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Original Research Article

Aliskiren attenuates oxidative stress and improves tubular status in non-diabetic patients with chronic kidney disease-Placebo controlled, randomized, cross-over study

Marcin Renke [a,*], Sławomir Lizakowski [b], Leszek Tylicki [b], Przemysław Rutkowski [b], Narcyz Knap [c], Zbigniew Heleniak [b], Maja Sławińska-Morawska [b], Ewa Aleksandrowicz-Wrona [d], Jacek Januszczyk [a], Małgorzata Wójcik-Stasiak [a], Sylwia Małgorzewicz [d], Michał Woźniak [c], Bolesław Rutkowski [b]

[a] Department of Occupational and Internal Medicine, Medical University of Gdansk, Gdansk, Poland
[b] Department of Nephrology, Transplantology and Internal Medicine, Medical University of Gdansk, Gdansk, Poland
[c] Department of Medical Chemistry, Medical University of Gdansk, Gdansk, Poland
[d] Department of Clinical Nutrition and Laboratory Diagnostics, Medical University of Gdansk, Gdansk, Poland

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ABSTRACT

Purpose: Pharmacological inhibition of the renin-angiotensin-aldosteron system (RAAS) may have a beneficial impact on proteinuria and chronic kidney diseases (CKD) progression. Despite recent progress by means of angiotensin-converting enzyme inhibitors (ACEI) and angiotensin II receptor blockers (ARB), there is still no optimal therapy which can stop progression of the nephropathy. Recently introduced aliskiren is the first orally bioavailable direct renin inhibitor approved for the treatment of hypertension. The purpose was to evaluate the extent of oxidative stress and tubular injury after the direct renin inhibitor, aliskiren compared with placebo and perindopril in patients with non-diabetic chronic kidney disease (NDCKD).

Material/methods: A randomized, double-blind, cross-over trial was performed in 14 patients receiving 300 mg aliskiren, 10 mg perindopril and placebo in random order. The end point was a change in the urinary excretion of N-acetyl-β-D-glucosaminidase (NAG) and α1-microglobulin (α1m) and 15-$F_{2\alpha}$-isoprostane.

Results: Aliskiren reduced excretion of 15-$F_{2\alpha}$-isoprostane ($p = 0.03$) and α1m ($p = 0.01$) as compared to placebo. There were no differences between aliskiren and perindopril in this regard. NAG urine excretion did not change after aliskiren and perindopril.

Conclusions: Aliskiren attenuates oxidative stress and may improve functional status of tubules in patients with NDCKD.

1. Introduction

Angiotensin II and aldosterone are the key players in the development of renal failure, either directly by promoting tissue fibrosis or indirectly through their action on glomerular hemodynamic and proteinuria. Therefore, pharmacological inhibition of the renin-angiotensin-aldosteron system (RAAS) may have a beneficial impact on proteinuria and chronic kidney diseases (CKD) progression [1–3].

Various studies have shown that treatment with angiotensin-converting enzyme inhibitors (ACEI) and angiotensin II receptor blockers (ARB) reduce both proteinuria and the rate of decline in the glomerular filtration rate in non-diabetic chronic kidney diseases (NDCKD) [4–6]. Despite recent progress, however, there is still no optimal therapy which can stop progression of these nephropathies. One possible reason of that is suboptimal suppression of RAAS activity via ACEI and ARB, because a compensatory increase in renin concentration and again increase angiotensin I and angiotensin II levels. Angiotensin II can also be formed using pathways that do not involve angiotensin converting

* Corresponding author at: Department of Occupational and Internal Medicine, Medical University of Gdansk, Powstania Styczniowego 9b, 81-519 Gdynia, Poland. Tel.: +48 58 699 8591; fax: +48 58 699 8402.
E-mail address: mrenke@gumed.edu.pl (M. Renke).

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enzyme [1]. Therefore, it is necessary to search for alternative therapeutic strategies blocking RAAS which can further improve renal outcome [7,8].

Recently, direct renin inhibitors, a new class of drugs that selectively inhibits angiotensin II formation at the first step of the RAAS cascade has been introduced to clinical practice. Aliskiren is the first orally bioavailable direct renin inhibitor approved for the treatment of hypertension. Blood pressure (BP)-lowering effect of aliskiren is associated with a decreased generation of angiotensin I, as it blocks its generation from angiotensinogen, by inhibiting the active enzymatic site of renin [9]. Once-daily oral treatment with aliskiren lowers BP effectively in hypertensive patients, with a safety and tolerability profile comparable to placebo [10,11]. In some recent trials, aliskiren has also shown renoprotective potential in patients with type 2 diabetes and albuminuria [12,13]. On the other hand 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 [14]. 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.

Quite recently, the authors demonstrated that aliskiren decreased proteinuria and profibrotic cytokines in NDCKD [15,16]. Since a progressive impairment of kidney function was found to correlate better with the extent of tubulointerstitial damage than with the degree of glomerular involvement, this aspect of renoprotective strategy is of particular relevance. Simultaneously oxidative stress was shown to contribute progressive kidney injury of different reasons. Consequently, as a subanalysis of the original study, in the present research we evaluated the effects of aliskiren on oxidative stress and markers of tubule injury in the same population.

2. Material and methods

2.1. Individuals

Patients were selected from the cohort that attended our renal outpatients department. The inclusion criteria were established as follows: age 18–65 years, chronic non-diabetic proteinuric nephropathy, normal or slightly impaired stable renal function expressed as estimated creatinine clearance above 30 ml/min, stable proteinuria above 500 mg/24 h, BP above 125/75 mmHg and below 150/95 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, subjects with an episode of malignant hypertension or stroke in the history and diabetics were excluded.

2.2. General protocol

The study was a randomized, double-blind, controlled, cross-over trial in which the effects of therapy with aliskiren (A), perindopril (P) and placebo (PLACEBO) were compared. It consisted of a 6-week run-in period, 12 weeks of active treatment with aliskiren (Rasilez, Novartis) or perindopril (Prestarium, Servier), 12 weeks of active treatment with the alternative medication separated by 12-week placebo administration (Fig. 1). At the beginning, subjects who met the inclusion criteria entered the 6 weeks run-in period during which any previously used hypotensive agents were stopped. At the end of the run-in period, patients were randomly allocated to one of the two treatment sequences: A/PLACEBO/P (sequence 1) or P/PLACEBO/A (sequence 2). Allocation was performed by a person that was independent of the research team according to a computer generated randomization list. For the first 6 week of the treatment period, aliskiren was used at a dose of 150 mg and perindopril was administered at a dose of 5 mg. The dosages were doubled for the next 6 weeks to the maximal recommended hypotensive dosages of both study medications i.e. aliskiren 300 mg and perindopril 10 mg. Drug compliance was assessed by tablet counts. Patients were instructed to take the study medication once daily in the morning. At the end of the run-in period, administration of placebo, perindopril 10 mg and aliskiren 300 mg 24-h ambulatory BP, serum creatinine, the urinary excretion of 15-$F_{2\alpha}$-isoprostane, N-acetyl-β-D-glucosaminidase (NAG) and α1-microglobulin (α1m) were determined and estimated creatinine clearance and glomerular filtration rate (GFR) were calculated. Patients were recommended not to change their usual daily protein and sodium intake during the study period. 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 and received a positive opinion (identifier: NCT01219413).

2.3. Procedures and laboratory analyses

A commercial ELISA kit (Cayman Chemical Co) was used to measure the urinary excretion of 15-$F_{2\alpha}$-isoprostane in the first morning urine sample. 15-F_2-Isoprostanes are considered the best available biomarkers of oxidative stress status and lipid peroxidation in vivo. They are frequently measured in urine, because it is noninvasive, isoprostanes are not formed artifactually by auto-oxidation in urine, and they are very stable in urine. There is no significant daily variability of urinary isoprostanes concentrations in healthy subjects, day-to-day variability in healthy and disease states is also relatively limited [17,18].

Creatinine clearance was calculated according to Cockcroft-Gault (CG) formula [19] and GFR by CKD-EPI formula [20]. NAG and α1m were analyzed in the second morning spot urine sample. NAG was determined by the spectrophotometric method according to Maruhn [21]. Incubation medium contained in a final volume of 0.4 ml, 5 nmol/l p-nitrophenyl-2-acetamido-β-D-glucopyranoside as a substrate in 50 mmol/l citrate buffer (pH 4.14). The reaction was started by the addition of 0.2 ml of undialysed urine, carried out for 15 min at 37 °C, and then terminated with 1 ml of glycine buffer, pH 10.5. Absorbance was measured at 405 nm against a sample terminated at time zero. The calculation of the NAG level was made from the molar extinction coefficient of the product of the reaction, p-nitrophenol, and equal to 18.5 $cm^2/\mu mol$. From the preliminary experiments it was clear that the dialysis of urine did not affect NAG level in urine. Immunoturbidimetric test (Tina-quant α1-microglobulin, Roche, Basel, Switzerland) was used for quantification of α1m in urine. The detection limit of the method was 2 mg/l. Urinary NAG, and α1m, were reported per mg or g of urine creatinine to correct for the variation in urine concentration. Creatinine level was measured in fresh blood samples drawn after fasting overnight for at least 12 h using standard laboratory techniques.

2.4. Statistical analysis

The primary end point of this study was a change in the urinary excretion of 15-$F_{2\alpha}$-isoprostane in measurements available for each patient. A sample size of 12 patients adequately allowed a power of 80% to detect a difference in variables equal to within patient standard deviation that is a standardized effect size of 1.0 at a significance level of 0.05 (two-tailed). Secondary end points included urine NAG and α1m excretions. Normality and homogeneity of the variances were verified by means of the Shapiro–Wilk test and Levene test, respectively. Because of their skewed