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Pharmacokinetics of Intramuscular Amopyroquin in Healthy Subjects and Determination of a Therapeutic Regimen for *Plasmodium falciparum* Malaria

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The disposition of amopyroquin was investigated in 10 healthy volunteers after a single 2-mg/kg (body weight) intramuscular dose of amopyroquin base. The major form of the drug in plasma and in whole blood was nonmetabolized amopyroquin, and only very low levels of its primary amine derivative were detected. After a rapid absorption phase (15 min), levels in plasma declined, following a tri-exponential model with a terminal elimination half-life of 129.6 ± 92.5 h. The apparent volume of distribution (*V*/F) and the systemic clearance (CL/F) were 238 ± 75 liters/kg and 2,063 ± 1,159 ml/min, respectively. The renal clearance, calculated by using urine excreted during the first 48 h, was 119 ± 99 ml/min and represented about 6% of the systemic clearance. About 1.2 and 0.2% of the amopyroquin dose was excreted in the urine during the first 48 h as nonmetabolized amopyroquin and its primary amine metabolite, respectively. Twenty-two *Plasmodium falciparum* malaria patients were studied after treatment with one of the following regimens of intramuscularly injected amopyroquin base: 3 mg/kg (body weight), 6 mg/kg, or 6 mg/kg followed by 3 mg/kg 24 h later. Parasitemia was cleared at day 7 in one of six, four of seven, and seven of nine patients, respectively. On the basis of this study, a regimen of 12 mg/kg (body weight) administered in two or three injections is suggested.

The 4-amino quinoline amopyroquin was proposed for antimalarial therapy nearly 30 years ago. It has been effective in the treatment of acute *Plasmodium falciparum* and *Plasmodium vivax* malaria (10, 11, 17). Moreover, amopyroquin seems more active in vitro than the other 4-amino quinolines (chloroquine or monodesethyl amodiaquine) against resistant strains of *P. falciparum* (15).

Amopyroquin is metabolized more extensively in rats than in rabbits to three metabolites, one of which is probably the primary amine derivative (15). In rats, amopyroquin is widely distributed in tissues and in plasma the drug concentrations are decreased, with a terminal half-life of 14 h (15, 18). But during the 8 h after a single 60- or 120-mg/kg (body weight) dose of amopyroquin given intraperitoneally, this half-life has been estimated as 2 h (6). In rabbits, a similar bioavailability, about 70%, was observed after oral and intramuscular (i.m.) administration, and terminal elimination half-lives were 18, 24, and 26 h for intravenous (i.v.), i.m., and oral routes, respectively (15). No study has described the metabolism and the pharmacokinetic behavior of amopyroquin in humans.

The aims of the present study were as follows: (i) to investigate the metabolism and the pharmacokinetic parameters of amopyroquin in healthy subjects after i.m. administration of amopyroquin base at 2 mg/kg (body weight) and (ii) to compare the following three i.m. therapeutic regimens in *P. falciparum* malaria patients on the basis of drug concentrations in the blood and in vivo efficacy: 3 mg/kg (body weight) (group 1), 6 mg/kg (group 2), and 6 mg/kg followed by 3 mg/kg 24 h later (group 3).

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MATERIALS AND METHODS

Subjects. (i) Volunteer study. The study was performed with 10 healthy volunteers (six men and four women) after their written informed consent was obtained. They were between 21 and 38 years old (mean, 27 ± 6 years) and weighed between 50 and 78 kg (mean, 65 ± 10 kg). A heparinized catheter was inserted into a forearm vein, and predose samples of blood and urine were obtained. A single 2-mg/kg (body weight) i.m. dose of amopyroquin base (Warner-Lambert Parke Davis, Courbevoie, France) (total dose, 100 to 150 mg) was injected. In a preliminary study, the first blood samples were taken at 0.5 h postinjection for subjects 1 and 2 and at 0.166, 0.333, and 0.5 h for the other eight subjects. Subsequently, blood samples were taken from all subjects at 1.0, 1.5, 2, 3, 4, 6, 10, 14, 24, 30, 36, 48, 56, 72, 96, 120, 144, 192, 360, and 396 h postinjection. For subjects 3 to 10, urine was collected at time intervals of 0 to 6, 6 to 12, 12 to 24, and 24 to 48 h postinjection. Additional urine samples were collected at days 15 and 30. For all subjects, routine measurements of hematological indices, liver enzymes, plasma electrolytes, urea, and creatinine were performed before injection and at days 15 and 30 postinjection. Blood pressure and heart rate were measured before injection and during the first 24 h postinjection.

(ii) Therapeutic study. Twenty-two *P. falciparum* malaria patients, including one from the Congo and one from Togo, admitted to Claude Bernard Hospital were included in this study from a larger therapeutic field study on the basis of denial of taking antimalarial drugs, absence of 4-amino quinolines in the urine (3), and absence of chloroquine, monodesethyl amodiaquine, and quinine in the blood samples corresponding to the amopyroquin assay (day 1 or day 2). The presence of sulfadoxinepyrimethamine was not tested. All patients had given informed consent. The country

of residence, age, weight, and degree of parasitemia at day 0 are listed below (see Table 3). At day 0, blood was withdrawn for the determination of the in vitro activity of chloroquine and amopyroquin for almost all isolates in the semi-microtest (12).

Amopyroquin base was injected at an i.m. dose of 3 mg/kg (body weight) in six patients (group 1), 6 mg/kg in seven patients (group 2), and 6 mg/kg followed by 3 mg/kg after 24 h in nine patients (group 3). Blood samples were collected (by pricking the finger) for parasite counts at days 1, 2, and 7 (0.25 μl of blood examined) and in EDTA-containing tubes (venous blood) for drug assay at day 2 (groups 1 and 3) or at day 1 (group 2).

Parasite densities were assessed at day 14 or later only for the two hospitalized patients since reinfestation could have occurred in the other 20 patients. Patients who were not cured with amopyroquin were treated with quinine.

Drug analysis. A portion of whole blood was put aside, and another was centrifuged at 1,500 × *g* for 15 min at room temperature. Plasma, whole blood, and urine were stored at −20°C until analysis.

In plasma, whole blood, and urine, amopyroquin concentrations were measured by a slight modification of the previously described high-performance liquid chromatographic method (13). Because of the absence of the less polar metabolite previously detected in the blood of rats and rabbits, it was possible to use the 4-amino quinoline 6-8-dichloro-4-(1-methyl-4-diethylamino-butylamino) quinoline as the internal standard. The coefficient of variation of repeated determinations of any one sample (intra-assay) was 4.5% for a concentration of 50 nmol/liter in the three biological media. The coefficient of variation of the primary amine metabolite (intra-assay) was 6% in urine (the only medium in which it was quantified) for a concentration of 50 nmol/liter. The limit of sensitivity was 10 nmol/liter for nonmetabolized drug (3.5 ng/ml) and metabolite (3 ng/ml). In urine samples, amopyroquin and its primary amine metabolite, 4-[(7-chloro-4-quinolylamino)]-2-(aminomethyl)phenol, were assayed in 500 and 1,000 μl of urine diluted in water (1:10).

Kinetics analysis. The concentration-time curves for amopyroquin in plasma and in whole blood were fitted by using a tri-exponential model with a first-order absorption with a linear least-squares computer, Graphakin, in conjunction with the Tektronix 4051 calculator. The pharmacokinetic parameters, including the area under the experimental concentration-time curve, the total extrapolated area, the half-life associated with each exponent ($t_{1/2\alpha}$, $t_{1/2\beta}$, $t_{1/2\gamma}$), the total systemic clearance (CL/F), and the volume of distribution (*V*/F), were computed from the equations describing the data by using standard procedures (8).

Renal clearance (CL_R) was calculated from urine and plasma data from the first 48 h on the basis of the following equation: CL_R = (amount of nonmetabolized excreted drug/time unit)/C_p, where C_p is the concentration in plasma at the midpoint of the urine collection interval.

The drug concentration-time curve in whole blood was obtained from the volunteer subjects' means. It was used to model the concentration-time profile of the various therapeutic regimens.

In vitro activity of the drugs. The in vitro activities of amopyroquin and chloroquine against the *P. falciparum* isolates were determined by using the [^{3}H]hypoxanthine semi-microtest (12).

Statistical analysis. The results were expressed as the mean ± 1 standard deviation. Data were analyzed by using Bartlett's test for equal variance. When significant differences between values were found, the two groups were compared by using the Mann-Whitney *U* test; otherwise, the means were compared by using Student's *t* test.

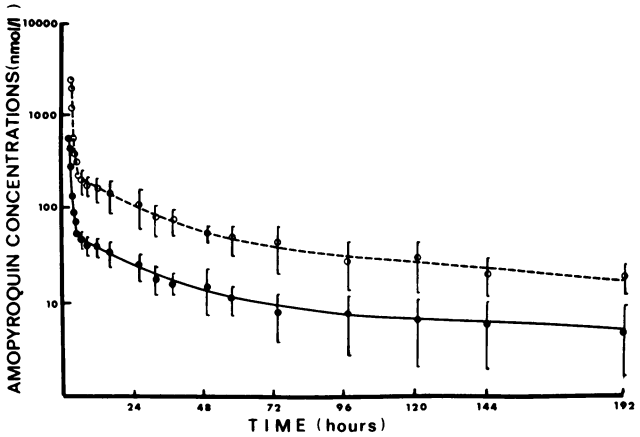

FIG. 1. Profiles of mean amopyroquin concentration-time curves obtained in plasma (●) and in whole blood (○) after a single i.m. injection of 2 mg of amopyroquin base per kg in healthy volunteers. Bars indicate 1 standard deviation.

RESULTS

Amopyroquin metabolites. After i.m. injection of amopyroquin into healthy subjects and malaria patients, amopyroquin and one metabolite, identified as the primary amine by chromatographic comparison with a standard, were detectable in both plasma and whole blood. However, the concentration of the metabolite was so low (about 5 to 10 nmol/ml) that it was difficult to quantify it precisely. Therefore, we decided not to consider these results. Nonmetabolized amopyroquin seems to be the major form of the drug in human blood. However, both amopyroquin and its metabolite could be quantified in urine during the first 48 h. Other metabolites (which are found in animals) were not found.

Amopyroquin kinetics in healthy volunteers. Amopyroquin appeared rapidly in plasma after i.m. administration; the time to maximum concentration in plasma (T_{max}) was 0.25 ± 0.08 h. The corresponding peak concentration in plasma varied between 296 and 883 nmol/liter (mean ± standard deviation, 536 ± 198 nmol/liter). This rapid absorption phase was followed by a tri-exponential decline (Fig. 1), and the concentration in plasma represented only 9.5, 4.5, and 2.5% of the peak concentration after 3, 24, and 48 h, respectively. At 360 h postinjection, amopyroquin concentrations were lower than the sensitivity limit of the analytical method. Calculated plasma pharmacokinetic parameters are summarized in Table 1. The half-lives of the three exponential phases were as follows: $t_{1/2\alpha}$, 0.34 ± 0.09 h (range, 0.17 to 0.48 h); $t_{1/2\beta}$, 10.7 ± 3.2 h (range, 7.3 to 17.5 h); and $t_{1/2\gamma}$, 129.6 ± 92.5 h (range, 31.6 to 268.0 h). *V*/F and CL/F were 238 ± 75 liters/kg and 2,063 ± 1,159 ml/min, respectively. The mean CL_R was 119 ± 99 ml/min (range, 23 to 342 ml/min).

The peak concentration of amopyroquin appeared simultaneously in whole blood and in plasma. The time course of amopyroquin concentrations in blood was not different from that in plasma, but concentrations were consistently higher ($P < 10^{-9}$) (Fig. 1). The ratio between the amopyroquin concentration in whole blood and that in plasma was 4.8 ±

TABLE 1. Pharmacokinetic parameters for amopyroquin in plasma after i.m. injection of a single 2-mg/kg (body weight) dose of amopyroquin base in healthy volunteers[a]

Subject	Wt (kg)	C_{max} (nmol/liter)	T_{max} (h)	$t_{1/2}$			$AUC_{0-\infty}$ (nmol · h/liter)	V/F (liters/kg)	CL/F (ml/min)	CL_R (ml/min)
				α (h)	β (h)	γ (h)				
1	78			0.37	13.9	218.6	8,609	198	817	
2	60			0.37	11.1	248.4	7,469	337	940	
3	70	646	0.33	0.48	9.4	73.6	2,385	251	2,750	90
4	75	378	0.33	0.21	11.8	68.4	2,495	223	2,817	98
5	74	697	0.33	0.35	17.5	37.7	1,945	160	3,617	342
6	54	883	0.17	0.31	11.2	31.6	1,477	175	3,450	157
7	60	455	0.33	0.35	7.3	34.0	1,883	147	2,991	42
8	57	375	0.17	0.37	9.7	166.0	4,283	314	1,248	23
9	73	555	0.17	0.39	7.4	150.0	5,604	219	1,230	93
10	50	296	0.17	0.17	8.2	268.0	6,160	356	767	108
Mean ± SD	65 ± 10	536 ± 198	0.25 ± 0.08	0.34 ± 0.09	10.7 ± 3.2	129.6 ± 92.5	4,231 ± 2,582	238 ± 75	2,063 ± 1,159	119 ± 99

[a] C_{max}, Maximum concentration of drug in plasma; T_{max}, time to C_{max}; $t_{1/2}$, half-life; $AUC_{0-\infty}$, area under the concentration-time curve from 0 h to infinity; V/F, volume of distribution; CL/F, total systemic clearance; CL_R, renal clearance.

2.0, with a wide variability among subjects (range, 2.2 to 7.8).

Urinary excretion. The amount of nonmetabolized amopyroquin excreted during the first 48 h after a single i.m. amopyroquin base injection of 2 mg/kg (body weight) was 4,546 ± 2,782 nmol, which represented 1.2% of the administered dose. The mean recovery of the primary amine metabolite was 0.2% during the first 48 h, with a wide variability among subjects (Table 2). Amopyroquin and its metabolite were still detectable in urine samples taken 30 days postinjection.

Side effects. The subjects did not report any major side effects, and there was no significant change in blood pressure or heart rate during the first 24 h or in biochemical or hematological parameters at days 15 and 30. Nevertheless, almost all the subjects (healthy subjects and patients) complained of a burning sensation at the injection site that persisted for 2 h postinjection.

Therapeutic study. The parasitemia at days 0, 1, 2, and 7, the amopyroquin levels in blood obtained with the three regimens, and the in vitro susceptibilities to chloroquine and amopyroquin of almost all of the *Plasmodium* isolates are summarized in Table 3. Parasitemia was cleared at day 7 in one subject of group 1 (3 mg/kg [body weight], $n = 6$), four subjects of group 2 (6 mg/kg, $n = 7$), and seven subjects of group 3 (6 + 3 mg/kg, $n = 9$). In all other subjects, only a temporary decrease in parasitemia was observed. The ranges of amopyroquin levels in blood were 62 to 123 nmol/liter at 48 h (group 1), 131 to 237 nmol/liter at 24 h (group 2), and 106 to 236 nmol/liter at 48 h (group 3). All amopyroquin 50% inhibitory concentrations (IC_{50}s) were ≤25 nmol/liter, which is much lower than those of chloroquine both for chloroquine-susceptible and chloroquine-resistant isolates. No difference could be found between the incidence of therapeutic efficacy and failure of treatment with regard to the parasite count at day 7, susceptibility to chloroquine (or amopyroquin when studied), and drug concentrations at 24 or 48 h (Table 3). In subjects infected with a chloroquine-susceptible strain (IC_{50}, ≤100 nmol/liter), efficacy at day 7 was associated with amopyroquin levels in blood ranging from 106 to 179 nmol/liter and failure of treatment was observed with levels in blood ranging from 62 to 149 nmol/liter. In subjects infected with a chloroquine-resistant strain (IC_{50}, >100 nmol/liter), efficacy was associated with levels in blood ranging from 143 to 237 nmol/liter and failure was observed with levels in blood ranging from 104 to 227 nmol/liter. Nonparametric tests have not shown any difference in concentrations in blood between efficacy and failure of treatment. Despite wide variability between the drug concentrations measured at the same time in the regimens for groups 1 and 3, a significant difference was

TABLE 2. Percentage of administered amopyroquin dose excreted in urine after a single i.m. injection of 2 mg of amopyroquin base per kg[a]

Subject	% Excreted at:							
	0–6 h		6–12 h		12–24 h		24–48 h	
	APQ	M	APQ	M	APQ	M	APQ	M
3	0.50	0.16	0.20	0.16	0.16	0.11	0.28	0.76
4	0.34	0.00	0.13	0.01	0.16	0.00	0.08	0.01
5	1.56	0.01	0.44	0.01	0.18	0.01	0.08	0.01
6	0.50	0.01	0.31	0.01	0.19	0.01	0.29	0.03
7	0.12	0.02	0.14	0.02	0.05	0.01	0.08	0.01
8	0.12	0.06	0.15	0.06	0.07	0.07	0.08	0.11
9	0.14	0.00	0.30	0.02	0.21	0.01	0.20	0.04
10	0.73	0.18	0.50	0.03	0.58	0.02	0.91	0.06
Mean ± SD	0.50 ± 0.48	0.06 ± 0.07	0.27 ± 0.14	0.04 ± 0.05	0.20 ± 0.16	0.03 ± 0.04	0.25 ± 0.28	0.13 ± 0.26

[a] APQ, Unchanged amopyroquin; M, primary amine derivative.

TABLE 3. Therapeutic study (decrease in parasitemia, IC_{50}s of chloroquine and amopyroquin for *P. falciparum* isolates, and levels of amopyroquin in blood with the three regimens)

Dose (mg/kg)	Subject	Wt (kg)	Age (yr)	Geographic origin	Parasitemia (parasites/μl of blood) on day:				IC_{50} (nmol/liter) of[a]:		Whole blood APQ level (nmol/liter)[b]
					0	1	2	7	Cq	APQ	
3	1	56	28	Gabon	10,000	90	1,400	2,400	520	25	104
	2	48	17	Gabon	68,000	1,200	700	300	40	5	62
	3	42	14	Gabon	12,500	7,500	0	0	110	5	123
	4	33	12	Gabon	7,700	2,400	4,200	40	20	7	108
	5	34	12	Gabon	2,800	2,900	1,000	4,400	240	9	113
	6	25	8	Gabon	92,000	1,800	2,000	400	320	14	110
6	1	15	4	Gabon	31,000	12,000	1,250	81,600			131
	2	17	4	Gabon	70,000	45,000	500	250	260		205
	3	33	9	Gabon	102,000	20,000	250	0			195
	4	45	14	Gabon	21,000	13,000	0	0	780		237
	5	20	7	Gabon	56,000	51,000	250	8			213
	6	14	3	Gabon	9,000	5,200	250	0	390		193
	7	24	5	Gabon	12,000	21,000	2,000	0	310	4	143
6 + 3	1	35	13	Gabon	11,000	9,000	250	0	30	7	106
	2	45	15	Gabon	112,000	47,000	125	170	310	23	227
	3	40	13	Gabon	3,700	6,000	750	0	390	4	172
	4	50	18	Gabon	15,000	0	0	0	220	0	188
	5	40	14	Cameroon	53,300	590	0	0			200
	6	55	65	Cameron	2,700	90	40	0	10	5	179
	7	15	10	Cameron	4,000	83,300	20	2,000	70	5	149
	8	81	32	Congo	29,400	19,600	430	0	30	7	178
	9	80	36	Togo	17,500	13,330		0			236

[a] Cq, Chloroquine; APQ, amopyroquin.
[b] In the 3- and 6- + 3-mg/kg regimens, the level was measured at 48 h; in the 6-mg/kg regimen, it was measured at 24 h.

observed between the respective means: 103 ± 21 and 182 ± 39 nmol/liter ($t = 4.443$, degrees of freedom = 13, $P < 10^{-3}$).

DISCUSSION

In a recent study, we have shown that amopyroquin undergoes greater biotransformation in rats than in rabbits (15). In humans, only the primary amine metabolite was detected and the disappearance of the other two metabolites permitted an internal standard to be used in the analytical method and the reproducibility to be increased. The levels of the primary amine in whole blood and in plasma were too low in humans to be taken into account, but in the first 48 h, about 0.2% of the amopyroquin dose was excreted in the form of this metabolite. The transformation of the diethylamino radical (present in chloroquine and amodiaquine) in a pyrrolidyl cycle seems to result in a greater stability in view of the degradation by the major desethylation pathway by which chloroquine and amodiaquine are metabolized (Fig. 2). In human blood, nonmetabolized amopyroquin seems to be the form of the drug that is responsible for the antimalarial activity. The in vitro activity of the primary amine against both chloroquine-susceptible and chloroquine-resistant strains was very low (16). In contrast, after oral administration of chloroquine the concentrations of mono- and bidesethyl chloroquine reached about 1/3 to 2/3 and 1/50 that of chloroquine, respectively (7, 19). When amodiaquine is given orally, relatively small amounts of the parent drug are present in blood (23). Hepatic biotransformation to monodesethyl amodiaquine is the main route of amodiaquine clearance, and this metabolite is the effective form of the drug (5, 14). Only a small amount of bidesethyl amodiaquine was detected in the blood and in urine (4, 14), and this amodiaquine metabolite was actually the primary amine derivative obtained by chemical reactions on the double bonds of the pyrrolidyl cycle of amopyroquin.

Comparison of amopyroquin pharmacokinetic parameters with those of chloroquine and monodesethyl amodiaquine is limited by the differences between the experimental designs of the previous studies: duration of sampling, sensitivity of the analytical methods, and the best model for fitting the concentration decreases. In humans, amopyroquin exhibits a very rapid absorption phase and the time to peak concentration in plasma (T_{max} = 15 min) was similar to that observed after a single i.m. 200-mg dose of chloroquine base (1) but was less than that observed in the rabbit after a 10-mg/kg (body weight) i.m. injection (15). Like chloroquine and monodesethyl amodiaquine, amopyroquin exhibits a triphasic decline in concentrations in plasma and blood and a long terminal elimination phase. Nevertheless, this terminal half-life (129.6 h) is shorter than that of chloroquine after a single oral dose in healthy subjects (792 h) (7) or after a single 200-mg i.m. dose in healthy African subjects (216 h) (1). It is also shorter than the terminal half-life of monodesethyl amodiaquine (311 h) after a single oral dose of amodiaquine of 10 mg/kg in healthy subjects (16). In contrast, following intravenous administration of amodiaquine, the monodesethyl derivative was not detected and the decay in amodiaquine concentrations in plasma followed a biphasic profile, with a terminal half-life of about 10 h (22). The terminal half-life of amopyroquin also seems longer in humans than in rabbits (26 h) by the i.m. route; this indicates interspecies variability (15). The very steep slopes of the first two phases of amopyroquin concentration decrease ($t_{1/2\alpha}$, 0.3 h; $t_{1/2\beta}$, 11 h) led to 10 and 3% of the peak concentration at the end of the two first phases (about 3 and 48 h). The third phase led to concentrations close to the limit of detection,

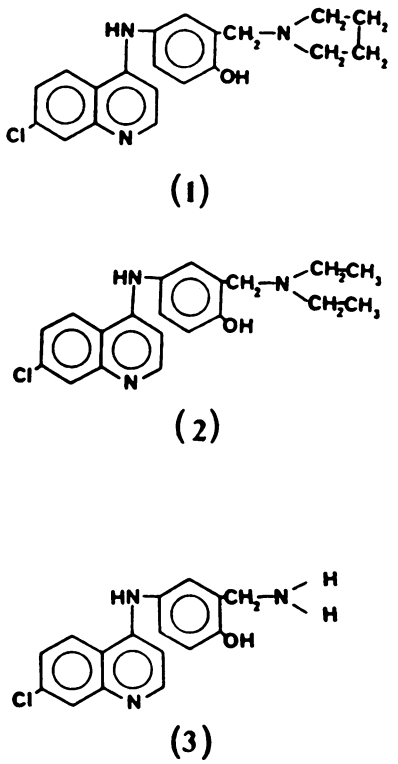

FIG. 2. Chemical structures of amopyroquin (no. 1), amodiaquine (no. 2), and the primary amine metabolite (no. 3).

and they probably do not result in antimalarial activity. The large volume of distribution of amopyroquin (240 ± 80 liters/kg) is in good agreement with that obtained through various routes of chloroquine administration: 181 ± 48 liters/kg after a single 200-mg i.m. dose (1), 261 ± 108 liters/kg after a 2-mg/kg (body weight) i.v. infusion (20), and about 800 liters/kg after a single 150-mg oral dose (7). In contrast, following i.v. administration the apparent volume of distribution of nonmetabolized amodiaquine (17.4 liters/kg) is much smaller (22). This large volume of distribution observed with both amopyroquin and chloroquine is probably due to the high concentrations of these drugs in tissue (15, 20). Slow redistribution between tissue stores and blood might account for the slow elimination half-life. Amopyroquin uptake by erythrocytes, estimated by the ratio of whole blood to that in plasma (4.8 + 2.0), is similar to that of monodesethyl amodiaquine (mean, 5.5) (16) but seems to be less than that of chloroquine (range, 8 to 10) (7). Amopyroquin total systemic clearance (CL/F = 2,100 ± 1,200 ml/min) appears to be greater than that of chloroquine after oral administration of 150 mg (about 1,200 ml/min) (7) and i.m. injection of 200 mg (660 ml/min) (1) but lower than that of amodiaquine after oral administration of 200 mg (163,000 ml/min) (24) or i.v. administration of 3 mg/kg (body weight) (about 10,800 ml/min) (22).

Renal clearance of amopyroquin (CL_R = 120 ± 100 ml/min) seems to correspond approximately to the rate of glomerular filtration. Chloroquine renal clearance (CL_R = about 400 ml/min) appears substantially greater than the glomerular filtration rate; this suggests that chloroquine renal excretion takes place by both glomerular filtration and tubular secretion (20). Renal clearance represents about 50% of systemic clearance for chloroquine (9, 20) but only 5 to 10% for amopyroquin; this suggests an associated biliary excretion pathway, as was previously described for amodiaquine (2). In addition, about 1% of the amopyroquin dose is excreted in urine during the first 24 h in the nonmetabolized and metabolized form. This renal elimination route appears to be less important for amopyroquin than for chloroquine and monodesethyl chloroquine, about 11% of the administered dose in the same period (20), but of similar importance for amodiaquine, about 2% of the oral dose as nonmetabolized drug and the monodesethyl metabolite (23).

In an attempt to evaluate the efficacy of amopyroquin against the parasite, three regimens were assessed by use on African malaria patients selected from a larger study on the basis of absence of other 4-amino quinolines and of quinine in their blood. As predicted by the low $t_{1/2\alpha}$ and $t_{1/2\beta}$ values, a single 3-mg/kg (body weight) i.m. injection was not sufficiently effective. The difference in efficacy between our results and those reported previously (10, 11, 17) might be partly explained by the facts that malaria patients were infected with both *P. vivax* and *P. falciparum* and, above all, that the drug resistance of the parasite was just emerging at that time. The results obtained with the other two regimens show that drug concentrations at 24 or 48 h and therapeutic efficacy at day 7 were the highest with the 6- + 3-mg/kg (body weight) regimen. The differences in the levels of amopyroquin in blood that were encountered with the same regimen could probably be explained by the wide variability among individuals in the pharmacokinetic parameters.

Nevertheless, complete efficacy was not obtained in these partly immune patients, in whom it was not possible to verify the occurrence of late recrudescence. Since parasiticidal drug concentrations in blood must be maintained for at least 4 days, i.e., for about 100 h (corresponding to two asexual cycles of development), to ensure total parasitemia clearance we considered an arbitrary high concentration (200 nmol/liter) that would be necessary to avoid therapeutic failure in both chloroquine-susceptible and chloroquine-resistant cases.

The modeled drug concentration in blood-time profiles (Fig. 3) derived from the volunteer study show that the 3-mg/kg regimen could maintain a concentration of 200 nmol/liter for only 15 to 20 h, that the 6-mg/kg regimen could maintain it for only 40 h, and that the 6- + 3-mg/kg regimen could maintain it for only 72 h. Subsequently, a 12-mg/kg regimen was modeled in two ways: a loading dose of 6 mg/kg (body weight) followed by either one additional dose of 6 mg/kg 24 h later or two doses of 3 mg/kg each 24 and 48 h later (Fig. 3). Although the concentration 200 nmol/liter could be maintained for about 100 h in both cases, the alternative profile of 6 mg/kg associated with an additional two injections, each consisting of 3 mg/kg at an interval of 24 h, would be more appropriate to reduce differences between peak and trough concentrations, yielding a maintenance concentration, and to lessen the possibility of transient excessively high concentrations, as have been described for amodiaquine (22) and chloroquine (21). However, this total dose of 12 mg/kg (body weight) is lower than that recently proposed for chloroquine (25 mg/kg in 3.5-mg/kg doses every 6 h) by the i.m. route (21).

To avoid three repetitive injections, an alternative, oral form of the drug could be chosen if bioavailability via i.m. and oral routes in humans is found to be similar to that previously demonstrated in rabbits (15).

In contrast to chloroquine and amodiaquine, amopyroquin is poorly metabolized in humans after i.m. administration. This difference could be explained by the presence of a pyrrolidyl cycle that protects it from the desethylation

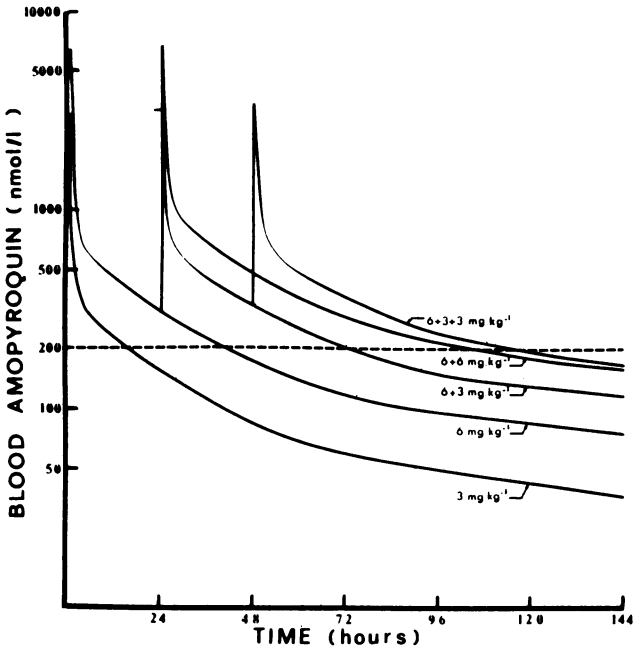

FIG. 3. Modeled concentration-time curves for various therapeutic regimens derived from the healthy volunteer curve.

pathway. Like the other two main 4-amino quinolines, amopyroquin probably is widely distributed in tissues but eliminated more rapidly. Urinary excretion seems to be one elimination route, probably associated with biliary excretion.

Although amopyroquin exhibits a high in vitro activity, as demonstrated by the low IC_{50}s, even against chloroquine-resistant isolates, the three therapeutic regimens tested do not give satisfactory in vivo efficacy. An alternative schedule of 12 mg/kg (body weight) (a loading dose of 6 mg/kg followed by 3 mg/kg both 24 and 48 h later) is suggested. But therapeutic multicenter studies should be conducted to confirm this point and to enable the optimal therapeutic regimen to be defined.

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