

Correlation of amodiaquine sensitivity to chloroquine and quinine against *Plasmodium falciparum* in vitro

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ABSTRACT

Soesanto Tjokrosonto - *Correlation of amodiaquine sensitivity to chloroquine and quinine against plasmodium falciparum In Vitro*

Amodiaquine, a 4-aminoquinoline derivative has been introduced since decades ago, and yet the use remains debatable for its side effects. Chloroquine, the least side effect of antimalarials possessing similarity in pharmacokinetic properties has been less attractive due to the development of resistance worldwide. The return to amodiaquine is debatable. Comparison of the level of sensitivity of 42 isolates to amodiaquine and chloroquine using in-vitro microtesting has been carried out. Amodiaquine was shown to be more sensitive to the isolates as compared to chloroquine. The reasonably high correlation with chloroquine ($r=0.62$; $p<0.05$) has referred the alternative treatment of chloroquine resistant falciparum to amodiaquine despite of side effects. The study concluded that amodiaquine remains an alternative drug to chloroquine resistant falciparum malaria in vitro.

Key words: falciparum malaria - amodiaquine - chloroquine - quinine - in vitro.

ABSTRAK

Soesanto Tjokrosonto - *Hubungan antara sensitivitas amodiakuin dan klorokuin dan kina terhadap plasmodium falciparum in vitro*

Amodiakuin, obat antimalaria derivat 4-aminoquinolin sudah dikenalkan sejak lama namun pemakaiannya tetap diperdebatkan disebabkan adanya efek samping. Klorokuin, obat antimalaria yang sudah sering dipakai dan mempunyai kesamaan farmakokinetik hanya menimbulkan sedikit efek samping, tetapi pemakaiannya telah mengalami masalah karena munculnya resistensi plasmodium terhadap obat ini di berbagai tempat di dunia. Perbandingan kemanjuran kedua obat ini terhadap 42 isolat dari penderita malaria falciparum telah dilakukan secara in vitro. Amodiakuin ternyata lebih baik dibandingkan dengan klorokuin dan menunjukkan adanya korelasi antara kedua obat tersebut ($r=0,62$; $p<0,05$). Penelitian ini menunjukkan bahwa amodiakuin merupakan obat alternatif dari klorokuin secara in vitro terhadap malaria falciparum dengan tanpa meninggalkan kemungkinan adanya efek samping. Amodiakuin dan klorokuin tidak menunjukkan korelasi yang bermakna dengan kina.

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INTRODUCTION

Amodiaquine as 7-chloro-4-(4-diethylamino-1-methylbutylamino) quinoline is a 4-aminoquinoline derivative and has been introduced since decades ago but the use in practice is hampered

by its side effects especially if used as prophylactic by travellers. Amodiaquine is prepared as tablets and suspension. The tablets contain 200 and 600 mg amodiaquine base as hydrochloride or 153.1 mg base as chlorhydrate. The suspension contains 10 mg/ml amodiaquine base as hydro-

chloride or chlorhydrate. Amodiaquine is more palatable than chloroquine and may be more effective. A systematic review of amodiaquine support the use of the drug for treatment of uncomplicated malaria, but it is not recommended as first-line treatment of malaria¹. It is a pro drug for the active malaria metabolite desethylamodiaquine possessing similarity in pharmacokinetic properties of chloroquine. The latter has been less attractive since the development of resistance worldwide. The most striking effect of both these 4-aminoquinolines is an inhibition of maturation at successively earlier stages of development as ring forms are exposed to increasing concentration of these drugs. Many pros and contras regarding the use of amodiaquine have been reported^{2,3} but the possible use to replace chloroquine remains open. In the endemic malaria where parasites resist the effect of chloroquine, quinine also has been the drug of choice after Fansidar (pyrimethamine-sulphadoxine). The side effects distract the use of quinine in practice. Despite the practical single dose, Fansidar should be used with care for the fear of the development of Stevens-Johnson syndrome. Based on problems occurring in the use of these drugs, therefore amodiaquine can be used as the alternative drug among quinine and Fansidar. Amodiaquine has not been introduced in Indonesia for some (political) reasons. It is as cheap as chloroquine and saver than quinine and Fansidar. In the light of the global debate over the use of amodiaquine, assessment of this drug on its association to chloroquine and the other antimalarial has been analyzed *in-vitro*.

MATERIALS AND METHODS

The study was conducted based on hospital subject with falciparum malaria in Jayapura hospital in 1984. The use of amodiaquine remains possible and debatable and to reinforce the knowledge of it the old data is therefore reopened and analyzed. It is always understood that the data remain valid to support current progress on discussion of antimalarial drugs.

An amount of 42 subjects met the inclusion criteria of malaria falciparum using microscopy were asked to contribute their blood to obtain the wild isolates for drug sensitivity testing. Exclu-

sion criteria included those suffering from malaria of other species and mixed malaria, and those who underwent complicated malaria. Subjects have been exposed to antimalarials for the past 14 days were also excluded from the study. To assure the absence of the drugs in the blood and urine therefore Dill-Glazko and Lignin tests^{3,4} have been applied. WHO *in-vitro* micro-testing technique⁵ was applied to measure the sensitivity of amodiaquine, chloroquine and quinine to *Plasmodium falciparum* drug impregnated microplates for the purpose in complete package including the instruction have been purchased directly from the WHO. For short, an amount of 100 ul blood was extracted from finger prick into a sterile anticoagulant treated micropipette of each isolate. Only subjects with parasitaemia of 500 asexuals or more were included (containing less than 0.01% *Plasmodium falciparum* parasites per microliter) or mixed infection. It was then mixed with 900 ul growth medium (provided in the package) in a vial and swirled to obtain a homogenized suspension of blood. An amount of 50 ul of the suspension was the placed in drug impregnated microplate wells in descending order from A to H. Each of isolate was tested in duplicates. The microplate were then incubated in 37°C for 40 hours in a candle jar to allow the parasite to develop schizont with more than two nuclei. All the procedure was conducted according to the WHO instruction. Results describing percentage of schizont maturation inhibition by different concentration of each drug after incubation period were then computed using WHO program to obtain the effective drug concentrations (ECs) causing 50%, 90% and 99% of growth inhibition of each drug to the corresponding wild isolates. The doses were expressed as EC-50, EC-90 and EC-99 per liter of blood respectively. Resistance was determined if the schizont growth was not inhibited at 1.14×10^{-6} mol/l for chloroquine, 0.8×10^{-6} mol/l for amodiaquine and 51.2×10^{-6} mol/l for quinine⁶. Correlation coefficient (r) was then computed to measure the association between ECs of the drugs.

RESULTS

An amount of 42 isolates were assessed in parallel *in vitro* for the sensitivity to amodiaquine,

chloroquine and quinine. The sensitivity levels of isolates to the drugs were analyzed and shown as tables. All tests to chloroquine were successfully carried out and the schizont growth of 4 isolates were completely inhibited at 1.14×10^{-9} mol/l blood. No ECs can be calculated at this stage since lack of data. The remaining 38 (90.48%) were all highly resistant to chloroquine (TABLE 1).

The tests to amodiaquine showed 40% of isolates were resistant to amodiaquine (TABLE 2) which is much less as compared to those to chloroquine.

When the corresponding 40 isolates were tested to quinine, 2 isolates failed to grow in the microculture and only 12.5% were recorded as resistant to quinine (TABLE 3).

Mole for mole, the activity of amodiaquine in vitro was greater than that of chloroquine against the parasites with a high level of resistance to chloroquine. There was significant association between amodiaquine and chloroquine ($r=0.62$; $p<0.05$). Non significant association were ob-

served between amodiaquine and quinine ($r=0.30$; $p>0.05$), and chloroquine to quinine ($r=0.33$; $p>0.05$).

DISCUSSIONS

The reasonably high association with chloroquine ($r=0.62$) has referred the alternative treatment of chloroquine resistant *falciparum* to amodiaquine despite the side effects that may encounter. Amodiaquine has been associated to hepatitis and agranulocytosis in human cases. Agranulocytosis is attributed to reactive intermediates triggered by oxydant discharge from the leucocytes⁷. The correlation is acceptable since both drugs are 4-aminoquinoline derivatives. In a randomized controlled trial on chloroquine, amodiaquine and Fansidar it was observed that the *Plasmodium falciparum* parasites showed more sensitivity to amodiaquine than chloroquine ($p=0.0009$) or Fansidar ($p=0.0001$)⁸. EC50s (50% inhibitory concentrations) of amodiaquine has exhibited a significant correlation with chloroquine

TABLE 1. - The effective concentrations (ECs) of chloroquine to the parasites population.

Inhibition level ($\times 10^{-9}$ mol/l blood)	No. isolates	ECs mol/l blood			Slope	Var	X ²
		50	90	99			
1.14	4	-	-	-	-	-	
1.60	10	.98	1.97	3.01	4.07	0.00012 $\times 10^{-9}$	
3.20	10	1.31	4.22	10.89	2.53	0.07 $\times 10^{-9}$	
6.40	18	1.63	5.26	13.21	2.56	0.01 1×10^{-9}	

X² <0.05 indicates significant heterogeneity

TABLE 2. - The effective concentrations (ECs) of amodiaquine to the parasites population (only 20 tests were done).

Inhibition level ($\times 10^{-9}$ mol/l blood)	No. isolates	ECs mol/l blood			Slope	Var	X ²
		50	90	99			
0.05	7	-	-	-	-	-	
0.4	5	0.11	0.18	0.32	5.16	1 $\times 10^{-9}$	
0.8	8	0.08	0.15	0.18	6.19	2 $\times 10^{-9}$	

X² <0.05 indicates significant heterogeneity

TABLE 3. - The effective concentrations (ECs) of quinine to the parasites population.

Inhibition level ($\times 10^{-9}$ mol/l blood)	No. isolates	ECs mol/l blood			Slope	Var	X ²
		50	90	99			
< 51.2	35	2.94	8.03	18.28	2.93	0.05 2×10^{-9}	
51.2	5	10.44	87.75	498.25	1.39	0.11 2×10^{-9}	

X² <0.05 indicates significant heterogeneity

and pyronaridine (an acridine derivative used against drug resistant falciparum malaria in China) as another alternative especially for drug resistant falciparum malaria⁹.

Amodiaquine was shown to be more inhibitive to the growth of falciparum schizont than chloroquine. This supported the suggestion of placing amodiaquine as the first line in malaria therapy. This probably accounts for amodiaquine greater inherent activity and also the differences in binding affinity of this receptor supporting the case that amodiaquine is more potent in-vitro as compared to chloroquine¹⁰. Changes of sensitivity to chloroquine may affect the sensitivity to amodiaquine in parasites population since both drugs share common genetic basis of resistance which is allelic status of *pfmdr1* locus at the codon-86 position (asparagine or tyrosine)¹¹. Four-aminoquinoline resistant mutant emerged after 10 years when a huge amount of drugs has been sold. The mutant existed in different speed and varies among parasite isolates populations. Nevertheless, resistance to amodiaquine remains scarce as compared to chloroquine. A systematic review of trials support the use of amodiaquine in the treatment of uncomplicated malaria particularly in African countries even there was partial cross resistance between chloroquine and amodiaquine^{12,13}.

Challenges to the emergence of resistance has led to the development of new antimalarial which is four quinoline di-Mannich base compound showing more activity than amodiaquine and chloroquine¹⁴.

CONCLUSIONS

This study concluded that amodiaquine is more active than chloroquine and remains an alternative drug to chloroquine resistant falciparum malaria in vitro. The use in practice should be limited to uncomplicated falciparum malaria cases and with great care.

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