4 抗原虫薬

マラリア

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はじめに

国内でマラリアを経験することは多いとはいえず，そのため診断や治療に関する準備ができていない医療機関も多い．しかしながら海外旅行の増加とともに，マラリア患者が，いつどの医療機関を受診するとも限らない．また，熱帯地域への旅行を予定している者からマラリア予防薬の相談を受けることも次第に増えるであろう．本稿は，マラリアおよび抗マラリア薬の原則的なことを確実に理解していただき，臨床的対応が適切に行われるようになることを主眼とするが，興味ある人のために新しい知見も述べることとする．

1 マラリアとその疫学

●概　説

マラリアはハマダラカ属の蚊により媒介される疾患であり，病原体は原虫に属する．ヒトのマラリアとしては熱帯熱マラリア（病原体は*Plasmodium falciparum*），三日熱マラリア（*P. vivax*），卵形マラリア（*P. ovale*），四日熱マラリア（*P. malariae*）の4種類がある．蚊の刺咬により体内に入ったマラリア原虫は肝細胞に侵入し（一次肝臓内ステージ），その後肝細胞内で分裂・増殖すると血中に放出され，赤血球内に侵入して分裂・増殖を繰り返す（赤血球内サイクル）．発熱その他の症状は，原虫が赤血球内に侵入してから生じるものである．三日熱，卵形マラリアではこれ以外に肝細胞内に長期間潜む原虫（休眠原虫）が形成され，再発の原因となる（図1）．

臨床的には，熱帯熱マラリアは治療開始が遅れると脳症，肺水腫/ARDS，腎症，重症貧血，出血傾向，低血糖，代謝性アシドーシスなどを生じて重症化，死亡の危険があり（重症マラリア），マラリアの中で最も重視すべきものである．

●世界的な状況

世界保健機関（WHO）によると，世界中での年間のマラリア罹患者は3～5億人，死亡者は150～270万人と推定されている．それらの大部分はサハラ以南アフリカで発生しており，死亡者についてはその地域の5歳未満の小児が9割以上を占めるとされている．

サハラ以南アフリカではほとんどが熱帯熱マラリアであるが，ときに卵形，四日熱マラリアもみられる．アジアでは全体的に三日熱マラリアが多く，特に中東ではマラリアのほとんどすべてを占める．しかし，タイ・ミャンマーあるいはタイ・カンボジア国境，中国の南部（特に雲南省，海南省），フィリピンのパラワン島その他，インドネシアのロンボク島およびそれ以東などでは熱帯熱マラリアも多くみられる．南太平洋諸島（パプアニューギニア，ソロモン，バヌアツ）では熱帯熱

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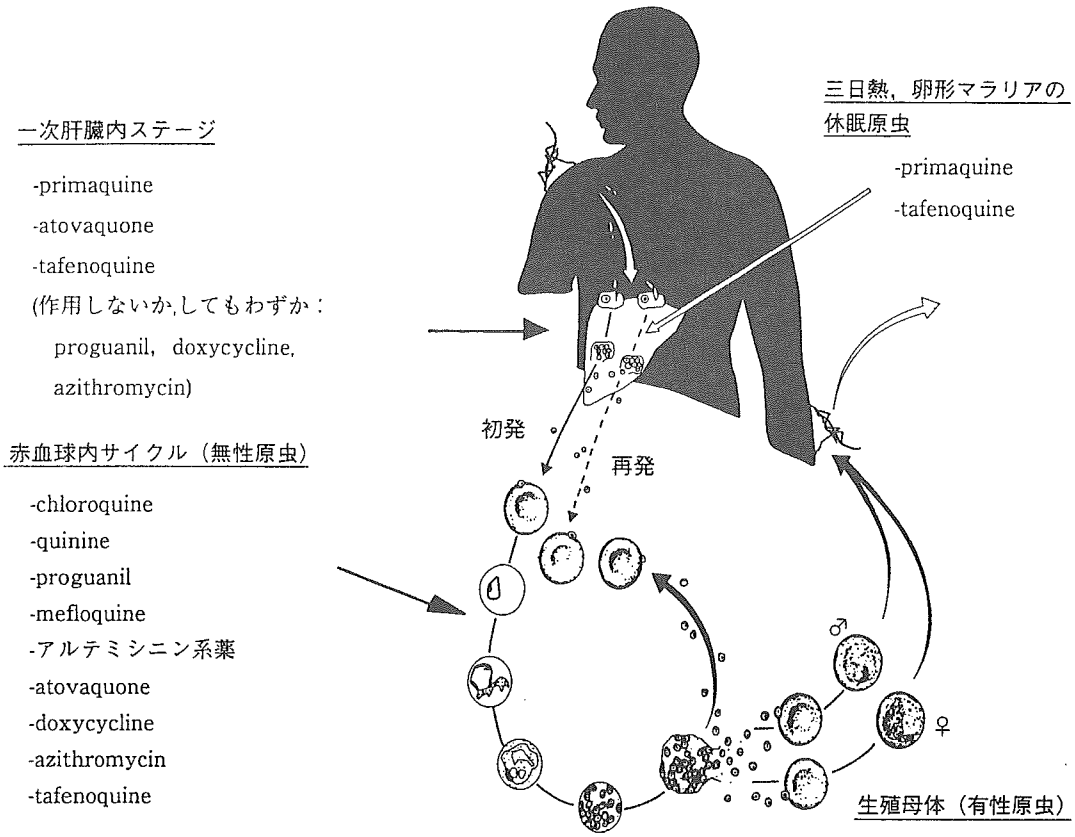

図1　抗マラリア薬が作用するマラリア原虫のステージ
文献1)より改変

マラリアと三日熱マラリアが多くみられる．中米ではほとんどが三日熱マラリアであるが，南米では特にアマゾン流域などで熱帯熱マラリアが多くみられる．

上記の地域をさらに細かくみるとマラリアの発生がみられない地域もあり，アジアではバンコク，シンガポール，ジャカルタ，クアラルンプール，マニラなどの首都，チェンマイ，パタヤ，プーケット島，バリ島，ペナン島などの観光地があげられる．これに対してサハラ以南アフリカの首都では，アディスアベバ，ナイロビ中心部などを除いては例外なくマラリアの発生がみられる．

●旅行者の状況

世界全体で年間約3万人の旅行者がマラリアに罹患していると推定され，実際にヨーロッパ全体では1万例近くが報告されている．アジアと比べてアフリカへの旅行者では罹患率が格段に高く，日本人旅行者の解析でも100倍以上を示している（加來私信）．パプアニューギニア，ソロモンではサハラ以南アフリカよりもマラリア罹患率は高いが，熱帯熱マラリアに限ると西アフリカの方が高くなる．西アフリカに続いて東アフリカでの罹患率が高いが，インド亜大陸（旅行者ではほとんどが三日熱マラリア）ではサハラ以南アフリカの20～30分の1程度となり，アジアの他の地域，南米，中米ではさらに低くなる．

日本人旅行者では，帰国後国内で発症する例が年間100例前後あるが，他に海外で医務官がほぼ同数を把握している．さらに，海外で発症しても医務官を受診しない例が相当数あると推測される．

❷国内における抗マラリア薬

本稿では国際的な視野で抗マラリア薬の解説を行うが，わが国で流通している薬剤は非常に限られている．それらは経口 quinine，sulfadoxine/pyrimethamine 合剤（Fansidar），mefloquine（わが国では Mephaquin）の 3 種類であり，mefloquine のみは予防（ただし健康保険の適応外）と治療の両者に承認されているが，他の 2 種類は治療のみである．それを補うべく「熱帯病に対するオーファンドラッグ開発研究」班（主任研究者:名和行文）が他の抗マラリア薬を国内導入し，22 カ所の医療機関に配備している[2]．詳細はホームページを参照されたい(http://www.ims.u-tokyo.ac.jp/didai/orphan/index.html)．

❸抗マラリア薬各論

ここでは種々の抗マラリア薬を分類して解説するが（表 1），それらがマラリア原虫のどのステージに作用するかも示す（図 1）．

●キノリン系および類似薬

この系統の薬剤は抗マラリア薬として主流の座を占めてきている．

Quinine，quinidine はシンコナアルカロイドに属する．前者の製剤としては二塩酸塩，硫酸塩，グルコン酸塩などさまざまであり，従来塩の量で用量を示してきたが，特に注射製剤の場合には塩基での表示に変わりつつある．Quinidine は quinine の立体異性体であり，抗マラリア作用は quinine より強いが，副作用として心毒性も強いのでほとんど使われない．しかし，米国では quinine 注射薬が認可されていないので，重症マラリアで使用されている．4-アミノキノリン系薬として chloroquine，amodiaquine があるが，これらは通常塩基の量で用量が示される．Amodiaquine は副作用のために先進国ではほとんど用いられない．Mefloquine はフッ素化 4-キノリンメタノール，halofantrine は 9-フェナントレンメタノール，lumefantrine（旧名 benflumetol）はラセミ化フルオレン誘導体である．Pyronaridine はベンゾナフチリジン誘導体，piperaquine はビスキノリン（キノリン骨格を 2 個）を母核とする抗マラリア薬である．Primaquine，tafenoquine（WR 238605 あるいは etaquine）はともに 8-アミノキノリン系薬である．Tafenoquine は臨床試験中の薬剤である．

本系統の薬剤の作用機序は完全に解明されたとはいいがたい．これらは弱塩基であり，原虫の酸性食胞に蓄積する性質があるが，抗マラリア原虫作用を説明できない．Chloroquine は DNA を intercalate するが，その作用の発現には原虫を殺滅する濃度よりも高濃度が必要であるともいわれる．Chloroquine はヘモグロビン分解産物であるフェリプロトポルフィリン IX と結合することにより，毒性のあるヘムの二量体化（すなわち無毒化）を抑制して原虫を殺滅するとの考えは有力である．また，原虫はグルタチオンが介在したヘムの分解も行うが，chloroquine はこれに競合的に阻害する．

●葉酸代謝拮抗薬

Pyrimethamine，proguanil（活性体はcycloguanil），trimethoprim，サルファ薬（特に sulfadoxine）があるが，前 3 者はジヒドロ葉酸還元酵素（DHFR）を阻害して，その結果，葉酸合成を抑制する．サルファ薬はそれより前の段階，すなわちジヒドロプテロイル酸合成酵素（DHPS）を阻害する．いずれの薬剤も作用点が明確であり，薬剤耐性機構（マラリア原虫の遺伝子変異の部位）はほとんど明らかにされている．

現在これらの薬剤は単独では使用されず，他の葉酸代謝拮抗薬あるいは別系統の抗マラリア薬と併用される．

●アルテミシニン系薬

Artemisinin（チンハオス qinghaosu）は

表1　抗マラリア薬の分類，作用機序，用法・用量

グループ	作用機序	薬剤	用法・用量（成人，経口投与を基本とする）		備考
			治療	予防	
キノリン系および類似薬	DNAのintercalation．フェリプロトポルフィリンIXと結合し，ヘムの無毒化を抑制	quinine	1.5～1.8 g/日・3～7日間　単独（7日間）あるいはdoxycyclineなどと併用．重症マラリアでは点滴静注で，8.3 mg塩基/kgを8～12時間間隔	適応なし	点滴静注では心毒性に注意．quinidineは立体異性体であるが，勧められない
		chloroquine	初回10 mg/kg，6，24，48時間後にそれぞれ5 mg/kg	300 mg塩基を週1回．ときにproguanilと併用	熱帯熱マラリアで耐性が多い．長期使用での網膜症
		amodiaquine	chloroquineに同じ	副作用のため勧められない	副作用（無顆粒球症，肝障害）の問題
		mefloquine	15あるいは25 mg/kgを単回，あるいは2～3回に分割	250 mg塩基を週1回	精神神経系副作用．アルテミシニン系薬との併用
		halofantrine	使用は勧められないが，500 mgを6時間間隔で3回，ときに1週間あけて2クール．	適応なし	QTc間隔延長，まれに心室性不整脈，心停止，死亡
		lumefantrine	artemether　20 mg/lumefantrine 120 mgの合剤を，0，8，24，36，48，60時間後にそれぞれ4錠	適応なし	QTc間隔延長はなし
		pyronaridine	総量1,800 mgを5日間で投与．最近では併用療法	適応なし	artesunateと併用で治験開始予定
		piperaquine	総量5,120 mgを0，8，24，48時間後に投与．最近では併用療法	適応なし	dihydroartemisinin/piperaquine，あるいはdihydroartemisinin/piperaquine/primaquine/trimethoprimの合剤
		primaquine	根治療法として，15 mg塩基（0.25 mg塩基/kg）/日・14日間，ときに増量	30 mg塩基（0.5 mg塩基/kg）/日	G6PD欠損症での溶血
		tafenoquine	根治療法薬としてまだ一般的でないが，例えば300 mg塩基/日・7日間	例えば，400 mg塩基/日・3日間＋400 mg塩基を週1回	現在試験中の薬剤．G6PD欠損症での溶血
葉酸代謝拮抗薬	葉酸代謝の阻害．サルファ薬以外はジヒドロ葉酸還元酵素の阻害	pyrimethamine	sulfadoxine 500 mg/pyrimethamine 25 mgの合剤3錠を単回投与	左記の合剤で副作用が多く，現在は使用しない	
		proguanil	atovaquone 250 mg/proguanil 100 mgの合剤，1日1回4錠を3日間	chloroquineとの併用では200 mg/日．左記の合剤としては1錠/日	活性型はcycloguanil
		trimethoprim	本薬剤の重要性は疑問	適応なし	piperaquineの項を参照
		サルファ薬	pyrimethamineの項を参照	pyrimethamineの項を参照	過敏性反応（特にHIV陽性者）

表1　抗マラリア薬の分類，作用機序，用法・用量(つづき)

グループ	作用機序	薬剤	用法・用量（成人，経口投与を基本とする）		備考
			治療	予防	
アルテミシニン系薬	ペルオキシド結合の切断で，フリーラジカルの生成，蛋白質のアルキル化	artemisinin	例えば1日目20 mg/kg，2〜5日目にそれぞれ10 mg/kg	適応なし	併用についてはmefloquine，lumefantrine，pyronaridine，piperaquineの項を参照．原虫殺滅作用は急速．動物実験では脂溶性薬剤(artemether，arteether)で中枢神経障害
		artemether	lumefantrineの項を参照．重症マラリアで注射として，初回3.2 mg/kg，その後1.6 mg/kg/日を24時間ごと，計5日間程度		
		arteether	重症マラリアで注射として，初回3.2 mg/kg，その後1.6 mg/kg/日を24時間ごと，計5日間程度		
		artesunate	重症マラリアで注射として，初回2.4 mg/kg，その後1.2 mg/kg/日を24時間ごと，計5日間程度		
		dihydroartemisinin	初日120 mg/日，その後60 mg/日を4〜6日間，いずれも1日1回		
atovaquone	ミトコンドリア電子伝達系チトクロームbc$_1$複合体の阻害	atovaquone	proguanilの項を参照	proguanilの項を参照	早くも耐性の出現
抗生物質	蛋白合成の阻害	doxycycline	quinineとの併用で，1日目200 mg，2〜7日目それぞれ100 mg	100 mg/日	抗マラリア作用は緩徐
		clindamycin	quinineとの併用で（両者とも静注），5 mg/kgを8時間ごと，3日間	適応なし	海外では妊婦にも安全とされる
		azithromycin	データは不十分	250 mg/日．今のところ，熱帯熱マラリアに対する効果は不十分	海外では妊婦にも安全とされる

Artemisia annua（和名：クソニンジン）から抽出され，中国で開発されたセスキテルペン・ラクトン過酸化物であり，水に難溶性である．側鎖をメチル化，エチル化，コハク酸エステル化，あるいはジヒドロ体に還元することで得られたartemether，arteether，artesunate，あるいはdihydroartemisininなどの誘導体が抗マラリア薬として用いられている．Artemether，arteether，artesunateは体内でdihydroartemisinin（artemisininの5〜10倍の抗マラリア活性を有する）に代謝される．

抗マラリア原虫作用の発現には分子内ペルオキシド結合部位が必須であり，これが炭素を中心にもつフリーラジカルを生成し，マラリア原虫の蛋白質をアルキル化して原虫殺滅を行うと考えられている．この薬剤は原虫の輪状体から早期分裂体にわたり，広い分裂周期に作用するので，他の薬剤と比べ効果が最も早く発現する．また，実質的に薬剤耐性の出現が報告されていない貴重な薬剤である．

●他の抗マラリア薬

Atovaquoneはヒドロキシナフトキノンであるが，トキソプラズマ，ニューモシスチス・カリニなどの原虫疾患の治療に用いられてきた．抗マラリア原虫作用は1940年に示されているが，実際の開発が行われたのは1990年代になってからである．ミトコンドリア電子伝達系のチトクロームbc$_1$複合体に作用すると考えられている．単独での治療では熱帯熱マラリアの約30%が耐性を獲得するため，proguanilとの合剤（Malarone）として用いられる．

抗生物質としてテトラサイクリン系薬（特にdoxycycline），マクロライド系（azithromycin）あるいは類似薬（clindamycin）なども抗マラリア作用がある．いずれも治療には併用で用いられるが，doxycyclineは単独で予防にも用いられる．

◆4 治療における抗マラリア薬

本稿では抗マラリア薬の用法・用量，禁忌，副作用などの系統的な記述は避けるので，それらについては他書を参照されたい．

●三日熱，卵形，四日熱マラリアの急性期治療薬

Chloroquineが第一選択薬である．ただし，1980年代末よりクロロキン耐性三日熱マラリアがパプアニューギニアで発見され，その後インドネシア領ニューギニア（イリアンジャヤ），ミャンマーや，南米のガイアナ，コロンビア[3]でも確認されている．根治療法として用いるprimaquineが赤内型原虫にも作用するので，それを早期に使用した場合にはクロロキン耐性を覆い隠してしまう可能性もある．Sulfadoxine/pyrimethamine合剤も通常効果があるが，タイの三日熱マラリアに対しては無効なことが多い[4]．

●三日熱，卵形マラリアの根治療法薬

Primaquineが使われてきているが，ニューギニア島の三日熱マラリアで低感受性の症例が知られていた．その後も東南アジア，中南米，ソマリアの三日熱マラリアで，primaquineの15 mg塩基/日・14日間の標準療法により再発をきたす症例がみられている．しかし，本当にプリマキン耐性が存在するかどうかの検討は不十分であり，体重換算した用量を用いていないことが関係するとの報告もある[5]．いずれにしても，治療抵抗性が問題となる場合には，標準療法後に再発がみられた後の再治療で増量したり，あるいは最初の根治療法から増量することも行われるが，その方法は一定していない．

なお，卵形マラリアで標準療法後の再発は余り知られていなかったが，最近報告がなされている．

●合併症のない熱帯熱マラリアの治療薬

Chloroquineにはもちろんのこと，sulfadoxine/pyrimethamine合剤にも耐性が増えており，両者ともに標準的治療薬とはみなされない．mefloquineに対しては，タイ・ミャンマーあるいはタイ・カンボジアの国境などで50%以上が耐性となっているが，他の地域では有効であり，今でも使用価値は高い．Halofantrineではほとんどの例で心電図でのQTc間隔延長がみられ，まれではあるが心室性不整脈による心停止，死亡例も報告されている．先天性にQTc間隔が延長している場合のみならず，投与前に心電図異常がない場合の死亡例[6]もあり，先進国では使用されない傾向である．

古典的薬剤のquinineはdoxycyclineとの併用で今でも広く使われており，治療効果も高い．Quinineは従来の7日間投与では耐容性が不良であったが，doxycyclineとの併用により3日間程度に短縮が可能である．また，quinineとclindamycin（両者とも静注）の3日間投与でも効果・耐容性において良好な成績が出ており[7]，嘔吐のために経口投与が不可能な症例などで有用であろう．

最近注目されている抗マラリア薬であるatovaquone/proguanil合剤[8]，あるいはarteme-

ther/lumefantrine 合剤 (Coartem, Riamet)[9] については，旅行者を対象とした大規模な試験は行われていないが，少数例ずつ学会発表も行われており，今のところ良好な成績が示されている．しかし前者については早くも，耐性変異が確認された熱帯熱マラリア原虫による治療不成功例が報告され[10]，耐性株の拡散が懸念される．後者については，artemether の迅速な効果と長期持続性の lumefantrine の効果が相補う利点がある．Lumefantrine は構造上 halofantrine と類似しており，また artemether は動物実験での大量投与で QTc 間隔延長を生ずることから，本合剤の心毒性についてヒトでの検討が注意深く行われているが，QTc 間隔延長を含む心電図異常はみられていない[11]．

●重症マラリアの治療薬

重症マラリアでは非経口薬剤を選択するのが原則である．

先進国では quinine 注射薬（静注）を使用することが多い．心毒性の注意が必要であるが，標準的な 1 回投与量（キニーネ塩基として 8.3 mg/kg）を守れば安全性は高い．場合により，初回投与のみ倍量（loading dose）を用いることもあるが，特に高齢者などでは心毒性に格別の注意が必要である．高度耐性マラリアであると予想される場合には，doxycycline などのテトラサイクリン系抗生物質との併用も行われる．注射投与の目的を達したら quinine 経口薬に変更したり，あるいは最終投与の終了から 12 時間以上あけて mefloquine の追加投与を行うなどの方法がある．

アルテミシニン系薬では artemether あるいは arteether（ともに筋注），artesunate（静注，筋注）などが用いられる．Dihydroartemisinin 坐剤も重症マラリアの初期治療に有用であるとする成績が出されているが[12]，今後さらに検討する必要がある．

アジア，アフリカ，パプアニューギニアなどで artemether と quinine との比較をランダム化して行った臨床試験 7 件のデータを集めて，メタ分析を行った成績によると，artemether 群は quinine 群と比べて多臓器不全を有する成人例での致死率が低く，全体的な致死率でも少なくとも同等であり，重篤な副作用も少なかった[13]．従来，アルテミシニン系薬は中国，ベトナム，タイなどで使われてきたが，欧米先進国でも徐々に使われつつある．今後，それらの製造が GMP（Good Manufacturing Practice）を遵守して行われ，副作用報告も欧米先進国と同等の精緻さで行われるようになると，従来の quinine 静注に取って代わることも考えられる．

◆5 予防における抗マラリア薬

●マラリア予防の原則

マラリア予防には，①蚊に刺されないための工夫，②予防内服，③スタンバイ治療（マラリアが疑われるときに，旅行者自身の判断で治療薬を服用すること）の 3 種類があるが，すべての場合に①が基本となる．しかし，マラリア罹患，あるいは重症化のリスクが高い場合には②あるいは（および）③のオプションも考慮する必要がある．一方では，薬剤による副作用も全く無視することはできないので，慎重さも要求される．予防内服を行うべきか，あるいはどの薬剤を用いるべきかについては，旅行目的国のマラリアの状況のみならず，旅行者の行動その他の多くの因子を考慮する必要がある[1]．このような問題は，新しい専門分野である「旅行医学」で活発に議論されている．

●Chloroquine 単独あるいは proguanil との併用

これらは熱帯熱マラリアの薬剤耐性の問題から，使用価値が低下している．したがって，chloroquine 単独の予防内服は中東，中米など，三日熱マラリアがほとんどを占める地域に限られる．Proguanil を併用すると熱帯熱マラリアに対する予防効果は高まるが，それでも最大 70%程

度とされている．Chloroquine と proguanil は使用の歴史が長く，副作用が少ないと思われがちであるが，ランダム化した二重盲検試験の結果からは必ずしも正しくない．また，chloroquine が週1回，proguanil が毎日1回の服用であり，併用の場合には服用方法の複雑さからコンプライアンスが低下しがちである．

●Mefloquine

タイ・ミャンマーあるいはタイ・カンボジア国境地域などの一部の地域を除けば，本薬剤の予防効果は90%を超え，今でも主要な予防薬である．諸外国で問題とされる精神神経系副作用については，メディアの過剰反応も考慮する必要がある．確かに，atovaquone/proguanil 合剤を対照としてのランダム化した二重盲検試験で，mefloquine は精神神経系副作用が多いことが示されている．しかし，副作用の中には不眠，“奇妙なあるいは鮮明な夢”をみることなど，容認可能な症状が多く含まれていることにも考慮が必要である[14]．入院を必要とするほどの重篤な副作用は10,000例に1例程度とされており[1]，精神疾患，てんかんの既往や家族歴がある場合には投与を避け，服薬期間中に大酒を避けるようにすれば相当程度副作用を避けることが可能である．すでに1500～2500万人が服用していると推測される．

●Doxycycline

Mefloquine に匹敵する効果が示されているが，mefloquine に比べるとデータの蓄積は少ない．しかし，世界的ににきびの治療に長期投与していることから，副作用に関する安心感がもたれ，データに現れない使用例はかなりあるものと推測される．前述のメフロキン耐性地域，mefloquine の禁忌あるいは服用を嫌う人（メディア情報に影響されることも多い），十分な準備期間がなく出かける人（1～2日前からの開始でよいので）などで使用価値がある．しかし，光線過敏症，消化器障害（食道潰瘍を含む），膣カンジダ症などの副作用もあり，8歳未満の小児，妊婦などは通常適応外となるなど，使用上の制限が多い．

●Atovaquone/proguanil 合剤

前述の予防薬は一次肝臓内ステージには作用せず，赤血球内サイクルに作用するので，現地を去ってから4週間の服用が必要である（抑制的予防薬）．しかし，atovaquone は一次肝臓内ステージに作用することから（原因的予防薬）（図1），現地を去ってから1週間の服用で済む利点がある．今まで熱帯アフリカ住民での予防効果が示されているものの，旅行者でのデータの蓄積は十分でなかったが，最近デンマーク人旅行者において，mefloquine とほぼ同等で chloroquine/proguanil より優れた効果，および安全性が報告された[15]．また，投与期間は従来4週間程度であったが，6カ月間投与での安全性と良好な耐容性が報告され[16]，投与期間を限定しない方向になりつつある．本薬剤の適応については，上述の doxycycline の適応が当てはまる．

⑥ 今後期待される抗マラリア薬

●キノリン系および類似薬

Primaquine 自体は新しくないが，すべてのマラリアに対する原因的予防薬として注目されている（図1）．以前には毒性が高い薬剤とされてきたが，予防において30 mg 塩基/日を1年間継続服用した報告もあり，毒性に関する考え方は変化している．インドネシア・イリアンジャヤで20週間服用したときの効果としては，熱帯熱マラリアに対して88%，三日熱マラリアに対して92%を超え，耐容性は良好で，軽度のメトヘモグロビン血症（平均3.4%）も2週間以内に回復していた[17]．Primaquine 類似薬の tafenoquine も原因的予防薬であり（図1），効果と副作用の面で primaquine より優れているとされる．消失半減期が2週間と長く，短期間の服用で長期間の予防効果が期待されているが，臨床試験が行われた地

域はまだ少なく，効果もやや不十分であるなど（最大89%）[18]，今後の検討が必要である．

Pyronaridineは多剤耐性熱帯熱マラリアに対する作用があり，始めは単独で用いられたが，現在ではartesunateとの併用で治療薬としての試験が予定されている．Piperaquineはdihydroartemisininとの2剤の合剤[19]，あるいはdihydroartemisinin，trimethoprim，primaquineとの4剤の合剤（CV8）で，治療薬として中国，ベトナム，カンボジアなどの流行地で使われており，優れた効果と安全性が報告されている．

●その他

Fosmidomycinは抗菌薬として開発された．イソペンテニル2リン酸の生合成を阻害し，その結果イソプレノイドの生成を抑制してマラリア原虫に選択的に作用する．ガボンとタイにおいて単剤による治験が行われ，作用発現は早く，副作用の問題も少なくて耐容性に優れていた．しかし再燃が多いことから，併用療法で行う必要があると思われる[20]．

おわりに

筆者（木村）が関係している前述の研究班が国内未承認抗マラリア薬を導入しており，国内の診療体制はかなりでき上がりつつある．しかしながら，相変わらず診断の遅れや不適切な治療から不幸な転帰をとる例もみられている．熱帯熱マラリアは迅速な対応が要求される疾患であり，専門医療機関に紹介すべき場合もある．そのような種々の相談を含めて，われわれ研究班のネットワークが活用されることを切望する．

本稿で述べた内容には，筆者が創薬等ヒューマンサイエンス総合研究事業「熱帯病に対するオーファンドラッグ開発研究」（KH42074），および国際医療協力研究委託事業「海外旅行者の健康管理及び疾病予防に関する研究」の分担研究者として行った臨床研究の成果が含まれる．

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Epidemiological and Clinical Aspects of Malaria in Japan

Mikio Kimura, Ai Suzaki, Yasuharu Matsumoto, Kazunori Nakajima, Yusuke Wataya, and Hiroshi Ohtomo

Worldwide, travel from industrialized countries to the Tropics is continuously expanding. Factors responsible for this trend include increasing levels of economic activity, cultural interests, academic investigations, and other factors targeting those countries. This trend is intensified by a recent development of large-scale air transport. However, the malaria situation in endemic countries has not improved drastically; rather it has even deteriorated in some areas, at least partly due to environmental changes and population movements. This may result in an increasing number of returning travelers with malaria and is therefore raising concerns about the appropriateness of clinical malaria management in travel donor countries.[1] Japan is not an exception to this; thus, in the year 2000, seven million Japanese travelers visited malarious areas and more than 100 malaria cases are recognized each year inside Japan. Clinically seeking medical advice by travelers, malaria diagnosis, and the initiation of appropriate treatments by physicians tend to be delayed, thus possibly resulting in a higher case fatality rate (CFR) than those reported from other industrialized countries. In addition to the malaria cases diagnosed inside Japan, about 100 cases per year are recognized among overseas Japanese citizens by embassy doctors.[2] Of note is Japan being far behind western countries in the practice of malaria chemoprophylaxis. Therefore, we attempted to review the epidemiologic situation and clinical management of malaria in Japan, including the activities of and data obtained by a research group, with the aim of clarifying promising strategies.

The Role of the Research Group on Chemotherapy of Tropical Diseases

Since 1980, a national Research Group on Chemotherapy of Tropical Diseases has been established, supported by different funding bodies, all of which were related to the Ministry of Health and Welfare (MHW),[3] which since January 2001, has been reorganized to the Ministry of Health, Labour and Welfare (MHLW). Two of the authors of this article (MK and HO) have been active members of this research group for years. The primary role of the research group was to make unlicensed drugs for tropical and parasitic diseases available when needed.

In Japan the production of chloroquine, which had been used for malaria and renal diseases, has been interrupted following development of retinopathy among users with renal diseases. Since then, oral quinine had been the only licensed drug for malaria treatment until 1987 when Fansidar was licensed with the support of the research group. However, in recent years, oral quinine, at least by itself, and Fansidar do not appear as major therapeutic antimalarials due to the emerging *Plasmodium falciparum* drug resistance to both drugs as well as the poor tolerability of oral quinine.[4] Recently, mefloquine has been licensed and marketed in October 2001 for both prophylaxis and treatment, again aided by the research group.

Since other antimalarials are not marketed, the research group has introduced chloroquine, atovaquone/proguanil combination, injectable quinine, primaquine, and recently, artesunate both as an oral and rectal form for the treatment of malaria cases.[3,5] Unlicensed drugs other than antimalarials are also made available from the research group, including diloxanide furoate and injectable metronidazole for amebiasis, sodium stibogluconate for leishmaniasis, ivermectin for strongyloidiasis,

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Table 1 Number of Malaria Cases by Year and Species-Research Group , 1990 to 2000

	Plasmodium *species*							
Year	*Pf*	*Pv*	*Po*	*Pm*	*Mixed*	*Unknown*	*Total*	*Fatality*
1990	40	62	3	0	5	6	116 (28*)	1
1991	43	63	0	3	3	2	114 (41)	2
1992	26	70	3	0	4	9	112 (38)	1
1993	40	60	5	2	3	2	112 (27)	0
1994	46	39	4	3	5	7	104 (28)	2
1995	56	58	6	1	4	0	125 (30)	1
1996	42	49	8	1	0	3	103 (36)	0
1997	46	53	2	1	3	7	112 (28)	0
1998	51	45	2	1	2	3	104 (20)	4
1999	40	66	4	1	4	4	119 (28)	3
2000	61	56	7	1	3	4	132 (27)	2
Total	491	621	44	14	36	47	1,253 (331)	16

Pf = *Plasmodium falciparum*; Pv = *Plasmodium vivax*; Po = *Plasmodium ovale*; Pm = *Plasmodium malariae*.

*Figures in parentheses represent the number of malaria cases in foreign citizens.

onchocerciasis and scabies, triclabendazole for fascioliasis and paragonimiasis, oral ribavirin for Lassa fever and other viral hemorrhagic fevers, suramin, melarsoprol, and eflornithine for African trypanosomiasis.

After obtaining permission for the importation and use of those drugs from the MHLW, their quality is checked at the National Institute of Health Sciences to ensure that they meet the Japanese Good Manufacturing Practice standards. The drugs are then distributed to 23 medical institutions throughout Japan, with the intent that they be accessed by any Japanese physician who needs the drugs. A written informed consent is obtained from each patient before use.

Those 23 medical institutions not only provide the drugs upon request, but also are willing to respond to consultations regarding diagnostic and therapeutic issues of tropical and parasitic disease cases, including microscopic examination of blood films for malaria parasites. In some urgent cases, a member physician even visits the hospital, sees the patient, and gives advice about the use of antimalarials and appropriate ancillary treatments. Some malaria cases might not have been cured without these activities of the research group.

The research group also analyzes data on the use of antimalarials and other drugs for their efficacy and adverse effects, especially focusing on the use in Japanese patients. Once a year, it conducts a questionnaire study on malaria cases, hosts a seminar on malaria treatment targeting a wide range of medical personnel, including physicians, nurses, laboratory staff, and pharmacists. Once every several years, it also publishes recommendations on the treatment of tropical and parasitic diseases, including recommended usages of the introduced drugs.

Statistics of Malaria Cases from the Past to the Present

According to the statistics of the MHW, now the MHLW, reported malaria cases counted at 12,000 in 1947. Most of the cases were due to *Plasmodium vivax* malaria that was imported by Japanese soldiers and their families who had been living abroad during World War II. Malaria was then transmitted among local Japanese people by indigenous *Anopheles* mosquitoes. However, after that year, the number of cases commenced to decline rapidly and 50 to 80 cases per year were recognized during the years 1990 to 1998, according to the MHW statistics.[6] In April 1999, the new infectious diseases control law was enacted, which simplified the reporting system. Probably for this reason, the number of malaria cases reported to the MHW tended to increase; thus, there were 153 cases in 2000.

The questionnaire-based study of the research group on malaria cases mentioned previously targets 1,800 hospitals throughout Japan,[3] and provides much valuable information on malaria cases, including reasons for travel, prophylactic antimalarials used and their efficacy, initial diagnoses presented and treatments given by physicians and the patients' outcomes and is thus not confined to simply compiling demographic data. The response rates were lower than expected, 60 to 65%, presumably because many hospitals without any malaria cases did not respond. As a result, the research group has identified 103 to 132 cases of malaria with zero to four deaths per year during the past 11 years (Table 1).[7] Therefore, until April 1999 it collected larger numbers of malaria cases than those reported to the MHW. It is obvious that the proportion of falciparum malaria cases is gradually increas-

Table 2 Malaria Cases Acquired Inside Japan

Year	*Age/Sex*	*Species*	*Cause*	*Outcome*
1971	83/Female	Pv	s/o Airport malaria	Cured
1973	21/Female	Pf	s/o Needle stick injury	Died
1974	55/Male	Pv	Unknown	Cured
1975	39/Male	Pv	s/o Whole blood transfusion	Cured
1976	61/Male	Pv	Unknown	Cured
1981	14 days/Female	Pv	Congenital	Cured
1983	19 days/Male	Pv	Exchange transfusion	Cured
1983	35/Male	Pf	Needle stick injury	Cured
1983	45/Female	Po	Platelet transfusion	Cured
1985	60/Male	Pv	Platelet transfusion	Cured
1991	70/Female	Pf	Platelet transfusion	Died

Pf = *Plasmodium falciparum*; Pv = *Plasmodium vivax*; Po = *Plasmodium ovale*; Pm = *Plasmodium malariae*.

ing, probably reflecting an increasing number of travelers to subSaharan Africa and Oceania, such as Papua New Guinea and the Solomon Islands. Thus, the proportion of falciparum malaria was the highest in 1994, 1998, and 2000.[6] The CFR due to falciparum malaria for the study period was 3.3% (16 of 491), a higher figure compared with those of many European countries except for Germany, which showed a CFR of 3.6%.[1]

The questionnaire-based study as well as literature searches identified malaria cases that were acquired inside Japan (Table 2). The definition of the case is that the patient had not visited any foreign country and not received transfusion except for transfusion-associated cases. One case was supposed to be airport malaria since the patient had no other risk factors and had lived near Tokyo (Haneda) International Airport. Five were related to transfusion of whole blood or platelet concentrate, among which one was fatal. Two cases had no known risk factors of malaria acquisition even with detailed investigation.

Blood Donor Selection

The transfusion-associated malaria cases prompted the Japanese Red Cross in 1995 to establish guidelines for blood donation based on travel history to malarious areas (Table 3). These areas are divided into nonendemic and endemic areas. An individual who visited an endemic area cannot donate blood within 1 month of return, regardless of the travel duration. Anyone who visited a rural or forested area in the evening or at night, regardless of the travel duration, or stayed for longer than 1 month in either type of malarious area should defer blood donation for 1 year, and anyone who stayed for longer than 3 months should defer for 3 years. Most strictly, anyone who has had malaria should defer donating blood indefinitely. After the implementation of these strict guidelines no transfusion-associated malaria case has been identified, despite uncertainty of its causal relationship in a strict sense.

Table 3 Blood Donor Selection after Travel to Malarious Areas—Japanese Red Cross, 1995

Malarious Area	*Condition*	*Blood Donation*
Nonendemic	Sightseeing or business travel of ≤1 month duration staying in urban or resort hotels No visit to rural or forested areas in the evening and at night	Possible
	Travel of >1 month duration	Defer for 1 year
	Visit to a rural or forested area in the evening or at night regardless of the travel duration	Defer for 1 year
	Staying for >3 months	Defer for 3 years
Endemic	<1 month of return	Impossible
	≥1 month of return Sightseeing or business travel of ≤1 month duration staying in urban or resort hotels. No visit to a rural or forested area in the evening and at night.	Possible
	Travel of >1 month duration	Defer for 1 year
	Visit to a rural or forested area in the evening or at night regardless of the travel duration	Defer for 1 year
	Staying for >3 months	Defer for 3 years

Those who have had malaria should be deferred indefinitely.

Table 4 Positive Travelers with the Malaria Check at International Airports

No.	*Date*	*Sex*	*Age*	*Travel Destination*	*Result*
1	8/8/1998	M	23	Senegal, Gambia, Guinea, Ghana	*Para*Sight F (+), microscopy *Pf* (+)
2	8/8/1998	F	25	Mali, Senegal	*Para*Sight F (+), microscopy (-)
3	8/23/1998	F	29	Malaysia	*Para*Sight F (+), microscopy (-)
4	10/15/1998	M	33	Nepal, India, Sri Lanka	*Para*Sight F (-), microscopy *Pv* (+)
5	10/27/1998	M	30	Kenya, Uganda, Congo	*Para*Sight F (+), microscopy (-)
6	5/7/1999	M	36	Central African Republic	*Para*Sight F (+), microscopy (-)
7	6/23/1999	M	31	Indonesia, China	*Para*Sight F (-), microscopy *Pv* (+)
8	10/7/2000	F	23	India	ICT Malaria P.f (+), microscopy *Pf* (+)
9	12/2/2000	M	27	Guinea, Mali, Senegal	ICT Malaria P.f (+), microscopy (-)
10	12/21/2000	M	23	Senegal, Mali, Guinea	ICT Malaria P.f (+), microscopy (-)
11	9/21/2001	M	42	Thailand, Myanmar	ICT Malaria P.f (+), microscopy (-)
12	1/9/2002	M	58	Uganda	ICT Malaria P.f (+), microscopy *Pf* (+)
13	3/30/2002	F	25	Malawi	ICT Malaria P.f (+), microscopy (-)

Pf = *Plasmodium falciparum*; Pv = *Plasmodium vivax*.

Malaria Checks at International Airports

In Japan, quarantine stations are indispensable for providing travelers with information on malaria and other travel-related illnesses.[8] Japan has also launched a malaria check system targeting febrile returnees from malarious areas. This system was first initiated at New Tokyo (Narita) and Kasai International Airports in October 1997, then at Fukuoka and Nagoya International Airports in April 1998. The inclusion criterion is that the traveler became febrile 7 or more days after staying for 1 or more nights in a malarious area and requests the testing of their own free will. A dipstick (*Para*Sight F, Becton Dickinson, Cockeysville, USA) or card-type (ICT Malaria P.f., Amrad ICT, French Forest, Australia) kit detecting *P.falciparum* antigen as well as the conventional microscopy of Giemsa-stained blood films are employed for the malaria check, and the results are later checked for their validity at specialized hospitals.

As of December 2001, a total of 365 travelers were subjected to this malaria check that identified 13 positive cases with either of the methods, including five acute cases with a positive microscopy result (Table 4). Those 13 positive travelers were immediately referred to specialized hospitals, which contributed to a favorable outcome in all cases.

Trends of Malaria Treatment

We analyzed the treatment of malaria in Japan, using data from the questionnaire-based study of the research group. In 1990, most cases of falciparum malaria were treated either with Fansidar, quinine, or chloroquine, each accounting for 64, 33 or 31% of the cases, respectively (some individuals were treated with two or more drugs giving a total percentage exceeding 100%), whereas those with mefloquine accounted for only 4.4% (Fig. 1).[5,7] Since then, however, the use of mefloquine has increased and accounted for 78% of the cases in 2000, whereas during the same year Fansidar and chloroquine treatment declined to 6.9% and 5.2%, respectively.[5] In contrast, quinine has been used invariably, throughout the years studied. Halofantrine was used in 14% of cases in 1993; however, after that year, its use declined, presumably influenced by cardiac adverse effects reported in the literature. Remarkably, the proportion of patients treated with artesunate rose to 36% in 2000. Minocycline was used exclusively in combination with quinine.

In the case of vivax malaria, 25% did not receive primaquine following initial schizontocidal drugs, and conversely, 15% of malariae malaria cases were unsuitably given primaquine due to the misconception of hypnozoite-form parasites remaining in the liver (data not shown).[7]

These data clearly showed that, especially during recent years, a substantial proportion of malaria cases were given antimalarials that were derived from the research group (i.e., chloroquine followed by primaquine in vivax and *Plasmodium ovale* malaria, mefloquine in noncomplicated falciparum malaria, and injectable quinine and artesunate in severe and/or drug-resistant falciparum malaria.

Novel Malaria Diagnostic Methods

Microscopy of Giemsa-stained blood films, the gold standard for malaria diagnosis and monitoring of antimalarial therapeutic outcomes, depends on the expertise of microscopists and thus can be misleading. In this context two novel malaria diagnostic methods developed in Japan are worth mentioning. They are: the acridine-orange method developed by Kawamoto, Nagoya University,[9] and a polymerase chain reaction (PCR) method developed by one of the authors (YW) and Yamane, Wakunaga Pharmaceutical Company (Mirashima, Japan).[10]

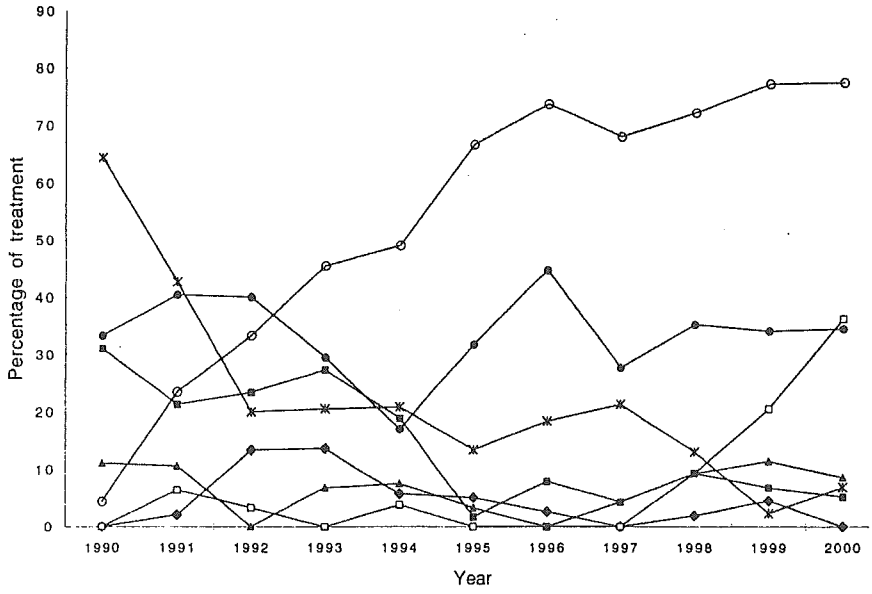

Figure 1 Antimalarial drugs used by year for treatment of falciparum malaria. Some individuals were treated with two or more drugs giving a total percentage exceeding 100%. Drugs include mefloquine (○), quinine (●), Fansidar (✱), artesunate (□), chloroquine (■), halofantrine (♦), and minocycline (△).

Although acridine-orange staining has been employed in the Becton Dickinson's QBC method, Kawamoto's method is unique in that thin blood films are stained on glass slides.[9] With this method, we could detect even a very small number of malaria parasites by examining for a shorter time than conventional microscopy if fully trained. Kawamoto also devised the method so that stained blood films can be viewed through a light microscope equipped with interference filters, rather than using an expensive fluorescence microscope.

Another is a species-specific PCR method targeting the 18S ribosomal ribonuclic acid gene of *Plasmodium* species. Reactions after PCR can be performed conveniently on microtiter plate wells, and the whole procedure can be accomplished in 6 hours.[10] It has proved useful for field studies in which a large number of samples need to be assayed. With clinical materials, we (MK and YW) have shown that the method has excellent sensitivity and specificity when compared with microscopy performed by well-experienced microscopists. Because of its excellent detection sensitivity, the PCR method could supplement microscopy in detecting low parasitemia and identifying *Plasmodium* species, for example, during early incubation stages of the illness or following antimalarial drug intake.[11] Predicting recrudescence of falciparum malaria could also be performed better with this PCR method than microscopy (manuscript in preparation).

Comments

The majority of Japanese travelers to the developing world visit sightseeing areas in Asian countries, such as Indonesia and India, for a short term where malaria risk is limited. Nevertheless, the number of those visiting highly malaria-endemic areas like sub-Saharan Africa or Oceania is steadily increasing. Consequently, countermeasures against a possible increase in the number of malaria cases especially that of falciparum malaria cases in the future should become a priority.

The fact that reporting of malaria cases to MHW might have improved since enactment of the new infectious diseases control law could be evaluated positively. However, in order to address important clinical issues of malaria, the questionnaire study conducted by the research group is essential and should continue in parallel with the national surveillance. So far, these two systems for detecting malarial cases are conducted independently; therefore, the possibility of linking these two should be taken into consideration in order to minimize unidentified malaria cases.

We have to mention a serious drawback to our immigration control system that lacks detailed statistics about countries Japanese travelers visit. These statistics are essential as reliable denominators for calculating malaria contraction rates by destination country. The only available data were derived from emigration cards on which only one major destination country was documented. Unfortunately, however, since July 2001 the emigration card itself has been abrogated.

The Japanese guidelines on blood donor selection appear stricter than the US guidelines. For instance, US guidelines permit blood donation from those with a past history of malaria after a 3-year deferral following completion of antimalarial therapy or departure from the area provided the individual has been asymptomatic.[12] The development of a reliable and cost-effective blood screening method for malaria parasites is desirable since such strict guidelines as ours based on travel history

might exclude a considerable number of potential blood donor candidates unnecessarily.

Encouraging more febrile returning travelers to receive the malaria check at the international airports is expected to lessen cases that develop into severe malaria or even become fatal. Educating travelers to specific high-risk destinations could be more efficacious if conducted individually at airport departure lounges.

Clinically, falciparum malaria in our county is characterized by a preferential use of mefloquine for treatment. However, the emergence of mefloquine-resistant cases and development of neuropsychiatric adverse effects should be monitored cautiously. The literature reported the drug resistance in a wide range of geographical areas especially along Thai-Burmese and Thai-Cambodian borders, and consequently the use of atovaquone/proguanil and introduction of artemether/lumefantrine might be recommended.[13] The neuropsychiatric adverse effects of mefloquine seem more frequent in treatment than prophylaxis.[14]

Diagnosis is another important issue that could help improve the clinical management of malaria. The development of novel malaria diagnostic methods, especially that of the PCR method, is advantageous to Japanese physicians as they have the potential to supplement the gold-standard microscopy in various clinical settings, and therefore, this should be advertised widely given the lack of experience in the microscopic diagnosis of malaria among Japanese clinical laboratory personnel.

The idea of malaria chemoprophylaxis has not been widely recognized in our country and therefore the recent approval of mefloquine could be a major advance. The next necessary step would be to educate physicians for which situation chemoprophylactic mefloquine should be prescribed. In this context, the establishment of guidelines on malaria prevention seems indispensable and one of the authors (MK) has launched an expert committee with this object. In the meantime, due to the steadily expanding resistance to mefloquine and ongoing arguments about neuropsychiatric adverse effects, we may have to prepare another chemoprophylactic regimen, that is, atovaquone/proguanil and doxycycline.[15]

Collectively, one of the most realistic strategies to improve the malaria situation in Japan, including the high CFR, is to strengthen the activities of the research group so that it would become a comprehensive network for clinical tropical and parasitic diseases. It should continue to provide useful information on the epidemiology, appropriate prophylactic, diagnostic and therapeutic means of malaria to medical personnel as well as to the general public. The recently publicized web page of the research group (http://www.ims.u-tokyo.ac.jp/didai/orphan, at present in Japanese only) could serve as a suitable tool in this regard.

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Structural Properties of Dibenzosuberanylpiperazine Derivatives for Efficient Reversal of Chloroquine Resistance in *Plasmodium chabaudi*

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For the purpose of developing chemosensitizers to reverse chloroquine (CQ) resistance in *Plasmodium chabaudi* in vivo, dibenzosuberanylpiperazine (1-(10,11-dihydro-5*H*-dibenzo[*a*,*d*]-cyclohepten-5-yl)piperazine) (DSP) and its piperazin-1-yl derivatives were synthesized systematically. DSP hydrochloride (**3**) was obtained from the reaction of dibenzosuberanyl chloride with piperazine in the presence of 1,8-diazabicyclo[5,4,0]-7-undecene (DBU). To understand the relationship between the substituent patterns of DSP derivatives and their biological activities, 13 hydroxyalkyl or hydroxyalkenyl derivatives were synthesized by an attack of the piperazine secondary amine of **3** on commercially available epoxides in the presence of triethylamine or DBU, and three alkyl or alkynyl derivatives were synthesized by the reactions of **3** with the corresponding organic chlorides in the presence of DBU. In both reactions, the yield was a maximum of 90%. The biological activities of the synthesized compounds were evaluated on the basis of two values: antimalarial activity and reversal activity. The values of antimalarial activities by single administration of 17 test compounds were not effective, being in the range 67−152% on day 4 after infection of *Plasmodium chabaudi* to mice except for the administration of 3-(dibenzosuberanylpiperazin-1-yl)-1-butene (**29**, 22%). On the other hand, administration of the seven test compounds (50 mg/kg dose) combined with CQ (3−4 mg/kg) gave high reversal activities, namely, low values (0% on day 4). The effective test compounds were those obtained by introducing the following substituents: 2-hydroxybutyl (**24**), 2-hydroxyhexen-5-yl (**27**), 2-hydroxybuten-3-yl (**28a**), 2-substituted 1-hydroxybuten-3-yl (**28b**), 4-acetoxybutyn-2-yl (**30**), 4-hydroxybutyn-2-yl (**31**), and 3-substituted buten-1-yl (**29**), which correspond to the nonbulky groups of hydroxyalkyl (C4), hydroxyalkenyl (C4−C6), hydroxyalkynyl (C4), or alkenyl (C4). These results may lead to the development of an approach to developing clinically applicable chemosensitizers for drug-resistant malaria.

Introduction

The spread of malaria, especially caused by the most deadly as well as the multidrug-resistant (MDR) *Plasmodium* (*P.*) *falciparum,* is becoming a serious problem in endemic countries in the tropical zones of Southeast Asia, Africa, and South America.[1] Furthermore, the inhabitants of malarial vectors (anopheline species) are expanding throughout the world through changes in global ecosystems and weather patterns due to global warming. However, *P. falciparum* promptly built up resistance to various types of antimalarial drugs (e.g., chloroquine, mefloquine, halofantrine, etc.).[2] To maintain MDR *P. falciparum* under control, the development of new MDR-sensitive drugs is essential for the treatment of MDR malaria. The recovery activity of chloroquine (CQ) resistance in *P. falciparum* by the use of verapamil, a calcium channel blocker,[3] chlorpromazine, and prochlorperazine of antipsychotics[4] and desipramine of tricyclic antidepressant drugs, etc.[5] has been reported. Recently, the design, synthesis, and in vitro bioevaluation of chemosensitizers such as phenothiazine, iminodibenzyl, iminostilbene, and diphenylamine derivatives against CQ-resistant *P. falciparum* have been reported.[6]

One of the present authors, Miyata, also found the recovery activity of drug sensitivity by some types of dibenzosuberanylpiperazine (1-(10,11-dihydro-5*H*-dibenzo[*a*,*d*]cyclohepten-5-yl)piperazine, DSP) derivatives against tumors, pathogenic microorganisms, and CQ-resistant rodent malaria.[7] The first DSP derivative against CQ-resistant malaria that did not exhibit sufficient reversibility was a compound substituted by 3-(7-chloro-4-quinolyl)thiopropan-2-ol. We then considered DSP derivatives bearing suitable substituents for MDR-reversing chemosensitizers in human malaria. As an effective bioavailable application of DSP derivatives, Suzuki et al. reported that a derivative of 4-quinolino-2-hydroxypiperazine (5-[3-{4-(10,11-dihydro-5*H*-dibenzo[*a*,*d*]cycloheptane-5-yl)piperazin-1-yl}-2-hydroxypropoxy]quinoline), MS-073, has high MDR reversal activity to tumors without significant toxicity.[8]

We have synthesized a large number of new DSP derivatives having piperazine N substituents and investigated in vivo the usefulness of these compounds for reversal of MDR *P. falciparum*. At first, we surveyed

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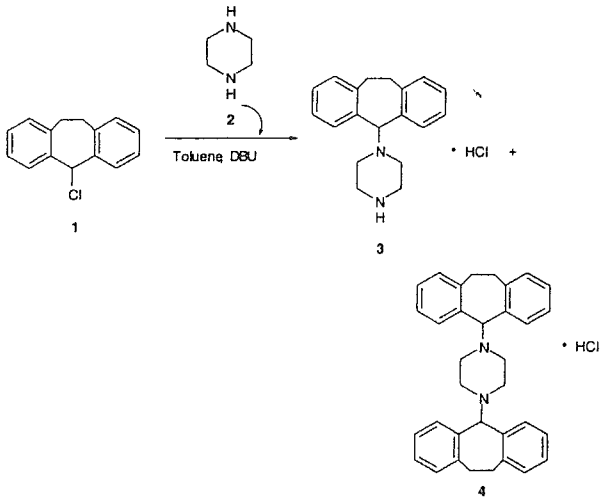
Scheme 1



the reversal effects[9] of CQ resistance in *Plasmodium* (*P.*) *chabaudi*[10,11] of rodent malaria in a search for the most promising substituents of the DSP derivatives as chemosensitizer. Many substituents were introduced to the secondary amine of the DSP piperazine residue by the ring opening of epoxides or by the reaction of organic chlorides. Some structural properties effective to reverse CQ resistance were examined and identified. The mechanism of drug resistance in malaria is not quite clear, even though some persuasive mechanisms suggesting an ATP-dependent efflux pump responsible for MDR observed in tumor cells and *P. falciparum* have been proposed.[12,13] However, if some promising chemosensitizers effective to reverse CQ resistance in *Plasmodium* could be obtained clinically, it would be helpful in achieving low-cost CQ therapy and developing new antimalarial drugs.

Chemical Results

DSP hydrochloride salt (**3**), a basic compound, was obtained from the reaction of dibenzosuberanyl chloride (**1**) with piperazine (**2**) catalyzed by 1,8-diazabicyclo-[5,4,0]-7-undecene (DBU) in toluene in 78% yield (Scheme 1). A byproduct of 1,4-bis(10,11-dihydro-*5H*-dibenzo[*a,d*]-cycloheptane-5-yl)piperazine (**4**) was simultaneously obtained as a hydrochloride salt in 22% yield.

To compare the reversal activities of CQ resistance with piperazine-N-substituted DSP derivatives synthesized by structural design, **3** was reacted with commercially available epoxides (**5**−**16**) in the presence of triethylamine (TEA) or DBU in methanol (MeOH) to produce the DSP derivatives (**17**−**28**) as shown in Scheme 2. The yield of products is also shown in Scheme 2. The use of TEA to produce **27** gave a low yield of 33%. However, the use of DBU to produce **27** raised the yield to 71%. This tendency was generally observed in small carbon-chain substituents probably because of the high basicity of DBU.

Reaction of epoxides **5**−**15** gave only **17**−**27**. By the reaction of **3** with epoxide **16**, isomer products **28a** and **28b** were obtained and separated by a silica gel column chromatography with yields of 48% and 27%, respectively.

3-Substituted buten-1-yl derivative (**29**) was synthesized by the reaction of **3** and 3-chloro-1-butene in the presence of DBU in 51% yield. The 4-acetoxybutyn-2-yl derivative (**30**) was prepared in 74% yield by the reaction of **3** and 4-acetoxy-1-chloro-2-butyne that was obtained from the reaction of 2-butyne-1,4-diol with acetic anhydride and then with thionyl chloride. Because the derivative **30** was syrupy just after being prepared and was unstable in standing at room temperature (changing from colorless to brown), it was converted to the crystalline deacetate **31** (70% yield) using K_2CO_3 as a base. However, syrupy **30** solidified when kept in a refrigerator for a while.

Biological Results

In addition to evaluating the antimalarial activity and the CQ-resistant reversal activity of the synthesized DSP derivatives, 4- to 5-week-old female mice (ICR Crj: CD-1, Charles River Japan, Inc.) were injected intravenously (iv) with 5×10^6 parasitized red blood cells (PRBC) of the CQ-resistant (3CQ) line of the AS strain of *P. chabaudi*.[11] These in vivo tests were carried out according to the method of Tanabe et al.[11b] except for injection of a CQ dose of 4 mg/kg. After 2 h of PRBC injection into the mice, the test compounds (5−50 mg/kg) and CQ (3 or 4 mg/kg) were subsequently injected intraperitoneally (ip) into the mice, and the injections were repeated for an additional 3 days (four total injections). The test compounds were dissolved in a physiological sodium chloride solution (saline) containing 10% (w/v) dimethyl sulfoxide (DMSO) for **4**, **17**−**23**, and **25**−**28** or in absolute DMSO for **3**, **24**, and **28**−**31**. CQ diphosphate salt (Sigma, C-6628) was dissolved in sterilized saline or dispersed in absolute DMSO. Four groups of mice were injected with either DMSO alone, CQ alone, test compound alone, or CQ combined with test compounds. Parasitemias (numbers of PRBC per 10 000 RBCs in Giemsa stained thin blood films) were microscopically monitored daily for 5 days.

The data of the antimalarial and the reversal activities were determined using the following two equations:

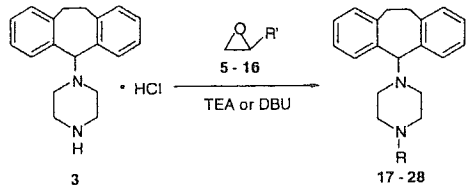
$$\text{value of antimalarial activity (\%)} = \text{(no. of PRBC by administering the compound only)/(no. of PRBC by no administering)} \times 100$$

$$\text{value of reversal activity (\%)} = \text{(no. of PRBC by administering the compound and chloroquine)/(no. of PRBC by administering chloroquine only)} \times 100$$

where the number of PRBC is per 10 000 RBCs in thin blood film. In both equations above, the lower the value (%) of antimalarial and reversal activities, the higher the antimalarial and reversal efficacies are expected.

Table 1 shows both values for the antimalarial and the reversal activities of 18 test compounds including 17 newly synthesized DSP derivatives at day 4 of administration (the values obtained on days 0−3 are not shown). As shown in the table, test compounds are classified into two groups mainly by the values of reversal activity. Group 1 includes 11 compounds, namely, **17**−**23**, **25**, **26**, **3**, and **4** showing generally high values of reversal activity (except for **3**, which shows a low value of reversal activity) obtained by a set of two mice. Group 2 includes seven compounds, namely, **24**

Scheme 2



	R'		R	Base	Yields (%)
5	$-CH_2O-C_6H_5$	17	$-CH_2CH(OH)-CH_2O-C_6H_5$	TEA	33
6	$-CH_2O-C_6H_4-OCH_3$	18	$-CH_2CH(OH)-CH_2O-C_6H_4-OCH_3$	TEA	38
7	$-(CH_2)_{15}CH_3$	19	$-CH_2CH(OH)(CH_2)_{15}CH_3$	TEA	82
8	$-(CH_2)_{13}CH_3$	20	$-CH_2CH(OH)(CH_2)_{13}CH_3$	TEA	76
9	$-(CH_2)_{11}CH_3$	21	$-CH_2CH(OH)(CH_2)_{11}CH_3$	TEA	81
10	$-(CH_2)_7CH_3$	22	$-CH_2CH(OH)(CH_2)_7CH_3$	TEA	42
11	$-(CH_2)_5CH_3$	23	$-CH_2CH(OH)(CH_2)_5CH_3$	TEA	57
12	$-CH_2CH_3$	24	$-CH_2CH(OH)CH_2H_3$	DBU	90
13	$-(CH_2)_6CH=CH_2$	25	$-CH_2CH(OH)(CH_2)_6CH=CH_2$	DBU	72
14	$-(CH_2)_4CH=CH_2$	26	$-CH_2CH(OH)(CH_2)_4CH=CH_2$	DBU	73
15	$-(CH_2)_2CH=CH_2$	27	$-CH_2CH(OH)(CH_2)_2CH=CH_2$	DBU	71
16	$-CH=CH_2$	28a	$-CH_2CH(OH)CH=CH_2$	DBU	75 (28a 28b=48%:27%)
		28b	$HOCH_2CH(OH)CH=CH_2$		

and **27**–**31** showing almost zero value of reversal activity (high reversal activity) obtained by more than four mice. Three and five mice were used to obtain the values of antimalarial activity of **28a** and **28b**, respectively. Therefore, the bioactivities of group 1 compounds have not been pursued further and the study of bioactivities was focused on group 2 compounds.

Each dose of the test compounds to the mice was 50 mg/kg except for **28b** (15 mg/kg). For administration of CQ, a dose of 3 or 4 mg/kg was chosen for **4**, **17**–**23**, and **25**–**27**, or for **3**, **24**, and **28**–**31**, respectively. The use of a higher CQ dose (4 mg/kg) would demonstrate the reversal efficacy more clearly. The order of the test compounds (with values less than 100%) effective for antimalarial action was **29**, **19** ~ **25** ~ **3**, **28a**, **23**, **24**. Other compounds gave values of antimalarial activity greater than 100%. Compounds **24**–**31** and **3** gave very low values of reversal activity when combined with the CQ dose.

To compare the reversal activity for CQ resistance with the carbon-chain length of piperazine N-substitutents of DSP derivatives, the values (%) of reversal activities for the derivatives with hydroxy-saturated carbon chains and with hydroxy-unsaturated carbon chains C 4–10 are shown in Figure 1. The values of reversal activities from day 0 to day 4 of the potent compounds **24**, **27**, **28a**, **29**, **30**, and **31** having high reversal effects are plotted in Figure 2.

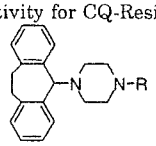
Compound **28** was isolated in two isomeric forms (**28a**, **28b**). The values of the reversal activity of **28a** at the different doses of 5, 10, 15, 30, 40, and 50 mg/kg are shown in Table 2 and Figure 3. Only the lower level doses (5, 10, and 15 mg/kg) of **28b** were administered to mice as shown in Table 2, since the higher level doses (30, 40, and 50 mg/kg) were expected to give 0% reversal activity.

Discussion

Compound **3** was isolated as the HCl salt and therefore was easy to handle. From this reaction, the sole byproduct formed was 1,4-bis(10,11-dihydro-*5H*-dibenzo[*a*,*d*]cycloheptane-5-yl)piperazine hydrochloride (**4**). The structure of **3** was confirmed by X-ray crystallographic analysis[14] as well as spectroscopic analysis. A free form of **3**, DSP, was easily liberated from **3** through Dowex 8 in 95% yield, and its spectral data were identical to those reported in previous papers.[8a,15] Even though the conventionally stable conformation of cycloheptane is a chair form,[16] X-ray analysis showed that the cycloheptane ring of **3** takes a boat form and the plane of the piperazine ring is almost perpendicular to that of the 1,2:4,5-dibenzocycloheptane skeleton. The structure of the piperazine ring was confirmed to be a chair form.

Thirteen hydroxy DSP derivatives were newly synthesized in satisfactory yields by an attack of the

Table 1. Values (%) of Antimalarial Activity and Reversal Activity for CQ-Resistant Malaria of Test Compounds



	Compd.	R	antimalarial activity (%) [a, b] 4 d	reversal activity (%) [a] CQ (mg/kg)	4 d
Group 1	**17**	-$CH_2CH(OH)CH_2O$-Ph	136 ± 17	3	113 ± 13
	18	-$CH_2CH(OH)CH_2O$-Ph-OCH_3	147 ± 4	3	90 ± 16
	19	-$CH_2CH(OH)(CH_2)_{15}CH_3$	67 ± 10	3	68 ± 6
	20	-$CH_2CH(OH)(CH_2)_{13}CH_3$	122 ± 18	3	110 ± 19
	21	-$CH_2CH(OH)(CH_2)_{11}CH_3$	138 ± 21	3	64 ± 21
	22	-$CH_2CH(OH)(CH_2)_7CH_3$	152 ± 21	3	84 ± 17
	23	-$CH_2CH(OH)(CH_2)_5CH_3$	92 ± 4	3	102 ± 6
	25	-$CH_2CH(OH)(CH_2)_8CH{=}CH_2$	68 ± 6	3	6.8 ± 2.8
	26	-$CH_2CH(OH)(CH_2)_4CH{=}CH_2$	108 ± 17	3	8.9 ± 1.5
	3 [c]	H	68 ± 16	4	2.0 ± 2.8
	4		108 ± 2	3	81 ±24
Group 2 [d]	**24**	-$CH_2CH(OH)CH_2CH_3$	99 ± 1	4	0
	27	-$CH_2CH(OH)(CH_2)_2CH{=}CH_2$	101 ± 1	3	0
	28a [e]	-$CH_2CH(OH)CH{=}CH_2$	82 ± 5	4	0
	28b [e]	$HOCH_2CH(OH)CH{=}CH_2$	103 ± 14	4	2.6 ± 0.8
	29	$CH_3CH(OH)CH{=}CH_2$	22 ± 15	4	0
	30	-CH_2-C≡C-CH_2OAc	116 ± 3	4	0
	31	-CH_2-C≡C-CH_2OH	121 ± 11	4	0

[a] Dose of 50 mg/kg except for **28b** (15 mg/kg). [b] Two mice were used. [c] Compound **3** was only dissolved in saline. [d] Reliability on reversal activity of group 2 obtained using more than four mice is for $P < 0.05$. [e] Three mice for **28a** and five for **28b** were used for antimalarial activity.

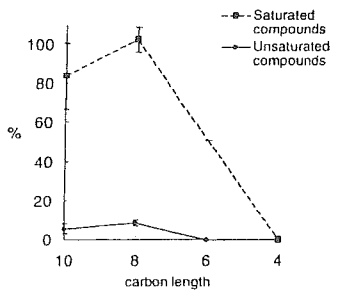


Figure 1. Reversal activities (%) of DSP derivatives with hydroxy-saturated substituents (**22**–**24**), and with hydroxy-unsaturated substituents (**25**–**28**) at 50 mg/kg dose of the test compounds combined with 3 mg/kg dose (**24** with 4 mg/kg) of CQ against carbon chain length.

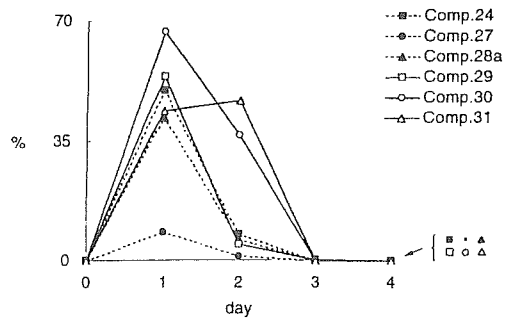


Figure 2. Preliminary results of reversal activities from day 0 to day 4 of potent compounds **24**, **27**, **28a**, **29**, **30**, and **31** at 50 mg/kg dose combined with 4 mg/kg dose of CQ (except **27**, with 50 mg/kg dose combined with 3 mg/kg dose of CQ).

secondary amine of **3** on the corresponding epoxides in the presence of TEA or DBU. Since the use of DBU tended to raise the yield of the DSP derivatives, DBU was usually used to synthesize the derivatives possessing a short hydroxy carbon chain. The synthesized