症例 1

アーテミシニン誘導体による溶血性貧血が疑われた重症熱帯熱マラリアの一例

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症例：1X 歳，日本人男性

主訴：発熱，頭痛，全身倦怠感

現病歴：200X 年 2 月 17 日に大学のインターンシップでカメルーンへ渡航した．2 月 25 日から発熱と水様性下痢が出現し，現地病院を受診したところ，マラリアあるいは腸チフスの可能性があると診断され現地で入院加療（キニーネやシプロフロキサシンを投与）を受けた．その後体調は改善し，現地で活動を行った．4 月 8 日帰国時に再度発熱を認め，経由地の検疫所で上気道炎と診断され投薬を受けた．帰国後も 39℃台の発熱が持続し，頭痛も出現したため当院救急外来を受診し，入院となった．

既往歴：気管支喘息

当院受診時の内服：アジスロマイシン，メトロニダゾール，セファレキシン，アセトアミノフェン，エトリコキシブ，オメプラゾール

嗜好歴：飲酒：なし　喫煙：なし

家族歴：特記すべき事なし

海外渡航歴

200X－4 年　韓国　5 日間
200X－3 年　サイパン　5 日間
200X－2 年　ラオス　1 週間
200X－1 年　北京　10 日間

カメルーンでの生活・予防

食べ物は現地の人と同じものを食し，飲用水はミネラルウォーター，生活用水は地下水を使用していた．蚊帳は使用せず蚊などの虫には多数刺されたという．動物との接触なし，性的交渉なし．今回の渡航にあたって A 型肝炎，破傷風，黄熱病を接種．マラリアの予防内服はなし．

Review of systems

陽性：悪寒を伴う発熱，頭痛，全身倦怠感

陰性：意識障害，けいれん，麻痺，咽頭痛，鼻汁，咳嗽，喀痰，呼吸困難，胸痛，動悸，腹痛，嘔気，便秘，下痢，排尿時痛，膿尿

入院時身体所見：

身長 175cm，体重 63kg，BMI 20.6，意識清明，体温 37.8℃，脈拍数 88 回毎分，整，血圧 102/50mmHg 呼吸回数 14 回毎分，SpO_2 97%（室内気）

頭頚部：眼球結膜黄染なし，眼瞼結膜貧血なし，リンパ節腫脹なし，項部硬直なし

胸部：呼吸音：全肺野で軽度の wheeze を聴取，心音正常 S1，S2，過剰心音聴取せず

腹部：平坦で軟，圧痛なし．腸蠕動音正常．脾腫あり

皮膚：軽度黄染あり．四肢には虫刺痕が多数あり

入院時諸検査：

Fig. 1　腹部造影 CT
脾腫を認める．

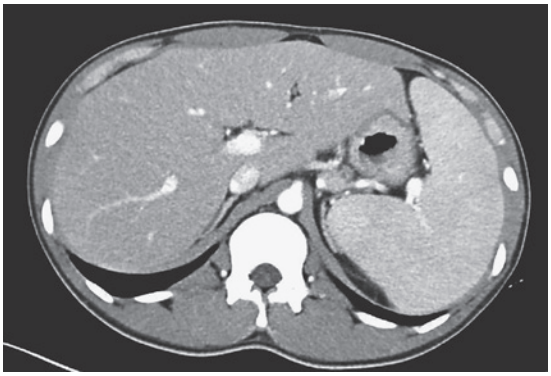


Fig. 2　末梢血塗抹標本
1 つの赤血球に複数の輪状体を認めるものもある．

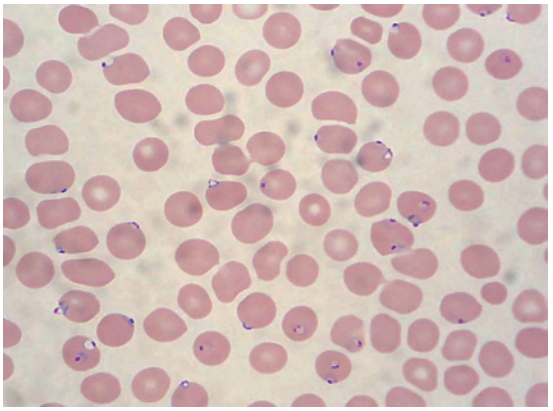
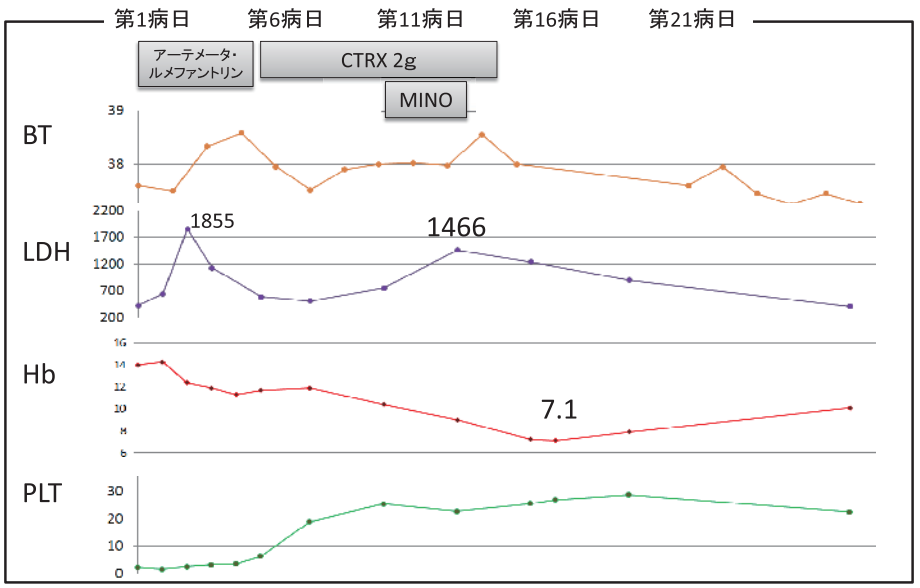


Fig. 3　入院後経過
BT：body temperature



血液検査：

末梢血：WBC 3,700/μL，（Stab 11.0 %，Seg 75.0%，Lym 12.0%，Eosin 2.0%），RBC 420×10^3/μL，Hb 14.0g/dL，Hct 39.9%，Plt 2.3×10^4/μL

止血：PT-INR 1.33，APTT 39.7sec，FDP 14.8μg/dL，D-dimer 7.9μg/mL

生化学：AST 71IU/L，ALT 62IU/L，LDH 421IU/L，ALP 416IU/L，T-Bil 2.7mg/dl，BUN 13mg/dL，Cre 0.79mg/dL，Na 137mEq/L，K 3.5mEq/L，CRP 9.0mg/dL

尿 pH 6.0，蛋白 100mg/dL，糖（－），潜血（3＋），ビリルビン（1＋）

インフルエンザ迅速検査：陰性

血液培養（2 セット）：陰性

腹部造影 CT（Fig. 1）

末梢血塗抹標本（Fig. 2）：原虫寄生率 20.6%

マラリア迅速キット（SD BIOLINE Malaria Ag P.f/Pan©）：Pf HRP-II（＋）/pan pLDH（＋）

入院後経過（Fig. 3）：

初診時に行ったマラリア迅速診断キットの結果から熱帯熱マラリアと考え，患者の状態が比較的安定していたためアーテメータ・ルメファントリン合剤を開始した．その後末梢血塗抹標本で原虫寄生率が高く重症熱帯マラリアと診断されたが，患者の状態は引き続き安定しており，また末梢血塗抹標本でも

原虫が速やかに消失したため，そのままアーテメータ・ルメファントリン合剤を継続した．

しかし第5病日になっても発熱が持続したため，血液培養は陰性であったが腸チフスやリケッチア感染症の可能性も考え，セフトリアキソン，ついでミノサイクリンを追加した．その後も発熱が持続したが，患者の状態は安定していたため，抗微生物薬はすべて中止し経過観察とした．

LDHは入院第3病日に1855IU/Lまで上昇し，一旦低下したものの入院第14病日に再度1466IU/Lまで上昇し，その後は徐々に低下した．ヘモグロビンは入院時より徐々に低下し，入院第18病日には7.1g/dLまで低下した．第14病日の血液検査で網赤血球14.6‰，間接ビリルビン2.4mg/dL，ハプトグロビン低値を認め，溶血性貧血と診断した．直接・間接クームス試験は陰性であった．

患者の自他覚所見は引き続き安定しており，その後LDHとヘモグロビンは徐々に改善傾向となり，38℃前後の発熱も第16病日まで持続したがその後は解熱傾向となり，退院，外来経過観察となった．

まとめ

重症熱帯熱マラリア患者をアーテメタ・ルメファントリンで治療し，マラリア治療が奏功したにもかかわらず発熱が遷延し，溶血性貧血が再増悪した1例を経験した．

“本症例の疑問点”から“研究的考察”へ

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1. マラリアとは？

本症例の“研究的考察”へ入る前に，マラリアについて概説する．

1）疫学

マラリアはサハラ以南アフリカ，東南アジア，南アジア，南太平洋諸島（パプアニューギニアやソロモンなど），中南米で流行している原虫疾患である．WHO[1)]は2012年に世界中で200億人以上のマラリア症例が発生し，そのうち63万人近くが死亡したと推定している．患者と死亡例の大多数はアフリカで，死亡例の77%は5才以下の小児が占めている．日本では4類感染症に分類され，最近は年間70例前後が届出されている．

2）病原体と生活環

マラリアの病原体は熱帯熱マラリア原虫（*Plasmodium falciparum*），三日熱マラリア原虫（*P. vivax*），四日熱マラリア原虫（*P. malariae*），卵形マラリア原虫（*P. ovale*）の4種とサルマラリア原虫の1種 *P. knowlesi* がある．最近，マレーシアで感染した日本人第1例目の *P. knowlesi* 感染症例が報告された[2)]．

マラリア原虫の生活環をFig. 4に示す[3)]．ヒトへの感染はハマダラカに媒介され，蚊に刺された際にスポロゾイトが体内に入り，まず血行性に肝臓へ到達して発育する（赤血球外発育）．肝細胞に侵入したスポロゾイトは分裂・発育してメロゾイトになると，肝細胞を破壊して血中の赤血球に侵入する．その後，

Fig. 4　マラリア原虫の生活環と薬剤の作用点（文献3より改変）
赤血球内発育中の原虫に作用する薬剤が治療の中心となる．ただしドキシサイクリンやクリンダマイシンは，適応外使用であることはもちろん，治療効果が出るまでに最低でも4日間かかるため第一選択にはならない．三日熱マラリアと卵形マラリアでは，根治治療にヒプノゾイトを殺滅するプリマキン投与が必要．またプリマキンは生殖母体にも作用するため，流行地では媒介蚊への伝播阻止効果を狙って熱帯熱マラリア患者に投与されることもある．アトバコン・プログアニルは赤血球内発育中の原虫だけでなく，赤血球外発育中の原虫（スポロゾイトから分裂体まで）にも作用することが特徴である．

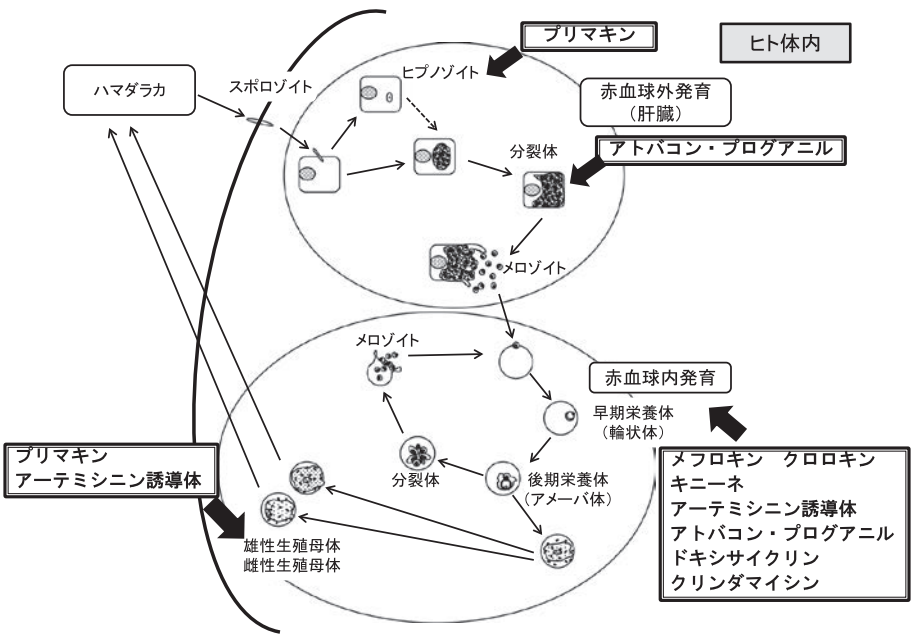

Table 1　重症熱帯熱マラリアの診断基準[4]

臨床徴候
意識障害 or 昏睡
衰弱（歩けない，手助けなしで起き上がれない）
食事がとれない
けいれん（24 時間以内に 2 回以上）
呼吸不全
循環不全・ショック
（成人；sBP ＜70mmHg, 小児；sBP ＜50mmHg）
黄疸＋臓器不全の徴候
ヘモグロビン尿
出血傾向
肺水腫（Cx-p）
検査所見
低血糖（＜40mg/dL）
代謝性アシドーシス（HCO_3 ＜15mEq/L）
重症正球性貧血（Hb ＜5g/dL）
ヘモグロビン尿
高原虫血症（＞2%；非流行地，＞5%；流行地）
高乳酸血症（5mmol/L）
腎不全（Cre ＞3.0mg/dL）

早期栄養体（輪状体）→後期栄養体（アメーバ体）→幼若分裂体→成熟分裂体となり，メロゾイトが完成すると赤血球を破壊して脱出し，新しい赤血球に侵入して同様のサイクルを繰り返す．これを赤血球内発育といいマラリアの症状に関連する．赤血球内発育を繰り返すうち，一部のメロゾイトは雄性生殖母体と雌性生殖母体になり，これらが蚊に吸われると蚊の体内で次世代のスポロゾイトが形成され，生活環が完成する．

以上の生活環はすべてのマラリア原虫に共通した経過であるが，三日熱・卵形マラリア原虫では肝細胞に侵入したスポロゾイトの一部がヒプノゾイト（肝内休眠型原虫）となり休止期に入る．ヒプノゾイトは数ヵ月後に分裂を開始し，赤内発育ステージへと移行するため再燃の原因となる．

3）症状

熱発作，貧血及び脾腫がマラリアの 3 主徴として知られているが，発症早期からすべてが揃うことはなく，患者の免疫状態（流行地で何度もマラリアに罹患している semi-immune 患者か，非流行地・旅行者の non-immune 患者か）によっても潜伏期や初発症状・自然経過が異なる．日常診療で遭遇するマラリア患者は流行地への渡航歴を持つ non-immune 患者であるが，特に熱帯熱マラリアでは脳症，急性呼吸窮迫症候群（ARDS），急性腎不全などを数日のうちに合併し，重症化・死亡することもある．Table 1 に重症マラリアの定義を示す[4]．マラリア患者の多くは発熱（＞92%）を主訴に受診し，その他，悪寒（79%），頭痛（70%），発汗（64%），めまい，倦怠感，腹痛，嘔気・嘔吐，下痢などの症状が見られる[5]．従って症状からマラリアを鑑別に挙げることは困難であり，早期診断には発熱患者に渡航歴の有無を聴取することが最も重要な第一歩である．

4）検査所見・診断

血液検査では血小板減少がもっとも多く（60%）．以下，高ビリルビン血症（40%），貧血（30%），トランスアミナーゼ高値（25%）と続く[5]が，マラリアに特異的な検査所見とは言えない．繰り返しになるが流行地への渡航歴を聴取し，潜伏期，症状，血液検査の結果からマラリアを鑑別に挙げ，確定診断のための検査を行わなければならない．マラリアの確定診断は末梢血塗抹標本でマラリア原虫を証明することである．染色に用いるギムザ液は pH を 7.2～7.4 に調整することが重要である．血液中のマラリア抗原を検出する迅速診断キットは入手可能であるが，あくまでも研究目的の使用である．原虫を検出する，種を同定する，あるいは重複感染の有無を検査するための遺伝子検査は専門施設に依頼する．

5）治療

マラリアの治療薬は原虫の種，感染地域の薬剤耐性，重症度を考慮して選択する．原虫の赤血球内発育が症状に関連しており，このステージに作用する薬剤で治療を開始する（Fig. 4）．WHO のガイドライン[4]では，アーテミシニン誘導体を key drug とし作用機序が異なる他の抗マラリア薬を併用する Artemisinin-based combination therapy（ACT）が熱帯熱マラリアの推奨治療である．非熱帯熱マラリアでは薬剤耐性がなければクロロキンが第一選択薬，その後，三日熱マラリアと卵形マラリアではヒプノゾイトを殺滅するためにプリマキンを投与する（根治療法）．しかし日本で承認されている抗マラリア薬はキニーネ末，メフロキン，アトバコン・プログアニル合剤の 3 剤だけである．承認薬以外の治療に必須の薬剤は，平成 25 年度厚生労働科学研究費補助金（医療技術実用化総合研究事業）「我が国における熱帯病・寄生虫症の最適な診断治療体制の構築」班（略称：熱帯病治療薬研究班）の薬剤保管機関が保管している．Table 2 に日本で使用できる薬剤を用いたマラリア治療例を示す．研究班，保管機関・連絡先，保管薬剤についてはホームページを参照の

Table 2　日本で使用できる薬剤を用いたマラリア治療例

熱帯熱マラリア（合併症なし）
①メフロキン ②アトバコン/プログアニル合剤 ③キニーネ末＋ドキシサイクリン[a] またはクリンダマイシン[a] ④アーテメーター/ルメファントリン合剤[b]
熱帯熱マラリア　重症
①グルコン酸キニーネ注射薬[b] 最終投与後 12 時間以上経過してからメフロキンを投与.
非熱帯熱マラリア
急性期治療 ①メフロキン ②リン酸クロロキン[b] 上記に加え，三日熱マラリア・卵形マラリアでは根治療法として ①リン酸プリマキン[b]

a：適応外使用，b：熱帯病治療薬研究班保管薬剤

こと（http://trop-parasit.jp/index.html，http://www.nettai.org）.

2. マラリアでみられる貧血の種類と機序は？

マラリア患者の検査所見で貧血に関連するものを列挙してみると，正球性正色素性貧血，網赤血球の低下，間接ビリルビン優位のビリルビン上昇，LDH 上昇，ビリルビン尿，脾腫であり，すなわち溶血性貧血である．その発症機序はメロゾイト放出時，脾機能亢進，マクロファージ活性化による原虫感染赤血球のクリアランス，原虫に感染していない赤血球のクリアランス，薬剤性あるいは G6PD 欠損による血管内溶血が報告されている[6]. さらにマラリア原虫感染により誘導されるサイトカイン，原虫産生物質により赤血球生成が抑制されること，骨髄で赤血球前駆細胞数の減少あるいは形態異常により異常赤血球が産生されることもマラリアに伴う重症貧血の発症機序と考えられている[6].

3. 本症例で見られた溶血性貧血の原因は？

マラリア患者の溶血性貧血の経過は，Camacho LH[7]らが報告している．これは重症熱帯熱マラリア患者をキニーネ＋テトラサイクリン，アーテスネートのみ，アーテスネート＋メフロキンで治療した群と非重症熱帯熱マラリア患者をアーテスネートのみ，メフロキンのみ，アーテスネート＋メフロキンで治療した群でヘモグロビン値，間接ビリルビン値などの推移を 7 日ごとに 28 日間観察したものである．その結果，どちらの群でもヘモグロビン値は治療後 7 日目が最低値で，重症患者群の方がその程度が強くその後は回復した．間接ビリルビン値は治療開始時が最も高値で重症患者群の方がその程度が高く，治療 7 日目には両群とも正常化していた．おそらくこれが典型的なマラリア患者の溶血性貧血の経過と思われるが，本症例ではそのような経過を取らず，治療開始後 14 日目に LDH の再上昇が見られ，18 日目にヘモグロビンが最低値となっており，マラリア治療後の自然経過とは考えにくい.

最近，マラリア治療後に見られる遅発性溶血性貧血の報告が相次いでおり，自験例を含め 23 例にのぼっている[8)~11)]．これらの症例はすべて重症熱帯熱マラリア患者であること，アーテスネート静注薬あるいはアーテメタ・ルメファントリンというアーテミシニン誘導体を含む治療レジメンを用いていることからアーテミシニン誘導体が遅発性溶血性貧血に関連していることが示唆される．興味深いことにアメリカから同様の症例は報告されていない．アメリカで用いられるアーテスネートはアメリカ国内で製造されていることから，他の地域で用いられている製剤との違いがあるのではないかと推測されている[8]. アーテミシニン誘導体は最高血中濃度に達するまでの時間と半減期が他の抗マラリア薬に比べて非常に短いことが特徴である．遅発性溶血性貧血を起こす時期が治療開始から数週間後であり，薬剤が直接赤血球に作用したとは考えにくい．実際に本患者の赤血球とアーテメタおよびルメファントリン溶液を反応させたが，溶血は起こらなかった．薬剤誘導性免疫性溶血性貧血や薬剤関連自己免疫性溶血性貧

血の可能性を考え，アーテメタ，ルメファントリンでそれぞれ処理した赤血球を患者血清と反応させたが，やはり溶血は起こらなかった．また本症例の経過中に投与したセフトリアキソン，ミノサイクリンは薬剤誘導性溶血性貧血の原因薬剤となる[12)13)]．抗マラリア薬以外の関与も検討しなければならない．

4. 今後の検討は？

第一に重症熱帯熱マラリア患者をアーテミシニン誘導体で治療した場合には，遅発性溶血性貧血が起こる可能性があることを念頭に経過観察するのが重要である．アーテミシニン誘導体関連の遅発性溶血性貧血が起きたら，患者検体を用いた検索がいつでもできるよう体制を整えておくことが理想的だが，症例数が少なく困難であろう．例えば *P. chaboudi* というマラリア原虫は熱帯熱マラリア原虫と同じく，マウスのすべてのステージの赤血球に感染する[6)]．本症例を動物モデルで再現することを試みてみたい．

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REVIEW ARTICLE

Artemisinin-based combination therapies and their introduction in Japan

Shigeyuki Kano

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Abstract Artemisinin was discovered in 1971 from a herb, *Artemisia annua*, which had been used for more than 2,000 years in China against intermittent fever. Now, the artemisinin and its derivatives have become essential components of artemisinin-based combination therapies (ACTs). The ACTs are the recommended first-line treatments of malaria because they are effective against all four human malarias, produce rapid parasite/fever clearance, and show fewer adverse effects. Some ACTs are particularly important in cases of severe and complicated falciparum malaria, including cerebral malaria. However, neither the artemisinin and its derivatives nor any ACTs are registered in Japan. Indeed, the only licensed drugs for the treatment of malaria in Japan are quinine, mefloquine, and sulfadoxine/pyrimethamine. Although indigenous malaria has been eradicated in Japan since 1959, 60–100 imported malaria cases have been reported annually for the past decade. Some of the patients were, in fact, dying of the severe complications. Thus, the introduction of the ACTs and their application to imported malaria patients in Japan are urgently needed. A few clinical studies using the ACTs have been reported in Japan. The first application of an ACT, intramuscular artemether plus mefloquine, was reported in 1988 to be very effective against cerebral malaria with coma. Five cases with intravenous artesunate plus mefloquine were reported through 2001–2007, for severe or drug-resistant falciparum cases, resulting in successful treatment with some side effects such as hemolytic anemia or postmalaria neurological syndrome. Currently, a fixed-dose ACT, artemether–lumefantrine, is prescribed successfully for uncomplicated falciparum cases, with a limited number of recrudescences.

Keywords Malaria · Artemisinin-based combination therapy (ACT) · Artemether · Artesunate · Dihydroartemisinin (DHA) · Drug resistance

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Introduction

Indigenous malaria has been eradicated since 1959 in Japan, and it is no longer a health threat for those who are residing within the Japanese islands. However, there are around 243 million people in the world who are reported to be contracting malaria, and more than 863,000 people are estimated to be dying from it [1]. In fact, expansion of the areas where multi-drug-resistant malaria prevails is a big issue for endemic people as well as those who are traveling to those areas.

About 16 million Japanese people go abroad in a year, and many of them are under risk of contracting malaria in the endemic areas. As it is, 60–100 imported malaria patients have been reported annually for the past decade in Japan, and some of them unfortunately died. One of the reasons why the imported malaria patients are not properly treated in Japan is the limited number of registered antimalarial drugs: only quinine, mefloquine, and sulfadoxine/pyrimethamine. That is to say, those travelers who might be infected in multi-drug-resistant malaria endemic regions such as the Thai–Myanmar or Thai–Cambodian borders cannot be cured effectively if they become ill after coming back to Japan (Fig. 1). Indeed, severe malaria patients who could be complicated with cerebral malaria or high parasitemia cannot rescued in Japan in any way.

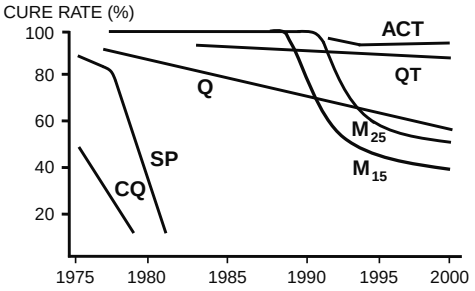


Fig. 1 Trends of drug resistance in Thai–Myanmar border. Yearly cure rate of falciparum malaria by antimalarial drugs such as chloroquine (*CQ*), sulfadoxine/pyrimethamine (*SP*), quinine (*Q*), mefloquine (*M*: M_{15}, 15 mg/body weight; M_{25}, 25 mg/body weight), quinine plus tetracycline (*QT*), and artemisinin-based combination therapy (*ACT*), which have been monitored at Faculty of Tropical Medicine, Mahidol University, Thailand (with compliments to the late Prof. S. Looareesuwan)

The selection of a new and/or alternative antimalarial medicine for use at the public health level within the context of national treatment guidelines in the endemic countries should be based on an average cure rate of >95%, as assessed in clinical trials [2]. Although there are no official treatment guidelines or any precise statistical records on drug-resistant cases in Japan, as it is not an endemic country, a change of an antimalarial medicine recommended in the malaria treatment policy should be initiated because the proportion of treatment failure is presumed to be increasing.

Artemisinin and its derivatives are now widely used as part of artemisinin-based combination therapies (ACTs) [3], not only in endemic countries but also in non-endemic developed countries. These drugs produce rapid parasite clearance and fever clearance, are clinically important, particularly in cases of cerebral and severe malaria [4], show fewer adverse effects, and have not been reported to produce significant resistance [5], Thus, introduction of the ACTs and their application to imported malaria patients in Japan are strongly needed. The importance of the ACTs in the context of travel medicine is discussed in this review article with summaries of reported cases treated with ACTs in Japan.

Artemisia in Chinese materia medica

Artemisinin (qinghaosu 青蒿素) is found from the plant sweet wormwood (*Artemisia annua*, *huang hua hao* 黄花蒿/*qing hao* 青蒿) (Fig. 2), which is believed to have been used for more than 2,000 years as a Chinese herbal medicine. It was first described in medical history by Ge Hong to recommend the herb extract for the treatment of "intermittent fever," which could be caused by malaria [6].

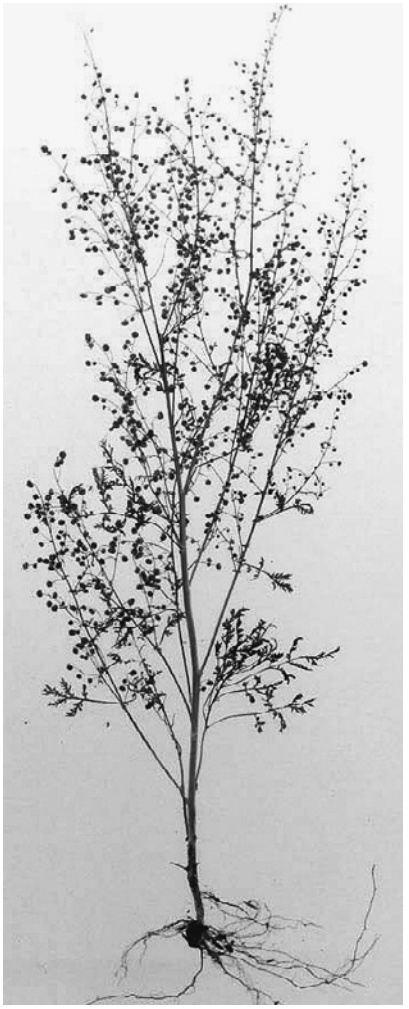


Fig. 2 *Artemisia annua*

He advised soaking the herb in water, and then wringing it out and ingesting the juice in its entirety (Fig. 3).

Late in the Tang Dynasty (618–907), a very interesting alternative therapeutic usage of *Artemisia* was recorded. In fact, they were soaking the entire plant in urine rather than water [4]. It could have been a very good way in case of emergency without fear of contamination from water-borne microbes, or the urine itself could have optimized the extraction of the effective substance, *Artemisia* sesquiterpenes, from the herb. *Artemisia* is also recommended to be used for acute convulsions or, interestingly, in case of contact with a dead body or possession by evil spirits [7]. We could imagine that those psychiatric disorders might be caused by complications from cerebral malaria, concluding that *Artemisia* may have been directly or indirectly useful because of its antimalarial effects.

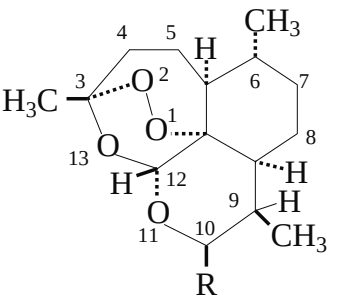
Fig. 3 First description of artemisinin for malaria in medical history [6] (with compliments to the late Prof. M. Aikawa)

Artemisinin and its derivatives

Artemisinin was discovered by a Chinese chemist in 1971, being extracted from *Artemisia annua* in ethyl ether, and was known practically to the world in 1979 [8]. It has a very complicated chemical structure with 15-carbon peroxide, sesquiterpene lactone, without a nitrogen-containing heterocyclic ring system (Fig. 4). In the early 1980s, it was soon reported that artemisinin was effective for both uncomplicated and severe malaria [9, 10]. Then, the parent drug artemisinin was replaced by dihydroartemisinin (DHA) and its water-soluble derivative, artesunate, and the oil-based formulation of artemether, which have greater antimalarial activity.

Artemisinin and its derivatives are effective against every stage of the asexual and sexual parasites in the erythrocytes, but do not affect the hypnozoite of *Plasmodium vivax* and *Plasmodium ovale*. The mechanism of action of the artemisinins is attributed to their endoperoxide bridge (see Fig. 4) [11, 12], and the mode of action is likely to be ion-dependent alkylation [13]. The primary target of *P. falciparum* is proposed to be the sarcoplasmic endoplasmic reticulum calcium adenosine triphosphatase (PfATPase 6) [14, 15]. However, these pharmacological properties still remain uncertain.

Peak plasma concentrations of artemisinin, artesunate, and artemether occur around 3–11, 0.5–1.5, and 2–3 h, respectively. Biotransformation is mediated via the cytochrome P450 enzyme CYP2B6 or CYP3A4. Elimination half-life of the three compounds is approximately 1 h, 45 min, and 1 h, respectively [16, 17].



R=O	Artemisinin
O H	Dihydroartemisinin
O CH_3	Artemether
O $CO(CH_2)_2COONa$	Sodiumartesunate

Fig. 4 Chemical structures of artemisinin and its main derivatives

Safety and tolerability has been well documented with artemisinin and its derivatives [18]. The only potential adverse effect was reported to be hypersensitivity reactions in approximately 1 in 3,000 patients [19]. Experimental neurotoxicity was reported in animal studies with high-dose administration with artemether, but this effect has not been substantiated in humans [20]. The one unresolved concern is in the safety of their use in the first trimester of pregnancy [21].

In the treatment of severe malaria, artemisinin and its derivatives have been used effectively. They reduce considerably the number of parasites that mature to schizonts to sequester in the capillaries, blocking the blood circulation [22, 23]. This effectiveness is directly connected to the life-saving benefit as compared to quinine [24]. Randomized trials comparing artesunate and quinine from South East Asia showed clear evidence of the benefit: mortality was reduced from 22% to 15% [25]. Now, the World Health Organization (WHO) recommends that artesunate is the treatment of choice for adults with severe malaria. Particularly, intravenous artesunate has been shown to significantly reduce the risk of death from severe malaria as compared to intravenous quinine with high-quality evidence [26].

Practical aspects of ACTs

Antimalarial combination therapy has its rationale in that the simultaneous use of two or more blood schizonticidal drugs with independent mode of action and different targets in the parasites is more effective, and the combination is expect to exert mutual protection preventing or delaying the emergence of parasite resistance against each drug. ACTs are the ways of administering drug combinations in

which one of the components is artemisinin and its derivatives (e.g., artesunate, artemether, DHA).

If artemisinins are given alone, 7-day regimens are required to maximize their cure rate because they are eliminated very rapidly. This long duration of treatment with the artemisinins can be reduced to 3 days if the combination partner in ACTs is a slowly eliminated antimalarial [27]. The complete clearance of all parasites is dependent on the partner medicine persisting effectively in the patient blood until the infecting parasites have been eliminated. In addition, this contributes to the protection from emergence of parasite resistance against the artemisinin component.

The first ACT to be evaluated was artesunate plus mefloquine [10, 28], because mefloquine has a long elimination half-life of around 21 days for healthy individuals and around 14 days for malaria patients. As it is, mefloquine can work synergistically with artesunate. The combination proved quite promising resolution on the Thai–Myanmar border where even mefloquine-resistant falciparum malaria had been reported [29–31]. This combination was also regarded to affect sexual-stage parasites, blocking the transmission of gametocytes, but a more effective gametocidal drug, e.g., primaquine, should be combined with this regiment to be useful [32]. Now, this ACT is usually available in endemic countries as blister packs with separate scored tablets containing 50 mg artesunate and 250 mg base mefloquine, respectively. A fixed-dose formulation of artesunate and mefloquine is at an advanced stage of development.

Practically, the first fixed-dose ACT was artemether–lumefantrine (Coartem®/Riamet®): a tablet containing 20 mg artemether and 120 mg lumefantrine. An advantage of this combination is that lumefantrine is not available as monotherapy, and it has never been used alone for the treatment of malaria [33]. It is absorbed and eliminated more slowly than artemether, with a terminal elimination half-life of 3–7 days [34]. Since Coartem® first received international licensing approval in 1999, it has been registered for use in almost 90 countries [35]. The efficacy and safety of its six-dose regimen were reported for treatment of uncomplicated *P. falciparum* malaria in adolescents and adults [36], in children and infants [37], and in non-immune populations [38]. Coartem® has been included in the WHO Model List of Essential Medicines since 2002 and has been approved by the Food and Drug Administration in the United States since 2009 [35]. Now, a formulation with improved palatability (Coartem® Dispersible) has been developed particularly for children that rapidly disperses in a small amount of water for ease of administration [39]. Meta-analysis of 32 comparative trials showed artemether–lumefantrine to be one of the most effective ACTs currently available and may help to limit the spread of resistance [40].

A relatively new ACT, dihydroartemisinin–piperaquine, is currently available as a fixed-dose combination (Artekin®) with tablets containing 40 mg DHA and 320 mg piperaquine. Oral DHA is the main active metabolite of the artemisinin derivatives and is rapidly absorbed from the gastrointestinal tract with marked interindividual variation, but the drug is usually detectable in plasma within 15 min of dosing and disappears thereafter from the systemic circulation within 3–8 h with elimination half-life of approximately 45 min [21, 41]. Artekin® was reported to be as effective and well tolerated as artesunate-mefloquine and can be used alternatively for the treatment of multi-drug-resistant *P. falciparum* malaria [42–44].

Artesunate plus amodiaquine (AQ) is sufficiently efficacious, whereas 28-day cure rates with AQ monotherapy only exceeded 80% [33]. AQ hydrochloride is readily absorbed from the gastrointestinal tract and converted in the liver to the active metabolite desethylamodiaquine. Data on the terminal plasma elimination half-life of desethylamodiaquine are not well known, but it is detected in the urine several months after its administration [21]. Now, Sanofi-Aventis and DND*i* have produced Artesunate Amodiaquine Winthrop® (ASAQ), a fixed-dose combination with a soluble formulation, specifically designed for children, to be granted a "prequalified" status by WHO.

Finally, an alternative short-course ACT for acute uncomplicated *P. falciparum* malaria was recently suggested with a fixed-dose combination of artemisinin–piperaquine–primaquine, Artequick®, with tablets containing 80:400:4 mg, respectively. The 28-day cure rate of the combination is 98.5%, and it is well tolerated. It could be used as an alternative treatment for multi-drug-resistant *P. falciparum* malaria in Southeast Asia [45].

Case reports and clinical research in Japan

As artemisinin and its derivatives are not officially licensed for the treatment of malaria or registered in Japan, management of drug-resistant malaria or severe/complicated malaria is quite difficult in Japanese medical settings. A research group, under a grant from the Ministry of Health, Labour and Welfare of Japan concerning appropriate preservation of orphan drugs and their application in Japan, has been importing and preserving those artemisinins for emergency therapy [46]. The artemisinins prepared for use are artesunate suppositories (Plasmotrim Rectocaps®; Mepha), and artemether–lumefantrine (Riamet®; Novartis). A few imported malaria cases treated with artemisinins have been reported by a limited number of researchers in Japan.

The first reported case with the ACT in Japan was a severe malaria patient successfully treated with intramuscular

Fig. 5 Intramuscular artemether in early days, 1980s [47]

artemether (Kunming Pharmaceutical Factory, Yunnan, China) (Fig. 5) [47]. A Japanese 28-year-old man was suffering from cerebral malaria with coma and peripheral blood parasitemia of 17% *P. falciparum*. He contracted malaria while he was traveling in several African countries in 1987–1988 and was finally hospitalized in Tokyo 10 days after he first manifested fever. Two hundred milligrams of artemether was administered as the first dose intramuscularly, followed by 100 mg/dose at intervals of 12 h, to a total of 1,000 mg. He recovered from his coma the following morning, and his consciousness became clear in 2 days. However, parasite clearance time (PCT) was 66 h and fever clearance time (FCT) was 124 h. Chloroquine, a total dose of 1,500 mg, followed after the in vitro chloroquine susceptibility test had showed its effectiveness. The artemether was proved to be effective and well tolerated, saving the life of the patient, but the total dose indicated in the instructions for use was much higher than is recommended now.

A case of recrudescence was reported in 1994 in Japan, after intramuscular artesunate monotherapy (60 mg/day for 5 days) was performed in Kenya [48]. *P. falciparum* was observed again after a 15-day interval. The recrudescence rate of artesunate monotherapy was reported to be 41.2–100% with a 3-day or shorter course and 0–28% with a 5-day or longer course [28, 49, 50]. Thus, the desperate need for combination therapies has been discussed and, as described above, effective ACTs are currently available. This Japanese patient was finally treated successfully with quinine hydrochloride plus minocycline for 7 days.

Intravenous artesunate (Artesunate for Injection®; Guilin Pharmaceutical No. 2 Factory, Guanxi, China) was used for the first time in Japan in 2000 against an imported *P. falciparum* malaria case of a 47-year-old Nigerian male whose parasitemia increased from 0.03% to 6.66% 1 day after mefloquine treatment [51]. His signs and symptoms were becoming sufficiently worse, with fever rising to 40°C, to be diagnosed as severe malaria according to WHO criteria [52]. Thus, 120 mg artesunate was initially administered intravenously, followed by 60 mg each at 24 and 48 h. Parasites were cleared within 20 h of the first administration of artesunate, but his fever persisted for 7 days. Worsening of hemolytic anemia was observed (RBC 210 × 10^4/μl, Hb 6.6 g/dl, T. bil 2.2 mg/dl, LDH 3,621 U/l) until red blood cell (RBC) transfusion was performed 3 days after the artesunate administration. No other partner drug was added because the in vitro test showed susceptibility of the parasites to mefloquine.

Four more falciparum cases treated with intravenous artesunate plus mefloquine were reported in Japan. (1) A 28-year-old Japanese woman coming back from India in 2001 showed 11% parasitemia. Her PCT was 24 h and FCT was 108 h. Her anemia worsened once after the artesunate administration [53]. (2) A 68-year-old Japanese woman coming back from Tanzania in 2001 showed 34.1% parasitemia, which once increased up to 45%, but all the parasites were dying within 24 h of the first administration of intravenous artesunate. However, her fever persisted on day 11 when severe hemolytic anemia and jaundice were observed. Blood transfusion was consequently required [54]. (3) A 54-year-old Japanese man coming back from several African countries in 2005 showed 10% parasitemia. His PCT and FCT of intravenous artesunate were both 24 h. However, hemolytic anemia started on day 10 and worsened until day 15 (Hb 4.4 g/dl, LDH 1,483 U/l) when RBC transfusion (12 U) was performed [55]. Finally, he successfully recovered from his severe malaria and was discharged on hospital day 36. Then, 3 weeks after discharge, he was readmitted to the hospital because of incoherent speech and markedly disturbed and uncooperative behavior without parasitemia. He was then diagnosed as postmalaria neurological syndrome [56]. (4) A 43-year-old Sudanese woman visiting Japan in 2006 showed 5.0% parasitemia with unconsciousness. Her PCT was 33 h and FCT was 26 h. Intravenous artesunate only was administered on admission and 12 h later, at a dose of 120 and 60 mg, respectively. No adverse effect was observed [57].

The first application of a fixed-dose ACT, artemether–lumefantrine (Coartem®), was reported in 2003 in Japan [58], to a 42-year-old Japanese woman who came back from Ghana in 2002, manifesting high fever with *P. falciparum* parasites at 0.27% parasitemia, but was not successfully treated with mefloquine alone. Thus, the treatment followed by Coartem® was with a six-dose regimen, four tablets (artemether 20 mg plus lumefantrine 120 mg/tablet) each at 0, 8, 24, 36, 48, and 60 h, which dosages are advised for multi-drug-resistant malaria. Eventually, the patient showed a good clinical course without any side effect.

Several cases with uncomplicated *P. falciparum* malaria have been treated with artemether–lumefantrine (Riamet®) successfully in Toyama Hospital, National Center for

Global Health and Medicine, Tokyo. However, the first case of treatment failure of imported malaria occurred in a 58-year-old Japanese man who showed recrudescence of *P. falciparum* after treatment with the ACT [59]. The recrudescence, which occurred 21 days after initial admission, may have been caused by the poor absorption of lumefantrine that resulted from not taking some fatty food concurrently with the drug. The patient was finally treated successfully with atovaquone–proguanil (Malarone®), another combination therapy.

Conclusions

The WHO states that malaria control in the world will be falling back to 10 years ago if drug resistance against the ACTs should emerge and spread widely. Proper monitoring and surveillance leading to appropriate containment measures of their resistance are urgently needed [60].

The ACTs are now first-line drugs for uncomplicated falciparum malaria, and millions of cases are being successfully treated in the world. Some ACTs are particularly effective for life-threatening severe and complicated malaria, including cerebral malaria. However, in Japan, only a limited number of patients have been treated with the ACTs, and some doctors are still suspicious of their application. Indeed, the Ministry of Health, Labour and Welfare of Japan has never tried to register those ACTs because a sufficient number of clinical trials have not been performed on "Japanese" patients. Pharmaceutical companies will never be interested in introducing the ACTs into Japan because no large profit will be expected for only 60–100 imported malaria cases per year.

However, some patients are suffering from drug-resistant malaria or, in fact, from severe malaria that cannot be successfully treated with those antimalarials registered in Japan. In this review, the efficacy of artemisinin and its derivatives, particularly of ACTs, is emphasized in the treatment of imported malaria patients in Japanese medical settings.

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