Interaction of alphamangostin and curcumin with dihydroartemisinin as antimalaria *in vitro*

S Tjahjani[1*], Syafruddin[2] and R Tjokropranoto[1]

[1]Maranatha Christian University, Bandung, Indonesia
[2]Hasanuddin University, Makassar, Indonesia
[*]Corresponding author: susy_tjahjani@yahoo.com

Abstract. To overcome malarial resistance tendency against the ACT (artemisinin-based combination therapy), several galenic preparations of *Garciniamangostana* L-rind and alphamangostin as the major xanthone in this rind have been studied, and they had antimalarial activity and showed its synergistic effect with artemisinin *in vitro.* Curcumin as anactive component of turmeric is also potentially to have antimalarial activity. This study aimed to evaluate the activity as antimalarial of curcumin and dihydroartemisinin, an active metabolite of all artemisinin derivates, and also to study the mechanism of action of aphamangostin, curcumin, and dihydroartemisinin as antimalaria.The interaction between them each other as the antimalarial *in vitro* was also investigated. The antimalarial activity was studied in *in vitro* 3D7 *Plasmodium falciparum* cultivation incubated with these compounds to look for the IC_{50} and $\sum FIC_{50}$ of them. The mechanism of action of these compounds was observed electron microscopically. The result of this promising study showed that these compounds were active antimalaria agents which inhibited hemozoin formation and there is synergistic antimalarial activity interaction between alphamangostin and dihydroartemisinin.

1. Introduction

Although from 2010 to 2015 there were decreasing tendencies of malaria morbidity and malaria mortality, globally in 2015, there were still 212 million new malaria cases and 429,000 malaria deaths, especially from falciparum malaria. ACTs are very effective against *P.falciparum* infection, but nowadays there are parasite drug resistance reports against the main component of ACTs, i.e., artemisinin in several countries.[1,2] Reducing antioxidant status and becoming oxidative stress condition because of malaria, as well as artemisinins,[3,4,5] is another problem. That's why malaria drug discovery, especially as artemisinin partner drug, is very urgent to overcome it and drug which has antioxidant properties is preferred.

G. mangostana L-rind had antimalarial activity in vitro and showed synergistic action with artemisinin[6], and this rind is also a highly active antioxidant.[7] It contains a lot of xanthones which are a potent antioxidant.[8] Hydroxyxanthones could inhibit hemozoin formation by formation of a soluble complex with heme which is considered to have antimalarial activity.[9,10] One of the mostxanthonesof this rind,i.e.,alphamangostin[11] which is a potent antioxidant[12] showed antimalarial activity.[13]

Curcumin, a natural yellow pigment, as one of the important curcuminoids, and as an active component of turmeric[14,15] is also an antioxidant.[16] Curcumin at low concentration in vitro produced ROS and inhibited histone acetylation enzyme which potentially inhibited transcription process in the parasite, and this might closely correlate with antimalarial activity.[17]

This study aimed to evaluate this in thevitro antimalarial activity of curcumin, and one derivate of artemisinin, i.e., dihydroartemisinin which is the active metabolite of all artemisinin derivate and also to study the mechanism of action of alphamangostin, curcumin, and dihydroartemisinin especially in inhibiting hemepolymerization. We also investigated the in the vitro antimalarial interaction between them.

2. Methods

Alphamangostin, curcumin, and dihydroartemisinin were bought from Biopurify Phytochemicals Ltd., Chengdu, China. *P. falciparum* 3D7 clone was obtained from The Malaria Laboratory, The Eijkman Institute for Molecular Biology, Jakarta, Indonesia. Several steps were done during this experiment. In vitro antimalarial activity analysis of curcumin and dihydroartemisinin compound was done as well as the antimalarial activity of combinations of alphamangostin-dihydroartemisinin, curcumin-dihydroartemisinin, and alphamangostin-curcumin in 1:1 ratio. The antimalarial activity interaction between each compound was analyzed according to a formula. The in vitro heme polymerization inhibition in parasitized red blood cell as one of the antimalarial mechanism of action was studied electron microscopically.

2.1. Preparation of alphamangostin, curcumin, and dihydroartemisinin stock solution

Alphamangostin, curcumin, and dihydroartemisinin were dissolved in DMSO (dimethyl sulfoxide, Sigma Aldrich, IL, USA) separately to make stock solutions. These stock solutions were put in the -20^{o}C freezer until used. *Plasmodium* culture medium was used to dilute these stock solutions to adjust the concentration needed for the experiment.

2.2. Parasite cultivation and determination of in vitro antimalarial activity of curcumin and dihydroartemisinin against P. falciparum 3D7 clone

Duplicate, *P. falciparum* 3D7 clone was cultivated in culture medium supplemented with 10% inactivated human serum in 24 wells culture plate which was incubated with a wide concentration range of each compound following the procedure described previously.[18] Red blood cells used in the experiment were a left over or outdated material from Indonesian Red Cross. Parasitemia was calculated before and after 48 hours cultivation by counting the parasite amount in 5000 red blood cells in Giemsa stained thin blood smear. Growth inhibition level was determined by comparing the parasitemia level between treated and non-treated control group. The IC_{50} (inhibition concentration 50) indicating the antimalarial activity was analyzed using probit analysis, and antimalarial activity level was classified according to available criteria described previously by Ramalhete el al.[19]

2.3. Determination of antimalarial activity of combination of alphamangostin, curcumin, and dihydroartemisinin each other and the interaction between the compounds against P. falciparum 3D7 clone in vitro

The parasite was cultivated duplicate and in the presence each of alphamangostin-dihydroartemisinin, curcumin-dihydroartemisinin, and alphamangostin-curcumin 1:1 ratio in a wide range of concentrations. Parasite growth, growth inhibition rate, and IC_{50} were evaluated as the procedure described above.

The interaction between these compounds in the combination regimens was evaluated by determination of the $\sum FIC_{50}$ (the sum of fractional inhibition concentration 50) using the formula: $A_C/A_S + B_C/B_S$. A_C and B_C is the IC_{50} of each compound in combination regimen where the A_S and B_S is the IC_{50} of each compound in the single regimen. It was concluded additive interaction if the sum was 1, whereas antagonistic interaction if it was > 1 and synergistic interaction if it was < 1.[20]

2.4. Heme polymerization inhibition analysis by transmission electron microscopy (TEM)
TEM analysis for the pellet of the culture was done in The Eijkman Institute for Molecular Biology through several processes, i.e., fixation, dehydration, infiltration, and embedding in resin to make a sample block to be cut by a diamond knife and observed under the electron microscope.

3. Results

3.1. Antimalarial activity of curcumin, and dihydroartemisinin against P. falciparum3D7 clone in vitro
Antimalarial activity is indicated by the IC_{50} of each compound which was >1µg/mL for curcumin and 0.001-0.0001µg/mL for dihydroartemisinin.

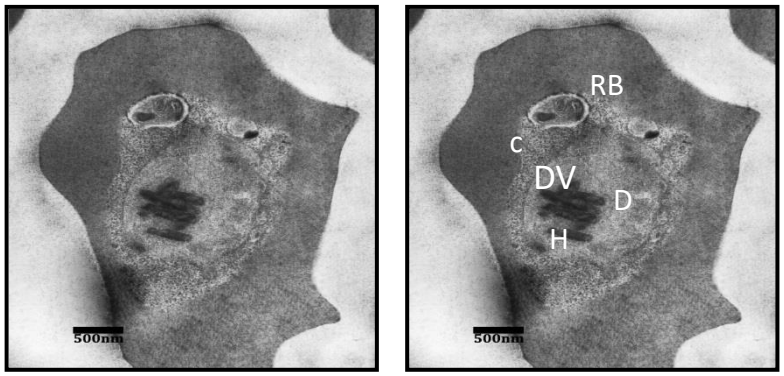
3.2. Antimalarial activity of combination of alphamangostin, curcumin, and dihydroartemisinin each other and the interaction between the compounds against P. falciparum 3D7 clone in vitro
The antimalarial activity (the IC_{50}) and the antimalarial interaction of these compounds which is indicated by the $\sum FIC_{50}$ are shown in the following table.

Table 1. The IC_{50} and the $\sum FIC_{50}$ of the combinations of compounds against *P. falciparum* 3D7 clone in vitro.

	IC_{50}	**$\sum FIC_{50}$**
Alphamangostin + dihydroartemisinin	0.0011	0.733
Curcumin + dihydroartemisinin	0.0047	2.35
Alphamangostin+ curcumin	0.27	45

It means that there is synergistic antimalarial interaction between alphamangostin-dihydroartemisinin 1:1, and antagonistic interaction between curcumin-dihydroartemisinin1:1 as well as alphamangostin-curcumin1:1.[20]

3.3. Heme polymerization inhibition analysis by transmission electron microscopy (TEM)



RBC : Red Blood Cell
Ct : Cytostome
DV : Digestive vacuole
Hz : Hemozoin
DVM: Digestive vacuole membrane

Figure 1. Hemozoin formation in parasite food vacuole without treatment.

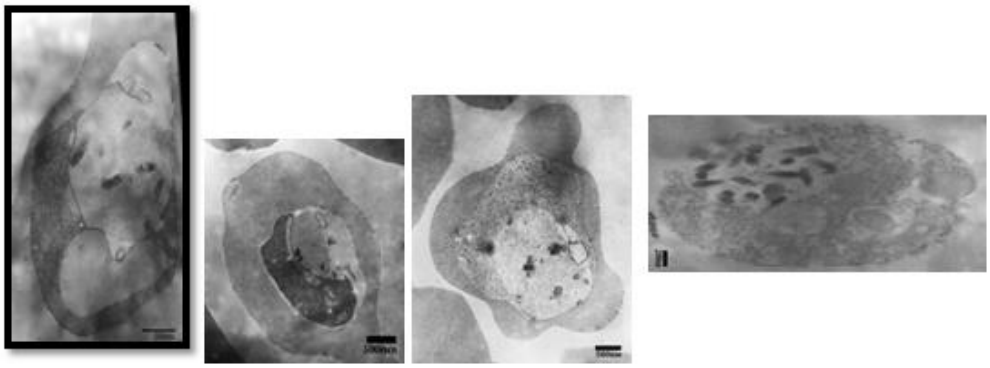


Figure 2. Lack of hemozoin formation in parasite food vacuole in several treatments consecutively from left to right side:alphamangostin (2 figures on the left), curcumin, dihydroartemisinin.

4. Discussion

4.1. Antimalarial activity of curcumin, and dihydroartemisinin against P. falciparum3D7 clone in vitro

IC_{50} of dihydroartemisinin was < 5 µg/mL which meant that it has a very active antimalarial property.[19] A similar study in Cameroon against Chloroquine sensitive *P. falciparum* also showed the highly active antimalarial activity of dihydroartemisinin.[19] From our experiment compared with another one,[20] it was concluded that the curcumin also had highly antimalarial activity. The antimalarial activity of curcumin may be correlated with perturbing of parasite's microtubules[21] and ROS formation as well as inhibition of histone acetylase enzyme of the parasite.[17] Other curcumin study showed the similar antimalarial activity which IC_{50} was 0.06 µM (2.21 µg/ mL).[22]

4.2. Antimalarial activity of combination of alphamangostin, curcumin, and dihydroartemisinin each other and the interaction between the compounds against P. falciparum 3D7 clone in vitro

Synergistic antimalarial activity between alphamangostin and dihydroartemisinin which is the active metabolite of all of the artemisinin derivates may be the basis of the synergistic interaction between several galenic preparations of *G. mangostana* L-rind and artemisinin shown in our previous study.[6] Other previous in vitro study showed the synergistic antimalarial activity between alphamangostin, gammamangostin, garcinone C, and garcinone D against artemisinin.[13] As shown in the TEM analysis, they have a similar action to inhibit heme polymerization, and it may correlate with the synergistic antimalarial activity of alphamangostin and dihydroartemisinin. But the in vitro study of the other two kinds of combinations showed antagonistic interaction as antimalarial although all of this compounds could inhibit heme polymerization. The reason for this kind of interaction needs to be studied further.

4.3. Heme polymerization inhibition analysis by transmission electron microscopy (TEM)

TEM images indicated that there was inhibition of heme polymerization in the dihydroartemisinin, alphamangostin, and curcumin treatment provided heme accumulation in the parasite food vacuole which is toxic to the parasites.

Inhibition of heme polymerization in parasite food vacuole by alphamangostin in our study is supported by the previous report that several xanthones showed inhibitory effect on heme polymerization in hemin solution .[9] Other study indicated that heme polymerization inhibition activity of a synthetic xanthone derivate even more than Chloroquine and might become a potential antimalaria drug.[23]

Curcumin can act as anantioxidant as well as aprooxidant in a concentration-dependent manner that's mean in low as an antioxidant but in higher one as a prooxidant. But against *Plasmodium falciparum*, it can already act as a prooxidant in as low as 1 µM in the presence of transitional metal

ions such as iron. The existence of this metal may be correlated with inhibition of heme polymerization.[17] This study supports our study.

Artemisinins can bind to heme as a product of hemoglobin digestion forming heart. Heart looks like heme which binds to PfHRPII (*P. falciparum* histidine-rich protein 2). While usually pfHRPII binds to heme forming hemozoin, binding of heart to PfHRPII can inhibit hemozoin formation as a product of heme polymerization.[24] This previous study supports our study in TEM analysis showing lack of hemozoin.

5. Conclusions

This study showed the promising synergistic alphamangostin-dihydroartemisinin to be studied furtherin the future as a novel malaria drug.

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