood pressure and laboratory results during the study

	Telsmisartan Perindopril (P1)	**Telmisartan Eplerenon (A)**	**Telmisartan Aliskiren (B)**	**Telmisartan 160 (C)**	Telsmisartan Perindopril (P2)	**ANOVA (A vs B vs C)**
Systolic BP (24h) *mmHg*	116.8±2.4	121.5±2.6	120.5±2.8	120.6±2.4	118.0±2.4	p=0.86
Diastolic BP (24h) *mmHg*	79.9±1.8	76.6±1.9	74.4±2.2	75.8±2.0	73.8±1.6	p=0.31
CrCl [C-G] *ml/min*	94.1±8.1	97.3±8.1	96.6±8.2	97.9 ±8.3	97.7±8.6	p=0.73
Daily protein intake *g/kg/24 h*	1.05±0.23	0.98±0.25	0.97±0.28	1.0±0.18	0.98±0.29	p=0.65
Sodium urine excretion *mmol/24 h*	169±21	170±17	194±22	194±22	173±20	p=0.25
Serum potassium *mmol/l*	4.47±0.1	4.28±0.08	4.56±0.13	4.45±0.1	4.43±0.11	p=0.58

Data are expressed as mean ± SEM. BP - blood pressure; ClCr CG – Creatinine clearance (Cockroft-Gault formula)

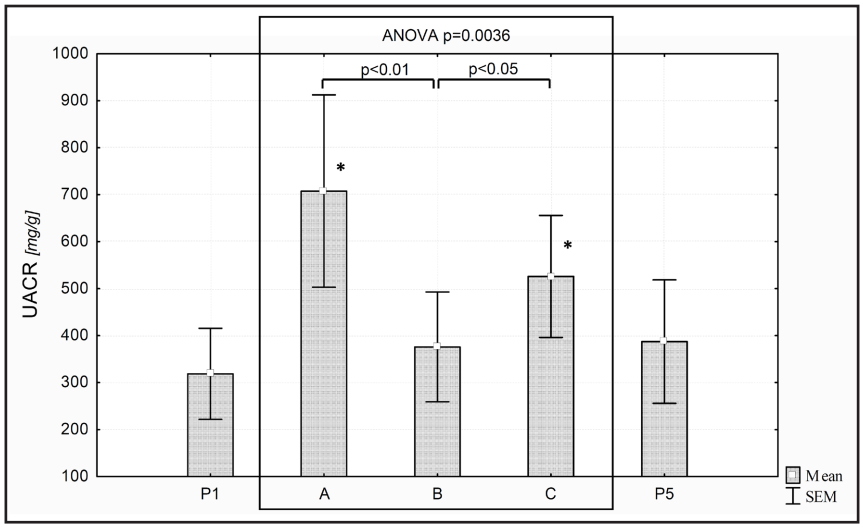

Fig. 2. Urinary albumin-to-creatinine (UACR) ratio during the study. P_1 and P_5 – telmisartan 80 mg + perindopril 10 mg; A - telmisartan 80 mg + eplerenone 50 mg, B - telmisartan 80 mg + aliskiren 300 mg, C - telmisartan 160 mg; *p<0.05 vs P_1(t-Student) and P_5 (t-Student).

Albuminuria

There was significant difference in UACR between studied therapies (ANOVA; p<0.01). The combination therapy with telmisartan plus aliskiren decreased UACR [376 mg/g (95% CI (286-686)] more effectively than the combination therapy with telmisartan plus eplerenone [707 mg/g (95% CI 502-1204)]; (post-hoc p<0.01) and monotherapy with telmisartan 160 mg [(525 (95% CI 318-763)]; (post-hoc p<0.05) (Figure 2).

In 10 of 18 (55.5%) patients, the lowest UACR was achieved with telmisartan plus aliskiren treatment, in further 5 subjects (27.8%) with telmisartan 160 mg therapy and in further 3 patients (17.2%) with the combination telmisartan plus eplerenone.

In secondary analyses, the background therapy with telmisartan and perindopril was found to decrease UACR [318 mg/g (95% CI; 237-539)] more effectively than the combination therapy with telmisartan plus eplerenone (t-Student; p<0.05) and monotherapy with telmisartan 160 mg (t-Student; p<0.05). There was no difference between the background therapy with telmisartan and perindopril and the combination therapy with telmisartan and aliskiren.

Renal function, sodium and protein intake

Renal function assessed by means of estimated creatinine clearance remained stable during the study. There were no differences in sodium and protein intake between treatment periods (Table 2).

Adverse effect

All therapies were well tolerated by patients. Adverse effects were not reported.

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Discussion

Several large randomized, controlled trials evidenced the renoprotective potential of the ACEIs and ARBs in nephropathies of almost any etiology [10]. In non-diabetic renal diseases, ACEIs are currently the best documented treatment to delay the progression of nephropathy. The strongest evidence was provided by the results of REIN study, in which, ramipril treatment resulted in a slower decline in GFR compared with placebo [14, 15]. The interim analysis of AASK trial demonstrated that the renoprotective effect of ACEI is superior to that of conventional antihypertensive regimens including β-blockers and calcium channel blockers [16]. Although several studies including the authors` findings have shown that treatment with ARBs significantly reduces proteinuria in the extent comparable to ACEIs [6, 17], an direct evidence for benefit from ARBs in nondiabetic renal disease has not been provided to date.

Despite great progress in the nephroprotecitve therapy of chronic nephropathies and a decrease in their progression rate by accurate RAAS blockade, it has not proved possible to inhibit their progress completely. The reasons may reflect the fact that monotherapy does not provide complete and persistent blockade of the RAAS system [18]. It has been also shown that constant treatment with an ACEIs or an ARBs eventually leads to the return of angiotensin II and aldosterone to their pretreatment level after some time of such therapy (escape phenomenon) [9].

Different methods of enhanced RAAS blockade involving the concomitant usage of two agents inhibiting RASS from different groups or increasing dosage of ACEI or ARB above their therapeutic range lead to more complete RAAS blockade and stronger antiproteinuric effects [10]. Several clinical studies have investigated dual RAAS blockade with ACEI and ARB in non-diabetic or mixed renal patients and documented a greater antiproteinuric effect of combined therapy with ACEIs and ARBs than with monotherapy with these agents [19-22]. The combination of a specific mineralocorticoid receptor antagonists, spironolactone or eplerenone and ACEI was more effective in reducing albuminuria/proteinuria in patients with nondiabetic CKD than ACEI alone [23, 24]. Previously, we showed that administration of mineralocorticoid receptor antagonist, spironolactone in addition to double RAAS blockade with an ACEI and ARB may reduce further residual proteinuria [25]. Similar properties seems to exert direct renin inhibitors as a part of combination strategies. Aliskiren as add-on treatment to standard therapy including the optimal dose of the ARB, losartan, in the AVOID study, reduced albuminuria and slowed development of renal dysfunction more than placebo across different levels of eGFR in patients with type 2 diabetes, hypertension, and nephropathy [26]. Adequate uptitration of the dose of ARBs to values well above those usually recommended to lower blood pressure is hypothesized to decrease proteinuria further and slow the progression glomerular filtration loss [27, 28]. It is not clear, however, which strategy is the best for the renal outcome.

The study involved nondiabetic proteinuric patients with hypertension and quite normal renal function. The study demonstrated that the combination with telmisartan and aliskiren produces a more profound decrease in albuminuria than monotherapy with telmisartan in high doses and the combination with telmisartan and eplerenon. Our study had enough power to detect significant difference in antiproteinuric effect between three studied therapies and it is unlikely that other factors might influence on the outcome. The three treatment periods did not differ with respect to blood pressure, patients' sodium and protein intake as well as renal function. In secondary analyses the antiproteinuric activity of the combination of telmisartan plus aliskiren was shown to be quite as effective as the combination telmisartan plus ACEI, perindopril. The later finding, however, should be confirmed in randomized studies.

The question arises as to whether the study results provides the answer for the question which the RAAS blockade is the best for long term renal outcome. The combination with telmisartan plus aliskiren seems to the best as long as surrogate marker of kidney injuries i.e. albuminuria is taken into account. Whether the stronger expressed reduction of albuminuria

translates into meaningful outcomes for CKD is unknown. On should also take into account the fact that the long-term effects of high doses of ACEI or ARB or addition of eplerenon, especially for fibrotic process, may be seen after longer period than observed in the study [29-31]. Until we know the answer to these questions, only those with very high level of proteinuria and normal renal function may be offered with the combination of telmisartan and aliskiren and need to be carefully monitored for hyperkalemia and worsening of kidney function. One should also remember that quite recently ALTITUDE study performed in diabetics was terminated early for lack of efficacy and risk of renal impairment, hyperkalemia and nonfatal stroke in patients taking aliskiren plus ACEI or ARB [32]. In response to these findings FDA recommended not to use such drug combination in patients with diabetes or renal insufficiency until results from other aliskiren trials will become available. Also, greater reduction in albuminuria not always translates into better long-term kidney outcome as was shown in ONTARGET study showing higher risk of acute kidney injuries and hyperkaliemia in patients treated with double RAAS blockade using ACEI and ARB [33].

The studied treatments were well tolerated. There were no differences in serum potassium concentration across studied therapies. One should take into account, however, that only patients with normal kidney function receiving low-potassium diet were included to the study. Despite these results one should be aware that pharmacological RAAS blockade may result in hyperkaliemia incidence, especially in case of enhanced RAAS blockade e.g. concomitant usage of two or three drugs [34, 35].

Conclusion

The study demonstrated that the combination therapy with ARB and renin inhibitor was more effective in albuminuria lowering than the concomitant usage of ARB and mineralocorticoid receptor antagonist as well as than ARB in doses two-fold higher than usually used in treatment of hypertension in patients with non-diabetic CKD and that this higher antiproteinuric efficacy was independent on changes in blood pressure.

Conflict of Interests

The authors have no conflicts of interest to declare.

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