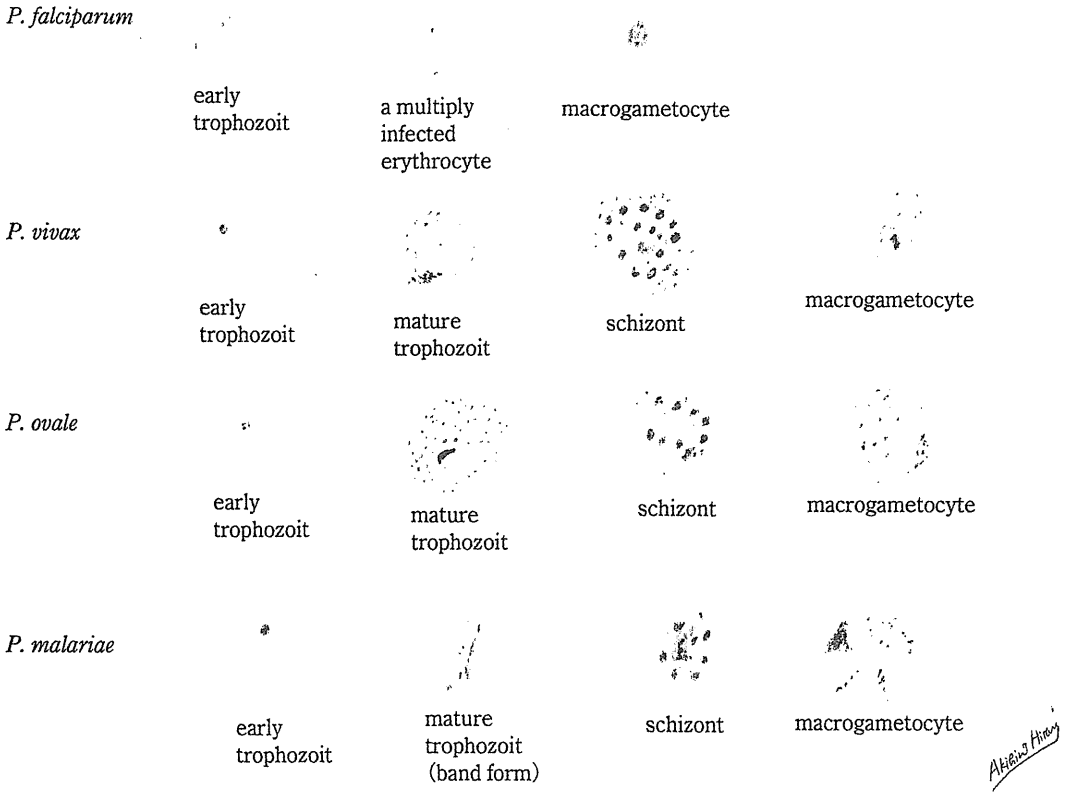

Figure 3 *Plasmodium* parasites: morphology of successive developmental stages in Giemsa-stained thin blood smears. (*This drawing is truly original and illustrated by the first author.)

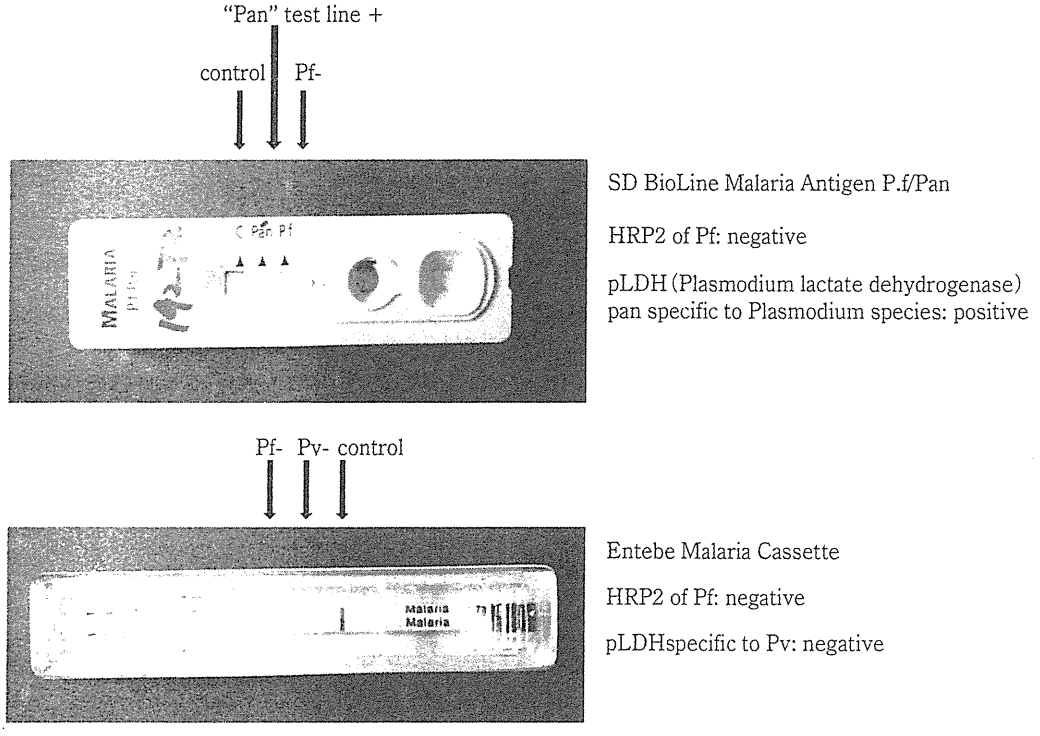

Figure 4 Reactivity to *Plasmodium ovale* malaria parasite in SD BioLine Malaria Antigen P.f/Pan and the Entebe Malaria Cassette.

バンドのみが薄く陽性に検出された。すなわちSDキットではPf陰性でPanが陽性，Entebeキットではpfとpvはともに陰性であった。したがってRDTのみによる解釈では，PfとPvではないマラリアということになる。この結果はnested PCRによる最終的な診断と一致した。本症例では，このようにRDTを組み合わせることにより，形態学的診断が難しいPoの診断の推測が可能であった。

最近，期待がもたれているRDTとしては，Binax NOW®がある。HRP-2とヒトに感染を起こす4種の原虫すべてに共通するaldolaseを検出する。このキットはサルマラリア原虫*Plasmodium knowlesi*のヒト感染の際もaldolaseを検出したとの報告がある[10]。他のRDTの操作も簡便であるが，このキットはさらに取扱いが易しく，判定までの時間は15分程度である。さらに試薬が一つであることが検査者の負担を軽減すると思われる。添付文書上，Pfの感度99.7%，特異度94.2%である。興味深いことに，Pfの原虫血症が高度な場合，それを半定量的に検出するマーカーとしての有用性が検証されつつある[11]。オランダのvan Goolらによる257名の海外渡航後のPf患者を対象とした研究によれば，栄養体が50,000/μl(赤血球の感染率＞1%)をこえる患者(n＝23)のすべてに，aldolaseとHRP2の両方が陽性に検出され，aldolase陰性でHRP2のみが陽性の場合は，「赤血球の感染率＞1%」を除外する信頼できるマーカーとなると示されている。本キットは我々も参加している「国内未承認薬の使用も含めた熱帯病・寄生虫症の最適な診療体制の確立」に関する研究班において既に使用されており，現在我が国の輸入症例に対して評価が行われている。帰国後診療を行う医療機関において将来的に導入することが望ましいと考えられる。

III. 結　　語

本症例のように種々の条件によりマラリア原虫の形態は変化に富む。今回我々は採血時期により早期栄養体が優勢に観察され形態学的診断が困難であった卵形マラリアを経験し，RDTの組み合わせにて診断を推測しnested PCRにて最終診断を行った。一般的にRDTの有用性は明らかであり顕微鏡法と組み合わせると診断能力の向上を図ることができる。これから増えていくと予測される帰国後診療を行う医療機関において，形態学的診断の更なる教育とRDTの導入を提案する。

nested PCRは国立国際医療研究センター研究所熱帯医学・マラリア研究部，狩野繁之先生にお願いしたので，ここに感謝する。

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帰国後診療機関を訪れた線虫症の一例

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Key Words: 糞線虫症，トラベルクリニック，帰国後診療，スクリーニング

諸言

糞線虫症は我が国では，奄美，沖縄になお多くの感染者が存在している．一方欧米では古典的に移民の疾患であった．しかし最近旅行者の輸入例が増加し注目されている．今回我々は，頻回に海外渡航を行う日本人旅行者に，感染経路を明白に決めることが困難な糞線虫症と思われる一例を経験したので報告する．

症例

症例：53 歳女性
主訴：鼓腸
既往歴：特記事項なし
現病歴：海外旅行が趣味で東南アジアを中心に年に 2−3 回の 2 週間程の旅行をしていた．2007 年にカナダ，南アフリカ，ジンバブエ，2008 年にベトナム，カンボジア，トルコ，台湾に渡航した．2008 年 10 月頃より腹部膨満感が出現したが特に気にしなかった．12 月に食品会社にパート勤務が決まり虫卵検査をおこなったところ，たまたま線虫が検出され聖隷横浜病院トラベルクリニックを受診した．
身体所見：特記事項なし
検査所見：WBC 4,380/mL，好酸球 12.1%以外に血液生化学で異常なし．HTLV-1 抗体（CLEIA）は陰性であった．新鮮な便を用いて集卵法を行い糞線虫の R 型幼虫，F 型幼虫と考えられる活発に動き回る虫体を数個確認した（×600 倍）．R 型幼虫と推測される虫体には短い口腔が確認され，食道には棍棒状の前部と狭窄した後中部，球部を観察することができた（図 1）．F 型幼虫と推測される虫体には長い食道を確認したが，撮影が不明瞭

A case of chronic strongyloidiasis in a Japanese tourist: the site where the infection was occurred was not identified

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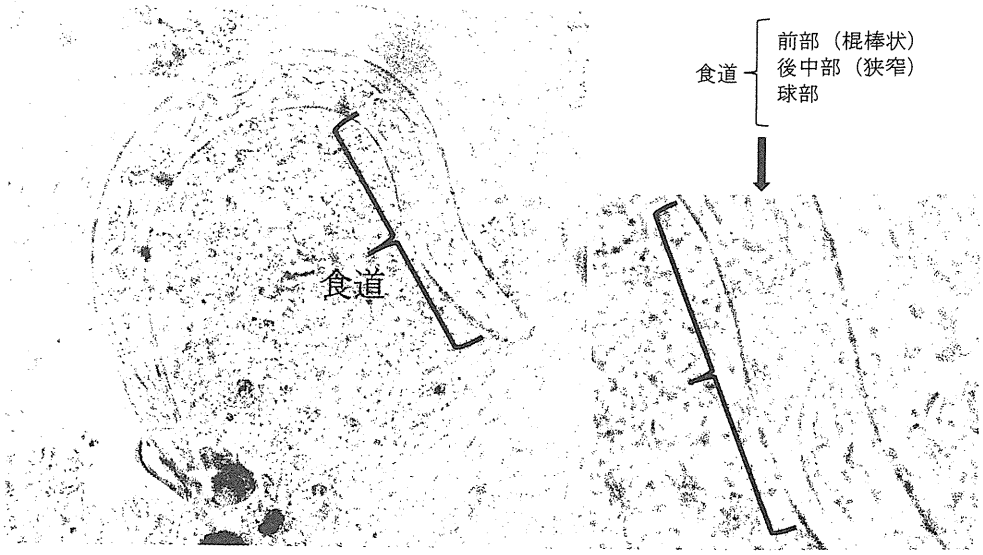
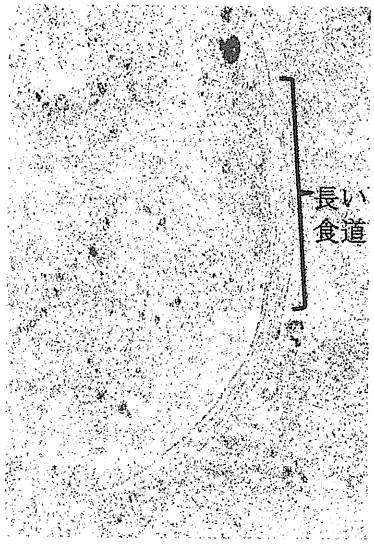


A: R 型幼虫疑い



B: F 型幼虫疑い

図 1: 本症例で検出した虫体

なため特徴的な尾端を確認できなかった（図 1）.

経過：写真にて，糞線虫症を扱う専門医療機関にコンサルテーションをおこなった．形態学的に糞線虫症の可能性が高いとのことで，ご本人に濾紙培養または寒天培養を勧告するも早期の治療を望まれた．2009 年 1 月 7 日と 21 日にイベルメクチン 12 mg を投与し，1 月 26 日の便より線虫は検出されなかった．

考察

本症例の診断と治療における問題点は，診断確定ができなかったことと感染経路が不明であった二点が挙げられる．まず観察された虫体は形態的に糞線虫の R 型幼虫と F 型幼虫と考えられたが，濾紙培養と寒天培養を施行できず診断の確定ができなかった．鑑別として鉤虫の幼虫または土壌

線虫（桿線虫）のcontaminationが考えられた．治療後の便より虫体は検出されなかったが，糞線虫症として治療を行ったため治療が効果的であったと断定することはできない．次に感染地域であるが，頻回の海外渡航と野菜が好きとのことで海外における感染の可能性を第一に考えたが，沖縄県出身とのことで区別が不可能であった．常に海外旅行に同行するという親友も検便を行ったが虫体は検出できなかった．

旅行者の糞線虫症に関して現時点で重要なことは，「①旅行者がリスク行動をあまり認知していない．②帰国後診療において医師が鑑別診断に想起することが少ない．」の二つである．

浜辺における行動のリスクは不明であるが，流行地で裸足やサンダルで歩くことはリスクがある[1]．最近出版された渡航医学の教科書では，「悪性腫瘍，ステロイド投与中，HTLV-1 感染などのリスクのある旅行者は裸足で歩かず特にぬかるみを避けるべきである[2]．」，「淡水，土壌との接触のある長期滞在した帰国者（若者）にとって重要な疾患[3]」との記載がある．最近のカナダ，スイスの調査より旅行者の例が増加しているが報告によりばらつきがある[4)-6)]．理由として便の直接塗抹法の感度が低く，また他の感度の高い検査法も普及していないため，診断確定が困難であることが考えられる．2008 年に新婚旅行から帰国したイタリア人 2 人が蕁麻疹様の皮疹，高熱，咳嗽，倦怠感にて入院となった．一人の便中にR 型幼虫が検出され血清学的に陽性となり，さらに二人とも便培養にて陽性であったため急性糞線虫症の輸入例と診断された．リスク行動としてたった一度のみタイのサムイ島にてバンガロー周囲の草地を裸足で歩行しており，それが原因として最も疑われた．同論文の考察では，今まで安全と考えられてきた地域での感染であり渡航前アドバイスの必要性を議論すべきであると提案されている[7]．

次にこの疾患を医師がどの程度認識しているかについて文献を検索すると，米国，ブラジル，シンガポール，タイの 363 名の研修医を対象とした調査において，研修医の糞線虫症の知識は乏しく特にその傾向は米国の研修医に顕著に見られた．喘息の既往がなく肺野に笛声音を聴取し好酸球増多を認める典型的な糞線虫症のシナリオが提示され，米国の研修医の 9%が寄生虫のスクリーニングが必要と答え，23%は更なる精査を行わずステロイドの使用を主張した．一方，その他の国の研修医の答えた比率はそれぞれ 56%，7%であり米国の研修医に比較して有意に良い成績であった（それぞれ $p<0.001$，$p=0.005$）[8]．我が国ではこのような調査は行われていないが，糞線虫症の啓蒙を行った方が適切と推測される．本疾患は診断確定が難しくステロイド投与などにて顕在化し致死的となるため，多くの専門家は，潜在的に重症糞線虫症を発症するリスクのある患者には presumptive treatment を推奨している[9, 10]．我々もこの点を踏まえ，診断確定は不可能であったが本症例に治療を行った．

今回，帰国者の診療において糞線虫症と考えられる症例を経験した．トラベルクリニックの帰国後診療において流行地からの帰国者では鑑別診断の一つとして検査を積極的に行うべきだが，スクリーニング検査をどの程度まで行うか明確な基準がないのが現状である．しかし一度感染すると潜在的に危険な疾患であるため積極的にスクリーニングを行っていく必要があると考えられる．また帰国後診療にあたる医師が診断を想起できるように生涯教育に組み入れ，さらに渡航前相談の際に旅行者にリスク行動について情報を提供するべきであると考える．

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病院薬剤師として服薬指導を行った アフリカ帰り卵形マラリアの一例

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Key Words: 卵形マラリア，メフロキン，プリマキン，病院薬剤師，渡航医学専門薬剤師

諸言

我が国のマラリア輸入症例は年間 52－109 例（2001-2008 年）であり，治療は特殊であるため，一般に医師・看護師をはじめとする医療従事者は治療に慣れていない．今回，我々は病院薬剤師が治療に介入することで医師・看護師・患者との情報の共有を達成し薬物治療の安全性を高めることに貢献した．一連の治療経過を報告し病院薬剤師が抗マラリア薬の服薬指導を行う際の一助としたい．

症例

46 歳男性

主訴：発熱，悪寒戦慄
既往：腎癌にて左腎摘出（35 歳）
職業：エンジニア（国際医療協力）

現病歴：

2011 年 5 月 4 日－31 日，ザンビアに滞在した．5 月 28 日より発熱あり 30 日に現地の医療機関を受診し迅速診断キットにてマラリア陰性，何らかの投薬を受けた．6 月 3 日に聖隷横浜病院トラベルクリニックを受診した．薄層塗抹標本にてマラリア原虫を認め（図 1），渡航地域と原虫の形態から卵形マラリアあるいは四日熱マラリアを考え入院となった．

A case of *Plasmodium ovale* malaria acquired in East Africa: the medication was supervised by the hospital pharmacist specialized in travel medicine

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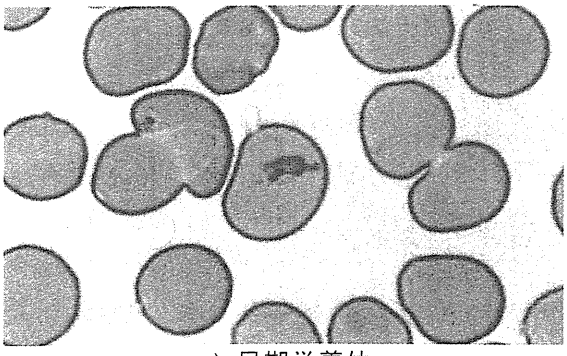
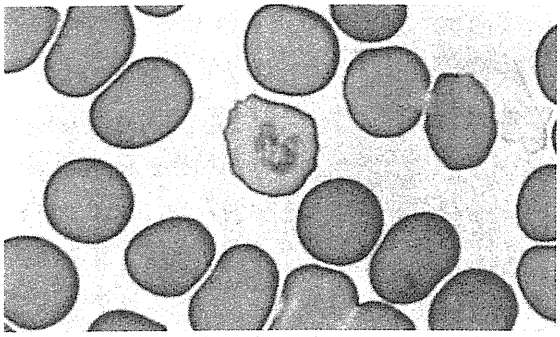


a) 早期栄養体



b) 後期栄養体（感染赤血球は卵形で鋸歯状縁を認めた.）

図1　本症例で観察された原虫の形態（×1000）

身体所見（入院時）：

体温 40.3℃，顔面の軽度浮腫あり，肝脾は触れず

検査所見（入院時）：

WBC:3870 /μL, RBC:396×10^4/μL, Hb:13.5 g/dL, Plt:3.3 × 10^4/μL, AST:29 IU/L, ALT:31 IU/L, γ-GTP:16 IU/L, ALP:163 IU/L, LDH:333 IU/L, CRP:3.1 mg/dL, BUN:15.2 mg/dL, Cre:1.39 mg/dL, parasite density:1857.6 /μL, 赤血球の原虫感染率:0.0469 %, 尿蛋白:1+, 尿潜血:陰性

経過と薬剤師の介入：

治療開始にあたり主治医と薬剤師でクロロキン入手について検討した．当院採用の抗マラリア薬はメフロキンのみであったこと，受診時の情報では服薬歴より熱帯熱マラリアとの混合感染が完全に否定できなかったことから，メフロキンで急性期治療を行い赤血球内の G6PD 活性を確認後プリマキンで根治療法を行うこととした．

メフロキン 275 mg 錠（塩基として 250 mg）を 3 錠－2 錠－1 錠と 8 時間おきに内服し症状は改善した．治療に伴い，parasite density の低下を認めた．メフロキン投与にあたり薬剤師は再度患者に既往歴と併用薬を聴取し禁忌事項の除外を行った．患者に本剤での治療の必要性，副作用の初期症状，十分な薬効を保つために食後に大量の水で服用することの重要性を伝えた．また主治医了承のもと看護師に副作用モニタリングとして心電図モニター装着，片腎であるため尿量と尿所見の確認を依頼した．

ヒト感染性マラリア原虫 4 種鑑別 nested PCR を行い卵形マラリア原虫の単純感染と診断された．根治療法に先立ち，赤血球内の G6PD 活性の測定を依頼し正常の活性であると確認した．プリマキンの使用に関しては当院の倫理委員会に治療内容を提示し承認された．12 月 19 日よりプリマキン塩基 15 mg/日を 14 日間投与し合併症は認められなかった．プリマキン投与にあたり薬剤師は患者に溶血発作のモニタリングとして尿の色を観察し尿が濃くなったら必ず受診すること，消化器症状副作用軽減のために必ず食後に服用することを指導した．

考察

本症例ではクレアチニン 1.39 mg/dL と腎機能の低下が問題となった．前述のごとく機能低下により溶血のリスクが高まるが 14 日間投与の後にとくに問題を認めなかった．頻繁に流行地に滞在する職種であり再度感染する可能性が高いと評価された．問診により今まではマラリア予防（防蚊対策，薬物予防）は行ってこなかったことが判明した．プリマキンの使用とマラリア罹患自体が

腎機能を悪化させる可能性があり，今後は防蚊対策と高度流行地では予防内服をおこなうように説明し服薬指導をおこなった．

プリマキンの副作用で最も重要なものはG6PD欠損症の罹患者に起こる溶血発作である．溶血の程度はG6PD欠損症の重症度とプリマキンの用量に依存する．発作は通常はself-limitedに治癒に至るが，G6PD活性が低いクラスⅡ（活性＜10%）の Gd^{Canton} 変異，クラスⅢ（10－60%の活性）の $Gd^{Mediterranean}$ 変異，GdA-変異では重症化することがあり注意が必要である[1]．日本人ではG6PD欠損症の頻度は0.1%と非常に低く，臨床症状を伴う例はさらに稀である[2]．アフリカ，東南アジア出身者ではG6PD欠損症の頻度が高く注意が必要である．肝機能，腎機能の低下と他の溶血を促す薬剤との併用によりさらに溶血をおこしやすくなる[1]．ときにG6PD活性の正常者においても溶血が起こることがある[3]．服薬指導の観点からは尿の色を観察し濃くなった場合は診察を受けるように指導をおこなう．さらに服薬期間が14日と長いことと悪心，嘔吐，腹痛などの消化器症状のためコンプライアンスの低下を招きやすい．消化器症状は空腹時に服用すると現れやすいので食後に服用するように服薬指導をおこなうとコンプライアンスを改善することができる．

今回，病院薬剤師として特殊な感染症に対する薬剤の管理と治療に参加し，より安全な治療を行うことに貢献できた．日頃から感染症専門医・指導医の在籍する医療機関と連携をとっておくことも大切である．渡航医学に興味がありスキルアップを計るならば日本渡航医学会のホームページを利用するのも良い[4]．スイス，ドイツのように薬剤師の渡航医学専門資格を設置している国もある．渡航医学と輸入感染症は今後我が国において薬剤師が力を発揮できる新しい分野であると考えられる．

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マラリアの最近の話題と院内伝播の危険性について

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Summary and Keywords

①ヒトに感染するマラリアは現在5種類ある．
②熱帯熱マラリアは致死的であり，診断に急を要する．
③マラリアの診断の基本はギムザ染色である．
④イムノクロマト法はマラリア診断の補助として有用である．
⑤針刺しによるマラリアの感染が報告されている．
⑥針刺し発生時には抗マラリア薬投与も考慮する．
⑦マラリアの予後判定にプロカルシトニンが有用である．

●マラリア　●イムノクロマト法　●針刺し　●抗マラリア薬　●プロカルシトニン

■マラリアとは？

マラリア原虫に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*），ヒトに感染するサルマラリア原虫（*P. knowlesi*）が知られている．ハマダラカ（*Anopheles* 属）によって媒介される．イエカやヤブカでは伝播しない．世界では100ヵ国以上で流行がみられ，死者は約80万人と推定されている．図1に示したように蚊からスポロゾイトが注入されるとすぐに肝細胞に寄生する．そこで分裂してメロゾイト（merozoite）と呼ばれる感染性の原虫が血中に放出され赤血球に感染する．三日熱マラリア原虫と卵形マラリア原虫は肝細胞内に休眠型（hypnozoite）が形成され再発の原因となる．熱帯熱や四日熱ではこの休眠型は作らない．赤血球内では発育し，赤血球が破壊されてメロゾイトが放出し，新しい赤血球に感染する．これら5種類のマラリアのうち熱帯熱マラリアは致死性であり，特にマラリア非流行地の免疫のない患者では注意を要する．

■マラリアの診断は？

マラリアの潜伏期は種によって異なるが熱帯熱では9～14日である．流行地から帰国後数ヵ月は注意が必要である（三日熱マラリアでは年単位）．発症初期はインフルエンザ様の症状，全身倦怠感，下痢などさまざまである．熱帯熱マラリアの発熱周期は24時間から48時間と一定していないことが多い．診断方法としては血液標本のギムザ染色が必須である．ギムザ染色はバッファーのpHを7.2～7.4で行う方が虫体の染まりがよい．抗原を検出するイムノクロマト法としては現在，数種類が入手可能である（輸入元：アリーア メディカル株式会社，株式会社東京未来スタイル，有限会社バイロテックなど）．どれも感度が高く少数寄生でも検出することができる．プロゾーン現象（原虫血症が多すぎて結果が陰性に出る現象）がみられることがあるので，ギムザ染色は必ず行う．ただし，イムノクロマトキットではサルマラリア

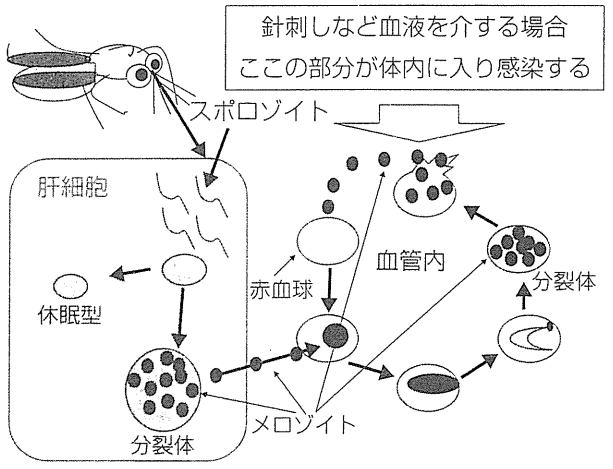

図1　マラリアの生活史

を別のマラリアと判定されるものがあるので注意が必要である．また現在マラリアのイムノクロマトキットは体外診断薬としては認可されていないことに注意する．あくまで補助として（研究用試薬）使用することが条件である．特殊な検査としてはポリメラーゼ連鎖反応（polymerase chain reaction，PCR）法があり，マラリアの種類の判別が難しい場合に専門機関に依頼することが可能である．

ICTが知っておくべき事柄

マラリアの感染は図1に示したようにハマダラカを介して伝播することが一般的であり，院内に生息する蚊（イエカなど）では伝播することは考えにくい．次に述べる通り，針刺しによる感染は起こりうるため注意する．

院内伝播の危険性

海外では実験室内での感染を含め針刺しによる感染の報告がなされている[1]．当然マラリア患者に対して生検などを行う場合にも注意が必要となる．マラリアに感染した血液が体内に入った場合，感染が成立する可能性が高い（図1）．実際米国ではマラリア患者の針刺しを通じて看護師が感染し，さらにその看護師が別の患者にマラリアを伝播した事例が報告されている[1]．またイタリアでも血糖測定器を介してのマラリアの患者間における病院感染事例もある[2]．したがって，マラリア患者あるいは疑い例が入院した場合には標準予防策，接触予防策を積極的に行う必要があると思われる．しかしながら，ほかの感染の合併がない限り（たとえば腸チフス），個室管理の必要はないと考えられる．

マラリア患者の血液に針刺しなどで曝露した場合の対応

マラリア患者の血液に曝露した場合の対応としては病院感染事例が少ないため定まったマニュアルはない．対処法として以下の3点が考えられる．

①マラリア以外の感染を除外することが重要である．海外からの帰国者の場合，腸チフスやデング熱，肝炎といった感染症の合併も想定されるため，これらに対する対応も考慮する．

②マラリアの感染を想定して予防的に治療を積極的に行う（表1）．

③経過観察を行い，発熱した場合にマラリアの診断を速やかに行い治療する．メフロキンによる治療（表1）のほか，国内では入手不可能な薬剤を「国内未承認薬の使用も含めた熱帯病・寄生虫症の最適な診療体制の確立」に関する研究

表1　薬剤の投与方法

抗マラリア薬はメフロキンを除いて，わが国では海外で使用されている薬剤は認可されていない．

＜処方例＞

メフロキン（Mefloquine：メファキン® [久光製薬株式会社]：275mg 錠 塩基 250mg）：成人 15～25mg 塩基 /kg を 2 回投与する（6～8 時間あける）．わが国では 15mg 塩基 /kg を初回投与し（体重 50kg で 3 錠）6～8 時間後に 10mg 塩基 /kg（同 2 錠）投与する場合が多い．小児は 25mg 塩基 /kg2 回投与（6～8 時間あける）[3]．

班[4]から薬剤を入手して使用する方法もある．ただし経過観察を行いマラリアの診断がつかずに重症化するおそれがある場合には，積極的に治療する方がよい場合もあると思われる．

マラリアの最近の話題

熱帯熱マラリア以外のマラリアでは経過も予後も良好であるが，熱帯熱マラリアでは診断治療の遅れで重症化し死亡する場合もある．早期の診断治療では後遺症を残さず完治する．最近，敗血症のマーカーとして注目されているプロカルシトニンの治療前の値が 25ng/mL 以上で予後が悪いとの報告もある[5]．

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ORIGINAL PAPER

Effect of Mirazid in *Schistosoma japonicum*-infected mice: parasitological and pathological assessment

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Abstract Conflicting reports are found in the literature about the antischistosomal efficacy of Mirazid (MZ), which is a special formulation of myrrh obtained from the stem of the plant *Commiphora molmol*. This initiated the present study to assess this drug for the first time in experimental schistosomiasis japonicum. Mice were divided into four groups: infected untreated control (I); infected treated with MZ, 500 mg/kg (II); infected treated with MZ, 250 mg/kg (III); and infected treated with praziquantel (PZQ), 200 mg/kg (IV). The drugs were given 7 weeks post-infection for five successive days. All animals were killed 3 weeks post-treatment. Results showed no signs of antibilharzial activity of MZ. Total worms, total tissue egg load, egg developmental stages, and granuloma area were not affected by any of the MZ treatment regimens as compared to the infected untreated group ($P > 0.05$ for all variables). These results were in contrast to those obtained in PZQ-treated animals in which 82.82 % total worm reduction, 94.62 % egg reduction, and 86.35 % granuloma area reduction were observed. Also, it significantly increased the percentage of dead ova and decreased the percentage of mature ova with complete absence of immature ones in comparison with the control group ($P<0.01$ for all variables). In conclusion, the results of the current study raise serious doubts about the antischistosomal activity of MZ.

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Introduction

Schistosomiasis is a chronic debilitating disease that continues to rank, following malaria, at the second position of the world's parasitic diseases in terms of prevalence, morbidity, and mortality rates. Currently, over 200 million people are estimated to be infected, while close to 800 million individuals are at risk of contracting the disease (Steinmann et al. 2006). At least 200,000 people die each year of schistosomiasis. The estimated burden of the disease is up to seven million disability-adjusted life years (King and Dangerfield-Cha 2008).

Due to the unavailability of a vaccine that is practically applicable to humans, the use of chemotherapy is the mainstay of schistosomiasis-associated morbidity control (Abdul-Ghani et al. 2009). For more than two decades, praziquantel (PZQ) has remained as the drug of choice for the treatment of the three common schistosome species, *Schistosoma mansoni*, *Schistosoma haematobium*, and *Schistosoma japonicum* (Doenhoff et al. 2008). Concern is mounting in public health medical circles that heavy reliance on a single drug for schistosomiasis control may promote the selection and spread of drug-resistant parasites (Caffrey 2007). There has been evidence of resistance to the praziquantel-based therapy and reports of acute disease manifestation; therefore, other drugs affecting different stages of the schistosome parasites life cycle and alternative therapeutic regimens should be developed and become accessible (Ribeiro-dos-Santos et al. 2006). As a result, the

need to develop alternative antischistosomal drugs has been stressed.

Mirazid (MZ) is a new antischistosomal drug introduced in the Egyptian market since 2001, in the form of gelatinous capsules produced by Pharco (Alexandria, Egypt). It is prepared from myrrh, which is an oleo-gum-resin obtained from the stem of the plant *Commiphora molmol* (Greene 1993). Myrrh contains the resin myrrhin (23–40 %), the volatile oil myrrhol (2–8 %), gum (40–60 %), and a bitter unidentified component (Claeson et al. 1991). It is a safe, natural flavoring substance and has been approved by the US Food and Drug Administration (Ford et al. 1992).

Myrrh is one of the oldest known medicines and was widely used by Ancient Egyptians (Badria et al. 2001). Traditionally, it has been used by Sumerians and Greeks to treat worms (Michie and Cooper 1991), by Chinese to relieve pain and swelling due to traumatic injury (lee and lam 1993), and by Somalians to treat stomach complaints, diarrhea, and wounds (Claeson et al. 1991; Michie and Cooper 1991). Tincture of myrrh is used for therapy of aphthous ulcer, treatment of sore throat and pharyngitis (Claeson et al. 1991), and reduction of cholesterol and triglycerides (Michie and Cooper 1991; Jain 1994). In experimental studies, the antitumor potential of myrrh was comparable with that of the standard cytotoxic cyclophosphamide (Al Harbi et al. 1994).

Many reports stated the efficacy of myrrh (Mirazid) as anthelmintic drug (Massoud et al. 2001, 2003, 2004, 2007; Sheir et al. 2001; Soliman et al. 2004; Al-Mathal and Fouad 2004; Haridy et al. 2004; Fathy et al. 2005) and antiprotozoa (Baghdadi and Al-Mathal 2010). Also, it has a molluscicide and cercaricide action (Allam et al. 2001; Massoud and Habib 2003) and mosquito larvicide action (Massoud and Labib 2000).

Over the last years, a debate is raised regarding the schistosomicidal effectiveness of MZ. Most of the published data documenting its antischistosomal activity against *S. mansoni* and *S. haematobium* consist of papers written by the discoverers of these properties (Badria et al. 2001; El Baz et al. 2003; Abo-Madyan et al. 2004; Massoud et al. 2004; Sheir et al. 2001; Soliman et al. 2004; Hamed and Hetta 2005). The mechanism of action of myrrh on the schistosome worms, as suggested by the manufacturer, is related to permanent loss of musculature of the worms leading to separation of male and female couples and their shift to the liver where destruction and phagocytosis take place (Badria et al. 2001). Only four groups of investigators reported low cure rates (Botros et al. 2004; Barakat et al. 2005; Ramzy et al. 2010; Osman et al. 2010).

Due to great controversy concerning the effect of myrrh on *S. mansoni* and *S. haematobium*, the current study was designed to assess the antischistosomal activity of MZ; in *S. japonicum*-infected mice in comparison with PZQ. Drug efficacy was evaluated on the basis of some parasitological and pathological criteria.

Materials and methods

Animals, parasites, and infection

All animal studies presented here were approved by the committee of animal rights and ethics, Tokyo Medical and Dental University, Tokyo, Japan, based on the institutional and national regulations for animal experimentation. Six-week-old female C57BL/6 mice were obtained from Japan SLC and used in this study. The animals were maintained on a standard commercial pellet diet and kept in air-conditioned animal house at 20–22 °C.

The life cycle of *S. japonicum* (Japanese strain) is maintained in our laboratory using ICR mice and *Oncomelania hupensis nosophora* (*Yamanashi* strain). Mice were percutaneously infected with 40 *S. japonicum* cercariae/mouse applied to the shaved abdomen.

Drugs

MZ (Pharco Pharmaceuticals Co., Alexandria, Egypt) was tested after resuspension of the content of the resinous capsules in 2 % Cremophore-EL (Sigma Chemical Co., St. Louis, MO, USA). Praziquantel (PZQ; Shin Poong Pharmaceutical Co., Ltd, Kyonggi, South Korea) was given as a freshly prepared suspension in 2 % Cremophore-EL.

Animal groups

In the present study, mice were randomly allocated into four groups each of seven mice:

Group I: Infected control and received the vehicle only.
Group II: Infected and treated with MZ 500 mg/kg.
Group III: Infected and treated with MZ-reduced dose 250 mg/kg.
Group IV: Infected and treated with PZQ 200 mg/kg.

All mice were deprived of food 1 h before treatment, drugs or vehicle were administrated orally using a ball-tipped feeding needle in a volume of 200 μl/mouse, and they were allowed to eat 1 h after treatment. The dosing protocols were given 7 weeks post-infection (p.i.) for 5 days. All animals were killed 3 weeks posttreatment. They were anesthetized using sodium thiopental and given heparin injection.

Study of parasitological criteria

After the mice were killed, hepatic and portomesentric vessels were perfused, using citrated saline. Recovered

schistosomes from each mouse were sexed and counted (Smithers and Terry 1965). The number of eggs per gram of hepatic and intestinal tissues was counted (Cheever 1968). Percentage of the different egg developmental stages (oogram pattern) was examined (Pellegrino et al. 1962).

Hepatic granuloma measurement

Liver specimens were fixed in 10 % formalin and processed to paraffin blocks. Sections (4 μm thick) were stained with hematoxylin and eosin. The size of granulomas was measured (30/mouse) using a VM-30 video micrometer (Olympus, Tokyo, Japan) and NIH image software (National Institute of Health, Bethesda, USA). Average granuloma area (square micrometer) was calculated for each mouse.

Statistical analysis

Comparison was made between each treated group and untreated control. The percentage of reduction between the treated group and the untreated control group was assessed using the formula: (mean value of the untreated group − mean value of the treated group) × 100/mean value of the untreated group. SPSS software version 17.0 was used for data analysis. Descriptive statistics including the mean ± standard deviation (SD) were used. Nonparametric Mann–Whitney test was used to test for significant differences between groups. The data were considered significant if P values were less than 0.05.

Results

Parasitological studies

Tables 1 and 2 show the effect of Mirazid using 500 and 250 mg/kg×5-dosing regimens in mice infected with the Japanese strain of *S. japonicum*. MZ did not show significant decrease in total females (mean ± SD=9.00±1.63 and 9.14±2.27 versus 8.29±1.98) or total worms (20.57±4.04 and 19.71±3.77 versus 19.15±4.22) when compared to the untreated control group, respectively. PZQ at a dose of 200 mg/kg×5 produced a highly significant female and total worm reduction (89.63 and 82.82 %, respectively). MZ regimens did not cause significant reduction in either the hepatic tissue egg count (55.02±20.82 and 82.74±22.83 versus 50.91±18.37/mg of tissue) or intestinal tissue egg count (199.52±113.44 and 189.33±62.26 versus 204.67± 90.55/mg of tissue) when compared to the untreated infected mice, respectively. While, PZQ reduced them significantly by 90.30 and 95.69 %, respectively. Eggs of all developmental stages were observed with MZ regimens. In the PZQ-treated group, no immature eggs were found, with marked reduction in mature eggs (9.86 %) and a marked increase in dead eggs (90.14 %) when compared with parallel values in the untreated controls (55.86, 35.0, and 9.14 %, respectively).

Granuloma measurements

Liver sections of infected untreated controls showed several cellular granulomas. Alternatively, when MZ was given using 500 mg/kg×5 regimen or the reduced dose, the liver pathology was similar to the infected untreated controls, with mean granuloma area of 150.14±8.07, 160.48±11.70, and 150.94±6.78 mm^2, respectively. However in the PZQ-treated group, the granulomas were less numerous and cellular compared with the control group, with highly significant reduction in mean granuloma area (20.60±1.33 78 mm^2), with percentage reduction of 86.35 % (Table 2).

Discussion

This work showed a striking discrepancy between the antischistosomal activity observed in this study and that reported by previous investigators for other schistosome species: *S. mansoni* (Badria et al. 2001; Sheir et al. 2001; Abo-Madyan et al. 2004; Massoud et al. 2004; Hamed and Hetta 2005) and *S. haematobium* (El Baz et al. 2003; Abo-Madyan et al. 2004). In the present study, we have tested two MZ dosing protocols (500 and 250 mg/kg for five consecutive days) in mice infected with *S. japonicum* (Japanese strain), and as a control it was compared with PZQ. Animals were treated 7 weeks p.i. and killed 3 weeks following treatment, which should allow the death of all drug-damaged worms.

Table 1 Effects of Mirazid (MZ) in comparison to praziquantel (PZQ) on worm burden in *S. japonicum*-infected mice 3 weeks posttreatment

Animal groups	Drug dose (mg/kg)	Total males	Total females	Total worms
Infected control	Vehicle	10.86±2.61	8.29±1.98	19.15±4.22
Infected + MZ	500×5	11.57±2.64*	9.00±1.63*	20.57±4.04*
Infected + MZ	250×5	10.57±1.72* (2.67)	9.14±2.27*	19.71±3.77*
Infected + PZQ	200×5	2.43±0.98** (77.62)	0.86±1.21** (89.63)	3.29±2.06** (82.82)

Values are expressed as means ± SD. Numbers between parentheses indicate the percentage reduction from infected control group

*P<0.01 (significant difference from PZQ 500×2 mg/kg); **P<0.01 (significant difference from infected control)

Table 2 Effects of Mirazid (MZ) in comparison to praziquantel (PZQ) on ova counts, oogram patterns, and granuloma area in *S. japonicum*-infected mice 3 weeks posttreatment

Animal groups	Drug dose (mg/kg)	Hepatic ova count×10^3	Intestinal ova count×10^3	Total tissue egg load×10^3	% immature ova	% mature ova	% dead ova	Granuloma area ($\mu m^2 \times 10^3$)
Infected control	Vehicle	50.91±18.37	204.67±90.55	255.58±90.54	55.86±2.41	35.00±1.91	9.14±1.46	150.94±6.78
Infected + MZ	500×5	55.02±20.82*, **	199.52±113.44** (2.52)	254.54±126.96 (0.41)	60.43±1.27**	29.57±0.53**	10.00±1.00**	150.14±8.07** (0.53)
Infected + MZ	250×5	82.74±22.83**, ***	189.33±62.26** (8.10)	272.07±73.47	59.29±1.11**	34.43±0.98**	6.30±0.95**	160.48±11.70**
Infected + PZQ	200×5	4.94±7.72**** (90.30)	8.82±14.28**** (95.69)	13.76±22.00**** (94.62)	0±0****	9.86±1.35****	90.14±1.35****	20.60±1.33**** (86.35)

Values are expressed as means ± SD. Numbers between parentheses indicate the percentage reduction from infected control group

*$P<0.05$ (significant difference from MZ 250×5 mg/kg); **$P<0.01$ (significant difference from PZQ 500×2 mg/kg); ***$P<0.05$; ****$P<0.01$ (significant difference from infected control)

In present work, no signs of antischistosomal activity of MZ were seen. No alterations in either the *S. japonicum* total worm burden or total tissue egg load were observed. Moreover, MZ failed to induce any alterations in the oogram pattern or in the granuloma area in comparison to those in untreated animals. These results are in agreement with few previous reports. Indeed, a multicenter investigation of the potential antischistosomal activity of different derivatives of the resin including the commercial preparation MZ was tested in mice and hamsters infected with Egyptian, Puerto Rican, or Brazilian *S. mansoni* strains. The drug was found toxic for mice at high doses and produced modest or no worm reduction at lower doses and the authors stated that they could not recommend the use of this drug in human cases of schistosomiasis (Botros et al. 2004). Moreover, Ramzy et al. (2010) reported that MZ given in a dose 500 mg/kg for 6 days to *S. haematobium*-infected hamsters showed very slight 3.4 % worm reduction. No change in the number of ova in tissues and slight reduction of 18.3 % in the number of stool eggs were found. Scanning electron microscopic examination of *S. haematobium* worms revealed intact tubercles, spines, and sensory bulbs and no effect of the ventral side

Our findings are in contrast with those of Badria et al. (2001), who reported 76 and 75 % worm reduction upon treatment of mice infected with an Egyptian strain of *S. mansoni* with myrrh at doses 250 and 500 mg/kg twice a day for 3 days. These investigators reported that these treatment regimens induced worm uncoupling and hepatic shift of female worms in a dose-dependent manner. They also revealed a marked increase (93 %) in mature eggs in MZ-treated mice with diminution up to complete absence of some of the immature egg stages. They did not report on the percentage of dead eggs, an increase of which can be considered as a hallmark effect for effective antischistosomals (Pellegrino et al. 1977). The percentages of dead eggs were 90.14 % in our PZQ-treated animals compared with 9.14 % in untreated controls and 6.30–10 % in those receiving MZ regimens. In other studies, when myrrh in a dose of 500 mg/kg/day for 5 days was given to *S. mansoni*-infected mice on the 21st or 45th day p.i., the percentage reduction of worm burden was 76.92 and 98.46 %, respectively, with marked reduction in the egg count in tissues. Significant decrease in the mean number and size of granulomas, paucity of eosinophils, decreased fibrosis and reticular fibers, and the restoration of the glycogen content in the hepatocytes were also reported (Massoud et al. 2004). Furthermore, oral dose of MZ as 600 mg/kg/day for 3 days, given to infected mice, also caused significant reduction in worm and ova count of 81.10 and 73.07 %, respectively (Hamed and Hetta 2005).

Although many observations concerning the antischistosomal activity of MZ appeared promising, we have failed to detect any antischistosomal activity with the commercially

obtained MZ in mice infected with the Japanese strain of *S. japonicum*. Therefore, this experimental study gives extra support to previously reported negative evaluation about the effectiveness of this drug in the treatment of schistosomiasis against many other published positive results. Based on the findings of this work, we cannot recommend the use of Mirazid in schistosomiasis patients.

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CD36-related protein in *Schistosoma japonicum*: candidate mediator of selective cholesteryl ester uptake from high-density lipoprotein for egg maturation

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ABSTRACT **Familial cholesteryl ester transfer protein (CETP) deficiency is more common in some East Asian populations than elsewhere, suggesting the possibility of a selective advantage of this genetic defect against regional infectious diseases. Historically, infection with the Asian blood fluke *Schistosoma japonicum* has been endemic in these regions, including Japan. We previously reported that eggs of *S. japonicum* require cholesteryl ester uptake from normal high-density lipoprotein (HDL) but not from CETP-deficient HDL for their maturation to miracidia, a critical step of the hepatic pathogenesis of schistosomiasis. Herein we show that cholesteryl ester uptake is selective from HDL, and identified CD36-related protein (CD36RP) as a candidate to mediate the reaction. CD36RP was cloned from the adult and the egg developmental stages of *S. japonicum*, with 1880 bp encoding 506 amino acid residues exhibiting the CD36 domains and two transmembrane regions. Using antibodies against recombinant peptides representing the potential extracellular domains of CD36RP, Western blotting detected a protein with a molecular mass of 82 kDa in the particulate fraction of the adult parasite cells, which was reduced to 62 kDa after *N*-glycanase treatment. The extracellular domain peptide bound human HDL, as established by immunoblots following nondenaturing gel electrophoresis. Antibodies against the extracellular domain suppressed HDL cholesteryl ester uptake and maturation of the eggs *in vitro*. CD36RP is a candidate receptor on eggs of *S. japonicum* that facilitates uptake of HDL cholesteryl ester necessary for egg embryonation and maturation.—Okumura-Noji, K., Miura, Y., Lu, R., Asai, K., Ohta, N., Brindley, P. J., Yokoyama, S. CD36-related protein in *Schistosoma japonicum*: candidate mediator of selective cholesteryl ester uptake from high-density lipoprotein for egg maturation. *FASEB J.* 27, 1236–1244 (2013). www.fasebj.org**

Key Words: CETP deficiency · miracidium · CETP · embryonation · hepatic granulomatosis · HDL

THE MAJOR AND FATAL PATHOGENESIS of schistosomiasis due to infection with *Schistosoma japonicum* or *Schistosoma mansoni* is ectopic implant of the eggs in the liver *via* the portal blood flow and their intrahepatic maturation to miracidia to cause hepatic granulomatogenesis and, accordingly, hepatic cirrhosis (1–5). Schistosomes take up lipids as their nutrient sources from the host blood plasma lipoproteins, and the receptors for low-density and very low density lipoproteins (LDLs and VLDLs) that mediate this interaction have been identified on their surfaces (6–9). On the other hand, it is not yet clear whether or not schistosomes use high-density lipoprotein (HDL) lipids as a nutrient source. In this regard, we previously showed that eggs of *S. japonicum* in culture require the presence of HDL to grow and develop to miracidia. Notably, maturation of schistosome eggs was significantly retarded when they were incubated with HDL from homozygous cholesteryl ester transfer protein (CETP)-deficient patients (10). In addition, expression of the CETP transgene significantly enhanced this process in mice that lack endog-

Abbreviations: apoA-I, apolipoprotein A-I; apoB-LP, apoB-containing lipoprotein; CD36RP, CD36-related protein; CETP, cholesteryl ester transfer protein; EndoH, endoglycosidase H; GSH, reduced glutathione; GST, glutathione *S*-transferase; HDL, high-density lipoprotein; LDL, low-density lipoprotein; *N*-acetylglucosaminidase, *N*-acetyl-β-D-glycosaminide-*N*-acetylglucosamino-hydrolase; *N*-glycanase, peptide-*N*-glycosidase F; PBS, phosphate buffered saline; SR-BI, scavenger receptor BI; VLDL, very low density lipoprotein

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enous CETP activity in plasma (10). These findings indicate that there is a specific pathway available to eggs of *S. japonicum* to utilize mammalian HDL lipid, including cholesterol or cholesteryl ester, to grow to miracidia. The abnormal HDL generated in the CETP-deficient plasma, large and cholesteryl ester-rich HDL, may not be a good substrate for such reactions. Thus, CETP deficiency may render humans resistant to hepatic maturation of eggs of *S. japonicum*, a phenomenon central to liver pathology characteristic of schistosomiasis japonica. We proposed that this could be a background behind the high prevalence of CETP deficiency in Far Eastern Asia (10–14).

Accordingly, we have undertaken an investigation to locate specific mediators of the schistosome parasite involved in interaction with human HDL lipids. We cloned a cDNA composed of 1880 bp encoding 506 aa that includes the CD36 domains and two transmembrane domains. Whereas functional expression of this protein remains to be accomplished, we now demonstrate binding of the proposed extracellular domain of a schistosome CD36-like glycoprotein to HDL, and suppression of cholesteryl uptake and maturation of the eggs by the antibody against this domain.

MATERIALS AND METHODS

Reagents commercially purchased or previously established

Anti-scavenger receptor BI (SR-BI) rabbit antisera (NB 400-101) was purchased from Novus Biologicals, Inc. (Littleton, CO, USA). Anti-apolipoprotein A-I (apoA-I) antibody was raised with rabbit against human apoA-I. Peptide-*N*-glycosidase F (*N*-glycanase) was purchased from PROzyme, Inc. (Hayward, CA, USA),endoglycosidase H (EndoH) was from Seikagaku Corp. (Tokyo, Japan), and *N*-acetyl-β-D-glycosaminide-*N*-acetylglucosamino-hydrolase (*N*-acetylglucosaminidase) was from Calbiochem (Berlin, Germany). Anti-glutathione *S*-transferase (GST) antibodies were purchased from GE Healthcare (Piscataway, NJ, USA), and anti-RGS-His antibodies were from Qiagen (Valencia, CA, USA).

Parasites and egg embryonation *in vitro*

S. japonicum (Yamanashi strain) was maintained by passage through *Oncomelania nosophora* and BALB/C mice (15,16). The pairs of adult worms were recovered from the portal vein of the infected mice and cultured as 1 pair/well of 12-well culture plates in RPMI 1640 medium supplemented with 5 or 10% human serum in 5% CO_2 atmosphere, as described previously (10). C57Black/6J mouse serum or lipoprotein-depleted serum was also used as a supplement in some experiments. For egg culture, the adult worms were removed from the wells after 2 d; eggs left in the wells were incubated further for 8 d in the same medium with or without 10% serum. After incubation for 10 d, the eggs were collected, the numbers of miracidia were counted microscopically, and the percentage of maturation was estimated as a maturation/embryonation rate.

Cholesterol uptake from [^{3}H], [^{14}C] double-labeled HDL and binding of [^{3}H]- or [^{125}I]-HDL by *S. japonicum* eggs

HDL and apoB-containing lipoprotein (apoB-LP, including LDL and VLDL) fractions were prepared from fresh human serum as density fractions at 1.063–1.21 g/ml and <1.063 g/ml, respectively, by sequential ultracentrifugation and labeled differentially with [1α,2α-^{3}H]cholesteryl ester and [4-^{14}C]cholesterol (Amersham, Piscataway, NJ, USA) as described previously (10). No [^{14}C] cholesteryl ester was detected in lipoproteins after labeling. ^{125}I-labeled HDL was prepared as described previously (17), using ^{125}I (Amersham) and iodine chloride. Total and free cholesterol in the labeled lipoproteins were measured by Determiner L reagents (Kyowa Medex Co. Ltd., Tokyo, Japan). *S. japonicum* eggs were collected from the homogenates of the liver and the intestine of the *S. japonicum*-infected BALB/C mice by the digestion method (18), and incubated with [^{3}H], [^{14}C] double-labeled lipoproteins (70–280 μg cholesterol/ml) in 0.5 ml RPMI 1640 in 12-well plates for 24 h at 37 or 4°C in 5% CO_2 atmosphere. The eggs were collected by centrifugation and washed with phosphate buffered saline (PBS), and the radioactivity of ^{14}C and ^{3}H in the egg pellet was analyzed. Uptake of free and esterified cholesterol was estimated by counting uptake of ^{14}C and ^{3}H radioactivity, respectively (10). Active uptake was determined by the difference between the results at 37 and 4°C. To observe selectivity of the lipid uptake, 10 vol of cold HDL or apoB-LP was added before adding the labeled HDL. HDL binding was also studied in parallel with [^{3}H] cholesteryl ester-labeled HDL and ^{125}I-labeled HDL. Use of human plasma lipoprotein was justified by institutional guidelines and approval of Nagoya City University.

RNA isolation, cDNA synthesis, and PCR amplification

Total RNA was extracted from the adult *S. japonicum* by using Isogen (Nippon Gene, Toyama, Japan). From 1 or 2 μg of total RNA, first-strand cDNA was synthesized by a SuperScriptII RT-PCR system (Invitrogen, Carlsbad, CA, USA) with random hexamer primers, according to the manufacturer's instruction. To search a new CD36 family protein in *S. japonicum*, the sequence of coding region of Sj-Ts2 protein consisting of 671 bp (Genbank AF291715), which appeared to have one of the CD36 domains defined by Prodom (release 2001.3; http://prodom.prabi.fr) analysis of the U.S. National Center for Biotechnology Information (NCBI) database, was used for preparation of the hybridization probes for Northern blot analysis and for screening of the *S. japonicum* adult cDNA library. First-strand cDNA was amplified by PCR using the specific primers for Sj-Ts2 protein: sense, 5′-TAATGAAATG-AATACAGTC-3′; antisense, 5′-AACAAACATATAATGACAAT-3′; and for GAPDH: sense, 5′-TGTACTCCGTGCAGCTTTTC-3′; antisense, 5′-AATGGATCCCTCTCGCAGTA-3′ (synthesized by Hokkaido System Science, Sapporo, Japan). The PCR products (488 and 198 bp) were purified by gel extraction and ligated in pGEM-T Easy vector (Promega, Madison, WI, USA). Ligation products were transformed into DH10B competent cells, and the sequences of inserts of positive clones were analyzed with T7 primer by using Applied Biosystems 3100 DNA sequencer (Applied Biosystems, Foster City, CA, USA). The 488-bp PCR product corresponded to nucleotide positions 5–492, consisting of coding region of Sj-Ts2 protein mRNA. For screening of the *S. japonicum* egg cDNA library, another probe was obtained by PCR using the specific primers for CD36-related protein (CD36RP): sense, 5′-CCGT-GAAAAACGTTTGAAGC-3′; antisense, 5′-AACATCATTGGATT-GATGGCTA-3′. The resulting PCR product size was 1177 bp. To analyze the size of the coding region of egg CD36RP, first-strand cDNA from *S. japonicum* adults or eggs was amplified by PCR using the 5′ primer with *Kpn*I site addition (5′-GCGTGGTACCTCTTGTACACGATGATATCTCG-3′) and GSP2 primer (below). PCR analysis with the center-region primer was also carried out: sense (L1), 5′-CCGTGAAAAACGT-

TTGAAGC-3′; antisense (R3), 5′-GTGCACCAGGTTGACATGA-3′. The PCR product corresponds to positions 321–1060 of CD36RP. Quantitative RT-PCR analysis was performed in a 7300 Realtime PCR System (Applied Bioscience) by using probe sets of 5′-primer GSP2 and L1-GSP2.

Northern blot analysis

Total RNA (10 μg) from mixed-sex adult *S. japonicum* worms was electrophoresed in 1.0% agarose-formaldehyde gel and transferred to nylon membranes. The 489-bp fragment of Sj-Ts2 cDNA and 198-bp fragment of *S. japonicum* GAPDH cDNA were purified by SigmaSpin column (Sigma-Aldrich, St. Louis, MO, USA) and labeled with [α-^{32}P]dCTP by using Klenow fragment, *Escherichia coli* DNA polymerase I (Takara Bio, Otsu, Japan), and hybridized to the membrane in a 5× SSPE hybridization solution containing 20% formamide at 42°C for 16 h. After washing, hybridization signals were detected by autoradiography on X-Omat film (Eastman Kodak, Rochester, NY, USA).

Screening and sequencing of CD36-related protein

Two cDNA-libraries (2×10^4 pfu) derived from *S. japonicum* adult (China) and eggs (Philippine) (19) were amplified using *E. coli* XL1-blue host cells (Stratagene, La Jolla, CA, USA) followed by plating and incubating at 37°C overnight. Plates were transferred to nylon membrane (Hybond-N+; Amersham), after which membranes were hybridized to the ^{32}P-labeled 488-bp probe (for *S. japonicum* adult) or 1177-bp probe (for *S. japonicum* eggs) in similar fashion as for the Northern blot analysis (above), except that 6× SSC solution containing 15% formamide was employed. Secondary screening was carried out after selection of positive phage plaques, and the final positive plaques were excised into SOLR cells by using the pBluescript II phagemid vector kit (Stratagene). Sequence analysis of the inserts was performed by using T3, T7, and the specific primer Sj-Ts2. For determination of 5′ end sequencing, 5′ RACE was carried out by the Gibco 5′RACE system, ver.2 kit (Gibco, Carlsbad, CA, USA), using two primers: GSP1, 5′-ATTGAATCCATGCGTTGACA-3′; GSP2, 5′-AGAAACCATGGCATTGAATTG-3′. The nucleotide sequence (1892 bp) and amino acid sequence (506 aa) of the product have been assigned Genbank accession no. AY496973 and termed CD36RP of *S. japonicum* (Supplemental Fig. S1).

Recombinant CD36RP

To prepare the antigens for anti-CD36RP antibodies, cDNA from *S. japonicum* adults was amplified by PCR with specific primers: sense, 5′-ATGGTAGTGATGGAACATT-3′; antisense, 5′-ATTGGTAGAAGAGTAGTTGA-3′. This PCR product, corresponding to the positions 797–1280 and coding the predicted extracellular half region, Ex160 (aa G249-P408, 160 residues) of CD36RP (see **Fig. 7**), was ligated first into pGEM-T Easy and then into the bacterial expression vector, pQE30 (Qiagen). For analysis of lipoprotein binding, a shorter discrete extracellular fragment of CD36RP, Ex121 (aa G249-Y369, 121 residues; see Fig. 7) was expressed as a GST-fusion protein in *E. coli*, BL21 with pGEX-6p vector (Amersham). GST-fusion proteins of full size CD36RP and Ex121 in the lysates of BL21 cells were adsorbed to reduced glutathione (GSH)-Sepharose gels, and the GST-free proteins were obtained in the supernatant in 50 mM TBS, 1 mM DTT, and 1 mM EDTA from GSH-Sepharose gels by treatment with PreScission Protease (GE Healthcare), according to the protocol of the kit.

Raising antibodies against CD36RP

The expression and purification of this His-tagged protein product (Ex160), as well as immunization of rabbits with Ex160, were carried out by Medical and Biological Laboratory. Co. Ltd. (MBL; Nagoya, Japan). An IgG fraction was affinity purified from polyclonal antiserum against Ex160 for Western blotting. Peptides 331-348, CQPGAPIVVSQPHFLNAN, amino acid residues 331-348 of CD36RP were synthesized and used by MBL to immunize rabbits.

Homology search

Homology and structural analysis of predicted polypeptides was carried out by using BLAST-X (20), BLOCKS (21), PROSITE motif analysis (22), and PHD searching (23) from the NCBI database.

Western blotting analysis

S. japonicum adult worms and mouse liver were sonicated for 5 s in hypotonic 50 mM phosphate buffer (pH7.4), containing protease inhibitor cocktail (Sigma-Aldrich). For preparation of the particulate fraction of *S. japonicum* eggs, freeze-thawing and sonication were repeated 10 times. After removing cell debris and nuclei by centrifugation at 1000 rpm for 5 min, the supernatant was further treated by centrifugation at 90,000 rpm for 30 min (Himac model CS120GX centrifuge; Hitachi, Tokyo, Japan). Both the pelleted particulate fraction and the supernatant cytosol fraction were solubilized in 1% SDS sample buffer and subjected to 10% SDS-PAGE, after which gel contents were transferred to polyvinylidene fluoride membrane (Bio-Rad). Western blotting analysis was carried out using 1:1,000 dilution of the rabbit anti-Ex160 IgG or anti-SR-BI antiserum and 1:10,000 horseradish peroxidase-conjugated goat anti-rabbit IgG secondary antibody. For detection of recombinant protein Ex121, a 1:500 dilution of rabbit anti-peptide 331-348 (anti-P) antibody was used. A 1:200,000 dilution of the anti-human apoA-I rabbit serum was used for detection of apoA-I in HDL.

Deglycosylation of particulate fractions

The particulate fractions of *S. japonicum* adults (48 μg protein) and mouse liver particulate fractions (100 μg protein) were suspended and heated at 100°C in the denaturing solution containing 0.1% SDS and 50 mM β-mercaptoethanol, and after addition of 0.75% Nonidet P-40 detergent, reacted with 10 mU *N*-glycanase in 50 μl for overnight at 37°C or room temperature, according to the manufacturer's protocol. In some cases, 35 mU *N*-acetyl-glucosaminidase was added to the reaction mixtures. The particulate fraction of *S. japonicum* eggs was incubated with 10 mU EndoH at 37°C for 1 h. Subsequently, reaction mixtures were solubilized with SDS sample buffer and subjected to SDS-PAGE for Western blot analysis.

Lipoprotein binding of recombinant Ex121

Ex121 preparation (1 or 2 μl) was incubated with 5–30 μg (protein) of human HDL or LDL in 8–12 μl PBS at room temperature for 30 min, and then mixed with native sample buffer (62 mM Tris-HCl, pH 6.8; 10% sucrose; and 0.1% bromphenol blue), followed by application to nondenaturing PAGE with 4%–20% gradient Tris-Gly gel (Invitrogen). Two parallel samples were run; one was analyzed with the anti-P antibody and the other with the anti-apoA-I antibody. The