COMMUNICATIONS TO THE EDITOR

In Vitro and *in Vivo* Antimalarial Activities of a Non-glycosidic 18-Membered Macrolide Antibiotic, Borrelidin, against Drug-resistant Strains of *Plasmodia*

Sir:

In the course of our screening program to discover antimalarial antibiotics from soil microorganisms which are active against drug-resistant parasites *in vitro* and *in vivo*, we previously reported that the polyether antibiotics X-206 and K-41 and other microbial metabolites exhibited potent antimalarial properties[1~3]. Thereafter, we found that a substance produced by an actinomycete strain OM-0060 had potent and selective antimalarial activities *in vitro* and *in vivo*. It was identified as borrelidin, a known non-glycosidic 18-membered macrolide antibiotic with a cyclopentanecarboxylic acid side chain (Fig. 1)[4,5]. We report here the antimalarial profile of borrelidin in comparison with those of clinically used antimalarial drugs.

Borrelidin was isolated from the cultured broth of an actinomycete strain OM-0060. *In vitro* activities against *Plasmodium falciparum* strains K1 (drug-resistant) and FCR3 (drug-sensitive), and cytotoxicity against human diploid embryonic cell line MRC-5 were measured as described previously[1]. Rodent malaria-derived strains for *in vivo* 4-days suppressive testing, *P. berghei* N (drug-sensitive) and *P. yoelii* ssp. NS (chloroquine-resistant) were used to assess *in vivo* antimalarial activities as described previously[1,2]. Test compounds were dissolved in 10% DMSO-Tween 80 aqueous solution and administered subcutaneously (s.c.) or orally (p.o.) to the mice two hours after infection with parasites (Day 0). Then the compounds were successively administered (s.c. or p.o.) to the infected mice once a day for 3 days (Days 1~3). On the day after the last treatment (Day 4), thin blood films were made from the tail blood of the mice, and the parasitaemia was determined as described previously[2].

Table 1 shows the *in vitro* antimalarial activities of borrelidin and the standard antimalarial drugs. Borrelidin showed more potent activity against the drug-resistant K1 strain of *P. falciparum* than the clinically used antimalarials, artemether, artesunate and chloroquine. Furthermore, borrelidin showed similar activity against the drug-sensitive FCR3 strain of *P. falciparum* to artemether and artesunate, and was more potent than chloroquine. The cytotoxicity of borrelidin against MRC-5 cells was relatively low (the IC_{50} value: 410 nM). Borrelidin showed high selectivity indexes with the ratios of 216 and 228 for the MRC-5 cells/K1 strain and MRC-5 cells/FCR3 strain, respectively.

Table 2 shows a preliminary comparison of the *in vivo* subcutaneous antimalarial activities of borrelidin and the standard antimalarial drugs. Borrelidin had antimalarial activity against both rodent malaria-derived *P. berghei* N and *P. yoelii* ssp. NS. Especially, borrelidin showed more potent subcutaneous antimalarial effects than artemether, artesunate and chloroquine against the chloroquine resistant strain (*P. yoelii* ssp. NS). The ED_{50} and ED_{90} values of borrelidin against *P. yoelii* ssp. NS were lower by factors 5.7~64 and 6.4~>125, respectively, than those of the other three drugs. Borrelidin also showed more potent

Fig. 1. Structure of borrelidin.

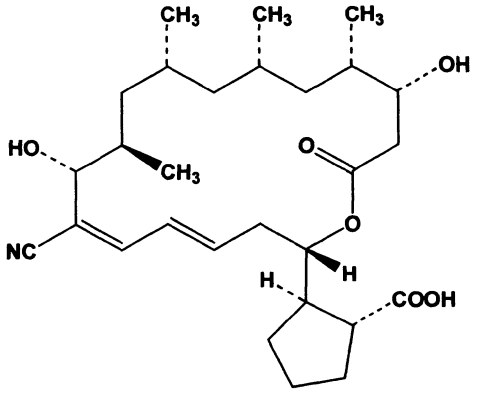

Table 1. Antimalarial activities of borrelidin and the antimalarial drugs against K1 and FCR3 strains of *Plasmodium falciparum*.

Compound	IC50 (nM)	
	K1 strain	FCR3 strain
Borrelidin	**1.9**	**1.8**
Artemether	**7.6**	**2.2**
Artesunate	**11**	**2.7**
Chloroquine	**357**	**29**

Table 2. *In vivo* subcutaneous antimalarial activities of borrelidin, artemether, artesunate and chloroquine against *P. berghei* N and *P. yoelii* ssp. NS.

Parasite	Compound	ED50 (mg/kg)	ED90 (mg/kg)
P. berghei* N	**Borrelidin**	**0.18**	**2.0**
	Artemether	**0.95**	**3.8**
	Artesunate	**1.7**	**10.0**
	Chloroquine	**1.5**	**2.5**
P. yoelii* ssp. NS*	**Borrelidin**	**0.07**	**0.8**
	Artemether	**1.1**	**5.1**
	Artesunate	**0.4**	**26.0**
	Chloroquine	**4.5**	**>100.0**

*** drug-sensitive strain ** chloroquine-resistant strain**

antimalarial effects than artemether, artesunate and chloroquine when the drugs were administered orally (Table 3). The ED_{50} and ED_{90} values of borrelidin were lower by factors 13~17 and 36~>91, respectively, than those of the other three drugs.

It is known that borrelidin has inhibitory activities against *Borrelia*[4], *Treponema*[6,7], viruses[8], certain micrococci[9], tumor cells[10], angiogenesis[11] and a yeast cyclin-dependent kinase[12], and that its mode of antibiotic action in sensitive microorganisms involves selective inhibition of threonyl-tRNA synthetase[13]. WAKABAYASHI *et al.* reported that it inhibits both threonyl-tRNA synthetase and protein synthesis in cultured rat cells[11]. However, the finding of the antimalarial activity of borrelidin is novel and the above data are the first report of such properties.

SCHNITZER *et al.*[14] and SINGH *et al.*[6] reported that the LD_{50} values (i.v., s.c. and p.o. in mice) of borrelidin were 39.0, 74.7 and slightly less than 400 mg/kg, respectively. We also determined that the acute subcutaneous toxicity of borrelidin (the LD_{50} value in mice) was >50 mg/kg. However, we did observe toxicity (loss of weight, mortality) when the compound was delivered by the p.o. route (preliminary results suggest an LD_{50} of 16.4 mg/kg). These effects are being investigated further.

The above results reveal that borrelidin is a promising lead compound for a new type of the antimalarial drug. Further investigation of the antimalarial potential of borrelidin is in progress.

Table 3. *In vivo* oral antimalarial activities of borrelidin, artemether, artesunate and chloroquine against *P. yoelii* ssp. NS.

Compound	ED50 (mg/kg)	ED90 (mg/kg)
Borrelidin	**0.3**	**1.1**
Artemether	**5.0**	**40.0**
Artesunate	**4.0**	**>50.0**
Chloroquine	**4.5**	**>100.0**

Acknowledgments

This work was supported, in part, by funds from the UNDP/World Bank/WHO Special Program for Research and Training in Tropical Diseases (grants ID 990806 and ID A10124), and Grants-in-Aid for Scientific Research (A) from the Ministry of Education, Culture, Sports, Science and Technology, Japan. A part of work was supported by The 21st Century COE program, the Ministry of Education, Culture, Sports, Science and Technology (MEXT), Japan. We are grateful to Dr. K. HATA, JPMW Coordination Center, for valuable discussion. We also thank Miss. A. KOHANA, C. MANABE and S. SIBATA, the Kitasato Institute, for the antimalarial assay, Mr. Y. YAMAGUCHI and Ms A. MATSUMOTO, Kitasato Institute for Life Sciences, Kitasato University, for technical assistance.

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(Received April 18, 2003)

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