Effect of Tacrolimus Levels on Cerebral Blood Flow and Cognitive Function: A Pilot Study

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Cognitive impairment is common in kidney transplant recipients (KTRs).[1] Potential mechanisms include advanced kidney disease and hemodialysis, immunomodulation, and immunosuppression. While some risk factors of cognitive impairment such as age are nonmodifiable, immunosuppression is modifiable. Tacrolimus, a calcineurin inhibitor (CNI) is widely used in KTRs. CNIs are potent vasoconstrictors[2] and cause endothelial dysfunction with decrease in vasodilators such as prostaglandins and nitric oxide and increase in vasoconstrictors such as endothelin and thromboxane.[3] While CNI-induced vasoconstriction is documented in other organs, CNI-induced cerebral vasoconstriction and its downstream effects are relatively unexplored. Cerebral vasoconstriction can decrease cerebral blood flow (CBF), which can cause cognitive impairment.[4]

Since CNI-induced vasoconstriction is dose dependent, we hypothesized that lowering tacrolimus goal levels can improve CBF and cognition. We conducted an open-label, single-center, prospective, pilot study (Institutional Review Board study 140594). After obtaining informed consent, we enrolled 39 KTRs with stable allograft function and on stable immunosuppression with high-dose immediate release tacrolimus and an antimetabolite, with or without prednisone. Patients in the intervention group and their treatment team were agreeable to starting everolimus for the study. Study assessments included a noncontrast magnetic resonance imaging to measure CBF and cognitive assessment at baseline and at 12 weeks. We measured CBF in the total gray matter of the brain and *a priori* defined regions using arterial spin labeling as previously used by us.[5] For cognitive assessments, we used a battery of standard neuropsychological (NP) tests.[6] At baseline, all participants had a tacrolimus trough goal of 7–10 ng/ml per institutional protocol. After the baseline visit, patients in the intervention group (n=16) were started on everolimus, tacrolimus was reduced to achieve a goal of 3–5 ng/ml, and the antimetabolite was stopped.

Our primary outcomes were change in total gray matter CBF and change in Logical Memory 1 and Logical Memory 2. These NP tests were chosen *a priori* based on prior cognitive data in KTR.[6] To estimate the effect of the intervention, we used an analysis of covariance model adjusted for baseline values. Since this was a pilot study and not powered to assess statistically significant difference at the level of $P < 0.05$, we examined trends in difference in change of outcome variables from baseline to 12 weeks in the two groups.[7]

Baseline demographics are described in Table 1. Baseline tacrolimus levels were consistent with the institutional protocols for post-transplant immunosuppression, and there was no difference in levels between the intervention and control groups (8.6±2.8 ng/ml intervention, 8.8±2.4 ng/ml control, $P = 0.7$). At 12 weeks, tacrolimus levels were lower in the intervention group (6.3±2.7 ng/ml) compared with the control group (8.6±3.3 ng/ml), ($P = 0.04$). The mean CBF in the total gray matter increased in the intervention group compared with the control group ($P = 0.03$) (Table 1). The individual regions of the brain also had a similar trend of increase in CBF in the intervention group. Logical Memory 1 ($P = 0.01$) improved in the intervention group (Table 1). Similarly, Logical Memory 2, Montreal cognitive assessment, Mini-Mental Scale Examination, Digit Symbol

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Table 1. Demographics of study participants and cerebral blood flow and neuropsychological test scores at baseline and at 12 weeks in the intervention and control groups

Participants Demographics (Intervention Group, n=16; Control Group n=23; Overall N=39)			
	Intervention	Control	Overall
Age (yr), mean±SD	54.7±10.9	50.3±14.6	52±13.3
Male sex, *No.* (%)	12 (75)	17 (73.9)	29 (74.3)
Race, *No.* (%)			
White	14 (87.5)	18 (78.3)	32 (82.1)
Black	1 (6.3)	2 (8.7)	3 (7.7)
Other	1 (6.3)	3 (13)	4 (10.3)
BMI, mean±SD	28.6±4.8	29.6±6.4	29.2±5.7
BP (mm Hg), mean±SD			
Systolic BP (sitting)	132±12.7	131±13.3	131±12.9
Diastolic BPs (sitting)	76±11.2	72.5±10	73.9±10.5
Time since transplant (yr), mean±SD	9.2±8.1	5.1±3.5	6.8±6.1
Primary cause of ESKD, *No.* (%)			
Polycystic kidney disease	5 (31.3)	5 (21.7)	10 (25.6)
GN	3 (18.8)	2 (8.7)	5 (12.8)
Hypertension	0 (0)	4 (17.4)	4 (10.3)
Diabetes	1 (6.3)	1 (4.3)	2 (5.1)
Other	6 (37.5)	11 (47.8)	17 (43.6)
Unknown	1 (6.3)	0 (0)	1 (2.6)

CBF (ml/100 g Tissue) (Intervention Group, n=16; Control Group n=20[a])							
Brain Region	Intervention		Control		Change		P Value
	Mean±SD		Mean±SD		Mean±SD		
	Baseline	12 wk	Baseline	12 wk	Intervention	Control	
Total gray matter[b]	74.7±12.8	79.5±16.4	77.9±13.2	74.4±10.4	4.78±10.5	−3.60±9.4	0.04
Anterior cingulate cortex	80.2±11.2	80.9±13.2	82.3±13.6	78.5±11.8	0.73+14.6	−4.05±7.9	0.28
Caudate	52±13.2	55.0±12.4	58.9±23.3	52.9±16.3	3.02±18.0	−7.34±26.7	0.43
Frontal	94.8±17.6	99.6±21.2	99.8±19.9	92.9±15.2	4.76±15.2	−7.81±26.7	0.05
Middle frontal	106±20.0	114±2.4	112±21.1	106±19.3	7.64±19.4	−6.13±14.2	0.03
Hippocampus	43.4±7.1	44.3±10.1	44.8±9.4	40.5±8.28	0.86±7.9	−4.37±9.3	0.15
Primary motor cortex	108±20.3	114±27.6	113±25.0	109±21.0	5.93±20.8	−4.25±26.5	0.23
Parietal	98.2±18.7	107±28.3	103±20.1	102±19.1	9.25±16.4	−1.25±11.9	0.03
Pallidum	39.2±5.88	40.3±6.68	39.5±9.76	39.0±10.1	1.20±9.1	−0.54±16.0	0.81
Precuneus	92.1±19.2	96.8±23.2	96.7±17.4	94.3±15.0	4.69±15.1	−2.97±13.3	0.1
Superior parietal	98.6±22.0	111±35.4	105±28.3	104±25.6	12.5±21.6	−1.07±18.2	0.04
Temporal	58.0±9.6	60.8±12.7	59.7±9.5	56.8±8.2	2.79±8.2	−3.23±7.7	0.11
Thalamus	58.3±10.9	58.8±12.1	59.7±12.4	54.6±10.4	0.44±11.2	−5.46±12.6	0.19
NP Test Scores (Intervention Group, n=19; Control Group n=20[a])							
Logical memory 1[b]	10.1±3.9	13.8±4.5	9.77±3.7	11.1±4.4	3.75±2.8	1.36±3.43	0.01
Logical memory 2[b]	9.25±3.7	13.0±4.2	8.95±4.2	10.6±4.2	3.75±3.5	1.68±3.9	0.07
MoCA	26.6±2.4	27.4±2.0	26.1±2.5	26.3±2.2	0.81±2.0	0.18±1.5	0.08
MMSE	28.6±1.5	29.2±1.05	28.4±1.2	28.5±1.5	0.62±1.3	0.09±1.5	0.13
DSST	49.1±11.9	53.5±11.6	52.5±11.3	54.5±12.0	4.38±2.6	1.95±6.9	0.2
Trailmaking A (s)	25.6±9.1	23.1±5.9	27.7±7.8	25.7±8.7	−2.5±3.8	−2.0±8.07	0.36
Trailmaking B (s)	68.2±19.8	61.1±21.6	69.5±17.2	68.6±23.0	−7.12±25.5	−0.91±15.3	0.42
Digit span forward	9.12±2.5	8.75±1.4	8.73±2.3	9.09±1.8	−0.37±1.7	0.36±1.1	0.37
Digital span backward	6.81±2.2	6.81±2.0	6.68±2.3	7.09±2.2	0.0±1.9	0.40±1.4	0.32
Category fluency animals	22.1±4.6	21.9±4.1	20.2±5.6	21.2±6.1	−0.12±3.8	1.0±4.5	0.86
Category fluency vegetables	15.0±2.9	14.2±4.0	12.0±2.9	14.3±2.7	−0.75±3.6	2.27±2.8	0.24
Block design	39.2±8.2	40.9±38.3	38.3±11.8	41.5±12.1	1.69±7.5	3.18±7.3	0.61
Stroop interference	45.6±25.9	42.1±8.5	40.0±8.0	39.6±9.2	−3.5±24.7	−0.47±4.8	0.47

CBF, cerebral blood flow; DSST, digital symbol substitution test; MMSE, Mini-Mental State Examination; MoCA, Montreal Cognitive Assessment; NP, neuropsychological.
[a]Three participants did not complete magnetic resonance imaging because of claustrophobia or scanner malfunction.
[b]Primary outcome based on prior cognitive data in kidney transplant recipient.

Substitution Test, and Trailmaking A and B showed higher improvements in the intervention group than the control group, but overall, the changes in NP tests were small; this may be as changes in cognition can take time and be affected by practice effects.

In summary, after reduction of tacrolimus and initiation of everolimus, there was an increase in CBF across all *a priori* defined brain regions and an improvement in cognitive function. CNI-induced vasoconstriction can explain these findings. Direct effects of tacrolimus or endothelial dysfunction could also contribute.[3] The intervention group was started on everolimus and in theory these changes could be attributed to everolimus. Everolimus is not known to influence CBF but has shown to protect against focal ischemia-reperfusion injury in rats. Whether this can alter CBF in all regions of the brain is not known.

These preliminary data are important and can guide future research. Longer and larger studies are needed to confirm these pilot data and further investigate the effect of tacrolimus on cognitive function. The greatest increases in CBF were observed in the frontal and parietal regions. The frontal cortex is involved in short-term memory which is consistent with improvement in Logical Memory 1 and Logical Memory 2; future studies should further explore this association.

Our study was limited by the small size, short-term follow-up, and lack of randomization. We did not randomize patients for this pilot trial as we did not want to change immunosuppression and risk allograft function for a proof-of-concept study without convincing preliminary data. Strengths of the study include longitudinal design, detailed cognitive assessments, use of analysis of covariance for comparisons (instead of commonly used *t* test, which does not account for baseline differences), and blinding of personnel assessing CBF.

In conclusion, tacrolimus levels may affect CBF which can affect cognitive function.

Disclosures

Disclosure forms, as provided by each author, are available with the online version of the article at http://links.lww.com/KN9/A530.

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Data Sharing Statement

Partial restrictions to the data and/or materials apply. Only anonymized data will be shared.

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