References

1. World Health Organization. Western Pacific Region. Accessed September 1, 2010 at http://www.wpro.who.int/countries/countries.htm.
2. Davis X, MacDonald S, Borwein S, et al. Short report: Health risks in travelers to China: the GeoSentinel experience and implications for the 2008 Beijing Olympics. Am J Trop Med Hyg 2008;79(1):4–8.
3. Mahara F. Rickettsioses in Japan and the Far East. Ann N Y Acad Sci 2006;1078:60–73.
4. World Health Organization. Western Pacific Region. Accessed September 1, 2010 at http://www.wpro.who.int/countries/2009/kor/health situation.htm.
5. Chien YC, Jan CF, Kuo HS, Chen CJ. Nationwide hepatitis B vaccination program in Taiwan: effectiveness in the 20 years after it was launched. Epidemiol Rev 2006;28(1):126–35.
   Ebright JR, Altantsetseg T, Oyungerel R. Emerging infectious diseases in Mongolia. Emerg Infect Dis 2003;9(12):1509–15.
   World Health Organization. Western Pacific Region. Accessed September 1, 2010 at http://www.wpro.who.int/countries/2009/mog/health_situation.htm.
   Ji Y, Zhang Y, Songtao X, et al. Measles resurgence associated with continued circulation of genotype H1 viruses in China, 2005. Virol J 2009;6:135.
   Arikawa J, Yoshimatsu K, Thang TU, Ninh TU. Hantavirus infection—typical rodent-borne viral zoonosis. Trop Med Health 2007;35:55–9.
   WHO. Multidrug and Extensively-Drug Resistant TB (M/XDR-TB): 2010 Global Report on Surveillance and Response. Geneva: WHO, 2010.
   Gao X, Nasci R, Liang G. The neglected arboviral infections in mainland China. PLoS Negl Trop Dis 2010;4(4):e624.
   Wang LH, Fu SH, Wang HY, et al. Japanese encephalitis outbreak, Yuncheng, China, 2006. Emerg Infect Dis 2007;13(7):1123–5.
6. Guan Y. National Institute of Parasitic Diseases, China CDC. Available from http://www.actmalaria.net/downloads/pdf/info/2009/China.pdf.
14. Mendsaikhan J, Watt JP, Mansoor O, et al. Childhood bacterial meningitis in Ulaanbaatar, Mongolia, 2002–2004. Clin Infect Dis 2009;1(48, Suppl. 2):141–6.
15. Tick-Borne Encephalitis among U.S. Travelers to Europe and Asia 2000–2009. MMWR. Case 4. Accessed August 5, 2010 at http://www.cdc.gov/mmwr/preview/mmwrhtml/mm5911a3.htm.
16. Tsang K, File T. Respiratory infections unique to Asia. Respirology 2008;13(7):937–49.
17. Chen J, Varma A, Diaz M, Litvintseva A, Wollenberg K, Kwon-Chung K. *Cryptococcus neoformans* strains and infection in apparently immunocompetent patients, China. Emerg Infect Dis 2008;14(5):755–62.
18. Zhou XN, Guo JG, Wu XH, et al. Epidemiology of schistosomiasis in the People's Republic of China, 2004. Emerg Infect Dis 2007;13(10):1470–6.
19. Tucker J, Chen XS, Peeling R. Syphilis and social upheaval in China. N Engl J Med 2010;362:1658–61.
20. Yu XJ, Liang MF, Zhang SY, et al. Fever with thrombocytopenia associated with a novel Bunyavirus in China. N Engl J Med 2011 (in press).
21. Zhang R, Eggleston K, Rotimi V, Zeckhauser F. Antibiotic resistance as a global threat: evidence from China, Kuwait and the United States. Global Health 2006;2:6.
22. Ding JG, Sun QF, Li KC, et al. Retrospective analysis of nosocomial infections in the intensive care unit of a tertiary hospital in China during 2003 and 2007. BMC Infect Dis 2009;9:115.
23. Kim S, Lee NY. Epidemiology and antibiotic resistance of group A streptococci isolated from healthy schoolchildren in Korea. J Antimicrob Chemother 2004;54(2):447–50.
24. Lee K, Jang SJ, Lee HJ, et al. Increasing prevalence of vancomycin-resistant *Enterococcus faecium*, expanded-spectrum cephalosporin-resistant *Klebsiella pneumoniae* and imipenem-resistant *Pseudomonas aeruginosa* in Korea: KONSAR Study in 2001. J Korean Med Sci 2003;18(5):655–61.

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Efficacy and safety of atovaquone–proguanil in treating imported malaria in Japan: The second report from the research group

Mikio Kimura [a,*], Michiko Koga [b], Tadashi Kikuchi [b], Toshiyuki Miura [b,1], Haruhiko Maruyama [c]

[a] Department of Internal Medicine, Shin-Yamanote Hospital, Japan Anti-Tuberculosis Association, Tokyo, Japan
[b] Division of Infectious Diseases, Advanced Clinical Research Center, Institute of Medical Science, University of Tokyo, Tokyo, Japan
[c] Department of Infectious Diseases, Division of Parasitology, Faculty of Medicine, University of Miyazaki, Miyazaki, Japan

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ABSTRACT

Malaria remains an important health risk among travelers to tropical/subtropical regions. However, in Japan, only 2 antimalarials are licensed for clinical use — oral quinine and mefloquine. The Research Group on Chemotherapy of Tropical Diseases introduced atovaquone–proguanil in 1999, and reported on its excellent antimalarial efficacy and safety for treating non-immune patients with uncomplicated *Plasmodium falciparum* malaria (20 adult and 3 pediatric cases) in 2006. In the present study, additional cases of malaria were analyzed to confirm the efficacy and safety of this antimalarial drug. Fourteen adult and 2 pediatric cases of *P. falciparum* malaria and 13 adult cases and 1 pediatric case of *P. vivax/ovale* malaria were successfully treated with atovaquone–proguanil, including 3 *P. falciparum* cases in which the antecedent treatment failed. Two patients with *P. vivax* malaria were treated twice due to primaquine treatment failure as opposed to atovaquone–proguanil treatment failure. Except for 1 patient with *P. falciparum* malaria who developed a moderate liver function disturbance, no significant adverse effects were observed. Despite the intrinsic limitations of this study, which was not a formal clinical trial, the data showed that atovaquone–proguanil was an effective and well-tolerated therapeutic option; licensure of this drug in Japan could greatly contribute to individually appropriate treatment options.

1. Introduction

Malaria is one of the most important, potentially fatal, health problems among travelers from industrialized countries who visit regions with endemic malaria, affecting approximately 10,000 European and North American travelers annually [1]. A study of imported malaria in selected European countries and the United States revealed case fatality rates of 0%–3.6%, averaging 1.6%, for *Plasmodium falciparum* malaria [2]. Treatment is becoming increasingly difficult due to the widespread drug resistance of *P. falciparum*, and the occasional drug resistance of *P. vivax*. Atovaquone–proguanil (Malarone, GlaxoSmithKline) is a fixed-dose combination of 250 mg of atovaquone and 100 mg of proguanil hydrochloride that was originally developed for treating drug-resistant *P. falciparum* malaria. Since its investigational use in endemic areas, such as Africa and Southeast Asia in the 1990s, this agent has been used extensively for prophylaxis and treatment of malaria among travelers. To date, this agent has been well tolerated and highly effective, with only occasionally reported cases of treatment failure.

In Japan, oral quinine and mefloquine are the only licensed antimalarial drugs, raising concerns that patients may follow an unfavorable clinical course if they do not tolerate these drugs or their illness responds poorly. The Research Group on Chemotherapy of Tropical Diseases, Japan, of which the authors are principal members, advocates the use of medicines that are not nationally licensed for tropical and parasitic diseases [3,4]. This system is indispensible for the appropriate treatment of Japanese patients who contract exotic diseases for which formal compassionate drug use protocols are not available [5]. Atovaquone–proguanil, which is one of those medicines, was imported in 1999, and our experience with this antimalarial drug was reported in 2006. Atovaquone–proguanil was found to be efficacious and safe when used in non-immune patients with uncomplicated *P. falciparum* malaria [4]. Here, we report on additional cases of malaria treated with atovaquone–proguanil, including those due to *P. vivax/ovale* infection.

2. Patients and methods

2.1. The research group and the use of medicines

The Research Group on Chemotherapy of Tropical Diseases was established in 1980 and is currently funded by the Ministry of Health,

* Corresponding author at: Department of Internal Medicine, Shin-Yamanote Hospital, Japan Anti-Tuberculosis Association, Suwa-cho 3-6-1, Higashi-Murayama, Tokyo 189-0021, Japan. Tel.: +81 42 391 1425; fax: +81 42 391 5760.
E-mail address: kimumiki@abox3.so-net.ne.jp (M. Kimura).
[1] Present address: Department of Medical Affairs, ViiV Healthcare K.K. Sendagaya 4-6-15, Shibuya-ku, Tokyo 151-8566, Japan.

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Labour and Welfare in Japan. The group has introduced nationally unlicensed medicines such as those against amebiasis, leishmaniasis, trypanosomiasis, fasciolosis, and cryptosporidiosis. In addition to atovaquone–proguanil (purchased from John Bell & Croyden Ltd., London, UK), the group has also introduced other antimalarial drugs, including chloroquine, artemether–lumefantrine, primaquine, injectable quinine, and rectal artesunate [3]. The quality of these medicines was examined at the National Institute of Health Sciences, Tokyo, especially upon their first introduction. Some antimalarials are readily available at 25 registered medical facilities distributed throughout the country. This enables the appropriate treatment of patients, without significant delay, in any area of the country. Other medicines are provided, upon request, to those registered facilities from a central storage facility at the Institute of Medical Science, University of Tokyo, Tokyo.

Members of the registered medical facilities have obtained approval for participating in this program from the research ethics committee of each facility. The use of these unlicensed drugs at the registered facilities is allowed only after obtaining the patient's informed consent that clearly states that the drugs are not licensed in Japan. In exceptional cases, when a patient cannot be referred to one of the registered facilities, e.g., due to disease severity, drugs have been used outside of the registered medical facilities on a humanitarian basis. Following treatment, the physicians-in-charge complete the patient records that were formulated by the research group. Since August 2009, those unlicensed medicines have been used in accordance with the Ethics Guidelines for Clinical Research, Ministry of Health, Labour and Welfare, Japan (July 31, 2008). Clinical research insurance is made available to cover unexpected health damages that may occur with the use of those medicines.

2.2. Patients and analysis

Cases were excluded if they were enrolled in our previous study [4]. Analyses were conducted using the patients' records; however, when necessary, direct contact with the physicians-in-charge was made in order to gain more detailed information. Each patient's physician was primarily responsible for the selection of the antimalarial (atovaquone–proguanil); in some cases, drug selection was aided by consultation with specialists in the aforementioned research group. Patients were excluded if they received other antimalarials at the same episode, as this could compromise evaluation of the test drug. An exception was made for the use of primaquine as a radical cure of *P. vivax/ovale* malaria.

Non-immune individuals were defined as those who lived in non-endemic countries for at least 1 year and who traveled to an endemic country and contracted malaria [6]. The effectiveness and adverse effects of the antimalarial were evaluated based on the physicians' descriptions, as well as by a review of the laboratory data shown in the patient records.

3. Results

3.1. Treatment of *P. falciparum* malaria

Patients who developed illness between 2003 and 2010 were enrolled (Table 1). Fourteen adult patients were treated, all of whom received 4 tablets, once daily for 3 successive days. Many of the patients were Japanese, most were non-immune, and all were infected while traveling to sub-Saharan African countries. Of those patients, 3 received atovaquone–proguanil after failure of an antecedent treatment, i.e., Case 4, after injectable quinine and mefloquine; Cases 6 and 14, after artemether–lumefantrine. Defervescence and malaria parasite clearance were confirmed in all or in almost all cases, respectively. Possible adverse effects were reported in 2 cases. One patient (Case 5) showed a liver function disturbance (aspartate aminotransferase (AST), 215 IU/L; alanine aminotransferase (ALT), 294 IU/L; total bilirubin (T-Bili), 2.0 mg/dL), leukopenia (2200/μL), and thrombocytopenia (48×10^3/μL), which worsened after start of the treatment and returned to normal within 4 weeks of treatment. The second patient (Case 11) developed a low-grade headache and diarrhea.

A seventeen-month-old girl (Case 15) developed *P. falciparum* malaria after visiting Guinea and was treated with 3/4 tablet, once daily for 3 days. Another 2-year-old girl (Case 16) developed *P. falciparum* malaria after visiting Uganda and was given 1 tablet, once daily for 3 days. Both of the children were cured without noticeable adverse effects.

3.2. Treatment of *P. vivax/ovale* malaria

Patients who developed illness between 2001 and 2008 were enrolled (Table 2). Thirteen adult patients, one of whom contracted *P. ovale* malaria, were treated with 4 tablets, once daily for 3 days; 2 patients were counted twice, as described below. Most of the cases were non-immune and many were foreign nationals who visited Papua New Guinea. The above 2 patients were counted twice due to demonstrated relapses of *P. vivax* malaria. One patient was infected

Table 1
Atovaquone–proguanil treatment of *P. falciparum* malaria.

Case	Age/sex	Body weight (kg)	Nationality	Semi-immune	Country of disease acquisition	Treatment results				Remarks
						Parasite clearance	Effectiveness	Outcome	Adverse events	
1	47/M	ND[a]	Ghana	Unknown	Ghana	+	++	ND	−	VFRs[b]
2	30/F	52	Japan	−	Mozambique	+	++	Cure	−	
3	40/M	82	Japan	−	Liberia	+	+	Cure	−	
4	44/M	81	Japan	−	Tanzania	ND	+	ND	−	Failure of antecedent therapy
5	29/F	50	Japan	−	Ghana	+	+	Cure	+	Headache, nausea, liver function disturbance, leukopenia, thrombocytopenia
6	58/M	83	Japan	−	Sierra Leone	+	++	ND	−	Failure of antecedent therapy
7	26/F	48	Japan	−	Kenya	+	+	Cure	−	
8	33/M	64	Japan	−	Kenya	+	+	Cure	−	
9	29/F	ND	Japan	−	Ghana	+	++	ND	−	
10	33/M	ND	Ghana	−	Ghana	+	++	Cure	−	VFRs
11	54/M	73	Japan	−	Ghana	+	++	Cure	+	Slight headache, diarrhea
12	52/F	ND	Japan	−	Niger	+	+	Cure	−	
13	39/M	61	Ghana	+	Ghana	ND	+	Cure	−	
14	29/M	65	Japan	−	Uganda/Tanzania	+	++	Cure	−	Failure of antecedent therapy
15	01/F	8.8	ND	−	Guinea	+	+	Cure	−	3/4 tablet/day for 3 days
16	02/F	13	Japan	−	Uganda	+	++	Cure	−	1 tablet/day for 3 days, VFRs

[a] ND; not described.
[b] VFRs; visiting friends and relatives.

Table 2
Atovaquone–proguanil treatment of *P. vivax/ovale* malaria.

Case	Age/sex	Body weight (kg)	Nationality	Semi-immune	Country of disease acquisition	Treatment results				Remarks
						Parasite clearance	Effectiveness	Outcome	Adverse events	
1	23/F	68	U.S.A.[a]	–	Thailand	ND[b]	++	Relapse	–	
2	23/F	68	U.S.A.	–	Thailand	+	++	Cure	–	Retreatment of Case 1
3	30/F	63	South Africa	Unknown	P.N.G.[c]	+	++	Cure	–	
4	62/M	61	Japan	–	P.N.G.	+	++	Cure	–	
5	22/F	66	U.S.A.	–	P.N.G.	+	+	Cure	–	
6	25/M	ND	U.K.[d]	–	P.N.G.	+	++	Relapse	–	
7	25/M	ND	U.K.	–	P.N.G.	+	++	Cure	–	Retreatment of Case 6
8	24/M	72	U.S.A.	–	P.N.G.	+	+	Cure	–	
9	26/M	72	U.K.	–	P.N.G.	+	+	Cure	–	
10	22/F	54	Japan	–	Honduras	+	++	Cure	–	
11	45/M	80	Madagascar	Unknown	Madagascar	+	++	Unknown	+	Slight skin itch
12	21/M	ND	Japan	–	Uganda	+	++	Cure	–	*P. ovale* malaria
13	24/M	66	Japan	–	Vanuatu	+	++	ND	–	
14	14/F	ND	Japan	–	P.N.G.	+	++	Cure	–	Adult dosage

All but Case 12 were due to *P. vivax* malaria.
[a] U.S.A.; the United States of America.
[b] ND; not described.
[c] P.N.G.; Papua New Guinea.
[d] U.K.; the United Kingdom.

in Thailand and initially received atovaquone–proguanil, followed by primaquine base 15 mg/day for 14 days, which led to defervescence (Case 1). However, because of a relapse occurring after 4 months, atovaquone–proguanil was administered again, followed by primaquine base 30 mg/day for 14 days, which resulted in complete cure (Case 2). A second patient was infected in Papua New Guinea and primaquine base 30 mg/day was given for 14 days, following acute-stage treatment with atovaquone–proguanil (Case 6). Due to a relapse that occurred after 3 months, atovaquone–proguanil was administered again, followed by the same daily dosage of primaquine for 28 days, leading to a complete cure (Case 7). Defervescence and malaria parasite clearance were confirmed in all or almost all cases, respectively. As a possible adverse effect, 1 patient reported a low-grade skin itch.

A 14-year-old girl (Case 14) contracted *P. vivax* malaria after visiting Papua New Guinea. She received the adult dosage of atovaquone–proguanil, followed by primaquine, and showed complete cure, without developing adverse effects.

4. Discussion

This study was not conducted as a formal clinical trial and is, therefore, subject to some limitations. One limitation is the non-uniform evaluation of the effectiveness and adverse effects of the drug. Categorization of the therapeutic effectiveness and outcome, as well as the determination of possible adverse effects may have been assessed differently between physicians. In addition, post-treatment follow-up periods may have varied between patients; for example, foreign visitors to Japan may have been observed only for a short period of time prior to their return to their home country. However, it is also plausible that the physicians established close relationships with their patients due to the unique nature of this trial, with the result that most of the unusual events, such as recrudescence/relapse of malaria or delayed adverse effects, were reported even after the patient record was fulfilled and submitted. Thus, despite these limitations, the data contribute to the evaluation of the efficacy and safety of atovaquone–proguanil in malaria treatment in Japan.

In our previously reported study [4], the efficacy and safety of atovaquone–proguanil were compared to those of mefloquine in non-immune patients with uncomplicated *P. falciparum* malaria. In that study, all 20 patients were cured with atovaquone–proguanil compared to 49 cures out of 50 cases treated with mefloquine. The mean fever clearance time and parasite clearance time appeared to be longer in the atovaquone–proguanil group, but the differences were not statistically significant. Adverse effects were significantly fewer in the atovaquone–proguanil group, with no patients reporting gastrointestinal or neuropsychiatric symptoms, such as dizziness and vivid dreams; these symptoms were occasionally reported by mefloquine recipients. The only reported adverse effect in the atovaquone–proguanil group was mild-to-moderate elevation of liver enzymes, which, however, seemed to be associated with the disease itself. In addition, 3 children with *P. falciparum* malaria (Ages: 1 year and 11 months, 4 years and 1 month, and 5 years and 8 months) were treated successfully and safely with reduced dosages of atovaquone–proguanil. The results of the current study reinforces the observation that atovaquone–proguanil is an effective and well-tolerated malaria treatment regimen in Japan.

Combining studies performed in the 1990s in malaria-endemic regions, such as Southeast Asia, Africa, and South America, atovaquone–proguanil has shown an overall cure rate of >98% for *P. falciparum* malaria [7]. The excellent efficacy of this agent against *P. falciparum* malaria was maintained when studied in Thailand during November 2004–December 2005 (97.8%) [8]. Regarding imported malaria, two studies in France that examined the drug's efficacy against *P. falciparum* malaria, one with 25 cases [6] and the other with 112 cases [9], confirmed the excellent efficacy and safety of atovaquone–proguanil. Another study in France reported the use of the agent in 72 patients with excellent tolerability, with only 1 treatment failure associated with digestive disorders [10]. A Danish study enrolled 50 *P. falciparum* malaria patients, most of whom seemed to be non-immune, and reported successful treatment in all patients and the absence of significant adverse effects [11]. A more recent French study also reported 48 pediatric patients with imported *P. falciparum* malaria who were treated successfully with atovaquone–proguanil [12]. The only reported adverse events were from 3 patients who discontinued the antimalarial because of vomiting. Thus, the available data support the safety and efficacy of atovaquone–proguanil for treating imported, uncomplicated *P. falciparum* cases. These data also provide a rationale for the recommended use of atovaquone–proguanil in malaria treatment guidelines in developed countries. In addition, due to the gradual increases in chloroquine resistance of *P. vivax*, a U.S. recommendation for treatment of malaria positioned this agent as the treatment of choice for the acute stage of *P. vivax* malaria acquired in Papua New Guinea or Indonesia [13].

Proguanil was postulated to exert anti-plasmodial activity after being metabolized to cycloguanil by CYP2C19 rather than in its native form. Because the CYP2C19-related poor metabolizers are more

frequent in East Asian populations, including Japanese [14], concern was raised that Japanese patients might not respond well to treatment with antimalarials containing proguanil. Although a phenotypic or genetic analysis was not conducted, the current study does not support that concern. Additionally, studies on African and Asian populations did not reveal any association between the poor metabolizers and breakthrough parasitemia or treatment failure associated with proguanil use [15].

The *P. falciparum* cytochrome *b* complex is thought to be the target of atovaquone, the major constituent of the combination drug. Since 2002, cases of genetically confirmed atovaquone–proguanil treatment failures have been reported for *P. falciparum*, each showing a modification of codon 268 (wild type, tyrosine) to serine, asparagine, or cysteine, which results in the inhibition of atovaquone binding to the complex, thus conferring resistance [16]. According to Rose et al. [17], most of the resistant parasites were found in cases contracted in sub-Saharan Africa, but cases acquired in other areas such as Comoros [18], South America [19], and the Indian subcontinent [20] were also reported. Other possible causes of treatment failure may include still unidentified mutations of the *Plasmodium* genes, impaired bioavailability of the drug, and heavier than normal patients [21]. Vigilance will necessarily be required to monitor the future occurrence of this drug-resistant form of malaria in travelers returning to Japan.

Generally, treatment with atovaquone–proguanil has been reportedly well-tolerated with fewer reported neuropsychiatric adverse events than those associated with mefloquine. Two reviews of antimalarial drugs' adverse effects mentioned occasional elevation of transaminases [22,23], which, however, often resolved within 4 weeks of atovaquone–proguanil treatment [22]. A systematic summary of studies with this drug indicated that liver function disturbance occurred in <5% of cases [24], while a study in Thailand showed elevation of ALT and AST in 16% and 13% of patients, respectively [25]. An anecdotal report has also been published on a traveler who was prescribed prophylactic atovaquone–proguanil and who developed an acute hepatitis-like illness, with increased levels of ALT (~700), AST (~>200), and with T-Bili levels indicative of jaundice [26]. The liver function disturbance reported in one of the present *P. falciparum* cases, however, may not be totally ascribed to the drug's adverse effects, rather it may have been malaria-related. More data are needed regarding this hepatotoxicity issue, especially when focusing on Japanese patients.

In conclusion, atovaquone–proguanil has, again, been shown to be an effective and well-tolerated therapeutic regimen for *P. falciparum* and *P. vivax* malaria. The licensing of this product in Japan, where only 2 antimalarials are licensed, could greatly contribute to offering individually appropriate treatment options.

5. Conflict of interest

None.

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References

[1] Nakato H, Vivancos R, Hunter PR. A systematic review and meta-analysis of the effectiveness and safety of atovaquone–proguanil (Malarone) for chemoprophylaxis against malaria. Journal of Antimicrobial Chemotherapy 2007;60:929–36.
[2] Muentener P, Schlagenhauf P, Steffen R. Imported malaria (1985–1995): trends and perspectives. Bulletin of the World Health Organization 1999;77:560–6.
[3] Kimura M, Suzaki A, Matsumoto Y, Nakajima K, Wataya Y, Ohtomo H. Epidemiological and clinical aspects of malaria in Japan. Journal of Travel Medicine 2003;10:122–7.
[4] Hitani A, Nakamura T, Ohtomo H, Nawa Y, Kimura M. Efficacy and safety of atovaquone–proguanil compared with mefloquine in the treatment of nonimmune patients with uncomplicated *P. falciparum* malaria in Japan. Journal of Infection and Chemotherapy 2006;12:277–82.
[5] Teraoka A, Tsutani K. Compassionate use of unapproved drugs — how can we fulfill Japanese patients' needs to access unapproved drugs? Japanese Pharmacology and Therapeutics 2010;38:109–50 (Article in Japanese).
[6] Bouchaud O, Monlun E, Muanza K, Fontanet A, Scott T, Goetschel A, et al. Atovaquone plus proguanil versus halofantrine for the treatment of imported acute uncomplicated *Plasmodium falciparum* malaria in non-immune adults: a randomized comparative trial. The American Journal of Tropical Medicine and Hygiene 2000;63:274–9.
[7] Looareesuwan S, Chulay JD, Canfield CJ, Hutchinson DBA. Malarone™ (atovaquone and proguanil hydrochloride): a review of its clinical development for treatment of malaria. The American Journal of Tropical Medicine and Hygiene 1999;60:533–41.
[8] Krudsood S, Patel SN, Tangpukdee N, Thanachartwet W, Leowattana W, Pornpininworakij K, et al. Efficacy of atovaquone–proguanil for treatment of acute multidrug-resistant *Plasmodium falciparum* malaria in Thailand. The American Journal of Tropical Medicine and Hygiene 2007;76:655–8.
[9] Malvy D, Djossou F, Vatan R, Pistone T, Etienne G, Longy-Boursier M, et al. Experience with the combination atovaquone–proguanil in the treatment of uncomplicated *Plasmodium falciparum* malaria–report of 112 cases. Médecine Tropicale : Revue du Corps de Santé Colonial 2002;62:229–31 (Article in French).
[10] Vatan R, Pistone T, Millet P, Etienne G, Mercié P, Longy-Boursier M, et al. Retrospective analysis of 107 imported adult cases of malaria. Experience report of uncomplicated falciparum malaria treatment in adults with oral atovaquone–proguanil. Presse Médicale 2006;35:571–7 (Article in French).
[11] Thybo S, Gjorup I, Ronn AM, Meyrowitsch D, Bygberg IC. Atovaquone–proguanil (Malarone): an effective treatment for uncomplicated *Plasmodium falciparum* malaria in travelers from Denmark. Journal of Travel Medicine 2004;11:220–4.
[12] Blondé R, Naudin J, Bigirimana Z, Holvoet L, Fenneteau O, Vitoux C, et al. Tolerance and efficacy of atovaquone–proguanil for the treatment of paediatric imported *Plasmodium falciparum* malaria in France: clinical practice in a university hospital in Paris. Archives de Pédiatrie 2008;15:245–52 (Article in French).
[13] Griffith KS, Lewis LS, Mali S, Parise ME. Treatment of malaria in the United States: a systemic review. JAMA 2007;297:2264–77.
[14] Man M, Farmen M, Dumaual C, Teng CH, Moser B, Irie S, et al. Genetic variation in metabolizing enzyme and transporter genes: comprehensive assessment in 3 major East Asian subpopulations with comparison to Caucasians and Africans. Journal of Clinical Pharmacology 2010;50:929–40.
[15] Kerb R, Fux R, Mörike K, Kremsner PG, Gil JP, Gleiter CH, et al. Pharmacogenetics of antimalarial drugs: effect on metabolism and transport. The Lancet Infectious Diseases 2009;9:760–74.
[16] Fivelman QL, Butcher GA, Adagu IS, Warhurst DC, Pasvol G. Malarone treatment failure and *in vitro* confirmation of resistance of *Plasmodium falciparum* isolate from Lagos, Nigeria. Malaria Journal 2002;1:1.
[17] Rose GW, Suh KN, Kain KC, Le Saux N, McCarthy AE. Atovaquone–proguanil resistance in imported falciparum malaria in a young child. Pediatric Infectious Disease Journal 2008;27:567–9.
[18] Savini H, Bogreau H, Bertaux L, Bouchiba H, Kraemer P, Parzy D, et al. First case of emergence of atovaquone–proguanil resistance in *Plasmodium falciparum* during treatment in a traveler in Comoros. Antimicrobial Agents and Chemotherapy 2008;52:2283–4.
[19] Legrand E, Demar M, Volney B, Ekala M-T, Quinternet M, Bouchier C, et al. First case of emergence of atovaquone resistance in *Plasmodium falciparum* during second-line atovaquone–proguanil treatment in South America. Antimicrobial Agents and Chemotherapy 2007;51:2280–1.
[20] Perry TL, Pandey P, Grant JM, Kain KC. Severe atovaquone-resistant *Plasmodium falciparum* malaria in a Canadian traveller returned from the Indian subcontinent. Open Medicine 2009;3:10–6.
[21] Durand R, Prendki V, Cailhol J, Hubert V, Ralaimazava P, Massias L, et al. *Plasmodium falciparum* malaria and atovaquone–proguanil treatment failure. Emerging Infectious Diseases 2008;14:320–2.
[22] Taylor WRJ, White NJ. Antimalarial drug toxicity: a review. Drug Safety 2004;27:25–61.
[23] AlKadi HO. Antimalarial drug toxicity: a review. Chemotherapy 2007;53:385–91.
[24] Marra F, Salzman JR, Ensom MHH. Atovaquone–proguanil for prophylaxis and treatment of malaria. The Annals of Pharmacotherapy 2003;37:1266–75.
[25] Looareesuwan S, Wilairatana P, Chalermarut K, Rattanapong Y, Canfield CJ, Hutchinson DBA. Efficacy and safety of atovaquone/proguanil compared with mefloquine for treatment of acute *Plasmodium falciparum* malaria in Thailand. The American Journal of Tropical Medicine and Hygiene 1999;60:526–32.
[26] Grieshaber M, Lämmli J, Marcus L. Acute hepatitis and atovaquone/proguanil. Journal of Travel Medicine 2005;12:289–90.

MALARIA JOURNAL

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Effects of anti-malarial drugs on the electrocardiographic QT interval modelled in the isolated perfused guinea pig heart system

Atsushi Kinoshita[1*], Harumi Yamada[2], Hajime Kotaki[2], Mikio Kimura[3]

Abstract

Background: Concern over the potential cardiotoxicity of anti-malarial drugs inducing a prolonged electrocardiographic QT interval has resulted in the almost complete withdrawal from the market of one anti-malarial drug - halofantrine. The effects on the QT interval of four anti-malarial drugs were examined, using the guinea pig heart.

Methods: The guinea pig heart was isolated, mounted on a Langendorff apparatus, and was then perfused with pyruvate-added Klebs-Henseleit solutions containing graded concentrations of the four agents such as quinidine (0.15 - 1.2 μM), quinine (0.3 - 2.4 μM), halofantrine (0.1 - 2.0 μM) and mefloquine (0.1 - 2.0 μM). The heart rate-corrected QaTc intervals were measured to evaluate drug-induced QT prolongation effects.

Results: Quinidine, quinine, and halofantrine prolonged the QaTc interval in a dose-dependent manner, whereas no such effect was found with mefloquine. The EC_{50} values for the QaTc prolongation effects, the concentration that gives a half-maximum effect, were quinidine < quinine ≈ halofantrine.

Conclusions: In this study, an isolated, perfused guinea pig heart system was constructed to assess the cardiotoxic potential of anti-malarial drugs. This isolated perfused guinea pig heart system could be used to test newly developed anti-malarial drugs for their inherent QT lengthening potential. More information is required on the potential variation in unbound drug concentrations in humans, and their role in cardiotoxicity.

Background

Worldwide, in 2006, an estimated 247 million (189-327 million) malaria cases occurred, with an approximated 881,000 (610,000-1,212,000) deaths [1]. *Plasmodium falciparum* is the species that can cause severe, complicated malaria and death. Intravenous (IV) quinine (a 4-quinoline methanol) has been the mainstay of treatment for such severe malaria, although in some countries, including the United States, quinidine, the dextrorotatory diastereoisomer of quinine, is used because of the non-availability of IV quinine. Parenteral forms of artemisinin derivatives are increasingly being used in developing countries and more recently also in industrialized countries. Cardiac toxicity has been a major concern with the use of IV quinine or quinidine, with quinidine considered to be more toxic than quinine [2,3]. The primary mechanism of cardiotoxicity caused by quinine or quinidine is the prolongation of the electrocardiographic (ECG) QT interval which can cause potentially fatal ventricular arrhythmias, including torsades de pointes, and even sudden death.

Since the 1960s, chloroquine-resistant and multidrug-resistant strains of *P. falciparum* have emerged in Africa and Southeast Asia and have spread worldwide. Newer anti-malarial drugs were developed including mefloquine, a 4-quinoline methanol similar to quinine, and halofantrine, a 9-phenanthrene methanol structurally related to quinoline anti-malarial drugs. Because both drugs are administered orally, their widespread use was anticipated for the treatment of uncomplicated cases of drug-resistant *P. falciparum* infection. However, in 1993, reports of severe and sometimes fatal cardiotoxicity associated with the use of halofantrine led the World Health Organization to limit its use [4], and as of

* Correspondence: atsushi@himeji-du.ac.jp
[1]Division of Drug Informatics, Faculty of Pharmaceutical Sciences, Himeji Dokkyo University, 7-2-1 Kamiono, Himeji, Hyogo, 670-8524 Japan
Full list of author information is available at the end of the article

2002, there were at least 20 reports of fatal cardiac complications relating to use of the drug [5]. These events were attributed to a QT prolongation effect of halofantrine, identified in several human studies of the drug [6,7]. These unexpected cardiac problems resulted in the withdrawal of the drug from the market in many countries except Pakistan and parts of West and Central Africa [8], and underlines the importance of examining the cardiotoxic potential of quinoline and other structurally related anti-malarial drugs before the wider marketing of newer drugs.

In this study, the effects on the QT interval of the following anti-malarials: quinidine, quinine, halofantrine, and mefloquine were examined, using an isolated perfused guinea pig heart model. The aim of this study was to clarify whether the results obtained from this model could be used to predict the cardiotoxicity of these anti-malarial drugs when used in clinical settings.

Methods

Chemical agents

Quinidine sulfate dihydrate and quinine hydrochloride dihydrate were purchased from Wako Pure Chemical Industries Ltd. (Tokyo, Japan). Mefloquine hydrochloride and halofantrine hydrochloride were kindly donated by Roche Co. Ltd. (Basel, Switzerland) and SmithKlein Beecham Co. Ltd. (Brentford, UK), respectively. All other chemicals used were of the reagent grade and were purchased commercially.

Isolation of guinea pig hearts

The isolated perfused guinea pig heart system used in this study was constructed using a method described elsewhere [9]. Male Hartley guinea pigs weighing 350 to 500 g were anesthetized with a mixture of urethane and α-chloralose (1.2 and 30 mg/kg, respectively, intraperitoneally), and then injected with heparin (500 U/body, intraperitoneally). After 30 min, the heart was promptly excised. After the aorta was cannulated, the heart was mounted on a Langendorff apparatus and perfused with the pyruvate-added Klebs-Henseleit solution composed of: NaCl, 118 mM; KCl, 4.7 mM; $CaCl_2$, 2.55 mM; $MgSO_4$, 1.18 mM; KH_2PO_4, 1.18 mM; $NaHCO_3$, 24.88 mM; glucose, 11.1 mM; sodium pyruvate, 2 mM; ascorbic acid, 0.14 mM; EDTA2Na, 0.5 mM. The solution was aerated with O_2: CO_2 (95:5) and kept at 37°C (pH 7.4 ± 0.01). The perfusion pressure was kept at 85 cm of water. The sinoatrial node of the heart was crushed after perfusion was commenced.

Electrocardiogram recording

An ECG recording of the epicardial surface was commenced immediately after attaching the heart to the Langendorff apparatus. A stimulator was seated at the right atrium and the heartbeats were artificially kept constant at 210 per min by 3.5 Hz stimuli. Two silver wire electrodes were placed on the epicardial surface. Signals from both electrodes were amplified by an electric amplifier (AB-621G, Nihon-Kohden, Tokyo), recorded on a personal computer (PC-9801VX, NEC, Tokyo) *via* an A/D converter (Analog-Pro Jr., Canopus Electric, Kobe), and analysed with WAVE MASTER II and WM Read (Canopus Electric, Kobe) as described previously [10,11].

QT interval measurement

Quinidine, quinine, and mefloquine were dissolved in Klebs-Henseleit solution at 0.15 - 1.2 μM, 0.3 - 2.4 μM, 0.1 - 2.0 μM, respectively. Halofantrine, which is poorly water-soluble, was first dissolved in polyethylene glycol 400 (PEG) and then in Klebs-Henseleit solution at 0.1 - 2.0 μM, with a final PEG concentration of 0.1% (v/v). The anti-malarial drug free 0.1% PEG Klebs-Henseleit solution served as a control for halofantrine treatment. Each heart was allowed to equilibrate with the drug-free solutions for 30 min. Measurements were performed after perfusion with the drug-containing solutions for 15 min. ECG parameters such as the heart rate, QT or QaT (from the beginning of the Q wave to the top of the T wave) intervals were obtained from the average wave shape of recordings for 10 sec.

Analysis of the QT interval prolongation

QTc and QaTc intervals were obtained after correction of QT and QaT intervals using the Bazett's formula [12], since the formula was shown to be applicable to the guinea pig heart [13]. The QT prolongation effects of each drug were fitted simultaneously according to the full nonlinear regression analysis for effect as expressed in the equation below.

$$E = \frac{E_{max}}{1 + \exp^{-\left(\frac{C - EC_{50}}{E_{min}}\right)}}$$

Where E is the change in QaTc interval, E_{max} is the maximum effect of the drug, E_{min} is the minimum effect of the drug, EC_{50} is the concentration that gives a half-maximum effect, and C is the concentration of drug. Each parameter was calculated by the simultaneous fitting using a nonlinear least-squares programme (MULTI) with the modified Marquardt method [14].

Statistics

Change in the QaTc interval is expressed as a mean ± standard error of the mean (SEM) and EC_{50} is expressed as a mean ± standard deviation (SD). Statistical analysis was performed using the Student's *t*-test.

Results

Perfusion with quinidine

Perfusion of the isolated guinea pig heart with the control solutions showed no QTc prolongation effect. To assess the optimal experimental conditions, the hearts were perfused with graded concentrations of quinidine (the prototype anti-malarial drug carrying cardiotoxic potential). Quinidine prolonged both the QTc and QaTc intervals at concentrations of 0.15 - 1.2 μM in a dose dependent manner. The EC_{50} for the QTc and the QaTc interval was 0.45 and 0.49 μM, respectively. Unexpectedly, the QTc interval proved difficult to measure at high drug concentrations as the end of the T-wave was unclear, overlapping the following P-wave. Therefore, it was decided to use the QaTc instead of the QTc interval to assess drug-induced QT lengthening throughout this study.

Effects of anti-malarial drugs on the QaTc interval

Figure 1 shows the relationship between concentrations of the four anti-malarial drugs and the changes in the QaTc interval. Quinidine and quinine prolonged the QaTc interval in a dose dependent manner within the range of concentrations described above. Statistically, the QaTc prolongation effect was significantly higher with quinidine than quinine at concentrations of 0.3 and 1.2 μM (p <0.05). Halofantrine also prolonged the QaTc interval in a dose dependent manner within the range of concentrations described above. The EC_{50} values of quinidine, quinine, and halofantrine were 0.49 ± 0.61, 1.68 ± 0.43, 1.59 ± 1.26 μM, respectively, and thus the *in vitro* QaTc prolongation effect was highest with quinidine, and those with quinine and halofantrine were similar and were lower than that with quinidine. In contrast, mefloquine did not prolong the QaTc interval within the range of concentrations of 0.5 - 2.0 μM.

Discussion

An isolated, perfused guinea pig heart system was constructed to assess the cardiotoxic potential of anti-malarial drugs. While the cardiotoxic effects of halofantrine have been studied previously using a similar (feline heart) model [15], there have been no previous studies reported where the cardiotoxic potential of several anti-malarial drugs were assessed simultaneously.

Quinidine, quinine, and halofantrine, all of which have been known to have potential cardiotoxicity in humans,

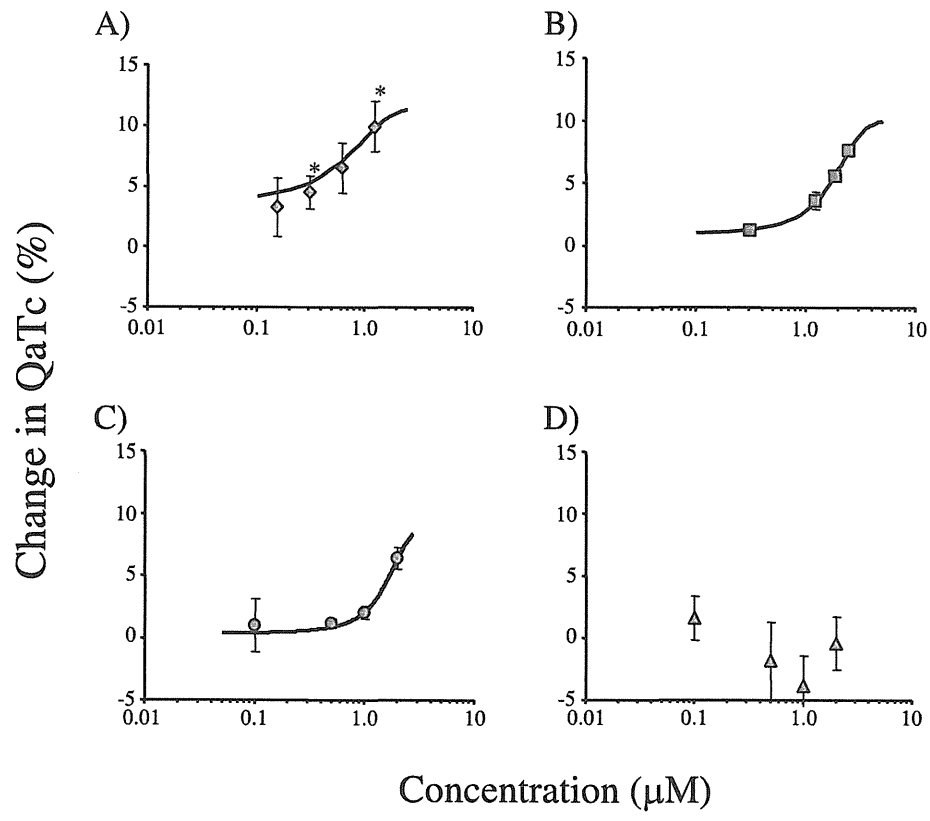

Figure 1 Relationships between QaTc changes and concentrations of quinidine (A), quinine (B), halofantrine (C), and mefloquine (D) in the isolated perfused guinea pig heart system. Each bar represents the mean ± SEM (n = 3 - 4). *Statistically significant compared with the same concentrations of quinine (p < 0.05).

exerted a QT prolongation effect in this experimental model. The effects of the three anti-malarial drugs were shown to be dose-dependent at the drug concentrations tested. In general, the biologically active component is considered to be the unbound drug, and the therapeutic range of unbound quinine in African children was shown to be 0.2 - 2.0 mg/mL (0.62 - 6.2 μM) [16]. This suggests that the quinine concentrations used in this experimental model may prove clinically relevant. The observed higher QT prolongation effect of quinidine (EC_{50} = 0.49 ± 0.61 μM) over quinine (1.68 ± 0.43 μM) is consistent with the findings in humans that the former is more cardiotoxic than the latter in terms of developing serious ventricular arrhythmias [2] and prolonging the QT interval [3]. An *in vitro* study was conducted on the inhibition of potassium channel currents on *Xenopus* oocytes expressing the human *ether-a-go-go*-related gene (hERG), which represents an underlying molecular mechanism of QT prolongation [17]. This study also showed that quinidine was more toxic than quinine with IC_{50} values of 4.6 μM and 57 μM, respectively. In addition to this inherent difference in cardiotoxic potential between quinidine and quinine, the former was reported to have a higher unbound fraction than the latter [18]. When assessing cardiotoxicity in human therapies, the possibility of finding differences in unbound fractions of an anti-malarial drug should also be considered. For example, the plasma unbound fraction of quinine was reported to be lower in cerebral malaria than in uncomplicated malaria [19], which may explain why severe quinine toxicity is unusual in severe falciparum malaria [20]. Moreover, studies are needed to investigate the various factors that could influence the total concentration and consequently the unbound concentration of an anti-malarial drug to ensure safer use of drugs.

Mefloquine did not prolong the QT interval within the range of concentrations of 0.5 - 2.0 μM. This agent is characterized by its very high protein binding, e.g, 98.3% shown in humans [21], with the unbound plasma mefloquine concentration in human therapy reported to be 0.05 μM [22]. This concentration is below the lowest concentration used in this study, making it very unlikely to be cardiotoxic in clinical settings. In fact, although Davis *et al* [23] suggested a mild and transient QTc lengthening in humans after mefloquine use, this was not evident in other studies [6,24], consistent with the expert's view that there is no convincing evidence for significant cardiotoxicity following mefloquine administration [2]. The possible mefloquine-induced cardiotoxicity might, however, be due to another mechanism, i.e., reduced contractility of cardiac muscles due to inhibition of the L type Ca^{2+} channel by mefloquine [25].

The findings of this study, which showed that the QT prolongation effect of halofantrine was lower than that of quinidine and similar to that of quinine, may not seem to be clinically relevant. There are, however, few studies in which the therapeutic levels of unbound halofantrine concentrations in humans are well defined. One study showed the average peak total halofantrine concentration to be 6.4 μM [26], and the serum protein binding rate of halofantrine was reported to be 83% [27]. Therefore, the average peak unbound halofantrine concentration was calculated as 1.2 μM.

Other reports showed that the unbound therapeutic plasma concentration was 0.57 μM [22]. According to the results of this study, these reportedly low concentrations in humans do not seem to lengthen the QT interval. However, halofantrine is characterized by its marked differences in plasma concentrations among individuals, with one individual showing a five-times higher peak concentration than the other [26]. It has also been reported that its absorption is significantly enhanced when administered with fatty food (6.6 times higher peak concentrations) [28] or grapefruit juice [29]. In addition, the metabolite desbutylhalofantrine was shown to have some QT interval prolongation effect in a rabbit model [30]. Alternatively, the inherent cardiotoxicity of halofantrine may be detected more sensitively in other experimental models. For example, another *in vitro* study of inhibition of the potassium channel currents on hERG transfected cells, the IC_{50} was as low as 0.04 μM for halofantrine whereas it was 2.6 μM for mefloquine [22].

To assess cardiotoxicity of an anti-malarial drug, factors other than the inherent QT prolongation potential as shown in this study and the plasma unbound drug concentration need to be considered. For instance, accumulation in the myocardium may differ between anti-malarial drugs [22]. Furthermore, one report showed an absence of QT prolongation with an increased fraction of unbound quinidine induced by heparin administration [31]. Therefore, caution must be exercised when simply applying findings of unbound drug levels to assess cardiotoxicity in clinical settings.

Conclusion

The results of this study were largely consistent with the reported cardiotoxicity of the four anti-malarial drugs in clinical use. This isolated perfused guinea pig heart system could be used to test newly developed anti-malarial drugs for their inherent QT lengthening potential. More knowledge is required on the variability of unbound anti-malarial drug concentrations in humans, as well as their impact on cardiotoxicity in clinical settings.

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Author details
[1]Division of Drug Informatics, Faculty of Pharmaceutical Sciences, Himeji Dokkyo University, 7-2-1 Kamiono, Himeji, Hyogo, 670-8524 Japan. [2]School of Pharmacy, International University of Health and Welfare, 2600-1 Kitakanemaru, Ootawara, Tochigi, 324-8501 Japan. [3]Shin-Yamanote Hospital, Japan Anti-Tuberculosis Association, 3-6-1 Suwa-cho, Higashi-Murayama, Tokyo, 189-0021 Japan.

Authors' contributions
AK and HY contributed to the study design, participated in the acquisition and interpretation of data, performed the statistical analysis, and helped draft the manuscript. HK and MK contributed to the acquisition and interpretation of data, and helped review the manuscript. All authors read and approved the final manuscript.

Competing interests
The authors declare that they have no competing interests.

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References

1. World Health Organization: **Estimated burden of malaria in 2006.** *World Malaria Report 2008* World Health Organization, Geneva; 2008, 9-15.
2. White NJ: **Cardiotoxicity of antimalarial drugs.** *Lancet Infect Dis* 2007, **7**:549-558.
3. White NJ, Looareesuwan S, Warrell DA: **Quinine and quinidine: a comparison of EKG effects during the treatment of malaria.** *J Cardiovasc Pharmacol* 1983, **5**:173-175.
4. World Health Organization: **Drug alert: halofantrine. Change in recommendations for use.** *Wkly Epidemiol Rec* 1993, **68**:269-270.
5. Bouchaud O, Bruneel F, Schiemann R, Peytavin G, Coulaud JP: **Severe cardiac toxicity due to halofantrine: importance of underlying heart disease.** *J Travel Med* 2002, **9**:214-215.
6. Nosten F, ter Kuile FO, Luxemburger C, Woodrow C, Kyle DE, Chongsuphajaisiddhi T, White NJ: **Cardiac effects of antimalarial treatment with halofantrine.** *Lancet* 1993, **341**:1054-1056.
7. Bindschedler M, Lefèvre G, Degen P, Sioufi A: **Comparison of the cardiac effects of the antimalarials co-artemether and halofantrine in healthy participants.** *Am J Trop Med Hyg* 2002, **66**:293-298.
8. Bouchaud O, Imbert P, Touze JE, Dodoo AN, Danis M, Legros F: **Fatal cardiotoxicity related to halofantrine: a review based on a worldwide safety data base.** *Malar J* 2009, **8**:289.
9. Uematsu T, Vožeh S, Ha HR, Follath F, Nakashima M: **Method for stable measurement of the electrocardiogram in isolated guinea pig heart. Evaluation of the RR-QT relationship and the effect of quinidine.** *J Pharmacol Methods* 1987, **18**:179-185.
10. Minematsu T, Ohtani H, Yamada Y, Sawada Y, Sato H, Iga T: **Quantitative relationship between myocardial concentration of tacrolimus and QT prolongation in guinea pigs: pharmacokinetic/pharmacodynamic model incorporating a site of adverse effect.** *J Pharmacokinet Pharmacodyn* 2001, **28**:533-554.
11. Ohtani H, Hanada E, Yamamoto K, Sawada Y, Iga T: **Pharmacokinetic-pharmacodynamic analysis of the electrocardiographic effects of terfenadine and quinidine in rats.** *Biol Pharm Bull* 1996, **19**:1189-1196.
12. Bazzet HC: **An analysis of the time-relations of electrocardiograms.** *Heart* 1920, **7**:353-370.
13. Hayes E, Pugsley MK, Penz WP, Adaikan G, Walker MJA: **Relationship between QaT and RR intervals in rats, guinea pigs, rabbits, and primates.** *J Pharmacol Toxicol Methods* 1994, **32**:201-207.
14. Yamaoka K, Nakagawa T, Tanaka H, Yasuhara M, Okumura K, Hori R: **A nonlinear multiple regression program, MULTI2 (BAYES), based on Bayesian algorithm for microcomputers.** *J Pharmacobiodyn* 1985, **8**:246-256.
15. Wesche DL, Schuster BG, Wang WX, Woosley RL: **Mechanism of cardiotoxicity of halofantrine.** *Clin Pharmacol Ther* 2000, **67**:521-529.
16. Winstanley P, Newton C, Watkins W, Mberu E, Ward S, Warn P, Mwangi I, Waruiru C, Pasvol G, Warrell D, Marsh K: **Towards optimal regimens of parental quinine for young African children with cerebral malaria: the importance of unbound quinine concentration.** *Trans R Soc Trop Med Hyg* 1993, **87**:201-206.
17. Sánchez-Chapula JA, Ferrer T, Navarro-Polanco RA, Sanguinetti MC: **Voltage-dependent profile of human *ether-a-go-go*-related gene channel block is influenced by a single residue in the S6 transmembrane domain.** *Mol Pharmacol* 2003, **63**:1051-1058.
18. Mihaly GW, Ching MS, Klejn MB, Paull J, Smallwood RA: **Differences in the binding of quinine and quinidine to plasma proteins.** *Br J Clin Pharmacol* 1987, **24**:769-774.
19. Silamut K, White NJ, Looareesuwan S, Warrell DA: **Binding of quinine to plasma proteins in falciparum malaria.** *Am J Trop Med Hyg* 1985, **34**:681-686.
20. White NJ, Looareesuwan S, Warrell DA, Warrell MJ, Bunnag D, Harinasuta T: **Quinine pharmacokinetics and toxicity in cerebral and uncomplicated falciparum malaria.** *Am J Med* 1982, **73**:564-572.
21. Mu JV, Israili ZH, Dayton PG: **Studies of the disposition and metabolism of mefloquine HCl (WR 142,490), a quinolinemethanol antimalarial, in the rat. Limited studies with an analog, WR 30,093.** *Drug Metab Dispos* 1975, **3**:198-210.
22. Traebert M, Dumotier B, Meister L, Hoffmann P, Dominiguez-Estevez M, Suter W: **Inhibition hERG K^+ currents by antimalarial drugs in stably transfected HEK293 cells.** *Eur J Pharmacol* 2004, **484**:41-48.
23. Davis TME, Dembo LG, Kaye-Eddie SA, Hewitt BJ, Hislop RG, Batty KT: **Neurological, cardiovascular and metabolic effects of mefloquine in healthy volunteers: a double-blind, placebo-controlled trial.** *Br J Clin Pharmacol* 1996, **42**:415-421.
24. Laothavorn P, Karbwang J, Na Bangchang K, Bunnag D, Harinasuta T: **Effect of mefloquine on electrocardiographic changes in uncomplicated falciparum malaria patients.** *Southeast Asian J Trop Med Public Health* 1992, **23**:51-54.
25. Coker SJ, Batey AJ, Lightbown ID, Díaz ME, Eisner DA: **Effects of mefloquine on cardiac contractility and electrical activity *in vivo*, in isolated cardiac preparations, and in single ventricular myocytes.** *Br J Pharmacol* 2000, **129**:323-330.
26. Ohrt C, Watt G, Teja-Isavadharm P, Keeratithakul D, Loesuttiviboon L, Webster HK, Schuster B, Fleckenstein L: **Pharmacokinetics of an extended-dose halofantrine regimen in patients with malaria and healthy volunteers.** *Clin Pharmacol Ther* 1995, **57**:525-532.
27. Cenni B, Meyer J, Brandt R, Betschart B: **The antimalarial drug halofantrine is bound mainly to low and high density lipoproteins in human serum.** *Br J Clin Pharmacol* 1995, **39**:519-526.
28. Milton KA, Edwards G, Ward SA, Orme ML, Breckenridge AM: **Pharmacokinetics of halofantrine in man: effects of food and dose size.** *Br J Clin Pharmacol* 1989, **28**:71-77.
29. Charbit B, Becquemont L, Lepère B, Peytavin G, Funck-Brentano C: **Pharmacokinetic and pharmacodynamic interaction between grapefruit juice and halofantrine.** *Clin Pharmacol Ther* 2002, **72**:514-523.
30. McIntosh MP, Batey AJ, Porter CJH, Charman WN, Coker SJ: **Desbutylhalofantrine: evaluation of QT prolongation and other cardiovascular effects after intravenous administration in vivo.** *J Cardiovasc Pharmacol* 2003, **41**:406-413.
31. Kessler KM, Wozniak PM, McAuliffe D, Terracall E, Kozlovskis P, Mahmood I, Zaman L, Trohman RG, Castellanos A, Myerburg RJ: **The clinical implication of changing unbound quinidine levels.** *Am Heart J* 1989, **118**:63-69.

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Short Report: Elevated Levels of Vascular Endothelial Growth Factor (VEGF) and Soluble Vascular Endothelial Growth Factor Receptor (VEGFR)-2 in Human Malaria

Takahisa Furuta,* Mikio Kimura, and Naohiro Watanabe

Division of Infectious Genetics, Department of Microbiology and Immunology, Institute of Medical Science, University of Tokyo, Tokyo, Japan; Shin-Yamanote Hospital, Japan Anti-Tuberculosis Association, Tokyo, Japan; Department of Tropical Medicine, Jikei University School of Medicine, Tokyo, Japan

Abstract. In cerebral malaria, the binding of parasitized erythrocytes to the cerebral endothelium and the consequent angiogenic dysregulation play a key role in pathogenesis. Because vascular endothelial growth factor (VEGF) is widely regarded as a potent stimulator of angiogenesis, edema, inflammation, and vascular remodeling, the plasma levels of VEGF and the soluble form of the VEGF receptor (sVEGFR)-1 and -2 in uncomplicated malaria patients and healthy adults were measured by enzyme-linked immunosorbent assay (ELISA) to examine their roles in malaria. The results showed that VEGF and sVEGFR-2 levels were significantly elevated in malaria patients compared with healthy adults. Moreover, it was confirmed that malarial parasite antigens induced VEGF secretion from the human mast cell lines HMC-1 or KU812 cell. This is the first report to suggest that the interaction of VEGF and sVEGFR-2 is involved in the host immune response to malarial infection and that malarial parasites induce VEGF secretion from human mast cells.

Malaria is a complex disease that results in the death of more than one million people every year.[1] The most lethal form of malaria is caused by infection by *Plasmodium falciparum* that induces severe anemia and/or cerebral malaria (CM), which are considered to be the most serious complications leading to mortality. Although the detailed pathophysiology of CM remains far from resolved, it is thought that the binding of parasitized erythrocytes to the cerebral endothelium and the consequent angiogenic dysregulation play a key role in the disease pathogenesis. Thus, the pathogenesis of severe malaria is closely related to pathophysiological changes of blood vessels such as endothelial cell activation, increased vascular permeability, and blood-brain barrier dysfunction. A recent study showed that vascular endothelial growth factor (VEGF) and its receptor-related molecules are overexpressed in the brain tissues of CM patients.[2] Increased levels of VEGF are often detected in tissues and biologic samples from malaria patients.

The VEGF was first identified as a potent stimulator of vascular endothelial permeability and was subsequently reported to promote the proliferation, migration, and survival of endothelial cells.[3,4] The VEGF is characterized by its highly specific mitogenic activity for endothelial cells and its angiogenic effect observed both *in vitro* and *in vivo*. In addition to its role in promoting endothelial permeability and proliferation, VEGF may also contribute to inflammation and coagulation.[5] *In vitro*, VEGF induces the expression of cell adhesion molecules, including E-selectin, intercellular adhesion molecule 1, and vascular cell adhesion molecule 1, in endothelial cells and promotes the adhesion of leukocytes.[6,7] Moreover, VEGF signaling up-regulates tissue factor mitochondrial RNA (mRNA), protein, and procoagulant activity.[8] The proinflammatory/procoagulant effects of VEGF are mediated, at least in part, by the activation of NF-κB, Egr-1, and nuclear factor of activated T cells (NFAT) transcription factors.[9–11] The VEGF has been implicated as a pathophysiological mediator in several human diseases, including rheumatoid arthritis, cancer, and inflammatory bowel disease.

*Address correspondence to Takahisa Furuta, Division of Infectious Genetics, Department of Microbiology and Immunology, Institute of Medical Science, University of Tokyo, 4-6-1 Shirokanedai, Minato-Ku, Tokyo 108-8639, Japan. E-mail: Furuta@ims.u-tokyo.ac.jp

Recently, it was reported that mast cells are the main source of VEGF and can rapidly release VEGF from a preformed pool, which is then sustained by the secretion of newly synthesized VEGF.[12,13] Mast cells play an important role in inflammation[14] and in the host defense against foreign pathogens.[15–17] Our recent reports have indicated the importance of mast cell activation through Toll-like receptor (TLR) 4 or the binding of $Fc\varepsilon R_I$ to IgE antibody for the protection from malarial infection.[18,19]

In this study, the level of VEGF and the soluble form of its receptors, vascular endothelial growth factor receptor (sVEGFR)-1 and -2, in the plasma from uncomplicated malaria patients and healthy adults was compared to examine the potential role of these molecules in the host immune response to malarial infection. The specific activity of malarial antigens on the secretion of VEGF by human mast cell lines was also studied.

A total of 73 malaria patients with uncomplicated malaria including 55 *P. falciparum* and 18 *Plasmodium vivax* patients were enrolled in this study. The 55 *P. falciparum* patients (parasite density: 5.2×10–$9.8 \times 10^5/\mu L$) consisted of 46 Asian (39 males and 7 females), 6 Africans (5 males and 1 female), and 3 Caucasians (2 males and 1 female), whereas the 18 *P. vivax* patients (parasite density: 7.2×10–$3.5 \times 10^4/\mu L$) consisted of 16 Asians (14 males and 2 females) and 2 Africans (2 males). The participants ranged from 18 to 67 years of age and were either tourists or business travelers visiting malaria-endemic countries in South-East Asia, South Asia, or Africa. The clinical manifestations of uncomplicated malaria were defined according to World Health Organization (WHO) criteria.[20] The plasma from 15 Asian patients with febrile illness without an obvious source of infection and from 26 healthy adults was also collected to use as a control. The study design was approved by a committee (headed by Dr. Mariko Honda) at Jikei University School of Medicine in Tokyo, where the experiments were performed. The informed consent was obtained from all of the participants. All plasma samples were collected before the treatment of the patient, and in addition, four plasma samples were also obtained from the Asian *P. falciparum* patients at convalescence after the treatment. The plasma was stored at −80°C until used. The VEGF, sVEGFR-1, and sVEGFR-2 levels in the plasma and culture supernatants were measured using a sandwich enzyme-linked immunosor-

bent assay (ELISA) kit (R&D Systems, Minneapolis, MN). The human mast cell/basophil line KU812 cells[21] and the human mast cell line HMC-1 cells[22] were maintained in RPMI M 1640 medium or IMDM (Invitrogen, Grand Island, NY). The KU812 or HMC-1 cells (10^6/mL) were treated with 20 μg/mL of soluble malarial parasite or normal erythrocyte crude antigen for 24 hr. The culture supernatants were then collected and analyzed by ELISA to determine the VEGF concentration. Soluble antigens of malarial parasites were prepared from *P. falciparum* (FCR-3 strain)-infected human erythrocytes cultured *in vitro* or from the blood of *P. vivax*-infected patients. Briefly, malarial parasite-infected erythrocytes were lysed with 0.5% saponin and then washed with phosphate buffered saline (PBS). After two rounds of freeze-thawing, the sample was sonicated at 5A for 30 sec, and then used as crude malarial parasite antigens in the experiments.[19] Normal erythrocyte crude antigen was also prepared from normal erythrocytes using the same method.

The total amount of VEGF was measured in patients infected with *P. falciparum* or *P. vivax* and compared with the levels in the febrile illness and healthy adult groups. The relationship between malarial infection and the VEGF response before drug administration was also examined. The total VEGF concentration in patients with *P. falciparum* or *P. vivax* infections was significantly higher than those in the febrile illness and healthy adult groups (Table 1). However, no significant difference in the VEGF, VEGFR-1, and -2 levels between the *P. falciparum* and *P. vivax* patients (Table 1) or among the different races was observed (data not shown). In addition, a significant association between the age or sex of the participants and the incidence of malaria was not observed. Moreover, there was no significant correlation between parasitemia and the levels of VEGF level (data not shown).

One potential ligand of VEGF is the soluble form of VEGFR-1 (sVEGFR-1 or sFlt-1), which is generated by the differential splicing of VEGFR-1 mRNA.[23] We measured the sVEGFR-1 and -2 levels in the plasma from the malaria patients, febrile illness patients, and healthy adult groups. The sVEGFR-1 plasma level in each of these groups was not significantly different and was very low compared with sVEGFR-2, suggesting that sVEGFR-1 might not participate in malarial infections. Interestingly, the sVEGFR-2 plasma level in malarial patients was dramatically increased compared with the febrile illness patients and healthy adults, whereas the amount of sVEGFR-2 in *P. falciparum* and *P. vivax* patients was not significantly different (Table 1). Finally, four post-treatment plasma samples were obtained from *P. falciparum*-infected patients, and the VEGF and VEGFR-2 levels in the plasma were determined. The results showed that their levels had returned to comparable levels with the febrile illness patients and healthy adult group after the treatment (data not shown).

To investigate the possibility that malarial antigens can induce VEGF production from mast cells, *in vitro* VEGF production in HMC-1 cells and KU812 cells was examined after their stimulation with malarial antigens. The HMC-1 and KU812 (1×10^6 cells/mL) were stimulated with 20 μg/mL of *P. falciparum*, *P. vivax*, or normal erythrocyte crude antigens overnight, and then the amount of VEGF in culture supernatants was measured by ELISA. A large amount of VEGF was observed in the supernatants of HMC-1 and KU812 cells after stimulation with both *P. falciparum* and *P. vivax* crude antigens (Table 2), which suggests that malarial parasites induce VEGF secretion from human mast cells during infection.

Recently, Muehlenbachset and others[24] reported that the plasma level of sVEGFR-1 was elevated in first-time mothers with either placental malaria, hypertension, or both. However, it has been reported that sVEGFR-1 is abnormally overexpressed in the placenta of preeclamptic patients and is reasonable for the major pathological symptoms on the maternal side such as hypertension and renal dysfunction.[25] Clark and others[26] reported that the serum from pregnant women contains sFlt-1 (sVEGFR-1), which was not present in the serum from men or nonpregnant women. The serum levels of placentally derived sVEGFR-1 are also elevated before and during preeclampsia.[25,27] It is still unclear whether the elevation of sVEGFR-1 observed in the placental malaria is caused by malarial infection or another serious disease affecting pregnancy. In this study, the observed plasma sVEGFR-1 levels in the malarial patients and control groups were low, which is consistent with findings previously reported for healthy volunteers under non-pregnancy-related conditions.[25] Generally, sVEGFR-2 has much higher plasma levels than sVEGFR-1, and the kinase activity of VEGFR-2 is approximately 10-fold higher than that of VEGFR-1, suggesting that VEGFR-2 plays a more important role in VEGF-mediated effects *in vivo*.[28]

Recently, Jain and others[29] examined the plasma levels of 30 biomarkers in human malaria using a multiplex bead-based cytokine immunoassay and found that VEGF was protective against CM-associated mortality. The VEGF is known to bind to two membrane-anchored receptors, VEGFR-1(Flt-1) and VEGFR-2 (KDR/FIK-1), on endothelial cells that result in the MAPK signaling cascade. To examine the role of these receptors in murine malaria, *Plasmodium berghei* ANAK was used to infect VEGFR-1 knockout (KO) mice and mice treated with an antagonistic peptide specific to VEGFR-2. Although increased parasitemia was observed in mice treated with the

TABLE 1

Plasma VEGF, sVEGFR-1, and sVEGFR-2 levels in malaria patients and healthy adults*

Subject	VEGF (pg/mL)	sVEGFR-1 (pg/mL)	sVEGFR-2 (pg/mL)
Plasmodium falciparum malaria	62.5 ± 19.9†	38.9 ± 15.9	4414 ± 428‡
Plasmodium vivax malaria	60.9 ± 20.4†	30.1 ± 9.3	3839 ± 587‡
Febrile illness§	13.1 ± 9.1	25.9 ± 8.7	2595 ± 324
Healthy adults	11.5 ± 8.6	20.9 ± 11.3	2576 ± 522

*VEGF = vascular endothelial growth factor; sVEGFR = soluble vascular endothelial growth factor receptor.
†$P < 0.01$ (vs. febrile illness and healthy adults).
‡$P < 0.01$ (vs. febrile illness and healthy adults).
§Patients with febrile illness without an obvious source of infection.

TABLE 2

Vascular endothelial growth factor (VEGF) production in human mast cell lines

Human mast cell	*Plasmodium falciparum* antigen (pg/mL)	*Plasmodium vivax* antigen (pg/mL)	Normal erythrocyte antigen (pg/mL)
HMC-1	612.1 ± 75.3*	375.4 ± 38.9*	56.2 ± 8.4
KU812	22494 ± 97.8*	1692.6 ± 193.5*	203.5 ± 79.2

*$P < 0.01$ (vs. normal erythrocyte antigen).
Plasmodium falciparum, *P. vivax*, or normal erythrocyte antigen: *P. falciparum*, *P. vivax*, and normal erythrocyte antigen were prepared according to a previously reported method.[7] Briefly, *P. falciparum*-infected, *P. vivax*-infected, or normal erythrocytes were lysed with 0.05% saponin in phosphate buffered saline (PBS) at 37°C for 20 min, washed with PBS and then centrifuged at 2,280 *g*. The pellet was sonicated and used for *in vitro* stimulation of the cells as crude malarial or normal erythrocyte antigens.

antagonistic peptide to VEGFR-2, parasitemia was much lower in the VEGFR-1 KO mice. These findings suggest the importance of VEGF and VEGFR-2 interaction in the host response to malarial infection (Furuta, unpublished data).

We have recently attempted to examine the possible roles of mast cells and VEGF in malaria using mast cell-deficient (W/W^v) and control littermate (+/+) mice. When W/W^v and +/+ mice were infected with *P. berghei* ANKA (PbA), +/+ mice showed lower parasitemia and higher VEGF levels when compared with W/W^v mice. The diminished resistance to infection in the W/W^v mice was considered to be caused by the lack of mast cells and the low amount of VEGF as W/W^v mice reconstituted with the bone marrow-derived mast cells (BMMCs) of +/+ mice recovered resistance to PbA infection and had high VEGF serum levels. Moreover, increased parasitemia was observed in antiVEGF antibody-treated mice compared with nontreated mice (Furuta, unpublished data). Although their precise role is currently unknown, these results clearly suggest the involvement of mast cells and mast cells-derived VEGF in malarial infection. With regard to the mechanism by which mast cells interact with malarial parasites, we have proposed the following explanation. Mast cells are derivatives of hematopoietic progenitor cells that migrate into virtually all vascularized tissues where they complete their maturation. Mature mast cells are normally located in perivascular tissues and close to blood vessels. It is probable that soluble malarial parasite antigens (or the debris of destroyed malarial parasites) leak out of blood capillaries into the perivascular tissues and activate mast cells. We also propose that the spleen is important for the interaction of mast cells and malarial parasites, as parasitized erythrocytes or damaged parasites are filtered from the blood stream in the red pulp of the spleen where mast cells are known to be located.

On the basis of similarities in lineage development and the activation as well as release of mediators, basophils and mast cells have long been considered to be closely related to one another. However, the role of basophils has not yet been explored in human malarial infections. Although mast cells are typically located in tissues and in close association with blood vessels, basophils normally circulate in the blood. In our previous study described above,[19] when mast cell-deficient W/W^v and control +/+ mice were infected with PbA, parasitemia in W/W^v mice was higher than +/+ mice. The resistance to PbA infection in W/W^v mice was recovered by the transfer of BMMCs of +/+ mice. This finding suggests a protective role for mast cells in murine malaria. As to the role of basophils in murine malaria, because basophils were present equally in W/W^v and +/+ mice, it suggests that basophils are not directly involved in the protection against malaria.

In this study, both HMC-1 and KU812 cells secreted VEGF after stimulation with malarial parasite antigens, suggesting the direct activation of mast cells by malarial parasites. As mast cells express TLR1, 2, 3, 4, 6, 7, and 9, it is possible that they are activated directly by molecules that interact with TLRs.[29] It has been reported that glycosylphosphatidylinositol and hemozoin derived from malarial parasites function as a ligand for TLR2 or TLR9 on dendritic cells,[30–32] and recently, we have reported that malarial peroxiredoxin recognizes TLR4 on mast cells and induces cytokine production.[18] Taken together, these findings suggest that malarial parasite components activate mast cells through TLRs to secrete various kinds of inflammatory mediators.

In conclusion, we have confirmed that plasma VEGF levels are elevated in malarial patients compared with febrile illness patients or healthy adults, and is accompanied with an increased level of sVEGFR-2, but not sVEGFR-1. *In vitro* studies showed that malarial parasite antigens induce VEGF secretion from the human mast cell lines KU812 and HMC-1. This is the first report to indicate that the interaction between VEGF and VEGFR-2 is involved in the host immune response to uncomplicated malaria. These findings may be useful for the development of diagnosis and prognostic markers for malarial infection, and will hopefully provide the basis for a new strategy in antimalarial therapy.

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Authors' addresses: Takahisa Furuta, Division of Infectious Genetics, Department of Microbiology and Immunology, Institute of Medical Science, University of Tokyo, Tokyo, Japan, E-mail: Furuta@ims.u-tokyo.ac.jp. Mikio Kimura, Shin-Yamanote Hospital, Japan Anti-Tuberculosis Association, Tokyo, Japan, E-mail: kimumiki@abox3.so-net.ne.jp. Naohiro Watanabe, Department of Tropical Medicine, Jikei University School of Medicine, Tokyo, Japan, E-mail: naohiro@jikei.ac.jp.

Reprint requests: Takahisa Furuta, Division of Infectious Genetics, Department of Microbiology and Immunology, Institute of Medical Science, University of Tokyo, 4-6-1 Shirokanedai, Minato-Ku, Tokyo 108-8639, Japan, Tel: 81-3-5449-5378, Fax: 81-3-5449-5410, E-mail: Furuta@ims.u-tokyo.ac.jp.

REFERENCES

1. Moorthy VS, Good MF, Hill AV, 2004. Malaria vaccine developments. *Lancet 363:* 150–156.
2. Deininger MH, Winkler S, Kremsner PG, Meyermann R, Schluesener HJ, 2003. Angiogenic proteins in brains of patients who died with cerebral malaria. *J Neuroimmunol 142:* 101–111.
3. Senger DR, Galli SJ, Dvorak AM, Perruzzi CA, Harvey AS, Dvorak HK, 1983. Tumor cells secrete a vascular permeability factor that promotes accumulation of ascites fluid. *Science 219:* 983–985.
4. Leung DW, Cachianes G, Kuang WJ, Goeddel DV, Ferrara N, 1989. Vascular endothelial growth factor is a secreted angiogenic mitogen. *Science 246:* 1306–1309.
5. Ferrara N, Houck K, Jakeman L, Leung DW, 1992. Molecular and biological properties of the vascular endothelial growth factor family of proteins. *Endocr Rev 13:* 18–32.
6. Kim I, Moon SO, Kim SH, Kim HJ, Koh YS, Koh GY, 2001. Vascular endothelial growth factor expression of intercellular adhesion molecule 1 (ICAM-1), vascular cell adhesion molecule 1 (VCAM-1), and E-selectin through nuclear factor-kappa B activation in endothelial cells. *J Biol Chem 276:* 7614–7620.
7. Reinders ME, Sho M, Izawa A, Wang P, Mukhopadhyay D, Koss KE, Geehan CS, Luster AD, Sayegh MH, Briscoe DM, 2003. Proinflammatory functions of vascular endothelial growth factor in alloimmunity. *J Clin Invest 112:* 1655–1665.
8. Lucerna M, Mechtcheriakova D, Kadl A, Schabbauer G, Schafer R, Gruber F, Koshelnick Y, Muller HD, Issbrucker K, Clauss M, Binder BR, Hofer E, 2003. NAB2, a corepressor of EGR-1, inhibits vascular endothelial growth factor-mediated gene induction and angiogenic responses of endothelial cells. *Biol Chem 278:* 11433–11440.
9. Kuenen BC, Levi M, Meijers JC, Kakkar AK, van Hinsbergh VW, Kostense PJ, Pinedo HM, Hoekman K, 2002. Analysis of coagulation cascade and endothelial cell activation during inhibition of vascular endothelial growth factor/vascular endothelial growth factor receptor pathway in cancer patients. *Arterioscler Thromb Vasc Biol 22:* 1500–1505.
10. Harada M, Mitsuyama K, Yoshida H, Sakisaka S, Taniguchi E, Kawaguchi T, Ariyoshi M, Saiki T, Sakamoto M, Nagata K,

Sata M, Matsuo K, Tanikawa K, 1998. Vascular endothelial growth factor in patients with rheumatoid arthritis. *Scand J Rheumatol 27:* 377–380.
11. Taha Y, Raab Y, Larsson A, Carlson M, Loof L, Gerdin B, Thorn M, 2004. Vascular endothelial growth factor (VEGF)–a possible mediator of inflammation and mucosal permeability in patients with collagenous colitis. *Dig Dis Sci 49:* 109–115.
12. Boesiger J, Tsai M, Maurer M, Yamaguchi M, Brown LF, Claffey KP, Dvorak HF, Galli SJ, 1998. Mast cells can secrete vascular permeability factor/vascular endothelial cell growth factor and exhibit enhanced release after immunoglobulin E-dependent upregulation of Fcεreceptor I expression. *J Exp Med 21:* 1135–1145.
13. Grützkau A, Krüger-Krasagakes S, Baumeister H, Schwarz C, Kögel H, Welker P, Lippert U, Henz BM, Möller A, 1998. Synthesis, storage, and release of vascular endothelial growth factor/vascular permeability factor (VEGF/VPF) by human mast cells: implications for the biological significance of VEGF206. *Mol Biol Cell 9:* 875–884.
14. Marshall JS, Bienenstock J, 1994. The role of mast cells in inflammatory reactions of the airways, skin, and intestine. *Curr Opin Immunol 6:* 853–859.
15. Echtenacher B, Mannel DN, Hultner L, 1996. Critical protective role of mast cells in a model of acute septic peritonitis. *Nature 381:* 75–77.
16. Galli SJ, Maurer M, Lantz CS, 1999. Mast cells as sentinels of innate immunity. *Curr Opin Immunol 11:* 53–59.
17. Malaviya R, Ikeda T, Ross E, Abraham SN, 1996. Mast cell modulation of neutrophil influx and bacterial clearance at sites of infection through TNF-alpha. *Nature 381:* 77–80.
18. Furuta T, Imajo-Ohmi S, Fukuda H, Kano S, Miyake K, Watanabe N, 2008. Mast cell-mediated immune responses through IgE antibody and Toll-like receptor 4 by malarial peroxiredoxin. *Eur J Immunol 38:* 1341–1350.
19. Furuta T, Kikuchi T, Iwakura Y, Watanabe N, 2006. Protective roles of mast cells and mast cell-derived TNF in murine malaria. *J Immunol 177:* 3294–3302.
20. WHO, 1990. Severe and complicated malaria. *Trans R Soc Trop Med Hyg 84:* 1–65.
21. Kishi K, 1985. A new leukemia cell line with Philadelphia chromosome characterized as basophil precursors. *Leuk Res 9:* 381–390.
22. Butterfield JH, Weiler D, Dewald G, Gleich GJ, 1988. Establishment of an immature mast cell line from a patient with mast cell leukemia. *Leuk Res 12:* 345–355.
23. Kendall RL, Wang G, Thomas KA, 1996. Identification of a natural soluble form of the vascular endothelial growth factor receptor, FLT-1, and its heterodimerization with KDR. *Biochem Biophys Res Commun 226:* 324–328.
24. Muehlenbachs A, Mutabingwa TK, Edmonds S, Fried M, Duffy PE, 2006. Hypertension and maternal-fetal conflict during placental malaria. *PLoS Med 3:* 214–230.
25. Maynard SE, Min J-Y, Merchan J, Lim K-H, Li J, Mondal S, Towia A, Libermann TA, Morgan JP, Sellke FW, Stillman IE, Epstein FH, Sukhatme VP, Karumanchi SA, 2003. Excess placental soluble fms-like tyrosine kinase 1 (sFlt1) may contribute to endothelial dysfunction, hypertension, and proteinuria in preeclampsia. *J Clin Invest 111:* 649–658.
26. Clark DE, Smith SK, He Y, Day KA, Licence DR, Crops AN, Lammoglia R, Charnock-Jones S, 1998. A vascular endothelial growth factor antagonist is produced by the placenta and released into maternal circulation. *Biol Reprod 59:* 1540–1548.
27. Levine RJ, Maynard SE, Qian C, Lim KH, England LJ, Kai F, Yu KF, Schisterman EF, Thadhani R, Sachs BP, Epstein FH, Sibai BM, Vikas P, Sukhatme VP, Karumanchi SA, 2004. Circulating antigenic factors and the risk of preeclampsia. *N Engl J Med 350:* 672–683.
28. Watenberger J, Claessen-Weish L, Siegbahn A, Shibuya M, Heldin CH, 1994. Different signal transduction properties of KDR and Flt-1, two receptors for vascular endothelial growth factor. *J Biol Chem 269:* 26988–26995.
29. Jain V, Armah HB, Tongren JE, Ned RM, Wilson NO, Crawford S, Joel PK, Singh MP, Nagpal AC, Dash AP, Udhayakumar V, Singh N, Stiles JK, 2008. Plasma IP-10, apoptotic and angiogenic factors associated with fatal cerebral malaria in India. *Malar J 7:* 83–98.
30. Marshall JS, 2004. Mast-cell responses to pathogens. *Nat Rev Immunol 4:* 787–799.
31. Krishnegowda G, Hajjar AM, Zhu J, Douglass EJ, Uematsu S, Akira S, Amina S, Woods AS, Gowda DC, 2005. Induction of proinflammatory responses in macrophages by the glycosylphosphatidylinositols of *Plasmodium falciparum*: cell signaling receptors, glycosylphosphatidylinositol (GPI) structural requirement, and regulation of GPI activity. *J Biol Chem 280:* 8606–8616.
32. Coban C, Ishii KJ, Kawai T, Hemmi H, Sato S, Uematsu S, Yamamoto M, Takeuchi O, Itagaki S, Kumar N, Horii T, Akira S, 2005. Toll-like receptor 9 mediates innate immune activation by the malaria pigment hemozoin. *J Exp Med 201:* 19–25.

熱帯病治療薬研究班（略称）の最近の動向

木村　幹男[*1]，丸山　治彦[*2]，古賀　道子[*3]，菊地　　正[*3]，
清水　少一[*3]，三浦　聡之[*3*4]

[*1] 結核予防会新山手病院内科
[*2] 宮崎大学医学部感染症学講座寄生虫学分野
[*3] 東京大学医科学研究所先端医療研究センター感染症分野
[*4] 現勤務先　ヴィーブヘルスケア株式会社メディカルアフェアーズ部，
　および長崎大学熱帯医学研究所（客員教授）

要　旨

熱帯病治療薬研究班（略称）は30年以上にわたって国内未承認の熱帯病・寄生虫症治療薬を導入し，国内で発病した当該疾患患者の適切な治療に貢献してきた．最近では，治療の前に必要な診断に関する支援も行なっている．導入薬剤としては抗マラリア薬が多いが，他の様々な疾患を対象に計19種類の薬剤を扱っている．近年，これらの薬剤の使用を臨床研究として位置づけ，倫理指針を遵守した薬剤使用を行なっている．薬剤の使用は正式な臨床試験ではないが，有効性と安全性の面から使用症例の検討を行い，適切なフィードバックを心がけている．最近，厚生労働省がドラッグ・ラグ解消の取り組みを開始したが，研究班のデータは，熱帯病・寄生虫症に対する国内未承認薬が国内承認を取得する上で，大いに役立つと思われる．

はじめに

熱帯病治療薬研究班（略称）は，その母体や名称は変りつつも1980年より30年以上にわたって存続しつづけ，国内で熱帯病・寄生虫症患者の適切な治療を行なう上で役立ってきた．しかし最近，研究班の薬剤は国内未承認薬であることから，その使用は臨床研究と位置付けられることとなり，そのために薬剤供給規程などの見直しが必要となった．また，国レベルでの“ドラッグ・ラグ”解消のための取り組みが開始されたことに伴い，研究班の協力が求められることになった．本稿ではそれらの最近の変化につき述べ，研究班保管薬剤による治療を必要とする症例において，薬剤が適切に使用されることを望むものである．

連絡先：木村　幹男
〒189-0021　東京都東村山市諏訪町3-6-1
結核予防会新山手病院内科
TEL：042-391-1425　FAX：042-391-5760
E-mail：kimumiki@abox3.so-net.ne.jp

研究班の目的・沿革

観光，企業活動，学術調査，途上国援助など種々の形で国際交流が活発化しており，大量航空機輸送の発達がさらに拍車をかけ，日本からの海外渡航者や海外長期滞在者が増加しつつある．渡航目的国のなかでも熱帯・亜熱帯地域や途上国が増えていることから，熱帯病・寄生虫症に罹患する症例の増加が危惧される．従って，国内においても当該疾患の治療薬剤の医療上の有用性は高くなっているが，多くの場合，患者数が期待される収益に見合うほど多くはないので，国内製薬企業はそれらの薬剤の国内開発に積極的でない．その問題が1980年当時の厚生省薬務局審査課を中心に検討され，研究班を発足させて国内未承認薬を導入し，熱帯病・寄生虫症患者に対して適切な治療を提供できる体制の確立を目指すこととなった．

それにより，1980年に厚生省研究事業「輸入熱帯病の薬物治療法に関する研究班」（代表者：東京大学医科学研究所・田中 寛）が発足し，クロロキン，スルファドキシン/ピリメタミン合剤（当時，国内未承認薬），キニーネ注射薬，プリマキンなどの抗マラリア薬を含む15種類の国内未承認薬の導入が始まった．そして，当時の国立

衛生試験所（現，国立医薬品食品衛生研究所）でそれらの薬剤の品質検査を行い，我が国の製剤基準に合致することを確認してから使用することとした．その後の研究班の母体は，厚生省新薬開発研究事業，厚生省オーファンドラッグ研究事業，創薬等ヒューマンサイエンス総合研究事業（代表者：東京慈恵会医科大学・大友 弘士，その後，宮崎大学・名和 行文），厚生労働科学研究費補助金政策創薬総合研究事業（代表者：名和 行文，その後，木村 幹男）と変遷を重ね，現在の研究班は，平成22年4月に発足した厚生労働科学研究費補助金創薬基盤推進研究事業「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」（代表者：木村 幹男）である．本稿では，これら全ての研究班を「熱帯病治療薬研究班（略称）」と呼ぶ．

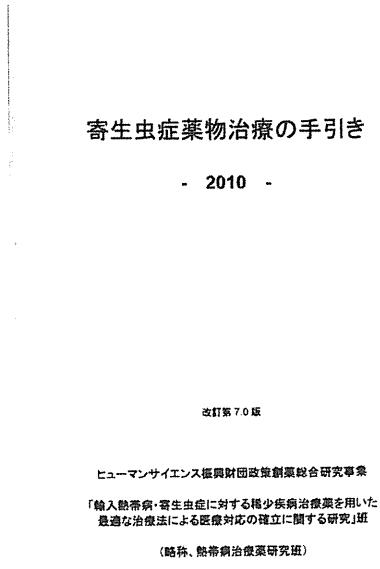
研究班の活動

研究班は現在，抗マラリア薬のアトバコン・プログアニル合剤，アーテメター・ルメファントリン合剤，抗赤痢アメーバ薬のメトロニダゾール注射薬，パロモマイシンその他，種々の疾患に対して計19品目の薬剤を保管し（**表1**），国内で発病する患者に対して，欧米先進国並みのレベルの治療を提供する体制を構築している．これらの薬剤の新規導入時には，国立医薬品食品衛生研究所の班員が品質検査を行い，問題がないことを確認する．さらに近年，全国の医療従事者からの診断や治療に関する問い合わせにも対応しているが，実際の症例の相談に際しては，血液塗抹顕微鏡写真，CT/MRIなどの画像，皮膚症状の写真などを添付した電子メールを利用して症例検討を行なっている．また，ほぼ3年に一度，「寄生虫症薬物治療の手引き」（現在は改訂7.0版，**図1**）を出版して，各種学術集会などで広く配付している．さらに，医療従事者に対する有用な情報提供の場として研究班ホームページ[1]を立ち上げ，随時更新しているが，そこでは「寄生虫症薬物治療の手引き」の電子版も掲載して随時その改訂を行なうなど，我が国での熱帯病・寄生虫症の総合的ネットワークとして機能してきた．

これらの臨床的活動を支えるものとして，研究班では熱帯病・寄生虫症の診断に関する研究その他，基礎的研究も行なっている．その一環として，薬剤の品質確保のために簡便で確実な分析法の開発を行なっている．また，宮崎大学医学部寄生虫学教室では長期間にわたって血清診断法を行なってきたが，毎年全国から400件を超える診断の依頼に対応しており，新規症例の35～40％で診断が得られている．そして，従来の粗抗原を用いた方法のみならず，組換えタンパクを用いた抗体測定により特異的な寄生虫診断を目指している．マラリアについては海外で市販されている抗原検出キットを導入し，その評価を行ないつつある．また，我が国における輸入住血吸虫

表1　研究班の保管薬剤（19品目）

一般名	投与	対象疾患
リン酸クロロキン	経口	マラリア
アトバコン/プログアニル合剤	経口	マラリア
アーテメター/ルメファントリン合剤	経口	マラリア
キニーネ注	注射	マラリア
アーテスネート	坐薬	マラリア
リン酸プリマキン	経口	マラリア
メトロニダゾール注	注射	赤痢アメーバ症
パロモマイシン	経口	赤痢アメーバ症
トリクラベンダゾール	経口	肝蛭症
ニタゾキサニド	経口	クリプトスポリジウム症
スチボグルコン酸ナトリウム	注射	リーシュマニア症
ミルテフォシン	経口	リーシュマニア症
スラミン	注射	アフリカトリパノソーマ症
メラルソプロール	注射	アフリカトリパノソーマ症
エフロールニチン	注射	アフリカトリパノソーマ症
ニフルチモックス	経口	アメリカトリパノソーマ症
スルファジアジン	経口	トキソプラズマ症
ピリメタミン	経口	トキソプラズマ症
イセチオン酸プロパミジン	点眼	アカントアメーバ角膜炎


寄生虫症薬物治療の手引き

- 2010 -

改訂第7.0版

ヒューマンサイエンス振興財団政策創薬総合研究事業

「輸入熱帯病・寄生虫症に対する稀少疾病治療薬を用いた最適な治療法による医療対応の確立に関する研究」班

（略称、熱帯病治療薬研究班）

図1　研究班作成の治療マニュアル．その後の修正・加筆があれば電子版にて改訂し，研究班ホームページ上で更新される．

症の治療の参考のために，アジアの流行地における治療法の検討を行なっているが，さらに，岡山大学綿矢らが抗マラリア薬として開発した新規合成化合物 N-89の住血吸虫に対する作用も調べている．

薬剤使用症例の解析

研究班が導入した薬剤の使用は正式な臨床試験ではないが，できるだけ薬剤使用症例の検討を行い，今後のために結果をフィードバックさせることが必要である．そのため，薬剤使用後に主治医から提出された治療報告書の内容を検討し，必要に応じて主治医に詳細を問い合わせ，有効性と安全性の面から薬剤の評価を行っている．その一つとして，筆者・木村が中心となり，アトバコン・プログアニル合剤（Malarone）の使用症例をまとめた[2]．そこでは，non-immune の成人で合併症のない熱帯熱マラリアを発症し，本薬剤での治療が行われた20例をメフロキンにより治療された49例と比べ，本薬剤の優れた有効性と安全性を報告した．また，小児の３例（１歳11ヶ月，４歳１ヶ月，５歳）でも問題ないことを示した．その後は，平成22年度研究報告書において，成人の熱帯熱マラリア14例，三日熱/卵形マラリア12例，小児の熱帯熱マラリア２例（１歳５ヶ月，２歳０ヶ月）においても，有効性と安全性に優れていること，副作用として報告されたものは，薬剤よりもマラリア自体による可能性が高いことを示した．

さらに，殆どが中等症～重症の赤痢アメーバ症（大腸炎，肝膿瘍，および両者の合併例）で，メトロニダゾール注射薬（Flagyl Inj.）が使われた28症例を検討した[3]．それらの中にはメトロニダゾール経口薬が使われ，大腸炎では結腸切除術が行われた症例も多かったが，本注射薬が顕著な効果を示したものも多くみられた．また，最終的に死亡した例は副作用ではなく原疾患の悪化によると考えられ，より早期に投与開始すれば有効であった可能性も考えられた．また平成19年度研究報告書では，肝蛭症でトリクラベンダゾール（Egaten）を使用した22例をまとめたが，全てにおいて著効が見られ，副作用として重篤なものはみられなかった．他にも少数例ではあるが，研究報告書レベルでマラリアにおけるアーテメター・ルメファントリン合剤（Riamet），リーシュマニア症におけるスチボグルコン酸ナトリウム（Pentostam），クリプトスポリジウム症におけるニタゾキサニド（Alinia）使用症例の評価も行ったが，これらの薬剤の有効性や安全性に特別な問題はみられていない．さらに現在，赤痢アメーバ症での急性期治療の後に再発予防の目的で，非吸収性薬剤パロモマイシン（Humatin）を用いた120例を超える症例を解析しつつある．

臨床研究としての位置づけ

研究班が保管する国内未承認薬は，わずかな例外を除いて欧米先進国で承認されており，世界的には標準的薬剤と位置づけられるが，国内未承認薬であるために健康被害に対して副作用被害救済制度が適用されない問題があり，その解決策を模索しつづけてきた．一方，平成20年７月31日には厚生労働省「臨床研究に関する倫理指針」の全面改正がなされ[4]，臨床研究を行なう際に守るべき種々の事柄が示された．

倫理指針では，「通常の診療を超えた医療行為」は「介入」として扱われる．国内未承認薬の使用は，国際的標準では通常の診療の範囲内と思われるが，我が国では国内未承認薬であることから「通常の診療を超えた医療行為」と考え，倫理指針が規定する「臨床研究」として扱うべきであると結論された．その結果，従来の薬剤使用説明書その他の書式の整備を行い，研究代表者である著者・木村は2010年７月末に，研究計画書を自らの所属機関（結核予防会新山手病院）の倫理審査委員会に提出し，承認を得た（No.10002）．また，倫理指針に則って臨床研究保険の契約を行なった．保険の適用範囲については**表２**に示したが，臨床研究に関連する医療行為によって

表２　臨床研究リスク*に対する臨床研究保険のカバー

健康被害の原因	過失の有無	
	有（賠償責任）	無（補償責任）
臨床研究関連の医療行為 （例，国内未承認薬の投与）	医師賠償責任保険	臨床研究保険
臨床研究関連で医療行為以外 （例，研究計画書の不備）	臨床研究保険	臨床研究保険

＊臨床研究のために研究計画書で使用することを定めた医薬品の投与，または医療機器の使用，研究計画書に定めた臨床上の介入，または手順などによるリスク．診療および治療のみを目的とした医療行為によるリスクは含まない．
保険の対象外として，機会原因に起因する健康被害（例：入院中の給食による食中毒，通院途中での交通事故など），因果関係を合理的に否定できる健康被害，原疾患の悪化による健康被害，被験者に対して本剤の予期した効果またはその他の利益を提供できなかった場合，および賠償責任・過失責任を問えるものがある場など．

健康被害が生じて，過失があった場合には通常の医師賠償責任保険を適用させるが，それ以外の場合については臨床研究保険によりカバーされることになった．実際に支払い対象となるのは，死亡あるいは後遺障害が発生した場合であり，保険金額は医薬品副作用被害救済制度を参考にして設定されている．研究計画書の中で重要な事項は後述するが，各薬剤使用機関の責任者には，筆者・木村が自らの組織に提出して承認を受けた研究計画書と同じ内容で，それぞれの機関の倫理審査委員会での承認を得ることを求めた．

その後，この種の臨床研究を対象とする「大学病院医療情報ネットワーク・臨床試験登録システム（UMIN-CTR)」に登録し，2010年９月より一般公開している（ID 000004125)[5]．さらに，同月には筆者・木村の所属機関（結核予防会）の利益相反委員会の審査を受け，「利益相反には該当しない」との審査結果を得ている（No.20)．

薬剤使用の実際

研究班保管薬剤の使用は倫理指針を遵守して厳格に行なうべきであり，薬剤使用が可能となるのは以下のいずれかの場合と規定した．

1）当該疾患/病態に対して国内承認薬がなく，研究班保管の国内未承認薬による治療が必要と判断される場合．

2）当該疾患/病態に対する国内承認薬があるが，効果や副作用を勘案し，国際的標準に照らしても国内未承認薬の方を選択すべきであると判断される場合．

3）当該疾患/病態に対して国内承認薬を用いたが，効果あるいは副作用の面から，国内未承認薬による再治療が必要と判断される場合．

また，薬剤は登録された医療機関（薬剤使用機関）（**図2**）で使用するのが原則である．従って，それ以外の医療機関を受診した患者については，薬剤使用機関に紹介あるいは搬送して，そこでの治療を依頼する．そして，実際の薬剤使用に際しては，薬剤使用機関の責任者が以下の如く行なうものとする．

1）研究班作成の「薬剤使用説明書」を患者に渡し，それを元に，患者が自由に質問できる状況下で十分な説明を行なう．

2）「薬剤使用承諾書」に患者の署名を得る．

3）「薬剤使用登録書」を東京大学医科学研究所の班員に郵送，ファクス，あるいは電子メール添付で送付する．

4）重篤有害事象がみられたら，ただちに「重篤有害事象報告書」を東京大学医科学研究所の班員にファクス送付する．

5）治療終了後は適切な時期に「治療報告書」（マラリア用，非マラリア用）に記載し，東京大学医科学研究所の班員に送付する．

患者の容態などから薬剤使用機関への搬送が不可能な場合などで，人道的観点から一般の医療機関で薬剤を使用せざるを得ない場合，研究班の３名（木村幹男，古賀

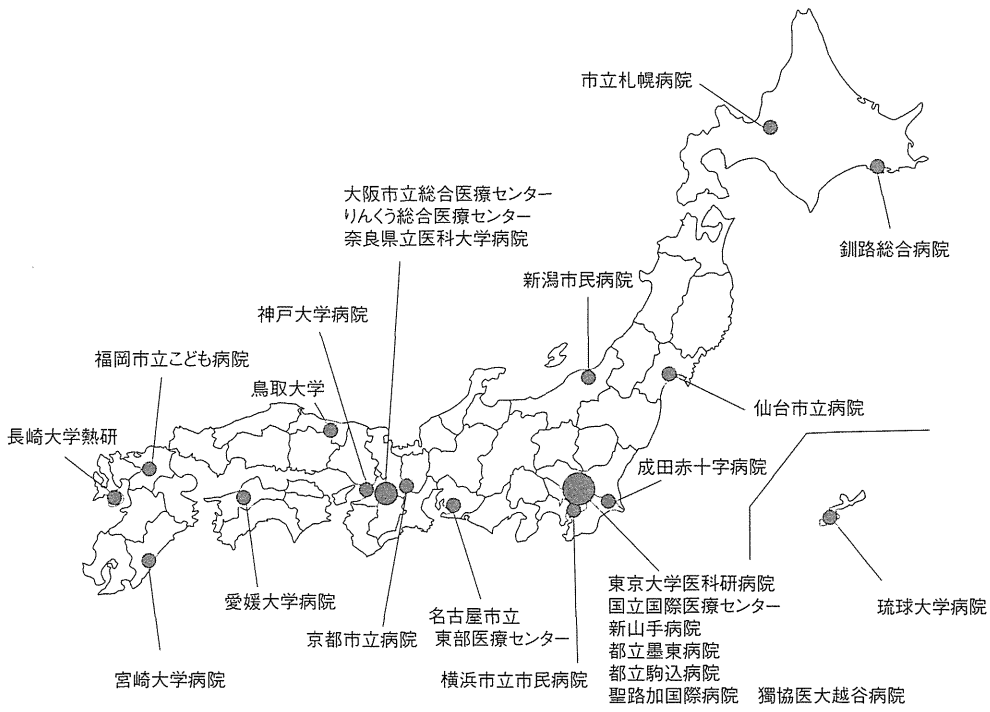


図２　熱帯病治療薬研究班（略称）の薬剤使用機関（全国25ヶ所）

道子，丸山治彦)のいずれかの許可を得なければならない．また，研究班が指定する対象疾患以外で投与せざるを得ない場合，「寄生虫症薬物治療の手引き」やその他に記載されている標準的治療法を大きく超える用法・用量を余儀なくされる場合においても，上記と同じ許可を得ることとした．

ドラッグ・ラグ解消の動き

我が国では，海外で広く使われている薬剤でも国内で使用可能となるのが遅く，その間に患者に大変な不利益をもたらしうることから，ドラッグ・ラグとして問題視されてきた．この問題を一挙に解決すべく，2009年に厚生労働省は学術団体や研究班，さらには患者団体をも対象に，国内未承認薬で国内承認が望まれるもの，あるいは既に国内承認薬であるが適応拡大が望まれるものの募集を行なった．本研究班は約30年間の長きにわたり，熱帯病・寄生虫症の分野で国内未承認薬を使用して経験やデータを蓄積しており，各薬剤の性質や必要性なども把握していることから，この動きに協力する義務があると判断した．そして2009年８月に３品目，すなわちアトバコン・プログアニル合剤，パロモマイシン，メトロニダゾール注射薬の国内承認，および国内承認薬であるメトロニダゾール経口薬の適応拡大の計４件の要望を提出した（**表３**）．厚生労働省は「医療上の必要性の高い未承認薬・適応外薬検討会議」にて提出された要望を検討し，2010年５月より一部の薬剤についてその国内開発を企業に要請し，あるいは開発企業の募集を行なっている．

アトバコン・プログアニル合剤は薬剤耐性熱帯熱マラリアの治療薬として重要であるが，三日熱マラリアのクロロキン耐性も問題となりつつあり，特に耐性が高頻度の地域で感染した三日熱マラリア症例に対しても推奨され始めている[6]．また，我が国ではマラリア予防のための承認薬はメフロキンのみである．しかし，タイ・ミャンマーおよびタイ・カンボジア国境地帯での熱帯熱マラリアではメフロキン耐性が高度であり，メフロキンの精神神経系副作用が危惧されることもあって，欧米先進国ではアトバコン・プログアニル合剤の予防薬としての評価が高く，それを裏付けるデータも出されている[7]．研究班は，本薬剤がマラリアの治療薬としてのみならず，予防薬としても国内承認されれば，国際化時代における国民の健康増進に大きく寄与すると考えた．

抗赤痢アメーバ薬のパロモマイシンは，大腸炎の軽症例での単独使用や，メトロニダゾールなどによる急性期治療の後に根治療法として使われ[8]，研究班保管薬剤の中では最も使用頻度が多い．以前にはフロ酸ジロキサニドを導入していたが，ある時期に入手困難となり，2004年よりパロモマイシンに変更したが，パロモマイシンの方が優れているとする論文[9]もみられる．HIV 感染症などの免疫不全患者では，再発後に急速に重症化する症例もありうるので，根治療法薬として必要性の高い薬剤である．また，知的障害者施設などで赤痢アメーバ症の集団発生が生じた場合には，無症候性保菌者の治療として本薬剤の投与が大変重要である．メトロニダゾール注射薬については，重症赤痢アメーバ症で経口摂取不可能な場合に唯一の治療薬であり，それだけでも要望するに値するが，嫌気性菌一般の感染症の治療薬としても世界的標準の薬剤であり，抗菌薬関連疾患で病院感染としても重要なクロストリジウム・ディフィシル腸炎の経口投与不能例においても効果が期待される．最後のメトロニダゾール経口薬の適応拡大については，以前から多くの関係者により切望されてきたことである．

最終的には，研究班が提出したこれら４件の要望全てが採択され，開発企業が選定されて国内開発が進行中である．なかでも前２者の薬剤の国内承認に関しては，研究班のデータ提供が重要な役割を果たすと思われる．

おわりに

本研究班の薬剤使用に当っては，以前と比べてより厳格さが必要とされる．また，薬剤の使用は臨床研究として位置づけられたことから，患者が全くの新薬の治験と誤解する可能性があり，丁寧な説明が必要となる．薬剤は原則として登録された薬剤使用機関で使用することの

表３　研究班が提出した国内承認・適応拡大の要望薬剤

薬剤	要望	対象疾患	他の要望団体	開発企業
アトバコン・プログアニル合剤	国内承認	マラリア（治療および予防）	なし	グラクソ・スミスクライン
パロモマイシン	国内承認	腸管アメーバ症（赤痢アメーバ症の一病型）	日本感染症学会	ファイザー
メトロニダゾール注射薬	国内承認	赤痢アメーバ症，嫌気性菌感染症	日本感染症学会，日本感染症教育研究会	ファイザー
メトロニダゾール経口薬	適応拡大	赤痢アメーバ症，ランブル鞭毛虫症，クロストリジウム・ディフィシル腸炎を含む嫌気性菌感染症	日本感染症学会，日本感染症教育研究会，東京 HIV 診療ネットワーク	塩野義製薬

理解も必要である．国レベルでのドラッグ・ラグ解消の動きは望ましいことであり，研究班は長期間蓄積してきた経験を元に協力しなければならないが，薬剤の安全上の問題を見逃したりすることがないよう，細心の注意が求められ，重大な責任を負っている．

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文　献

1）http://www.med.miyazaki-u.ac.jp/parasitology/orphan/index.html
2）Hitani A, Nakamura T, Ohtomo H, Nawa Y, Kimura M. Efficacy and safety of atovaquone-proguanil compared with mefloquine in the treatment of nonimmune patients with uncomplicated *P. falciparum* malaria in Japan. J Infect Chemother 2006;12:277-82.
3）Kimura M, Nakamura T, Nawa Y. Experience with intravenous metronidazole to treat moderate-to-severe amebiasis in Japan. Am J Trop Med Hyg 2007;77:381-5.
4）http://www.mhlw.go.jp/general/seido/kousei/i-kenkyu/rinsyo/dl/shishin.pdf
5）http://www.umin.ac.jp/ctr/index-j.htm
6）Griffith KS, Lewis LS, Mali S, Parise ME. Treatment of malaria in the United States. JAMA 2007;297:2264-77.
7）Nakato H, Vivancos R, Hunter PR. A systematic review and meta-analysis of the effectiveness and safety of atovaquone-proguanil (Malarone) for chemoprophylaxis against malaria. J Antimicrob Chemother 2007;60:929-36.
8）Petri WA Jr, Haque R. *Entamoeba* species, including amebiasis. In: Mandell GL, Bennett JE, Dolin R ed. Mandell, Douglas, and Bennett's Principles and Practice of Infectious Diseases, 7th ed. Elsevier (Churchill Livingstone), 2009;p.3411-25.
9）Blessmann J, Tannich E. Treatment of asymptomatic intestinal *Entamoeba histolytica* infection. N Engl J Med 2002;347:1384.