

**STUDIES ON THE MOLECULAR BIOLOGY OF
THALASSEMIA AND MALARIA**

การศึกษาดูชีววิทยาของโรคธาลัสซีเมียและมาลาเรีย

Project No: RTA/02/1539

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Executive Summary

The high prevalence in Thailand of two diseases, thalassemia and malaria, presents both an opportunity and a challenge to scientists: opportunity to obtain clinical specimens for studies at the molecular level and challenge to apply the knowledge gained towards the search of novel therapeutics. Both thalassemia and malaria, although affecting a large proportion of the world's population, are "neglected diseases" as judged from the extent of interest shown by scientists in the developed countries to these two diseases.

Anemia and related pathophysiology of thalassemia is not due to the reduction of hemoglobin in red cells *per se* but results from binding to red cell membrane of unmatched globin chains which causes oxidative damage to the phospholipid and protein components. Such red cells are rapidly removed by the body's reticuloendothelial system or may never even leave the bone marrow. The properties of the affected red cell membrane can be attributed to the species of globin chains bound to it.

Data from this project indicate that binding of Hb Constant Spring (CS; the most common nondeletional α -thalassemic mutation and an important cause of Hb H-like disease in Southeast Asia) to red cell membrane affects both spectrin and protein 4.1 but attachment of Hb H alone perturbs only the extrinsic membrane protein 4.1. Although thalassemic red cells are microcytic, Hb H/CS red cells are more normal in size when compared with Hb H cells. One controlling system of red cell volume is regulation by K-Cl cotransport activity. Presence of increased potassium efflux of thalassemic cells may contribute to their dehydration state, but this phenomenon did not account for the relative hyperhydration of Hb H/CS red cells.

One of the hallmarks of thalassemic red cells is the increase in rigidity (reduced deformability) of the plasma membrane, resulting in their premature destruction by the body's reticuloendothelial system. In normal red cells spectrin phosphorylation is inversely correlated with membrane deformability. Our studies showed that the level of steady state level of spectrin phosphorylation at serine and threonine residues in thalassemic red cells was not significantly different from that in normal red cells, indicating that oxidative damage to thalassemic red cells is independent of serine/threonine phosphorylation. In the presence of vanadate (an inhibitor of protein tyrosine phosphatase), tyrosine phosphorylation in β -spectrin was observed in both normal and thalassemic red cell membrane, a phenomenon not hitherto reported. However, the level of β -spectrin tyrosine phosphorylation could not be correlated with phenotypic severity of anemia nor with degree of oxidative damage to the thalassemic red cell membrane. Increased deformability of thalassemic red cells may also be due to an increased activity of transglutaminase which covalently crosslinks proteins through the formation of $\epsilon(\gamma$ -glutamyl) lysine linkages. Changes in transglutaminase were observed with thalassemic samples which reflected the status of endogenous red cell calcium level rather than an alteration to or amount of the enzyme.

One of the effects of oxidative stress to thalassemic red cell membrane is the increase in intracellular calcium concentration. Previous study from our laboratory showed that, contrary to expectation, thalassemic red cell membrane Ca^{2+} -ATPase is not impaired, but had full activity which, however, did not respond to calcium-calmodulin stimulation and also may be uncoupled to calcium transport. To gain further insight into this unexpected phenomenon, normal red cells were treated with protein oxidants (diamide and *tert*-butylhydroperoxide) in order to obtain an *in vitro* model of thalassemic red cells. After exposure to these oxidants, Ca^{2+} -ATPase activity and its stimulation by calmodulin were inhibited but this effect could be reversed upon treatment with dithiothreitol, indicating that Ca^{2+} -ATPase in thalassemic red cell membrane is not modified through sulfhydryl oxidation.

It is well known that oxidatively damaged red cell membranes or those with activated complement components on their surfaces will shed part of their plasma membrane as small vesicles. By employing the technique of laser flow cytometry and using fluorochrome-labeled anti-glycophorin antibody (which is specific to red cell membrane) significant amounts of circulating red cell vesicles were found in thalassemic subjects. These vesicles were devoid of spectrin (the major component of membrane cytoskeleton), contained phosphatidylserine on the outer surface, were able to shorten clotting time when incubated with recalcified plasma, could efficiently activate the alternate complement pathway and had bound complement C3b. These findings are unique and significant as they serve to link red cell membrane perturbations with a group of clinical complications in thalassemia, viz. repeated infections, abnormalities in platelet and coagulation functions, platelet microthrombi in pulmonary vasculature (in severe cases), and deficiency in complement. Although a number of details of the molecular changes to the thalassemic red cell membrane have emerged from these studies, the oxidative damage inflicted by membrane-bound

globin remains enigmatic, and further research are needed in order to obtain information which will provide directions leading to an introduction of effective and appropriate therapeutic intervention measures which would ameliorate the pathological consequences brought about by the presence of thalassemic red cells.

It has been previously reported that when human umbilical cord vein endothelial cells are incubated with either α - or β -thalassemic sera, cell proliferation is decreased. Current studies now indicated that cell death was via the apoptotic pathway based on both morphological (condensation of nuclei and cytoplasm, margination of nuclear matrices, presence of membrane blebbing, and appearance of apoptotic bodies) and biochemical criteria (cleavage of nuclear DNA into 50-300 kb fragments, and presence of phosphatidylserine on the cell outer surface). The propensity of sera from thalassemic individuals to cause endothelial cells to undergo apoptosis may be a contributing factor to the onset of thromboembolic episodes in such patients.

Patients with β -thalassemia/Hb E have a remarkable variability in severity of anemia which cannot be attributed to the nature of the mutations in the β -globin gene. Severity reflects the imbalance in α - to β -globin produced. It is becoming more apparent that eukaryotic mRNAs containing nonsense mutations upstream to the normal termination codon are degraded in order to prevent the synthesis of truncated proteins which could be toxic to the cell. Using a novel "real time" quantitative reverse transcriptase polymerase chain reaction technique to measure mRNA levels in human reticulocytes, α/β globin mRNA ratios were obtained from β^0 -thalassemia/Hb E subjects revealing an imbalance in α/β globin ratios. Interestingly, a balanced mRNA ratio was seen in normal red cells although there are twice as many α -globin genes relative to β -globin genes. Thus the imbalance in α/β globin ratios in thalassemic red cells is due to both reduced β -globin chain synthesis and decreased β -globin mRNA level.

One of the most pressing need in malaria control is to find new antimalarial drugs to replace existing compounds to which the parasite, *P. falciparum*, is rapidly developing resistance. Instead of screening at random (as been done in the past) for potential inhibitors of parasite growth, another approach is to identify parasite targets to which can be specifically targeted a variety of chemicals which have minimum or no toxicity against human cells. Derivatives of 9-anilinoacridine, an antitumor drug, have been shown to inhibit *P. falciparum* growth in culture and to inhibit parasite DNA topoisomerase II activity *in vitro*. Current studies demonstrated that these compounds were also capable of inhibiting malaria parasite DNA topoisomerase II *in situ*. A report that pyronaridine, 9-anilino-aza-acridine, inhibits heme polymerization, a process unique to the malaria parasite, led to the discovery that an analogue of 3,6-diamino-9-anilinoacridine was also capable of this function. It should be possible, in the future, to design anilinoacridine antimalarials which are capable of inhibiting both parasite DNA topoisomerase II and heme polymerization.

The ability to generate *P. falciparum* gametocytes (the sexual forms) *in vitro* allowed for assays of the gametocytocidal effects of available acridine analogues. Pyronaridine was the most effective gametocytocidal drug, and this together with its schizontocidal activity should make pyronaridine highly attractive for clinical use.

In order to increase the efficacy of antimalarials and to prevent or delay the emergence of drug resistance and thereby extend the useful lifespan of antimalarial drugs (the few that are available), chemotherapy will increasingly involve drug combinations. *In vitro* studies of drug combinations indicated that combining pyronaridine with chloroquine, quinine, mefloquine or artesunate was permissible but not with the antifolates (pyrimethamine and cycloguanil) nor tetracycline. The fluoroquinolone antibiotics could be used in combination with quinine or mefloquine but not together with pyronaridine or artesunate.

The sensitivity of *P. falciparum* to fluoroquinolone antibiotics suggested their target as being mitochondrial DNA topoisomerase II. The enzyme was purified from *P. falciparum* mitochondria and found to be more resistant to these inhibitors than a preparation from the whole parasite extract, suggesting that other isoforms of the enzyme may be present whose inhibition accounts for the sensitivity of the parasite to these antibiotics. DNA polymerase γ -like was also isolated from *P. falciparum* mitochondria and found to differ from the human homologue in being more resistant to dideoxythymidine-5'-triphosphate. This dissimilarity could be exploited in the design of antimalarials directed to DNA γ -polymerase.

Another potential parasite target is telomerase, an enzyme used in the repair of DNA ends. This study demonstrated the presence of *P. falciparum* telomerase activity in parasites maintained under continuous culture, thereby accounting for the ability of *P. falciparum* K1 strain to be maintained for more than 20 years without any signs of cessation of parasite cell division. Berberine, extracted from *Arcangelisia flava* (L.) Merr., was able to inhibit parasite telomerase activity.

An antisense DNA strategy for inhibition of malaria parasite growth was initiated. Advantage was taken of our previous knowledge of *Plasmodium falciparum* topoisomerase II gene sequence to design a number of antisense oligodeoxynucleotide complementary to unique regions of the parasite gene. The antisense oligodeoxynucleotides exhibited antimalarial properties, albeit the inhibition was not better than chloroquine used as control in all experiments. Nevertheless, antisense strategy holds promise as a potential tool in the development of novel antimalarials against drug-resistant parasites which are expected to be continually emerging from the field in response to antimalarial drug pressure.

Previous investigations from our laboratory showed that *Plasmodium falciparum* infecting alpha-thalassemic erythrocytes (Hb H or Hb H/Hb CS) is resistant to artemisinin derivatives. 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, current studies in this project revealed inactivation of the drug by both thalassemic and normal erythrocytes. Incubation with either the hemolysate or the membrane fraction from these erythrocytes also resulted in preferential inactivation of the drug. The inactivating principle appears to reside in the high molecular weight cytosolic fraction and is heat labile for normal red cells but is heat stable for Hb H or Hb H/Hb CS cells. Although this artemisinin inactivation property is observed under *in vitro* conditions, *in vivo* it may allow ineffective antimalarial artemisinin to be present in *P. falciparum*-infected thalassemic individuals which may afford an explanation for the recrudescence often seen during drug treatment and might possibly lead to the appearance of artemisinin-resistant malaria parasites in areas where thalassemia is prevalent.

The original scope of the project was to study thalassemia and malaria. However, the realization that mutations in the red cell membrane anion exchanger 1 (AE1, also known as band 3) can be responsible for the kidney disease, distal renal acidosis (dRTA), prompted investigations of its possible role in the etiology of endemic distal renal acidosis found in Thailand. Recessive inheritance of *AE1* mutations in dRTA associated with Southeast Asian ovalocytosis (SAO) in three unrelated Thai families have been identified. Affected individuals in two families were compound heterozygotes for SAO (deletion of 27 base pairs) and G701D (glycine to aspartic acid) mutations, and in the other family for SAO and R602H (arginine to histidine). These incidences of autosomal recessive inheritance of dRTA suggest that carriers for *AE1* mutations (other than SAO) must be polymorphic in the

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INTRODUCTION

Research in my laboratory has been guided by the belief that it should be possible to explain living processes in chemical and physical terms. In other words, there is a molecular logic underlying the observed phenomena of cells and organisms, both normal and pathological. Of the myriad of questions that can be addressed, successful research in Thailand depends on two factors: identification of relevant problems and the existence of advantage over other workers in solving the selected problems.

Originally two diseases found in Thailand, thalassemia (a hereditary anemia) and malaria, were chosen for study but the interests of the

research group has been expanded to cover kidney diseases, one of which, familial distal renal tubular acidosis, may have an association with malaria.

Thalassemia and malaria are not unrelated diseases as it is believed (but still not definitively proven) that the existence of thalassemic genes in the human population at polymorphic frequencies (i.e. greater than 1%) is due to protection against malaria conferred by these genes to heterozygotes. The carrier frequency of thalassemia in the Thai population has been estimated to be between 30-40% (18-24 million), with about 500,000 individuals having the disease. On the other hand, although malaria no longer poses a life-threatening menace to the Thai population, the most virulent species, *Plasmodium falciparum* (accounting to about 50% of all malaria cases), is fast becoming resistant to almost all currently used antimalarial drugs, and thus has the potential to become an important cause for increase in mortality.

1. THALASSEMIA

Anemia and related pathophysiology of thalassemia is not due to the reduction of hemoglobin in red cells *per se* but results from binding to red cell membrane of unmatched globin chains which causes oxidative damage to the phospholipid and protein components. Such red cells are rapidly removed by the body's reticuloendothelial system or may never even leave the bone marrow. The properties of the affected red cell membrane can be attributed to the species of globin chains bound to it. Although a number of details of the molecular changes have emerged from our previous studies and others, further research are needed in order to obtain

information which will provide directions leading to an introduction of effective and appropriate therapeutic intervention measures which would ameliorate the pathological consequences brought about by the presence of thalassemic red cells.

1.1 Studies of thalassemic red cell membrane: sulfhydryl content and K-Cl cotransport

(Investigators: Ms. Nopparat Saeier (M.Sc. candidate), Dr. Prapon Wilairat)

Hemoglobin Constant Spring (Hb CS) is the most common nondeletional α -thalassemic mutation and an important cause of Hb H-like disease in Southeast Asia. Anemia is more severe in individuals with Hb H/CS than Hb H individuals. The decrease in the level of α^{CS} -globin in red cells may be attributed to both a decrease in α^{CS} -mRNA levels and the binding of Hb CS on the cell membrane. Membrane bound globin chains result in oxidative damage and pathology to the red cells. One indicator of the oxidative condition is that of reduced sulfhydryl content on membrane proteins such as spectrin and protein 4.1. In this study we have compared the oxidative damage to red cell membrane between these two types of α -thalassemic red cells. Membrane proteins were radiolabelled with [3 H] N-ethymaleimide which bind to sulfhydryl groups and the extent of radiolabeling were determined. Specific activity of radiolabeling (cpm/mg) of spectrin in normal (n=4), Hb H (n=5) and Hb H/CS (n=6) red cell membranes ranged from 322 - 594, 311 - 410 and 227 - 337, respectively; the only significant difference was between normal and Hb H/CS samples ($p = 0.025$). Protein 4.1 specific activities in normal samples were between 511-834, in Hb H between 308-575 and in Hb H/CS between

253-725; significant differences were both seen between normal and hemoglobin H cells and between normal and Hb H/CS samples ($p < 0.05$). These data indicate that binding of Hb CS to red cell membrane affects both spectrin and protein 4.1 but attachment of Hb H alone perturbs only the extrinsic membrane protein 4.1.

Although thalassemic red cells are microcytic, Hb H/CS red cells are more normal in size when compared with Hb H cells. One controlling system of red cell volume is regulation by K-Cl cotransport activity. Atomic absorption spectrophotometry was utilized to measure potassium (K^+) efflux. Measurements of normal red cells ($n=7$) showed K^+ efflux was in the range 2.1-10.6 mM K/l cell/h, for Hb H cells ($n=14$) 11.9-51.6 mM K/l cell/h and for Hb H/CS cells ($n=13$) 4.52-23.89 mM K/l cell/h; there was significant difference between normal and Hb H samples ($p<0.05$) and between normal and Hb H/CS cells ($p<0.05$), but not between the two types of α -thalassemia. For comparison, studies were also conducted on β -thalassemia/Hb E red cells which showed significant increased activity in splenectomized cases ($n=4$) (21.5-65.6 mM K/l cell/h) and less pronounced increase in non-splenectomized samples ($n=6$) (11.37-26.38 mM K/l cell/h). Increased K efflux of thalassemic cells may contribute to their dehydration state, but this phenomenon did not account for the relative hyperhydration of Hb H/CS red cells.

1.2 Studies of thalassemic red cell membrane: spectrin phosphorylation and transglutaminase

(Investigators: Ms. Pompimon Methenukul (M.Sc. candidate), Dr. Rutaiwan Tohtong, Dr. Prapon Wilairat)

One of the hallmarks of thalassemic red cells is the increase in rigidity (reduced deformability) of the plasma membrane, resulting in premature destruction by the body's reticuloendothelial system. This is caused by oxidation of red cell membrane through the binding of unmatched globin chains (α -globin in the case of β -thalassemia and β -globin in the case of α -thalassemia) to the cytoplasmic side of the membrane.

In normal red cells spectrin phosphorylation is inversely correlated with membrane deformability. The possibility exists that spectrin phosphorylation may have been increased in thalassemic red cells. However, our studies showed that the level of steady state level of spectrin phosphorylation at serine and threonine residues in thalassemic red cells was not significantly different from that in normal red cells, despite the presence of okadaic acid, an inhibitor of serine/threonine phosphatase. These results indicate oxidative damage to thalassemic red cells is independent of serine/threonine phosphorylation, although alterations in phosphorylation status of other minor membrane proteins cannot be excluded.

In the presence of vanadate (an inhibitor of protein tyrosine phosphatase), tyrosine phosphorylation in β -spectrin was observed in both normal and thalassemic red cell membrane, a phenomenon not hitherto reported. The levels of β -spectrin tyrosine phosphorylation were significantly higher than

normal controls in Hb H ($p < 0.01$), Hb H/CS ($p < 0.05$) and β -thalassemia/Hb E (nonsplenectomized, nsp x) ($p < 0.05$) subjects, but not in β -thalassemia/Hb E (splenectomized, sp x) cases. After density fractionation through Percoll, both top (light) and bottom (dense) fractions of β -thalassemia/Hb E (sp x) samples exhibited similar levels of tyrosine phosphorylation in β -spectrin, ruling out the possibility that a red cell population with significant increase in β -spectrin tyrosine phosphorylation may have been masked by a population with lower level of tyrosine phosphorylation. The level of β -spectrin tyrosine phosphorylation could not be correlated with phenotypic severity of anemia nor with degree of oxidative damage to the thalassemic red cell membrane. This is in contrast with the situation in sickle cell anemia in which deoxygenation leads to increased tyrosine kinase activity and phosphorylation of red cell membrane band 3, resulting in enhanced susceptibility to lipid peroxidation and protein thiol oxidation. On the other hand, β -spectrin tyrosine phosphorylation may be associated with other phenomena, such as change in cell shape, ion transport activity, and maintenance of membrane lipid asymmetry.

Increased deformability of thalassemic red cells may also be due to an increased activity of transglutaminase which covalently crosslinks proteins through the formation of $\epsilon(\gamma$ -glutamyl) lysine linkages. Transglutaminase activity in red cells from both Hb H and Hb H/CS patients was significantly lower than that in normal cells ($p < 0.1$), possibly due to a reduced amount of the enzyme based on immunoblot analysis ($p < 0.05$). On the other hand, transglutaminase activity was increased in red cells from β -thalassemia/Hb E (sp x) patients ($p < 0.05$), but immunoblot analysis did not show any increase in enzyme amount. However, when calcium

concentration in the transglutaminase assay (transglutaminase is a calcium-dependent enzyme) was raised to 4 mM, transglutaminase activity of all thalassemic red cell samples was not different from that of normal control. These data indicate that the changes in transglutaminase observed with thalassemic samples reflect the status of endogenous red cell calcium level rather than an alteration to or amount of the enzyme. The level of red cell calcium concentration in β -thalassemia/Hb E (spx) is increased 7-fold over that in normal red blood cells. Whether elevated transglutaminase activity in β -thalassemia/Hb E (spx) red cells contributes in any significant way to membrane rigidity and loss of deformability awaits further analysis.

1.3 Studies of thalassemic red cell membrane: red blood cell vesicles

(Investigators: Mr. Prapat Suriyaphol (Ph.D. candidate), Dr. Prida Malasit (Medical Molecular Biology Unit, Faculty of Medicine Siriraj Hospital, Mahidol University) Dr. Prapon Wilairat)

It is well known that oxidatively damaged red cell membranes or those with activated complement components on their surfaces will shed part of their plasma membrane as small vesicles. By employing the technique of laser flow cytometry and using fluorochrome-labeled anti-glycophorin antibody (which is specific to red cell membrane) significant amounts of circulating red cell vesicles were found in thalassemic subjects. The amount was highest in splenectomized β -thalassemia/Hb E (24736 ± 5491 particles/mm³, n = 21), followed by nonsplenectomized β -thalassemia/Hb E (24736 ± 3777 particles/mm³, n = 15) and then nonsplenectomized α -thalassemia (18960 ± 4026 particles/mm³, n = 9); normal controls had values of 9661 ± 1206 particles/mm³, n = 14. Erythrocytes from

thalassemic patients produced significantly more vesicles *in vitro* when cells were incubated in buffer containing Ca^{2+} , whereas a negligible amount was generated when normal red cells were used. The vesicles were spherical in shape, with an average diameter of 0.3 μm when examined under the electron microscope. SDS-PAGE analysis of the vesicles showed them to be devoid of spectrin, the major component of membrane cytoskeleton. Vesicles contained phosphatidylserine (PS) on the outer surface as revealed by flow cytometry employing fluorochrome-labeled annexin V, a PS-specific binding protein. Thalassemic vesicles were able to shorten clotting time when incubated with recalcified plasma, indicating the presence of procoagulant activity on the outer surface. Vesicles could efficiently activate the alternate complement pathway as shown by the production of C3b in autologous serum in the presence of EGTA- Mg^{2+} . Vesicles also bound C3b as demonstrated by flow cytometry and immunoblot analysis of membrane.

These findings are unique and significant as they serve to link red cell membrane perturbations with a group of clinical complications in thalassemia. Thalassemic patients, especially those splenectomized, are highly susceptible to repeated infections, show abnormalities in platelet and coagulation functions, have platelet microthrombi in pulmonary vasculature (in severe cases), and are deficient in complement. The presence in thalassemic subjects of circulating red cell vesicles with the properties described may account for the above phenomena.

1.4 Studies of thalassemic red cell membrane: calmodulin response of red cell membrane Ca^{2+} -ATPase to oxidants

(Investigators: Namphaung Pengpad (M.Sc. candidate), Udompun Khansuwan. (Department of Biochemistry, Faculty of Medicine, Chiang Mai University), Dr. Prapon Wilairat)

One of the effects of oxidative stress to thalassemic red cell membrane is the increase in intracellular calcium concentration. Our previous study showed that, contrary to expectation, thalassemic red cell membrane Ca^{2+} -ATPase is not impaired, but had full activity which, however, did not respond to calcium-calmodulin stimulation and also may be uncoupled to calcium transport. To gain further insight into this unexpected phenomenon, the effects of sulfhydryl oxidizing agent, diamide and *tert*-butylhydroperoxide on the Ca^{2+} -ATPase activity and its response to (bovine brain) calmodulin were studied in calmodulin-deficient normal human erythrocyte membrane preparations. Ca^{2+} -ATPase activity was determined by a pyruvate kinase/lactate dehydrogenase coupled enzyme method. Calmodulin in the presence of 10 μM free Ca^{2+} stimulated ATPase activity some 3-fold. After exposure to these oxidants, Ca^{2+} -ATPase activity and its stimulation by calmodulin were inhibited but this effect could be reversed upon treatment with dithiothreitol. These studies indicate that treatment of normal red cell membrane with sulfhydryl oxidants did not mimic the property of Ca^{2+} -ATPase found in the oxidized thalassemic red cell membrane. Studies of other oxidizing conditions need to be conducted.

1.5 Studies on apoptosis in thalassemia

(Investigators: Dr. Ratana Banjerdpongchai (Ph.D. candidate), Dr. Ahnond Bunyaratvej (Department of Pathology, Faculty of Medicine Ramathibodi Hospital, Mahidol University), Dr. Prapon Wilairat)

Multicellular organisms have evolved a genetically controlled mechanism for self destruction when the cells are no longer needed or are damaged. This process is known as programmed cell death or apoptosis. One of the well known signals of apoptosis is oxidative stress.

It has been previously reported that when human umbilical cord vein endothelial cells (HUVEC) are incubated with either α - or β -thalassemic sera, cell proliferation is decreased. Current studies now indicated that cell death was via the apoptotic pathway based on both morphological (condensation of nuclei and cytoplasm, margination of nuclear matrices, presence of membrane blebbing, and appearance of apoptotic bodies) and biochemical criteria (cleavage of nuclear DNA into 50-300 kb fragments, and presence of phosphatidylserine on the cell outer surface). DNA damage *in situ* was confirmed by the use of terminal deoxynucleotidyl transferase-mediated dUTP-fluorescein nick end labeling (TUNEL) assay. Similar results were obtained by treating HUVEC with low density lipoprotein (LDL) extracted from β -thalassemia/Hb E plasma. Addition of noncytotoxic levels of cycloheximide (inhibitor of protein synthesis) prevented apoptosis of HUVEC induced by both thalassemic sera and LDL, confirming that the cell death observed required active gene expression. The propensity of sera from thalassemic individuals to cause endothelial cells to undergo apoptosis may be a contributing factor to the onset of thromboembolic episodes in such patients.

1.6 Quantitation of mRNA in β -thalassemia/Hb E reticulocytes
(Investigators: Dr. Yuwadee Watanapokasin (TRF Post-doctoral Fellow, Department of Biochemistry, Faculty of Medicine, Srinakharinvirot University), Dr. Pranee Winichagoon and Dr. Suthat Fucharoen (Thalassemia Research Center, Institute of Science and Technology for Research and Development, Mahidol University), Dr. Prapon Wilairat)

Patients with β -thalassemia/Hb E have a remarkable variability in severity of anemia which cannot be attributed to the nature of the mutations in the β -globin gene. Severity reflects the imbalance in α - to β -globin produced. It is becoming more apparent that eukaryotic mRNAs containing nonsense mutations upstream to the normal termination codon are degraded in order to prevent the synthesis of truncated proteins which could be toxic to the cell, a phenomenon known as nonsense mRNA degradation. Using a novel "real time" quantitative reverse transcriptase polymerase chain reaction (PCR) technique to measure mRNA levels in human reticulocytes, α/β globin mRNA ratios were obtained for β^0 -thalassemia/Hb E (2.4-9.4, n = 20), β^+ -thalassemia/Hb E (3.1-5.1, n = 11), Hb E trait (1.7-2.6, n = 8) and normal (1.0-1.2, n = 6) subjects. Thus the imbalance in α/β globin ratios in thalassemic red cells is due to both reduced β -globin chain synthesis and decreased β -globin mRNA level. Interestingly, a balanced mRNA ratio was seen in normal red cells although there are twice as many α -globin genes relative to β -globin genes. This was not due to an artifact of the technique since the α/β globin genomic DNA measured using the "real time" quantitative PCR method yielded the expected ratio of 2. This suggests that the balanced α/β globin mRNA level in normal red cells is

regulated at the transcription stage of gene expression, and not at the translation step as previously believed.

2. MALARIA

One of the most pressing need in malaria control is to find new antimalarial drugs to replace existing compounds to which the parasite, *P falciparum*, is rapidly developing resistance. Instead of screening at random (as been done in the past) for potential inhibitors of parasite growth, another approach is to identify parasite targets to which can be specifically targeted a variety of chemicals which have minimum or no toxicity against human cells. Attention has been focused on the malaria parasite DNA replicating enzymes (including telomerase) as the parasite divides rapidly within the human host cells (both liver and erythrocyte). Since DNA replication is a basic requirement for survival, malaria parasites should find it difficult to circumvent this process following drug treatment. In order to increase the efficacy of antimalarials and to prevent or delay the emergence of drug resistance and thereby extend the useful lifespan of antimalarial drugs (the few that are available), chemotherapy in the future will involve drug combinations. *In vitro* studies of possible drug combinations which have synergistic effects have thus been initiated. Preliminary work on using an antisense DNA strategy for inhibition of malaria parasite growth is also reported. In addition, the project undertook further investigation into the mechanism of resistance to artemisinin of malaria parasites infecting thalassemic red cells.

2.1 Cleavage of DNA induced by 9-anilinoacridine inhibitors of topoisomerase II in the malaria parasite *Plasmodium falciparum*.

(Investigators: Saranya Auparakkitanon (M.Sc. candidate), Dr. Prapon Wilairat)

Derivatives of 9-anilinoacridine, an antitumor drug, have been shown to inhibit *P. falciparum* growth in culture and to inhibit parasite DNA topoisomerase II activity *in vitro*. Using KCl-SDS precipitation assay to detect the presence of protein-DNA complexes within parasite cells, an indicator of DNA topoisomerase II inactivation, derivatives containing 3,6-diNH₂ substitutions with 1'-electron donating (NMe₂, CH₂NMe₂, NHSO₂Me, OH, OMe), and 1'-electron withdrawing (SO₂NH₂) groups produced protein-DNA complexes. However, the antimalarial pyronaridine, 9-anilino-aza-acridine, did not generate protein-DNA complexes although it was capable of inhibiting *P. falciparum* DNA topoisomerase II activity *in vitro*. These results should prove useful in future designs of novel antimalarial compounds directed against parasite DNA topoisomerase II.

2.2 The effects of 3,6-diamino-anilinoacridines on heme polymerization

(Investigators: Kritchai Pooncharoen (M.Sc. candidate), Saranya Auparakkitanon (Ph.D. candidate), Dr. Prapon Wilairat)

Recognition of the ability of pyronaridine to inhibit heme polymerization, a process unique to the malaria parasite, led us to investigate whether the related anilinoacridine compounds (see section 2.1) could also exhibit this property. Heme polymerization was performed by incubating heme (0.4

mM) overnight under acid condition, and the heme polymer quantitated by dissolving the insoluble pellet in alkali. Of the four C1'-analogues of 3,6-diamino-9-anilinoacridine (OMe, CH₂NMe₂, NHSO₂Me and NMe₂), only the latter was capable of inhibiting heme polymerization at an IC₅₀ concentration (0.77 mM) which was comparable to that of pyronaridine (IC₅₀ = 0.26 mM) and chloroquine (IC₅₀ = 0.25 mM). It should be possible to design anilinoacridine antimalarials which are capable of inhibiting both parasite DNA topoisomerase II and heme polymerization.

2.3 Gametocytocidal effects of pyronaridine and DNA topoisomerase II inhibitors

(Investigators: Dr. Porntip Chavalitshewinkoon-Petmitr and Ganokwan Pongvilairat (Department of Protozoology, Faculty of Tropical Medicine, Mahidol University), Saranya Auparakkitanon (M.Sc. candidate), Dr. Prapon Wilairat)

Gametocytocidal activities of pyronaridine and DNA topoisomerase II inhibitors against two isolates of multidrug-resistant *Plasmodium falciparum*, KT1 and KT3, were determined. After sorbitol treatment, pure gametocyte cultures of *Plasmodium falciparum* containing mostly young gametocytes (stage II and III) obtained on day 11 were exposed to the drugs for 48 h. The effect of the drugs on gametocyte development was assessed by counting gametocytes on day 15 of culture. Pyronaridine was the most effective gametocytocidal drug against *P. falciparum* isolates KT1 and KT3 with IC₅₀ of 6 and 20 nM, respectively. Moreover the IC₅₀ value of pyronaridine was lower than that of primaquine which is the only drug used to treat malaria patients harboring gametocytes. Prokaryotic (norfloxacin) and eukaryotic (amsacrine and etoposide) DNA

topoisomerase II inhibitors were only effective against the asexual but not the sexual stages of the malaria parasites. Thus pyronaridine has both schizontocidal and gametocytocidal activities against the human malaria parasite, and this dual role should make pyronaridine highly attractive for clinical use.

2.4 Gametocytocidal effects of 9-anilinoacridines

(Investigators: Dr. Pornpip Chavalitshewinkoon-Petmitr and Ganokwan Pongvilairat (Department of Protozoology, Faculty of Tropical Medicine, Mahidol University), Dr. R.K. Ralph and Dr. W.A. Denny (Cancer Research Laboratory, University of Auckland Medical School), Dr. Praon Wilairat)

Pure gametocyte cultures of *Plasmodium falciparum*, isolate KT1 and KT3, containing mostly young gametocytes (stage II, III and IV) obtained on day 11 were exposed to 13 analogs of 9-anilinoacridine for 48 h. The effect of the drugs on gametocyte development was assessed by counting gametocytes on day 15 of culture. The IC_{50} values ranged from 0.6 μ M to over 100 μ M, with the best compound having C1'-CH₂NMe₂ substitution. Anilinoacridines with 3,6-diamino moiety had reduced gametocytocidal activity in contrast to their enhancing effect against the sexual forms, possibly reflecting differences in drug uptake property or intracellular compartmentalization.

2.5 Studies of gyrase and DNA topoisomerase II inhibitors on *Plasmodium falciparum* mitochondria topoisomerase II

(Investigators: Dr. Pornpip Chavalitsheewinkoon-Petmitr and R. Worasing (Department of Protozoology, Faculty of Tropical Medicine, Mahidol University), Dr. Prapon Wilairat)

P. falciparum DNA topoisomerase II (partially purified) is sensitive to both eukaryotic and fluoroquinolone antibiotic inhibitors. As the malaria parasite contains mitochondrion, the possibility exists that the observed sensitivity to fluoroquinolone antibiotics may be due to the presence of the mitochondrial form of the enzyme. Purified mitochondrial *P. falciparum* DNA topoisomerase II was prepared and tested against a battery of eukaryotic and prokaryotic inhibitors. Minimum inhibitory concentrations (MIC) of ciprofloxacin, norfloxacin and ofloxacin were in the range 10-100 mM, whereas that of the eukaryotic inhibitor etoposide and amsacrine was > 0.5 mM and 10 mM, respectively. These MIC values were higher than those previously reported for *P. falciparum* DNA topoisomerase II obtained from total parasite extract. The lack of sensitivity of *P. falciparum* mitochondrial topoisomerase II to fluoroquinolone inhibitors suggest that other isoforms of the enzyme may be present whose inhibition accounts for the sensitivity of the parasite to these antibiotics.

2.6 Partial purification of mitochondrial DNA polymerase from *Plasmodium falciparum* and its sensitivity to inhibitors

(Investigators: Dr. Pornpip Chavalitshewinkoon-Petmitr and Srisucha Chawprom (Department of Protozoology, Faculty of Tropical Medicine, Mahidol University), Dr. Prapon Wilairat)

Eukaryotic DNA polymerases can be divided into 5 different types: α , β , γ , δ and ϵ . So far, only the α -like and γ -like (mitochondrion) DNA polymerases have been identified from total extract of *P. falciparum*. The γ -like (mitochondrion) DNA polymerase of *P. falciparum* has been shown to be highly sensitive to the nucleotide analogue (s)-1-[3-hydroxy-2-phosphonylmethoxypropyl]adenine diphosphate (HPMPApp). To further characterize *P. falciparum* DNA polymerase γ , mitochondria were isolated from mature trophozoites by differential centrifugation and DNA polymerase purified from such a preparation using fast protein liquid chromatography (FPLC). The partially purified parasite enzyme was characterized as a γ -like DNA polymerase based on its sensitivity to the inhibitors aphidicolin, N-ethylmaleimide and 1- β -D-arabinofuranosyladenosine-5'-triphosphate. The enzyme was, however, strongly resistant to dideoxythymidine-5'-triphosphate, and in this respect differed from the human homologue. Furthermore, *P. falciparum* DNA polymerase γ was also resistant to HPMPApp ($IC_{50} > 1$ mM), indicating that the mitochondrial DNA polymerase was not the target of this drug.

2.7 Studies on drug combinations against *Plasmodium falciparum* in vitro

(Investigators: Sugunya Utaida (M.Sc. candidate), Phattanapong Konthiang, Sunan Nakornchai, Dr. Prapon Wilairat)

When 9-anilinoacridines (1'-O-methyl- and 1'-NHSO₂Me-3,6-diamino derivatives), which have previously been shown to inhibit malaria parasite growth by inhibiting DNA topoisomerase II activity resulting in the production of protein-DNA complexes within parasite cells (see section 2.1), were used in combination with cysteine proteinase inhibitors (TLCK, leupeptin) or cycloheximide (inhibitor of eukaryotic protein synthesis) additive effects on parasite growth in culture were observed for all combinations of the compounds. Additive phenomena were also seen in the combination of artemisinin, an endoperoxide antimalarial, with antibiotics (tetracycline, chloramphenicol), cycloheximide or actinomycin D (anticancer drug). Synergism was obtained through the combination of hydrogen peroxide and protein synthesis inhibitors (prokaryotic and eukaryotic), with a marked synergy in the case of chloramphenicol/hydrogen peroxide combination. However, hydrogen peroxide combined with actinomycin D or with chloroquine produced an additive effect. If hydrogen peroxide production in malaria parasites could be augmented, this would improve the efficacy of chloramphenicol which is already used in combination with other antimalarials.

Fluoroquinolone is a potent antibacterial drug that is commonly used for treatment of gastrointestinal tract or urinary tract infection. Fluoroquinolone acts by inhibiting bacterial DNA topoisomerase II (DNA gyrase). Fluoroquinolones are able to kill malaria parasites with IC₅₀ = 10

μM . Norfloxacin and ciprofloxacin are more effective than ofloxacin and efloxacin against *P. falciparum*, strain K₁. The trophozoite and schizont stages are more sensitive to norfloxacin than the ring stage. Drug combinations of fluoroquinolone and currently used antimalarial drugs were studied. Quinine showed an additive effect with norfloxacin, ofloxacin and ciprofloxacin. Mefloquine also showed an additive effect in combination with norfloxacin or ciprofloxacin whereas combination of mefloquine and ofloxacin showed a synergistic effect. An antagonistic effect was produced for combinations of pyronaridine or artesunate with all the fluoroquinolones tested. Using a kinetoplast DNA decatenation assay, partially purified *P. falciparum* topoisomerase II was tested against the fluoroquinolones; no inhibition of the enzyme was observed at concentrations as high 10^{-3} M. Employing the TUNEL technique to identify fragmented DNA strand breaks *in situ* (an indicator for inhibition of topoisomerase II) fluoroquinolone produce a slight increase in the number of TUNEL⁺ cells. These results demonstrated that fluoroquinolone can be used in combination with quinine or mefloquine but not in combination with pyronaridine or artesunate. Although fluoroquinolones did not inhibit malaria parasite (nuclear) topoisomerase II activity *in vitro* they may inhibit the parasite mitochondrial or plastid enzyme *in situ*.

Combination studies of pyronaridine with other antimalarials revealed antagonistic effect with the antifolates (pyrimethamine and cycloguanil) and tetracycline, and additive effect with chloroquine, quinine, mefloquine and artesunate. Since pyronaridine is already in clinical use, these data will be of benefit in the proper choice of drug combinations. The mechanisms for these observed phenomena need to be investigated.

2.8 Studies on the telomerase of the human malaria parasite, *Plasmodium falciparum*.

(Investigators: Ms. Nongluk Sriwilajareon (M.Sc. candidate), Dr. Apiwat Mutirangura (Department of Anatomy, Faculty of Medicine, Chulalongkorn University), Dr. Songsak Petmitr (Department of Tropical Nutrition and Food Science, Faculty of Tropical Medicine, Mahidol University), Dr. Mathurose Ponglikitmongkol, Dr. Prapon Wilairat)

Telomeres are repeating DNA sequences at the ends of linear eukaryotic chromosomes that preserve genome stability and cell viability by preventing aberrant recombination and degradation of DNA. Telomeric repeats are progressively shortened after each cycle of DNA replication due to the inability of DNA polymerase to replace the RNA primers located at the 5'-ends, resulting in a limit to the number of times a cell can divide. However germline and immortalized cells can overcome this problem by expressing telomerase, a ribonucleoprotein enzyme which synthesizes telomeres using its RNA component as a template. *P. falciparum* K1 strain has been maintained in continuous culture for more than 20 years without any signs of cessation of parasite cell division indicating that the parasite must have the capacity to repair telomeres present in its 14 chromosomes. Using a TRAP assay, telomerase activity was detected in parasite extracts obtained from all stages of the erythrocytic cycle. Telomerase activity was sensitive to inhibition by RNase A and was enhanced in the late stages compared with the early ring forms indicating a relationship between activity and development within the red cell. Among the four dideoxyribonucleoside triphosphates tested, ddGTP was the most potent telomerase inhibitor. Berberine, extracted

from *Arcangelisia flava* (L.) Merr., could also inhibit parasite telomerase activity. These data indicate that *P. falciparum* telomerase may provide a novel target for future malaria chemotherapy.

2.9 Antisense oligodeoxynucleotide inhibition of *Plasmodium falciparum*

(Investigators: Wirat Nimitsantiwong (M.Sc. candidate), Dr. Wilai Noonpakdee, Dr. Prapon Wilairat).

Advantage was taken of our previous knowledge of *Plasmodium falciparum* topoisomerase II gene sequence to design a number of antisense (AS) oligodeoxynucleotide (ODNs) complementary to unique regions of the parasite gene. ASODNs were used to test for their ability to inhibit growth of *P. falciparum* K1 strain in culture over 48 h period. Inhibition of parasite growth was determined by both microscopic examination and [³H] hypoxanthine incorporation measurement. ASODNs added exogenously to the parasite cultures were found to be able to stimulate hypoxanthine uptake, and therefore [³H] hypoxanthine incorporation could not be used directly to determine the parasite inhibition effects of ASODNs. However, microscopic examinations revealed that ASODNs against *P. falciparum* topoisomerase II gene could inhibit parasite growth as well as ASODN directed against *P. falciparum* DHFR gene.

ASODNs have been reported to be easily degraded by nuclease in the culture medium and therefore their efficacy may have been compromised. Conversion of phosphodiester linkage to phosphorothioate confers resistance to nucleases. Four antisense phosphorothioate oligodeoxynucleotides (PSASODNs) against *P.*

falciparum topoisomerase II mRNA were designed to target the cap site, the translation initiation site and 2 internal coding regions of the gene, with one non-sequence specific oligodeoxynucleotide as control. The antimalarial activities of these PSASODNs were tested against *P. falciparum* K1 strain in culture. The parasites were exposed to antisense oligodeoxynucleotides for 48 hours and growth was determined by microscopic examination and [³H] hypoxanthine incorporation. Microscopic examinations showed that all PSASODNs at 10 µM inhibited growth of parasites in a target independent manner. However, at lower concentrations (0.01-0.1 µM) two PSASODNs (targeted at cap site and initiation site) significantly inhibited growth of parasites compared with control PSASODNs containing 9 mismatches. Results from [³H] hypoxanthine incorporation could not be used to measure growth inhibition of parasites as PSASODNs (similar to ASODN) stimulated hypoxanthine uptake.

To confirm the results obtained from microscopic examinations, fluorescence-activated cell sorter assay was employed to measure parasitemia. Some differences between the two methods were noted. At low concentration (0.01-0.5 µM) two PSASODNs (targeted at the initiation site and an internal coding region close to the initiation site) significantly inhibited growth of parasites compared with control PSASODNs containing 9 mismatches.

In summary, the studies indicated that the antisense oligodeoxynucleotides that we had designed exhibited antimalarial properties, albeit the inhibition was not better than chloroquine used as control in all experiments. The inhibitory concentrations measured

depended on the assay technique (microscope or fluorescence-activated cell sorter) reflecting the nonspecific effects that these highly polyanionic molecules may have on cells in culture. Nevertheless, antisense strategy holds promise as a potential tool in the development of novel antimalarials against drug-resistant parasites which are expected to be continually emerging from the field in response to antimalarial drug pressure.

2.10 Inactivation of artemisinin by normal and variant erythrocytes

(Investigators: Juree Charoenteeraboon (M.Sc. candidate), Tatsanee Chuchue (M.Sc. candidate), Napawan Ponmee (M.Sc. candidate), Dr. Phantip Vattanaviboon (Department of Clinical Microscopy, Faculty of Medical Sciences, Mahidol University), Dr. Sumalee Kamchonwongpaisan and Dr. Yongyuth Yuthavong (NSTDA) Dr. Prapon Wilairat)

Plasmodium falciparum infecting alpha-thalassemic erythrocytes (Hb H or Hb H/Hb CS) 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 alpha-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. The inactivating principle appears to reside in the high molecular weight cytosolic fraction (MW > 10 kDa) and is heat labile for normal red cells but is heat stable for Hb H or Hb H/Hb CS cells. Hemin, but not free iron, also has the capability of inactivating artemisinin.

Although this artemisinin inactivation property is observed under *in vitro* conditions, it may allow ineffective antimalarial artemisinin to be present in *P. falciparum*-infected thalassemic individuals which may afford an explanation for the recrudescence often seen during drug treatment and might possibly lead to the appearance of artemisinin-resistant malaria parasites in areas where thalassemia is prevalent.

3. KIDNEY DISEASES

3.1 Red blood cell band 3 and distal renal tubular acidosis

(Investigators: Dr. Peti Thuwajit (Ph.D. candidate), , Dr. Prida Malasit and Dr. Pa-thai Yenchitsomanus (Medical Molecular Biology Unit, Faculty of Medicine Siriraj Hospital, Mahidol University), Dr. Somkiat Vasuvattakul and Dr. Sumalee Nimmannit (Renal Division, Faculty of Medicine Siriraj Hospital, Mahidol University), Dr. Prayong Vachuanichsanong, Dr. Charoen Kaitwatcharchai and Dr. Vichai Laosombat (Songklanakarin Hospital, Prince of Songkla University), Dr. Prapon Wilairat).

The kidney maintains acid-base homeostasis by reclaiming all filtered bicarbonate and excreting acid sufficient in amount to that produced by systemic metabolism. This acid secretion occurs in the distal nephron, mediated largely by type A intercalated cells (IC) of the cortical and outer medullary collecting ducts. Inherited defects in this urinary acid secretion process is known as primary distal renal tubular acidosis (dRTA), and is manifested by inappropriate alkaline urine in the presence of systemic metabolic acidosis or in response to an imposed acid loading test. Primary dRTA can be inherited in both autosomal dominant and recessive patterns. Autosomal dominant dRTA has previously been shown to be associated with mutations in the *AE1* anion exchanger gene. This gene encodes for both the erythroid (eAE1) and the kidney (kAE1) isoforms of band 3 protein. Kidney AE1 exchanges bicarbonate for chloride across the basolateral membrane of the type A IC during urinary acidification, which requires proton to be transported across the apical membrane. More recently, *AE1* mutations linked to a recessive syndrome of dRTA in two

Thai siblings have been identified. We have now identified recessive inheritance of *AE1* mutations in dRTA associated with Southeast Asian ovalocytosis (SAO) in three unrelated Thai families. SAO individuals have abnormal shaped red cells and are heterozygous for a band 3 deletion. Affected individuals in two families were compound heterozygotes for SAO and G701D, and in the other family for SAO and R602H. Sulfate uptake of dRTA red cells from probands in the former two families did not differ from those of SAO heterozygotes (about half of normal controls) but in the latter family sulfate uptake of proband was half of that of SAO red cells. These incidences of autosomal recessive inheritance of dRTA suggest that carriers for *AE1* mutations (other than SAO) must be polymorphic in the Thai population. It is hypothesized that these carriers for *AE1* mutations have a selective advantage by being protected against malaria, possibly by reduction of cytoadherence of *P. falciparum*-infected red cells to capillary vessels, and thereby are less likely to have fatal cerebral malaria.

3.2. Studies of mutations of polycystic kidney disease 1 (*PKD1*) gene

(Investigators: Ms. Wanna Thongnoppakhun (Ph.D. candidate), Dr. Pa-thai Yenchitsomanus (Medical Molecular Biology Unit Faculty of Medicine Siriraj Hospital, Mahidol University), Dr. Prapon Wilairat).

Autosomal dominant polycystic kidney disease (ADPKD) occurs mainly from mutations of polycystic kidney disease 1 (*PKD1*) gene. A novel mutation of the *PKD1* gene due to a nucleotide substitution in the splice-receptor site of IVS13 (AG → TG) was identified by analysis of cDNA

prepared from *PKDI*-mRNA transcript by long RT-PCR and of *PKDI*-specific genomic DNA. The IVS13-2 A→T substitution resulted in an inactivation of this splice site and utilization of a cryptic splice acceptor site in exon 14, causing a 74-nucleotide deletion of this exon in the *PKDI*-mRNA transcript. The abnormal transcript was present ectopically in lymphocytes of the patients. The deletion leads to a frameshift in translation and introduces an in-frame termination signal at codon 1075, producing a truncated protein about one quarter of the full length polycystin, which would most likely be inactive. Thus the effect of this mutation seems to be a "loss of function". Allele-specific amplification (ASA) was developed to detect this mutation in DNA samples from other members of the affected family. The mutation was present in 11 affected but absent in 13 unaffected family members, corresponding to the results of linkage analysis, and it was not observed in 23 unrelated healthy individuals.

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- 1*. Petmitr P, Chawprom S, Wilairat P. Partial purification of DNA polymerase from *Plasmodium falciparum* mitochondria and its sensitivity to inhibitors.

- 2*. Petmitr P, Pongvilairat G, Ralph RK, Denny WA, Wilairat P. Inhibitory effects of 9-anilinoacridines on *P. falciparum* gametocytes.
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ABSTRACTS OF PRESENTATIONS

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นักศึกษาระดับปริญญาเอกที่สำเร็จการศึกษา
จากภาควิชาชีวเคมี คณะวิทยาศาสตร์ มหาวิทยาลัยมหิดล
โดยการสนับสนุนของเงินทุนวิจัยของ ศ.ดร.ประพนธ์ วิไลรัตน์
ที่ได้รับทุนเมธีวิจัย สกว.

ชื่อ-นามสกุล	วิทยานิพนธ์ปริญญาเอก	สถานที่ทำงานปัจจุบัน
นางปานทิพย์ วัฒนวิบูลย์	Artemisin binding components in α -thalassemic erythrocytes (สำเร็จการศึกษา 31 ต.ค. 2540)	อาจารย์ภาควิชาจุลทรรศน์คลินิก คณะเทคนิคการแพทย์ มหาวิทยาลัย มหิดล
น.ส. รัดนา บรรเจิดพงษ์ชัย	Thalassaemic sera and apoptosis of human umbilical cord vein endothelial cells (สำเร็จการศึกษา 15 ธ.ค. 2540)	อาจารย์ภาควิชาชีวเคมี คณะแพทย ศาสตร์ มหาวิทยาลัยเชียงใหม่
นายประทีปณ์ สุริยมงคล	RBC vesicles and their possible roles in pathogenesis of thalassemia (สำเร็จการศึกษา 14 ก.ค. 2541)	คณะแพทยศาสตร์ศิริราชพยาบาล มหาวิทยาลัยมหิดล
นายนิติ จูวงศ์	Mutations in the Cl-HCO ₃ exchange gene AE1 cause autosomal recessive distal renal tubular acidosis (สำเร็จการศึกษา 20 ก.ย. 2542)	อาจารย์ภาควิชาชีวเคมี คณะแพทย ศาสตร์ มหาวิทยาลัยขอนแก่น

นักศึกษานิพนธ์ปริญญาโทที่สำเร็จการศึกษา
จากภาควิชาชีวเคมี คณะวิทยาศาสตร์ มหาวิทยาลัยมหิดล
โดยการสนับสนุนของเงินทุนวิจัยของ ศ.ดร.ประพนธ์ วิไลรัตน์
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ชื่อ-นามสกุล	วิทยานิพนธ์ปริญญาโท	สถานที่ทำงานปัจจุบัน
น.ส.ภรณ์ทิพย์ กัณหา	Evaluation of bisquaternary quinolinium compounds against <i>P. falciparum</i> in culture (สำเร็จการศึกษา 14 พ.ค. 2540)	ข้าราชการภาควิชาจุลทรรศน์คลินิก คณะเทคนิคการแพทย์ มหาวิทยาลัยขอนแก่น
น.ส.ศรียุภา ชูปรีภักคานนท์	Study on the mechanism of action of <i>P.falciparum</i> DNA topoisomerase II inhibitors (สำเร็จการศึกษา 26 ธ.ค. 2540)	ศึกษาปริญญาเอก ภาควิชาชีวเคมี คณะวิทยาศาสตร์ มหาวิทยาลัยมหิดล
น.ส.สุกัญญา ชูทัยดา	Studies on drug combination against <i>P.falciparum</i> in vitro (สำเร็จการศึกษา 1 มิ.ย. 2541)	ศึกษา Ph.D. ที่ Illinois State University, Illinois U.S.A.
น.ส.พรวิมล แซ่ซือ	Membrane studies of thalassemic red cell:sulphydryl content and K-Cl co-transport (สำเร็จการศึกษา 8 มิ.ย. 2541)	อาจารย์ คณะเทคนิคการแพทย์ มหาวิทยาลัยหัวเฉียว
น.ส.พรพิมล เมธีบุญถน	Role of spectrin phosphorylation and transglutaminase on the properties of thalassemic erythrocyte membrane (สำเร็จการศึกษา 9 มิ.ค. 2542)	ศึกษาปริญญาเอก ภาควิชาชีวเคมี คณะวิทยาศาสตร์ มหาวิทยาลัยสุรนารี
น.ส.สุรีย์ เจริญธีรบูรณ์	Inactivation of artemisinin by normal and variant erythrocytes (สำเร็จการศึกษา 13 พ.ค. 2542)	อาจารย์ คณะเภสัชศาสตร์ มหาวิทยาลัยศิลปากร
น.ส.นงลักษณ์ ศรีวิไลเจริญ	Studies on telomerase of the human malaria parasite, <i>P.falciparum</i> (สำเร็จการศึกษา 17 พ.ค. 2542)	ศึกษาปริญญาเอก ภาควิชาชีวเคมี คณะวิทยาศาสตร์ มหาวิทยาลัยมหิดล
นายกฤษชัย ชูสงเจริญ	Study on the effects of 3,8-diamino anilinoacridines on heme polymerization (สำเร็จการศึกษา 13 พ.ค. 2543)	อาจารย์ คณะเกษตรศาสตร์ มหาวิทยาลัยเกษตรศาสตร์ กำแพงแสน

นักศึกษาระดับปริญญาโทในปัจจุบัน
 ของภาควิชาชีวเคมี คณะวิทยาศาสตร์ มหาวิทยาลัยมหิดล
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ชื่อ-นามสกุล	วิทยานิพนธ์ปริญญาโท
น.ศ. ไร่ศิริศิลป์ ศรีชัยภักดิ์	The effects of cycloguanil derivatives on <i>P. falciparum</i> Dihydrofolate reductase and on parasite growth (กำลังจะส่งฉบับของวิทยานิพนธ์)
นายวิฑูรย์ นิยมสันติวงศ์	Antimalarial effect of antisense oligonucleotides directed against plasmodial topoisomerase II <i>in vitro</i>
น.ศ. พัทธนิ รุธีร	Studies on the mechanism of inactivation of artemisinin and its derivatives by normal and genetically variant red blood cells
น.ศ. นันทิมา เตชะภาค	Studies on plasmamembrane Ca^{2+} -ATPase of oxidized red cells
น.ศ. นภาพรรณ ผลมี	Inactivation of artemisinin by red blood cells

นักศึกษาระดับปริญญาเอกในปัจจุบัน
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ชื่อ-นามสกุล	วิทยานิพนธ์ปริญญาเอก
น.ส.ศรัญญา อูปภิทธิตานนท์	Development of novel anilinoacridine compounds directed at multiple targets in the malaria <i>Plasmodium falciparum</i>

PUBLICATIONS