抗マラリア剤効果と人間、原虫、媒介蚊の遺伝的多様性に関する研究

Study on antimalarial efficiency and and genetic diversities of human, parasites and mosquitoes

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はしがき

マラリアは現在なお、熱帯諸国を中心に年間二百万人に達する犠牲者を出しており、グローバル・アジェンダ上で重要課題として取り上げられている。一方、地球温暖化に伴う日本を含むマラリア流行地域の拡大が危惧されている。1992年のマラリアサミット以降、新たな対策戦略(Global Malaria Control Strategy)が試みられ、WHOは新たに“Roll Back Malaria”キャンペーンを展開しているが必ずしも成果が上がっていない。

有効なマラリアワクチン開発が達成されていない現状においてマラリア化学療法は最も重要な対策法である。抗マラリア剤治療効果は従来原虫薬剤耐性の観点から主に検討されてきたが、患者における薬物代謝も重要であることが解ってきた。一方、マラリア伝播サイクル関わる人間、原虫、および媒介蚊の遺伝的多様性が明らかにされつつある。これらは環境に応じ進化学的に変異してきた結果、現在の地理的差異として認められ、マラリア対策や化学療法剤治療効果に様々な影響を及ぼしていると考えられる。当研究班はこれらの新しい観点からマラリア対策に貢献することを究極的な目標とし掲げてきた。

一方現在国内においては「橋本イニシアチブ」のもとにグローバルな寄生虫対策への貢献が模索されており、その線上において様々な分野の研究者がマラリア研究に参画してきているのは大変喜ばしいことである。その多くは実験室における基礎研究であり、新たな技術・方法論により研究の飛躍的推進が期待されよう。ただ、この局面においてもマラリア研究の原点は流行地患者であり、研究の方向性はそこから出てくるべきものであると筆者は信じる。本研究班はその研究戦略として、流行地における観察を実験室内の解析と効果的に繋ぎ合わせることにより新たな事実を見出すことを試みてきた。

ここに上梓する報告書について様々な分野の方から御批判を頂ければ幸いである。

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— *SUMMARY*

STUDY ON ANTIMALARIAL EFFICACY AND GENETIC DIVERSITIES OF HUMAN, PARASITES AND MOSQUITOES

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Aims of the study

The genetic diversities displayed by human and malaria parasites at different frequencies in different geographical areas represent a major obstacle for the development of malaria control strategies including malaria chemotherapy and malaria vaccine. Factors affecting their distribution patterns include transmission and parasite population dynamics, human travel and migration patterns, and human behavior.

The islands of the Pacific offer diverse human habitats with a certain diversity of malaria endemicity. Vanuatu consists of 80 islands in Melanesia. These isolated islands provide suitable conditions to investigate the relations between the genetic diversities and the effectiveness of the malaria control strategies.

With the overall ultimate aim of contributing to an optimal malaria control strategy on the islands, the following specific objectives were designed within the present project:

1. To determine and possibly explain the distribution of the CYP2C19 mutations on the islands of Vanuatu (*paper I*), and to investigate possible implications for proguanil (PG) metabolism and antimalarial efficacy of PG (*papers II & III*).

2. To study the distribution patterns of dihydrofolate reductase (*dhfr*) mutations in *P. falciparum* infections and the implications for the efficacy of malaria therapy by pyrimethamine-sulfadoxine and PG (*paper IV*).

3. To study the long term effects of temporary mass drug administration (MDA) and permanent use of permethrin-impregnated bed nets on malaria transmission on an isolated island (*paper V*).

Major findings

A. The frequencies of the cytochrome P450 (CYP) 2C19 mutant alleles are uniformly greater in populations of Vanuatu than in previous populations surveyed. However there are differences between populations from different islands. Comparisons of genetic, linguistic and geographic patterns suggest that short range gene flow is largely responsible for the current distribution of *CYP2C19* alleles in Vanuatu. Together with previous studies of nuclear genetic loci of Pacific island populations, these data predict that the poor metabolizer (PM) genotype is common throughout Polynesia and Micronesia and may be even more prevalent in western Melanesia than in Vanuatu. This suggests that the majority of Pacific Islanders metabolize a wide variety of clinically important drugs to a significantly lower degree than other geographical populations, e.g. the average Europeans, who have been subject to most studies on the pharmacological properties of modern drugs.

B. Our data in malaria patients demonstrate an association between *CYP2C19* mutations and poor metabolism of PG to cycloguanil (CG), a strong DHFR inhibitor, and may suggest a gene dose effect relationship. Mild adverse events (mainly gastro-intestinal symptoms) were often reported, but the incidence was similar in PMs vs extensive metabolizers (EMs), although positively correlated with PG concentrations.

C. Restricted diversity in the *dhfr* gene was showed in a total of 140 *P. falciparum* cases on 4 isolated islands of Vanuatu. All isolates had the same Asn-108 mutation, and all, except 3 in Gaua, also had Arg-59 mutation, normally associated with moderate resistance to DHFR-inhibiting drugs. The 3 remaining isolates in Gaua had His-51, a change not previously reported in the literature. Despite these partly resistant variants, PS treatment was still highly effective. The restricted diversity of the *dhfr* gene in unstable malaria endemicity on Vanuatu islands may be at least partly explained by a loss in heterozygosity of the parasite populations derived from periodically interrupted transmission and isolation with limited gene flows between islands.

D. Mutations in human *CYP2C19* and parasite *dhfr* genes, related to poor metabolism of PG and resistance to CG respectively, have both been assumed to be

associated with poor antimalarial effect by PG. We however observed a high antimalarial efficacy of PG also in patients with *CYP2C19*-related PM and *dhfr*-related moderate CG resistant genotypes. This suggested that the parent compound PG has an intrinsic efficacy independent of the metabolite CG, which may possibly further enable its use also against parasites with *dhfr* mutations.

E. On Aneityum island with low-to-moderate transmission, we have most probably interrupted transmission by combining MDA with impregnated bed nets and achieved sustained and significant gains. Periodic spleen and blood surveys for the past nine
years have showed complete absence of *Plasmodium falciparum* after the MDA and *P. vivax* from 1996 onwards, with the exception of two instances of imported infections (one mixed infection in 1993 and one *P. vivax* infection in 1999), suggesting that malaria can be eliminated if the intervention is conducted over the whole area of transmission and the risk of importation of malaria is controlled within a framework of community consent and participation.

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MAIN ORIGINAL PAPERS

The copies of the following papers are presented hereinafter.
They will be referred to by their roman numerals.

I. Kaneko A, Lum JK, Yaviong J, Takahashi N, Ishizaki T, Bertilsson L, Kobayakawa T, Björkman A. High and variable frequencies of *CYP2C19* mutations: medical consequences of poor drug metabolism in Vanuatu and other Pacific islands. *Pharmacogenetics* 1999; 9: 581-590.

II. Kaneko A, Bergqvist Y, Taleo G, Kobayakawa T, Ishizaki T, Björkman A. Proguanil disposition and toxicity in malaria patients from Vanuatu with high frequencies of *CYP2C19* mutations. *Pharmacogenetics* 1999; 9: 317-326.

III. Kaneko A, Kalkoa M, Bergqvist Y, Takechi M, Nishiyama A, Kobayakawa T, Björkman A. Restricted diversity and novel mutations in the *Plasmodium falciparum* dihydrofolate reductase gene on isolated islands of Vanuatu. In manuscript.

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