

CHAPTER 4

RESULTS

Assay Validation

The assay validation of this experimental method showed that the calibration curve was linear in the quinine concentration range of 0.5 to 10 $\mu\text{g/ml}$ (Figure 9) with the correlation coefficient (r) of 0.999. The coefficient of variations (CV) for intra-assay and inter-assay were 1.05 to 3.48% and 3.57 to 6.67%, respectively (Table 3-4). The recovery of standard quinine in blank plasma was ranged from 89.38 to 94.03% (Table 5). The chromatograms illustrated that the peak of quinine was well separated from the peak of quinidine, used as internal standard and the other peaks in plasma (Figure 5-8). There was no interference from the peak of itraconazole and ketoconazole in the chromatograms of this method. The mean plasma concentration-time profiles of quinine after receiving quinine alone and pretreatment with ketoconazole or itraconazole were fitted to one compartment open model (Figure 10).

Pharmacokinetics

The pharmacokinetic parameters showed wide interindividual variations. The mean plasma concentration-time profiles of quinine after pretreatment with ketoconazole or itraconazole were shown in Figure 10. All pharmacokinetic parameters calculated (mean \pm S.D.) were shown in Table 6.

The results indicated that AUC, Ka, Ke, $t_{1/2}$, C_{max} , T_{max} , Vd/f and CL/f of quinine after pretreatment with 400 mg ketoconazole for 4 days were significantly different ($P < 0.01$) when compared with the administration of quinine alone, whereas only the parameters AUC, Ke, $t_{1/2}$, and CL/f of quinine after pretreatment with 200 mg itraconazole for 4 days were significantly different ($P < 0.01$) when compared with the administration of quinine alone. The values (mean \pm S.D.) of AUC, Ka, Ke, $t_{1/2}$, T_{max} , C_{max} , Vd/f and CL/f after administration of quinine alone were 36.06 ± 14 mg/l.hr, 3.63 ± 2.87 hr⁻¹, 0.08 ± 0.02 hr⁻¹, 9 ± 2.13 hr, 1.76 ± 0.59 hr, 2.35 ± 0.52 mg/l, 1.97 ± 0.61 l/kg and 0.17 ± 0.09 l/hr/kg, respectively, whereas those after pretreatment with 400 mg ketoconazole and 200 mg itraconazole for 4 days were 74.76 ± 26.9 mg/l.hr, 1.85 ± 0.72 hr⁻¹, 0.05 ± 0.02 hr⁻¹, 15.26 ± 4.9 hr, 2.8 ± 0.83 hr, 3.14 ± 0.54 mg/l, 1.53 ± 0.27 l/kg and 0.077 ± 0.032 l/hr/kg, respectively; and 70.8 ± 35.76 mg/l.hr, 2.09 ± 1.41 hr⁻¹, 0.051 ± 0.02 hr⁻¹, 15.4 ± 7.2 hr, 2.23 ± 0.87 hr, 2.84 ± 0.76 mg /l, 1.75 ± 0.62 l/kg and 0.096 ± 0.067 l/hr/kg, respectively. All pharmacokinetic parameters of quinine after pretreatment with ketoconazole were not significantly different from itraconazole.

Pharmacodynamics (Blood pressure, pulse rate and QT_c)

Blood pressure, pulse rate and EKG were also monitored in this study. In the phase of quinine alone, all pharmacodynamic parameters were not significantly different from control, except the decreased in pulse rates at 0.5 and 1.5 hours (73 vs 68, $P < 0.05$)

After pretreatment with ketoconazole or itraconazole, the blood pressure at time measured in all treatment were not significantly different from control, except the systolic blood pressure at 0.5 hour (116 vs 109, $P < 0.05$) after pretreatment with itraconazole and the diastolic blood pressure at 2.5 hour (74 vs 66, $P < 0.05$) after pretreatment with ketoconazole were significantly different from control (Figure 11; Table 7). Pulse rates were significantly different between group of quinine alone and pretreatment with ketoconazole at 0.5, 1, 2.5, 4 and 24 hours (68 vs 62, 71 vs 63, 79 vs 72, 78 vs 71 and 72 vs 64, respectively $P < 0.05$) (Figure 12; Table 8). The QT_c in all phases were not significantly different, (Figure 13; Table 9). However, none of the subjects complained heart irregularity and syncope after quinine administration in any phases.

Adverst effect

Three of nine subjects complained nausea after the administration of ketoconazole, but they did not require any specific treatment. However, all subjects were well tolerated and completed the study. Other adverse effects were not reported in this study.

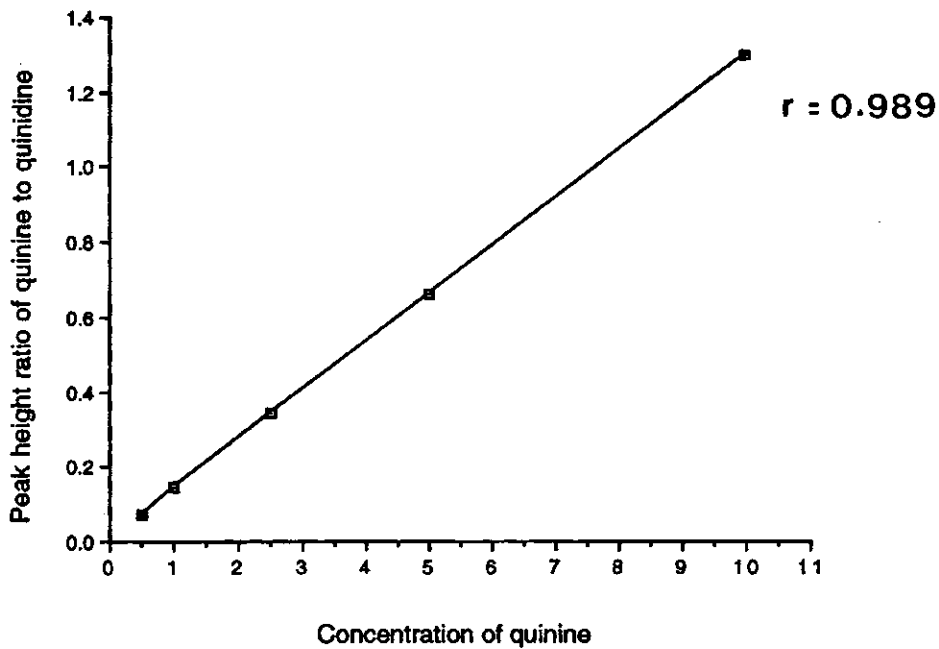


Figure 9 Mean calibration curve of standard quinine in plasma.

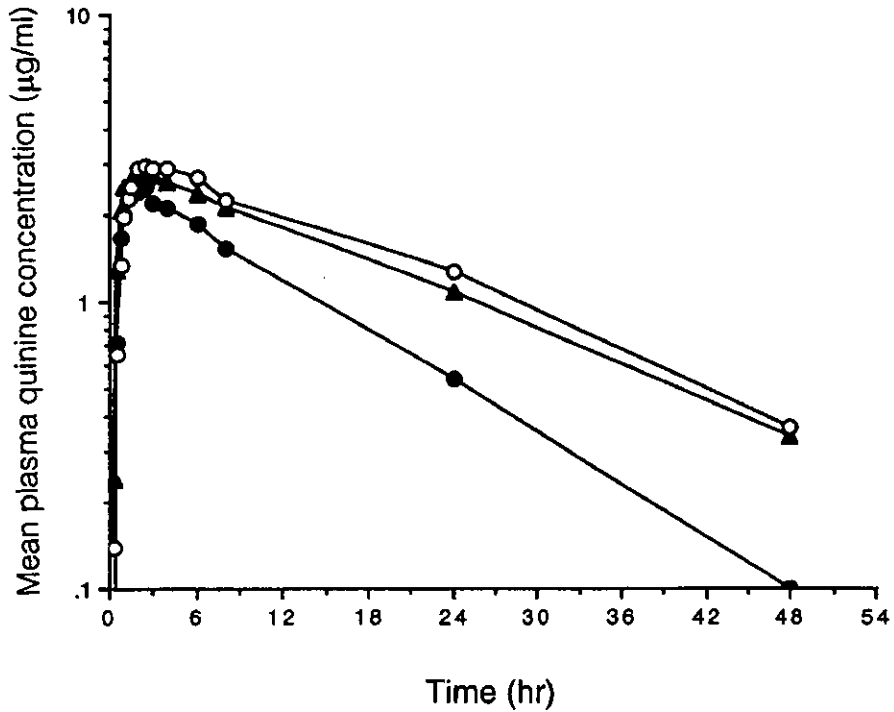


Figure 10 Semi-logarithmic mean plasma quinine concentration at 0-48 hours after a single oral dose of 300 mg quinine alone (O) and pretreatment with 400 mg ketoconazole (●) or 200 mg itraconazole (▲) orally for 4 days.

Table 6 Pharmacokinetic parameters (mean \pm S.D.) of quinine in nine subjects receiving a single oral dose of 300 mg quinine alone and after pretreatment with 400 mg ketoconazole or 200 mg itraconazole orally for 4 days.

Parameter	Quinine alone	Quinine + Ketoconazole ^a	Quinine+ Itraconazole ^a
AUC (mg/l.hr)	36.06 \pm 14	74.76 \pm 26.9**	70.80 \pm 35.76**
Ka (hr ⁻¹)	2.63 \pm 1.7	1.85 \pm 0.72	2.09 \pm 1.41
Ke(hr ⁻¹)	0.08 \pm 0.02	0.05 \pm 0.02**	0.051 \pm 0.02**
t _{1/2} (hr)	9.00 \pm 2.13	15.26 \pm 4.9**	15.40 \pm 7.2**
T _{max} (hr)	1.76 \pm 0.59	2.80 \pm 0.83**	2.23 \pm 0.87
C _{max} (mg/l)	2.35 \pm 0.52	3.14 \pm 0.54**	2.84 \pm 0.76
Vd/f (l/kg)	1.97 \pm 0.61	1.53 \pm 0.27**	1.75 \pm 0.62
CL/f (l/hr/kg)	0.17 \pm 0.09	0.077 \pm 0.03**	0.096 \pm 0.067**

*P < 0.05, **P < 0.01, significantly different compared with the administration of quinine alone.

^a There were no significant differences in all pharmacokinetic parameters in quinine - ketoconazole phase compared to those of quinine - itraconazole phase.

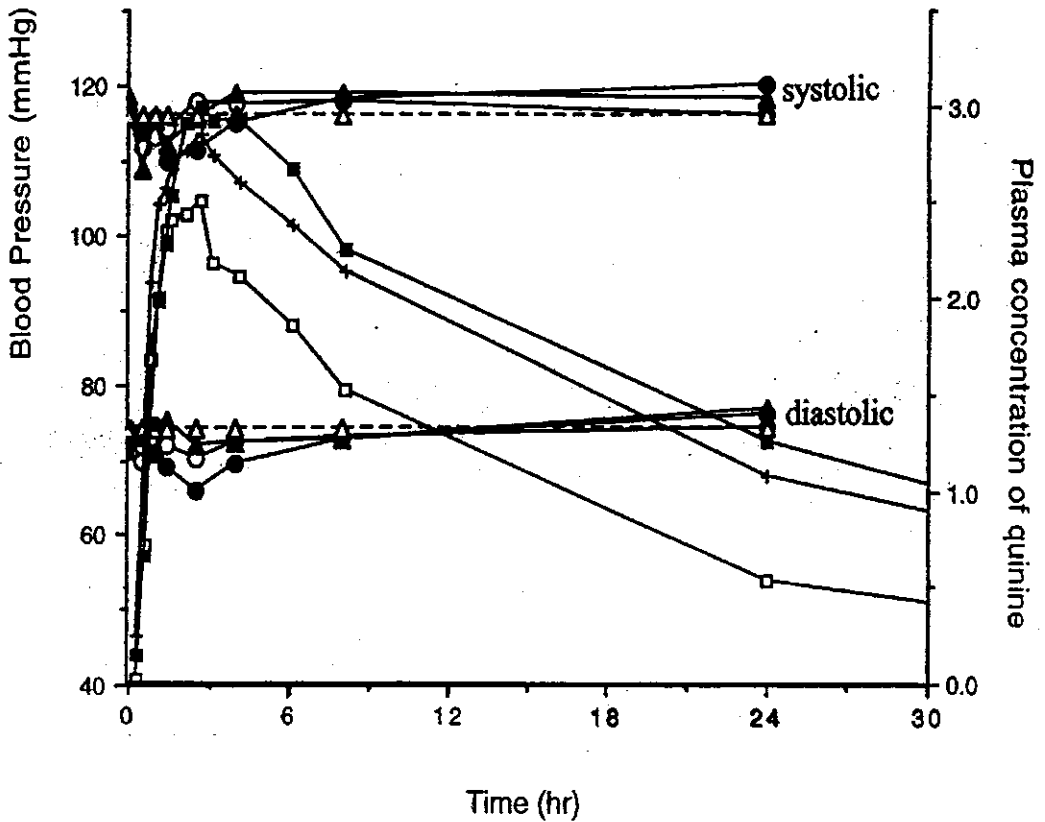


Figure 11 The mean systolic and diastolic blood pressure before (control, Δ) and after receiving a single oral dose of quinine alone (\circ), and pretreatment with 400 mg ketoconazole (\bullet) or 200 mg itraconazole (\blacktriangle) orally for 4 days in corresponding with plasma concentration in various times after receiving a single oral dose of quinine alone (\square) and pretreatment with 400 mg ketoconazole (\blacksquare) or 200 mg itraconazole (\times) orally for 4 days.

Table 7 The systolic and diastolic blood pressure (mmHg, Mean \pm S.D.) before (control) and after receiving a single oral dose of 300 mg quinine alone, and pretreatment with 400 mg ketoconazole or 200 mg itraconazole orally for 4 days.

Time (hr)	Systolic / Diastolic blood pressure (mmHg)			
	Control	Quinine alone	Quinine + Ketoconazole ^a	Quinine + itraconazole ^a
0	116 \pm 8 / 74 \pm 7	116 \pm 8 / 74 \pm 7	117 \pm 12 / 72 \pm 12	118 \pm 9 / 71 \pm 7
0.5	-	112 \pm 8 / 70 \pm 7	117 \pm 12 / 74 \pm 12	109 \pm 10* / 73 \pm 7
1	-	113 \pm 8 / 72 \pm 9	116 \pm 13 / 75 \pm 13	115 \pm 9 / 71 \pm 8
1.5	-	114 \pm 7 / 72 \pm 6	110 \pm 11 / 69 \pm 11	112 \pm 5 / 75 \pm 8
2.5	-	117 \pm 8 / 70 \pm 8	111 \pm 9 / 66 \pm 8*	116 \pm 7 / 72 \pm 9
4	-	116 \pm 6 / 72 \pm 9	115 \pm 10 / 70 \pm 9	119 \pm 5 / 72 \pm 8
8	-	118 \pm 10 / 73 \pm 9	118 \pm 10 / 73 \pm 11	119 \pm 8 / 73 \pm 8
24	-	117 \pm 7 / 74 \pm 7	120 \pm 7 / 76 \pm 10	118 \pm 8 / 77 \pm 7

*P < 0.05, significantly different compared with control

^aThere were no significant differences in all blood pressure in quinine - ketoconazole or itraconazole phase at the time measured compared with quinine alone.

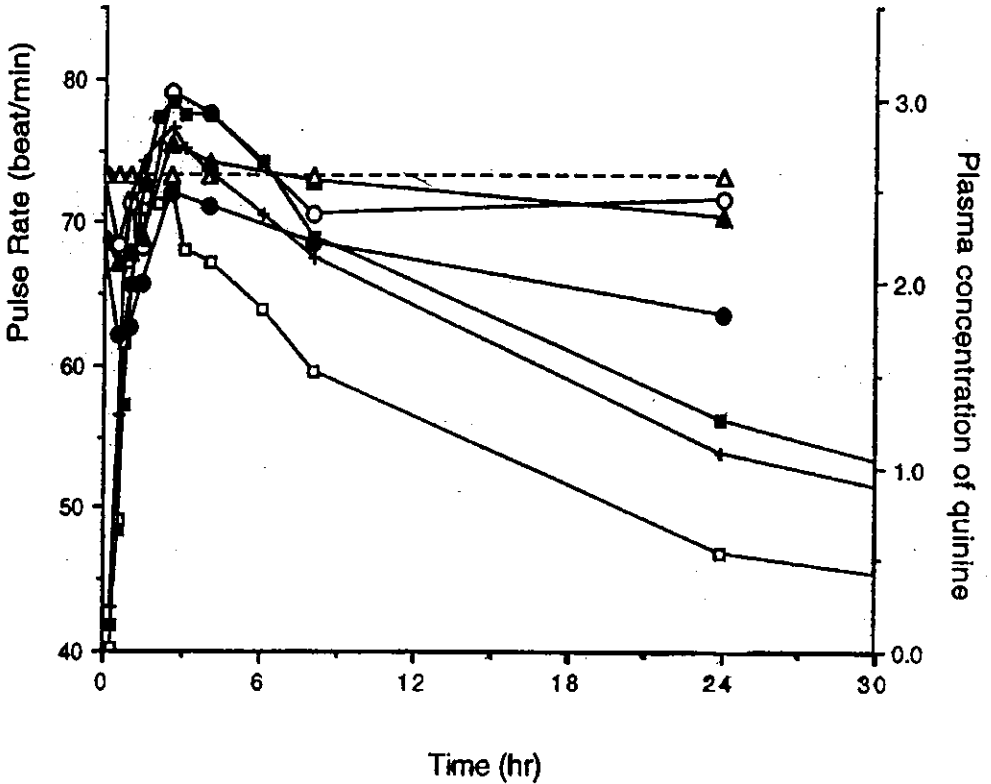


Figure 12 The pulse rate (beat/min, mean) before (control, Δ), after receiving 300 mg a single oral dose of quinine alone (\circ), pretreatment with 400 mg ketoconazole (\bullet) or 200 mg itraconazole (\blacktriangle) orally for 4 days in corresponding with plasma concentration in various times after receiving a single oral dose of quinine alone (\square) and pretreatment with 400 mg ketoconazole (\blacksquare) or 200 mg itraconazole (\times) orally for 4 days.

Table 8 The pulse rate (beat/min, mean \pm S.D.) before (control) and after receiving 300 mg a single oral dose of quinine alone and after pretreatment with 400 mg ketoconazole or 200 mg itraconazole orally for 4 days.

Time (hr)	Pulse rate (beat/min)			
	Control	Quinine alone	Quinine+ Ketoconazole ^a	Quinine + Itraconazole ^a
0	73 \pm 10	73 \pm 10	69 \pm 9	69 \pm 9
0.5	-	68 \pm 6*	62 \pm 5* [†]	67 \pm 6
1	-	71.25 \pm 9.9	63 \pm 6** [†]	68 \pm 6
1.5	-	68.22 \pm 3.38*	66 \pm 5*	69 \pm 7
2.5	-	79 \pm 7.62	72 \pm 7 ^{††}	75 \pm 6
4	-	77.67 \pm 5.87	71 \pm 4 ^{††}	74 \pm 5
8	-	70.56 \pm 6.69	69 \pm 6	74 \pm 9
24	-	71.67 \pm 7.19	64 \pm 7** [†]	70 \pm 7

*P < 0.05, **P < 0.01, significantly different compared with control.

[†]P < 0.05, ^{††}P < 0.01, significant difference compared with quinine alone at the time measured.

^aThere were no significant differences in pulse rates between quinine-ketoconazole and quinine-itraconazole phase at the time measured.

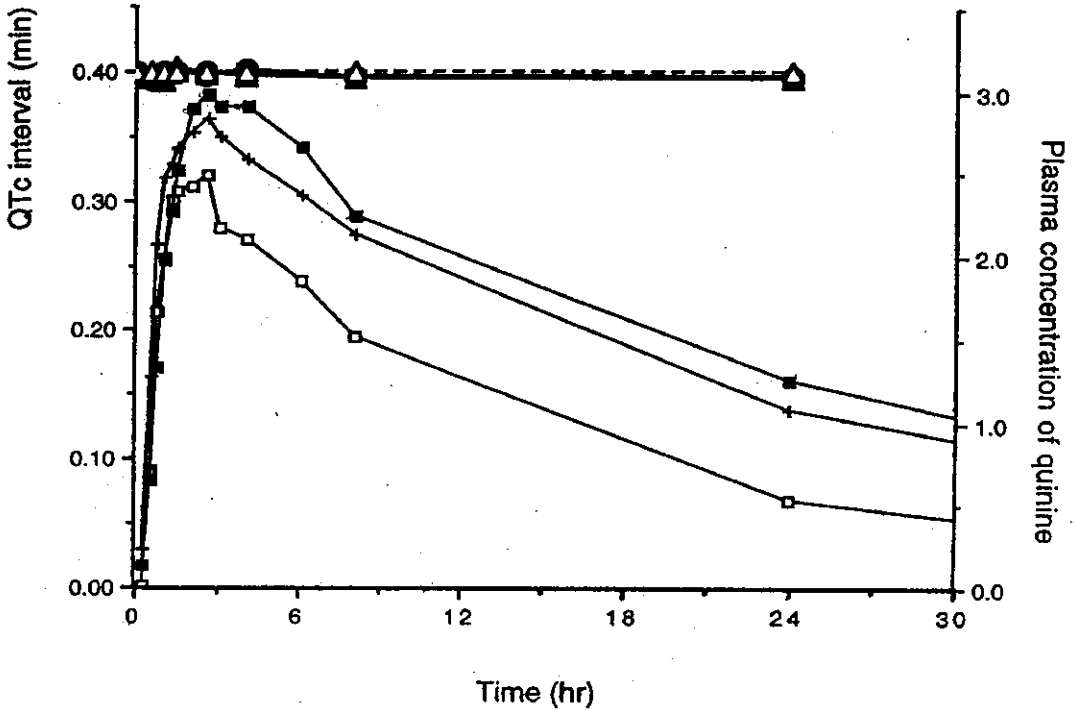


Figure 13 The QT_c (mean) interval before (control, Δ) and after receiving a single oral dose of 300 mg quinine alone (\circ) and pretreatment with 400 mg ketoconazole (\bullet) or 200 mg itraconazole (\blacktriangle) orally for 4 days in corresponding with plasma concentration in various times after receiving a single oral dose of quinine alone (\square) and pretreatment with 400 mg ketoconazole (\blacksquare) or 200 mg itraconazole (\times) orally for 4 days.

Table 9 The QT_c (mean ± S.D.) interval before (control) and after receiving a single oral dose of 300 mg quinine alone, pretreatment with 400 mg ketoconazole or 200 mg itraconazole orally for 4 days.

Time (hr)	QT _c interval (min)			
	Control	Quinine alone	Quinine + Ketoconazole ^a	Quinine + Itraconazole ^a
0	0.40 ± 0.01	0.40 ± 0.01	0.40 ± 0.01	0.40 ± 0.01
0.5	-	0.40 ± 0.01	0.40 ± 0.08	0.40 ± 0.02
1	-	0.40 ± 0.01	0.40 ± 0.01	0.39 ± 0.02
1.5	-	0.40 ± 0.01	0.40 ± 0.01	0.40 ± 0.01
2.5	-	0.40 ± 0.11	0.40 ± 0.01	0.40 ± 0.01
4	-	0.40 ± 0.01	0.40 ± 0.01	0.40 ± 0.01
8	-	0.40 ± 0.01	0.39 ± 0.01	0.40 ± 0.01
24	-	0.40 ± 0.01	0.39 ± 0.01	0.40 ± 0.01

^aThere were no significant differences in QT_c in quinine - ketoconazole or quinine - itraconazole phase at the time measured compared with quinine alone.

Table 10 Pharmacokinetic parameters of a single oral dose of 300 mg quinine alone in nine healthy volunteers at time interval of 0-48 hours.

Parameter	Subject No.									Mean \pm S.D.
	1	2	3	4	5	6	7	8	9	
AUC (mg/l.hr)	51.7	26.81	31.00	24.50	32.64	16.49	33	51.37	56.88	36.06 \pm 14
Ka (hr ⁻¹)	3.65	2.90	1.0	1.79	0.52	1.81	3.82	2.10	6.07	2.63 \pm 1.7
Ke(hr ⁻¹)	0.057	0.11	0.09	0.09	0.075	0.105	0.075	0.064	0.06	0.08 \pm 0.02
t _{1/2} (hr)	12.13	6.28	7.36	7.74	9.27	6.60	9.24	10.91	11.36	9.00 \pm 2.13
T _{max} (hr)	1.50	1.50	0.84	2.40	2.71	2.08	1.42	2.12	1.23	1.76 \pm 0.59
C _{max} (mg/l)	2.77	2.60	2.79	1.87	2.06	1.46	2.28	2.93	3.31	2.35 \pm 0.52
Vd/f (l/kg)	1.67	1.69	2.29	2.18	1.79	3.33	2.07	1.51	1.24	1.97 \pm 0.61
CL/f (l/hr/kg)	0.10	0.19	0.21	0.194	0.134	0.35	0.152	0.096	0.075	0.17 \pm 0.08
Lag time(hr)	0.43	0.33	0.37	0.65	0.10	0.43	0.371	0.403	0.462	0.39 \pm 0.14

Table 11 Pharmacokinetic parameters of a single oral dose of 300 mg quinine after pretreatment with 400 mg ketoconazole orally for 4 days in nine healthy volunteers at time interval of 0-48 hours.

Parameter	Subjects No.									Mean±S.D.
	1	2	3	4	5	6	7	8	9	
AUC (mg/l.hr)	105.35	58.00	87.00	37.26	59.43	45.62	70.70	112.34	97.06	74.76 ± 26.9
Ka (hr ⁻¹)	2.83	1.85	0.87	0.95	1.15	1.81	2.27	2.45	2.48	1.85 ± 0.72
Ke(hr ⁻¹)	0.034	0.05	0.043	0.073	0.05	0.066	0.068	0.031	0.034	0.05 ± 0.02
t _{1/2} (hr)	20.27	13.50	15.98	9.49	14.51	10.50	10.19	22.36	20.564	15.26 ± 4.9
T _{max} (hr)	2.26	2.56	4.00	4.07	3.31	2.33	1.72	2.14	2.85	2.80 ± 0.83
C _{max} (mg/l)	3.41	2.69	3.23	2.21	2.47	2.67	4.3	3.31	2.99	3.14 ± 0.54
Vd/f (l/kg)	1.38	1.72	1.70	1.78	1.52	1.91	1.04	1.42	1.31	1.53 ± 0.27
CL/f (l/hr/kg)	0.047	0.086	0.073	0.128	0.073	0.13	0.071	0.044	0.044	0.077 ± 0.032
Lag time (hr)	0.69	0.57	0.38	1.15	0.429	0.432	0.125	0.34	0.235	0.48 ± 0.30

Table 12 Pharmacokinetic parameters of a single oral dose of 300 mg quinine and after pretreatment with 200 mg itraconazole orally for 4 days in nine healthy volunteers at time interval of 0-48 hours.

Parameter	Subject No.									Mean±S.D.
	1	2	3	4	5	6	7	8	9	
AUC (mg/l.hr)	82.37	44.08	68.98	34.00	61.96	23	77.09	129.86	116.78	70.80 ± 35.76
Ka (hr ⁻¹)	4.39	4.13	1.69	0.68	3.39	1.73	2.82	1.47	1.51	2.09 ± 1.41
Ke(hr ⁻¹)	0.043	0.06	0.052	0.065	0.056	0.08	0.051	0.022	0.033	0.051 ± 0.02
t _{1/2} (hr)	16.29	11.67	13.47	10.66	12.29	8.66	13.59	31.50	20.81	15.40 ± 7.2
T _{max} (hr)	1.23	1.46	2.38	3.84	1.53	1.99	1.75	3.15	2.74	2.23 ± 0.87
C _{max} (mg/l)	3.34	2.57	3.19	1.73	3.22	1.59	3.65	2.70	3.56	2.84 ± 0.76
Vd/f (l/kg)	1.40	1.90	1.79	2.15	1.27	3.13	1.28	1.73	1.12	1.75 ± 0.62
CL/f (l/hr/kg)	0.06	0.114	0.093	0.14	0.071	0.25	0.065	0.038	0.037	0.096 ± 0.067
Lag time (hr)	0.166	0.43	0.243	0.015	0.31	0.127	0.3	0.261	0.155	0.22 ± 0.12