J. Antibiot. 60(3): 220–222, 2007

COMMUNICATIONS TO THE EDITOR

THE JOURNAL OF ANTIBIOTICS

Selective and Potent *In Vitro* Antimalarial Activities Found in Four Microbial Metabolites

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Received: November 22, 2006 / Accepted: March 5, 2007

Abstract Antimalarial activities have been identified in four microbial metabolites through a screening programme of existing compounds in the Kitasato Institute chemical library. Hedamycin showed selective and potent activity against both drug-resistant and drug-sensitive strains of *Plasmodium falciparum*. Simaomicin α exhibited remarkably strong antimalarial activity, although its activity against a drug-resistant strain was weaker than that against a drug-sensitive strain. The antimalarial effects of triacsins C and D are also reported.

Keywords antimalarial antibiotics, hedamycin, simaomicin α, triacsin, *Plasmodium falciparum*, drug-resistant strain

As a result of our on-going program of screening soil microorganisms and renewed testing of compounds lodged in the antibiotic library of the Kitasato Institute for Life Sciences, we have previously reported on various microbial metabolites that exhibit potent antimalarial activities [1～5]. We have now discovered four more compounds that possess antimalarial characteristics. Hedamycin, simaomicin α, triacsin C and triacsin D, all from the antibiotic library of the Kitasato Institute, display potent antimalarial activity *in vitro*. We report here the antimalarial profiles of these four compounds (Fig. 1) [6～8] in comparison with those of clinically-used antimalarial drugs.

In vitro activities against *Plasmodium falciparum* strains K1 (drug-resistant) and FCR3 (drug-sensitive), and cytotoxicity against human diploid embryonic cell line MRC-5 of these compounds, were measured as described previously [1].

Hedamycin showed weak activity against both the K1 and FCR3 strain of *P. falciparum*, similar to the activity of chloroquine for K1 strain (Table 1). The IC_{50} value of chloroquine to the K1 strain is 10-fold higher than to the FCR3 strain, indicating that the antimalarial action of hedamycin is different from that of chloroquine. Although the compound does not meet the UNICEF/UNDP/World Bank/WHO Special Programme for Research and Training in Tropical Diseases (TDR) criteria for whole cell screening (IC_{50}<0.1 μg/ml for *P. falcipurum* K1 strain), it could be a promising lead if the activity could be improved since it has low cytotoxicity (IC_{50}>25 μg/ml).

Simaomicin α showed the most potent activity against both drug-resistant K1 and drug-sensitive FCR3. The IC_{50} values of the compound were remarkably lower than those of the clinically-used antimalarial drugs, artemether, artemisinin and chloroquine. The cytotoxicity of the compound was weaker (IC_{50}=4 ng/ml) than its antimalarial activities. Simaomicin α was 4.6-fold less active against the K1 strain than the FCR3 strain. Although simaomicin α showed considerably more potent antimalarial activity than that of the other compounds, the presence of a polycyclic xanthone structure containing an isoquinoline moiety may

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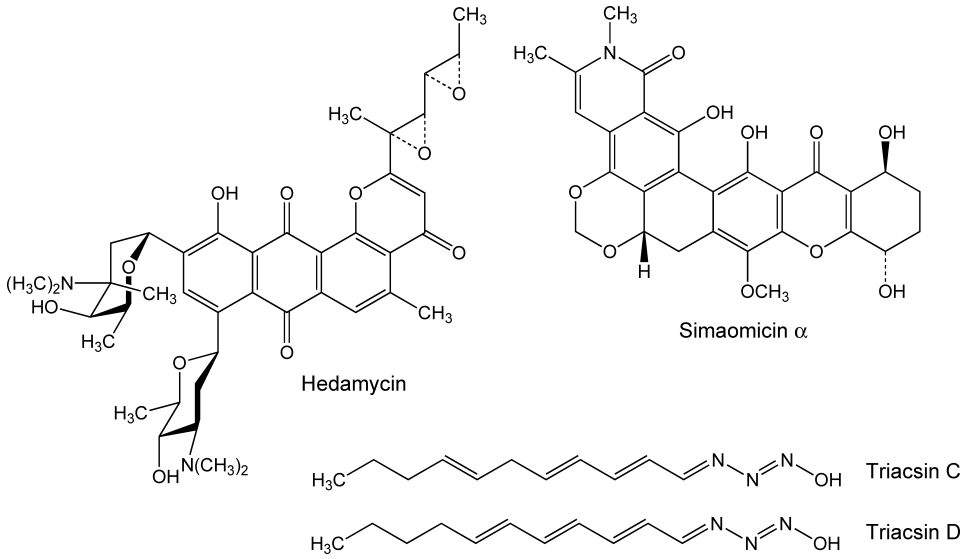

Fig. 1. Structures of hedamycin, simaomicin α, triacsin C and triacsin D.

Table 1. *In vitro* antimalarial activity against K1 and FCR3 strains of *Plasmodium falciparum* of four microbial metabolites and some commonly-used antimalarial drugs

Compound	IC_{50} (ng/ml)	
	K1 strain	FCR3 strain
Hedamycin	230	170
Simaomicin α	0.045	0.0097
Triacsin C	6.0	8.0
Triacsin D	770	560
Artemether	2.3	0.7
Artemisinin	6.8	5.1
Chloroquine	184	15.0

explain its lower potency against drug-resistant (K1) strains than that of the drug-sensitive (FCR-3) strains. Recently, we reported similar antimalarial activities of the plant products magnoline and magnolamine, which contain the isoquinoline moiety, against K1 and FCR3 strains [9].

Triacsins C and D exhibited antimalarial activity against both K1 and FCR3 strains of *P. falciparum*. The activity of triacsin C is comparable to that of artemisinin, while triacsin D was 70-fold less potent against FCR3 and 128-fold less potent against K1 than triacsin C. The difference of the IC_{50} values between antimalarial activities and cytotoxicity (C: 90 ng/ml, D: 2,900 ng/ml) was almost 100-fold. The greater potency of triacsin C is probably due to the small molecular structure difference at the unsaturated position seen between these compounds.

Hedamycin is an anthraquinone antibiotic, and is reported to have antitumor, antibacterial and anti-*Tetrahymena pyriformis* (anti-protozoal) activity [10]. The mode of action of anthraquinone antibiotics as inhibitors of nucleic acid synthesis is well documented [10]. Hedamycin may inhibit *Plasmodia* in the same manner and detailed studies of the mode of action need to be undertaken to clarify this.

Simaomicin α is a polycyclic xanthone antibiotic, and it is known that it exhibits inhibitory activities against Gram-positive bacteria and coccidia [7]. However, its mode of action is not clearly understood. Recently, we reported that it inhibits bleomycin-induced G2 checkpoints in Jurkat cells [11]. While the molecular controls of the *Plasmodium* life-cycle are poorly understood, Arnot & Gull, however, reported a clear correspondence between cellular events occurring during *Plasmodium* schizogony and the G1, S, G2 and M phases of the "classical" cell cycle have not been established [12]. Therefore, it may be possible to study the *Plasmodium* life-cycle using simaomicin α as a cell-cycle effector.

Triacsin C has been reported to be a potent competitive inhibitor of rat acyl-CoA synthetases (ACS) 1 and 4 [13]. Triacsins were found to be lethal to animal cells but not to microorganisms [14]. With regard to *Plasmodia*, Matesanz *et. al.*, recently reported that *P. falciparum* fatty acyl-CoA synthetases (PfACS) were different from those of human ACS, and suggested that PfACS might be considered as molecular targets for chemotherapeutic antimalarial drugs [15]. Our data for triacsins suggest that it may prove profitable to synthesize triacsin derivatives with higher

selectivity indexes.

Further studies, including *in vivo* testings, and characterization of other biological activities of hedamycin, simaomicin α, triacsin C and triacsin D are in progress.

Acknowledgments This work was supported, in part, by funds from the UNICEF/UNDP/World Bank/WHO Special Programme for Research and Training in Tropical Diseases (grants ID A10124), and a grant of the 21st Century COE program, the Ministry of Education, Culture, Sports, Science and Technology, Japan. Part of the work was supported by the grant for All Kitasato Project Study (AKPS).

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