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Inactivation of Artemisinin by Thalassemic Erythrocytes

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ABSTRACT. *Plasmodium falciparum* infecting α-thalassemic erythrocytes (Hb H or Hb H/Hb Constant Spring) is resistant to artemisinin derivatives. Similar resistance, albeit at a much lower level, is shown by the parasite infecting β-thalassemia/Hb E erythrocytes. The resistance is due to host-specific factors, one of which is the higher uptake of the drugs by thalassemic erythrocytes than normal erythrocytes, due to binding with Hb H. In addition to higher drug binding, incubation of artemisinin with α-thalassemic erythrocytes resulted in preferential inactivation of the drug. Both thalassemic and normal erythrocytes have the capability to inactivate the drug. Addition of serum can protect against inactivation by normal erythrocytes, but not by thalassemic erythrocytes. Incubation with either the hemolysate or the membrane fraction from these erythrocytes also resulted in preferential inactivation of the drug. The drug was also inactivated by purified Hb H. It is concluded that the ineffectiveness of artemisinin derivatives against *P. falciparum* infecting thalassemic erythrocytes is due partly to competition of the host cell components for binding with the drugs, and partly to inactivation of the drugs by the cell components. BIOCHEM PHARMACOL **59**;11:1337–1344, 2000. © 2000 Elsevier Science Inc.

KEY WORDS. *Plasmodium falciparum*; malaria; thalassemia; artemisinin; erythrocytes; artemisinin inactivation

Falciparum malaria continues to afflict increasing millions across the tropical latitudes of the world. It remains one of the most lethal and widespread diseases due to the emergence of parasites resistant to most available antimalarial drugs [1]. Artemisinins, highly effective antimalarial drugs derived from *Artemisia annua* Linn., have played an important role for many years in the treatment of these resistant parasites, with no significant occurrence of resistance as yet [2]. *In vitro* evidence for artemisinin resistance was found when α-thalassemic erythrocytes, both Hb H‖ and Hb H/Hb CS, with genotypes of α-thal1/α-thal2 (--/-α) and α-thal1/Hb CS (--/α^{cs}α), respectively, were used as parasite hosts [3–5]. Similar reduction in artemisinin sensitivity of the parasite was also found in the old cell fractions of α-thal trait (αα/-α) and β-thal trait erythrocytes, compared with the same cell fraction of normal erythrocytes [6]. This host-dependent artemisinin resistance may be of epidemiological significance, since α-thalassemic genes are found in malaria endemic areas, and, thus, are potential sources of drug resistance [7]. Therefore, it is important to understand the factors responsible for this host-dependent artemisinin resistance.

We have shown previously that the apparent artemisinin resistance of *Plasmodium falciparum* infecting α-thalassemic erythrocytes is due mainly to the higher capacity of uninfected α-thalassemic erythrocytes for drug accumulation as compared with that of genetically normal erythrocytes [4]. This phenomenon results in depletion of the drug from the parasite environment. Subsequently, we showed that Hb H accounts for the increased binding capacity of Hb H erythrocytes, and that the binding capacity of Hb H is 5–7 times that of Hb A [5]. There is also an increase in these cells of other intraerythrocytic components, such as heme and non-heme irons [8, 9]. These react with artemisinins [2] and may also interfere with drug effectiveness. In this report, we present evidence for preferential artemisinin inactivation by α-thalassemic erythrocytes as an additional mechanism for this host-dependent artemisinin resistance.

MATERIALS AND METHODS

Materials

Artemisinin was purchased from the National Center of Natural Science and Technology, Hanoi, Vietnam. Artemisinin was recrystallized from a methylene chloride/hex-

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‖ *Abbreviations*: Hb, hemoglobin; Hb CS, hemoglobin Constant Spring; α-thal, α-thalassemia; β-thal, β-thalassemia; and K_d, dissociation constant.

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ane mixture to give white needles (m.p. 154–156°). Dihydroartemisinin was prepared from artemisinin by reduction with sodium borohydride [10] and recrystallized from a methylene chloride/hexane mixture to give white needles (m.p. 151–153°). Radioactive 15-[^{14}C]artemisinin was a gift from the Research Triangle Institute. The specific activity of [^{14}C]artemisinin was 26.1 Ci/mol. 2,8-[^{3}H]Hypoxanthine was purchased from Moravek Biochemicals. The specific activity of [^{3}H]hypoxanthine was 20–30 Ci/mmol.

Subjects

Thalassemic blood samples were obtained from the Division of Hematology, Department of Medicine, Faculty of Medicine, Siriraj Hospital, Mahidol University, where hemoglobin types were identified. Subjects had the following genotypes: Hb H (α-thal1/α-thal2), Hb H/Hb CS (α-thal1/Hb CS), and β-thalassemia with Hb E (β-thal/Hb E). All were non-splenectomized and had received no blood transfusions for at least 3 months before the collection of blood. Citrate–phosphate–dextrose solution was used as an anticoagulant.

In Vitro *Culture of* P. falciparum

A chloroquine-resistant strain of *P. falciparum* (K1) was obtained from an infected individual in the Kanchanaburi province of Thailand [11]. Parasites were maintained continuously in human erythrocytes using RPMI 1640 medium supplemented with 25 mM HEPES, pH 7.4, 0.2% $NaHCO_3$, 40 μg/mL of gentamicin, and 10% human serum [12]. Parasite growth was synchronized at the ring stage by 5% sorbitol treatment [13], and the schizont stage was collected by Percoll centrifugation [14]. Washed thalassemic blood cells were co-cultivated with schizont-infected erythrocytes for at least 96 hr before determining antimalarial sensitivity of the infecting parasites.

Antimalarial Activity Assay

In vitro antimalarial activity was determined by using the [^{3}H]hypoxanthine incorporation method [15]. Briefly, 25-μL aliquots of drug solutions of different concentrations were placed in a 96-well plate together with 200 μL of a 1.5% cell suspension of parasitized erythrocytes containing 1–2% parasitemia at the early ring stage. The mixtures were incubated in a candle jar at 37°. After 24 hr of incubation, 25 μL (0.25 μCi) of [^{3}H]hypoxanthine was added to each well. The mixtures were incubated further under the same conditions for 18–24 hr. DNA of parasites was harvested onto glass filter paper (Unifilter®, Packard). The filters were dried, and liquid scintillation fluid was added for radioactivity measurement in a 6-probe liquid scintillation counter (Packard). An IC_{50} value was determined from the sigmoid curve of percent [^{3}H]hypoxanthine incorporation against drug concentration.

In some experiments, to explore the effect of Hb H on the antimalarial activity of dihydroartemisinin, isolated Hb A and Hb H prepared from lysate of Hb H erythrocytes [5] were added to the parasites together with dihydroartemisinin. Final concentrations of Hb H and Hb A in the tests ranged from 7.5 to 60 μM.

Effect of Intact Erythrocytes on Artemisinin Inactivation

An aliquot (70 μL) of washed erythrocytes was incubated at 37° with 630 μL of [^{14}C]artemisinin in culture medium without serum (incomplete medium) for 2 hr. The final concentration of the radiolabelled drug was 1 μM. At the end of the incubation, the cell mixture was centrifuged at 10,053 *g* (Hettich, Mikro 24–48R centrifuge) for 30 sec at 4° to separate free drug from intact cells. The supernatant was used for determining artemisinin effectiveness, and the cell pellet was used for measuring artemisinin accumulation.

In some experiments, to determine the protective effect of serum on artemisinin inactivation by erythrocytes, medium containing from 10 to 100% serum was used in place of incomplete medium. In these experiments, cells were incubated with the radiolabelled drug, and then the drug effectiveness was determined from the supernatants of the cell mixtures at various time points for up to 4 hr.

Determination of Artemisinin Effectiveness

Supernatant was diluted to appropriate concentrations with culture medium. Antimalarial activity of diluted samples was determined in triplicate as described above. An aliquot (100 μL) of the undiluted supernatant was mixed with 900 μL of water and 4 mL of Triton X-100-based liquid scintillation fluid for determination of radioactivity. Then the concentration of [^{14}C]artemisinin was calculated from its specific activity. The drug effectiveness index was defined as IC_{50} of control/IC_{50} of sample.

Determination of [^{14}C]Artemisinin Accumulation in Intact Erythrocytes

After the incubation, cells were washed three times with cold 10 mM PBS solution to remove excess drug. Cells were lysed with 10 vol. of hypotonic solution (10 mM phosphate, pH 7.4). An aliquot of the lysate (500 μL) was incubated with an equal volume of 2% SDS solution at 60° for 1 hr. Then the mixture was bleached with 2 mL of 15% hydrogen peroxide at 60° for 12 hr. Next, 4 mL of Triton X-100-based liquid scintillation fluid was added for measurement of radioactivity in a liquid scintillation counter (Beckman). The results were expressed as amount of the drug (picomoles) per 10^9 cells.

TABLE 1. Amount of artemisinin accumulation in intact cells, cytosol (D_C), and membrane (D_M) compartments of uninfected normal and thalassemic erythrocytes

Erythrocyte Compartment	[^{14}C]Artemisinin (pmol/10^9 cells)			
	Normal	Hb H	Hb H/Hb CS	β-thal/Hb E
Intact cells	57.0 ± 10.0	337.1 ± 129.9*	657.0 ± 122.5*	223.8 ± 45.3*
Cytosol (D_C)	63.8 ± 11.3	304.1 ± 103.9*	509.2 ± 124.8*	207.9 ± 44.7*
$\%D_C$†	88.6 ± 1.6	85.2 ± 5.0‡	83.3 ± 8.1‡	88.9 ± 3.4‡
Membrane (D_M)	8.3 ± 2.0	58.3 ± 35.2*	95.2 ± 22.9*	26.2 ± 10.1*
$\%D_M$§	12.1 ± 2.5	14.8 ± 5.0‡	16.7 ± 8.1‡	11.1 ± 3.4‡
D_C/D_M Ratio	7.5 ± 1.7	6.4 ± 2.8‡	5.8 ± 2.7‡	8.6 ± 2.7‡

Intact erythrocytes, membrane, and cytosol were incubated at 37° for 2 hr, with 1 μM of [^{14}C]artemisinin in culture medium without serum as a 10% cell suspension or equivalent. After incubation, free drug was separated from the intact cells, membrane, and cytosol by centrifugation for intact cells and membrane, and by ultracentrifugation through 10-kDa cut-off membranes for cytosol. After washing, the pellets and the cytosol-retentate were treated as described in the text for measurement of radioactivity. The results (means ± SEM), expressed as amounts of the drug per 10^9 cells, are from three duplicate experiments. Statistical analyses were performed by using a non-parametric Mann-Whitney U test.

*$P = 0.05$, N = 3.

† $\%D_C = D_C \times 100/(D_C + D_M)$.

‡ Not significantly different, N = 3.

§ $\%D_M = D_M \times 100/(D_C + D_M)$.

Effect of Cytosol and Membrane on Artemisinin Inactivation

One milliliter of 50% (v/v) erythrocyte suspension was lysed with 3.5 mL of hypotonic solution with freeze-thawing to obtain complete lysis. An aliquot (630 μL) of the lysate was centrifuged at 22,620 *g* for 10 min at 4°. The pellet was washed with 10 mM phosphate buffer, pH 7.4, five times, to remove bound hemoglobin. Supernatant and pellet were used as cytosol and membrane samples, respectively.

Cytosol and membrane preparations equivalent to 10% (v/v) of red blood cells were incubated with 1 μM [^{14}C]artemisinin at 37° for 2 hr. The cytosol sample was centrifuged in a Centricon 10® tube to separate free drug from hemolysate. The filtrate was used for determining the drug effectiveness index. The retentate was adjusted to its original volume with hypotonic solution and treated with SDS and hydrogen peroxide to determine artemisinin accumulation as described. Free drug was separated from membrane by centrifugation at 22,620 *g*. Drug effectiveness index and accumulation then were determined as described.

Statistical Analysis

The Mann–Whitney U test was used for comparing the data from normal and variant erythrocytes based on independent random samples.

RESULTS AND DISCUSSION

Artemisinin Resistance of P. falciparum *Infecting Variant Erythrocytes*

Resistance to artesunate of normally susceptible *P. falciparum* when infecting thalassemic erythrocytes was first reported in 1989 [3]. Similar findings of resistance to artemisinin [4] and dihydroartemisinin [5] were reported subsequently. In this study, the resistance of *P. falciparum* to artemisinin was confirmed again in Hb H-containing erythrocytes *in vitro*. The IC_{50} values were 42.3 ± 29.5 nM for Hb H (N = 9) and 62.5 ± 43.3 nM for Hb H/Hb CS (N = 6), approximately 12 and 17 times higher than that of *P. falciparum* infected normal erythrocytes (3.6 ± 1.8 nM, N = 5). This host-specific resistance also was found with parasites infecting β-thal/Hb E erythrocytes, albeit with a lower IC_{50} value (9.0 ± 1.8 nM, N = 4). The IC_{50} values for artemisinin against the parasites in these variant erythrocytes were significantly higher than those in normal erythrocytes ($P < 0.01$) for the three variants. These findings are also in line with a recent report where artemisinin sensitivities of the parasites were found to be reduced in the old cell fractions of α- and β-thal trait erythrocytes as compared with the same fraction of normal controls [6].

Increased Artemisinin Accumulation in Thalassemic Erythrocytes

Preferential accumulation [4, 5] and increased binding of artemisinin in Hb H-containing erythrocytes due to the presence of Hb H [5] are the major factors that contribute to the apparent resistance of parasites to the drug. The increase in artemisinin accumulation was also confirmed in this study. As shown in Table 1, after incubation of [^{14}C]artemisinin with variant erythrocytes, the amounts of artemisinin accumulated in Hb H and Hb H/Hb CS erythrocytes were, respectively, 5.9 and 11.5 times that for normal erythrocytes. This phenomenon was also observed, but to a lesser extent, in β-thal/Hb E erythrocytes. The levels of [^{14}C]artemisinin accumulation of these variant erythrocytes were significantly higher than that of normal erythrocytes ($P < 0.05$).

Artemisinin Inactivation by Erythrocytes

The differences between the fold increase in IC_{50} values and in drug accumulation of artemisinin in α-thalassemic erythrocytes compared with normal erythrocytes indicated the presence of an additional mechanism for decreasing artemisinin effectiveness. We hypothesize that this mechanism involves preferential inactivation of the drug by the thalas-

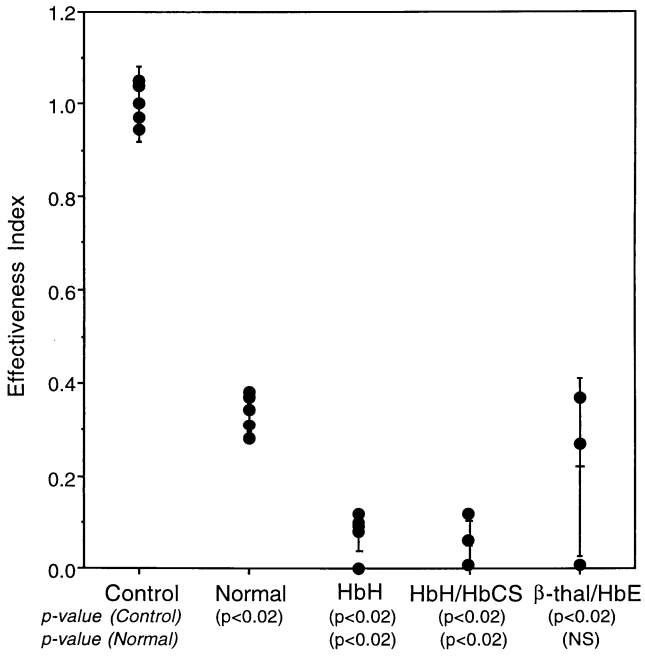

FIG. 1. Effectiveness index of [^{14}C]artemisinin following exposure to thalassemic and normal erythrocytes for 2 hr. Data points and means ± SEM are shown. Numbers of samples in the tests were 6 for control and normal, 5 for Hb H, 4 for Hb H/Hb Constant Spring (Hb CS), and 3 for β-thal/Hb E.

semic erythrocytes. To explore this artemisinin-inactivation hypothesis, the effectiveness of the drug, defined as IC_{50} for artemisinin in control incubated without erythrocytes/IC_{50} in the sample, was determined after incubation of [^{14}C]artemisinin with intact erythrocytes from normal and thalassemic individuals in incomplete medium for 2 hr. The results are shown in Fig. 1. The effectiveness index of [^{14}C]artemisinin following exposure to normal erythrocytes decreased significantly to 0.34 ± 0.04 (N = 6) as compared with control without erythrocytes (1.00 ± 0.08). This decrease in [^{14}C]artemisinin effectiveness was not due to instability of the drug, since the effectiveness index of [^{14}C]artemisinin incubated in the medium remained unchanged up to 12 hr of incubation (data not shown). The effectiveness indices of [^{14}C]artemisinin following exposure to both types of Hb H-containing erythrocytes significantly decreased to a greater extent than that of [^{14}C]artemisinin exposed to normal erythrocytes: 0.08 ± 0.04 for Hb H (N = 5) and 0.05 ± 0.05 for Hb H/Hb CS (N = 4). The effectiveness index of [^{14}C]artemisinin exposed to β-thal/Hb E erythrocytes (0.22 ± 0.19, N = 3) was not significantly different from that when exposed to normal erythrocytes. These results indicated that artemisinin was inactivated by all types of erythrocytes, but preferentially by Hb H-containing erythrocytes.

Protective Effect of Human Serum against Artemisinin Inactivation

Since artemisinin and its derivatives are effective against malarial parasites *in vitro* and *in vivo*, where serum is present, it is possible that serum exerts a protective effect against inactivation of artemisinin by the erythrocytes. To investigate the effect of serum on artemisinin activity upon exposure to erythrocytes, similar incubations were carried out in the presence of increasing amounts of serum. The data are shown in Fig. 2. Upon exposure to normal erythrocytes, [^{14}C]artemisinin effectiveness was retained in the presence of serum (Fig. 2A). The presence of 50% serum resulted in full retention of artemisinin effectiveness for up to 4 hr of incubation. However, for Hb H-containing erythrocytes, the presence of serum did not improve the effectiveness index of artemisinin, only delaying the inactivation process (Fig. 2, B and C). These data indicate that serum protects against artemisinin inactivation by decreasing artemisinin entry into the erythrocytes.

The role of human serum in protecting or stabilizing the drug against inactivation by normal erythrocytes could be due to the presence of proteins in serum. It has been reported that serum albumin, the major protein in serum, can bind non-covalently to many drugs and small molecules [16, 17] including artemisinin [18]. In addition, α_1-acid glycoprotein, an acute phase protein found at high levels during infection, has also been reported to bind artemisinin non-covalently with a greater binding affinity than albumin [19]. Binding of the drug to these serum proteins may account for the protection of the drug *in vitro* by decreasing the rate of drug transportation into uninfected normal erythrocytes or by competing for the drug with components in the cells.

In Hb H-containing erythrocytes, human serum was not able to protect artemisinin from inactivation. The results also showed a faster rate of artemisinin inactivation by both Hb H and Hb H/Hb CS erythrocytes than by normal erythrocytes. This indicates that there was competition for artemisinin by components within the cells (e.g. Hb H and Hb A) and those outside (e.g. serum components such as serum albumin and α_1-acid glycoprotein). The binding affinities of four major binding components can be listed as follows: α_1-acid glycoprotein with arteether (K_d = 4.4 ± 0.4 μM), human serum albumin with arteether (K_d = 84 ± 7 μM), Hb H with dihydroartemisinin (K_d = 66 ± 17 μM), and Hb A with dihydroartemisinin (K_d = 224 ± 15 μM) [5, 19]. There are two artemisinin binding sites in α_1-acid glycoprotein and Hb H, and one in human serum albumin and Hb A [5, 19]. Although α_1-acid glycoprotein may possess stronger drug binding affinity than Hb H, assuming that artemisinin derivatives have similar binding characteristics, the glycoprotein is present in much lower concentration in normal human serum, thus driving the binding towards Hb H. Binding of artemisinin to Hb H probably is also favored over binding to human serum albumin. These considerations argue for favored localization of artemisinin in Hb H-containing cells and its subsequent inactivation there. Even if artemisinin does not bind to Hb H with as high affinity as to the serum proteins, inactivation would still result if it could dissociate from the serum proteins and penetrate the erythrocytes.

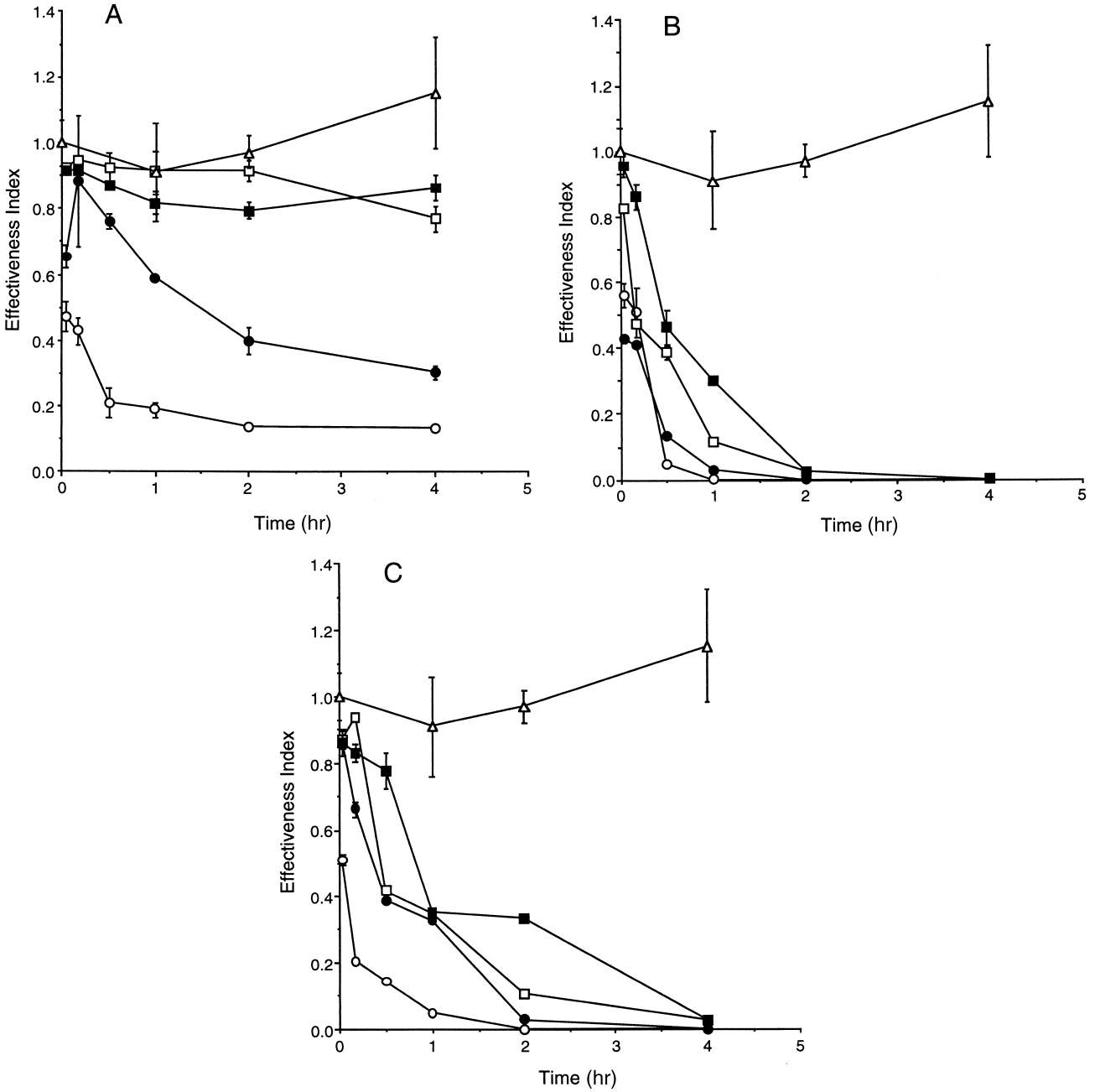

FIG. 2. Effectiveness index of [^{14}C]artemisinin following exposure to erythrocytes in the absence (○) and presence of 10% (●), 50% (□), and 100% (■) human serum, and (△) in the control medium without cells and serum. (A) Normal erythrocytes, (B) Hb H erythrocytes, and (C) Hb H/Hb CS erythrocytes. The experiments were performed twice using two different sets of samples. The data shown here are means ± SD from one of the two experiments where similar results were obtained.

Effect of Cytosolic and Membrane Compartments on Artemisinin Inactivation

Distributions of [^{14}C]artemisinin in erythrocyte membrane and cytosolic fractions were investigated. As shown in Table 1, the amount of drug was higher in the cytosol compartment than in the membrane fraction of both normal and variant erythrocytes. Cytosol and membrane from both types of Hb H-containing erythrocytes accumulated [^{14}C]artemisinin to a higher extent than those of normal and β-thal/Hb E erythrocytes. The ratios of the drug in the two compartments for these variant erythrocytes were not significantly different from normal. These values were also in line with the previous report using dihydroartemisinin [5].

To investigate further the role of the two compartments in inactivating artemisinin, radiolabelled drug was incubated with washed membrane and cytosol of normal and thalassemic erythrocytes, and free drug fractions were separated for determination of drug effectiveness. The results of the effects of cytosolic fractions and of membrane

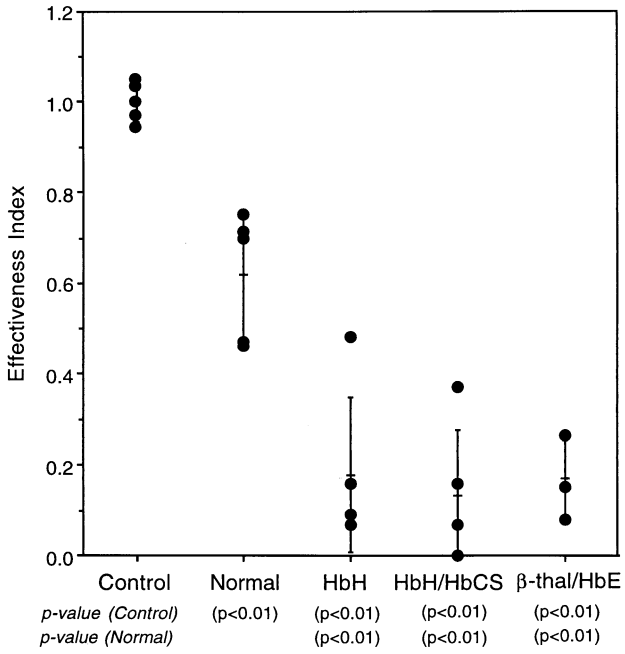

FIG. 3. Effectiveness index of free [^{14}C]artemisinin obtained from thalassemic and normal hemolysates after a 2-hr incubation. Data points and means ± SEM are shown. Numbers of samples in the tests were 5 for all.

fractions are shown in Figs. 3 and 4, respectively. The effectiveness index of free artemisinin extracted after 2 hr of incubation with the cytosolic fraction of Hb H, Hb H/Hb CS, β-thal/Hb E, and normal erythrocytes decreased markedly from 1.0 in control to 0.18 ± 0.17, 0.14 ± 0.14, 0.17 ± 0.08, and 0.62 ± 0.14, respectively. These effectiveness indices of the drugs from the cytosolic fractions of variant erythrocytes were also significantly less than the index from normal erythrocytes (Fig. 3). Upon incubation with membrane fraction, drug effectiveness was also decreased significantly in the presence of Hb H and Hb H/Hb CS membranes (the indices were 0.41 ± 0.17 for Hb H and 0.41 ± 0.09 for Hb H/Hb CS), and to a lesser extent in the presence of membrane from β-thal/Hb E (0.67 ± 0.26) and normal erythrocytes (0.81 ± 0.05). Both cytosolic and membrane components of normal and thalassemic erythrocytes inactivated artemisinin, and the inactivation was most pronounced in the cytosolic fraction of Hb H-containing erythrocytes.

Role of Hb H on Artemisinin Inactivation

The role of Hb H in Hb H-containing erythrocytes on artemisinin antimalarial activity has been suggested to be due to preferential binding of artemisinin and its derivatives to Hb H, thereby causing the drugs to accumulate at high concentrations in these variant erythrocytes [5]. To determine whether Hb H could also inactivate the drug, purified Hb H was added to the culture medium, and the antimalarial activity of dihydroartemisinin was tested. Addition of Hb H to the malaria culture caused a large increase in the dihydroartemisinin IC_{50} value, whereas addition of Hb A showed little effect (Fig. 5). When the concentrations of dihydroartemisinin were corrected for the portion of the drug bound to added Hb H (K_d = 66 μM) [5], IC_{50} values were still anomalously high, indicating a role of Hb H in dihydroartemisinin inactivation.

Since artemisinin inactivation occurred in normal as well as β-thal/Hb E erythrocytes, with the cytosolic fraction playing a major role in the inactivation process, factors other than Hb H alone must also be responsible for drug inactivation. It has been reported that antioxidant enzymes, e.g. catalase, glutathione peroxidase, glutathione reductase, and superoxide dismutase, are present in increased amounts in thalassemic erythrocytes [20, 21]. Iron is also found at high levels in both α- and β-thalassemic erythrocytes [8, 9]. Interaction of artemisinin with some of these cytosolic components may lead to its inactivation. In addition, in the presence of iron [2], artemisinin binding and/or alkylation of proteins in the cells can also lead to a decrease in drug availability. This hypothesis is supported by various reports on the interaction of artemisinin with iron, redox metal, heme, and proteins [2, 22–27].

In a study of the pharmacokinetics of artesunate in α-thalassemic subjects, it was found that plasma drug concentrations of biologically active drug metabolites in the plasma of the thalassemic subjects are higher than normal, and the volume of distribution is 15-fold lower [28]. Although this result is surprising, since higher uptake of the drug by thalassemic erythrocytes [4, 5] would be expected to lead to lower plasma concentrations and higher volume of distribution, possible explanations were given as slow re-

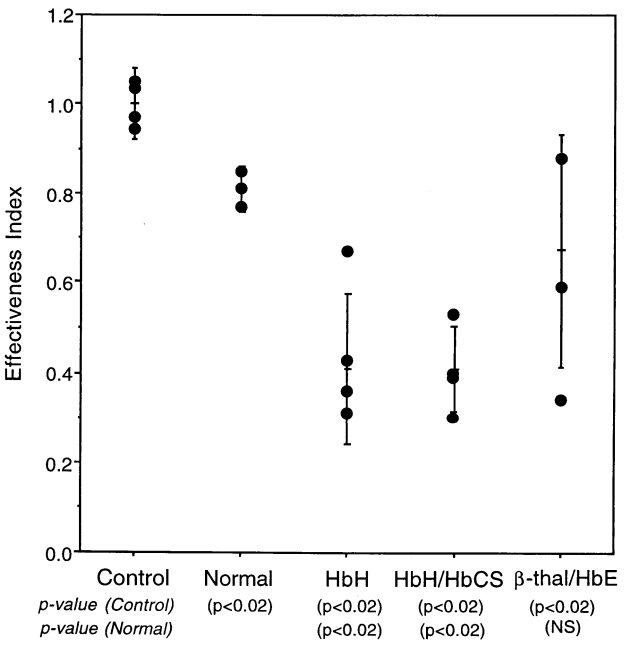

FIG. 4. Effectiveness index of free [^{14}C]artemisinin obtained from thalassemic and normal membranes after a 2-hr incubation. Data points and means ± SEM are shown. Numbers of samples in the tests were 4 for all.

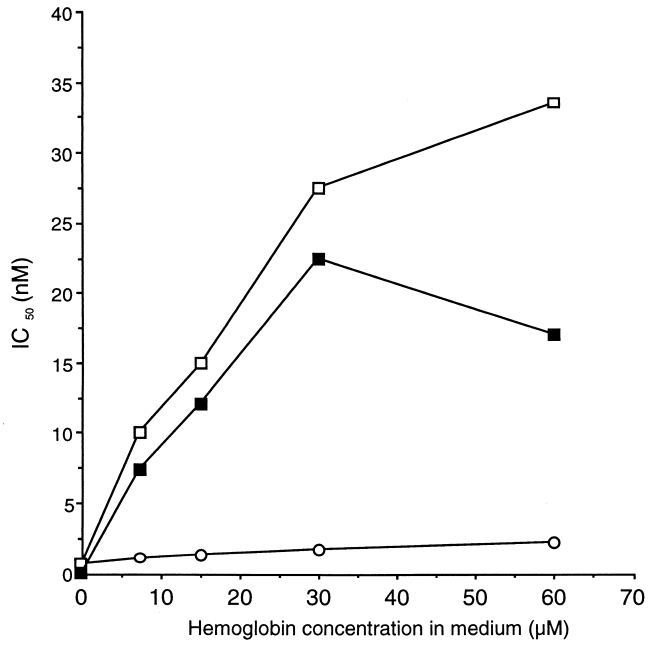

FIG. 5. Effect of Hb H on the IC_{50} values of artemisinin against *P. falciparum in vitro*. Key: (○) Hb A; (□) Hb H; and (■) corrected Hb H. The data were averaged from a triplicate measurement.

lease of the drug and metabolites into the plasma, and differences in drug metabolism. Our present report of artemisinin inactivation by thalassemic erythrocytes is not necessarily in conflict with the pharmacokinetic studies on artesunate [28], since different drugs were used, and plasma concentrations are determined by complex factors not limited to drug inactivation.

In conclusion, the results reported here show the presence of an additional mechanism responsible for resistance to artemisinin by *P. falciparum* infecting α-thalassemic red cells *in vitro*, namely, the inactivation of artemisinin. The host-specific resistance, resulting both from drug binding to Hb H and other erythrocyte components and from its inactivation, may be an important consideration in the clinical use of the drug for malaria treatment, especially in areas with a high frequency of thalassemic genes. Although no artemisinin-resistant malaria parasites have been detected to date, drug-resistant parasites and high recrudescence rates may result if artemisinin and its derivatives are used without awareness of the significance of this host-specific effect.

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