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Effect of the Antimicrobial Peptide Gomesin Against Different Life Stages of *Plasmodium* spp

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Abstract

While seeking strategies for interfering with *Plasmodium* development in vertebrate/invertebrate hosts, we tested the activity of gomesin, an antimicrobial peptide isolated from the hemocytes of the spider *Acanthoscurria gomesiana*. Gomesin was tested against asexual, sexual and pre-sporogonic forms of *P. falciparum* and *P. berghei* parasites. The peptide inhibited the *in vitro* growth of intraerythrocytic forms of *P. falciparum*.When gomesin was added to *in vitro* culture of *P. berghei* mature gametocytes, it significantly inhibited the exflagellation of male gametes and the formation of ookinetes. *In vivo*, the peptide reduced the number of oocysts of both *Plasmodium* species in *Anopheles stephensi* mosquitoes, and did not appear to affect mosquito. These properties make gomesin an excellent candidate as a transmission blocking agent for the genetic engineering of mosquitoes.

Index descriptors

Antimicrobial peptide; gomesin; Malaria; Mosquito; Ookinete; Oocyst; Exflagellation

1. Introduction

Over 3 billion inhabitants of tropical regions are at risk for malaria. According to the World Health Organization, at least 500 million people contracted malaria in 2004, resulting in more than 3 million deaths. Although usually curable and preventable, the incidence of malaria cases

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is expanding as parasites developed resistance to the most commonly used drugs, and mosquitoes became resistant to insecticides (WHO, 2005). The search for new anti-malarial drugs and the development of alternative strategies of disease control are crucial to successfully decrease morbidity, mortality, and transmission.

Several naturally occurring antimicrobial peptides have been tested as potential anti-*Plasmodium* agents, including scorpin, dermaseptin S3 and S4, magainin 2, cecropin B, and defensin (Gwadz et al., 1989; Shahabuddin et al., 1989; Ghosh et al., 1997; Conde et al., 2000), as well as synthetic peptides, e.g. Vida 1–3, P2WN, ILF, SM1, SB-37, and Shiva-1 and 3 (Jaynes et al., 1988; Rodriguez et al., 1995; Possani et al., 1998; Ghosh et al., 2001; Arrighi et al., 2002). These peptides have a broad spectrum of action and permeate cell membranes by incompletely understood mechanisms. The lipid composition and the charge of the target cell membrane influence their activity, making it possible to selectively target pathogens but not their host cells. For example, studies with dermaseptin S4 derivatives demonstrated that antimicrobial peptides can be engineered to act specifically on the membrane of intracellular parasites, with the anti-*Plasmodium* effect of the original peptide being mediated by host cell lysis (Dagan et al., 2002; Efron et al., 2002). This is an important achievement since it demonstrates the potential use of antimicrobial peptides as chemotherapeutic agents for malaria treatment as well as other intracellular pathogens.

Strategies for interfering with *Plasmodium* development in the mosquito, such as transmission blocking vaccines, paratransgenesis, and transgenic mosquitoes, have been proposed as alternative methods to control malaria (Riehle et al., 2003). Proof of concept that transgenic mosquitoes can be used to block parasite transmission has been demonstrated (Ito et al., 2002; Moreira et al., 2002; Kim et al., 2004; Abraham et al., 2005). In addition to the antimicrobial peptides mentioned above, anti-*Plasmodium* effector candidates include anti-circumsporozoite (CS) antibody and phospholipase-A_2 (de Lara Capurro et al., 2000; Zieler et al., 2001). Because most of these agents are only partially effective, and because parasites readily overcome agents that interfere with their survival, it is imperative that multiple transmission-blocking effector molecules be used simultaneously. The search for additional, highly active, effector molecules is an important goal toward the implementation of mosquito-blocking strategies.

The antimicrobial peptide gomesin, isolated from the hemocytes of the tarantula spider *Acanthoscurria gomesiana*, is a potent agent against different strains of bacteria, filamentous fungi, and yeast, as well as *Leishmania amazonensis* (Silva et al., 2000). Here we report on the evaluation of gomesin as an anti-*Plasmodium* agent, and discuss its potential use as a transmission blocking molecule for expression in genetic modification of mosquitoes.

2. Material and Methods

2.1. Parasites and mosquitoes

Plasmodium berghei ANKA strain (rodent malaria), clone 2.34, was maintained by cyclic passages in Swiss Webster mice and *Anopheles stephensi* mosquitoes, and used between the first and fifth direct mouse passage.

Plasmodium falciparum (human malaria) was grown in continuous culture using RPMI 1640 medium supplemented with 10% inactivated human serum, and 5% hematocrit with an adapted candle jar method (Jensen and Trager, 1977). Parasitemia was monitored daily by counting infected red blood cells in Giemsa stained blood smears. After reaching 5% parasitemia, the cultures were synchronized with D-Sorbitol to obtain ring-form stages, as described previously (Lambros and Vanderberg, 1979).

The effect of gomesin towards intraerythrocytic forms was tested on two *P. falciparum* strains: W2 (Oduola et al., 1988) and 3D7-GFP (MRA-317, MR4; ATCC, Manassas, VA), whereas *P. falciparum* stage V gametocytes from NF54 strain (Ponnudurai et al., 1981) were used to test the peptide action towards oocyst formation.

An. stephensi mosquitoes were reared at 27°C and 80% humidity under a 12 h light/dark cycle, and adults were fed on 10% sucrose solution. *P. berghei* infected mosquitoes were maintained at 20°C, whereas *P. falciparum* infected insects were kept at 26°C.

2.2. Gomesin and artesunate

Gomesin and its linear analogue (without disulfide bridges) were manually synthesized by solid-phase methodology using the t-Boc strategy, as described previously (Fazio et al., 2006). Sodium artesunate was synthesized and provided by the medical division of FIOCRUZ/FARMANGUINHOS (Rio de Janeiro, Brazil).

2.3. Gomesin activity against *P. falciparum* asexual erythrocytic forms

The *in vitro* activity of gomesin against W2 and 3D7-GFP strains was assessed by the inhibition of [^{3}H] hypoxanthine incorporation (Desjardins et al., 1979). Synchronized ring forms, diluted to a parasitemia of 2%, were transferred to 96-well microtiter plates. Different concentrations of gomesin (3.1 to 200 μM) and artesunate (1.1 to 35.4 nM), as a control, were added per well in triplicate, in a total volume of 200 μL, and the plates were incubated at 37°C. After 24 h 0.5 μCi of [^{3}H] hypoxanthine (20 μL) was added to each culture and 18 h later the cells were harvested (Cell Harvester Mach II), transferred to filters and the radioactivity determined with a liquid scintillation counter (1450 Microbeta Wallac Trilux; Perkin Elmer). Three independent experiments were performed with artesunate and two with gomesin. The IC50 value was calculated by using the Origin version 5.0 software (Microcal Software, Inc.).

2.4. *In vitro* effect of gomesin on the ability of male gametes to exflagellate

One μl of *P. berghei* gametocyte-positive mouse blood was incubated with 9 μl RPMI 1640 (Gibco, USA), pH 7.5, containing 25 mM Hepes, 2 mM glutamine, 1 μM xanthurenic acid, 0.37 mM hypoxanthine and 0.2% sodium bicarbonate for 10 min at 20°C. Gomesin at the desired concentration or 1x PBS (control) in a volume of 1 μl was added to the incubation mixture. The effect of the peptide on male exflagellation was assessed by scoring centers of movement in 5 random microscope fields at 400x magnification after 10 minutes incubation at room temperature. The IC50 value was calculated by the Origin version 5.0 software (Microcal Software, Inc.).

2.5. *In vitro* activity of gomesin on the ability of gametocyte cultures to develop to oocysts

P. berghei mature gametocytes were cultured *in vitro* as described (Sinden et al., 1985). Briefly, gametocyte-positive mouse blood was collected by heart puncture and immediately submitted to a five-fold dilution in RPMI 1640 (Gibco, USA), pH 7.5, containing 25 mM Hepes, 2 mM glutamine, 1 μM xanthurenic acid, 0.37 mM hypoxanthine and 0.2% sodium bicarbonate. Ninety μL of diluted blood were added to wells of a 96-well microtiter plate and incubated for 16 h at 20°C, for ookinete formation. Various concentrations of gomesin in a volume of 10 μl were added at the beginning of the culture (time zero) and 4 and 8 h later. The number of ookinetes per 10,000 red blood cells was determined, 16 h after the beginning of the ookinete culture with the use of a hemocytometer, under 400x magnification.

2.6. Evaluation of *Plasmodium* oocyst formation after gomesin-enriched blood meal

Individual *P. berghei* gametocyte-positive mice were used to infect control mosquitoes. Immediately after this feeding, the different mice were injected with 200 μL of different

concentrations of gomesin into the tail vein, to obtain the final concentration of 0–200 μM of peptide in the mouse blood. In order to calculate the amount of gomesin to inject, we assumed that the mouse circulating blood is 8% of its weight, and expected to have the peptide equally distributed in the blood (Hoff, 2000).The oocysts were counted at day 15 and the inhibitory effect was calculated [(mean oocyst number per gut of mosquitoes fed on gomesin-injected mouse)/(mean oocyst number per gut of mosquitoes fed on the mouse prior to gomesin injection) x 100] and the numbers were compared between control and gomesin exposed mosquitoes fed on the same mouse.

P. falciparum NF54 stage V gametocytes mixed with 0–200 μM gomesin were membrane fed to mosquitoes, and oocysts were counted in mercurochrome-stained midguts, 8 days after feeding. The inhibitory effect was calculated as (mean oocyst number per gut of mosquitoes fed on gomesin)/(mean oocyst number per gut of mosquitoes fed on 1x PBS) x 100.

2.7. Effect of gomesin on the fitness of *An. stephensi* mosquitoes

Three day–old female *An. stephensi* were membrane fed with mouse blood mixed with 0, 25 or 200 μM gomesin. Engorged females were separated and placed in 500 mL paper cups (2 females per cup, 30 cups per treatment) and 10% sucrose was offered *ad libitum*. The number of dead mosquitoes was recorded daily. Egg collections in moist paper filter-lined small plastic cups were set up 2 d after the blood meal. Eggs were collected after an additional 2 d, counted and placed in water for hatching. The number of larvae was counted 5 d after hatching.

2.8. Statistical analysis

The effect of gomesin on oocyst formation was analyzed with the non-parametric Mann-Whitney test for *P. berghei* and the t-test for *P. falciparum*. The Kruskal-Wallis non-parametrical test was used to analyze the data of gomesin effects on mosquitoes.

3. Results

3.1. Effect of gomesin on *Plasmodium* asexual erythrocytic forms

The effect of gomesin on *P. falciparum* intraerythrocytic stage proliferation was studied by the radioactive hypoxanthine incorporation method, which is directly correlated with parasite growth. Gomesin inhibited parasite development of both chloroquine-sensitive (3D7-GFP) and chloroquine-resistant (W2) parasites (Fig. 1). The calculated IC50 values ranged from 75.8 (W2) to 86.6 μM (3D7-GFP) in independent experiments. By comparison, the IC50 for artesunate, used as a positive control, ranged from 5.1 nM (3D7-GFP) to 25 nM (W2).

3.2. Effect of gomesin on *Plasmodium* pre-sporogonic stages

The effect of gomesin on *P. berghei* sexual and pre-sporogonic stages was determined through *in vitro* experiments. As shown in Table 1, gomesin significantly inhibited male gamete exflagellation. At 50 μM the peptide promoted approximately 58% reduction in the exflagellation of *P. berghei* male gametes, and 68% inhibition was obtained with 100 μM. However, 100 μM linear (inactive) gomesin (Fazio et al., 2006) or 1x PBS used to solubilize the peptide had no inhibitory effect (data not shown). The calculated IC50 value is 46.8 μM, which is about half of the calculated concentration for asexual intraerythrocytic forms

To determine the effect of gomesin on ookinete formation, *P. berghei* gametocyte cultures were incubated in the presence of different concentrations of the peptide. Gomesin was added at 3 different time points (0, 4, and 8 h after the beginning of the culture) and inhibition was scored 16 h later (Table 2). When gomesin was added at the beginning of culture, 12.5 μM inhibited 50 to 100% ookinete formation, while 25 μM inhibited 67 to 100% and 50 μM led to 100% inhibition. When gomesin was added 4 or 8 h after the beginning of the culture, 75%

inhibition or higher was observed with 12.5 μM, and 100% and 75–100% mortality with 50 μM, at 4 and 8 h respectively. Although gomesin impaired *P. berghei* early sporogonic stage development we were unable to pinpoint if it targets all the stages between gametes and mature ookinetes. It is well known that *Plasmodium* development varies between parasite species and the mosquito vector, but in general, at 0 h after an infectious blood meal, one expects to see gametocytes and gametes within the mosquito midgut, at 4 h zygotes and at 8 h immature ookinetes should be present (Baton and Ranford-Cartwright, 2005).

3.3. *Plasmodium* oocyst reduction after gomesin-enriched blood meal

To check whether the addition of gomesin to the mosquito blood meal would reflect on reduction of oocyst numbers we tested the peptide in two different systems. *An. stephensi* mosquitoes were blood fed before (control) and after injection of gomesin into the tail vein of a *P. berghei* infected mouse. As shown in Table 3, gomesin-enriched blood meal resulted in *P. berghei* oocyst reduction. At 50 μM, the average number of oocysts per midgut was 53% lower than the control, whereas 86% inhibition was achieved when 100 μM was given to the mouse. Gomesin also inhibited *P. falciparum* oocyst formation; 50 μM reduced oocysts formation by 56% while complete blockage was obtained with 100 μM (Table 4). It is important to emphasize that lower number of oocysts could be reflected by the action of gomesin towards earlier parasite stages (gametes, zygotes and/or ookinetes).

3.4. Effect of gomesin on mosquito fitness

To analyze the effect of gomesin on mosquito fitness, female *An. stephensi* mosquitoes were fed mouse blood containing different concentrations of the peptide through an artificial membrane. Fitness was determined by measuring mosquito survival, fecundity (egg laying), and fertility (larvae production). Gomesin had no effect on female survival. Gomesin did not affect fecundity nor fertility at 50 μM, but at 200 μM it decreased fertility by approximately 50% and significantly reduced larval production (Table 5).

4. Discussion

In this study we analyzed the effect of the antimicrobial peptide gomesin, on the development of the malaria parasite, to address its possible utility as either a new therapeutic agent and/or as an effector molecule for expression in transgenic mosquitoes. Gomesin inhibited the growth of intraerythrocytic forms of two different *P. falciparum* lines, with an IC50 of 76–87 μM. Lysis of red blood cells was observed when gomesin was added to the cultures. A recent study reported that at a gomesin concentration of 1 μM the hemolysis rate is 16%, and reaches approximately 40% when the concentration is raised to 100 μM (Fazio et al., 2006). It is not known whether gomesin kills the malaria parasite by direct interaction with its membrane, or indirectly by lysis of the host cell. Due to its hemolytic activity, further *in vivo* assays should be done in order to evaluate the potential of this peptide as an anti-malarial drug.

The frog skin antimicrobial peptide, dermaseptin S4, lyses *Plasmodium*-infected erythrocytes at a 30-fold-lower concentration than it lyses non-infected cells (Dagan et al., 2002). Within the blood malaria parasites modify the erythrocyte surface and cytoplasm, displaying parasite-encoded receptors and solute channels on the erythrocyte surface (Templeton and Deitsch, 2005), and this could explain the different susceptibility of infected versus non-infected cells to dermaseptin S4. It is possible that the same observation is valid for gomesin activity; however this hypothesis still has to be investigated.

Gomesin also impaired the development of *Plasmodium* sexual and early sporogonic stages *in vitro* and *in vivo*. Under *in vitro* conditions we observed an inhibition of male gamete exflagellation , and a parasiticidal effect towards ookinetes. However, we were unable to

compare the blocking efficiencies towards different pre-sporogonic stages because it is not possible to rule out a cumulative effect from gametes through ookinetes. Similar studies with other antimicrobial peptides reported different inhibitory effects on distinct *Plasmodium* sexual stages for a fixed peptide concentration (Conde et al., 2000; Arrighi et al., 2002), likely due to changes in the repertoire of surface proteins (Carter and Kaushal, 1984; Kaushal and Carter, 1984), and possibly changes in lipid composition during parasite development, as described in other protozoa (Buscaglia et al., 2004). Exchange of surface lipids or proteins can alter the surface charges and hydrophobicity of cells, thus affecting the activity of antimicrobial peptides (Powers and Hancock, 2003). We believe that the action mechanism of gomesin is through the permeabilization of *Plasmodium* membrane, as previously determined for the parasites *Trypanosoma cruzi* and *Leishmania* spp. (S. Daffre, unpublished data). In the *in vivo* experiments, mosquitoes fed with infected blood containing gomesin developed fewer oocysts or none. The reduced number of oocysts can be probably attributed to fewer ookinetes formed, although a direct effect on oocysts should not be ruled out. Although high variation in oocyst number has been observed in all experiments, this is consistent with results of previous studies which deal with *Plasmodium* oocysts in mosquitoes (Bhatnagar et al., 2003; Kim et al., 2004; Shahabuddin et al., 1995, 1998; Zieler et al., 1999, 2001).

To further determine its use as a transmission blocking agent, we investigated the effect of gomesin on some fitness parameters of adult female anophelines. Gomesin did not increase mosquito mortality; 50 μM had no deleterious effect on mosquito fecundity and fertility, whereas 200 μM significantly reduced egg laying and larval survival. The concentration of endogenous antimicrobial peptides in the hemolymph of immune activated insects ranges from 1 to 100 μM (Hetru et al., 1998), and this compares with the gomesin concentrations used in our anti-parasite experiments. The cationic nature of gomesin suggests that its affinity to prokaryotic membranes is higher than to eukaryotic cell membranes (Silva et al., 2000). We will test more physiologically relevant levels between 50 and 200 μM to determine where the effect begins, and may use these data to somehow limit expression in transgenic mosquitoes.

The broad effect of gomesin on the mosquito stages of *Plasmodium*, and its low toxicity to mosquitoes when administered *per os*, make this peptide an interesting candidate to be expressed in the midgut of genetically engineered mosquitoes, driven by blood-meal induced midgut promoters such as carboxypeptidase (AgCP) or peritrophic matrix protein 1 (AgAper 1) (Edwards et al., 1997; Abraham et al., 2005). The latter, which encodes a perithrophic matrix protein, can direct the secretion of transgenic proteins immediately upon mosquito blood feeding (Devenport et al., 2005). Other antimicrobial peptides were successfully expressed within the hemolymph or midgut of transgenic mosquitoes. The *Aedes aegypti* defensin, when driven by the fat body promoter vitelogenin was present within the insect hemolymph up to three weeks after a blood meal and when extracted from transgenic mosquitoes inhibited the growth of the bacteria *Micrococcus luteus* (Kokoza et al., 2000). Cecropin A from *Anopheles gambiae* was overexpressed in this mosquito species by using the *Ae. aegypti* carboxypeptidase promoter and was able to significantly reduce *Plasmodium berghei* oocyst numbers (Kim et al., 2004).

Assuming that gomesin had already effect towards early ookinetes (Table 2) and based on the parasite life cycle on mosquito midgut, there is a relatively broad timing (from minutes up to 24 h post infective blood meal) that gomesin could work as an anti-parasitic molecule, when present within the insect midgut lumen.

In conclusion, gomesin is a promising anti-*Plasmodium* effector candidate for expression in transgenic mosquitoes to be added to the arsenal of other effector molecules.

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Abbreviations

GFP
: green fluorescent protein

P. berghei
: *Plasmodium berghei*

P. falciparum
: *Plasmodium falciparum*

An. stephensi
: *Anopheles stephensi*

RPMI-1640
: Roswell Park Memorial Institute medium

t-Boc
: tert-butyloxycarbonyl

MR4
: Malaria Research and Reference Reagent Resource Center

ATCC
: American Type Culture Collection

FIOCRUZ
: Fundação Oswaldo Cruz

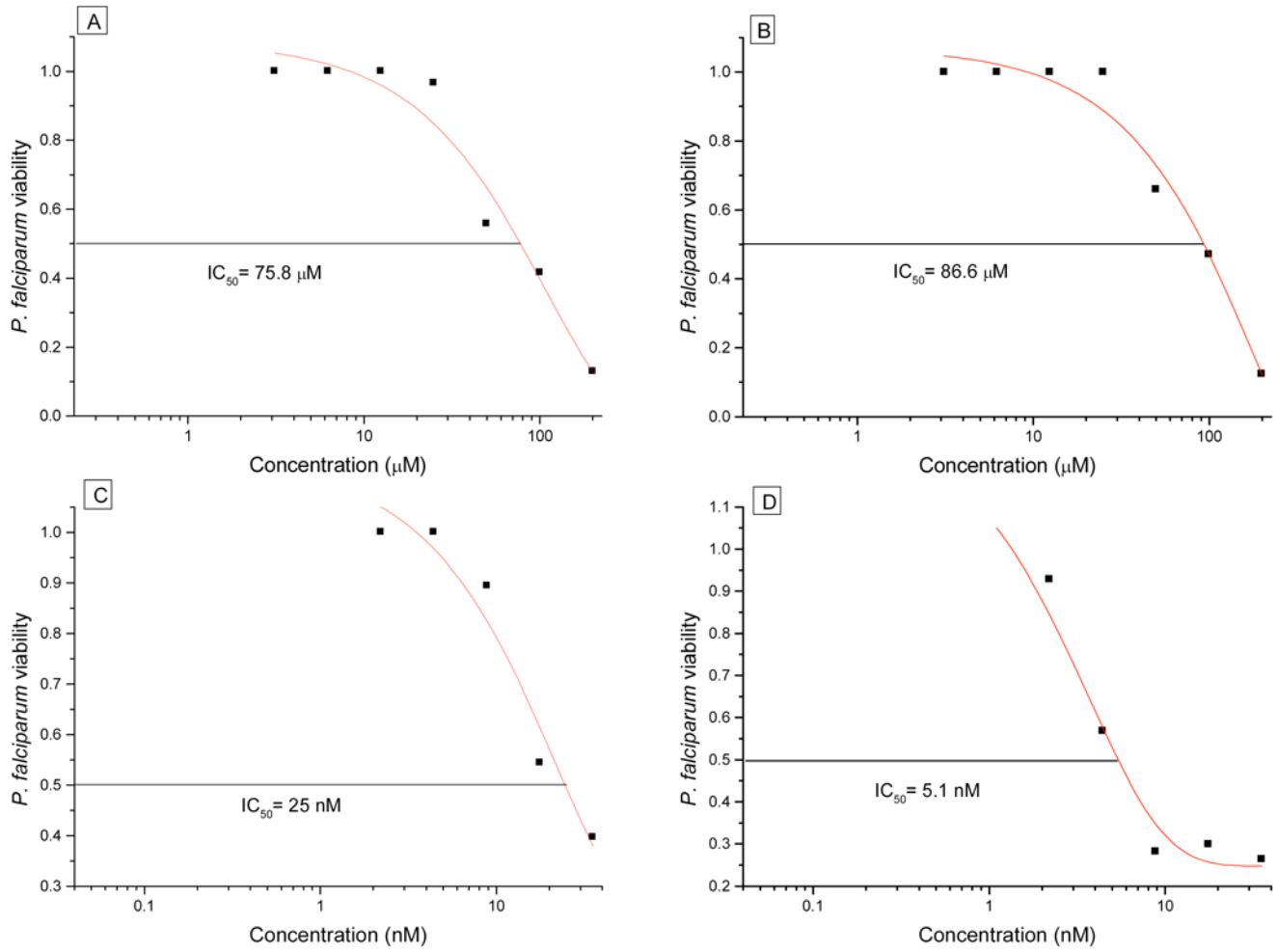

Figure 1.

Dose-response curves of parasite viability in the presence of gomesin (A, B; in μM) and artesunate (C, D; in nM) for *in vitro* cultures of the *P. falciparum* lines W2 (A and C) and 3D7-GFP (B and D). Data are representative of three independent experiments using artesunate as a control, and two independent experiments with gomesin, each experiment done in triplicate.

Table 1

In vitro effect of gomesin on *P. berghei* male exflagellation

Gomesin concentration	# exflagellations/ # microscope fields					Average % inhibition
	Exp. 1	Exp. 2	Exp. 3	Exp. 4	Exp. 5	
0 μM	146/5	200/5	246/5	108/5	81/5	0
6.25 μM	135/5	154/5	170/5	96/5	78/5	18.9
12.5 μM	135/5	120/5	156/5	85/5	68/5	27.7
25 μM	123/5	101/5	90/5	74/5	68/5	41.6
50 μM	79/5	64/5	82/5	59/5	42/5	58.2
100 μM	74/5	43/5	61/5	49/5	21/5	68.2

Gametocyte-positive mouse blood was diluted and incubated for 10 min at 20ºC in the presence of gomesin, as described in Material and Methods. Exflagellation was scored by counting centers of movement in 5 random microscope fields (400x magnification). Data from 5 independent experiments.

Table 2

In vitro effect of gomesin on the formation of *P. berghei* ookinetes

	Gomesin concentration	Number of ookinetes/ 10,000 RBC (% inhibition)		
		Gomesin added 0 h after the start	Gomesin added 4 h after the start	Gomesin added 8 h after the start
Exp. 1	0 μM	21	24	20
	12.5 μM	10 (54%)	6 (75%)	4 (80%)
	25 μM	7 (67%)	6 (75%)	2 (90%)
	50 μM	0 (100%)	0 (100%)	0 (100%)
Exp. 2	0 μM	16	15	16
	12.5 μM	4 (75%)	1 (93%)	0 (100%)
	25 μM	0 (100%)	0 (100%)	0 (100%)
	50 μM	0 (100%)	0 (100%)	0 (100%)
Exp. 3	0 μM	4	4	4
	12.5 μM	0 (100%)	1 (75%)	1 (75%)
	25 μM	0 (100%)	0 (100%)	0 (100%)
	50 μM	0 (100%)	0 (100%)	0 (100%)
Exp. 4	0 μM	23	22	23
	50 μM	0 (100%)	0 (100%)	5 (78%)
Exp. 5	0 μM	17	15	17
	50 μM	0 (100%)	0 (100%)	2 (88%)

Gametocyte-positive mouse blood was placed in culture and gomesin was added at 0, 4 and 8 h after the start of gametogenesis. The number of ookinetes per 10,000 red blood cells (RBC) was determined, after 16 h of the start of the culture, with the use of a hemocytometer, under 400x magnification. Data from 5 independent experiments.

Table 3

In vivo inhibition of *P. berghei* oocyst formation by gomesin

Exp.	Gomesin concentration	Oocysts per Midgut[a]	Oocyst-positive mosquitoes[b]	% Oocyst inhibition
1	25 μM	72.1 (0–225)	93.3 (14/15)	45
	control	131.4 (0–352)	91.7 (11/12)	
2	50 μM	14.5 (0–162) *	65.6 (21/32)	53
	Control	30.8 (0–122)	83.3 (25/30)	
3	50 μM	74.4 (0–244) *	94.1 (16/17)	80
	Control	375.8 (190–592)	100 (5/5)	
4	100 μM	2.4 (0–14) **	40 (14/35)	86
	Control	17.6 (0–72)	85.7 (30/35)	
5	200 μM	85.3 (3–188) *	100 (11/11)	69
	Control	274 (95–510)	100 (5/5)	
6	200 μM	19.2 (0–92) *	97 (33/34)	70
	Control	65.7 (0–284)	81.3 (26/32)	

For each experiment, control *An. stephensi* mosquitoes were fed on anaesthetized *P. berghei*-infected mouse. A second group of experimental mosquitoes was fed on the same mouse about 10 min after injection of gomesin into its tail vein. Mosquitoes were kept at 21°C and the number of oocysts per midgut was determined on day 15. Data from 6 independent experiments.

[a] Mean oocyst number per mosquito midgut. The range of observed values is indicated in parentheses.

[b] Percentage of mosquitoes that had oocysts in their midgut. These values were derived from the number of oocyst-positive mosquitoes over the total number of mosquitoes examined (shown in parentheses).

* $p<0.05$;

** $p<0.0001$ in comparison to the control by the Mann-Whitney test.

Table 4

In vivo inhibition of *P. falciparum* oocyst formation by gomesin

Gomesin concentration	Oocysts per Midgut[a]	Oocyst-positive mosquitoes[b]	% Oocyst inhibition
control	23±23 (0–99)	78.8 (41/52)	-
25 μM	17±21 (0–89)*	63.4 (33/52)	26
50 μM	10±12 (0–51)**	71.2 (37/52)	56
100 μM	0.5±1.2 (0–5)**	25 (13/52)	99.9
200 μM	0**	0 (0/52)	100

P. falciparum stage V gametocytes mixed with gomesin were membrane fed to *An. stephensi* mosquitoes. Mosquitoes were kept at 26°C and the number of oocysts per midgut was determined on day 8.

[a] Mean oocyst number per mosquito midgut. The range of observed values is indicated in parentheses.

[b] Percentage of mosquitoes that had oocysts in their midgut. These values were derived from the number of oocyst-positive mosquitoes over the total number of mosquitoes examined (shown in parentheses).

* $p<0.05$;

** $p<0.0001$ in comparison to the control by the t- test.

Table 5

Effect of gomesin on the fitness of *An. stephensi* female mosquitoes

Gomesin concentration (number of mosquitoes)	Survival (mean number of days ± SD)	Eggs (mean ± SD)	Larvae (mean ± SD)
0 μM (n=31)	23.7 ± 4.2	72.6 ± 34	43.3 ± 32.6
50 μM (n=30)	26.9 ± 6	57.7 ± 23.5	33.9 ± 21
200 μM (n=30)	24.9 ± 6	27.1 ± 15.6*	5.5 ± 5.4*

Female mosquitoes were membrane-fed on mouse blood containing different concentrations of gomesin and the mean and standard deviation of mosquito survival, fecundity (number of eggs) and fertility (number of larvae) were recorded. Control mosquitoes ('0') were artificially fed on mouse blood mixed with the same volume of 1x PBS.

*Statistically significant difference of means compared to control using the Kruskal-Wallis test ($p < 0.0001$).