Su RB *et al* / Acta Pharmacol Sin 2003 Sep; 24 (9): 918-922

Antimalarial effect of agmatine on *Plasmodium berghei* K173 strain

SU Rui-Bin[1], WEI Xiao-Li[2], LIU Yin, LI Jin

Beijing Institute of Pharmacology and Toxicology, Beijing 100850;
[2]Beijing Institute of Microbiology and Epidemiology, Beijing 100071, China

KEY WORDS agmatine; difluoromethylornithine; antimalarials; spermidine; *Plasmodium berghei*

ABSTRACT

AIM: To study the antimalarial effect of agmatine (Agm) on chloroquine-susceptible *Plasmodium berghei* K173 strain (S strain) and the *P berghei* K173 resistant strain (R strain). **METHODS:** The antimalarial effects of Agm on *P berghei* K173 S strain and R strain were evaluated by Peters 4-d suppression test in mice. **RESULTS:** Agm (12.5-200 mg/kg, ig, daily) decreased the parasitemia for both *P berghei* K173 S strain (IC_{50}=139 mg/kg) and R strain (IC_{50}=126 mg/kg) in mice. Subcutaneous injection (sc) of Agm (5-40 mg/kg, tid) showed relatively stronger antimalarial effect than intragastric gavage (IC_{50}=30 mg/kg) in *P berghei* K173 S strain. Spermidine antagonized the antimalarial effect of Agm for *P berghei* K173 S strain and R strain. Agm did not reverse the chloroquine resistance of *P berghei* K173 S strain. *dl*-α-Difluoromethylornithine (DFMO, sc) decreased the parasitemia of *P Berghei* K173 S strain and this effect was antagonized by spermidine. **CONCLUSION:** Agm has an antimalarial effect and the mechanism is related to its inhibition of polyamine synthesis.

INTRODUCTION

Spermidine is an important precursor for the biosynthesis of hypusine and homospermidine in eukaryotes, and interference with polyamine biosynthesis by inhibition of ornithine decarboxylase (ODC) and *S*-adenosylmethionine decarboxylase (AdoMetDC) has been discussed as a potential chemotherapy of cancer and parasitic infection[1]. Blocking spermidine and spermine synthesis in *P falciparum*-infected erythrocytes with irreversible inhibitors of AdoMetDC prevents the growth of the parasite *in vitro*. *D,L*-α-Difluoromethylornithine (DFMO), an inhibitor of ODC, which can inhibit the synthesis of spermine and spermidine and influence the content of the polyamines, is able to decrease the growth rate and proliferation of *Leishmania mexicana promastigote*, and parasite multiplication could be resumed by addition of exogenous polyamines[2]. Furthermore, the addition of DFMO to human *P falciparum*-infected red cells in continuous culture decreased parasite growth and intracellular polyamine concentrations[3]. DFMO also blocked exoerythrocytic schizogony of *Plasmodium berghei* in mice and in cultured human hepatoma cells and these effects were also reversed by administration of exogenous spermidine[4]. 1,7-Diaminoheptane, an inhibitor of deoxyhypusine synthase, which could decrease the intracellular spermidine content, inhibits the proliferation of malaria parasites of *P falciparum* NF54 strain *in vitro*; this effect could be reversed by spermidine[5]. All these results extend previous observations that the polyamines, especially spermidine, are crucial for differentiation and proliferation of malarial parasites, thus interference with polyamine biosynthesis may be a viable chemotherapeutic target of malaria parasites.

Agmatine (Agm) is a primary amine formed by

[1] Correspondences to SU Rui-Bin, PhD. Phn 86-10-6693-1621. Fax 86-10-6821-1656. E-mail Ruibinsu@yahoo.com
Received 2002-08-06 Accepted 2003-01-21

decarboxylation of *L*-arginine by an enzyme, *L*-arginine decarboxylase. Agm was a constituent of bacteria, plants and many invertebrates, and was believed to be a metabolic intermediate in the formation of spermine and spermidine[6]. Agm is proved to inhibit NOS[7] and ODC activity and hold back cell proliferation[8]. Agm is also a moderate inhibitor of homospermidine synthase and suppresses the parasitemia of the chloroquine-susceptible NF54 strain and the less chloroquine-susceptible R strain *in vitro*[5]. In addition, Agm is able to deplete polyamine in hepatocytes by inducing spermidine/spermine acetyltransferase[9]. All these results inferred that Agm might antagonize the effect of spermidine on cell proliferation. In the present study we investigated the effect of Agm on *P Berghei* K173 strain and the *Plasmodium berghei* K173 resistant strain (RCQ/K173) in mice and analyzed whether this effect was related to the inhibition of polyamine synthesis.

MATERIALS AND METHODS

Animals and malarial parasites Male and female (1:1) Kunming mice [20 g±2 g, Grade II, Certificate No 01-3023, provided by the Experimental Animal Center of Academy of Military Medical Sciences (AMMS)] were used in all experiments. Animals were randomly assigned to treatment groups and maintained on a 12-h light/dark cycle and given *ad libitum* access to food and water, strictly in compliance with the guidelines established for the use of experimental animals by the European Community. Ten mice were used in each group, and each animal was used only for one treatment.

P berghei K173 S strain was introduced in 1983 from the Peters Laboratary of London Academy of Tropical Medicine and Hygiene. *P berghei* K173 R strain was cultured to be highly resistant to chloroquine by using high concentration chloroquine on *P berghei* K173 S strain continuously for 200 generation, index of resistance (RI) was 110.

Mice were injected with 1×10^7 malarial parasite intraperitoneally (ip). One hour later, drugs were injected alternately for a 4-d suppression test. Four days later, tail blood was used to make thin blood slice and parasitemia was counted by microscopy. The inhibitory rate of drugs was expressed as [(Parasitemia in control group – Parasitemia in drug treated group)/(Parasitemia in control group)×100 %].

Drugs and administration Agm sulphate and spermidine were obtained from Sigma (St Louis, MO, USA). DFMO hydrochloride was purchased from Merck KgaA (Darmstadt, Germany). All drugs were dissolved in normal saline and freshly prepared on the experimental day. Agm was injected ig or sc. Spermidine and DFMO were given sc.

Antimalarial effect of Agm on *P berghei* K173 S strain Mice were ip injected with red blood cells infected by *P berghei* K173 strain. One hour later, the mice were continuously treated with different doses of Agm by ig (12.5-200 mg/kg, daily) or sc (5-40 mg/kg, tid) injection for 4 d. To study the influence of spermidine on the antimalarial effect of Agm, spermidine (30 mg/kg, sc, daily) was coadministered with Agm (40 mg/kg, ig).

Antimalarial effect of Agm on *P berghei* K173 R strain Mice were intraperitoneally injected with red blood cells infected with *P berghei* K173 R strain. One hour later, mice were continuously treated with Agm (12.5-200 mg/kg, ig, daily) for 4 d. To investigate whether Agm could reverse the chloroquine resistance of malarial parasites, mice were co-pretreated with Agm (100 mg/kg, ig) and chloroquine (0.22-3.5 mg/kg, ig). In another experiment, Agm (12.5-200 mg/kg, ig) was coadministered with chloroquine (1.75 mg/kg, ig). To study the influence of spermidine on the antimalarial effect of Agm, spermidine (5-60 mg/kg, sc, daily) was coadministered with Agm (100 mg/kg, ig).

Antimalarial effect of DFMO on *P berghei* K173 S strain Mice were ip injected with red blood cells infected by *P berghei* K173 S strain. One hour later, the mice were continuously treated with DFMO (10-50 mg/kg, sc, daily) for 4 d. To study the influence of spermidine on the antimalarial effect of DFMO, spermidine (30 mg/kg, sc, daily) was coadministered with DFMO (10-50 mg/kg, sc, daily). Tail blood was used to make thin blood slice and parasitemia was counted by microscopy in all antimalarial experiments.

Statistics The parasitemia in red blood cells were expressed as mean±SD. ANOVA was used to analyze the statistical significance of differences between groups. Difference was significant at $P<0.05$.

RESULTS

Antimalarial effect of Agm on *P berghei* K173 S strain In Peters 4-d suppression test, the average parasitemia of *P berghei* K173 S strain was 222 per 1000 red blood cells. Agm (12.5-200 mg/kg, ig, daily) decreased the parasitemia dose-dependently, the parasitemia reduced to 7 % at 200 mg/kg ($n=6$, $P<0.01$ *vs* control), the inhibitory rate reached 68.5 % at the dose

of 200 mg/kg, and IC_{50} was about 139 mg/kg (Tab 1). The antimalarial effect of Agm (10-40 mg/kg, sc, tid) was increased, the inhibitory rate reached 68.9 % at the dose of 40 mg/kg and the IC_{50} was 30 mg/kg (Tab 2). These results first demonstrate that Agm had an antimalarial effect *in vivo*. Spermidine (30 mg/kg, sc) itself did not exhibit any antimalarial effect, however, the effect of Agm on parasitemia was inhibited significantly by coadministration of spermidine (30 mg/kg), Indicating that the effect of Agm is related to its influence on synthesis of polyamines.

Tab 1. Antimalarial effect of Agm (ig, daily) on *P berghei* K173 S strain in mice. *n*=6. Mean±SD. $^bP<0.05$, $^cP<0.01$ *vs* NS.

Drugs	Dose/mg· kg^{-1}	Parasitemia/%	Inhibitory rate/%
NS	-	22.2±3.6	
Agm	12.5	21.3±2.4	3.9
	25	20.5±3.7	7.7
	50	16.2±2.5^b	27.2
	100	13.5±2.7^b	39.2
	200	7.0±2.5^c	68.5

NS: normal saline; Agm: agmatine.

Tab 2. Antimalarial effect of Agm (sc, tid) on *P berghei* K173 S strain in mice and the influence of spermidine (sc, qd) on the effect of Agm. *n*=6. Mean±SD. $^bP<0.05$ *vs* NS, $^eP<0.05$ *vs* Agm 40 mg/kg.

Drugs	Dose/mg· kg^{-1}	Parasitemia/%	Inhibitory rate/%
NS	-	19.6±3.5	
Agm	5	19.3±6.9	1.5
	10	16.9±6.4^b	13.7
	20	13.2±6.0^b	32.6
	40	6.1±5.3^b	68.9
Agm+Spd	40+30	14.0±3.6^{b,e}	28.5
Spd	30	18.1±4.9^e	7.5

NS: normal saline; Agm: agmatine; Spd: spermidine.

Antimalarial effect of Agm on *P berghei* K173 R strain Agm (12.5-200 mg/kg, ig, daily) showed a dose-dependent inhibitory effect on the proliferation of *P berghei* K173 R strain, the inhibitory rate reached 70.2 % at the dose of 200 mg/kg, and the IC_{50} was 126 mg/kg (Tab 3). This effect of Agm was the same as that on *P berghei* K173 S strain. Chloroquine at 1.75 mg/kg had no inhibitory effect on *P berghei* K173 R strain. Coadministration of different doses of chloroquine (0.22-3.5 mg/kg) with Agm (100 mg/kg) did not increase the antimalarial effect of Agm (Tab 4). Agm (12.5-200 mg/kg) did not enhance the antimalarial effect of chloroquine (1.75 mg/kg) too (Tab 5). These results indicated that Agm could not reverse chloroquine resistance of malaria parasites. Spermidine (5-60 mg/kg) significantly inhibited the antimalarial effect of Agm (100 mg/kg) on *P berghei* K173 R strain in a dose-dependent manner (*n*=6, *P*<0.05 *vs* Agm 100 mg/kg; Tab 6), the inhibitory rate of Agm decreased from 44.0 % (Agm 100 mg/kg) to 8.9 % in the presence of spermidine 60 mg/kg. This result further proved that the effect of Agm was exerted through the inhibition of spermidine synthesis.

Tab 3. Antimalarial effect of Agm (ig, daily) on *P berghei* K173 R strain in mice. *n*=6. Mean±SD. $^bP<0.05$ *vs* NS.

Drugs	Dose/mg· kg^{-1}	Parasitemia/%	Inhibitory rate/%
NS		14.2±1.7	
Agm	12.5	14.4±1.5	0
	25	13.3±2.0	6.4
	50	10.6±1.6^b	25.4
	100	6.3±1.4^b	55.3
	200	4.2±1.4^b	70.2

NS: normal saline; Agm: agmatine.

Antimalarial effect of DFMO on *P berghei* K173 strain DFMO (10-50 mg/kg, sc, daily) inhibited

Tab 4. Influence of Agm (100 mg/kg, ig, daily) on the antimalarial effect of chloroquine (0.22-3.5 mg/kg, ig, daily) on *P berghei* K173 R strain in mice. *n*=6. Mean±SD. $^bP<0.05$ *vs* Agm 100 mg/kg.

Drugs	Dose/mg· kg^{-1}	Parasitemia/%	Inhibitory rate/%
NS		14.2±1.7^b	
Agm	100	6.3±1.4	55.3
Agm+CQ	100+0.22	6.5±1.5	54.1
	100+0.44	6.8±0.7	52.2
	100+0.88	6.7±1.1	52.5
	100+1.75	6.6±1.5	53.6
	100+3.50	6.9±1.1	51.7

NS: normal saline; Agm: agmatine; CQ: chloroquine.

Tab 5. Influence of Agm (12.5-200 mg/kg, ig, daily) on the antimalarial effect of chloroquine (1.75 mg/kg, ig, daily) on *P berghei* K173 R strain in mice. *n*=6. Mean±SD. [b]*P*<0.05 *vs* CQ 1.75 mg/kg.

Drugs	Dose/mg· kg^{-1}	Parasitemia/%	Inhibitory rate/%
NS		14.0±2.1	
CQ	1.75	14.1±1.4	0
CQ+Agm	1.75+12.5	14.3±1.4	0
	1.75+25	13.2±1.9	6.1
	1.75+50	10.5±1.6[b]	24.9
	1.75+100	6.7±1.9[b]	52.5
	1.75+200	4.3±1.8[b]	69.1

NS: normal saline; Agm: agmatine; CQ:chloroquine.

Tab 6. Influence of spermidine (5-60 mg/kg, sc, daily) on the antimalarial effect of Agm (100 mg/kg, ig, daily) on *P berghei* K173 R strain in mice. *n*=6. Mean±SD. [b]*P*<0.05 *vs* NS, [e]*P*<0.05 *vs* Agm 100 mg/kg.

Drugs	Dose/mg· kg^{-1}	Parasitemia/%	Inhibitory rate/%
NS	-	16.8±2.3	
Agm	100	9.3±27[b]	44.9
Agm+Spd	100+5	10.2±2.2[b]	39.6
	100+10	12.2±1.9[b]	27.7
	100+20	13.7±1.8[be]	18.8
	100+40	14.7±1.9[be]	12.5
	100+60	15.3±2.5[be]	8.9

NS: normal saline; Agm: agmatine; Spd: spermidine.

the proliferation of *P berghei* K173 S strain significantly, the inhibitory rate reached 73.1 % at the dose of 50 mg/kg, IC_{50} was 19 mg/kg (*n*=6, *P*<0.05 *vs* control, Tab 7). Spermidine (30 mg/kg, sc) itself had no influence on parasitemia of *P berghei* K173 S strain, however, coadministration of spermidine with DFMO antagonized the effect of DFMO. In the presence of spermidine (30 mg/kg, sc), the inhibitory rate of DFMO was decreased from 73.1 % to 53.8 % (*n*=6, *P*<0.05 *vs* DFMO 50 mg/kg), indicating that the antimalarial effect of DFMO was also exerted through the inhibition of spermidine synthesis.

DISCUSSION

Agm, a product of arginine decarboxylation in mammalian cells, can be metabolized by agmatinase to putrescine, and the latter can be synthesized to spermidine and spermine. Spermidine and spermine were reported to increase the activity of malarial DNA polymerase[9] and play important roles in the translation of the DNA polymerase mRNA and regulating the cell cycle of the malarial parasite. And DFMO, an inhibitor of ODC, which can inhibit the synthesis of spermine and spermidine, inhibited proliferation of *P falciparum*. Therefore, spermine and spermidine are essential for the differentiation and proliferation of cells and malarial parasites, and the use of specific inhibitors of spermidine metabolism might be a novel strategy for the design of new antimalarials. As far as the important role of spermidine in cell proliferation, it is possible that Agm may be important in promoting the proliferation of malarial parasites too. However, Agm is believed to govern cell polyamines by inducing antizyme which in turn suppresses ODC activity and polyamine uptake *in vitro* and *in vivo*[10] and in addition, Agm was able to deplete polyamine in hepatocytes and inhibit cell proliferation[11]. Thus it is also possible that Agm may have an opposite effect like spermidine, which is to inhibit the differentiation and proliferation of cells and malarial parasites. Agm was reported to inhibit the proliferation of malarial parasites of *P falciparum* NF54 strain *in vitro*[5]. Consistent with this study, the result in our present experiment demonstrated that Agm had an antimalarial effect in mice *in vivo*, and spermidine antagonized the effect of Agm. This result further proved the negative effect of Agm on spermidine content.

Tab 7. Antimalarial effect of DFMO (sc, daily) on *P berghei* K173 strain in mice and the influence of spermidine (sc, daily) on the effect of DFMO. *n*=6. Mean±SD. [b]*P*<0.05 *vs* NS; [e]*P*<0.05 *vs* DFMO 50 mg/kg group.

Drugs	Dose/mg· kg^{-1}	Parasitemia/%	Inhibitory rate/%
NS		31.0±5.2	
DFMO	10.0	18.2±2.3[b]	41.4
	25.0	13.0±2.8[b]	58.1
	50.0	8.3±4.0[b]	73.1
DFMO+Spd	50+30	14.3±2.9[be]	53.8
Spd	30.0	28.0±5.7[e]	9.7

NS: normal saline; DFMO: *D,L*-α-difluoromethylornithine; Spd: spermidine.

DFMO, an irreversible inhibitor of mammalian ODC activity, caused polyamine depletion in the infected cells

and inhibited synthesis of DNA in the malarial parasites grown in cultured human erythrocytes, at the same time, the growth rate of the parasites was inhibited as well[12]. In our experiment sc injection of DFMO inhibited proliferation of malarial parasites, and this effect was also reversed by spermidine. These results inferred that both Agm and DFMO might exert their antimalarial effect through inhibition of ODC activity and spermidine synthesis.

The resistance of *P berghei* to many drugs is thought to correlate with the alteration of polyamine metabolism in the malarial parasites. In the mice infected with drug resistant malarial parasites, the contents of spermidine and spermine in the infected erythrocytes increased significantly, and spermidine was thought to be a very important factor that led to drug resistance[13]. Both CQS and CQR infections produced similar changes in polyamine profiles of various tissues. An increase in spermidine level was more prominent as compared to putrescine and spermine, leading to an overall increase in spermidine/spermine ratio. This ratio is an important index of cellular proliferation[14]. Normal erythrocytes contained traces of polyamine while the erythrocytes loaded with malarial parasites of *P berghei* exhibited appreciably higher polyamine levels. Again, CQS as well as CQR *P berghe*i, exhibited qualitatively and quantitatively similar polyamine profiles thus ruling out a role of polyamines in CQ-resistance in malaria. Although spermidine was proved to inhibit the uptake of chloroquine in malarial parasites, spermidine combined with chloroquine did not influence the antimalarial effect of chloroquine in chloroquine resistant malarial parasites, thus chloroquine resistance is not merely attributed to the insufficient quantity of chloroquine in the drug resistant parasites[15]. In the present study, Agm inhibited the proliferation of malarial parasites of *P berghei* K173 R strain as well as the S strain, and spermidine antagonized these effects, these results further supported that spermidine was related to the proliferation of malarial parasites. However, Agm did not reverse the effect of chloroquine on the R strain, thus spermidine is not a factor that is related to chloroquine resistance of the R strain.

In conclusion, for the first time we found that Agm had an antimalarial effect on both *P berghei* K173 S strain and R strain, the mechanism is through inhibition of the synthesis of polyamine especially spermidine. Spermidine is not related to chloroquine resistance of *P berghei* K173 R strain and Agm could not reverse chloroquine resistance of malarial parasites.

REFERENCES

1 Wright PS, Byers TL, Cross-Doersen DE, McCann PP, Bitonti AJ. Irreversible inhibition of *S*-adenosylmethionine decarboxylase in *Plasmodium falciparum*-infected erythrocytes: growth inhibition *in vitro*. Biochem Pharmacol 1991; 41: 1713-8.

2 Gonzalez NS, Sanchez CP, Sferco L, Algranati ID. Control of *Leishmania mexicana* proliferation by modulation of polyamine intracellular levels. Biochem Biophys Res Commun 1991; 180:797-804.

3 Bitonti AJ, McCann PP, Sjoerdsma A. *Plasmodium falciparum* and *Plasmodium berghei*: effects of ornithine decarboxylase inhibitors on erythrocytic schizogony. Exp Parasitol 1987; 64:237-43.

4 Hollingdale MR, McCann PP, Sjoerdsma A. Plasmodium berghei: inhibitors of ornithine decarboxylase block exoerythrocytic schizogony. Exp Parasitol 1985; 60: 111-7.

5 Kaiser A, Gottwald A, Wiersch C, Lindenthal B, Maier W, Seitz HM. Effect of drugs inhibiting spermidine biosynthesis and metabolism on the *in vitro* development of *Plasmodium falciparum*. Parasitol Res 2001; 87: 963-72.

6 Lortie, MJ, Novetny, WF, Peterson, OW, Vallon, V. Agmatine, a bioactive metabolite of arginine. J Clin Invest 1996; 97: 413-20.

7 Li J, Li X, Pei G, Qin BY. Correlation between inhibitions of morphine withdrawal and nitric-oxide synthase by agmatine. Acta Pharmacol Sin 1999; 20: 375-80.

8 Ishizuka S, Cunard R, Poucell-Hatton S, Wead L, Lortie M, Thomson SC, *et al*. Agmatine inhibits cell proliferation and improves renal function in anti-thy-1 glomerulonephritis. J Am Soc Nephrol 2000; 11: 2256-64.

9 Bachrach U, Abu-Elheiga L Effect of polyamines on the activity of malarial alpha-like DNA polymerase. Eur J Biochem 1990; 191: 633-7.

10 Babal P, Ruchko M, Campbell CC, Gilmour SP, Mitchell JL, Olson JW, et al. Regulation of ornithine decarboxylase activity and polyamine transport by agmatine in rat pulmonary artery endothelial cells. J Pharmacol Exp Ther 2001; 296: 372-7.

11 Vargiu C, Cabella C, Belliardo S, Cravanzola C, Grillo MA, Colombatto S. Agmatine modulates polyamine content in hepatocytes by inducing spermidine/spermine acetyltransferase. Eur J Biochem 1999, 259: 933-8.

12 Whaun JM, Brown ND. Ornithine decarboxylase inhibition and the malaria-infected red cell: a model for polyamine metabolism and growth. J Pharmacol Exp Ther 1985; 233: 507-11.

13 Shi X, Ye X, Cai Y, Ha S, Yao J. Determination of polyamines in pyronaridine-sensitive and -resistant *Plasmodium berghei*-infected erythrocytes. Chin J Parasitol Parasit Dis 1998; 16: 109-12.

14 Mishra M, Chandra S, Pandey VC, Tekwani BL. Polyamine metabolism in various tissues during pathogenesis of chloroquine-susceptible and resistant malaria. Cell Biochem Funct 1997; 15: 229-35.

15 Chang HL, Wang QM, Lan QX, Qiu CP. Effect of spermidine on uptake of chloroquine by *Plasmodium berghei*. Acta Pharmacol Sin 1993; 14: 440-2.