

Therapeutic drug monitoring of modified release once daily tacrolimus in *de novo* renal transplant with conversion to a twice daily generic in the stable period

Sir,

Management of transplant patients is a challenge in developing countries in view of the availability of multiple generics and different formulations in the market and financial constraints faced by the patients. Advagraf® (Astellas Pharma US, Inc.) is available as a once daily modified release (MR) tacrolimus. Comparable clinical outcome, trough concentration and tacrolimus area under concentration time curve (AUC_{0-24}) between twice a day, tacrolimus innovator (Prograf®) and tacrolimus MR in renal transplant recipients have been

reported.^[1] Studies used Prograf® as *de novo* and found a comparable AUC_{0-24} after patients were converted later to tacrolimus MR (Advagraf®).^[2] In comparison to the conventional release formulation (Pangraf) of tacrolimus, the modified release formulation (Advagraf) is subject to wet granulation and capsule filling to delay drug release of tacrolimus.^[3]

PanGraf® (tacrolimus twice daily, generic) is widely prescribed and used successfully in this population from the advent of tacrolimus into the Indian market in 2005. The cost of 1 mg of tacrolimus is INR 100 for the innovator, Prograf® (Astellas Pharma US, Inc.) in comparison to INR 43 for PanGraf® (Panacea Biotec Ltd., India).

We wish to report an experience with therapeutic drug monitoring (TDM) of tacrolimus in four *de novo* renal transplant recipients who were initiated on Advagraf®, once daily prior to renal transplantation and subsequently converted to the twice daily, PanGraf® in the stable post transplant period.

Patients (case no. 1, 2, 3 and 4) were initiated on Advagraf®, tacrolimus MR after giving written informed consent, two days prior to transplantation (dose between 0.192 and 0.202 mg/kg/day), along with prednisolone and mycophenolate mofetil. Patients (case no. 1, 3 and 4) were from the North east of India and case no. 2 was from the South of India. Whole blood concentration of tacrolimus was measured by the LC-MS/MS. Reversed phase chromatography was performed using a

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Nova-Pak C18 (2.1 × 10 mm) cartridge. The mobile phase was 2 mM ammonium acetate in water, with 0.1% formic acid and 2 mM ammonium acetate in methanol, with 0.1% formic acid, at a flow rate of 0.4 ml/min. It was run using a gradient where the organic phase was increased from 50% to 100% at 0.6 min and reduced back to 50% at 1.2 min of each run.

The mass spectrometer was operated in positive ion mode. The transition ions were m/z 821.5/768.2 for tacrolimus and m/z 809.5/756.2 for the internal standard, ascomycin. The MS/MS set up parameters included capillary voltage of 1.00 kV, source temperature was 150°C, desolvation temperature was 350°C, desolvation gas (N_2) flow was 900 L/h and cone gas (N_2) flow was 50 L/h. The cone voltage was 27 V and 28 V for tacrolimus and ascomycin respectively and collision voltage was 20 V for both tacrolimus and ascomycin. Whole blood specimens were treated with zinc sulfate for extraction of tacrolimus and further precipitated using acetonitrile. The chromatogram for tacrolimus and ascomycin (internal standard) is shown in Figure 1.

Sampling time points for estimation of tacrolimus AUC_{0-24} was trough, 0.5, 1, 1.5, 2, 2.5, 3, 4, 6, 8, 10, 12, 14 and 24 hours after administration of Advagraf® and was performed a week after transplant (visit 1). The trough concentration targeted during the early post transplant period was 8–12 ng/ml.^[4] Daily dose of tacrolimus MR at visit 1 was 0.208, 0.196, 0.208 and 0.15 mg/kg, respectively. Case no. 4 had a delayed visit 1 performed 3 weeks after transplant

because of antibody-mediated rejection which was treated with plasmapheresis, after which he completely recovered. Tacrolimus trough concentrations were 5.0, 16.7, 11.0 and 11.4 ng/ml in each of the four cases respectively and AUC_{0-24} was 286.7, 620.6, 397.8 and 332.4 $\mu\text{g.h/L}$, respectively.

Frequent TDM was performed during the post transplant period while on Advagraf®, by measuring tacrolimus trough measurements. The mean (sd) total daily dose, as noted during the TDM visits when on Advagraf® was 8.6 (1.9), 9.9 (3.5), 11.0 (0.4) and 8.1 (1.8) mg/day for each of the cases, respectively. The mean (sd) of all the tacrolimus trough concentrations monitored in each of the four patients during this period was 10.4 (3.8), 11.2 (3.4), 8.4 (1.5) and 11.96 (2.7) ng/ml, respectively. The within patient variability, as % CV of dose normalized trough concentrations when on Advagraf® ranged from 16.6% to 45.7%. With twice daily tacrolimus also, there was an earlier report of a wide range in within patient variability from <5% to >50%.^[5]

The second AUC_{0-24} determination on Advagraf® was done in all patients at 3 months after transplantation (visit 2) and the range of tacrolimus AUC_{0-24} was from 280.9 $\mu\text{g.h/L}$ (this unit is correct to the best of our knowledge) to 454.6 $\mu\text{g.h/L}$. In visit 2, based on earlier tacrolimus trough concentrations, case no. 1, 2 and 4 had a dose reduction of 47.1%, 50.5% and 58.0% compared to visit 1 (except case no. 3 whose dose was increased by 1.9% in visit 2).

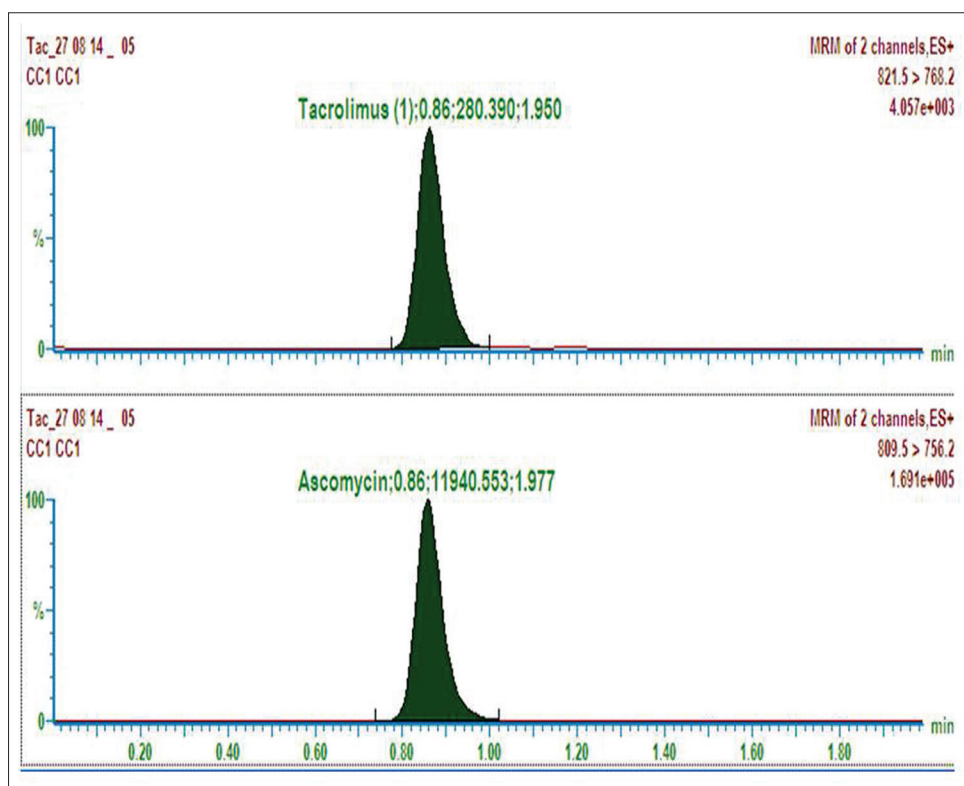


Figure 1: The chromatogram for tacrolimus and ascomycin (internal standard) (dimensions: 1195 × 754)

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Case no. 1, 2 and 4 showed an improvement in the exposure measured as dose normalized AUC_{0-24} , 3 months after transplant to 165%, 48% and 101.2% of the visit 1 AUC_{0-24} (except case no. 3 who showed a reduction of 19.7% in dose normalized tacrolimus AUC_{0-24}). With Advagraf[®], an interpatient variability of 32.3% to 40.0% in the AUC_{0-24} and 45.8% to 45.97% in the trough, respectively (normalized to the mg/kg) was observed.

After which the patient was immediately changed to PanGraf[®], tacrolimus BD generic on an equivalent milligram to milligram basis total daily dose, after obtaining written informed consent from the patient. The next sampling was performed 7–10 days after initiating PanGraf[®] (visit 3) and the sampling time points were trough, 0.5, 1, 1.5, 2, 2.5, 4, 6, 8 and 12 hours, performed both after morning and evening tacrolimus doses. Case no. 2 was unwilling to discontinue tacrolimus MR at 3 months and was converted to PanGraf[®] at 6 months post transplant. So this patient had an additional AUC_{0-24} done on Advagraf[®], at 6 months and thereafter repeated again after conversion to PanGraf[®]. Figure 2 demonstrates the area under concentration time profile during all three visits.

Table 1 shows details of exposure and % change in trough and AUC_{0-24} with tacrolimus prior to conversion in the stable post transplant period and also after conversion to PanGraf[®]. The C_{max} with Advagraf[®] was lower by 6.4% to 50.5% compared to PanGraf[®]. Three patients showed a lower tacrolimus trough and AUC_{0-24} with Advagraf[®] compared to PanGraf[®]. None of the above-mentioned patients experienced any significant serious adverse events while on Advagraf[®] or after conversion to PanGraf[®].

When initiated on doses of Advagraf[®] similar to the routine practice with PanGraf[®], given twice daily, three of our patients (except case no. 2) on Advagraf[®] had AUC_{0-24} below that recommended by Scholten *et al.* in the early post renal transplant visit 1 (AUC_{0-12} of 210 $\mu\text{g.h/L}$).^[4] Jelassi *et al.* suggested that up to 25% higher doses of Advagraf[®] would be needed in the first weeks after transplant.^[6] But a high tacrolimus AUC_{0-24} in case no. 2 would imply that we need to exercise caution in initiating higher dose of Advagraf[®] for all *de novo* renal transplant patients. The changing post transplant dynamics may be responsible for this variability between patients. Franck Saint-Marcoux *et al.* reported an

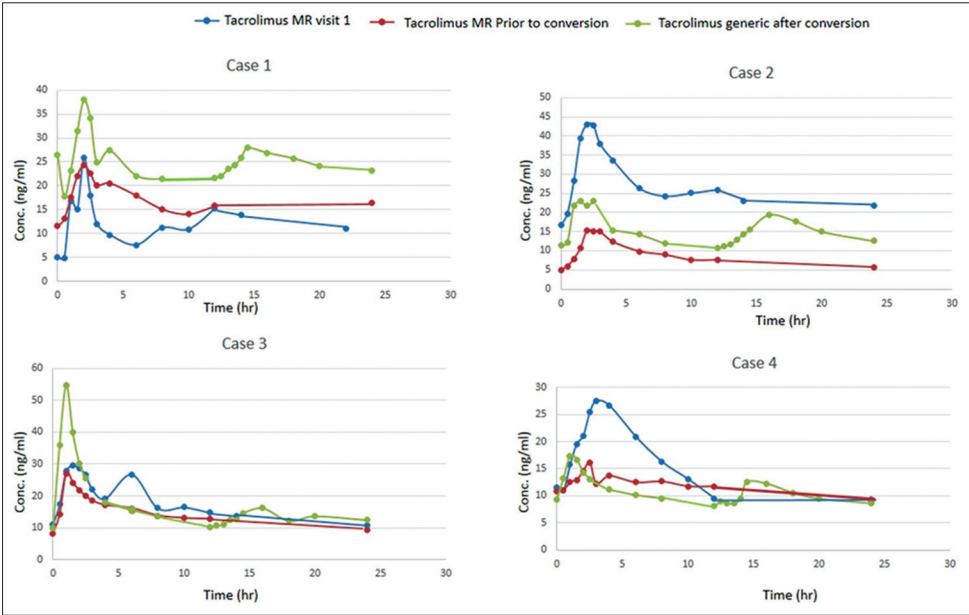


Figure 2: Area under concentration time profile with tacrolimus MR Visit 1, tacrolimus MR prior to conversion and tacrolimus twice daily generic post conversion—four cases (dimensions: 1195 × 754)

Table 1: Dose, AUC_{0-24} and trough measurements prior to after conversion to PanGraf [®] in the stable post transplant period							
Patient identity/sex	Dose of Advagraf [®] and PanGraf [®] (mg/kg)	AUC_{0-24} with Advagraf [®] ($\mu\text{g.h/L}$)	AUC_{0-24} with PanGraf [®] ($\mu\text{g.h/L}$)	Trough with Advagraf [®] (ng/ml)	Trough with PanGraf [®] (ng/ml)	(%) change in trough with PanGraf [®]	(%) change in AUC_{0-24} with PanGraf [®]
1/Male	0.11	401.8	588.4	11.6	26.4	Increase by 56.1%	Increase by 31.7%
2/Male (at 6 month)	0.05	198.6	360.8	5.01	11.5	Increase by 56.4%	Increase by 44.9%
3/Female	0.212	325.7	392	8.2	9.9	Increase by 17.2%	Increase by 16.9%
4/Male	0.063	280.9	250.6	10.8	9.3	Decrease by 16.2%	Decrease by 12.1%

AUC=Area under concentration time curve

interpatient variability of 34.3% to 36.2% for AUC_{0-24} with Advagraf®.^[7]

In the stable 3 month post renal transplant period, tacrolimus AUC_{0-24} with Advagraf® measured between 280.9 and 454.6 µg.h/L. Based on the recommended AUC_{0-12} of tacrolimus of 125 µg.h/L by Scholten *et al.* in the stable post transplant period, the dose of Advagraf® could be further reduced when used in our patients in this period.^[4]

Diez Ojea *et al.* suggested a different conversion rate may be necessary for Advagraf® compared to tacrolimus twice daily in the stable post transplant period.^[8] de Jonge *et al.* reported a reduction above 20% in trough concentration in 38.3% patients when converted on a 1:1 basis to Advagraf®.^[9] Advagraf® produced a lower C_{max} which may have a role to reduce side effects such as diabetes and hyperlipidemia in patients who would benefit with a reduction in the cardiovascular risk factors.^[10]

While conversion from Advagraf® to PanGraf® most likely may improve exposure, in one of our patients (case no. 4), a reduction in AUC_{0-24} was observed with PanGraf®. Because of a difference in the quantum of pharmacokinetic exposure in patients on Advagraf® compared to PanGraf®, a common conversion factor cannot be applied when switching to twice daily generic tacrolimus. However, we recommend that more clinical trials be performed to confirm this finding. This confirms that when converting to PanGraf®, it may be necessary to do an AUC_{0-24} , to assist in dose optimization. In patients without financial constraints and where compliance is an issue, Advagraf® tacrolimus MR may be used with strict monitoring both in the early and stable post transplant period.

**Binu Susan Mathew, Ratna Prabha, Gopal Basu¹,
Pradeep Rajkumar, Veerasamy Tamilarasi¹,
Denise Helen Fleming**

Department of Pharmacology and Clinical Pharmacology, Clinical Pharmacology Unit, ¹Department of Nephrology, Christian Medical College, Vellore, Tamil Nadu, India

Address for correspondence:

Binu Susan Mathew, Clinical Pharmacology Unit,
Christian Medical College Hospital, Vellore, Tamil Nadu, India.
E-mail: binusmathew@yahoo.co.in

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