われているが、予防内服と比べてまとまったデータが示されることは少なく、有用性・安全性などの評価が不十分である。

一九九〇年代前半のスイス人旅行者一一八七人における研究では、発熱した者が一〇・四%であったが、スタンバイ治療を行った六名のうち、後日、抗体検査でマラリアが確定したのは一名で、スタンバイ治療が過度に行われることの危険性が指摘された[11]。

また、一九九三年のドイツ人旅行者二八六七人を対象とした研究では、旅行中に発熱した者が八・一%、スタンバイ治療（ほとんどが予防内服のバックアップとして）を行った者が一・四%であったが、発熱した者がマラリアでないと考えた理由はほとんどの場合正当であると思われた。スタンバイ治療を行った者の中で、後日、抗体測定にて熱帯熱マラリアと判明したのは一〇・八%であったが、これを特別過度とみなしてはいない[16]。

スタンバイ治療はその性格上、ある程度過剰に行われるのは不可避であるが、その許容範囲についてのコンセンサスは得られていない。

わが国では、筆者（木村）らが日本人海外渡航者に対するアンケート調査を行った結果、時期的に早すぎるスタンバイ治療、迅速に医療機関を受診できる状況でのスタンバイ治療、スタンバイ治療実施後に迅速に医療機関を受診していない、などの問題点が明らかになった[17]。今後、わが国でもスタンバイ治療を積極的に導入する際には、この種の問題が起きないよう、旅行者および医療従事者に対する効果的な啓発を行う必要がある。

六、迅速診断キット

マラリア原虫抗原の検出法として、海外ではhistidine-rich protein 2（HRP2）を検出するNow® Malaria（Binax社）、pLDHを検出するOptiMAL-IT（DiaMed社）が発売されている。最も重要な熱帯熱マラリア原虫の検出感度については、HRP2検出系のほうがやや優れているとされている。迅速診断キットの使用・判定が適切に行われれば、スタンバイ治療の質が高まると予想されるので、一般の旅行者がこれを用い、その結果を基にスタンバイ治療を行う方法に期待が持たれた[11]。しかし、前もって血液と試薬を反応させたキットを渡し、説明書に従って判定させたところ、かなりの間違いがあることが示された[18]。

また、ケニアにおいて発熱している旅行者九八名にHRP2検出系キットを渡し、添付の説明書のみで検査・判定を行わせたところ、正確に検査を行うことができたのは六八%で、最終的に判明した一一名の熱帯熱マラリア患者のうち、自分で陽性の判定ができたのはわずか一名であった[19]。

さらに、帰国後の有症状旅行者一五三名を対象とした研究では、途中から筆者らが詳細な説明書を作成し、しかも採血のために特殊なランセットを使うことになった。全体として九一%の旅行者が正確に検査を行い、二一名の熱帯熱マラリア患者のうち一名を除き、他はすべて熱帯熱マラリアと判定できたが、旅行者自身によるキットの使用は時期尚早との意見であった[20]。

迅速診断キットの検出感度は優れているが[21]、旅行者が添付の説明書のみで実施するのは危険にもなりかねない。一般の旅行者に使用を勧めるためには、今後、その条件をより明らかにする必要がある[19)~21)]。

七、スタンバイ治療薬

スタンバイ治療薬としては、一般の薬剤と同様に効果が大きいこと、副作用が少ないこと、小児、妊婦、授乳婦に対して安全であることなどのほかに、服用法が簡単であること[10]が要求される。しかし完璧なものはなく、「効果が大きい」「副作用が少ない」のどちらにどの程度の重きを置くかにより評価が異なってくる。いずれにしても、スタンバイ治療薬の選択に当たっては、現地におけるマラリアの種類、流行度、薬剤耐性、旅行者の持病、常用薬剤などのさまざまな情報が不可欠である。

わが国でスタンバイ治療薬の候補となるのは、キニーネ経口薬、スルファドキシン／ピリメタミン

合剤（ファンシダール），メフロキン（メファキン「エスエス」）の三種類のみである（表3）。しかし，キニーネ経口薬では服用期間が長く，コンプライアンスが悪化しやすいこと，また，単独では効果が低く，ドキシサイクリンなどとの併用により服用法が複雑になること[12]，スルファドキシン／ピリメタミン合剤では効果が低下しており[10,14]，予防の場合よりは少ないが中毒性表皮壊死症やスティーヴンス・ジョンソン症候群などの重篤な副作用があること，メフロキンでは時に精神神経系副作用が問題となること[12,14]など，いずれも理想的なスタンバイ治療薬とはいえないのが残念である。

海外のマラリア予防ガイドラインでは他の薬剤も対象となっている（表4）。いち早くスタンバイ治療の導入を行ったスイスの場合，クロロキン，メフロキン，アトバコン／プログアニル合剤（Malarone），アーテメター／ルメファントリン合剤（Riamet）が挙げられている[22]。

英国では，スイスのガイドラインに加えてキニーネ単独あるいはドキシサイクリンとの併用が挙げられている（ただし，メフロキンに関しては消極的）[14]。英国の医療機関では熱帯熱マラリアの治療薬としてキニーネが中心であることの反映と思われる。

スイスと英国いずれの場合でも，クロロキンが勧められる地域は限定されており，スルファドキシン／ピリメタミン合剤は両国のガイドラインから削除されている。ヨ

表3　国内でスタンバイ治療薬の候補になる薬剤の概要

一般名	商品名	投与量（成人を基本）	禁忌*	備考
メフロキン	メファキン「エスエス」	15mg/kgの単回投与，メフロキン耐性地域では6～24時間後に10mg/kgの追加で計25mg/kg	精神疾患あるいは痙攣性疾患，重症な精神神経疾患の既往，ハロファントリンの併用治療，過去4週間以内のメフロキン治療	キニーネの最終服用後12時間以内には使用しない．類似薬（キニーネ，キニジン，クロロキン）の同時服用では心毒性の増強，痙攣のリスク増加のため，厳重な監視が必要．β遮断薬，Ca拮抗薬，抗ヒスタミン薬（H_1拮抗薬を含む），フェノチアジンとの同時服用ではQTc間隔延長の可能性
スルファドキシン／ピリメタミン合剤	ファンシダール	500mg/25mg錠剤を3錠単回	高度の肝障害・腎障害，巨赤芽球性貧血	治療効果は低下しており，スティーヴンス・ジョンソン症候群，中毒性表皮壊死症などの重症の皮膚疾患（HIV感染者では特に高頻度）もありうるので，使われない傾向
キニーネ	硫酸キニーネ，塩酸キニーネ	キニーネ塩1,800mg/日・分3で5～7日間	耳鳴，視神経炎，溶血，重症筋無力症	キニーネ高度耐性地域では単独でなく，ドキシサイクリン，クリンダマイシンなどとの併用．副作用としていわゆる“cinchonism”，小児，妊婦，重症疾患では低血糖の誘発
キニーネ・ドキシサイクリン** 併用	硫酸キニーネ，塩酸キニーネ	キニーネ塩1,800mg/日・分3で3日間	上記キニーネの項参照	上記のキニーネの項参照
	ビブラマイシン，他	200mg/日・分2で7日間	肝機能異常	消化器症状（食道潰瘍を含む），光線過敏，腟カンジダ症

* いずれも当該薬剤に対するアレルギーでは禁忌．
スルファドキシン/ピリメタミン合剤では，特にサルファ剤アレルギーに注意が必要．

**マラリアの治療薬としては認可されていない．

ーロッパではアーテメター／ルメファントリン合剤の評価が高まっているが，non-immuneのマラリア患者でのまとまったデータとしてはまだ少ない[23]。

これに対し米国では予防内服が中心で，スタンバイ治療に重きを置いていない。また，スタンバイ治療薬としてもアトバコン／プログアニル合剤のみである[15]。

このような国による違いの理由として，薬剤の認可状況のみならず，予防内服およびスタンバイ治療の位置づけ，副作用に対する評価や考え方の違いがありうる。

表4　海外でスタンバイ治療薬として使用されている薬剤*の概要

一般名	商品名	投与量（成人を基本）	禁　忌*	備　考
クロロキン	Nivaquine, Aralen, Resochin, Avloclor など	クロロキン塩基として初回10 mg/kg，6，24，48時間後にそれぞれ5mg/kgで，計25mg/kg	てんかんの既往，乾癬	通常は三日熱，卵形，四日熱マラリアが対象．熱帯熱マラリアで効果があるのは中米のみ
アトバコン／プログアニル合剤	Malarone	250mg/100mgの合剤4錠を1日1回・3日間	高度腎障害（クレアチニンクリアランス30mℓ/分未満）	Non-immuneでの治療経験は少ない．リファンピシン，リファブチン，メトクロプラミド，テトラサイクリンとの同時服用でアトバコンの血漿中濃度の低下．一部に耐性の報告
アーテメター／ルメファントリン合剤	Riamet	20mg/120mgの合剤4錠を0，8，24，36，48，60時間後の計6回	現在のところ特になし	Non-immuneでの治療経験は少ない．QTc間隔延長を含む心電図異常は認められていない

* 表3に示した薬剤を除く．
**いずれも当該薬剤に対するアレルギーでは禁忌．

おわりに

マラリアのリスクが高い場合には予防内服が勧められるが，リスクが低い場合，また，リスクが高い場合でも特殊な例ではスタンバイ治療が重要なオプションと考えられる。しかし，スタンバイ治療に伴うリスクも十分認識することが必要であり，熟練した医療従事者のみが処方すべきであると思われる。今後，日本人旅行者のマラリア予防をどのように行うべきか，その中でスタンバイ治療をどう位置づけるべきかについて，考える機会になればと望むものである。

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CLINICAL CHARACTERISTICS OF IMPORTED MALARIA IN JAPAN: ANALYSIS AT A REFERRAL HOSPITAL

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Abstract. Imported malaria remains an important problem in Japan. We have reviewed the medical records of 170 cases of malaria in our hospital, which corresponds to 14.9% of the total cases in Japan. The predominant malarial species was *Plasmodium falciparum* (52.3%), and the most frequent area of acquisition was Africa (54.2%), followed by Asia (20.9%) and Oceania (19.6%). The most common reason for travel among Japanese patients was business. A significant proportion (22.2%) of vivax malaria cases experienced relapse despite standard primaquine therapy. Most primaquine failures were from Oceania. We also found that a substantial number of Japanese patients contracted malaria without chemoprophylaxis and consulted medical facilities with an unfavorably long delay from initial symptoms (median: 3.0 days). Direct education of travelers and travel companies, in addition to health care providers, is likely necessary to improve outcomes of imported malaria.

INTRODUCTION

Forty-one percent of the world's population live in areas where malaria is transmitted, and an estimated 700,000–2.7 million persons die of malaria each year. (http://www.cdc.gov/malaria/facts.htm WorldMalaria.) In Japan, indigenous malaria was eradicated in 1961.[1] Recently, an increasing number of Japanese have been traveling to malaria-endemic countries for business and vacation. People from countries with malaria endemicity have also been visiting Japan for education. This global travel has resulted in 100–160 cases of imported malaria per year.[1–3] The number of physicians, however, who can diagnose malaria and treat it appropriately has fallen in Japan as the current priority of medical research and education has moved to chronic diseases such as cancer or cardiovascular diseases. Most technologists are also not trained to diagnose malaria on properly prepared thin and thick blood smears. Diagnostic delay with falciparum malaria can result in increased mortality, especially among nonimmune travelers. It is therefore important for physicians to have a low index of suspicion for malaria as a cause of acute febrile illness among Japanese travelers and immigrants from malaria-endemic countries.

Recently, two reports were published describing the global statistics of imported malaria in Japan based on data from the national surveillance system.[1,2] To further evaluate clinical problems in the management of malaria in Japan, a detailed review of individual patient medical records is necessary. The Institute of Medical Science (IMS) Hospital at the University of Tokyo has been a referral center for cases of malaria in Japan for decades. Malaria cases managed at IMS Hospital account for 10–20% of the total cases in Japan. In this study, we reviewed patient medical records of 170 malaria cases seen at IMS Hospital from 1992 to 2001 and analyzed their demographic and clinical data.

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MATERIALS AND METHODS

All confirmed cases of malaria at IMS Hospital from January 1992 to December 2001 were reviewed. The following parameters were included: age, sex, suspected area of contraction, nationality, *Plasmodium* species, antimalarial treatment, duration of symptoms prior to seeking medical care, use of chemoprophylaxis, complications, prognosis, and evidence of relapse or recrudescence. Patients who were treated abroad and visited IMS Hospital for follow-up after clearance of parasitemia were excluded unless their blood smears performed in Japan were positive. All cases were diagnosed with conventional microscopic examination of Giemsa staining of thin and thick blood films by technicians or physicians with expertise in tropical medicine, and the diagnosis was always confirmed by polymerase chain reaction (PCR) in indeterminant cases.[4] Collected data were analyzed using EpiInfo2002 downloaded from the Web site of the U.S. Centers for Disease Control and Prevention. All statistical analyses were performed with two-tailed test, and $P < 0.05$ was considered statistically significant.

RESULTS

Overall, there were 170 confirmed cases of malaria at IMS Hospital from January 1992 to December 2001. There were no indigenously acquired cases. According to national surveillance systems, the annual number of malaria cases in Japan has remained stable at 103–156 cases for the decade.[1,2] The number of cases at IMS Hospital has accounted for 9.0–21.4% (average 14.9%) of all cases in Japan. Seventeen of the 170 cases were relapses or recrudescence (4 episodes from 2 subjects with *Plasmodium ovale*, 12 episodes from 9 subjects with *Plasmodium vivax*, and 1 episode from a subject with *Plasmodium falciparum*) after treatment at IMS Hospital and were excluded from analysis to avoid redundancy. Of 153 cases, 101 were Japanese citizens and 52 were foreigners (Table 1). Seven of the 52 foreigners were from industrialized countries without indigenous malaria and had traveled to malaria endemic tropical countries prior to visiting Japan. The remaining 45 people were from malaria-endemic countries. There was no significant difference in demographic data between Japanese and foreign patients (Table 1). *Plasmodium*

TABLE 1

Characteristics of imported malaria in the past decade in IMS hospital

	All	Japanese	Foreigner	*P* value
Number of all cases	170	116	54	–
Number of new cases*	153	101	52	–
Age (mean ± SD)	34.8 ± 11.2	35.4 ± 12.7	33.8 ± 7.7	0.42†
Male:female	124:29	84:17	40:12	0.35‡
Species *N* (%)§				
Pf	80 (52.3)	50 (49.5)	30 (57.7)	
Pv	55 (35.9)	38 (37.6)	17 (32.7)	
Po	14 (9.2)	11 (10.9)	3 (5.8)	0.46‡
Pm	3 (2.0)	1 (1.0)	2 (3.8)	
Pf/Pv	1 (0.7)	1 (1.0)	0 (0.0)	
Total	153 (100)	101 (100)	52 (100)	–
Suspected contraction areas *N* (%)¶				
Africa	83 (54.2)	51 (50.5)	32 (61.5)	
Asia	32 (20.9)	19 (18.8)	13 (25.0)	
Oceania	30 (19.6)	25 (24.8)	5 (9.6)	
South America	3 (2.0)	1 (1.0)	2 (3.8)	0.15‡
Africa/Asia	3 (2.0)	3 (3.0)	0 (0.0)	
Asia/Oceania	1 (0.7)	1 (1.0)	0 (0.0)	
EU	1 (0.7)	1 (1.0)	0 (0.0)	
Total	153 (100)	101 (100)	52 (100)	–

* Excluding relapse and recrudescence after treatment of initial event.
† Student *t* test.
‡ χ^2 test.
§ Pf, Pv, Po, and Pm represent *P. falciparum*, *P. vivax*, *P. ovale*, and *P. malariae*, respectively. Pf/Pv means mixed infection of Pf and Pv.
¶ "Africa/Asia" and "Asia/Oceania" mean that patients traveled around more than one area, and it was impossible to determine the estimated contraction area.

falciparum was the leading species among both Japanese and foreign patients, followed by *Plasmodium vivax*, *Plasmodium ovale*, and *Plasmodium malariae* (Table 1). The proportion of *P. falciparum* cases tended to increase between the first and second half of the decade. Nevertheless, there was no statistical significant difference (47.6% versus 58.6%, $P = 0.176$, χ^2 test). Africa was the most frequent area of acquisition in both groups throughout the decade, followed by Oceania among Japanese patients and by Asia among foreign patients. Overall, Oceania overtook Asia as the second most common area of malaria acquisition in the second half of the decade (Asia versus Oceania: 21.7% versus 15.7% in the first half decade, 20.0% versus 24.3% in the second half decade).

P. falciparum infection accounted for 74.4% of cases from Africa, 40.0% from Oceania, 12.5% from Asia, and 0% from South America. These proportions remained stable through the decade (data not shown).

The purpose of travel to malaria-endemic areas was reported for all of Japanese patients with malaria. Contrary to our expectation, the proportion of travelers for sightseeing has not increased in the second half decade (first versus second half decade: 27.6% versus 27.9%), and business was the most common purpose through the decade (first versus second half decade: 70.7% versus 69.8%). On the other hand, visiting friends and relatives (VFR) accounted for only 1.0% in the decade.

The use of chemoprophylaxis was available for 100 of 101 Japanese patients. The percentage of Japanese patients with malaria who had taken chemoprophylaxis decreased dramatically between 1992–1996 and 1997–2001 (48.3 versus 14.3%, $P = 0.0003$). Although chloroquine was the leading agent used between 1992 and 1996 (Table 2), no patients took it from 1997 to 2001. Four patients acquired vivax malaria despite taking mefloquine for chemoprophylaxis. These four cases

TABLE 2

Comparison of chemoprophylaxis between 1992–1996 and 1997–2001

Chemoprophylaxis among Japanese patients *N* (%)	1992–1996	1997–2001	*P* value*
Chloroquine	18 (31.0)	0 (0.0)	
CP	2 (3.4)	0 (0.0)	
Mefloquine	1 (1.7)	3 (7.1)	0.0006
SP	1 (1.7)	0 (0.0)	
Others	6 (10.3)	3 (7.1)	
None	30 (51.7)	36 (85.7)	
Total	58 (100)	42 (100)	–

* χ^2 test between 1992–1996 and 1997–2001.
CP, chloroquine/proguanil; SP, sulfadoxine/pyrimethamine.

were thought to represent relapses, as none had taken primaquine after leaving the malarious areas.

Mefloquine has been the most frequently prescribed antimalarial treatment against *P. falciparum* infection (82.5%, data not shown). Chloroquine was used in several cases in the early 1990s but has not been used since 1997. Atovaquone/proguanil and artesunate have been used in a limited number of patients. Of 80 patients with falciparum malaria, only 4 cases had severe complications; 1 case of cerebral malaria, 2 cases of renal failure, and 1 case of severe anemia. There were, however, no deaths attributable to malaria. Of 38 cases of vivax malaria among Japanese patients, 36 were prescribed primaquine to prevent relapse after successful treatment with schizonticides. Despite primaquine prophylaxis, eight cases (22.2%) experienced relapse. Six of these cases (75.0%) contracted malaria in Oceania; mostly Papua New Guinea (Table 3). In cases in which relapse occurred after standard primaquine treatment (15 mg base per day for 14 days), modified regimens with larger doses or longer periods of primaquine were administered with favorable results (Table 3).

We analyzed the length from onset of symptoms to first medical consultation among Japanese patients (Figure 1). Data was available for 99% of Japanese patients. The mean and median duration was 4.7 days (95% confidence interval [CI]: 3.7–5.7) and 3.0 days (interquartile range [IQR]: 2.0–5.0 days), respectively. The mode was 2.0 days (18.9%). In cases of falciparum malaria, the mean and median duration were 4.1 days (95% CI: 2.5–5.7) and 2.0 days (IQR: 1.0–5.0), respectively. There were 12 falciparum malaria patients who visited clinics 5 days or more after onset of illness. Nine patients had taken chemoprophylaxis (chloroquine: 4; unknown: 1) and/or had self-administered antimalarials (chloroquine: 5; halofantrine: 1) prior to presentation. Of the three patients with delayed presentation who had not received antimalarials, one had renal failure (6 days) and the other two had no complications (7 and 11 days).

DISCUSSION

This retrospective review of malaria cases at a national referral hospital corresponds to 14.9% of the total cases in Japan over 10 years. This study represents the largest review of clinical characteristics and outcomes of patients with malaria in Japan. Falciparum malaria accounted for about 50% of total malaria cases at the IMS Hospital. The proportion of *P. falciparum* cases is relatively high in France (around 80%), moderate in Germany and the United Kingdom (around

TABLE 3
List of vivax malaria cases that had relapses after primaquine treatment

Case	Sex	Age	Year of first episode	Contracted countries	Episode	Schizonticides	Primaquine regimens
1	M	30	1992	Malaysia	1st	CRQ	15 mg base for 14 days
					2nd	CRQ	30 mg base for 7 days
					3rd	CRQ	15 mg base for 14 days × 2 courses
2	M	23	1993	Papua New Guinea	1st	CRQ	15 mg base for 14 days
					2nd	CRQ	15 mg base for 14 days × 2 courses
3	M	44	1993	Papua New Guinea, Indonesia	1st	CRQ	15 mg base for 14 days
					2nd	CRQ	15 mg base for 14 days
					3rd	CRQ	15 mg base for 14 days × 2 courses
4	M	22	1997	Papua New Guinea	1st	CRQ	15 mg base for 14 days
					2nd	CRQ	15 mg base for 14 days × 2 courses
					3rd	CRQ	30 mg for 11 days
5	M	32	1997	Papua New Guinea, Solomon Island	1st	MEF	15 mg base for 9 days
					2nd	CRQ	Lost to follow-up
6	M	34	1998	Philippines	1st	CRQ	15 mg base for 14 days
					2nd	CRQ	15 mg base for 14 days
7	M	49	2001	Papua New Guinea	1st	CRQ	15 mg base for 14 days
					2nd	CRQ	30 mg base for 14 dyas
					3rd	CRQ	30 mg base for 28 days
8	M	24	2001	Vanuatu	1st	CRQ	15 mg base for 14 days
					2nd	MAL	30 mg base for 14 days

CRQ, chloroquine; MAL, atovaquone/proguanil.

60%), and low in the United States (around 40%).[5] The proportion of falciparum malaria cases in Japan seems to lie between European countries and the United States. European countries are geographically close to Africa where *P. falciparum* is a dominant species, whereas the United States is closer to Central and South America where *P. vivax* is predominant. Japanese travel to both Africa and Asia/Oceania likely accounts for this midrange proportion of *P. falciparum* in IMS Hospital.

VFR, which is one of the most common reasons for travel to malarious areas in Western countries, accounted for only 1.0% of Japanese travelers. In the United Kingdom, where immigrants compose a substantial percentage of populations, VFR, holidays, and business accounted for 56%, 12%, and 6.5%, respectively.[6] Because Japan is racially homogeneous, VFR is not likely to be a major reason for travel to malaria-endemic countries. Business travels accounted for 70% among Japanese patients throughout the decade. In Germany, where the number of immigrants from malarious countries is small like Japan, however, business travel accounted for only 18%, and 75% was holiday travels.[7] This contrasting result might be explained by the difference in the number of sightseeing travelers to malarious areas between Japan and Germany. Nevertheless, detailed investigation would be required to clarify it. Improving travel advisements to overseas employees is likely to contribute to reduction in the number of imported malaria in Japan.

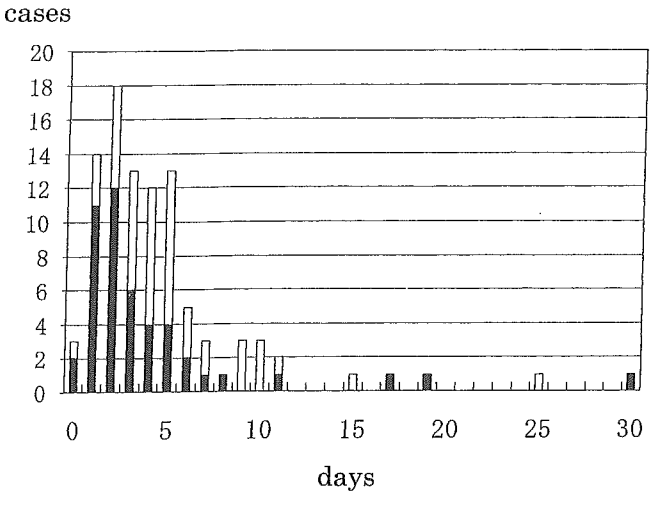

FIGURE 1. Distribution of period from the onset of illness to first medical consultation. Horizontal axis represents days from onset of symptoms to first medical consultation of malaria patients. Longitudinal axis indicates the number of cases. The bar represents total number of cases, and the black portion in each bar corresponds to the cases of falciparum malaria.

There are no national guidelines for malarial prophylaxis or treatment in Japan. At IMS Hospital, mefloquine is used to treat falciparum malaria without complications, intravenous quinine for severe falciparum malaria, and chloroquine for nonfalciparum malaria. Although we have not experienced mefloquine-resistant falciparum malaria, it is well-known that multidrug-resistant falciparum malaria has emerged in Southeast Asia, especially at the border between Thailand and Myanmar and between Thailand and Cambodia.[8,9] Because many Japanese visit Thailand, health care providers must be aware of potential resistance when treating patients returning from these areas. Fortunately, we experienced no deaths from malaria at our institution. However, we previously reported that the case fatality rate (CFR) nationally from falciparum malaria is 3.3%.[2] This CFR is as high as that of Germany (3.6%) and much higher than that of France (1.98%), the United States (1.01%), and the United Kingdom (0.65%).[5] A high CFR could be attributable to better mortality reporting compared with total case reporting,[5] a high proportion of patients without immunity to malaria,[10] or poor management of complicated malaria.[10] These factors may explain the discrepancy of CFR between the IMS Hospital and the rest of Japan. Alternatively, travelers who are aware of their malaria risk may present earlier to reference hospitals for tropical medicine. Unawareness of malarial risk will lead to delayed

infectious diseases physician consultation and result in unfavorable outcomes.

P. vivax infection generally causes nonfatal disease. Primaquine administration after treatment with schizonticides is required to eradicate the dormant form of *P. vivax* in the liver. Because primaquine is not always effective against hypnozoites, relapse is occasionally observed even after adequate primaqine therapy.[11,12] Of the cases of vivax malaria that relapsed after primaquine therapy at IMS Hospital, 75% were from Oceania. Both primaquine and chloroquine resistant *P. vivax* have emerged in the same area.[13] We have shown that the number of malaria cases from Oceania has increased from 1997 to 2001. And further increases are likely, as Japanese travel to Papua New Guinea is increasing. We, therefore, are more likely to encounter imported cases of primaquine and/or chloroquine resistant *P. vivax.* Cases of vivax malaria from Oceania, therefore, require careful observation during treatment of the acute febrile phase and consideration for a modified dosage or duration of primaquine (for example, a longer duration or higher dose of primaquine therapy).[14]

Although there had been neither guidelines nor approved drugs for malaria chemoprophylaxis in Japan until 2001, travelers used to collect information and get drugs in a variety of ways. We unexpectedly found that the proportion of Japanese patients who were taking chemoprophylaxis had dropped drastically in 1997–2001. The difference may reflect the absence of chloroquine failures, as this drug was used less frequently for prophylaxis. This suggests that Japanese travelers are correctly informed of chemoprophylactic regimens that successfully prevent malaria acquisition. In support of this speculation, the annual number of imported malaria cases at the IMS Hospital and nationally has remained stable in the past decade despite record high levels of travel by both Japanese people and foreigners in 2000 (see http://www.immi-moj.go.jp/toukei/index.html). However, given the substantial number of travelers who still contract malaria without chemoprophylaxis, further efforts must be made to educate travelers.

The interval between the onset of symptoms and presentation to a hospital is another concern because any delay in diagnosis can lead to increased mortality with falciparum malaria. Kain and others reported that the mean duration from onset of symptoms until first medical consultation in Canadian travelers was 3.6 days (95% CI: 2.5–4.7) in hospitals without expertise in tropical medicine and 3.8 days (95% CI: 2.3–5.3) in hospitals with a tropical medicine unit.[15] The mean duration in our cases was 4.7 days (95% CI: 3.7–5.7). However, because the duration in our cases was not normally distributed (Figure 1), a precise comparison with our data is difficult. Nevertheless, even a median duration of 3.0 days in our cases is an unacceptable length that could cause severe malaria or death in a nonimmune population. Delayed diagnosis despite early presentation to hospitals is another common problem that can increase mortality of falciparum malaria at health care facilities lacking an infectious diseases unit. However, because almost all cases in the IMS Hospital were diagnosed on the first day of presentation, the data shown above also represents the duration from the onset of symptoms to diagnosis.

This retrospective study reveals clinical problems relevant to malaria imported to Japan. A high frequency of relapse of vivax malaria despite primaquine administration in patients from Oceania must be relayed to health care providers. In addition, the absence of chemoprophylaxis and the delay in initial medical consultation suggest a continued ignorance of travelers regarding malarial risk. To reduce morbidity and mortality due to malaria, travelers must be informed of malaria risks, the necessity of chemoprophylaxis, and the importance of immediate medical consultation if fever develops. To this end, it is important to develop travel medicine referral centers to coordinate the education of health care providers and travel companies regarding malaria.

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NOTE

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Questionnaire-based analysis of mefloquine chemoprophylaxis for malaria in a Japanese population

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Abstract Although mefloquine is the only drug licensed for malaria chemoprophylaxis in Japan, there have been few reports describing the effects of and adverse events in the prophylactic usage of mefloquine in a Japanese population. We therefore performed a questionnaire-based study in 21 travelers who were prescribed mefloquine for malaria chemoprophylaxis between October 2001 and December 2003. The study revealed that only 8 out of 21 (38.1%) of the travelers could complete the prophylaxis schedules. Another 8 travelers (38.1%) with incomplete adherence stated that they did not take mefloquine because of either actually experienced or anticipated adverse events. Twelve of the 16 travelers (75.0%) who took mefloquine complained of at least one adverse event probably related to mefloquine. As an overall impression about mefloquine chemoprophylaxis, 14 of the 21 travelers stated that they would take mefloquine again for the next travel to malaria-endemic areas, although 5 of them were concerned about adverse events. These results suggest that, although mefloquine is an indispensable drug for malaria prevention, other effective and well-tolerated chemoprophylactic antimalarials should be available for Japanese travelers who do not tolerate mefloquine.

Key words Malaria · Mefloquine · Chemoprophylaxis
Travel medicine · Adverse effect · Adherence

In Japan, 100–160 cases of imported malaria have been reported annually for the past 10 years.[1–3] Although caution against mosquito bites is an essential preventive measure, chemoprophylaxis using antimalarial drugs is important when travelers stay in highly endemic areas for a substantial period. Mefloquine is a quinine-related compound with strong antimalarial activity, and has been proven to be highly effective not only for the treatment of the acute phase of malaria but also for its prophylaxis.[4–7] In Japan, mefloquine was approved, in November 2001, for both the treatment and prophylaxis of malaria, and it was the only drug licensed for malaria chemoprophylaxis as of October 2004. However, several studies have shown that mefloquine is not well tolerated, due to adverse events.[4,8–10] To our knowledge, only two clinical trials in Japanese subjects have reported the effects of and adverse events in mefloquine chemoprohylaxis, and these trials were in members of the Japan Self Defense Force.[6,7] Although several groups in other countries have reported clinical data on mefloquine chemoprophylaxis in travelers,[5,8,11] information in Japanese travelers is essential for its safe and effective use for chemoprophylaxis in Japan.

The subjects chosen for the present study were 62 Japanese travelers who were prescribed mefloquine as malaria chemoprophylaxis at the hospital of the Institute of Medical Science, University of Tokyo, between October 2001 and December 2003. The travelers were informed of the risks and benefits of malaria chemoprophylaxis, and mefloquine was prescribed based on the travelers' decision. The schedule of mefloquine chemoprophylaxis was 250 mg (one tablet of Mephaquin; Mepha Aesch BL, Switzerland) per week, starting from 2–3 weeks before arrival in the malarious area and continuing until 4 weeks after leaving the area. We recommended starting chemoprophylaxis from 2–3 weeks before arrival, because it has been shown that many adverse events of mefloquine occurred within three doses from starting.[12] Questionnaires were mailed all together at the end of December 2003, with a notification that there would be a 3000-yen (approximately US$ 26) gratuity for responders. Information that was requested in the questionnaires included age, sex, present and past illnesses, destination, duration of travel, purpose of travel, adherence to mefloquine, details of adverse events, and whether they would consider taking mefloquine when they

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next traveled to a malarious area. The degree of severity of adverse events caused by mefloquine was defined as "mild" for trivial, "moderate" for those that lowered travel quality, and "severe" for those compelling travelers to change their schedule of travel.

Questionnaires were mailed to 62 travelers, and 21 of them (33.9%) responded to the questionnaires. None of the responders had a neuropsychiatric disease, epilepsy, or hypertensive disease for which a Ca blocker or a β blocker had been prescribed. As for adherence to mefloquine, only 8 of the 21 respondents (38.1%) could complete the course of chemoprophylaxis (Table 1). Six respondents terminated the course earlier than scheduled, and three of them stated that the reason for early termination was adverse events. Other early terminations occurred inadvertently or for unspecified reasons, including one person who had taken mefloquine twice, but cancelled travel after that. Surprisingly, five respondents (23.8%) did not take mefloquine at all.

Of the 16 respondents who took mefloquine with some adherence to the prescription, 12 (75.0%) experienced at least one adverse event (Table 2). One respondent suffered from severe dizziness, nausea, headache, anxiety, and anorexia for about 1 month after the first dose and had to delay leaving for 1 month. All adverse events occurred within three doses from the start of administration, and the durations of the symptoms varied. The most frequent adverse events were dizziness, nausea, headache, and unpleasant dreams. For their next travel to malaria-endemic areas, 14 of the 21 respondents (66.7%) answered that they would take mefloquine again, although 5 (23.8%) of them stated that they would do so with concern about adverse events. Four respondents (19.0%) stated they would not take mefloquine again because of adverse events.

Because the data for this study were collected through mailed questionnaires, the data obtained were limited. In addition, the response rate to the questionnaires was low (33.9%), which may have caused bias in the sampling data from the mother population. Travelers who have experienced adverse events may respond to questionnaires more readily than those without such events. However, the data described here should be useful, because it was urgent to obtain data on the safe and effective administration of mefloquine chemoprophylaxis in a Japanese population.

Table 1. Results of mefloquine chemoprophylaxis

	Number (%)
Adherence (n = 21)	
Completed	8 (38.1%)
Skip	2 (9.5%)
Early termination	6 (28.6%)
Did not take at all	5 (23.8%)
Reasons for "skip" (n = 2)	
Inadvertently	2
Reasons for "early termination" (n = 6)	
Adverse events	3
Inadvertently	1
Not specified	2
Reasons for "did not take at all" (n = 5)	
Concerned about adverse events	2
Kept for stand-by therapy	3
Contraction of malaria in travelers	
With chemoprophylaxis with any adherence (n = 16)	0
With no chemoprophylaxis (n = 5)	1

Table 2. Characteristics of adverse events in travelers who took mefloquine for chemoprophylaxis

Characteristics of adverse events	Number (%)
Frequency (n = 16)	
Any	12 (75.0%)
None	4 (25.0%)
Severity (n = 11)	
Mild	7 (43.8%)
Moderate	3 (18.8%)
Severe	1 (6.3%)

Symptoms (n = 16)	Number (%)	Details of adverse events								
		Severity			Time of onset (after the)			Duration (recovered within)		
		Mild	Moderate	Severe	First dose	Second or 3rd dose	Later	24 h	Several days	More
Dizziness[a]	5 (31.3%)	3	1	1	3	1		1	1	2
Nausea	5 (31.3%)	2	2	1	5			3		2
Unpleasant dreams[a]	3 (18.8%)		2		2	1		2	1	
Headache	3 (18.8%)	1	1	1	1	2		1	1	1
Anxiety	1 (6.3%)			1	1					1
Abdominal pain	1 (6.3%)		1		1					1
Anorexia	1 (6.3%)			1	1					1
Fever	1 (6.3%)		1		1				1	
Palpitation	1 (6.3%)		1		1			1		
Somnolence[a]	1 (6.3%)									
Insomnia	1 (6.3%)	1				1		1		
Irritability	1 (6.3%)	1				1		1		
Tinnitus	1 (6.3%)	1				1		1		

[a]Totals for details do not agree with numbers because a respondent did not answer appropriately

Previous studies in other countries have shown that the frequency of adverse events caused by mefloquine varied from 18.8% to 67.3%.[5,9–11] We can not explain why the incidence of adverse events was higher in our study (75%). We speculate that most of our participants took mefloquine for the first time, and may have been particularly aware of the drug's adverse effects, which we informed them of when prescribing. Mefloquine is really necessary and is an indispensable drug for the prevention of falciparum malaria, although our study showed poor adherence to the prescribed mefloquine. Education of travelers regarding the risk of malaria and the importance of adherence to chemoprophylaxis is important. In addition, our study indicates the necessity for the use in Japan of other effective and well-tolerated chemoprophylactic antimalarial drugs; for example, proguanil plus chloroquine; atovaquone plus proguanil; and doxycycline.[1,8–9,11]

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トラベラーズワクチンの現状と課題


大友　弘士

4．ワクチン接種・治療の実際
1）マラリア

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はじめに

マラリアは有史以前から人類の健康を損ない，その生命をも奪ってきた疾患である．しかも，いまなお熱帯，亜熱帯各地を猖獗してやまず[1)]，年間3億人以上が罹患し，幼児を中心に200万人以上が犠牲になっており，非流行地では熱帯地からの輸入マラリアの増加に悩まされ，その対策に有効なマラリアワクチンの開発が望まれている．しかし，叡智を尽くした研究が推進されて久しいが，未だ実用的なマラリアワクチンは開発されておらず，その治療は化学療法に依存せざるを得ないのが現状である．

マラリアワクチン開発の曙光

一概に感染症といっても，その病因はウイルスから多細胞の真核生物の蠕虫まで多種多様である．このうち，天然痘や黄熱などは重篤な疾患ではあるが，その獲得免疫は長期間存続し，宿主を次の感染ないしは発症抑止に導くことから，弱毒生ワクチンまたは不活化ワクチンの接種による防御が応用されている．さらに，他の多くのウイルス性疾患，細菌性疾患には，その種別により経口・非経口生ワクチン，不活化ワクチン，生菌，生・死菌ワクチン，リコンビナント，蛋白多糖体，トキソイドなど，多種多様なワクチンが開発され，既に実用化されている．しかし，真核生物であるマラリア原虫の場合は，ウイルスや細菌よりも高度に寄生適応しており，宿主の免疫応答を回避するエスケープ機構を有することが，ワクチン開発のネックになっている．

一方，これまでのマラリアワクチン研究の成果をみると，大阪大学微生物病研究所（阪大微研）の堀井俊宏教授らが人工遺伝子を用いて熱帯熱マラリア原虫のSERA蛋白質の*N*-末端領域を大腸菌に発現させ，それがマラリア原虫に対する標的抗原の1つであることを明らかにしたことが注目され[2)]，ワクチン開発に向けての研究の今後の発展が期待されている．いずれにしても，マラリア原虫の遺伝子解析により，原虫増殖を抑制する抗体の標的抗原の遺伝子多型や多重族遺伝子群形成による抗原性の変化のほか，マラリア患者の血清中には多数の原虫抗原に対する多量の抗体が産生されるが，その多くは防御に働かないこともワクチン開発を困難にしている．しかし，マラリアワクチン開発は，その有効性と持続期間はもとより，安全性と他の予防接種に干渉しないことが重要な要件である．

マラリア治療の原理と問題点

人類の宿願であったマラリアワクチン開発の曙光がみえてきたとはいえ，マラリアの感染・発症を抑制するワクチンは現段階では未開発の状態にあり，マラリア制圧の重要な一翼を担っているのは治療であり，化学療法が基本である．なかでも，この化学療法では，全種マラリアの赤血球内無性原虫をターゲットにした

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急性期の発熱抑止療法と，三日熱と卵形マラリアでは発熱抑止療法に引き続き，肝細胞内発育環の休眠原虫(ヒプノゾイト)を殺滅して以降の再発阻止を図る根治療法が重要である．

マラリアの発熱抑止療法に適用される化学療法薬を殺シゾント薬と呼び，最初に使用されたのはキナ樹皮で，17世紀中葉に原産地のペルーからヨーロッパに導入され，300年以上もマラリア治療に使用されてきた．このキニーネに代わる合成薬として1934年にドイツで初めて開発されたクロロキンは，抗マラリア活性が高く，キニーネよりも毒性が低く，小児や妊婦にも投与できるため，第二次世界大戦後に広範に使用されるようになり，1950年代には画期的な抗マラリア薬として不動の地位を確立していた．しかし，1957年にはタイ，1960年にはコロンビアにおいて，4種マラリア原虫の中で最も悪性の熱帯熱マラリア原虫のクロロキン耐性株が出現して以降，急速に熱帯各地にも出現または拡散し[3]，今日ではクロロキンに感受性を示すのは中近東と中米の一部に分布する熱帯熱マラリア原虫に限られ，熱帯熱マラリア治療の隘路になっている．そこで，この薬剤耐性マラリアの克服のため，1970年代以降に新規開発されたスルファドキシン・ピリメタミン合剤，メフロキン，ハロファントリン，アルテミシニンおよびその誘導体(アーテメター，アーテスネートなど)，アトバコン・プログアニル合剤，アーテメター・ルメファントリン合剤などのほか[4]，1970年代にその効果が再評価された古典的な硫酸キニーネとテトラサイクリン系抗生物質との併用などによる治療が現在は行われている[5]．

しかし，タイと周辺諸国との国境地帯，アマゾン川流域，東アフリカ諸国の特定地域では，既にクロロキンと新規薬剤との交差耐性株も出現しており，その治療は一層困難になっている．また，これらの多剤耐性マラリアにはキニーネの感受性が高いとされてきた．しかし，最近はその感受性低下も東南アジアやブラジルなどから報告されているが，未だ，1990年代に開発されたアトバコン・プログアニル合剤やアーテメター・ルメファントリン合剤のほか，中国最古の医書である黄帝内経に記載され，古くから民間療法に用いられてきたヨモギ科の植物から1970年代に有効成分が確定された青蒿素(quinghaosu＝artemisinine)とその誘導体の治療効果が高いとされている．なお，新規薬剤のハロファントリンは，治療効果は高いが，心電図上のQT延長から心室性不整脈の出現による死亡例の発生などの有害反応があり，次第に使用されなくなっている．

合併症を併発した熱帯熱マラリアの重症例には，キニーネ塩基8.3 mg/kgを200～500 mLの5％ブドウ糖液や生理食塩水に溶解，4時間かけて点滴静注し，必要に応じて8～12時間ごとに繰り返す非経口療法の救命率が高く，軽快したらキニーネの最終投与12時間後にメフロキンなどを経口投与する．さらに，最近は即効性の高いアルテミシン誘導体のアーテメターの筋注，アーテスネートの静注もしくは座薬も効果的であるとする報告が多い．なお，最近の新規抗マラリア薬の中には，従来の薬剤と化学構造，作用機序や薬物動態，特に消失半減期が著しく異なる合剤が少なくないが，これは治療効果の増強を図ると同時に，耐性株出現の阻止，あるいはできるだけ遅延させることを狙ったものである．

三日熱，四日熱，卵形マラリアに対する発熱抑止療法の選択薬はクロロキンである．しかし，1989年にパプア・ニューギニアから三日熱マラリア原虫のクロロキン耐性株の出現が報告され，1990年代にはインドネシア，ミャンマー，バヌアツなどでも耐性株の出現が確認され，クロロキンによる治療が奏効しないときは，メフロキンによる治療に変更する．

さらに，肝細胞発育環のヒプノゾイトを有する三日熱と卵形マラリアでは，クロロキンなどによる発熱抑止療法後にプリマキンでそれを殺滅しないと，その後の再発阻止が困難になる．しかし，パプア・ニューギニアには1950年代からプリマキン低感受性のChesson株の分布が知られており，最近は東南アジア各地からも低感受性株の存在が報告されている．そのため，プリマキンの標準投与(15 mg塩基/日，14日間)を1カ月の間隔を置いてさらに1クール繰り返したり，1日当たりの投与量を22.5 mgに増量する方法などが案出されている[6]．しかし，増量によりG6PD欠損症患者以外にも溶血を誘導する危険があり，より安全性の高い用法・用量の検討のほか，効果的で安全性の高い新規薬剤の開発が望まれている．いずれにしても，抗マラリア薬は，原虫の種類とその発育環に特異的に作用する特性を念頭に置き，特に熱帯熱マラリアの場合は，患者の病態，合併症発現の有無，推定感染地における薬剤耐性株の分布状況を考慮して，最も適切な薬剤の選択による迅速な治療を開始するか否かが，患者の予後に直接関与することを肝に銘ずるべきである．

表1　国内で入手可能な抗マラリア薬とその用法

商品名・剤型	一般名・含量	用法・用量(成人量)
Ⅰ．合併症のない薬剤耐性熱帯熱マラリアに対する発熱抑止療法		
ファンシダール，錠	スルファドキシン500 mg/ピリメタミン25 mg	3錠単回服用
メファキン，錠	塩酸メフロキン275 mg	4錠単回服用，または6～8時間間隔で2分服(体重45 kg以下では3錠に減量)
硫酸キニーネ，末	硫酸キニーネ	1.5～1.8 gを分3，7日間服用 テトラサイクリン系薬剤1 g，分4，7日間併用
マラロン*，錠	アトバコン250 mg/塩酸プログアニル100 mg	1日1回4錠，3日間，食事または乳製品とともに服用
リアメット*，錠	アーテメター20 mg/ルメファントリン100 mg	初回，8，24，36，60時間後に各4錠服用
プラスモトリム*，錠・座薬	アーテスネート50 mg，200 mg	初日200 mg 2錠，分2 第2～5日1内服または直腸内挿入
Ⅱ．重症熱帯熱マラリアに対する発熱抑止療法		
キニマックス*，注	2 mL中にグルコン酸キニーネ：キニーネ塩基240 mg，他のアルカロイドを含む全塩基250 mg	キニーネ塩基8.3 mg/kgを200～500 mLの5％ブドウ糖液や生理食塩水に希釈し，4時間かけて点滴静注．必要に応じ8～12時間ごとに繰り返し，軽快したら経口薬に切り替える． なお，1回量を算出するには，体重÷15.1(単位mL)をアンプルから吸い，5％ブドウ糖液で希釈すればよい．
プラスモトリム*，座薬	アーテスネート500 mg	用法・用量は上記と同じ
Ⅲ．三日熱，四日熱，卵形マラリアに対する発熱抑止療法		
ニバキン*	硫酸クロロキン150 mg塩基	クロロキン塩基を初回600 mg，6，24，48時間後に各300 mg服用
ファンシダール3錠単回服用，硫酸キニーネ1.2～1.5 gの3～5日間内服でもよい．		
Ⅳ．三日熱，卵形マラリアに対する根治療法		
プリマキン*，錠	リン酸プリマキン15 mg塩基	1日1回，15 mg塩基，14日間服用

*：「熱帯病治療薬研究班」から入手可能．

治療の実際と注意点

重要なことは，治療中は患者の病状経過を厳重に監視すると同時に，経時的に血液塗抹ギムザ染色標本の鏡検により赤血球原虫感染率(虫血症)の推移と赤血球内無性原虫の変性像を観察，さらに原虫消失時間(PCT)と発熱消失時間(FCT)を確認することが，治療効果の判定に不可欠である．特に熱帯熱マラリアの場合は，少なくとも1日数回はこの血液検査を行い，化学療法を開始して2～3日以内に虫血症の減少・消失が認められなかったり，増加するときは選択薬に耐性と判断し，薬剤の変更を考慮する．ただし，選択薬により，即効性のものと遅効性のものとがあり，一般にスルファドキシン・ピリメタミン合剤は遅効性，アトバコン・プログアニル合剤はやや遅効性，アルテミシニン誘導体は即効性であるので，選択薬の薬剤特性を理解しておくことも必要である．

表1に国内で入手可能な抗マラリア薬の用法・用量を示したが，薬剤によりその特性が異なる．

1．スルファドキシン・ピリメタミン合剤

東南アジア，アフリカ，南米の一部などに耐性株が存在し，本剤の治療効果は期待できない．本剤過敏症，妊婦，授乳婦，新生児，G6PD欠乏症患者には禁忌．スルホンアミドと薬物相互作用．まれに中毒性表皮壊死，Stevens-Johnson症候群などの重篤な副作用．薬価は1錠484.2円．

2．メフロキン

治療効果は高いが，既に耐性株出現．本剤・キニーネ過敏症，新生児，妊婦，痙攣既往者，精神病患者には禁忌．抗不整脈薬，Ca拮抗薬，β遮断薬などとの併用注意．副作用としてめまい，ふらつき，頭痛，悪夢，まれに中毒性表皮壊死．経口腸チフスワクチン，狂犬病ワクチンに対する干渉．CYP3A阻害薬，CYP3A誘導薬と薬物相互作用．薬価は1錠854.8円．

3．アトバコン・プログアニル合剤

薬剤耐性熱帯熱マラリアを含む全種マラリアに有効．リファンピシンとの併用でアトバコンのAUC(血中濃度－時間曲線下面積)は約50%低下．腹痛，悪心，嘔吐などの副作用があるが，概して軽度．国内未発売．

4．アーテメター・ルメファントリン合剤

薬剤耐性熱帯熱マラリアに有効．腹痛，下痢，頭痛，めまいなどの副作用．グレープフルーツジュースでの服用で吸収低下．国内未発売．

5．アーテスネート

アルテミシニンのコハク酸塩で，多剤耐性マラリアや重症マラリアの治療に速やかに反応．副作用は少なく，小児，妊婦にも使用できる．しかし，消失半減期が短いことから作用時間が短く，再燃率が高いので，メフロキンなどでの追加療法が有効[7]．薬物相互作用としてはCYP3A4の阻害薬．国内未発売．

6．キニーネ

経口投与には硫酸塩，時に塩酸塩，静脈内投与には二塩酸キニーネまたはグルコン酸キニーネを使用．常用量の副作用として可逆性の耳鳴，高音性難聴，視覚障害，頭痛，悪心，大量投与によりキニーネ中毒，低血糖，血圧降下．G6PD欠乏症には溶血を誘導．本剤過敏症，妊婦には禁忌．薬物相互作用としては，強心配糖体，リトナビル，ワルファリンとの併用により，その血漿濃度を高める．薬価は硫酸キニーネ1g 130.9円，塩酸キニーネ1g 152.8円．二塩酸キニーネ，グルコン酸キニーネの注射薬は国内未発売．

7．テトラサイクリン系薬剤

薬剤耐性熱帯熱マラリアに対するキニーネと併用し，キニーネの治療効果増強．単独では使用しない．また，予防内服には塩酸ドキシサイクリンの単独使用．光線過敏症，めまい，胃腸障害，菌交代症などの副作用．過敏症には禁忌，妊婦，8歳以下の小児には投与しない．ドキシサイクリンによる予防内服は12週を超えてはならない．保険適用外．

8．クロロキン

クロロキン感受性熱帯熱を含む全種マラリアの熱発作治療に有効であるが，熱帯熱マラリア原虫の生殖母体，三日熱と卵形マラリア原虫の休眠原虫には無効．比較的安全性の高い薬剤であるが，胃腸障害，羞明，瘙痒感，長期投与により視覚異常，網膜炎，錯乱などの副作用．てんかん，重症筋無力症患者には禁忌．G6PD欠乏症患者では溶血を起こす．薬物相互作用では，金剤またはフェニルブタゾンとの併用により皮膚炎，アミオダロンとの同時投与では心室性不整脈を倍加させ，ジゴキシンやシクロスポリンとの併用により血漿濃度の増加．国内未発売．

9．プリマキン

三日熱と卵形マラリアの再発阻止を図る根治療法薬であるが，熱帯熱マラリア原虫の生殖母体の駆除にも有効．時に腹痛，腹部不快感を起こす．本剤により急性溶血を誘導するG6PD欠乏症患者と妊婦には禁忌．国内未発売．

重症マラリアに対する支持療法

熱帯熱マラリアは，臨床経過中に高度虫血症，脳症，急性腎不全，ARDS/肺水腫，代謝性アシドーシス，出血傾向，循環不全，重度貧血などの重篤な合併症を発現する危険性が高い．WHOは重症マラリアの判定基準とその治療のガイドラインを策定し，随時改訂している[8]．

この重症マラリアには，上述の特異療法だけでなく，病態に応じた支持療法の強化が患者救命に不可欠である．

国内未承認抗マラリア薬の入手法

現在，国内に流通している抗マラリア薬は，従来からのキニーネ，1987年承認のファンシダール錠(スルファドキシン・ピリメタミン合剤)，2001年承認のメファキン錠(塩酸メフロキン)のみである．そこで，本文中に述べた抗マラリア薬が患者治療に必要な場合は，厚生労働科学研究費創薬等ヒューマンサイエンス総合研究事業「熱帯病治療薬研究班(略称)」(http://ims.u-tokyo.ac.jp/didai/orphan/index.html)にアクセスすれば無償供与されることを知っておくとよい．

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Antimalarial Agents: Current Perspective

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Malaria is still the most serious global protozoan disease in the tropical and subtropical regions. Additionally, the risk of malaria infection in persons traveling in the epidemic area is increasing, but is seldom known in detail. Development of malaria vaccine for preventing infection, disease, and death is urgently needed and now some vaccines are carefully in trials.

The main framework for malaria prevention and control is a larger choice of antimalarial drugs. Chemotherapy of malaria is limited by established drug resistance and back of novel targets. The drug resistance becomes a public health problem worldwide.

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Potent Plasmodicidal Activity of a Heat-Induced Reformulation of Deoxycholate-Amphotericin B (Fungizone) against *Plasmodium falciparum*

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The emergence and spread of drug-resistant *Plasmodium falciparum* continue to pose problems in malaria chemotherapy. Therefore, it is necessary to identify new antimalarial drugs and therapeutic strategies. In the present study, the activity of a heat-treated form of amphotericin B (HT-AMB) against *P. falciparum* was evaluated. The efficacy and toxicity of HT-AMB were also compared with those of the standard formulation (AMB). HT-AMB showed significant activity against a chloroquine-resistant strain (strain K-1) and a chloroquine-susceptible strain (strain FCR-3) in vitro. The 50% inhibitory concentrations of HT-AMB were 0.32 ± 0.03 μg/ml for strain K-1 and 0.33 ± 0.03 μg/ml for strain FCR-3. In the presence of 1.0 μg of HT-AMB per ml, only pyknotic parasites were observed after 24 h of incubation of early trophozoites (ring forms). However, when late trophozoites and schizonts were cultured with 1.0 μg of HT-AMB per ml, those forms multiplied to ring forms but the number of infected erythrocytes did not increase. These results indicate that HT-AMB possesses potent antiplasmodial activity and that the drug is more effective against the ring-form stage than against the late trophozoite and schizont stages. HT-AMB was observed to have little cytotoxic effect against a human liver cell line (Chang liver cells). In conclusion, the results suggest that HT-AMB has promising properties and merits further in vivo investigations as a treatment for falciparum malaria.

Malaria infection due to *Plasmodium falciparum* is a major public health problem in many tropical and subtropical areas. Sporozoites, the infective form of the parasite, are transferred to the human host during a bite by female *Anopheles* mosquitoes, invade hepatocytes, and develop into liver schizonts, which contain large numbers of merozoites. The asexual blood-stage cycle of *P. falciparum* commences when the merozoites released from hepatocytes enter the blood circulation and invade red blood cells (RBCs). In this phase of the cycle merozoites initially develop within RBCs as ring forms and then progress to trophozoites and eventually to schizonts, which rupture and release a new wave of merozoites that invade a new batch of RBCs. Chloroquine (CQ), a blood-stage schizonticidal drug, has been the drug of choice for the treatment of falciparum malaria for several decades, but its clinical utility has been greatly reduced in most areas where CQ-resistant malaria is endemic (1, 22). However, CQ remains the most widely used first-line antimalarial drug because it is well tolerated, safe for pregnant women and young children, efficacious against susceptible strains of *P. falciparum* and the other three human malaria species, and inexpensive. At present, the development of new antimalarial drugs and the use of preexisting drugs in combination are the most important approaches to overcoming the problem of drug resistance.

Amphotericin B (AMB) is a heptaene macrolide antibiotic that is active against fungi and yeasts. Fungizone, the commercially available deoxycholate salt form of AMB, is the drug that is the most widely used for the treatment of deep-seated mycotic infections. This drug is also the recommended second-line treatment for visceral leishmaniasis when conventional tetravalent antimony therapy is inappropriate or ineffective (11, 15). Unfortunately, intravenously (i.v.) administered AMB causes acute side effects, which limit its more extensive clinical use.

One approach to decreasing the toxicity of AMB has been to develop new derivatives or formulations with greater aggregation. Some investigators have reported that heat treatment of Fungizone leads to an increase in the size of aggregated AMB. Heating of AMB at 70°C for 20 min (heat-treated AMB [HT-AMB]) induces a superaggregated form that leads to a new equilibrium. This novel formulation has been associated with reduced toxicity in mammalian cells, while its antifungal activity is retained in vitro and in vivo (4, 10). This formulation is inexpensive and can be used to improve the therapeutic index of AMB against candidiasis and cryptococcosis and to encourage the more widespread use of AMB (13).

The aim of this study was to evaluate the antimalarial activity of HT-AMB against blood-stage parasites of *P. falciparum* in vitro. The efficacy and toxicity of HT-AMB were compared with those of the standard AMB formulation.

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MATERIALS AND METHODS

Cultivation of *P. falciparum*. A CQ-resistant *P. falciparum* strain, strain K-1, which was originally from Thailand, and a CQ-susceptible strain, strain FCR-3, which was originally from The Gambia, were grown asynchronously by the

modified method of Trager and Jensen (17). We used RPMI 1640 medium with glutamine supplemented with 10% human type O serum, 25 mM HEPES, 25 μg of gentamicin (Sigma-Aldrich, St. Louis, Mo.) per ml, sodium bicarbonate, and human type O RBCs in disposable sterile dishes and a controlled atmosphere of 5% CO_2–5% O_2–90% N_2 at 37°C.

Antifungal agents. AMB (injectable Fungizone) was purchased from Bristol Pharma Co. (Tokyo, Japan). A stock solution of AMB was reconstituted in sterile water according to the instructions of the manufacturer. HT-AMB was prepared by heating AMB solutions for 20 min in a water bath at 70°C, as described by Petit et al. (13).

Evaluation of in vitro plasmodicidal effect of HT-AMB. The following procedures were used to evaluate the antimalarial activities of HT-AMB and AMB. Asynchronously cultivated malaria parasites were used. RPMI 1640 medium was supplemented with 0 (control), 0.5, 1.0, 5.0, or 10.0 μg of HT-AMB or AMB per ml. The antifungal drug-supplemented medium was changed every 24 h. Five hundred microliters of a parasitized RBC (pRBC) suspension was placed in each well of a 24-well flat-bottom culture plate (Sumiron; Sumitomo Bakelite Co., Ltd., Tokyo, Japan) with a hematocrit of 5% and an initial parasitemia level of 0.1%. Thin-smear specimens stained with Giemsa solution were made every 24 h, and the level of parasitemia was determined by counting the number of parasites per 10,000 RBCs.

Determination of HT-AMB and AMB IC_{50}s. In vitro drug susceptibility tests were performed as described previously (16). Briefly, synchronous pRBCs showing a parasitemia level of 1% were placed in 24-well culture plates. Synchronization was achieved by treating the pRBCs with 5% D-sorbitol for 30 min at room temperature. Twenty microliters of drug-supplemented medium was added to each well to give a series of doubling dilutions from 0.10 to 100.00 μg/ml. After 24 h of incubation in an atmosphere of 5% CO_2–5% O_2–90% N_2 at 37°C, the control wells were checked for parasite growth. When the schizonts in the control wells were fully grown, the culture plates were removed from the incubator. Thin-smear specimens were prepared and stained with Giemsa solution. The numbers of RBCs in the control smears were counted under a microscope until 50 schizonts were encountered. The effects of the drugs on parasite growth were evaluated by the observation of decreased numbers of schizonts per equal numbers of RBCs counted previously in the control cultures. The growth inhibition effect (in percent) was calculated as follows: (test well schizont count/control well schizont count) × 100. The 50% inhibitory concentrations (IC_{50}s) of AMB and HT-AMB were calculated by the probit method.

Effect of HT-AMB on a hepatic cell line. Cells of the Chang human liver cell line were a kind gift from Takeaki Nagamine, Gunma University School of Health Sciences (Gunma, Japan). The cells were grown continuously in complete Dulbecco modified Eagle's medium (Sigma-Aldrich) supplemented with 10% fetal bovine serum in a 5% CO_2 atmosphere at 37°C. Prior to exposure to the drugs, the cells were seeded at 10^5 cells/ml in 96-well culture plates and incubated for 72 h in 0.2 ml of Dulbecco modified Eagle medium supplemented with AMB or HT-AMB. Cell death was evaluated by a lactate dehydrogenase release assay (CytoTox 96 assay kit; Promega Corp., Madison, Wis.), according to the protocol recommended by the manufacturer (5). All of the test compounds were assayed at each concentration in triplicate.

Detection of hemolysis caused by treatment with HT-AMB. The level of hemolysis was determined by measuring the amount of hemoglobin that eluted into the medium by the sodium lauryl sulfate method (hemoglobin B test; Wako Pharmaceuticals, Osaka, Japan) described previously (16). Briefly, after exposure of pRBCs or RBCs to 1.0 to 100.0 μg of HT-AMB per ml, the samples were centrifuged at 1,000 × *g* for 5 min at 20°C, and the supernatant was collected and analyzed.

Data analysis. The data are presented as the means ± standard errors of the means from at least three sets of independent experiments. Student's *t* test was used for statistical analysis. A *P* value of less than 0.05 was considered statistically significant.

RESULTS

HT-AMB inhibits *P. falciparum* growth in vitro. To confirm the plasmodicidal activities of AMB and HT-AMB, a CQ-resistant *P. falciparum* strain (strain K-1) and a CQ-susceptible strain (strain FCR-3) were exposed to medium containing 0.5 to 10.0 μg of AMB or HT-AMB per ml for 72 h, and parasite growth and multiplication were monitored (Fig. 1). AMB at concentrations equal to or greater than 1.0 μg/ml induced marked decreases in the levels of parasitemia. HT-AMB at concentrations equal to or greater than 1.0 μg/ml also induced decreases in the levels of parasitemia, but the decrease was slower than that obtained with AMB. At these concentrations, complete inhibition of parasite multiplication was attained within 72 h of incubation.

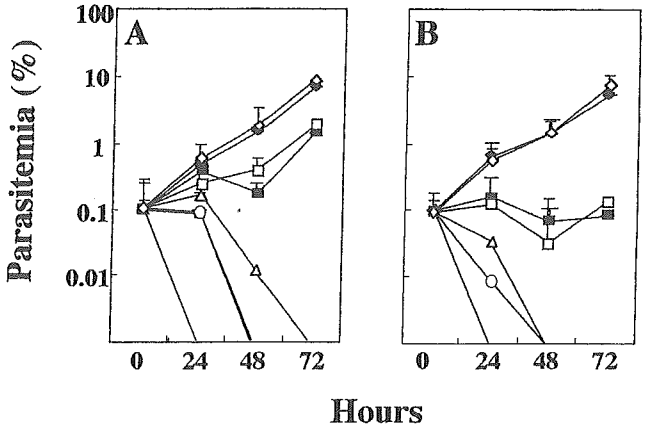

FIG. 1. Effect of HT-AMB on *P. falciparum* parasitemia in vitro. The time- and concentration-dependent effects of continuous incubation with AMB (closed symbols) or HT-AMB (open symbols) on levels of parasitemia caused by CQ-resistant strain K-1 (A) and CQ-susceptible strain FCR-3 (B) of *P. falciparum* are shown. All cultures were started with asynchronized parasites. Parasitemia was measured at the beginning of incubation (0 h) and every 24 h thereafter for 72 h. Parasites were incubated in the presence of drug at concentrations of 0 (diamonds), 0.5 (squares), 1.0 (triangles), and 5.0 (circles) μg/ml.

The results of the in vitro drug susceptibility assay were as follows. The IC_{50}s of AMB were 0.95 ± 0.10 μg/ml for K-1 and 0.89 ± 0.26 μg/ml for FCR-3. The IC_{50}s of HT-AMB were 0.32 ± 0.03 μg/ml for K-1 and 0.33 ± 0.03 μg/ml for FCR-3. The IC_{50}s of HT-AMB were threefold lower than those of AMB. There were no significant differences between the IC_{50}s for CQ-resistant strain K-1 and those for CQ-susceptible strain FCR-3 ($P > 0.05$).

HT-AMB alters *P. falciparum* morphology and interferes with parasite development. The effects of HT-AMB on the morphology and development of *P. falciparum* parasites were evaluated with synchronized cultures of the two strains (CQ-resistant strain K-1 and CQ-susceptible strain FCR-3). The effects against both strains were similar, and the parasites grew to mature stages after 24 h of incubation without HT-AMB (Fig. 2A). When 1.0 μg of HT-AMB per ml was added to synchronized ring-form parasites, pyknotic parasites inside and outside of the RBCs were observed after 24 h of incubation (Fig. 2B). When HT-AMB was added to synchronized late-stage trophozoites and schizonts in culture, parasites that had multiplied but that had altered morphologies at the ring stage were observed after 24 h of incubation (data not shown).

Effect of HT-AMB on Chang liver cells. As shown in Fig. 3, AMB at 12.5 μg/ml showed slight cytotoxicity for Chang liver cells ($P < 0.05$), and AMB at concentrations equal to and greater than 25.0 μg/ml showed strong cytotoxicity, whereas HT-AMB at the same concentrations showed no cytotoxicity ($P < 0.01$).

Hemolysis of pRBCs by HT-AMB. To detect hemolysis as an index of cytotoxicity, the concentration of hemoglobin in the

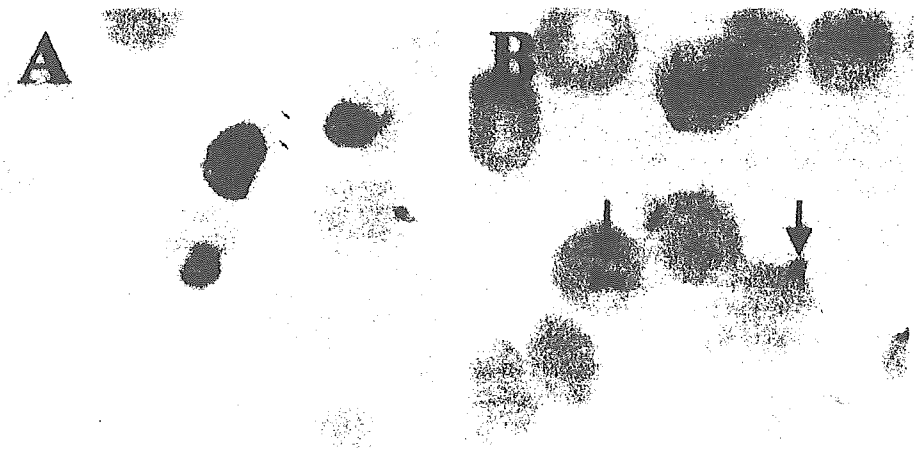

FIG. 2. Morphology of *P. falciparum* K-1 after 24 h of incubation with HT-AMB. Parasites were synchronized at the ring stage. The morphologies of cells in Giemsa-stained thin blood smears from drug-free cultures (A) and cultures incubated with 1.0 μg of HT-AMB per ml (B) for 24 h are shown. Note the parasite pyknotic changes and the prevalence of ring forms in the HT-AMB-treated culture (arrows in panel B). Magnifications, ×1,000.

pRBC or RBC culture medium was determined. In the presence of 1.0 μg of HT-AMB per ml, the concentration of hemoglobin in the pRBC culture medium was significantly higher (0.949 ± 0.192 g/dl) than that in the RBC culture medium (0.138 ± 0.048 g/dl) ($P < 0.05$). Similar results were observed in the case of AMB treatment.

DISCUSSION

Identification of the antimalarial effects of drugs that have been used for other purposes is an attractive approach to overcoming the increasing threat of drug-resistant malaria. AMB is one of the antifungal agents that is the most effective and widely used for the treatment of systemic fungal infections commonly found in immunocompromised patients. However, it can show dose-dependent renal toxicity, which is not predictable by monitoring of the serum drug concentration (19, 20). Several lipid AMB formulations have been developed to decrease this toxicity.

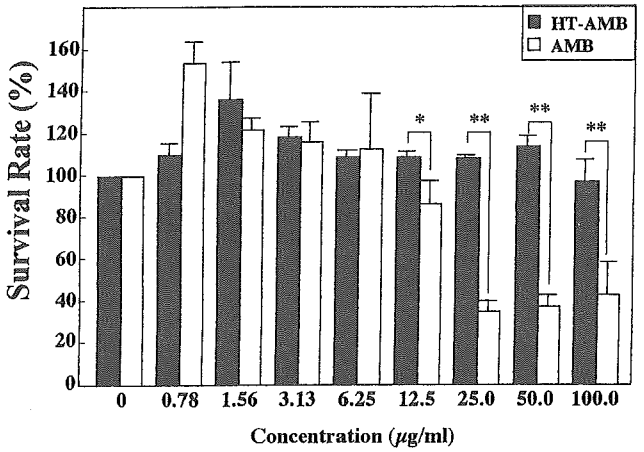

FIG. 3. Effect of HT-AMB on Chang liver cells in vitro. Chang liver cells were cultured for 72 h in the presence of HT-AMB. The viability of the cells was determined with a CytoTox 96 assay kit, which quantitatively measures the amount of lactate dehydrogenase released into the culture medium upon cell death. *, $P < 0.05$; **, $P < 0.01$.

As an inexpensive alternative, simple AMB was treated with moderate heat (70°C for 20 min) to produce a new, self-aggregated state. It has been reported that in vitro HT-AMB exhibits significantly lower levels of toxicity for mammalian renal cells and fewer hemolytic effects against RBCs than the standard formulation and that HT-AMB also shows increased toxicity for fungal cells (4, 10, 13). Our study showed that in vitro HT-AMB has a greater plasmodicidal effect than AMB against both CQ-resistant and CQ-susceptible *P. falciparum* strains. The growth curves of asynchronous parasites showed that AMB has a greater inhibitory effect than HT-AMB, but significant differences were observed only at high concentrations that would not be applicable to treatment for malaria. We confirmed that HT-AMB showed no cytotoxicity for Chang liver cells, as expected, and that HT-AMB showed much less hemolytic activity than AMB. In fact, we conducted in vivo studies of the efficacies of AMB and HT-AMB against malaria parasites using *Plasmodium berghei* NK65 and 20 female ICR/Jcl mice (age, 6 weeks), which consisted of 5 control mice, 5 mice treated with 0.5 mg/kg of body weight i.v., 5 mice treated with 1.0 mg/kg i.v., and 5 mice treated with 2.0 mg/kg i.v. However, no significant difference in parasite growth or the survival rate of the mice was observed (data not shown). AMB and HT-AMB were also not observed to have hemolytic activity. We learned from these experiments that maintenance of effective drug concentrations in the peripheral blood is very important (10), but we were not able to maintain effective drug concentrations by the administration of a single i.v. dose to mice. Further in vivo experiments are still needed before our findings on the effectiveness of AMB and HT-AMB in in vitro studies can be applied to the treatment of both drug-resistant and -susceptible human *P. falciparum* malaria.

Most antimalarial drugs, including CQ, have been reported to show schizonticidal activity in blood. One of the schizonticidal mechanisms of CQ is inhibition of heme polymerization in vitro (2). Another antimalarial drug, quinoline, also inhibits

heme polymerization (2, 7, 8, 12). In this study, pyknotic parasites were observed inside and outside of RBCs when HT-AMB was added to synchronized ring-form cultures at 1.0 μg/ml for 24 h (Fig. 2B). In contrast, when HT-AMB was added to synchronized mature-stage (late trophozoite and schizont) cultures, ring-form parasites that had multiplied successfully invaded and remained inside the RBCs for 12 to 24 h of incubation, indicating that HT-AMB has a greater hemolytic effect against pRBCs than it does against non-pRBCs.

It is generally assumed that the permeabilizing effects of AMB are related to its ability to form transmembrane channels, whereas the lytic effect is due to the peroxidative action of AMB at the membrane level (3, 14, 18). The oxidation of unsaturated fatty acids leads to a change in the membrane, which becomes more sensitive to the osmotic shock induced by channel formation. Autoxidation of AMB in solution as well as AMB-induced peroxidation of unsaturated fatty acids in the RBC membrane is assumed to be triggered by the reactive oxygen species that may be produced by AMB. It has also been reported that increased amounts of reactive oxygen species are generated during malaria infection, leading to RBC membrane damage (6, 9). This may explain the higher levels of plasmodicidal activity and hemolytic activity of HT-AMB against pRBCs, the greater effect of HT-AMB against ring forms than against late trophozoites and schizonts, and the apparently different antimalarial mechanism of HT-AMB compared with those of quinoline antimalarial drugs.

In conclusion, the results of the present study suggest that HT-AMB has promising properties and merits further in vivo investigations for the treatment of falciparum malaria.

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