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Renal dopamine in kidney transplant recipients on cyclosporin A (CsA) therapy

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ABSTRACT

Background: The recovery of renal function early post kidney transplantation is accompanied by an enhanced ability of the proximal tubules to produce dopamine. Cyclosporin A therapy is accompanied by tubulointerstitial injury and a compromised renal dopaminergic activity has been reported in rat models of Cyclosporin A nephrotoxicity.

Study design: The present study examined the 24h urinary excretion (nmol/24h) of free dopamine and metabolites, 3,4-dihydroxyphenylacetic acid and homovanillic acid in 74 renal transplant recipients with different post transplant times receiving triple immunosuppressive therapy with prednisolone, cyclosporin A and azathioprine. The patients were divided into three groups according to the time elapsed post surgery (Group I: Under six months, n=28; Group II: six months-one year, n=19; Group III: one year-three years, n=27). The relationship between Cyclosporin A trough levels and renal dopamine production was evaluated in the three groups.

Results: Despite similar creatinine clearance values (Group I: 75.8±21.7; Group II: 82.5±22.1; Group III: 74.6±20.5 ml/min/1.73m^2) and similar urinary sodium excretion (Group I: 231.6±91.4; Group II: 238.9±97.8; Group III: 215.3±83.0 mmol/24h) among the three groups, the urinary dopamine output progressively increased over time (Group I: 534.1±303.7; Group II: 890.5±472.4 and Group III: 1240.1±401.7 nmol/24h, p<0.001). This was accompanied by a parallel decrease in Cyclosporin A trough levels (ng/ml) (Group I: 292.3±71.9; Group II: 243.3±72.9 and Group III: 200.1±56.0 ng/ml, p< 0.001). A close relationship was observed between the urinary dopamine excretion and the Cyclosporin A trough levels (adjusted R2=0.19) in these 74 renal transplant recipients.

Conclusions: We conclude that renal dopamine production in renal transplant recipients progressively increases over time independent of the graft function. It is also suggested that Cyclosporin A nephrotoxicity in renal transplant recipients may contribute to the impairment of renal dopamine production in renal tubules.

Key-Words:
Cyclosporin A; Dopamine; DOPAC; HVA; Renal transplantation.

INTRODUCTION

Renal dopamine has long been recognised to behave as an endogenous natriuretic hormone[1], contributing significantly to the natriuresis that accompanies an acute or a chronic sodium load[2]. Up to 60% of sodium excretion is mediated by dopamine D1 receptors and it has been suggested that the loss or absence of intrarenal dopamine function plays a

role in the pathogenesis of sodium sensitive hypertension and oedema formation[3-5]. In the kidney, dopamine is synthesised mainly in the epithelial cells of proximal tubules which are endowed with a high aromatic *L*-amino acid decarboxylase activity (AADC)[6] and produce dopamine from filtered or circulating L-3,4-dihydroxyphenylalanine (L-DOPA)[7]. At least 90% of the urinary dopamine results from local kidney production[8,9] and the amount of dopamine produced in renal proximal tubules is related to the number of well functioning nephrons endowed with AADC. In the kidney, newly formed dopamine may undergo deamination to 3,4-dihydroxyphenylacetic acid (DOPAC), by monoamine oxidase (MAO) and deamination plus methylation to homovanillic acid (HVA) by both MAO and catechol-O-methyltransferase (COMT)[10].

In chronic renal parenchymal diseases, renal dopamine output decreases in proportion to the loss of renal function[11]. Conversely, in renal transplant recipients the recovery of renal function early post kidney transplantation is accompanied by an enhanced ability of renal tubules to synthesise dopamine[12]. Taken together, these observations suggest that in addition to the physiological importance of endogenous renal dopamine, urine dopamine output can be used as a marker of renal function as well as an index of the viability of the transplanted kidney.

Cyclosporin A (CsA) induced nephrotoxicity is characterised by tubulointerstitial injury[13], as evidenced by apoptosis of the renal tubular cells and tubulointerstitial fibrosis[14,15]. As CsA nephrotoxicity is accompanied by an increase of blood pressure with sodium sensitive characteristics[16,17] it is reasonable to postulate that renal dopaminergic activity might be compromised by CsA therapy. Supporting this view are the results in both man and rat showing that dopamine[18] and dopamine agonists[19] may completely reverse the haemodynamic and tubular disturbances induced by CsA. Although a reduced renal dopamine synthesis was observed in a rat model of CsA nephrotoxicity[20], the influence of CsA therapy on renal dopamine production in human subjects still remains to be elucidated.

The present study aimed to examine the 24h urinary excretion (nmol/24h) of free dopamine and metabolites, DOPAC and HVA in 74 renal transplant recipients with well-preserved renal function up to three years post surgery. All patients were receiving triple immunosuppressive therapy with prednisolone (PND), azathioprine (AZA) and CsA and were divided into three groups according to the time elapsed post surgery. The relationship between CsA therapy and renal dopamine production was also evaluated.

PATIENTS AND METHODS

Seventy-four Caucasian adult patients aged 38.5±12.4 years (18 to 64 years), 48 male and 26 female, were enrolled in the study. They were all first time recipients of a cadaver renal transplant. The immunosuppressive therapy in all patients consisted of PND and AZA in combination with CsA. The patients selected had no proteinuria and presented a well-preserved and stable renal function with plasma creatinine levels below 2 mg/dl. Delayed graft function was defined as the requirement of dialysis early post transplantation in the absence of rejection. Early acute rejection was assumed in patients who had biopsy-proven diagnosis up to the sixth month post transplantation.

For the purpose of this study, the patients were divided into three groups according to the time elapsed post surgery (Group I: under six months, n=28; Group II: six months-one year, n=19; Group III: one year-three years, n=27). Twenty-four hour urine specimens were collected from the patients to quantify daily urinary excretion of Na^+, K^+, phosphate, creatinine, free dopamine, DOPAC and HVA. The 24-hour urine samples were collected in containers with 15 ml hydrochloric acid (6M) to avoid the spontaneous oxidation of the amines and its derivatives. The levels of amines and its derivatives in the urine were determined by means of high-pressure liquid chromatography with electrochemical detection (HPLC-ECD). The CsA trough levels of each patient on the day of the urine collection were determined via TDx Cyclosporine Monoclonal Whole Blood assay (Abbott Laboratories, USA) using fluorescence polarisation immunoassay (FPIA) technology. The other measurements were performed by standard laboratory methods. Blood pressure was measured in all patients between 8.30 and 9.30 a.m. on the day

of the urine collection by means of a DINAMAP system and after a 5 min rest.

Statistics

The results are expressed as the mean ± standard deviation (SD). A *p* value below 0.05 defined statistical significance.

Analysis of Variance (ANOVA) was used to examine the significance of differences between the three groups for the variables analysed. Having established the existence of differences between the three groups, we performed all pair wise comparisons between group means using the Student's *t*-test. For categorical data, the comparison between groups was performed by means of the chi-square test. The graphical representation used a box-plot chart with the medians and the 25^{th} to 75^{th} percentiles. Regression analysis was used to evaluate the relation of dopamine excretion with CsA trough levels and with creatinine clearance.

RESULTS

The characteristics of the study population from the three groups are given in Table I. The chi-square test evidenced that the proportion of patients with delayed graft function was identical among the three groups. In addition, the incidence of acute rejection did not differ among the groups. Equally, no significant differences were observed in the demographic parameters of either recipients or donors among the three groups and the mean HLA mismatches were also similar among the three groups.

The mean daily doses of PND, AZA and CsA in the three groups are given in Table I. As can be observed, both the steroid and CsA daily doses were reduced over time and differed among the three groups. By

Table I.

General characteristics of renal transplant recipients and donors of the three groups of patients

	Group 1	Group 2	Group 3
Gender (M/F)	15/13	15/4	18/9
Age (years)	36.2 ± 11.0	43.5 ± 13.9	37.4 ± 12.3
Time post-transplant (months)	3.7 ± 4.4	8.3 ± 3.1	32.6 ± 7.8
Cause of ESRD			
Hypertension	6	1	3
Diabetes mellitus	2	0	0
Chronic GN	6	5	6
Polycystic Kidney	2	2	3
Others	12	11	15
Delayed graft function (%)	3.5	10.5	14.8
Early acute rejections (%)	3.5	5.2	3.3
Antihypertensive medication			
Calcium channel blockers (%)	78.5	89.4	33.3*#
β-blockers (%)	0.0	5.2	11.1
IECA (%)	3.5	15.7	14.8
Immunosuppression			
PND (mg/day)	17.9 ± 5.8	11.4 ± 3.5*	7.3 ± 2.4*#
AZA (mg/day)	70.6 ± 24.1	63.2 ±22.1	63.6 ± 12.7
CsA (mg/day)	417.4±114.1	340.8 ±50.7*	259.6 ± 40.1*#
Mean HLA mismatches	2.8 ± 1.0	2.4 ± 0.9	2.6 ± 0.9
Donor demographics			
Mean donor age (years)	27.8 ± 13.9	29.9 ± 13.9	32.0 ± 12.1
Female donor (%)	14.2	15.7	25.9

* Significantly different in comparison with the corresponding values for Group I.
# Significantly different in comparison with the corresponding values for Group II.
IECA, converting enzyme inhibitors.

Table II

Baseline clinical characteristics of renal transplant recipients of the three groups of patients

	Group 1	Group 2	Group 3
CsA level (12-hour trough, ng/ml)	292.3 ± 71.9	243.3 ± 72.9*	200.1 ± 56.1*#
Creatinine clearance (ml/min/1.73m2)	75.8 ± 21.7	82.5 ± 22.1	74.6 ± 20.5
Serum creatinine (mg/dl)	1.36 ± 0.2	1.41 ± 0.2	1.42 ± 0.2
Haematocrit (%)	35.2 ± 6.2	40.2 ± 6.8*	40.6 ± 5.5*
Urinary Na+ (mmol/24h)	231.6 ± 91.4	238.9 ± 97.8	215.3 ± 83.0
FENa+ (%)	1.53 ± 0.5	1.56 ± 0.9	1.32 ± 0.6
Systolic blood pressure (mmHg)	137.6 ± 13.4	138.8 ± 16.5	134.8 ± 14.8
Diastolic blood pressure (mmHg)	82.1 ± 11.9	82.7 ± 9.8	84.5 ± 8.2

* Significantly different in comparison with the corresponding values for Group I.
# Significantly different in comparison with the corresponding values for Group II.

contrast, the AZA daily doses were similar in all groups.

Both the systolic and diastolic blood pressure values were similar among the three groups (Table II). The proportion of patients treated with angiotensin converting enzyme inhibitors and β-blockers was similar among the groups. However, there was a smaller proportion of patients receiving calcium antagonists in group III than in the other two groups (Table I).

All the patients selected presented with stable graft function and the creatinine clearance values were similar among the three groups (Fig. 1 and Table II). Urinary excretion of sodium and fractional excretion of sodium were also similar among the three groups (Table II). However, as expected, the haematocrit values were lower in group I patients than in those from the other two groups (Table II).

As Fig. 2 shows, the urinary dopamine output progressively increased over time (Group I: 534.1±303.7; Group II: 890.5±472.4 and Group III: 1240.1±401.7 nmol/24h). Using Analysis of Variance (ANOVA), the twenty-four hour urinary dopamine excretion was significantly different among the three groups, with p=0.000. Further analysis revealed statistically significant differences in dopamine excretion between every two groups (Fig. 2). However, daily urinary excretion of the amine metabolites DOPAC and HVA did not differ among the three groups (DOPAC: Group I: 5166.0±1698, Group II: 6114.9±3162 and Group III: 4918.3±2124 nmol/24h; HVA: Group I: 19353.0±14537, Group II: 23554.2±8147 and Group III: 20131.1±6162 nmol/24h).

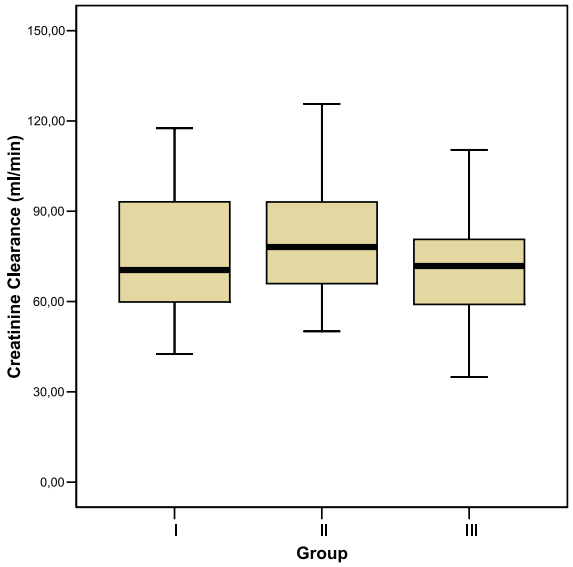

Figure 1

Creatinine clearance (ml/min) in three groups of renal transplant recipients, group I (Less than six months, n=28), group II (Six months to one year, n=19), group III (More than one year post transplant, n=27).

The increase in renal dopamine output over time was accompanied by a progressive decrease in CsA trough levels (Fig. 3 and Table II). The CsA trough levels were significantly different among the three groups, with p=0.000, and all pairwise comparisons between groups were statistically different (Fig. 3). A close relationship was observed in these 74 renal transplant recipients between the urinary dopamine excretion and the CsA trough levels (adjusted R^2=0.19; Fig. 4).

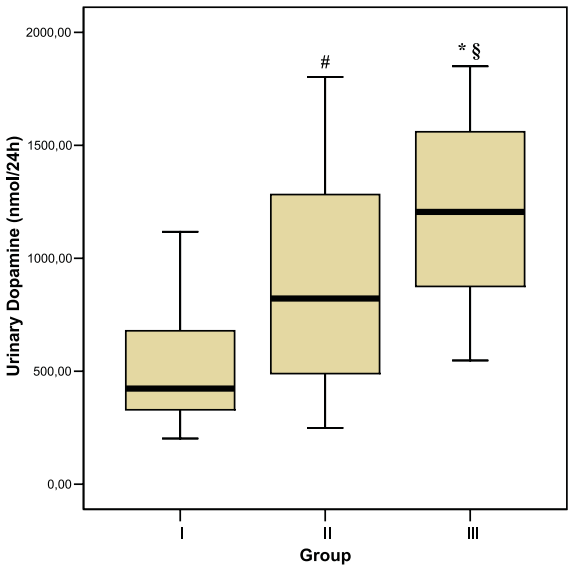

Figure 2

Twenty-four hour urinary dopamine excretion (nmol/24h) in three groups of renal transplant recipients, group I (Less than six months, n=28), group II (Six months to one year, n=19), Group III (More than one year post transplant, n=27).
Significantly different in comparison to group I (# p=0.008); significantly different in comparison to group I (* p=0.000) and significantly different in comparison to group II (§ p=0.001).

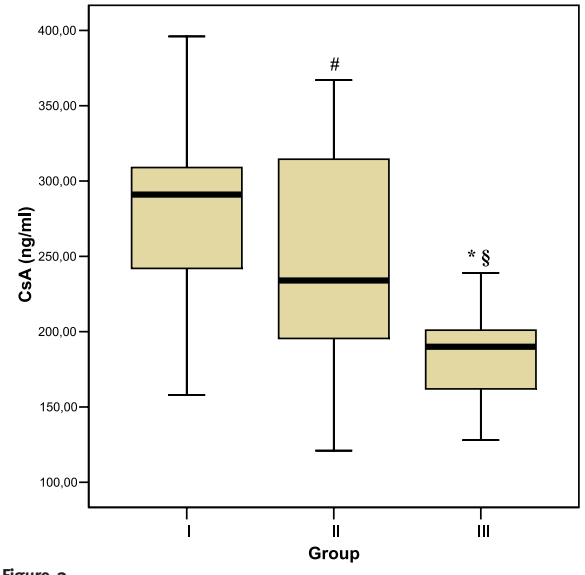

Figure 3

CsA trough levels (ng/ml) in three groups of renal transplant recipients, group I (Less than six months, n=28), group II (Six months to one year, n=19), group III (More than one year post transplant, n=27).
Significantly different in comparison to group I (# p=0.017); significantly different in comparison to group I (* p=0.000) and significantly different in comparison to group II (§ p=0.03).

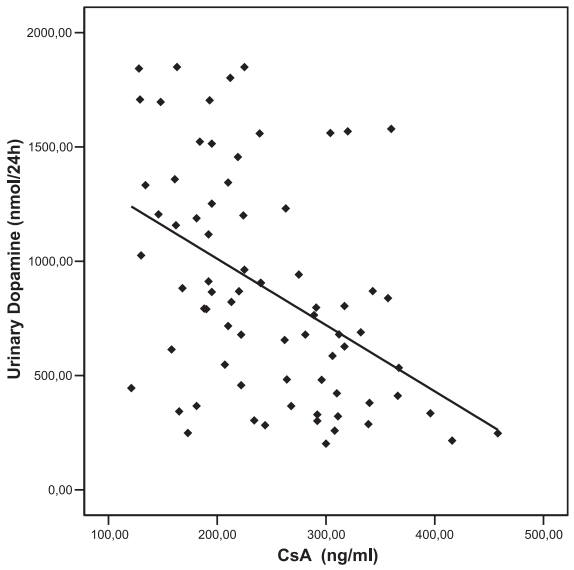

Figure 4

Reciprocal plot of CsA trough levels (ng/ml) and urinary dopamine excretion (nmol/24h) in renal transplant recipients (n=74).

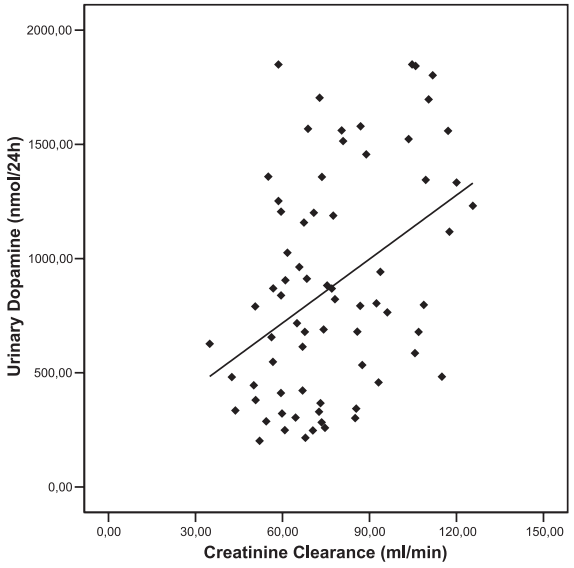

Figure 5

Reciprocal plot of creatinine clearance (ml/min) and urinary dopamine excretion (nmol/24h) in renal transplant recipients (n=74).

Although all 74 patients presented well-preserved renal function with creatinine blood levels below 2 mg/dl, a positive relationship was observed between creatinine clearance and renal dopamine excretion

(adjusted R^2=0.16). No significant relationship was observed between CsA trough levels and creatinine clearance.

DISCUSSION

This study aimed to examine the renal dopaminergic system activity in three groups of renal transplant recipients with well-preserved renal function up to three years post kidney transplantation. We found an increase in renal dopamine production in renal transplant recipients which was related to the time elapsed post surgery. The findings that both the creatinine clearance values and the urinary sodium excretion were similar among the three groups of patients with different post transplant times provides evidence favouring the view that the differences observed in renal dopamine production might be related to dysfunction of renal tubular cells. The relationship observed between the CsA trough levels and the urinary dopamine excretion in these 74 renal transplant recipients suggests that the impairment of renal dopamine production by proximal tubules might be related to CsA nephrotoxicity.

In addition to the well-recognised physiological importance of endogenous renal dopamine in the control of renal sodium excretion[1,2], evidence has been gathered that urine dopamine output is closely related to renal function[11,12]. In patients with chronic renal parenchymal diseases, the decline of renal function goes hand in hand with a decrease in renal dopamine production[5,11]. Conversely, the recovery of renal function early post kidney transplantation is accompanied by an enhanced ability of the renal tubules to synthesise dopamine being minimal in patients with acute tubular necrosis[12]. The results of our study in 74 renal transplant recipients with creatinine blood levels below 2 mg/dl also provide evidence for a relationship between renal dopamine production and renal function and further support the view that renal dopamine may be a useful index of the viability of the transplanted kidney.

However, the time dependent increase in renal dopamine production observed in renal transplant recipients in the present study cannot be explained by differences in the graft function, as the three groups of patients presented similar creatinine clearance values. Also, the urinary sodium excretion was similar among the three groups of patients with different post transplant times, thus excluding the possible influence of sodium intake in the overtime increase in renal dopamine production.

In addition to renal function and sodium intake, renal dopamine production can also be influenced by the metabolic processes involved in the degradation of the amine (deamination to DOPAC and deamination plus methylation to HVA) and it has been suggested that the urinary excretion of these two amine metabolites may be useful for the assessment of the renal dopamine system activity[12]. The finding that urinary excretion of both DOPAC and HVA was similar among the three groups of renal transplant recipients with different post transplant times suggests that changes in both the deamination and methylation of dopamine cannot account for the differences observed in urine dopamine excretion among the three groups.

Another possible influence on renal dopamine production in the present study might be related to differences in steroid therapy among the three groups, given that chronic steroid therapy has been associated with hyperdopaminuria[21]. Against this possibility is the finding that the patients from group I presenting with lower urine dopamine output were receiving a higher steroid daily dose. The finding that rats treated with high steroid doses presented with no changes in urinary L-DOPA and dopamine excretion[22,23] further excludes the possible involvement of steroid therapy on the differences in renal dopamine excretion observed among the three groups of renal transplant recipients in the present study.

It has been suggested that calcium channel blockers (CCB) may influence urinary dopamine excretion, as nifedipine therapy in non-Caucasian salt sensitive and salt resistant normotensive and hypertensive subjects is associated with a short-term increase in urinary dopamine, L-DOPA and DOPAC excretion[24]. However, the finding that fewer patients from group III in the present study were receiving CCB negates the possible influence of these anti-hypertensive drugs in the overtime increase in urinary dopamine excretion.

It is well recognised that proximal tubules, where renal dopamine is synthesised, are directly susceptible to calcineurin inhibitor nephrotoxicity, with

pathology characterised by isometric vacuolisation, tubular cytoplasmic inclusion bodies, abnormal giant mitochondria, tubular microcalcification and striped fibrosis[25]. In addition, a reduced renal dopamine production was observed in a rat model of chronic CsA nephrotoxicity[19]. Although histological analysis was not available in our patients, one can hypothesise that the differences observed in renal dopamine production in the present study are related to CsA therapy. In agreement with this hypothesis is the relationship observed in these 74 renal transplant recipients between CsA trough levels and renal dopamine production as well as the decrease in CsA trough levels over time.

In conclusion, our results provide evidence favouring the view that renal dopamine production in renal transplant recipients progressively increases over time up to one year post kidney transplantation, independent of the graft function. It is also suggested that this might result from selective tubulointerstitial injury related to CsA nephrotoxicity.

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Conflict of interest statement. None declared.

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