Chapter V

Results and Discussion

1. Demographic and Pharmacokinetic data

There were 25 patients participated in the study. Eighteen patients received first cadaver, 1 received second cadaver, and 6 received living-related donor renal transplant. The median time after transplantation (range) was 2 years and 7 months (6 months to 8 years and 10 months). Their characteristics are shown in table 5.1 and 5.2. Twelve patients were treated with dual drug regimen (CsA and prednisolone), only 2 patients received CsA as monotherapy while the other 11 patients were treated with triple immunosuppressive therapy; CsA, prednisolone, and azathioprine (8 patients) or mycophenolate mofetil (3 patients). The CsA doses ranged from 100 to 400 mg/day (1.39 to 5.41 mg/kg/day) with a median value of 175 mg/day (3.182 mg/kg/day).

All patients were not smoking and only two patients were drinking occasionally. Twenty four patients (96%) had hypertension as a concomitant disease. Other concomitant diseases are presented in table 5.3.

Table 5.4 displays concomitant used drugs which could cause either pharmacokinetic and pharmacodynamic drug interactions in the patients. There were 15 patients who used a second drug which could effect the CsA pharmacokinetic profile. CsA-sparing agents which had been used include diltiazem (10 patients), verapamil (3 patients), and ketoconazole (2 patients). Drugs which could cause pharmacodymamic drug interactions were detected in 6 patients.

Twenty-five pharmacokinetic profiles were analyzed. Figure 5.1 shows their mean concentration and 95% confidence interval at different time points. It was found that majority of patients (96%) reached the maximum concentration within 2 hours after dosing. The CsA whole blood levels and 12-hour area under the concentration versus time curve (AUC) of the patients were illustrated in table 5.5.

Table 5.6 demonstrates the patients pharmacokinetic parameters. The CsA pharmacokinetic parameter of Thai patients included in this study were compared to those western values (Kovarik et al., 1994; Foradori et al., 1995;

Masri et al., 1996; Rial et al., 1997). The result suggested that oriental pharmacokinetic parameters were not significantly different from those of the western . Therefore, applying proposed pharmacokinetic strategy to monitoring CsA therapy in Thai patients should be feasible.

Two trough levels were measured, before administration (C_0) and 12-hour after drug dosing (C_{12}). Paired T Test was performed to determine any significance different between those two trough levels (Table 5.7). It was shown that both trough levels were not significantly different at $\alpha = 0.05$ with the mean paired difference ($\pm$S.E.) equal to 6.35 ($\pm$5.15). No significantly difference was found between two trough levels, before administration (C_0) and 12-hour after drug dosing (C_{12}), indicated that steady state was reached. Since at steady state, the amount lost in each interval equals to the amount taken, the minimum level at the beginning of the interval should be similar to the level at the end of the interval if C_{12} was significantly higher than C_0, it indicated that accumulation occurred and steady state was not achieved yet.

Table 5.1 Characteristics of patients

Patient number	Gender	Age (years)	Type of donor	Time after transplantation (months)
1	m	40	CD	16
2	f	27	CD	13
3	m	42	CD	30
4	m	53	CD	6
5	m	40	LRD	38
6	m	37	CD	9
7	m	45	CD	10
8	m	36	CD	48
9	f	39	CD	31
10	f	32	CD	105
11	m	36	CD	21
12	m	59	CD	100
13	f	42	CD	71
14	f	42	LRD	8
15	f	41	CD	32
16	m	35	LRD	7
17	f	49	CD	25
18	f	39	LRD	56
19	m	34	LRD	24
20	m	36	CD	15
21	f	33	CD	39
22	f	51	CD	40
23	m	32	CD	74
24	f	35	LRD	12
25	f	42	CD	94
mean (±SE)	f = 12 m = 13	39.88 (±1.45)	CD=19 LRD=6	36.96 (±6.04)

Abbreviations: f = female; m = male; CD = Cadaveric donor; LRD = living related donor

Table 5.2 Characteristics of patients

Patient number	Weight (kg)	Height (cm)	CsA dose (mg/12hr)	CsA dose (mg/kg/12hr)	Immunosuppressive drug regimen
1	70	170	125	3.57	Triple
2	47	155	125	5.32	Triple
3	52	173	100	3.85	Dual
4	72	168	100	2.78	Dual
5	80	167	75	1.88	Dual
6	82	170	88	2.13	Triple
7	74	174	113	3.04	Triple*
8	60	169	100	3.33	Dual
9	55	157	50	1.82	Dual
10	67	157	88	2.61	Triple*
11	75	164	150	4.00	Triple
12	72	178	50	1.39	Mono
13	52	154	75	2.88	Mono
14	63	162	75	2.38	Dual
15	50	163	75	3.00	Dual
16	74	175	200	5.41	Triple
17	52	167	88	3.37	Triple
18	55	158	75	2.73	Dual
19	55	170	88	3.18	Triple
20	65	169	125	3.85	Triple
21	44	158	88	3.98	Dual
22	69	156	75	2.17	Dual
23	47	165	75	3.19	Dual
24	65	165	125	3.85	Dual
25	48	153	113	4.69	Triple*
mean (±SE)	61.8 (±2.27)	164.68 (±1.43)	195 (±12.99)	3.22 (±0.20)	Mono = 2 Dual = 12 Triple = 8 Triple*= 3

Abbreviations: Mono = Monotherapy with cyclosporin (CsA); Dual = Dualtherapy with CsA + Steroid; Triple = Tripletherapy with CsA + Steroid + Azathioprine; Triple = Tripletherapy with CsA + Steroid + Mycophenolate mofetil*

Table 5.3 Social habit and concomitant diseases of patients

Patient number	Smoking habit	Drinking habit	Concomitant disease
1	-	-	HTN
2	-	-	HTN, Hyperlipidemia
3	-	-	HTN
4	-	-	HTN, IDDM
5	-	-	HTN
6	-	-	HTN
7	-	-	HTN
8	-	-	HTN, Hyperuricemia
9	-	-	HTN
10	-	-	HTN
11	-	-	HTN
12	-	-	HTN
13	-	-	HTN
14	-	Occasionally	HTN
15	-	-	-
16	-	-	HTN
17	-	-	HTN
18	-	Occasionally	HTN
19	-	-	HTN
20	-	-	HTN, IDDM
21	-	-	HTN
22	-	-	HTN
23	-	-	HTN
24	-	-	HTN
25	-	-	HTN

Abbreviations: HTN = Hypertension; IDDM = Insulin Dependent Diabetes Mellitus

Table 5.4 Pharmacokinetic and pharmacodynamic drug interactions

Patient number	Pharmacokinetic Drug interaction (s)	Pharmacodynamic Drug interaction (s)
1	-	-
2	Diltiazem	Simvastatin
3	Diltiazem	-
4	Diltiazem	-
5	-	-
6	Diltiazem	Acyclovir
7	Diltiazem	Enalapril + Minoxidil
8	Diltiazem	-
9	Ketoconazole	-
10	Verapamil	Enalapril
11	-	Enalapril
12	Verapamil	-
13	Verapamil	-
14	Diltiazem	-
15	-	-
16	-	Minoxidil
17	Diltiazem	-
18	Ketoconazole	-
19	-	-
20	-	-
21	-	-
22	Diltiazem	-
23	Diltiazem	-
24	-	-
25	-	-

Fig 5.1 The mean concentration at different time points.
The bars represent 95% Confident Interval.

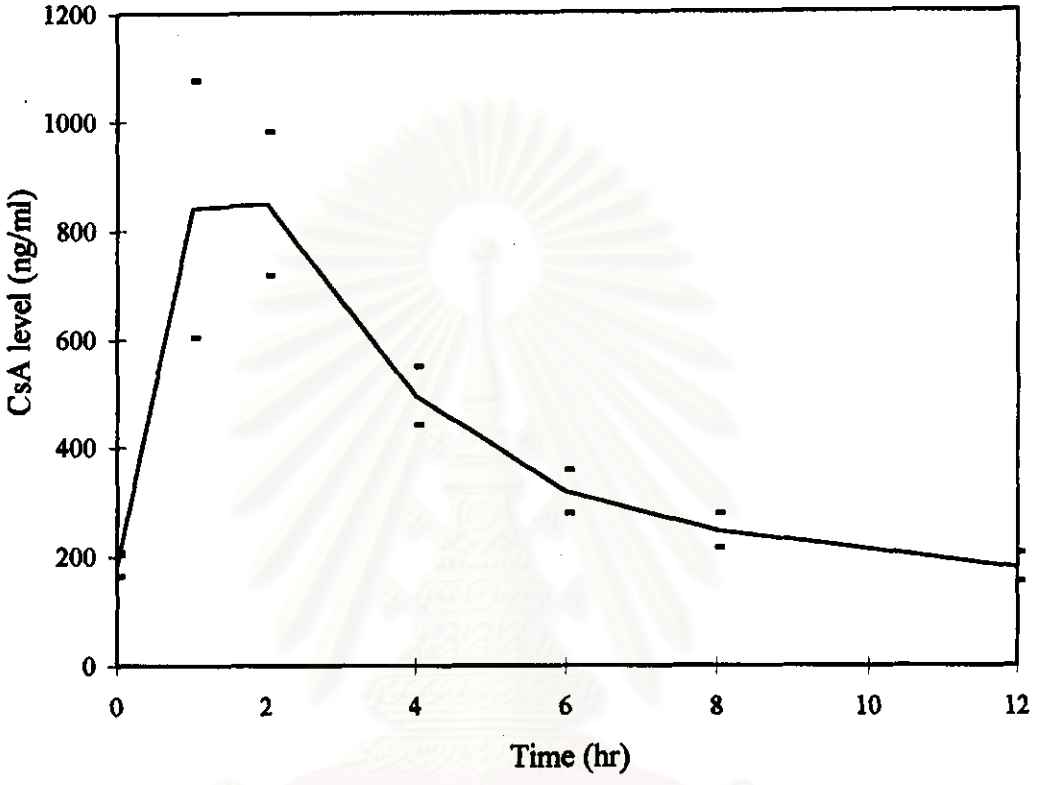

Table 5.5 Cyclosporin(CsA) dose, whole blood levels, 12-hr AUC, and C_{ssav} of patients

Patient number	CsA dose (mg/12hr)	CsA dose (mg/kg/12hr)	C_0 (ng/ml)	C_1 (ng/ml)	C_2 (ng/ml)	C_4 (ng/ml)	C_6 (ng/ml)	C_8 (ng/ml)	C_{12} (ng/ml)	AUC (ng*hr/ml)	Cssav (ng/ml)
1	125	3.57	187.79	510.30	1062.23	679.76	-	293.72	203.43	5735.040	477.92
2	125	5.32	209.67	405.39	1129.65	506.65	319.03	314.90	191.22	5183.200	431.93
3	100	3.85	263.12	233.77	351.26	697.67	468.65	294.89	217.21	4543.950	378.66
4	100	2.78	327.50	344.43	1084.69	712.15	440.53	380.34	349.88	6281.355	523.45
5	75	1.88	140.89	238.42	592.29	493.07	246.11	172.29	122.42	3437.370	286.45
6	88	2.13	147.35	504.26	758.01	421.94	302.03	249.12	182.84	4275.930	356.33
7	113	3.04	174.11	262.33	588.86	584.73	313.22	233.36	159.87	4048.395	337.37
8	100	3.33	183.71	1476.98	1555.92	581.14	383.82	280.89	185.81	7046.925	587.24
9	50	1.82	174.64	301.86	674.38	566.60	319.35	258.14	189.12	4325.310	360.44
10	88	2.61	201.55	1101.74	1147.98	446.06	282.47	252.18	198.63	5535.345	461.28
11	150	4.00	187.00	604.77	844.42	481.96	498.03	274.71	156.61	5062.231	421.85
12	50	1.39	138.61	363.81	373.71	347.76	197.13	151.51	116.13	2770.250	230.85
13	75	2.88	152.65	1391.64	717.65	351.65	263.13	191.75	146.01	4641.270	386.77
14	75	2.38	210.99	1043.53	868.74	511.09	315.90	256.17	186.27	5247.165	437.26
15	75	3.00	195.86	340.03	553.29	458.87	329.56	260.50	171.56	3969.375	330.78
16	200	5.41	238.09	1535.46	1129.04	591.61	352.36	255.13	189.38	6380.155	531.68
17	88	3.37	153.75	1815.30	1395.03	509.11	332.68	270.69	185.33	6851.030	570.92
18	75	2.73	310.80	1470.84	1285.67	780.47	564.55	488.50	370.86	8452.005	704.33
19	88	3.18	163.53	-	583.68	445.37	245.13	203.86	135.46	3632.378	302.70
20	125	3.85	146.06	-	596.78	433.69	296.79	203.74	137.19	3843.660	320.31
21	88	3.98	183.34	1846.38	1112.64	414.61	288.47	222.47	158.64	5997.860	499.82
22	75	2.17	168.33	1112.29	642.31	313.29	224.93	187.58	152.50	4104.100	342.01
23	75	3.19	135.90	565.94	538.76	358.12	227.29	158.92	120.89	3331.390	277.62
24	125	3.85	136.81	602.89	772.60	369.19	227.67	172.47	137.93	3817.185	318.10
25	113	4.69	96.69	1245.60	862.80	297.79	184.73	132.67	104.91	4161.015	346.75

Abbreviations and symbols: Cx = x hour(s) after CsA administration; Cssav = AUC/τ ; - = missing value

Table 5.6 The pharmacokinetic parameters of all 25 patients.

Patient number	tmax (hr)	Cmax (ng/ml)	Cmax/dose (ng/ml/mg)	CL/F (l/hr)	Vd/F (l)	β (hr^{-1})	$T_{1/2}$ (hr)
1	2	1062.23	8.50	21.7958	204.85	.10640	6.51
2	2	1129.65	9.04	24.1164	293.03	.08230	8.42
3	4	697.67	6.98	22.0073	160.75	.13690	5.06
4	2	1084.69	10.85	15.9201	423.41	.03760	18.43
5	2	592.29	7.90	21.8190	180.02	.12120	5.72
6	2	758.01	8.66	20.4634	243.32	.08410	8.24
7	2	588.86	5.23	27.7888	242.06	.11480	6.04
8	2	1555.92	15.56	14.1906	114.35	.12410	5.58
9	2	674.38	13.49	11.5599	131.21	.08810	7.87
10	2	1147.98	13.12	15.8075	269.29	.05870	11.81
11	2	844.42	5.63	29.6312	139.57	.21230	3.26
12	2	373.71	7.47	18.0489	200.77	.08990	7.71
13	1	1391.64	18.56	16.1594	159.52	.10130	6.84
14	1	1043.53	13.91	14.2934	160.96	.08880	7.80
15	2	553.29	7.38	18.8947	172.55	.10950	6.33
16	1	1535.46	7.68	31.3472	293.24	.10690	6.48
17	1	1815.30	20.63	12.8448	131.34	.09780	7.09
18	1	1470.84	19.61	8.8736	126.59	.07010	9.89
19	2	583.68	6.63	24.2266	246.20	.09840	7.04
20	2	596.78	4.77	32.5211	241.97	.13440	5.16
21	1	1846.38	21.10	14.5885	143.87	.10140	6.83
22	1	1112.29	14.83	18.2744	281.14	.06500	10.66
23	1	565.94	7.55	22.5131	205.41	.10960	6.32
24	2	772.60	6.18	32.7466	383.90	.08530	8.12
25	1	1245.60	11.07	27.0367	277.30	.09750	7.11
mean (±SE)	1.72 (±0.14)	1001.73 (±84.26)	10.89 (±1.01)	20.70 (±1.35)	219.92 (±15.67)	0.10 (±.01)	7.68 (±0.56)

Abbreviations: tmax = Time to maximum concentration; Cmax = Maximum concentration; CL/F = Clearance/Bioavailability; Vd/F = Volume of distribution/Bioavailability; β = Elimination rate constant; $T_{1/2}$ = Half-Life

Table 5.7 Comparison the trough level measured just before drug administration to the trough level measured twelve hours after drug administration

Patient number	C_0 (ng/ml)	C_{12} (ng/ml)	Difference (ng/ml)
1	187.79	203.43	-15.64
2	209.67	191.22	18.45
3	263.12	217.21	45.91
4	327.50	349.88	-22.38
5	140.89	122.42	18.47
6	147.35	182.84	-35.49
7	174.11	159.87	14.24
8	183.71	185.81	-2.10
9	174.64	189.12	-14.48
10	201.55	198.63	2.92
11	187.00	156.61	30.39
12	138.61	116.13	22.48
13	152.65	146.01	6.64
14	210.99	186.27	24.72
15	195.86	171.56	24.30
16	238.09	189.38	48.71
17	153.75	185.33	-31.58
18	310.80	370.86	-60.06
19	163.53	135.46	28.07
20	146.06	137.19	8.87
21	183.34	158.64	24.70
22	168.33	152.50	15.83
23	135.90	120.89	15.01
24	136.81	137.93	-1.12
25	96.69	104.91	-8.22
mean (±SE)	185.15 (± 10.75)	178.80 (± 12.48)	6.346 (± 5.146)

$t_{difference} = 1.23$ $(p = 0.229)$

$t_{.05, 24} = 2.064$

2. Optimum sampling time points for predicting CsA AUC

2.1 Correlation between single blood levels and CsA AUC

The correlation coefficients between the sampling time concentrations and AUC was demonstrated in table 5.8. Our result confirmed the previous reports that trough level was not the best representative value for total drug exposure over the dosing interval (r^2=0.6469) (Serino et al., 1994; Foradori et al., 1995; Tsang et al., 1996; Serafinowicz, et al, 1996; Amante and Kahan, 1996; Rial, 1997).

Besides, no single-point CsA concentration within the seven-point profile could explain more than 90% of the variability described by the measured AUC calculated by the trapezoidal rule using all seven data points. The single blood concentration that showed the best correlation with AUC was the level measured at 2 hours after drug administration (r^2=0.8845).

Table 5.8 Correlation coefficients between single CsA level and AUC[a] of all 25 patients

	12-hr AUC (ng*hr/ml)
C_0	0.6469 (P<0.05)
C_1	0.6289 (P<0.01)
C_2	0.8845 (P<0.01)
C_4	0.6424 (P<0.01)
C_6	0.6901 (P<0.01)
C_8	0.7885 (P<0.01)
C_{12}	0.7416 (P<0.01)
AUC	1.0000 (P<0.01)
C_{ssav}	1.0000 (P<0.01)

[a] Pearson product-moment correlation coefficients (P value)

2.2 Multiple linear regression analysis

Table 5.9 presented the model equation employ 1, 2, 3, or 4 time points that showed the highest correlation coefficient to the observed AUC value. Single time point, namely at 2 hour after dosing, estimate correlation which was lower than 0.9 (r^2=0.8845). Stepwise multiple linear regression analyze showed that using two time points, namely at 2 and 6 hour after administration, appreciably improved the correlation (r^2=0.9638) and prediction accuracy. These two sampling times were the same as those proposed by Kahan et al. (1995). Using three or four time points slightly improved the correlation coefficients (r^2=0.9823, and r^2=0.9959, respectively). The predicted AUC from those equations are displayed in table 5.10.

Table 5.9 The model equations and optimum sampling time points chosen by stepwise multiple linear regression analysis

No. of time points	Selected time points (hr after dosing)	Model equations: Predicted 12 hr-AUC =	r^2	Absolute prediction error (%) Mean ± SE
1	2	$3.727C_2 + 1760.619$	0.8845	9.10 ± 1.64
2	2,6	$3.085C_2 + 6.019C_6 + 376.893$	0.9638	5.40 ± 0.88
3	1,2,6	$0.738C_1 + 2.112C_2 + 7.02C_6 + 263.108$	0.9823	3.01 ± 0.81
4	1,2,6,12	$0.858C_1 + 1.725C_2 + 4.375C_6 + 5.974C_{12} + 261.108$	0.9959	2.20 ± 0.33

Table 5.10 Comparison of measured AUC and predicted AUC calculated by regression equations

Patient Number	Measured AUC (ng/ml)	Predicted AUC_2 (prediction error)	Predicted $AUC_{2,6}$ (prediction error)	Predicted $AUC_{1,2,6}$ (prediction error)	Predicted $AUC_{1,2,6,12}$ (prediction error)
1	5735.040	5719.55 (-0.27)	-	-	-
2	5163.200	5970.82 (15.20)	5782.10 (11.55)	5187.70 (0.09)	5095.68 (-1.69)
3	4543.950	3069.77 (-32.44)	4281.33 (-5.78)	4467.41 (-1.68)	4415.56 (-2.83)
4	6281.355	5803.26 (-7.61)	6374.71 (1.49)	5900.68 (-6.06)	6445.22 (2.61)
5	3437.370	3968.08 (15.44)	3685.44 (7.22)	3417.67 (-0.57)	3295.44 (-4.13)
6	4275.930	4585.72 (7.25)	4533.27 (6.02)	4356.42 (1.88)	4415.00 (3.25)
7	4048.395	3955.30 (-2.30)	4078.80 (0.75)	3899.18 (-3.69)	3827.37 (-5.46)
8	7046.925	7559.53 (7.27)	7487.12 (6.25)	7333.64 (4.07)	7001.56 (-0.64)
9	4325.310	4274.03 (-1.19)	4379.52 (1.25)	4152.01 (-4.01)	4210.37 (-2.66)
10	5535.345	6039.14 (9.10)	5618.60 (1.50)	5483.67 (-0.93)	5609.09 (1.33)
11	5062.231	4907.77 (-3.05)	5979.57 (18.12)	5989.01 (18.31)	5351.09 (5.71)
12	2770.250	3153.44 (13.83)	2716.31 (-1.95)	2704.73 (-2.37)	2774.11 (0.14)
13	4641.270	4435.32 (-4.44)	4174.64 (-10.05)	4653.00 (0.25)	4716.55 (1.62)
14	5247.165	4998.41 (-4.74)	4958.36 (-5.50)	5085.63 (-3.08)	5149.87 (-1.85)
15	3969.375	3822.73 (-3.69)	4067.41 (2.47)	3996.11 (0.67)	3974.00 (0.12)
16	6380.155	5968.55 (-6.45)	5980.84 (-6.26)	6254.38 (-1.97)	6199.06 (-2.84)
17	6851.030	6959.90 (1.59)	6682.96 (2.45)	6884.52 (0.49)	6787.70 (-0.92)
18	8452.005	6552.31 (-22.48)	7741.21 (-8.41)	8027.06 (-5.03)	8426.29 (-0.30)
19	3632.378	3935.99 (8.36)	3652.98 (0.57)	-	-
20	3843.660	3984.82 (3.67)	4004.34 (4.18)	-	-
21	5997.860	5907.43 (-1.51)	5545.69 (-7.54)	6000.69 (0.05)	5974.38 (-0.39)
22	4104.100	4154.51 (1.23)	3712.27 (-9.55)	4019.55 (-2.06)	4218.54 (2.79)
23	3331.390	3768.58 (13.12)	3407.03 (2.27)	3414.21 (2.49)	3392.64 (1.84)
24	3817.185	4640.10 (21.56)	4130.71 (8.21)	3938.02 (3.17)	3931.17 (2.99)
25	4161.015	4976.27 (19.59)	4150.52 (-0.25)	4301.40 (3.37)	4253.09 (2.21)
AUC (Mean±SE)	4906.96 ± 271.96	4924.45 ± 237.47	4880.24 ± 269.98	4975.76 ± 292.06	4975.63 ± 296.08
Absolute prediction error (Mean±SE)	-	9.10 ± 1.64	5.40 ± 0.88	3.01 ± 0.81	2.20 ± 0.33

2.3 Linear trapezoidal rule

1) Select 1 and 2 sampling time points

Table 5.11 showed the trapezoidal equation and the prediction error of the AUC predicted from 1 and 2 sampling times as compared to the observed AUC (calculated from 7 sampling time points). Because at steady state $C_{min,ohr}$ was considered to be nearly equal to $C_{min,12hr}$, to decrease the number of blood samples, only one trough level ($C_{min,ohr}$) was chosen. It can be seen that the single concentration at 2 hours post dose could predict AUC with least prediction error. Besides the predicted AUC from this time point correlated best with the actual AUC, which was not surprising since blood sample collected at 2 hours post dose was the peak level and the peak level is theoretically known to be correlated well with AUC.

Table 5.12 displayed comparison between the observed AUC and the predicted trapezoidal AUC which were calculated from 1 and 2 sampling time points of each individual patient.

Table 5.11 The prediction error of the predicted AUC from 1 and 2 time points using trapezoidal rule from the measured AUC

Selected time points (hr after dosing)	Trapezoidal Equations: Predicted 12 hr-AUC =	Absolute prediction error (%) Mean ± SE	r^2
0	$C_0 * 12$	53.68 ± 2.03	0.6469
1	$0.5*C_1*12$	44.66 ± 5.28	0.6289
2	$0.5*C_2*12$	13.68 ± 2.50	0.8845
4	$0.5*C_4*12$	37.86 ± 2.89	0.6424
6	$0.5*C_6*12$	60.16 ± 1.81	0.6901
8	$0.5*C_8*12$	69.59 ± 1.10	0.7885
0,2	$0.5*2*(C_0+C_2) + 0.5*10*(C_2+C_0)$	26.81 ± 2.45	0.9298
1,2	$0.5*1*C_1 + 0.5*1*(C_1+C_2) + 0.5*10*C_2$	19.15 ± 3.17	0.8730
2,4	$0.5*2*C_2 + 0.5*2*(C_2+C_4) + 0.5*8*C_4$	14.77 ± 1.66	0.9221
2,6	$0.5*2*C_2 + 0.5*4*(C_2+C_6) + 0.5*6*C_6$	15.92 ± 1.33	0.9629
2,8	$0.5*2*C_2 + 0.5*6*(C_2+C_8) + 0.5*4*C_8$	9.47 ± 1.57	0.9442

Table 5.12 Comparison of measured AUC and predicted AUC calculated from 1 and 2 time-point trapezoidal rule.

Patient Number	Measured AUC (ng/ml)	Predicted AUC_0 (prediction error)	Predicted AUC_1 (prediction error)	Predicted AUC_2 (prediction error)	Predicted AUC_4 (prediction error)	Predicted AUC_6 (prediction error)	Predicted AUC_8 (prediction error)
1	5735.040	2253.48 (-60.71)	3061.80 (-46.61)	6373.38 (11.13)	4078.56 (-28.88)	-	1762.32 (-69.27)
2	5183.200	2516.04 (-51.46)	2432.34 (-53.07)	6777.90 (30.77)	3039.90 (-41.35)	1914.18 (-63.07)	1889.40 (-63.55)
3	4543.950	3157.44 (-30.51)	1402.62 (-69.13)	2107.56 (-53.62)	4186.02 (-7.88)	2811.90 (-38.12)	1769.34 (-61.06)
4	6281.355	3930.00 (-37.43)	2066.58 (-67.10)	6508.14 (3.61)	4272.90 (-31.97)	2643.18 (-57.92)	2282.04 (-63.67)
5	3437.370	1690.68 (-60.81)	1430.52 (-58.38)	3553.74 (3.39)	2958.42 (-13.93)	1476.66 (-57.04)	1033.74 (-69.93)
6	4275.930	1768.20 (-58.65)	3025.56 (-29.24)	4548.06 (6.36)	2531.64 (-40.79)	1812.18 (-57.62)	1494.72 (-65.04)
7	4048.395	2089.32 (-48.39)	1573.98 (-61.12)	3533.16 (-12.73)	3508.38 (-13.34)	1879.32 (-53.58)	1400.16 (-65.41)
8	7046.925	2204.52 (-68.72)	8861.88 (25.76)	9335.52 (32.48)	3486.84 (-50.52)	2302.92 (-67.32)	1685.34 (-76.08)
9	4325.310	2095.68 (-51.55)	1811.16 (-58.13)	4046.28 (-6.45)	3399.60 (-21.40)	1916.10 (-55.70)	1548.84 (-64.19)
10	5535.345	2418.60 (-56.31)	6610.44 (19.42)	6887.88 (24.43)	2676.36 (-51.65)	1694.82 (-69.38)	1513.08 (-72.67)
11	5082.231	2244.00 (-55.67)	3628.62 (-28.32)	5066.52 (0.08)	2891.76 (-42.88)	2988.18 (-40.97)	1648.26 (-67.44)
12	2770.250	1663.32 (-39.96)	2182.86 (-21.20)	2242.26 (-19.06)	2086.56 (-24.68)	1182.78 (-57.30)	909.06 (-67.18)
13	4641.270	1831.80 (-60.53)	8349.84 (79.90)	4305.92 (-7.23)	2109.90 (-54.54)	1578.78 (-65.98)	1150.50 (-75.21)
14	5247.165	2531.88 (-51.75)	6261.18 (19.33)	5212.44 (-0.66)	3066.54 (-41.56)	1895.40 (-63.88)	1537.02 (-70.71)
15	3969.375	2350.32 (-40.79)	2040.18 (-48.60)	3319.74 (-16.37)	2753.22 (-30.64)	1977.36 (-50.18)	1563.00 (-60.62)
16	6380.155	2857.08 (-55.22)	9212.76 (44.40)	6774.24 (6.18)	3549.66 (-44.36)	2114.16 (-66.86)	1530.78 (-76.01)
17	6851.030	1845.00 (-73.07)	10891.80(58.98)	8370.18 (22.17)	3054.66 (-55.41)	1996.08 (-70.86)	1624.14 (-76.29)
18	8452.005	3729.60 (-55.87)	8825.04 (4.41)	7714.02 (-8.73)	4682.82 (-44.60)	3387.30 (-59.92)	2931.00 (-65.32)
19	3632.378	1962.36 (-45.98)	-	3502.08 (-3.59)	2672.22 (-26.43)	1470.78 (-59.51)	1223.16 (-66.33)
20	3843.660	1752.72 (-54.40)	-	3580.68 (-6.84)	2602.14 (-32.30)	1780.74 (-53.67)	1222.44 (-68.20)
21	5997.860	2200.08 (-63.32)	11078.28(84.70)	6675.84 (11.30)	2487.66 (-58.52)	1730.82 (-71.14)	1334.82 (-77.75)
22	4104.100	2019.96 (-50.78)	6673.74 (62.61)	3853.86 (-6.10)	1879.74 (-54.20)	1349.58 (-67.12)	1125.48 (-72.58)
23	3331.390	1630.80 (-51.05)	3395.64 (1.93)	3232.56 (-2.97)	2148.72 (-35.50)	1363.74 (-59.06)	953.52 (-71.38)
24	3817.185	1641.72 (-56.99)	3617.34 (-5.24)	4635.60 (21.44)	2215.14 (-41.97)	1366.02 (-64.21)	1034.82 (-72.89)
25	4161.015	1160.28 (-72.12)	7473.60 (79.61)	5176.80 (24.41)	1786.74 (-57.06)	1108.38 (-73.36)	796.02 (-80.87)
AUC (Mean ±SE)	4906.96 ± 271.96	2221.80 ± 128.96	5039.47 ± 684.02	5093.37 ± 382.29	2965.04 ± 155.88	1905.89 ± 117.28	1478.52 ± 91.76
Absolute prediction error (Mean ±SE)	-	53.68 ± 2.03	44.66 ± 5.28	13.68 ± 2.50	37.86 ± 2.89	60.16 ± 1.81	69.59 ± 1.10

Table 5.12 Comparison of measured AUC and predicted AUC calculated from 1 and 2 time-point trapezoidal rule (Continuing)

Patient Number	Measured AUC (ng/ml)	Predicted $AUC_{0,2}$ (prediction error)	Predicted $AUC_{1,2}$ (prediction error)	Predicted $AUC_{2,4}$ (prediction error)	Predicted $AUC_{2,6}$ (prediction error)	Predicted $AUC_{2,8}$ (prediction error)
1	5735.040	7500.12 (30.78)	6352.57 (10.77)	5523.26 (-3.69)	-	5717.52 (-0.31)
2	5183.200	8035.92 (55.04)	6618.47 (27.69)	4792.55 (-7.54)	4984.10 (-3.84)	6093.10 (17.55)
3	4543.950	3686.28 (-18.87)	2165.70 (-52.34)	4190.87 (-7.77)	3397.03 (-25.24)	2879.49 (-36.63)
4	6281.355	8473.14 (34.89)	6310.23 (0.46)	5730.13 (-8.78)	5456.72 (-13.13)	6240.46 (-0.65)
5	3437.370	4399.08 (27.98)	3496.02 (1.71)	3649.93 (6.18)	3007.42 (-12.51)	3230.61 (-6.02)
6	4275.930	5432.16 (27.04)	4673.32 (9.29)	3625.72 (-15.21)	3784.18 (-11.50)	4277.64 (0.04)
7	4048.395	4577.82 (13.08)	3501.06 (-13.52)	4101.37 (1.31)	3332.68 (-17.68)	3522.24 (-13.00)
8	7046.925	10437.78(48.12)	10034.54(42.40)	6017.54 (-14.61)	6586.86 (-6.53)	7628.13 (8.25)
9	4325.310	5094.12 (17.77)	4010.95 (-7.27)	4181.76 (-3.32)	3619.89 (-16.31)	3988.22 (-7.79)
10	5535.345	8097.18 (46.28)	7415.63 (33.97)	4526.26 (-18.23)	4856.29 (-12.27)	5852.82 (5.74)
11	5062.231	6188.52 (22.25)	5249.08 (3.69)	4098.64 (-19.03)	5023.41 (-0.77)	4751.23 (-6.14)
12	2770.250	3073.92 (10.96)	2419.22 (-12.6)7	2486.22 (-10.25)	2106.78 (-23.95)	2252.39 (-18.69)
13	4641.270	5221.82 (12.51)	5338.74 (15.03)	3193.56 (-31.19)	3468.61 (-25.27)	3829.37 (-17.49)
14	5247.165	6478.38 (23.46)	5821.60 (10.95)	4292.93 (-18.19)	4185.72 (-20.23)	4755.81 (-9.36)
15	3969.375	4494.90 (13.24)	3383.13 (-14.77)	3400.93 (-14.32)	3307.67 (-16.67)	3515.66 (-11.43)
16	6380.155	8202.78 (28.57)	7745.18 (21.39)	5216.13 (-18.24)	5148.92 (-19.30)	5791.81 (-9.22)
17	6851.030	9292.68 (35.64)	9487.97 (38.49)	5335.61 (-22.12)	5848.49 (-14.63)	6933.57 (1.20)
18	8452.005	9578.82 (13.33)	8542.03 (1.07)	6473.69 (-23.41)	6679.76 (-20.97)	7585.18 (-10.26)
19	3632.378	4483.26 (23.42)	-	3394.21 (-6.56)	2976.69 (-18.05)	3354.02 (-7.66)
20	3843.660	4457.04 (15.96)	-	3362.01 (-12.53)	3274.29 (-14.81)	3405.82 (-11.39)
21	5997.860	7775.88 (29.64)	7965.90 (32.81)	4298.33 (-28.34)	4780.27 (-20.30)	5562.91 (-7.25)
22	4104.100	4863.84 (18.51)	4645.00 (13.18)	2851.07 (-30.53)	3051.58 (-25.65)	3507.14 (-14.55)
23	3331.390	4047.96 (21.51)	3529.12 (5.94)	2868.12 (-13.91)	2752.73 (-17.37)	2949.64 (-11.46)
24	3817.185	5456.46 (42.94)	4852.19 (27.11)	3391.15 (-11.16)	3456.15 (-9.46)	3952.75 (3.55)
25	4161.015	5756.94 (38.35)	5991.00 (43.98)	3214.55 (-22.75)	3512.05 (-15.60)	4114.55 (-1.12)
AUC (Mean ±SE)	4906.96 ± 271.96	6204.27 ± 408.52	5632.55 ± 453.82	4168.66 ± 212.72	4108.26 ± 250.86	4627.68 ± 302.62
Absolute prediction error (Mean ±SE)	-	26.81 ± 2.45	19.15 ± 3.17	14.77 ± 1.68	15.92 ± 1.33	9.47 ± 1.57

The result from table 5.11 and 5.12 indicated that sampling blood levels at 2 and 8 hours after CsA administration and predicted AUC using trapezoidal rule accordingly to these two blood samples would result in least prediction error. However, when considering the correlation between the measured and the predicted trapezoidal AUC using two sampling time points in table 5.11, the result indicated that blood level collected at 2 and 6 hours after dosing could predict AUC which were most correlated to the observed AUC.

These non-parallel results could partly be explained by the known error when applying trapezoidal rule to calculate AUC (appendix A), i.e., during the absorption phase of the blood level-time curve, the convex portion of the AUC will be excluded by the trapezoid while during the distribution and the elimination phase of the blood level-time curve (figure 1), extra area in the concave portion of the curve will be included by the trapezoid. These minus and plus error areas could be best counter balance simultaneously when the blood sample was collected at 8 hours after CsA administration resulted in least AUC prediction error with the 2, 8 sample pair even though the predicted AUC from this sample pair was not actually correlated best with the actual AUC.

In contrary, the 2, 6 blood sample pair will result in lesser plus area than minus area because the concave portion of the blood level-time curve was small when compared with the convex portion, therefore the prediction error was higher than the 2, 8 blood sample pair even though this blood sample pair correlated best with the observed AUC.

2) Select 3 sampling time points

Three sampling time points were selected in the same manner as previous section. Table 5.13 displayed the correlation between the measured AUC and the predicted AUC from 3 time-point using trapezoidal rule. The levels which showed the best correlation with AUC were 0, 2, and 6 hours after dosing. Table 5.14 demonstrated the AUC which were estimated by 3 time-point trapezoidal rule.

From figure 5.1, one could see that the second hour after CsA administration was the peak time and the sixth hour after drug administration was the time where the blood level-time curve turned from the distribution phase into the elimination phase. Trapezoids calculated at these time points will result in predicted AUC which was least affected by the error caused by the convex and concave of the blood level-time curve, therefore, the 0, 2, 6 time points could be used to predict trapezoidal AUC with least prediction error as shown in table 5.13 and table 5.14. At the same time, the 0, 2, 6 time points also correlated most with the measured AUC due to the same reason.

Table 5.13 The prediction error of the predicted AUC from 3 time points using trapezoidal rule from the measured AUC

Selected time points (hr after dosing)	Trapezoidal Equations: Predicted 12 hr-AUC =	Absolute prediction error (%) Mean ± SE	r^2
0,1,2	$0.5*1*(C_0+C_1) + 0.5*1*(C_1+C_2) + 0.5*10*(C_2+C_0)$	33.28 ± 3.10	0.9213
0,2,4	$0.5*2*(C_0+C_2) + 0.5*2*(C_2+C_4) + 0.5*8*(C_4+C_0)$	10.50 ± 1.48	0.9118
0,2,6	$0.5*2*(C_0+C_2) + 0.5*4*(C_2+C_6) + 0.5*6*(C_6+C_0)$	4.94 ± 0.81	0.9695
0,2,8	$0.5*2*(C_0+C_2) + 0.5*6*(C_2+C_8) + 0.5*4*(C_8+C_0)$	7.65 ± 1.46	0.9578
1,2,4	$0.5*1*C_1 + 0.5*1*(C_1+C_2) + 0.5*2*(C_2+C_4) + 0.5*8*C_4$	7.38 ± 0.84	0.9835
1,2,6	$0.5*1*C_1 + 0.5*1*(C_1+C_2) + 0.5*4*(C_2+C_6) + 0.5*6*C_6$	9.07 ± 1.41	0.9645
1,2,8	$0.5*1*C_1 + 0.5*1*(C_1+C_2) + 0.5*6*(C_2+C_8) + 0.5*4*C_8$	9.81 ± 1.69	0.9422
2,4,6	$0.5*2*C_2 + 0.5*2*(C_2+C_4) + 0.5*2*(C_4+C_6) + 0.5*6*C_6$	18.93 ± 1.40	0.9578
2,4,8	$0.5*2*C_2 + 0.5*2*(C_2+C_4) + 0.5*4*(C_4+C_8) + 0.5*4*C_8$	14.61 ± 1.54	0.9458
2,6,8	$0.5*2*C_2 + 0.5*4*(C_2+C_6) + 0.5*2*(C_6+C_8) + 0.5*4*C_8$	14.17 ± 1.29	0.9653

Table 5.14 Comparison of measured AUC and predicted AUC calculated from 3 time-point trapezoidal rule.

Patient Number	Measured AUC (ng/ml)	Predicted $AUC_{0,1,2}$ (prediction error)	Predicted $AUC_{0,2,4}$ (prediction error)	Predicted $AUC_{0,2,6}$ (prediction error)	Predicted $AUC_{0,2,8}$ (prediction error)	Predicted $AUC_{1,2,4}$ (prediction error)
1	5735.040	7385.41 (28.78)	6462.21 (12.68)	-	6280.89 (9.52)	5502.45 (-4.06)
2	5183.200	7771.65 (49.94)	5840.90 (12.69)	5822.78 (12.34)	6722.11 (29.69)	4633.12 (-10.61)
3	4543.950	3612.86 (-20.49)	5506.47 (21.18)	4449.51 (-2.08)	3668.85 (-19.26)	4249.01 (-6.49)
4	6281.355	8111.48 (29.14)	7367.63 (17.29)	6766.72 (7.73)	7222.96 (14.99)	5532.22 (-11.93)
5	3437.370	4270.91 (24.25)	4354.38 (26.68)	3570.98 (3.89)	3653.28 (6.28)	3592.21 (4.50)
6	4275.930	5483.74 (28.25)	4362.47 (2.02)	4373.58 (2.28)	4719.69 (10.38)	3750.98 (-12.28)
7	4048.395	4458.67 (10.13)	4971.92 (22.81)	4029.12 (-0.48)	4044.57 (-0.09)	4069.27 (0.52)
8	7046.925	11044.95 (56.73)	6936.09 (-1.57)	7321.70 (3.90)	8179.26 (16.07)	6716.56 (-4.69)
9	4325.310	4971.47 (14.94)	5054.96 (16.87)	4318.45 (-0.16)	4512.14 (4.32)	4146.43 (-4.14)
10	5535.345	8524.16 (54.00)	5534.01 (-0.02)	5662.49 (2.30)	6457.47 (16.66)	5054.01 (-8.70)
11	5062.231	6277.58 (24.01)	5033.64 (-0.56)	5771.41 (14.01)	5312.23 (4.94)	4281.20 (-15.43)
12	2770.250	3181.57 (14.85)	3179.27 (14.76)	2661.22 (-3.94)	2668.22 (-3.68)	2663.18 (-3.87)
13	4641.270	6178.31 (33.12)	3956.81 (-14.75)	4079.21 (-12.11)	4287.32 (-7.63)	4226.37 (-8.94)
14	5247.165	6982.05 (33.06)	5347.88 (1.92)	5029.68 (-4.14)	5388.78 (2.70)	4902.09 (-6.58)
15	3969.375	4460.36 (12.37)	4380.23 (10.35)	4091.11 (3.07)	4103.24 (3.37)	3464.32 (-12.72)
16	6380.155	9054.68 (41.92)	6406.58 (0.41)	6101.28 (-4.37)	6506.08 (1.97)	6187.07 (-3.03)
17	6851.030	10333.59 (50.83)	6104.36 (-10.90)	6463.49 (-5.66)	7394.62 (7.94)	6453.40 (-5.80)
18	8452.005	10251.43 (21.29)	8027.69 (-5.02)	7922.96 (-6.26)	8517.58 (0.78)	7301.70 (-13.61)
19	3632.378	-	4211.86 (15.95)	3630.81 (-0.04)	3844.61 (5.84)	-
20	3843.660	-	4092.31 (6.47)	3858.53 (0.39)	3844.00 (0.01)	-
21	5997.860	8974.27 (49.62)	5215.03 (-13.05)	5513.63 (-8.07)	6112.93 (1.92)	5588.39 (-6.83)
22	4104.100	5570.81 (35.74)	3692.72 (-10.02)	3724.90 (-9.24)	4012.13 (-2.24)	3642.21 (-11.25)
23	3331.390	4276.57 (28.37)	3547.62 (6.49)	3296.33 (-1.05)	3357.34 (0.78)	3164.68 (-5.00)
24	3817.185	5604.65 (46.83)	4075.20 (6.76)	4003.39 (4.88)	4363.18 (14.30)	3607.74 (-5.49)
25	4161.015	6522.80 (56.76)	3698.00 (-11.13)	3898.81 (-6.30)	4404.62 (5.85)	4028.75 (-3.18)
AUC (Mean ±SE)	4906.96 ± 271.96	6865.39 ± 473.71	5094.41 ± 253.25	4848.42 ± 279.21	5183.13 ± 320.09	4641.62 ± 253.71
Absolute prediction error (Mean ±SE)	-	33.28 ± 3.10	10.50 ± 1.48	4.94 ± 0.81	7.65 ± 1.46	7.38 ± 0.84

Table 5.14 Comparison of measured AUC and predicted AUC calculated from 3 time-point trapezoidal rule (Continuing)

Patient Number	Measured AUC (ng/ml)	Predicted $AUC_{1,2,6}$ (prediction error)	Predicted $AUC_{1,2,8}$ (prediction error)	Predicted $AUC_{2,4,6}$ (prediction error)	Predicted $AUC_{2,4,8}$ (prediction error)	Predicted $AUC_{2,6,8}$ (prediction error)
1	5735.040	-	5696.71 (-0.67)	-	5338.62 (-6.91)	-
2	5183.200	4824.67 (-6.92)	5933.67 (14.48)	4548.72 (-12.24)	5038.85 (-2.78)	5290.74 (2.07)
3	4543.950	3455.17 (-23.96)	2937.63 (-35.35)	3972.46 (-12.58)	3975.09 (-12.52)	3344.40 (-26.40)
4	6281.355	5258.81 (-16.28)	6042.55 (-3.80)	5355.80 (-14.73)	5827.19 (-7.23)	5716.68 (-8.99)
5	3437.370	2949.70 (-14.19)	3172.89 (-7.69)	3155.16 (-8.21)	3352.95 (-2.46)	3032.07 (-11.79)
6	4275.930	3909.44 (-8.57)	4402.90 (2.97)	3568.02 (-16.56)	3778.32 (-11.64)	3927.48 (-8.15)
7	4048.395	3300.58 (-18.47)	3490.14 (-13.79)	3600.06 (-11.07)	3865.35 (-4.52)	3406.32 (-15.86)
8	7046.925	7285.88 (3.39)	8327.15 (18.17)	5809.40 (-17.56)	5978.82 (-15.16)	6661.89 (-5.46)
9	4325.310	3584.56 (-17.13)	3952.89 (-8.61)	3759.36 (-13.08)	4081.12 (-5.65)	3755.61 (-13.17)
10	5535.345	5384.04 (-2.73)	6380.57 (15.27)	4317.96 (-21.99)	4642.86 (-16.12)	5047.89 (-8.81)
11	5062.231	5205.97 (2.84)	4933.79 (-2.54)	4644.88 (-8.24)	4233.56 (-16.37)	4851.48 (-4.16)
12	2770.250	2283.74 (-17.56)	2429.35 (-12.31)	2231.46 (-19.45)	2396.74 (-13.48)	2167.05 (-21.77)
13	4641.270	4501.43 (-3.01)	4862.18 (4.76)	3191.13 (-31.24)	3257.26 (-29.82)	3517.60 (-24.21)
14	5247.165	4794.88 (-8.62)	5364.97 (2.25)	4023.26 (-23.33)	4295.43 (-18.14)	4322.43 (-17.62)
15	3969.375	3371.06 (-15.07)	3579.05 (-9.83)	3342.56 (-15.79)	3525.19 (-11.19)	3430.05 (-13.59)
16	6380.155	6119.86 (-4.08)	6762.75 (6.00)	4850.74 (-23.97)	5053.43 (-20.79)	5209.59 (-18.35)
17	6851.030	6966.28 (1.68)	8051.36 (17.52)	5139.00 (-24.99)	5400.15 (-21.18)	5995.20 (-12.49)
18	8452.005	7507.77 (-11.17)	8413.19 (-0.46)	6390.48 (-24.39)	6866.75 (-18.76)	7016.16 (-16.99)
19	3632.378	-	-	3038.62 (-16.35)	3318.91 (-8.63)	3098.01 (-14.71)
20	3843.660	-	-	3248.10 (-15.49)	3309.59 (-13.89)	3291.93 (-14.35)
21	5997.860	6070.33 (1.21)	6852.97 (14.26)	4208.38 (-29.84)	4358.99 (-27.32)	4870.74 (-18.79)
22	4104.100	3842.72 (-6.37)	4298.28 (4.73)	2810.92 (-31.51)	2974.81 (-27.52)	3164.46 (-22.90)
23	3331.390	3049.29 (-8.47)	3246.20 (-2.56)	2702.92 (-18.87)	2787.56 (-16.32)	2774.91 (-16.70)
24	3817.185	3672.74 (-3.78)	4169.34 (9.23)	3194.26 (-16.32)	3342.65 (-12.43)	3518.22 (-7.83)
25	4161.015	4326.25 (3.97)	4928.75 (18.45)	3060.10 (-26.46)	3149.65 (-24.31)	3540.60 (-14.91)
AUC (Mean ±SE)	4906.96 ± 271.96	4621.14 ± 313.01	5140.40 ± 363.45	3923.49 ± 212.90	4165.99 ± 223.11	4206.31 ± 260.86
Absolute prediction error (Mean ±SE)	-	9.07 ± 1.41	9.81 ± 1.69	18.93 ± 1.40	14.61 ± 1.54	14.17 ± 1.29

2.4 Apply the published models to the present data of all patients

Since some studies have made similar regression analyses of the pharmacokinetic profile, those previously proposed equations were tested for their ability to estimate the measured AUC obtained in Thai patients. Table 5.15 displayed the model equations which were used to calculate AUC in this present study and their correlation coefficients. The predicted AUC obtained by applying the above models to present data was shown in table 5.16.

Table 5.15 Model equations has been previously proposed

Authors	Proposed Model Equations: Predicted 12 hr-AUC =	r^2 Proposed data	r^2 Present data	Absolute prediction error (%) Mean ± SE
Lindholm et al., 1993	$4.44C_0 + 2.42C_2 + 5.91C_6 + 83$	0.96	0.9578	5.84 ± 0.93
Kahan et al., 1995	$2.4C_2 + 7.7C_6 + 195.8$	0.938	0.9510	6.62 ± 1.18
Serafinowicz et al., 1996	$9.131C_0 + 0.784C_1 + 2.617C_2 + 193.561$	0.954	0.9669	5.81 ± 1.01
The present study (Two sampling times)	$3.085C_2 + 6.019C_6 + 376.893$	-	0.9638	5.40 ± 0.88
The present study (Three sampling times)	$0.738C_1 + 2.112C_2 + 7.02C_6 + 263.108$	-	0.9823	3.01 ± 0.81

Applying the previously proposed regression models to our present data also demonstrated good correlation coefficient between the predicted and the full AUC, namely r^2 were equal to 0.9578, 0.9510, and 0.9669 for the equations proposed by Lindholm, Kahan, and Serafinowicz respectively. However, among the regression equations using three sampling times, the one that derived from the present data exhibited the best correlation as well as the best predictive accuracy. Similarly, the regression equation employed two sampling times which was derived from the present data also displayed better correlation as well as more predictive accuracy than the model proposed by Kahan et al.

Table 5.16 Comparison of measured AUC and predicted AUC calculated from previously proposed models.

Patient Number	Measured AUC (ng/ml)	Predicted $AUC_{Lindholm}$ (prediction error)	Predicted AUC_{Kahan} (prediction error)	Predicted $AUC_{Sawchowicz}$ (prediction error)	Predicted $AUC_{2,6}$ (prediction error)	Predicted $AUC_{1,2,6}$ (prediction error)
1	5735.040	-	-	5088.20 (-11.28)	-	-
2	5183.200	5633.16 (8.68)	5363.49 (3.48)	5382.18 (3.84)	5782.10 (11.55)	5187.70 (0.09)
3	4543.950	4871.02 (7.20)	4647.43 (2.28)	3698.63 (-18.60)	4281.33 (-5.78)	4467.41 (-1.68)
4	6281.355	6765.58 (7.71)	6191.14 (-1.44)	6292.63 (0.18)	6374.71 (1.49)	5900.68 (-6.06)
5	3437.370	3596.40 (4.63)	3512.34 (2.18)	3216.97 (-6.41)	3685.44 (7.22)	3417.67 (-0.57)
6	4275.930	4356.62 (1.89)	4340.66 (1.51)	3918.07 (-8.37)	4533.27 (6.02)	4356.42 (1.88)
7	4048.395	4132.22 (2.07)	4020.86 (-0.68)	3530.07 (-12.80)	4078.80 (0.75)	3899.18 (-3.69)
8	7046.925	6932.38 (-1.63)	6885.42 (-2.29)	7100.81 (0.76)	7487.12 (6.25)	7333.64 (4.07)
9	4325.310	4377.76 (1.21)	4273.31 (-1.20)	3789.71 (-12.38)	4379.52 (1.25)	4152.01 (-4.01)
10	5535.345	5425.39 (-1.99)	5125.97 (-7.40)	5901.94 (6.62)	5618.60 (1.50)	5483.67 (-0.93)
11	5062.231	5900.13 (16.55)	6057.24 (19.66)	4585.04 (-9.43)	5979.57 (18.12)	5989.01 (18.31)
12	2770.250	2767.84 (-0.09)	2610.61 (-5.76)	2722.44 (-1.73)	2716.31 (-1.95)	2704.73 (-2.37)
13	4641.270	4052.59 (-12.68)	3944.27 (-15.02)	4556.55 (-1.83)	4174.64 (-10.05)	4653.00 (0.25)
14	5247.165	4989.12 (-4.92)	4713.21 (-10.18)	5211.73 (-0.68)	4958.36 (-5.50)	5085.63 (-3.08)
15	3969.375	4239.28 (6.80)	4061.31 (2.32)	3696.50 (-6.87)	4067.41 (2.47)	3996.11 (0.67)
16	6380.155	5954.84 (-6.67)	5618.67 (-11.94)	6526.06 (2.29)	5980.84 (-6.26)	6254.38 (-1.97)
17	6851.030	6107.76 (-10.85)	6105.51 (-10.88)	6671.44 (-2.62)	6682.96 (2.45)	6884.52 (0.49)
18	8452.005	7910.76 (-6.40)	7628.44 (-9.74)	7549.21 (-10.68)	7741.21 (-8.41)	8027.06 (-5.03)
19	3632.378	3670.30 (1.04)	3484.13 (-4.08)	-	3852.98 (0.57)	-
20	3843.660	3929.74 (2.24)	3913.36 (1.81)	-	4004.34 (4.18)	-
21	5997.860	5294.48 (-11.73)	5087.36 (-15.18)	6226.98 (3.82)	5545.69 (-7.54)	6000.69 (0.05)
22	4104.100	3714.11 (-9.50)	3469.31 (-15.47)	4283.54 (4.37)	3712.27 (-9.55)	4019.55 (-2.06)
23	3331.390	3333.48 (0.06)	3238.96 (-2.77)	3288.10 (-1.30)	3407.03 (2.27)	3414.21 (2.49)
24	3817.185	3905.66 (2.32)	3803.10 (-0.37)	3937.33 (3.15)	4130.71 (8.21)	3938.02 (3.17)
25	4161.015	3692.03 (-11.27)	3688.94 (-11.35)	4310.94 (3.60)	4150.52 (-0.25)	4301.40 (3.37)
AUC (Mean ±SE)	4906.96 ± 271.96	4814.69 ± 263.65	4657.71 ± 255.62	4847.18 ± 286.93	4880.24 ± 269.98	4975.76 ± 292.06
Absolute prediction error (Mean ±SE)	-	5.84 ± 0.93	6.62 ± 1.18	5.81 ± 1.01	5.40 ± 0.88	3.01 ± 0.81

3. Pharmacokinetic drug interaction

Effect of pharmacokinetic drug interaction on pharmacokinetic parameters

CsA dose, AUC, C_{max}, t_{max} and CsA trough level for a group of ten patients did not use CsA-sparing agents were compared with the other group of fifteen patients using CsA-sparing agents using student T Test. It was shown that the administration of CsA-sparing agents was normally associated with a lower dosage of CsA. The mean difference in CsA dose (± S.E.) was 31.27 (±11.87) mg and 95% Confident interval for difference was 6.72 to 55.82 mg. CsA dose and pharmacokinetic parameters in both groups were shown in table 5.17 to table 5.24.

Table 5.17 Comparison of the CsA dose between patients not using CsA-sparing agents with patients using CsA-sparing agents

Dose (mg)		Dose (mg/kg)	
Not using CsA-sparing agent	Using CsA-sparing agent	Not using CsA-sparing agent	Using CsA-sparing agent
125	125	3.57	5.32
75	100	1.88	3.85
150	100	4.00	2.78
75	88	3.00	2.13
200	113	5.41	3.04
88	100	3.18	3.33
125	50	3.85	1.82
88	88	3.98	2.61
125	50	3.85	1.39
113	75	4.69	2.88
116.30 ± 12.21[a]	75	3.74 ± 0.30	2.38
	88		3.37
	75		2.73
	75		2.17
	75		3.19
	85.03 ± 5.35		2.87 ± 0.24
$t_{difference} = 2.64$ (p=.015) $t_{.05,23} = 2.069$		$t_{difference} = 2.27$ (p=.033) $t_{.05,23} = 2.069$	

[a] *Mean ± SE*

Table 5.18 Comparison of the CsA AUC between patients not using CsA-sparing agents with patients using CsA-sparing agents

AUC (ng*hr/ml)		AUC/dose (ng*hr/ml/mg)	
Not using CsA-sparing agent	Using CsA-sparing agent	Not using CsA-sparing agent	Using CsA-sparing agent
5735.040	5183.200	45.88	41.47
3437.370	4543.950	45.83	45.44
5062.231	6281.355	33.75	62.81
3969.375	4275.930	52.93	48.87
6380.155	4048.395	31.90	35.99
3632.378	7046.925	41.28	70.47
3843.660	4325.310	30.75	86.51
5997.860	5535.345	68.55	63.26
3817.185	2770.250	30.54	55.41
4161.015	4641.270	36.99	61.88
4603.627 ± 344.610[a]	5247.165	41.838 ± 3.81	69.96
	6851.030		77.85
	8452.005		112.69
	4104.100		54.72
	3331.390		44.42
	5109.175 ± 391.318		62.12 ± 5.12
$t_{difference}$ = -0.91 (p=.374) $t_{.05, 23}$ = 2.069		$t_{difference}$ = -2.89 (p=.008) $t_{.05, 23}$ = 2.069	

[a] *Mean ± SE*

Table 5.19 Comparison of the CsA trough level between patients not using CsA-sparing agents with patients using CsA-sparing agents

C_0 (ng/ml)		C_0/dose (ng/ml/mg)	
Not using CsA-sparing agent	Using CsA-sparing agent	Not using CsA-sparing agent	Using CsA-sparing agent
187.79	209.67	1.50	1.68
140.89	263.12	1.88	2.63
187.00	327.50	1.25	3.28
195.86	147.35	2.61	1.68
238.09	174.11	1.19	1.55
163.53	183.71	1.86	1.84
146.06	174.64	1.17	3.49
183.34	201.55	2.10	2.30
136.81	138.61	1.09	2.77
96.69	152.65	0.86	2.04
167.60 ± 12.46[a]	210.99	1.55 ± 0.173	2.81
	153.75		1.75
	310.80		4.14
	168.33		2.24
	135.90		1.81
	196.85 ± 15.46		2.40 ± 0.20
$t_{difference} = -1.36$ (p=.188) $t_{.05, 23} = 2.069$		$t_{difference} = -3.00$ (p=.006) $t_{.05, 23} = 2.069$	

[a] *Mean ± SE*

Table 5.20 Comparison of the CsA peak level between patients not using CsA-sparing agents with patients using CsA-sparing agents

C_{max} (ng/ml)		C_{max}/dose (ng/ml/mg)	
Not using CsA-sparing agent	Using CsA-sparing agent	Not using CsA-sparing agent	Using CsA-sparing agent
1062.23	1129.65	8.50	9.04
592.29	697.67	7.90	6.98
844.42	1084.69	5.63	10.85
553.29	758.01	7.38	8.66
1535.46	588.86	7.68	5.23
583.68	1555.92	6.63	15.56
596.78	674.38	4.77	13.49
1846.38	1147.98	21.10	13.12
772.60	373.71	6.18	7.47
1245.60	1391.64	11.07	18.56
963.27 ± 142.65[a]	1043.53	8.68 ± 1.49	13.91
	1815.30		20.63
	1470.84		19.61
	1112.29		14.83
	565.94		7.55
	1027.36 ± 106.97		12.37 ± 1.26
$t_{difference}$ = -0.37 (p=0.718) $t_{.05, 23}$ = 2.069		$t_{difference}$ = -1.87 (p=0.074) $t_{.05, 23}$ = 2.069 $t_{.1, 23}$ = 1.714	

[a] *Mean ± SE*

Table 5.21 Comparison of the time to peak level between patients not using CsA-sparing agents with patients using CsA-sparing agents

t_{max} (hr)	
Not using CsA-sparing agent	Using CsA-sparing agent
2	2
2	4
2	2
2	2
1	2
2	2
2	2
1	2
2	2
1	1
1.70 ± 0.15[a]	1
	1
	1
	1
	1
	1
	1.73 ± 0.80

$t_{difference}$ = -0.12 (p=0.907)
$t_{.05, 23}$ = 2.069

[a] *Mean ± SE*

Table 5.22 Comparison of half-life between patients not using CsA-sparing agents with patients using CsA-sparing agents

$t_{1/2}$ (hr)	
Not using CsA-sparing agent	Using CsA-sparing agent
6.51	8.42
5.72	5.06
3.26	18.43
6.33	8.24
6.48	6.04
7.04	5.58
5.16	7.87
6.83	11.81
8.12	7.71
7.11	6.84
6.26 ± 0.42[a]	7.80
	7.09
	9.89
	10.66
	6.32
	8.52 ± 0.86

$t_{difference} = -2.04$ ($p=0.053$)
$t_{.05, 23} = 2.069$
$t_{.1, 23} = 1.714$

[a] *Mean ± SE*

Table 5.23 Comparison of clearance between patients not using CsA-sparing agents with patients using CsA-sparing agents

Cl/F (l/hr)	
Not using CsA-sparing agent	Using CsA-sparing agent
21.7958	24.1164
21.8190	22.0073
29.6312	15.9201
18.8947	20.4634
31.3472	27.7888
24.2266	14.1906
32.5211	11.5599
14.5885	15.8075
32.7466	18.0489
27.0367	16.1594
25.46 ± 1.96[a]	14.2934
	12.8448
	8.8736
	18.2744
	22.5131
	17.52 ± 1.32

$t_{difference} = 3.5$ (p=0.002)
$t_{.05, 23} = 2.069$

[a] *Mean ± SE*

Table 5.24 Comparison of volume of distribution between patients not using CsA-sparing agents with patients using CsA-sparing agents

Vd/F (l)	
Not using CsA-sparing agent	Using CsA-sparing agent
204.85	293.03
180.02	160.75
139.57	423.41
172.55	243.32
293.24	242.06
246.20	114.35
241.97	131.21
143.87	269.29
383.90	200.77
277.30	159.52
228.35 ± 24.11[a]	160.96
	131.34
	126.59
	281.14
	205.41
	209.54 ± 21.73

$t_{difference} = 0.57$ (p=0.576)
$t_{.05, 23} = 2.069$

[a] *Mean ± SE*

A group of 15 patients using CsA-sparing agents showed a lower dosage of CsA, higher AUC/mg dose, higher C_0/mg dose, higher C_{max}/mg dose (α=0.1), longer $t_{1/2}$ (α=0.1), and lower Cl/F than a group of 10 patients did not use CsA sparing agents, while t_{max} and Vd were not significantly different in both groups.

Higher AUC/mg dose, C_0/mg dose and C_{max}/mg dose could result from higher absorption or lesser elimination. Since AUC/mg dose, C_0/mg dose and C_{max}/mg dose were significantly higher while t_{max} were similar indicated that the pharmacokinetic interaction occurred in the absorption part (if any) should resulted in increasing amount of absorption without any effects on the rate of absorption (t_{max}).

Lower Cl/F and longer $t_{1/2}$ indicated that those CsA-sparing agents were associated with reducing metabolism of CsA. These interactions were confirmed by many studies that ketoconazole, diltiazem, and verapamil inhibit the cytochrome P450 system. (Yee and McGuire, 1990; Campana et al; 1996; Jones, 1997)

Effect of pharmacokinetic drug interaction on optimum time points for predicting CsA AUC

Correlation between single blood levels and CsA AUC

The correlation coefficients between single blood concentrations and AUC for a group of ten patients did not use CsA-sparing agents were compared with the other group of fifteen patients using CsA-sparing agents using student T Test as shown in table 5.25. Two-hour post dose level exhibited the best correlation with AUC in both groups (r^2=0.9322 and 0.8750), while trough level could not explain more than 70% of the variability described by the full AUC.

Table 5.25 The correlation coefficient between CsA levels and AUC[a] for patients not using CsA-sparing agents and patients using CsA-sparing agents

	12-hr AUC (ng*hr/ml)	
	Not using CsA-sparing agent	Using CsA-sparing agent
C_0	0.6937 (P<0.05)	0.6121 (P<0.05)
C_1	0.6712 (P=0.068)	0.6368 (P<0.05)
C_2	0.9322 (P<0.05)	0.8750 (P<0.05)
C_4	0.5388 (P=0.108)	0.6621 (P<0.05)
C_6	0.4705 (P=0.201)	0.7632 (P<0.05)
C_8	0.6090 (P=0.062)	0.8321 (P<0.05)
C_{12}	0.7307 (P<0.05)	0.7547 (P<0.05)
AUC	1.0000 (P<0.01)	1.0000 (P<0.01)
C_{ssav}	1.0000 (P<0.01)	1.0000 (P<0.01)

[a] *Pearson product-moment correlation coefficients (P value)*

Multiple linear regression analysis

Stepwise multiple linear regression analysis was done in both groups of patients. The model equations that showed the highest correlation coefficient to measured AUC were shown in table 5.26. It was found that optimum sampling time patients which were chosen by stepwise regression analysis varied with the set of data, namely using two sampling times, 2 and 8 hours after dosing for patients did not use CsA-sparing agent and 2 and 6 hours after dosing for patients used CsA-sparing agent. The predicted AUC calculated by these regression equations in patients did not use CsA-sparing agent and used CsA-sparing agent are demonstrated in table 5.27 and 5.28, respectively.

Table 5.26 The model equations and optimum sampling time points chosen by stepwise multiple linear regression analysis

Data set and No. of time points	Selected time points (hr after dosing)	Model equations: Predicted 12 hr-AUC =	r^2	Absolute prediction error (%) Mean ± SE
I. Not using CsA-sparing agent				
1	2	$4.602C_2 + 832.409$	0.9322	7.21 ± 1.86
2	2,8	$4.262C_2 + 8.390C_8 - 669.417$	0.9808	3.97 ± 0.96
II. Using CsA-sparing agent				
1	2	$3.563C_2 + 1994.526$	0.8750	10.11 ± 2.26
2	2,6	$2.743C_2 + 7.379C_6 - 274.110$	0.9781	5.02 ± 0.81
3	1,2,6	$0.714C_1 + 1.970C_2 + 8.146C_6 + 105.942$	0.9955	2.22 ± 0.37

Table 5.27 Comparison of measured AUC and predicted AUC calculated by regression equations in 10 patients did not use CsA-sparing agent

Patient Number	Measured AUC (ng/ml)	Predicted AUC_2 (prediction error)	Predicted $AUC_{2,8}$ (prediction error)
1	5735.040	5720.79 (-0.25)	6322.12 (10.24)
5	3437.370	3558.13 (3.51)	3300.44 (-3.98)
11	5062.231	4718.43 (-6.79)	5234.32 (3.40)
15	3969.375	3378.65 (-14.88)	3874.30 (-2.40)
16	6380.155	6028.25 (-5.52)	6283.09 (-1.52)
19	3632.378	3518.50 (-3.13)	3528.61 (-2.86)
20	3843.660	3578.79 (-6.89)	3583.44 (-6.77)
21	5997.860	5952.78 (-0.75)	5939.18 (-0.98)
24	3817.185	4387.91 (14.95)	4070.43 (6.63)
25	4161.015	4803.01 (15.43)	4120.94 (-0.96)
Mean $\pm$SE	4603.63 $\pm$ 344.61	4564.53 $\pm$ 333.39	4625.69 $\pm$ 378.51

Table 5.28 Comparison of measured AUC and predicted AUC calculated by regression equations in 10 patients used CsA-sparing agent

Patient Number	Measured AUC (ng/ml)	Predicted AUC_2 (prediction error)	Predicted $AUC_{2,6}$ (prediction error)	Predicted $AUC_{1,2,6}$ (prediction error)
2	5183.200	6019.47 (16.13)	5726.86 (10.49)	5219.62 (0.70)
3	4543.950	3246.07 (-28.56)	4695.78 (3.34)	4782.46 (5.25)
4	6281.355	5859.28 (-6.72)	6500.09 (3.48)	6077.26 (-3.25)
6	4275.930	4695.32 (9.81)	4582.01 (7.16)	4419.60 (3.36)
7	4048.395	4092.63 (1.09)	4200.60 (3.76)	4004.79 (-1.08)
8	7046.925	7538.27 (6.97)	7374.21 (4.64)	7352.27 (4.33)
9	4325.310	4397.34 (1.67)	4480.42 (3.59)	4251.42 (-1.71)
10	5535.345	6084.78 (9.93)	5507.37 (-0.51)	5455.11 (-1.45)
12	2770.250	3326.05 (20.06)	2753.82 (-0.59)	2707.73 (-2.26)
13	4641.270	4551.53 (-1.93)	4184.27 (-9.85)	4656.81 (0.33)
14	5247.165	5089.85 (-3.00)	4988.09 (-4.94)	5135.76 (-2.12)
17	6851.030	6965.02 (1.66)	6555.52 (-4.31)	6860.29 (0.14)
18	8452.005	6575.37 (-22.20)	7966.52 (-5.74)	8287.72 (-1.94)
22	4104.100	4283.08 (4.36)	3695.72 (-9.95)	3997.75 (-2.59)
23	3331.390	3914.13 (17.49)	3429.10 (2.93)	3422.88 (2.75)
Mean ±SE	5109.17 ± 391.32	5109.21 ± 342.41	5109.36 ± 382.78	5108.76 ± 389.50

The correlation between the predicted AUC (computed by regression equations derived from data of 25 patients) and the observed AUC was also separately determined for both groups of patients (not using and using CsA-sparing agent) as exhibited in table 5.29.

Table 5.29 The correlation between the measured AUC and the predicted AUC computed by regression equations derived from data of 25 patients.

Data set and No. of time points	Selected time points (hr after dosing)	Model equations: Predicted 12 hr-AUC =	r^2	Absolute prediction error (%) Mean ± SE
I. Not using CsA-sparing agent				
1	2	$3.727C_2 + 1760.619$	0.9322	8.36 ± 2.45
2	2,6	$3.085C_2 + 6.019C_6 + 376.893$	0.9269	6.09 ± 1.80
3	1,2,6	$0.738C_1 + 2.112C_2 + 7.02C_6 + 263.108$	0.9552	4.02 ± 2.43
II. Using CsA-sparing agent				
1	2	$3.727C_2 + 1760.619$	0.8750	9.59 ± 2.26
2	2,6	$3.085C_2 + 6.019C_6 + 376.893$	0.9738	4.99 ± 0.94
3	1,2,6	$0.738C_1 + 2.112C_2 + 7.02C_6 + 263.108$	0.9932	2.54 ± 0.46

Linear trapezoidal rule

Using 1, 2 and 3 sampling time points to calculate AUC by trapezoidal rule were performed in the same manner as previously mentioned. The predicted AUC calculated by these methods for individual patients were the same as shown in table 5.12 and 5.14. The absolute prediction error and correlation coefficient between the predicted AUC and the observed AUC were separately computed for both groups of patients (not using and using CsA-sparing agent) and displayed in table 5.30 and 5.31.

The single blood concentration that exhibited the best prediction of AUC was the level at 2 hours after administration for both groups of patients. Using 2 sampling times, levels at 2 and 8 hours after administration resulted in least prediction error while using 3 sampling times, the 0, 2, 6 sampling time points predicted trapezoidal AUC with best accuracy for both groups of patients. It should be noted that the trapezoidal equations that showed the best prediction accuracy did not always exhibited the best correlation between predicted and measured AUC. The reason was the same as that previously mention.

Table 5.30 The prediction error of the predicted AUC from 1 and 2 time points using trapezoidal rule from the measured AUC

Selected time points (hr after dosing)	Trapezoidal Equations: Predicted 12 hr-AUC =	Absolute prediction error (%) Mean ± SE	r^2
I. Not using CsA-sparing agent			
0	$C_0 * 12$	55.60 ± 2.78	0.6937
1	$0.5*C_1*12$	49.48 ± 9.13	0.6712
2	$0.5*C_2*12$	10.47 ± 2.56	0.9322
4	$0.5*C_4*12$	37.70 ± 4.41	0.5388
6	$0.5*C_6*12$	59.66 ± 3.48	0.4705
8	$0.5*C_8*12$	70.93 ± 1.90	0.6090
0,2	$0.5*2*(C_0+C_2) + 0.5*10*(C_2+C_0)$	27.31 ± 2.89	0.9698
1,2	$0.5*1*C_1 + 0.5*1*(C_1+C_2) + 0.5*10*C_2$	19.53 ± 5.18	0.8863
2,4	$0.5*2*C_2 + 0.5*2*(C_2+C_4) + 0.5*8*C_4$	14.28 ± 2.49	0.8695
2,6	$0.5*2*C_2 + 0.5*4*(C_2+C_6) + 0.5*6*C_6$	14.16 ± 2.01	0.9420
2,8	$0.5*2*C_2 + 0.5*6*(C_2+C_8) + 0.5*4*C_8$	6.41 ± 1.22	0.9780
II. Using CsA-sparing agent			
0	$C_0 * 12$	52.40 ± 2.85	0.6121
1	$0.5*C_1*12$	42.09 ± 6.61	0.6368
2	$0.5*C_2*12$	15.82 ± 3.78	0.8750
4	$0.5*C_4*12$	37.96 ± 3.95	0.6621
6	$0.5*C_6*12$	60.46 ± 2.10	0.7632
8	$0.5*C_8*12$	68.69 ± 1.32	0.8321
0,2	$0.5*2*(C_0+C_2) + 0.5*10*(C_2+C_0)$	26.47 ± 3.68	0.9193
1,2	$0.5*1*C_1 + 0.5*1*(C_1+C_2) + 0.5*10*C_2$	18.95 ± 4.14	0.8754
2,4	$0.5*2*C_2 + 0.5*2*(C_2+C_4) + 0.5*8*C_4$	15.09 ± 2.33	0.9360
2,6	$0.5*2*C_2 + 0.5*4*(C_2+C_6) + 0.5*6*C_6$	16.97 ± 1.75	0.9691
2,8	$0.5*2*C_2 + 0.5*6*(C_2+C_8) + 0.5*4*C_8$	11.51 ± 2.38	0.9377

Table 5.31 The prediction error of the predicted AUC from 3 time points using trapezoidal rule from the measured AUC

Selected time points (hr after dosing)	Trapezoidal Equations: Predicted 12 hr-AUC =	Absolute prediction error (%) Mean ± SE	r^2
I. Not using CsA-sparing agent			
0,1,2	$0.5*1*(C_0+C_1) + 0.5*1*(C_1+C_2) + 0.5*10*(C_2+C_0)$	35.57 ± 5.44	0.9316
0,2,4	$0.5*2*(C_0+C_2) + 0.5*2*(C_2+C_4) + 0.5*8*(C_4+C_0)$	10.40 ± 2.44	0.8769
0,2,6	$0.5*2*(C_0+C_2) + 0.5*4*(C_2+C_6) + 0.5*6*(C_6+C_0)$	5.00 ± 1.41	0.9475
0,2,8	$0.5*2*(C_0+C_2) + 0.5*6*(C_2+C_8) + 0.5*4*(C_8+C_0)$	5.40 ± 1.31	0.9870
1,2,4	$0.5*1*C_1 + 0.5*1*(C_1+C_2) + 0.5*2*(C_2+C_4) + 0.5*8*C_4$	6.90 ± 1.64	0.9687
1,2,6	$0.5*1*C_1 + 0.5*1*(C_1+C_2) + 0.5*4*(C_2+C_6) + 0.5*6*C_6$	6.45 ± 2.15	0.9755
1,2,8	$0.5*1*C_1 + 0.5*1*(C_1+C_2) + 0.5*6*(C_2+C_8) + 0.5*4*C_8$	8.58 ± 2.06	0.9482
2,4,6	$0.5*2*C_2 + 0.5*2*(C_2+C_4) + 0.5*2*(C_4+C_6) + 0.5*6*C_6$	17.85 ± 2.51	0.9079
2,4,8	$0.5*2*C_2 + 0.5*2*(C_2+C_4) + 0.5*4*(C_4+C_8) + 0.5*4*C_8$	14.43 ± 2.49	0.9116
2,6,8	$0.5*2*C_2 + 0.5*4*(C_2+C_6) + 0.5*2*(C_6+C_8) + 0.5*4*C_8$	13.17 ± 1.57	0.9663
II. Using CsA-sparing agent			
0,1,2	$0.5*1*(C_0+C_1) + 0.5*1*(C_1+C_2) + 0.5*10*(C_2+C_0)$	32.06 ± 3.86	0.9213
0,2,4	$0.5*2*(C_0+C_2) + 0.5*2*(C_2+C_4) + 0.5*8*(C_4+C_0)$	10.56 ± 1.92	0.9118
0,2,6	$0.5*2*(C_0+C_2) + 0.5*4*(C_2+C_6) + 0.5*6*(C_6+C_0)$	4.91 ± 1.02	0.9746
0,2,8	$0.5*2*(C_0+C_2) + 0.5*6*(C_2+C_8) + 0.5*4*(C_8+C_0)$	9.15 ± 2.22	0.9517
1,2,4	$0.5*1*C_1 + 0.5*1*(C_1+C_2) + 0.5*2*(C_2+C_4) + 0.5*8*C_4$	7.63 ± 0.97	0.9895
1,2,6	$0.5*1*C_1 + 0.5*1*(C_1+C_2) + 0.5*4*(C_2+C_6) + 0.5*6*C_6$	10.29 ± 1.77	0.9681
1,2,8	$0.5*1*C_1 + 0.5*1*(C_1+C_2) + 0.5*6*(C_2+C_8) + 0.5*4*C_8$	10.47 ± 2.37	0.9419
2,4,6	$0.5*2*C_2 + 0.5*2*(C_2+C_4) + 0.5*2*(C_4+C_6) + 0.5*6*C_6$	19.57 ± 1.70	0.9642
2,4,8	$0.5*2*C_2 + 0.5*2*(C_2+C_4) + 0.5*4*(C_4+C_8) + 0.5*4*C_8$	14.72 ± 2.03	0.9552
2,6,8	$0.5*2*C_2 + 0.5*4*(C_2+C_6) + 0.5*2*(C_6+C_8) + 0.5*4*C_8$	14.77 ± 1.86	0.9669

Apply the published models to the present data of each patient group

The correlation between the predicted AUC computed by the previously proposed models and the observed AUC was independently determined for both groups of patients (not using and using CsA-sparing agent) as presented in table 5.32.

Table 5.32 The correlation between the measured AUC and the predicted AUC computed by previously proposed models

Authors	Proposed Model Equations: Predicted 12 hr-AUC =	r^2	Absolute prediction error (%) Mean ± SE
I. Not using CsA-sparing agent			
Lindholm et al., 1993	$4.44C_0 + 2.42C_2 + 5.91C_6 + 83$	0.9030	7.03 ± 1.74
Kahan et al., 1995	$2.4C_2 + 7.7C_6 + 195.8$	0.8537	7.65 ± 2.33
Serafinowicz et al., 1996	$9.131C_0 + 0.784C_1 + 2.617C_2 + 193.561$	0.9603	5.86 ± 1.14
The present study (Two sampling times)	$3.085C_2 + 6.019C_6 + 376.893$	0.9269	6.09 ± 1.80
The present study (Three sampling times)	$0.738C_1 + 2.112C_2 + 7.02C_6 + 263.108$	0.9552	4.02 ± 2.43
II. Using CsA-sparing agent			
Lindholm et al., 1993	$4.44C_0 + 2.42C_2 + 5.91C_6 + 83$	0.9704	5.13 ± 1.07
Kahan et al., 1995	$2.4C_2 + 7.7C_6 + 195.8$	0.9766	6.01 ± 1.32
Serafinowicz et al., 1996	$9.131C_0 + 0.784C_1 + 2.617C_2 + 193.561$	0.9692	5.78 ± 1.45
The present study (Two sampling times)	$3.085C_2 + 6.019C_6 + 376.893$	0.9738	4.99 ± 0.94
The present study (Three sampling times)	$0.738C_1 + 2.112C_2 + 7.02C_6 + 263.108$	0.9932	2.54 ± 0.46

Overall comparison of predictive accuracy of previously discussed regression equations (table 5.29 and 5.32) in both groups of patients, we found a tendency to predict more accurate in a group using CsA sparing agents than not using. The equation derived from our data, all 25 patients might predict more accurate in the using CsA sparing agents group because our data was obtained from 25 patients, which consisted of 15 patients (60%) using CsA sparing agents compared to 10 patients not using CsA sparing agents.

The different sampling time points were selected by stepwise multiple linear regression in both groups (table 5.26), namely 2 and 8 hours after dosing for patients did not use CsA-sparing agent and 2 and 6 hours after dosing for patients used CsA-sparing agent. Our results supported that finding of Gaspari et al. (1993) which mentioned that the time points which best correlated with measured AUC varied with the set of data. It should be noted that the highest correlation coefficient always found with the predicted AUC calculated by the derived equation from that data set (table 5.26, 5.29, and 5.32).

Predicted AUC calculated by trapezoidal rule from two sampling points at 0 and 4 hours after dosing resulted in least prediction error in both groups of patients while levels at 0 and 2 hours after administration exhibited best correlation with the actual AUC as previously discussed. Three sampling points, which showed the best predictive accuracy in both groups of patients was found to be obtained from sampling time at 0, 2, and 6 hour-post dose. Although sampling time at 0, 2, and 8 hour-post dose resulted in best correlation between predicted and actual AUC in a group not using CsA-sparing agents. However, there was not much difference in prediction error obtained from sampling time points at 0, 2, and 8 hours versus 0, 2, and 6 hours after administration.

4. Correlation between CsA levels and dose

There were poor correlation between dose and CsA level at any single time points including the trough level, which is usually used to guide dosing (table 5.33). The level that showed the best correlation coefficient with dose is 2 hours post dose (r^2=0.2847). A better correlation between CsA dose and levels were noted when a group using CsA-sparing agents was excluded as shown in Table 5.34. Even though the correlation still considered to be low, AUC was found to be best correlate with dose (r^2=0.5911) and among the single point drug concentration, 2 hours after dosing correlate best with dose (r^2=0.5646).

Table 5.33 Correlation coefficient between CsA level and dose[a] of all 25 patients.

	CsA dose (mg)
C_0	0.1417 (P=0.499)
C_1	0.1269 (P=0.564)
C_2	0.2847 (P=0.168)
C_4	0.1931 (P=0.355)
C_6	0.2362 (P=0.266)
C_8	0.0920 (P=0.662)
C_{12}	0.0040 (P=0.985)
AUC	0.2381 (P=0.252)
C_{ssav}	0.2381 (P=0.252)

[a] *Pearson product-moment correlation coefficients (P value)*

Table 5.34 Correlation coefficient between CsA level and dose[a] of 10 patients not using and using CsA-sparing agents

	CsA dose (mg)	
	Not using CsA-sparing agent	Using CsA-sparing agent
C_0	0.4485 (P=0.194)	0.3039 (P=0.271)
C_1	0.3392 (P=0.411)	-0.1012 (P=0.720)
C_2	0.5646 (P=0.089)	0.3481 (P=0.204)
C_4	0.3653 (P=0.299)	0.3488 (P=0.203)
C_6	0.4571 (P=0.216)	0.3151 (P=0.253)
C_8	0.3280 (P=0.355)	0.3251 (P=0.237)
C_{12}	0.4128 (P=0.236)	0.2055 (P=0.463)
AUC	0.5911 (P=0.072)	0.2803 (P=0.312)
C_{ssav}	0.5911 (P=0.072)	0.2803 (P=0.312)

[a] *Pearson product-moment correlation coefficients (P value)*

It can be seen from table 5.33 and 5.34 that pharmacokinetic drug interaction is one of factors affecting the relationship between levels and dose. However, the present study showed that trough level is a poor guide to dosage adjustment. Although a group using CsA-sparing agents was excluded, the correlation was not much improved. This finding contrast with pharmacokinetic study of Kahan, et al. (1995) which reported that trough level correlated with AUC with r^2 equaled to 0.823. Moreover our result suggested that dosage can be adjusted base on either AUC or C_2. Based on economic reason, if the only one sample must be collected, C_2 correlated with CsA dosage better than trough.

5. Relationship between AUC, blood levels and clinical effects

It is difficult to assess the degree of immunosuppressive efficacy because there is no simple parameter for assessment. Moreover, due to the shortage of time duration of the present study, renal rejection which is the primary efficacy end point of CsA was not found. Therefore this study did not determine the correlation of concentration measurements with clinical efficacy. In attempt to find the relationship between C_0, C_2, and Cssav (Cssav = AUC/τ), and CsA adverse effects, the possible CsA adverse effects were collected by patient interview and medical history.

To determine the effect of the trough level on possible CsA adverse events, patients were divided into three groups: CsA trough level below 150 ng/ml (low level, n=7), between 150 and 200 ng/ml (intermediate level, n=11), and level above 200 ng/ml (high level, n=7) as demonstrated in table 5.35. It was found that higher trough concentration showed a tend to correlate with an increased percentage of patients who had hirsutism.

Table 5.35 Correlation between CsA trough level and some possible CsA-adverse events

C_0 (ng/ml)	n	Hyperuricemia n (%)	Hyperlipidemia n (%)	Hirsutism n (%)	Gingival Hyperplasia n (%)	Hypertension n (%)
<150	7	4 (57)	3 (43)	5 (71)	2 (29)	7 (100)
150-200	11	5 (46)	3 (27)	10 (91)	5 (45)	10 (91)
>200	7	3 (43)	5 (71)	7 (100)	3 (43)	7 (100)

To determine the effect of the level at 2 hour post dose on possible CsA adverse events, patients were divided into three groups: CsA level below 600 ng/ml (low level, n=8), between 600 and 1000 ng/ml (intermediate level, n=8), and level above 1000 ng/ml (high level, n=9) as shown in table 5.36. It was demonstrated that higher concentration at 2 hour post dose showed a tend to correlate with an increased percentage of patients who had hyperlipidemia, gingival hyperplasia and hypertension.

Table 5.36 Correlation between CsA level at 2 hours after dosing and some possible CsA-adverse events

C_2 (ng/ml)	n	Hyperuricemia n (%)	Hyperlipidemia n (%)	Hirsutism n (%)	Gingival Hyperplasia n (%)	Hypertension n (%)
<600	8	5 (63)	3 (38)	8 (100)	2 (25)	7 (88)
600-1000	8	2 (25)	3 (38)	6 (75)	4 (50)	8 (100)
>1000	9	5 (56)	5 (56)	9 (100)	5 (56)	9 (100)

Likewise, to determine the effect of the average steady state level on possible CsA adverse events, patients were divided into three groups: CsA level below 350 ng/ml (low level, n=10), between 350 and 450 ng/ml (intermediate level, n=7), and level above 450 ng/ml (high levels, n=8) as shown in table 5.37. It was illustrated that higher average steady state level showed a tend to correlate with an increased percentage of patients who had hirsutism, and hypertension.

Table 5.37 Correlation between average steady state level and some possible CsA-adverse events

C_{ssav} (ng/ml)	n	Hyperuricemia n (%)	Hyperlipidemia n (%)	Hirsutism n (%)	Gingival Hyperplasia n (%)	Hypertension n (%)
<350	10	5 (50)	2 (20)	8 (80)	4 (40)	9 (90)
350-450	7	2 (29)	5 (71)	7 (100)	2 (29)	7 (100)
>450	8	6 (75)	4 (50)	8 (100)	5 (63)	8 (100)

Our result did not show the obvious relationship between CsA AUC, levels and those possible CsA adverse events. Besides, most of adverse events which were presented here did not likely to be depend on CsA concentration. However, a better relationship was found from C_2 and Cssav. The possible explanation of the poor results we obtained could be due to (a) too small number of patients were studied, (b) the difficult to assess the degree of those adverse effects. It should be noted that those adverse events presented here were not classified in term of their severity. For example, hirsutism, many patients informed that this adverse event had been improved after decreasing CsA dosage. However, since it was difficult to assess the degree of hirsutism, the improvement in this symptom was not recorded to be analyze here. (c) the difficult to evaluate the adverse event causality such as hypertension.