

Aliskiren reduces albuminuria after kidney transplantation*

Leszek Tylicki¹✉, Alicja M. Debska-Slizien¹, Sławomir Lizakowski¹, Milena Przybylska¹, Zbigniew Heleniak¹, Marcin Renke², Andrzej L. Chamienia³, Bogdan Biedunkiewicz¹, Przemysław Rutkowski³, Sylwia Małgorzewicz⁴ and Bolesław Rutkowski¹

¹Department of Nephrology, Transplantology and Internal Medicine; Medical University of Gdansk, Gdańsk, Poland; ²Department of Occupational and Internal Medicine, Medical University of Gdansk, Gdańsk, Poland; ³Department of General Nursery, Medical University of Gdansk, Gdańsk, Poland; ⁴Department of Clinical Nutrition, Medical University of Gdansk, Gdańsk, Poland

Background: The renoprotective effects of the direct renin inhibitor, aliskiren, in renal transplant recipients have been supposed, but not finally proven. We performed an exploratory double-blind, losartan controlled, cross-over study to evaluate the influence of aliskiren, direct renin inhibitor, on albuminuria and other surrogate markers of kidney injury in patients after renal transplantation. The safety of this therapy was also evaluated. **Method:** 16 of 18 patients (12 M, 4 F), 48.3±9.0 years, 57.7±9.1 months after kidney transplantation, with hypertension and stable serum creatinine 1.4±0.08 mg/dl without proteinuria, completed the protocol. Each patient underwent two 8-week treatment periods (one with 150 mg of aliskiren, and one with 50 mg of losartan) in random order, allowing an 8-week placebo wash-out between them. **Results:** There were no differences in albuminuria, transforming growth factor β -1 and 15-F2t-isoprostanes urine excretion between aliskiren and losartan. Creatinine serum level, eGFR, 24 h systolic and 24 h diastolic blood pressure were stable through the study. There were no differences in haemoglobin and potassium serum concentration between studied drugs. **Conclusion:** Aliskiren decreases albuminuria in renal transplant recipients with clinically minimal side effects. The effect does not differ from that of losartan.

Key words: albuminuria, aliskiren, kidney transplantation, renoprotection

Received: 29 March, 2016; **revised:** 21 November, 2016; **accepted:** 08 January, 2017; **available on-line:** 28 April, 2017

✉ e-mail: leszek.tylicki@gumed.edu.pl

*Information on the same subject was presented at the 52nd ERA-EDTA Congress (London, 28–31 May, 2015)

Abbreviations: ACEI, angiotensin converting enzyme inhibitors; ARA, angiotensin II receptor antagonists; CAN, chronic allograft nephropathy; IF/TA, Interstitial fibrosis and tubular atrophy; RAAS, renin-angiotensin-aldosterone system

INTRODUCTION

Interstitial fibrosis and tubular atrophy (IF/TA) in renal allografts, previously termed chronic allograft nephropathy (CAN), is a major cause of long-term renal graft dysfunction and loss (Solez *et al.*, 1998). Although the functional and morphological findings in this pathology are well characterised, no effective therapy has been developed so far to prevent, limit, or reverse graft lesions (Paul, 1999). Agents inhibiting renin-angiotensin-aldosterone system (RAAS) prevent and retard the progression of both diabetic and non-diabetic native kidney disease (Tylicki *et al.*, 2005). Although the fundamental role of angiotensin II in the processes associated with

the development of IF/TA, such as hypertension, arteriosclerosis, atherosclerosis and calcineurin inhibitor agents (cyclosporine, tacrolimus) toxicity, has been proven, the nephroprotective benefits of drugs inhibiting RAAS in renal transplant recipients remain unclear (Tylicki *et al.*, 2003). Indeed, analyses of large databases and meta-analyses of small prospective clinical studies have provided conflicting results showing improved outcomes, detrimental effects or null effects of post-transplantation angiotensin converting enzyme inhibitors (ACEI) or angiotensin II receptor antagonists (ARA) therapy compared with other antihypertensive agents (Heinze *et al.*, 2006; Opelz *et al.*, 2006). Undoubtedly, ACEI and ARA are effective in reducing albuminuria or proteinuria in a similar manner as in the population with chronic native kidney disease (Tylicki *et al.*, 2006). Consequently, according to the recent KDIGO recommendations, drugs that block the RAAS should be considered for use in all kidney transplant subjects with albuminuria after stable graft function is obtained (Roberts, 2014). Given the fact that microalbuminuria and macroalbuminuria are considered to be powerful independent predictors of end stage renal disease and death in renal transplant recipients, their use may offer both kidney and cardiovascular disease protection.

Direct renin inhibitor (DRI), aliskiren, is a representative of the newest class of agents inhibiting RAAS axis with a (similar as placebo) adverse reaction profile in monotherapy (Liu *et al.*, 2013; Lizakowski *et al.*, 2013b). Data on its renal effects in transplant patients is very limited. To shed more light on this issue we performed a randomized, controlled study to check influence of aliskiren on albuminuria and some other surrogate markers of kidney injury in this population. Losartan, ARA which was earlier evidenced to decrease albuminuria in renal transplant recipients, served as the control.

MATERIAL AND METHODS

Patients. Renal transplant recipients were recruited from a cohort that successively attended our outpatient department. The inclusion criteria were as follows: post-transplantation period above 6 months, calcineurin inhibitor (cyclosporine or tacrolimus) based immunosuppression, stable cyclosporine or tacrolimus trough level in the last three months (no variations above 25%), stable renal function defined as eGFR > 30 ml/min (no variations above 5 ml/min/1.73 m² in the last 3 months), arterial hypertension treated with one or two antihypertensive agents or blood pressure (BP) > 130/80 mmHg in patients not treated yet, albuminuria > 30 mg/g creati-

nine. In none of the patients was a graft biopsy available. Patients with graft artery stenosis, 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.

General protocol. The study was an exploratory randomised, double-blind, controlled cross-over trial in which the renal effects of therapy with aliskiren (A) and losartan (L) were compared. It consisted of an 8-week run-in period, 8 weeks of active treatment with aliskiren or losartan (period 1), 8 weeks of active treatment with the alternative medication (period 2), and an 8-week placebo-washout (W) period between them (Fig. 1). At the beginning, subjects who met the inclusion criteria entered the 8-week run-in screening period. All the hypotensive group of drugs were allowed with the exception of ACEI, ARA, DRI and mineralocorticoid receptor antagonists. The target BP was an office trough BP of 140/90 mmHg or less. At the end of the run-in period patients were randomly allocated to one of the two treatment sequences: L/W/A (sequence 1) or A/W/L (sequence 2). Allocation was performed independently of the research team member, according to a computer-generated randomization list. The study medications were introduced as single hypotensive drugs, or added to the current hypotensive agents, the dosage of which, once adjusted in the run-in period, was left unchanged throughout the study. Losartan was used at a dose of 50 mg, and aliskiren was administered at a dose of 150 mg. Drug compliance was assessed by tablet counts. Placebo preparation and both drugs blinding were performed at the Department of Pharmaceutical Technology, Medical University of Gdansk. Patients were recommended not to change their usual daily protein and sodium intake during the study period (there were no differences in sodium urine excretion between aliskiren and losartan). Dosage of cyclosporine or tacrolimus was not allowed to be modified either. Before and after each of the treatment periods, office trough BP, 24-h ambulatory BP, albuminuria, serum creatinine and potassium, haemoglobin, urine excretion of N-acetyl- β -D-glucosaminidase (NAG), transforming growth factor β -1 (TGF- β -1) and 15-F2t-isoprostanes (isoprostanes) were determined. eGFR was calculated. Patients were also asked to fill in a questionnaire for assessment of patient-reported side effects. The study was approved by the local ethical committee and the investigated patients all gave their informed consent.

Methods. The office trough BP was measured by mercury sphygmomanometer in a sitting position after 10 minutes of rest, and expressed as a mean value of two consecutive measurements taken 2 minutes apart. 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 office BP measurements were analysed for systolic (SBP) and diastolic (DBP) values; those of ambulatory BP measurements for 24 h SBP, 24 h DBP. Albumin excretion was measured in the first morning spot urine sample, as commonly recommended (Bailie *et al.*, 2005). The first morning urine specimen is preferred, because it correlates best with 24-hour protein excretion, and is required to avoid postural albuminuria. The authors calculated the ratio of albumin to creatinine (UACR) to correct for variations in urinary concentration due to hydration. The concentration of albumin was measured by enzyme-linked immunosorbent

assay (ELISA) using an *Albumin* (Immunodiagnostic AG, Bensheim, Germany) kit in accordance with the manufacturer's recommendations. The intra-assay and inter-assay coefficients of variations for this assay were 5.0% and 8.0%, respectively. Haemoglobin, serum creatinine and potassium levels were measured by the standard laboratory techniques. eGFR was calculated according to the CKD-EPI formula. Adverse effects were recorded at each visit in response to questionnaires.

The first morning urine sample was collected for the determination of NAG, TGF- β -1 and isoprostanes. The samples were stored at -75°C until assayed. TGF- β -1 urine concentration was analyzed using a commercially available solid-phase enzyme-linked immunosorbent assay (Quntykine; R&D Systems, Minneapolis, MN). The minimum level of TGF- β -1 detectable with the test was 7 pg/ml. NAG was determined by the spectrophotometric method according to Maruhn (8). Incubation medium contained, in a final volume of 0.4 ml, 5 mmol/l *p*-nitrophenyl-2-acetamido-2deoxy- 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 done from the molar extinction coefficient of the product of the reaction, *p*-nitrophenol, equal to $18.5\text{ cm}^2/\mu\text{mol}$. From the preliminary experiments, it was clear that the dialysis of urine did not affect NAG level in urine. A commercial ELISA kit (Cayman Chemical Co.) was then used to measure the urinary excretion of 15-F2t-isoprostanes. Urinary NAG, TGF- β -1 and isoprostanes were reported per mg of urine creatinine to correct the variation in urine concentration.

Statistics. The primary end point of the study was a difference in UACR in the measurements available for each patient. The secondary end point was the presence of differences in NAG, TGF- β -1 and isoprostanes urine excretion in the measurements available for each patient. In addition, a safety analysis of the therapy with aliskiren, involving its influence on serum creatinine, potassium, and haemoglobin level, was performed.

The normality and homogeneity of the variances were verified by the Shapiro-Wilk test and the Levene test, respectively. Because of their skewed distribution, values of UACR, NAG, TGF- β -1 were logarithmically transformed before statistical analysis, and expressed as geometric means and 95% confidence intervals. Other results were expressed as means \pm S.D. In the per-protocol design, the variable differences between aliskiren and losartan were assessed by *t*-test. A $P < 0.05$ (two-tailed) was considered statistically significant. Data was evaluated using STATISTICA (version 6.0 Stat Soft Inc.) software package.

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 two different therapy sequences with the three treatment periods (Fig. 1). Equal number of patients ($n=9$) per sequence was randomised. Since 2 patients were prematurely withdrawn, this balance was fully respected at the study end. To prevent or limit the risk of "carryover" effect, we planned each treatment period for 8 weeks, and placebo treatment between them. Previous studies demonstrated that the effects of RAAS blocking agents on albuminuria and glomerular permselectivity are fully reversible within

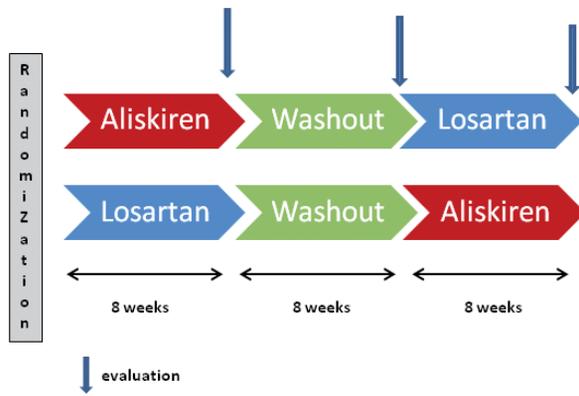


Figure 1. Study design.

4 weeks (Gansevoort *et al.*, 1994). Thus, prolonging each treatment period to 8 weeks and introducing washout-placebo therapy between them, allowed us to completely rule out any residual effect of previous treatment at the end of the eighth week of second period, when UACR was measured.

RESULTS

Of the 18 patients who entered the study, 16 (88.8%) completed the protocol. Their characteristics are presented in Table 1A and 1B. Two patients were withdrawn from the study, one subject from each of the 2 treatment sequences. One person was excluded due to important deviation from the study protocol. Another one resigned from participation in the study for personal reasons. Before data analysis, the “period effect” and “carryover effect” were tested and found to be not significant.

There were no differences in UACR between aliskiren and losartan treatment (Table 2). Both decreased albuminuria to the same extent. Individual results in UACR are presented in Fig. 2. Losartan decreased NAG excretion more effectively than aliskiren ($p=0.019$) (Table 2). There were no differences in TGF- β -1 and isoprostanes urine excretion between aliskiren and losartan (Table 2). There were no differences in 24-h SBP (124.9 ± 11.2 vs. 123.7 ± 7.3 mmHg; $P=0.75$) and 24-h DBP (82.4 ± 7.3 vs.

Table 1A. Characteristics of patients.

N	16
Age (min-max) years	48.3 ± 8.8 (35-64)
Gender female/male n	4/12
BMI kg/m ²	27.5 ± 4.3
Serum creatinine mg/dl	1.39 ± 0.33
eGFR CKD EPI ml/min	59.3 ± 18.1
UACR (min-max) mg/g creatinine	149.9 ± 193 (13-791)
Arterial hypertension (Y/N) n	12/4
Mean office systolic blood pressure mm Hg	130.3 ± 8.5
Mean office diastolic blood pressure mm Hg	82.0 ± 7.0
Number of hypotensive drugs n (without RAAs blockers)	1.75 ± 1.53

UACR, urine albumin/creatinine ratio; RAAS, renin angiotensin aldosterone system. Data are expressed as mean ± S.D. Note: To convert serum creatinine in mg/dL to μ mol/L, multiply by 88.4; eGFR CKD EPI in mL/min/1.73 m² to mL/s/1.73 m², multiply by 0.01667

Table 1B. Characteristics of patients.

Reasons of KTx:	
Chronic glomerulonephritis	7
Chronic pyelonephritis	1
ADPKD	4
Unknown	4
Methods of treatment before KTx (HD/PD/preemptive)	10/2/4
Post-transplantation period (months)	57.7 ± 36.0
Time of RRT before KTx	16.3 ± 15.4
Immunosuppressive therapy (n)	
Cyclosporin A	4
Tacrolimus	12

Data are expressed as mean ± S.D. RRT, renal replacement therapy; KTx, kidney transplantation; PD, peritoneal dialysis; HD, haemodialysis

81.3 ± 5.6 ; $P=0.64$) between aliskiren and losartan treatment.

Renal function assessed by means of serum creatinine and eGFR remained stable during the study periods. There was no difference in potassium concentration be-

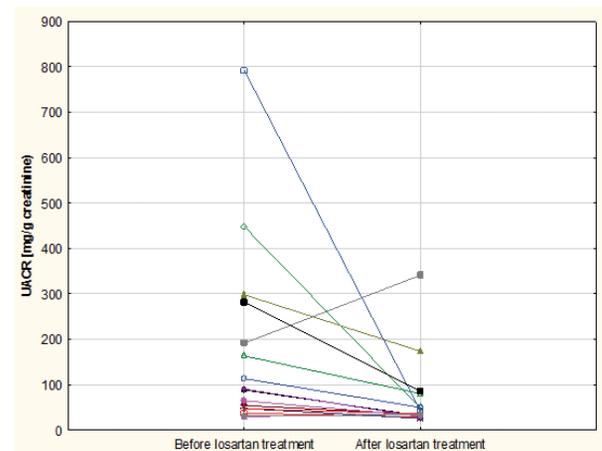
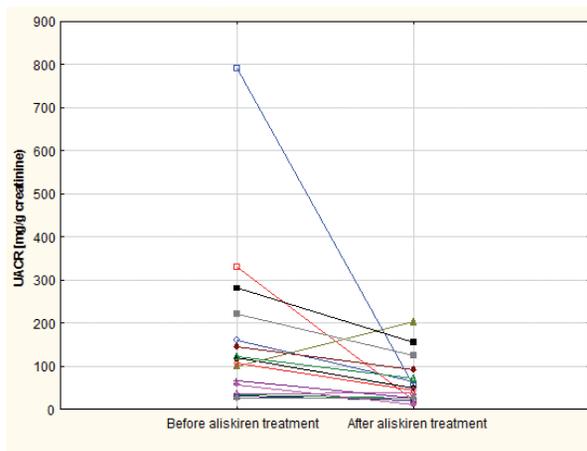


Figure 2. Pre- and post-treatment individual UACR values.

Table 2. Influence of therapy on the renal function, haemoglobin level, potassium, UACR and urine NAG, isoprostanes and TGF- β -1 level.

	Aliskiren post-treatment and Δ	Losartan post-treatment and Δ	P
Creatinine mg/dl	1.53 \pm 0.1 [0.08 \pm 0.19]	1.47 \pm 0.1 [0.03 \pm 0.16]	NS (P=0.49)
eGFR CKD-EPI ml/min	54.6 \pm 4.7 [-2.8 \pm 7.4]	56.8 \pm 4.9 [-1.4 \pm 7.8]	NS (P=0.6)
Haemoglobin g/dl	13.4 \pm 0.4 [-0.32 \pm 0.83]	13.3 \pm 0.4 [-0.45 \pm 0.64]	NS (P=0.61)
Potassium mmol/l	4.25 \pm 0.49 [0.19 \pm 0.48]	4.16 \pm 0.52 [0.18 \pm 0.36]	NS (P=0.9)
UACR mg/g	63.3 (31.2–95.4) [-106.4 \pm 195.0]	64.8 (20.2–109.4) [-106.2 \pm 212.6]	NS (P=0.99)
NAG IU/mg creatinine	2.76 (1.82–3.69) [0.28 \pm 1.9]	2.08 (1.67–2.49) [-1.6 \pm 2.34]	P=0.019
Isoprostanes ng/mg creatinine	5.06 \pm 0.49 [0.63 \pm 2.28]	5.20 \pm 0.37 [-0.21 \pm 1.05]	NS (P=0.32)
TGF- β -1 pg/mg creatinine	5.48 (5.79–8.18) [-0.44 \pm 6.81]	5.09 (1.83–8.34) [-3.40 \pm 13.09]	NS (P=0.45)

Data is provided as the post-treatment results and Δ (differences between post-treatment and pre-treatment values). Data is expressed as mean \pm S.D. or geometric mean (95% confidence interval). NAG, N-acetyl- β -D-glucosaminidase; TGF- β -1, transforming growth factor β -1; Isoprostanes, 15-F2t-isoprostanes

tween the treatments (Table 2). In none of the patients was a potassium level above 5.5 mmol/L observed. Haemoglobin level decreased numerically after aliskiren and losartan. This, however, was not associated with anaemia symptoms, and none of the patients needed treatment for anaemia. There were no differences between aliskiren and losartan in this regard (Table 2). Side-effects reported *de novo* during treatments are showed in Table 3. There were no significant differences in their frequency between the three treatments.

DISCUSSION

Some years ago, DRI, a new class of drugs that selectively inhibits angiotensin II formation at the first step of the RAAS cascade, were introduced to clinical practice. Aliskiren was the first orally bioavailable DRI approved for the treatment of hypertension. Once-daily oral treatment with aliskiren lowers BP effectively, with a safety and tolerability profile comparable with that of placebo (Liu *et al.*, 2013; Lizakowski *et al.*, 2013b). In our previous papers, we demonstrated that aliskiren significantly reduced proteinuria in nondiabetic chronic kidney disease, and this antiproteinuric effect was similar to that of ACEI in equivalent hypotensive dosages (Lizakowski

et al., 2013a). In a double-blind, randomised, crossover study Persson *et al.* demonstrated the same in diabetics (Persson *et al.*, 2009). Szeto *et al.* found that aliskiren has anti-proteinuric effect in patients with IgA nephropathy and persistent proteinuria despite ACEI or ARA (Szeto *et al.*, 2013). Enthusiasm for the use of aliskiren declined after the ALTITUDE study that prompted the FDA to issue the recommendation not to use aliskiren, combined with other RAAS inhibiting agents, in patients with diabetes or moderate to severe renal impairment. The reason of such statement was the risk of hypotension, acute renal impairment, and hyperkalaemia during such combination therapy (Parving *et al.*, 2012). Nevertheless, much evidence of the effectiveness and safety of aliskiren in monotherapy for the treatment of hypertension and proteinuria in different populations leads to the conclusion that aliskiren may be considered as an equivalent alternative to ACEI or ARA in certain conditions.

There are no controlled studies analysing the effects of aliskiren in renal transplant recipients so far. In experimental models of renal transplantation aliskiren had no effect on renal function (i.e. proteinuria, creatinine clearance), or on renal morphological changes (i.e. collagen deposition in glomerulosclerosis, myofibroblast accumulation and macrophage infiltration) determined 24 weeks after transplantation (Rusai *et al.*, 2011). On the contrary, histopathological and ultrastructural studies showed that aliskiren may attenuate tacrolimus-induced renal damage in rats, implying that aliskiren may counteract nephrotoxicity associated with calcineurin inhibitors applied after renal transplantation (Al-Harbi *et al.*, 2014).

In our previous report, we showed that angiotensin receptor antagonist, losartan, decreases albuminuria in renal transplant recipients (Tylicki *et al.*, 2006). In this exploratory short-term study, we demonstrated, for the first time to our knowledge, no differences between losartan and aliskiren in this regard. The measurement of urinary albumin excretion provides a sensitive marker of glomerular injury with impaired permselectivity (Komena *et al.*, 2014). In renal transplant recipients, proteinuria is regarded as an excellent marker of poor long-term allograft prognosis (Fernandez-Fresnedo *et al.*, 2004; Ibra-

Table 3. Side-effects detected *de novo* after treatment with studied drugs

Symptoms	Aliskiren	Placebo	Losartan
Rash, itch	1	0	1
Gastrointestinal disorders	1	1	2
Cough	0	1	2
Sexual dysfunction	1	2	1
Worsening of mood	0	2	1
Sleep disorders, nightmares	1	1	1
Fatigue	3	4	1
Swelling	0	2	0

him *et al.*, 2014). Albuminuria is also considered as an important risk factor for cardiovascular complications (Komenda *et al.*, 2014).

Considering the fact that the extent of tubulointerstitial damage is a fundamental predictor of kidney outcome, tubular cells have become a renal site of particular interest. To evaluate tubulointerstitial effects of our interventions, urine excretion of NAG, enzyme of the hydrolase class which is abundant in the kidney, predominantly in the lysosomes of the proximal tubular cells, was analysed as a secondary end point. It is physiologically excreted in low amounts in urine as a consequence of the normal exocytosis process (Price, 1982). The increased excretion of NAG is thought to be a specific marker of tubular injury in many renal pathologies (Sherman *et al.*, 1983). Considering non-hemodynamic mechanisms of possible benefits of aliskiren i.e. antagonizing the proinflammatory and profibrotic effects of angiotensin II, we also evaluated the influence of aliskiren on urine excretion of TGF- β 1 and isoprostanes. TGF- β 1 is a key profibrotic cytokine involved in the pathogenesis of IF/TA in renal allografts. A significant correlation between intragraft TGF- β 1 mRNA and renal allograft interstitial fibrosis was reported previously. Furthermore, TGF- β 1 urine excretion is increased in overt IF/TA (Djamali *et al.*, 2009; Sharma *et al.*, 1996).

The urinary excretion of 15-F2t-isoprostanes is a reliable and sensitive marker of intrarenal oxidative stress, the process known to be involved in the inflammation and kidney fibrosis (Renke *et al.*, 2013). Losartan decreased NAG urine excretion to a greater extent than aliskiren in studied patients. One may assume that the greater sodium intake observed during aliskiren therapy might affect the results. No differences between aliskiren and losartan were found in TGF- β 1 and 15-F2t-isoprostanes excretion. Small sample size and relatively short term of observation do not allow drawing final conclusions from these observations.

The question, whether potentially beneficial effects of aliskiren on albuminuria translate into the long-term renal graft outcome, remains open. Evidence may be provided only by histological examinations, or controlled studies focused on graft function. Until now, there has been no such report for aliskiren. Moreover, the nephroprotective benefits of either ACEI or ARA in renal transplant recipients remain unclear as well. Some years ago, we demonstrated that losartan decreases albuminuria and surrogates for tubular damage and graft fibrosis (Tylicki *et al.*, 2007). Since then, the research on this issue has not progressed too far. The Study on Evaluation of Candesartan Cilexetil after Renal Transplantation (SECRET) was not able to evidence the beneficial influence of ARA, candesartan, on graft failure/patients all-cause mortality/cardiovascular morbidity, but confirmed that candesartan reduced proteinuria (Philipp *et al.*, 2010). The recent study of Ibrahim *et al.* was also inconclusive. They conducted a double-blind, randomized, placebo-controlled trial, to determine whether losartan prevents the expansion of the cortical interstitial compartment, the precursor of fibrosis. Treatment with losartan did not lead to a statistically significant reduction in a composite of interstitial expansion or ESRD from IF/TA in kidney transplant recipients. On the other hand, in a secondary analysis, losartan seemed to reduce the risk of a composite of doubling of interstitial volume, or all-cause ESRD (Ibrahim *et al.*, 2014). A retrospective analysis by Heinze *et al.* found evidence supporting the benefit of RAAS blockers in kidney transplant recipients (Heinze *et al.*, 2006), whereas the Collaborative Transplant Study

report developed by Opelz and coworkers (2006) revealed no improvement of patient or graft survival in transplant recipients treated with ACEI or ARA (Opelz *et al.*, 2006). Quite recently, a large retrospective analysis showed that the rate of cardiovascular death in kidney transplantation recipients receiving ACEI/ARA, or other antihypertensive medications, is virtually identical (Opelz *et al.*, 2014).

A potential limitation of our study was the small sample size. Lack of repeated measurements of UACR at each study visit may be also recognized as a limitation. Calculation of albumin/creatinine ratio in the urine does not completely eliminate the variability of the urine albumin excretion. Treatment periods may be too short, and potentially beneficial renal effects may not yet fully develop. The effects for tubules and interstitium in the study were extrapolated only from presumptive early surrogates. It should also be taken into account, that the authors chose for their study a series of stable renal transplant recipients with excellent graft function. Given these together, the study results allow a conclusion only of pilot character.

In conclusion, aliskiren decreases albuminuria in renal transplant recipients with clinically minimal side effects. The renal effects do not differ from that of ARA, losartan. Given the strong prognostic value of albuminuria for graft outcome (Fernandez-Fresnedo *et al.*, 2004), and the fact that albuminuria is also considered as an important risk factor for cardiovascular complications (Fernandez-Fresnedo *et al.*, 2002), the present study may support the hypothesis that aliskiren improves allograft and patient outcome.

Acknowledgements

The authors are indebted to Professor Malgorzata Sznitowska from the Department of Pharmaceutical Technology for placebo preparation and drug blinding.

The study was supported by a grant from the Polish Committee for Scientific Research (KBN) via the Medical University of Gdansk (ST-4).

REFERENCES

- Al-Harbi NO, Imam F, Al-Harbi MM, Iqbal M, Nadeem A, Al-Shahrah OA, Korashy HM, Al-Hosaini KA, Ahmed M, Bahashwar S (2014) Treatment with aliskiren ameliorates tacrolimus-induced nephrotoxicity in rats. *J Renin Angiotensin Aldosterone Syst.* doi: 10.1177/1470320314530178
- Baillie GR, Uhlig K, Levey AS (2005) Clinical practice guidelines in nephrology: evaluation, classification, and stratification of chronic kidney disease. *Pharmacotherapy* 25: 491–502
- Djamali A, and Samaniego M (2009) Fibrogenesis in kidney transplantation: potential targets for prevention and therapy. *Transplantation* 88: 1149–1156. doi: 10.1097/TP.0b013e3181bcceca
- Fernandez-Fresnedo G, Escallada R, Rodrigo E, De Francisco AL, Cortoruelo JG, Sanz De Castro S, Zubimendi JA, Ruiz JC, Arias M (2002) The risk of cardiovascular disease associated with proteinuria in renal transplant patients. *Transplantation* 73: 1345–1348
- Fernandez-Fresnedo G, Plaza JJ, Sanchez-Plumed J, Sanz-Guajardo A, Palomar-Fontanet R, Arias M (2004) Proteinuria: a new marker of long-term graft and patient survival in kidney transplantation. *Nephrol Dial Transplant* 19, iii47–iii51
- Gansevoort R, De Zeeuw D, De Jong P (1994) Is the antiproteinuric effect of ACE inhibition mediated by interference in the renin-angiotensin system? *Kidney Int* 45: 861–867
- Heinze G, Mitterbauer C, Regele H, Kramar R, Winkelmayer WC, Curhan GC, Oberbauer R (2006) Angiotensin-converting enzyme inhibitor or angiotensin II type 1 receptor antagonist therapy is associated with prolonged patient and graft survival after renal transplantation. *J Am Soc Nephrol* 17: 889–899. doi: 10.1681/ASN.2005090955
- Ibrahim HN, Jackson S, Connaire J, Matas A, Ney A, Najafian B, West A, Lentsch N, Ericksen J, Bodner J, Kasiske B, Mauer M (2014).

- Angiotensin II blockade in kidney transplant recipients. *J Am Soc Nephrol* **24**: 320–327. doi: 10.1681/ASN.2012080777
- Komenda P, Rigatto C, Tangri N (2014). Estimated glomerular filtration rate and albuminuria: diagnosis, staging, and prognosis. *Curr Opin Nephrol Hypertens* **23**: 251–257. doi: 10.1097/01.mnh.0000444910.55665.e8
- Liu Y, Chen K, Kou X, Han Y, Zhou L, Zeng C (2013) Aliskiren and amlodipine in the management of essential hypertension: meta-analysis of randomized controlled trials. *PLoS One* **8**: e70111. doi: 10.1371/journal.pone.0070111
- Lizakowski S, Tylicki L, Renke M, Rutkowski P, Heleniak Z, Slawinska-Morawska M, Aleksandrowicz E, Lysiak-Szydłowska W, Rutkowski B (2013a) Effect of aliskiren on proteinuria in non-diabetic chronic kidney disease: a double-blind, crossover, randomised, controlled trial. *Int Urol Nephrol* **44**: 1763–1770
- Lizakowski S, Tylicki L, Rutkowski B (2013b) Direct renin inhibition – a promising strategy for renal protection? *Med Sci Monit* **19**: 451–457. doi: 10.12659/MSM.883949
- Opelz G, Dohler B (2014) Cardiovascular death in kidney recipients treated with renin-angiotensin system blockers. *Transplantation* **97**: 310–315. doi: 10.1097/01.TP.0000437672.78716.28
- Opelz G, Zeier M, Laux G, Morath C, Dohler B (2006) No improvement of patient or graft survival in transplant recipients treated with angiotensin-converting enzyme inhibitors or angiotensin II type 1 receptor blockers: a collaborative transplant study report. *J Am Soc Nephrol* **17**: 3257–3262. doi: 10.1681/ASN.2006050543
- Parving HH, Brenner BM, McMurray JJ, de Zeeuw D, Haffner SM, Solomon SD, Chaturvedi N, Persson F, Desai AS, Nicolaides M, Richard A, Xiang Z, Brunel P, Pfeffer MA (2012) Cardiorenal end points in a trial of aliskiren for type 2 diabetes. *N Engl J Med* **367**: 2204–2213. doi: 10.1056/NEJMoa1208799
- Paul LC (1999). Chronic allograft nephropathy: An update. *Kidney International* **56**: 783–793
- Persson F, Rossing P, Reinhard H, Juhl T, Stehouwer CD, Schalkwijk C, Danser AH, Boomsma F, Frandsen E, Parving HH (2009) Renal effects of aliskiren compared with and in combination with irbesartan in patients with type 2 diabetes, hypertension, and albuminuria. *Diabetes Care* **32**: 1873–1879. doi: 10.2337/dc09-0168
- Philipp T, Martinez F, Geiger H, Moulin B, Mourad G, Schmieder R, Lievre M, Heemann U, Legendre C (2010) Candesartan improves blood pressure control and reduces proteinuria in renal transplant recipients: results from SECRET. *Nephrol Dial Transplant* **25**: 967–976. doi: 10.1093/ndt/gfp581
- Price R (1982) Urinary enzymes, nephrotoxicity and renal disease. *Toxicol* **23**, 99–134.
- Renke M, Knap N, Tylicki L, Rutkowski P, Lizakowski S, Wozniak M, Rutkowski B (2013) Isoprostanol – important marker of the oxidative stress estimation in patients with chronic kidney disease. *Pol Merkur Lekarski* **34**: 14–17 (in Polish)
- Roberts MA (2014) Commentary on the KDIGO Clinical Practice Guideline for the management of blood pressure in chronic kidney disease. *Nephrology (Carlton)* **19**: 53–55. doi: 10.1111/nep.12168
- Rusai K, Schmaderer C, Hermans JJ, Lutz J, Heemann U, Baumann M (2011) Direct renin inhibition in a rat model of chronic allograft injury. *Transplantation* **92**: 999–1004. doi: 10.1097/TP.0b013e318230c05b
- Sharma VK, Bologa RM, Xu GP, Li B, Mouradian J, Wang J, Serur D, Rao V, Suthanthiran M (1996) Intragraft TGF-beta 1 mRNA: a correlate of interstitial fibrosis and chronic allograft nephropathy. *Kidney Int* **49**: 1297–1303
- Sherman R, Drayer D, Leyland-Jones B, Reidenberg M (1983) N-acetyl-D-glucosaminidase and beta-2-microglobulin; their urinary excretion in patients with renal parenchymal disease. *Arch Intern Med* **143**: 1183–1185
- Solez K, Vincenti F, Filo RS (1998) Histopathologic findings from 2-year protocol biopsies from a U.S. multicenter kidney transplant trial comparing tacrolimus versus cyclosporine: a report of the FK506 Kidney Transplant Study Group. *Transplantation* **66**: 1736–1740
- Szeto CC, Kwan BC, Chow KM, Leung CB, Li PK (2013) The safety and short-term efficacy of aliskiren in the treatment of immunoglobulin a nephropathy – a randomized cross-over study. *PLoS One* **8**: e62736. doi: 10.1371/journal.pone.0062736
- Tylicki L, Biedunkiewicz B, Chamienia A, Wojnarowski K, Zdrojewski Z, Aleksandrowicz E, Lysiak-Szydłowska W, Rutkowski B (2007) Renal allograft protection with angiotensin II type 1 receptor antagonists. *Am J Transplant* **7**: 243–248. doi: 10.1111/j.1600-6143.2006.01588.x
- Tylicki L, Biedunkiewicz B, Chamienia A, Wojnarowski K, Zdrojewski Z, Rutkowski B (2006) Randomized placebo-controlled study on the effects of losartan and carvedilol on albuminuria in renal transplant recipients. *Transplantation* **81**: 52–56
- Tylicki L, Habicht A, Watschinger B, Horl W (2003). Treatment of hypertension in renal transplant recipients. *Curr Opin Urol* **13**: 91–98. doi: 10.1097/01.mou.0000058634.64616.08
- Tylicki L, Larczynski W, Rutkowski B (2005) Renal protective effects of the renin-angiotensin-aldosterone system blockade: from evidence-based approach to perspectives. *Kidney Blood Press Res* **28**: 230–242. doi: 10.1159/000087842