COMMUNICATIONS TO THE EDITOR

In Vitro* and *In Vivo* Antimalarial Activities of a Carbohydrate Antibiotic, Prumycin, against Drug-resistant Strains of *Plasmodia

Sir:

In the course of our program to discover antimalarial antibiotics active against drug-resistant parasites, by screening soil microorganisms and antibiotic library of the Kitasato Institute for Life Sciences, we previously reported on various microbial metabolites exhibiting potent antimalarial properties¹⁻⁴). Now, we find that prumycin⁵⁻⁷), a compound in the antibiotic library of our institute, has potent and moderately selective antimalarial activity *in vitro* and *in vivo*. We report here the antimalarial profiles of prumycin and its derivatives (Fig. 1) in comparison with those of clinically used antimalarial drugs, and also present some conclusions on structure-activity relationships.

Prumycin was purified from the cultured broth of *Streptomyces* sp. strain No. F-1028^{5,6)}. The derivatives (2-4) were synthesized according to our previous report⁷⁾. Benzyl 4-amino-2-(benzyloxycarbonyl) amino-2,4-dideoxy- α -L-arabinopyraside (5) was a generous gift of Nippon Kayaku Co. LTD., (Japan). The compounds (6-8) were prepared from 5 with the protected amino acids (DCC, CH₂Cl₂, r.t.), followed by deprotections.

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 previously¹⁾. 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* efficacy as reported previously^{1,2)}. Prumycin was dissolved in water, while other test compounds were solubilized in 10% DMSO-Tween 80 aqueous solution. The formulated samples were administered subcutaneously (s.c.) to mice two hours after infection with parasites (Day 0), and then 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 previously²⁾.

Table 1 shows the *in vitro* antimalarial activities of prumycin, its derivatives and some standard antimalarial drugs. Prumycin and α -prumycin had similar activity to choroquine against the drug-resistant K1 strain of *P. falciparum*, but were less potent than the clinically used antimalarials artemether, artemisinin and artesunate. Furthermore, prumycin and α -prumycin showed similar activities against the drug-sensitive FCR3 strain of *P. falciparum*. However, the IC₅₀ values of prumycin derivatives (3-8) against the K1 strain were >12.5 μ g/ml.

Fig. 1. Structures of prumycin and its derivatives.

Table 1. Antimalarial activities of prumycin, its derivatives and the antimalarial drugs against K1 and FCR3 strains of *Plasmodium falciparum*.

Compound	IC ₅₀ (μg/ml)	
	K1 strain*	FCR3 strain**
Prumycin (1)	0.16	0.17
α-Prumycin (2)	0.34	0.14
3	>12.5	ND***
4	>12.5	ND
5	>12.5	ND
6	>12.5	ND
7	>12.5	ND
8	>12.5	ND
Artemisinin	0.01	0.006
Artemether	0.0023	0.0007
Artesunate	0.0042	0.001
Chloroquine	0.3	0.02

* drug-resistant strain ** drug-sensitive strain *** not determined

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

Parasite	Compound	ED ₅₀ (mg/kg)	ED ₉₀ (mg/kg)
<i>P. berghei</i> N*	Prumycin	6.2	17.0
	Artemether	0.95	3.8
	Artesunate	1.7	10.0
	Chloroquine	1.5	2.5
<i>P. yoelii</i> ssp. NS**	Prumycin	10.2	27.8
	Artemether	1.1	5.1
	Artesunate	0.4	26.0
	Chloroquine	4.5	>100.0

* drug-sensitive strain ** chloroquine-resistant strain

We then investigated the cytotoxicities of prumycin and α-prumycin against MRC-5 cells and found them to be, respectively, 3.6 and 1.2 μg/ml. Prumycin and α-prumycin showed moderate to low selectivity indexes, with ratios in the ranges of 22.5~3.5 and 21.3~8.6 for the MRC-5 cells/K1 strain and MRC-5 cells/FCR3 strain, respectively.

The lack of antimalarial activity of prumycin derivatives (3~8) in comparison to prumycin provides interesting information on structure-activity relationships. Thus introduction of a methyl group on the hydroxyl (to give 3) and acetylating the two primary amino groups (resulting in 4) destroys activity. Furthermore, replacement of the L-alanine group on the amino moiety at C-4 with, respectively, L-glycine (6), L-proline (7), and L-isoleucine (8) also destroys activity. These results suggest that a free anomer at C-1, amino groups at C-2, and the methyl group at L-alanine all play an important role in the antimalarial activity of prumycin.

Further studies are necessary to extend the structure-activity relationships of prumycin-related antimalarial compounds.

Table 2 shows a preliminary comparison of the *in vivo* subcutaneous antimalarial activities of prumycin and the standard antimalarial drugs. Prumycin had moderate activity against both rodent malaria-derived *P. berghei* N and *P. yoelii* ssp. NS, but was less effective than the clinically used antimalarials artemether and artesunate. The ED₉₀ value of prumycin (27.8 mg/kg) against the chloroquine-resistant strain (*P. yoelii* ssp. NS) was much lower than that of chloroquine (>100 mg/kg), and was

similar to that of artesunate.

It is known that prumycin has inhibitory activities against phytopathogenic fungi such as *Sclerotinia sclerotiorum* and *Botrytis cinerea*^{5,6)}, and against tumor cells^{8~11)}, and that its mode of anti-fungal action in growing cells of *B. cinerea* involves selective inhibition of protein synthesis¹²⁾. Furthermore, we previously reported that it inhibits both protein synthesis and DNA synthesis in cultured HeLa S3 cells⁸⁾. However, the finding of the antimalarial activities of prumycin and its derivative is novel and the above data are the first report of such properties.

We previously reported that the LD₅₀ values (i.v. and i.p., in mice) of prumycin were 144 and 155 mg/kg, respectively. However, we did observe toxicity (loss of weight, mortality) when the compound was delivered by the s.c. route at >30 mg/kg×4. These effects are being investigated further.

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

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References

- 1) OTOGURO, K.; A. KOHANA, C. MANABE, A. ISHIYAMA, H. UI, K. SIOMI, H. YAMADA & S. ŌMURA: Potent antimalarial activities of polyether antibiotic, X-206. *J. Antibiotics* 54: 658~663, 2001
- 2) OTOGURO, K.; A. ISHIYAMA, H. UI, M. KOBAYASHI, C. MANABE, G. YAN, Y. TAKAHASHI, H. TANAKA, H. YAMADA & S. ŌMURA: *In vitro* and *in vivo* antimalarial activities of the monoglycoside polyether antibiotic, K-41 against drug resistant strains of *Plasmodia*. *J. Antibiotics* 55: 832~834, 2002
- 3) OTOGURO, K.; H. UI, A. ISHIYAMA, N. ARAI, M. KOBAYASHI, Y. TAKAHASHI, R. MASUMA, K. SIOMI, H. YAMADA & S. ŌMURA: *In vitro* antimalarial activities of the microbial metabolites. *J. Antibiotics* 56: 322~324, 2003
- 4) OTOGURO, K.; H. UI, A. ISHIYAMA, M. KOBAYASHI, H. TOGASHI, Y. TAKAHASHI, R. MASUMA, H. TANAKA, H. TOMODA, H. YAMADA & S. ŌMURA: *In vitro* and *in vivo* antimalarial activities of a non-glycoside 18-member macrolide antibiotic, borrelidin, against drug-resistant strains of *Plasmodia*. *J. Antibiotics* 56: 727~729, 2003
- 5) HATA, T.; S. ŌMURA, M. KATAGIRI, K. ATSUMI, J. AWAYA, S. HIGASHIKAWA, K. YASUI, H. TERADA & S. KUYAMA: A new antifungal antibiotic, prumycin. *J. Antibiotics* 24: 900~901, 1971
- 6) ŌMURA, S.; M. KATAGIRI, J. AWAYA, K. ATSUMI, R. ŌIWA, T. HATA, S. HIGASHIKAWA, K. YASUI, H. TERADA & S. KUYAMA: Production and isolation of a new antifungal antibiotic, prumycin, and taxonomic studies of *Streptomyces* sp., strain No. F-1028. *Agr. Biol. Chem.* 37: 2805~2812, 1973
- 7) ŌMURA, S.; M. KATAGIRI, K. ATSUMI, T. HATA, A. A. JAKUBOWSKI, E. B. SPRINGS & M. TISHILER: Structure of prumycin. *J. Chem. Soc., Perkin Trans. I.* 1974: 1627~1631, 1974
- 8) OKUBO, S.; N. NAKAMURA, K. ITO, H. MARUMO, M. TANAKA & S. ŌMURA: Antitumor activity of prumycin. *J. Antibiotics* 32: 347~354, 1979
- 9) OKUBO, S.; N. NAKAMURA, M. MORIMOTO, K. MINEURA, H. MARUMO & S. ŌMURA: Studies on antitumor activity of prumycin. II. Studies on distribution and excretion of prumycin. *J. Antibiotics* 33: 221~225, 1980
- 10) OKUBO, S.; N. NAKAMURA, M. MORIMOTO, K. MINEURA, H. MARUMO & S. ŌMURA: Studies on antitumor activity of prumycin. III. Mode of action of prumycin on HeLa S-3 cells. *J. Antibiotics* 33: 226~230, 1980
- 11) OKUBO, S.; M. MORIMOTO, K. MINEURA, H. MARUMO & S. ŌMURA: Studies on antitumor activity of prumycin. IV. Effect of prumycin on mouse immune system. *J. Antibiotics* 33: 231~235, 1980
- 12) SCHWARTZ, J. L.; M. KATAGIRI, S. ŌMURA & M. TISHILER: The mechanism of prumycin action. *J. Antibiotics* 27: 379~385, 1974

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