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PHANTIP VATTANAVIBOON : ARTEMISININ BINDING COMPONENTS IN α-THALASSEMIC ERYTHROCYTES. THESIS ADVISOR: PRAPON WILAIRAT, Ph.D., YONGYUTH YUTHAVONG, D.Phil., SUTHAT FUCHAROEN, M.D., AHNOND BUNYARATVEJ, Ph.D. 149 p. ISBN 974-589-187-8

Artemisinin and its derivatives are relatively ineffective against *Plasmodium falciparum* infecting α-thalassemic erythrocytes, namely hemoglobin (Hb) H or Hb H/Hb Constant Spring (CS) erythrocytes, as compared with those infecting genetically normal as well as β-thalassemia/Hb E erythrocytes. The α-thalassemic erythrocytes accumulate radiolabelled dihydroartemisinin (DHART, one of artemisinin derivatives) to much a higher extent than the normal and β-thalassemic erythrocytes. The accumulated drug in α-thalassemic erythrocytes was retained after extensive washing, in contrast to the drug in normal and β-thalassemic erythrocytes which was mostly removed. DHART accumulation capacities of normal and α-thalassemic erythrocytes depended on the drug concentration in medium. The maximal difference of drug accumulation capacity between normal and α-thalassemic erythrocytes was observed at 1 nM level, and the accumulation showed saturation at 100 μM. At initial drug concentration of 1 μM, most (82-88%) of the drug was found in cytosol fraction of both variant and normal erythrocytes. Binding of the drug to Hb accounted for 50-80 % of the total drug uptake. Hb H, accounting for 11-12 % of total Hb in both types of α-thalassemic erythrocytes, bound 22-29 % of total drug. The drug/Hb ratio of Hb H was 5-7 times that of Hb A. At binding equilibrium, Hb H showed maximum drug binding capacity (B_{max}) = 1.7 ± 0.2 mol/mol Hb, and the dissociation constant (K_d) = 66 ± 17 μM, and for Hb A, B_{max} = 0.7 ± 0.2 mol/mol Hb, and K_d = 224 ± 15 μM. Relative increment of IC_{50} value of DHART was found to depend on Hb H concentration.

Another factor accounting for the apparent drug ineffectiveness is drug preactivation and subsequent inactivation on passing through the α-thalassemic erythrocytes. Study of drug accumulation in phenylhydrazine-treated normal, α- and β-thalassemic erythrocytes suggested that oxidized hemoglobin and α-thalassemic membrane and also oxidative stress accounted for drug ineffectiveness. It can be concluded that the apparent resistance of *P. falciparum* infecting α-thalassemic erythrocytes against artemisinin and its derivatives was due to high binding affinity of Hb H and drug inactivation when passing through these erythrocytes. The presence of inactive form of the drug resulted in prolonged sub-optimal concentration of active drug exposure to the parasite which could lead to the induction of drug resistance in the future. This finding is of importance for the application in clinical treatment of falciparum malaria in thalassemic patients.