A Reduction of Asymmetric Dimethylarginine in Renal Transplant Recipients Receiving Sirolimus-Based Regimen

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Objective: *Sirolimus may be of benefit in terms of a reduction of cardiovascular disease in renal transplant recipients. The aim of the present study was to investigate cardiovascular risk markers in renal transplant recipients receiving calcineurin inhibitors (CNI-based regimen), as compared to those receiving sirolimus (SRL-based regimen).*
Material and Method: *42 patients were recruited (21 patients for each regimen). Plasma concentrations of cardiovascular risk markers, including asymmetric dimethylarginine (ADMA), nitric oxide (NO), homocysteine (Hcy), and total antioxidant status (TAOS) were measured.*
Results: *Plasma ADMA concentrations were lower in patients with SRL-based regimen, as compared to those with CNI-based regimen (0.52±0.02 and 0.60±0.02 μmol/L, p = 0.027). There were no statistically significant differences seen in NO, Hcy, and TAOS between the two treatments.*
Conclusion: *As compared to CNI-based regimen, cardiovascular risk marker (ADMA) levels are lower in patients with SRL-based regimen.*

Keywords: *Asymmetric dimethylarginine, Nitric oxide, Homocysteine, Total antioxidant status, Sirolimus, Renal transplant*

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Cardiovascular morbidity and mortality are markedly increased in renal transplantation; a result that leads to both a deterioration in renal graft function and a major cause of death in transplant recipients[1,2]. Calcineurin inhibitors (CNIs) are immunosuppressants that are used to prevent graft rejection. However, CNIs are associated with nephrotoxicity and thus may impair long-term graph survival[3]. CNIs are also associated with increased risk of cardiovascular disease[2,4]. Sirolimus (SRL), an inhibitor of mammalian target of rapamycin (mTOR), is an alternative immunosuppressant for renal transplant recipients. The efficacy of SRL is equivalent to CNIs, but with less nephrotoxicity[5,6]. A study of Thai renal transplant recipients on an SRL-based regimen with minimal CNIs showed favorable outcomes in terms of graft and patient survival[7]. In addition, SRL may be of benefit in terms of cardiovascular disease reduction, as it is characterized in part by its anti-proliferative and anti-atherogenic properties[8,9].

Endothelial dysfunction, the impairment of vascular regulatory functions of the endothelium, plays a pivotal role in the pathogenesis of cardiovascular disease in renal patients. A reduction of nitric oxide (NO) production or NO activity is a major mechanism of endothelial dysfunction. Asymmetric dimethylarginine (ADMA) is an endogenous nitric oxide synthase inhibitor generated by methylation of arginine residues within the cells via enzyme protein arginine methyltransferase[10]. ADMA levels are closely linked to endothelial dysfunction and the pathogenesis of hypertension and atherosclerosis[10]. Importantly, ADMA has been shown to be an independent risk marker in the cardiovascular outcomes of cardiac[11] and renal transplant recipients[12,13]. Oxidative stress in renal transplant recipients also occurs mainly due to endothelial dysfunction. Homocysteine (Hcy) is one of the markers of oxidative stress that is increased in

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renal patients with cardiovascular disease[14].

The objective of the present study was to investigate cardiovascular risk markers, including ADMA, NO, Hcy, and total antioxidant status (TAOS), in Thai renal transplant recipients receiving a maintenance regimen of SRL-based immunosuppressants, as compared to patients receiving a CNI-based maintenance regimen.

Material and Method

Ethical considerations

The present study protocol was approved by the Ethics Committee for Human Research, Faculty of Medicine, Chulalongkorn University, Bangkok, Thailand. All subjects gave their written informed consent. The present study was conducted in accordance with the Declaration of Helsinki of the World Medical Association and International Conference on Harmonization guidelines for Good Clinical Practice.

Study design, subjects and blood sample collection

This was a cross-sectional study. Forty-two Thai renal transplant recipients from the renal unit, King Chulalongkorn Memorial Hospital were recruited. Of these, 21 patients received a CNI-based regimen and the other 21 patients received an SRL-based regimen as their maintenance post-transplantation immunosuppressive therapy, and were clinically stable on their treatment regimens for at least 3 months. All patients displayed stable renal function and were without clinical evidence of acute or chronic graft rejection episodes. Venous blood samples of each patient were collected into EDTA-containing tubes and centrifuged at 500 g, 4°C, for 10 minutes. Plasma samples were then transferred into aliquots and stored at -70°C until analysis.

Determination of ADMA, NO, Hcy, and TAOS in human plasma

Plasma ADMA and related arginine metabolites were quantified using an optimized and fully validated high performance liquid chromatography[15]. Plasma NO and Hcy were measured using commercially available assay kits (Cayman Chemicals, Ann Arbor, MI, USA and Abbot Diagnostics, North Chicago, IL, USA, respectively). The plasma TAOS level was measured using ferric reducing ability of plasma (FRAP) assay[16].

Statistical analyzes

Statistical analyzes were performed using SPSS version 21.0. Continuous data were presented as mean $\pm$ standard error of the mean. Comparisons between groups were performed using Mann Whitney U test, with $p<0.05$ indicating statistical significance.

Results

Both groups were comparable in their baseline characteristics (Table 1) Cardiovascular risk markers for CNIs-based and SRL-based groups are presented in Table 2. The group of renal transplant patients receiving the CNIs-based regimen showed a significant

Table 1. Baseline characteristics of renal transplant recipients who were on calcineurin inhibitors-based and sirolimus-based regimens

Parameters	Calcineurin inhibitors-based regimen (n = 21)	Sirolimus-based regimen (n = 21)
Male/female (n)	13/8	17/4
Age (years)	50.50±2.44	49.57±2.51
Hemoglobin (g/dL)	12.95±0.38	12.93±0.28
Systolic blood pressure (mmHg)	130.24±3.38	132.19±3.49
Diastolic blood pressure (mmHg)	77.33±2.40	80.71±2.69
Blood urea nitrogen (mg/dL)	17.90±0.93	17.57±1.54
Serum creatinine (mg/dL)	1.17±0.07	1.31±0.10
Total cholesterol (mg/dL)	170.75±5.35	197.19±7.93
Triglyceride (mg/dL)	114.88±12.80	149.33±11.13
Low-density lipoprotein cholesterol (mg/dL)	93.69±4.59	114.38±8.12
High-density lipoprotein cholesterol (mg/dL)	54.13±2.89	53.80±2.64
Underlying hypertension (n (%))	4 (19)	12 (57)
Underlying diabetes mellitus (n (%))	19 (90)	10 (48)
Underlying ischemic heart disease (n (%))	20 (95)	10 (48)

increase in plasma ADMA concentrations, as compared to SRL-based patients (0.60±0.02 vs. 0.52±0.02 µmol/L, p = 0.027). ADMA levels in renal transplant patients with various underlying cardiovascular diseases were further investigated. Patients with underlying diabetes mellitus, ischemic heart disease, and dyslipidemia, who were on the SRL-based regimen, had lower levels of ADMA (diabetes mellitus: 0.60±0.03 vs. 0.49±0.02 µmol/L, p = 0.003; ischemic heart disease: 0.60±0.11 vs. 0.48±0.02 µmol/L, p = 0.003; dyslipidemia: 0.59±0.02 vs. 0.50±0.02, p = 0.015). Patients with underlying hypertension showed no differences (0.56±0.02 vs. 0.55±0.01, p = 0.808) (Fig. 1).

Discussion

The results of the present study showed that renal transplant recipients receiving the SRL-based regimen as their maintenance immunosuppressive therapy had lower plasma ADMA concentrations, as compared to those receiving the CNI-based regimen.

Moreover, the results shown in this study are consistent with a previous study by Potena et al that reported ADMA levels as being associated with coronary intimal hyperplasia in heart transplantation recipients and SRL treatment as being associated with low levels of ADMA, thus reducing the risk of accelerated cardiac allograft vasculopathy[11]. Many previous studies in renal transplantation have shown that increased plasma ADMA is associated with endothelial dysfunction and is an independent risk marker for cardiovascular outcomes[12,13,17,18].

SRL was associated with a reduction in the incidence of cardiovascular disease in renal transplant patients with chronic allograft nephropathy, as compared with those receiving CNIs. This is due to SRL having less nephrotoxicity and not being associated with mechanisms that cause hypertension[19]. The exact mechanism for the reduction of cardiovascular disease with SRL treatment is not yet known. However, it has been suggested that SRL inhibits cell cycle functions during cell division stages, from G1 phase to S phase, resulting in an anti-proliferative effect[20,21].

Arginine is converted to NO by endothelial nitric oxide synthase (eNOS). NO helps to dilate blood vessels and increase the flexibility of arteries[10]. The results of the present study also showed a trend that patients with SRL-based regimen had higher concentrations of arginine. With regard to NO, a study of chronic kidney disease patients being treated with SRL for a period of 10 days reported an increase in nNOS levels in the brain and a reduced eNOS levels in the kidneys. These results showed that the levels of NOS correspond specifically to the types of tissue[22]. A study in mice showed that SRL reduced eNOS expression in carotid arteries under high shear stress[23]. This was in contrast to our study in which NO levels were reduced in the SRL group. This may be explained by the fact that plasma NO may not be an optimal marker for endothelial function. Additionally, the present study showed a trend of

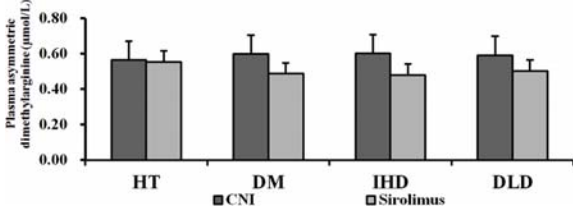

Fig. 1 Plasma asymmetric dimethylarginine concentrations in SRL-based regimen patients with various underlying diseases (HT = hypertension; DM = diabetes mellitus; IHD = ischemic heart disease; DLD = dyslipidemia; CNI = calcinuerin inhibitor. *p<0.05).

Table 2. Cardiovascular risk markers of renal transplant recipients who were on calcineurin inhibitors-based and sirolimus-based regimens

Cardiovascular risk markers	Calcineurin inhibitors-based regimen (n = 21)	Sirolimus-based regimen (n = 21)	p-value
Arginine (µmol/L)	96.48±3.14	111.30±7.18	0.122
Asymmetric dimethylarginine (µmol/L)	0.60±0.02	0.52±0.02	0.027*
Nitric oxide (µmol/L)	138.68±28.91	82.01±9.46	0.116
Homocysteine (µmol/L)	14.34±0.87	17.33±1.65	0.137
Total antioxidant status (µmol/L)	1072.40±51.67	1000.51±65.15	0.163

*p < 0.05

increased Hcy levels in SRL-based patients, unlike a previous study by Farsetti et al that reported lower levels of Hcy and a slight reduction in vitamin B6 in renal transplant recipients on everolimus[9]. Hcy levels are highly affected by dietary status and this may confound the results.

The levels of ADMA in renal transplant patients with related cardiovascular co-morbidity were also analyzed. Those with diabetes mellitus, ischemic heart disease, and dyslipidemia who received SRL-based regimen had low concentrations of ADMA. Despite the fact that the two groups had comparable cholesterol levels and levels of statin usage, the SRL-based patients had significantly lower ADMA levels.

The limitations of the present study include: 1) the cross-sectional design may show associations, but cannot prove causal relationships, 2) the sample size was relatively small and, 3) blood nitric oxide (NO) level measurement may not be specific or sensitive enough to distinguish differences between the two treatments.

Conclusion

SRL-based regimen is associated with low plasma ADMA concentrations suggesting the role of SRL in the reduction of cardiovascular complications in renal transplant recipients. The results may be of benefit to renal transplant patients suffering from cardiovascular complications after transplantation. Larger prospective studies are needed to clarify the impact of these cardiovascular risk markers, especially ADMA, on morbidity and mortality from cardiovascular diseases in renal transplant recipients.

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Potential conflicts of interest

None.

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ระดับอะซิมเมทริกไดเมทิลอาร์จินีนลดลงในผู้ป่วยที่ได้รับปลูกถ่ายไตและได้รับการรักษาด้วยยาสิโรลิมัส

ณัฐพัชร นามจัด, ปาจรีย์ จริยวิลาศกุล, ณัฐวุฒิ โตวน่ำชัย, สุพีชา วิทยเลิศปัญญา

วัตถุประสงค์: *เพื่อศึกษาตัวชี้วัดโรคหัวใจและหลอดเลือดในผู้ป่วยที่ได้รับการปลูกถ่ายไตและได้รับการรักษาด้วยยาแคลซินูริน อินฮิบิเตอร์ (CNI-based regimen) เปรียบเทียบกับกลุ่มที่ได้รับยาสิโรลิมัส (SRL-based regimen)*

วัสดุและวิธีการ: *ผู้ป่วย 42 รายได้รับการคัดเลือกเข้าสู่โครงการวิจัย (มีผู้ป่วย 21 รายต่อกลุ่มการรักษา) ทำการวัดระดับของตัวชี้วัดโรคหัวใจและหลอดเลือด ได้แก่ อะซิมเมทริกไดเมทิลอาร์จินีน (ADMA), ไนตริกออกไซด์ (NO), โฮโมซิสเทอีน (Hcy) และโททอลแอนติออกซิแดนท์สเตตัส (TAOS) ในพลาสมา*

ผลการศึกษา: *ระดับ ADMA ในพลาสมาของผู้ป่วยกลุ่มที่ได้รับ SRL-based regimen มีค่าต่ำกว่ากลุ่มผู้ป่วยที่ได้รับ CNI-based regimen อย่างมีนัยสำคัญทางสถิติ (0.52±0.02 and 0.60±0.02 μmol/L, p = 0.027) ไม่พบความแตกต่างอย่างมีนัยสำคัญทางสถิติของ NO, Hcy และ TAOS ในการรักษาทั้งสองแบบ*

สรุป: *ระดับของ ADMA ผู้ป่วยที่ได้รับการปลูกถ่ายไตที่ได้รับ SRL-based regimen น้อยกว่าผู้ป่วยที่ได้รับ CNI-based regimen*