ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, May 2007, p. 1753–1759 Vol. 51, No. 5
0066-4804/07/$08.00+0 doi:10.1128/AAC.01288-06

Antiplasmodial Activity of Lauryl-Lysine Oligomers[▽]

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Received 15 October 2006/Returned for modification 13 December 2006/Accepted 11 February 2007

The ever evolving resistance of the most virulent malaria parasite, *Plasmodium falciparum*, to antimalarials necessitates the continuous development of new drugs. Our previous analysis of the antimalarial activities of the hemolytic antimicrobial peptides dermaseptins and their acylated derivatives implicated the importance of hydrophobicity and charge for drug action. Following these findings, an oligoacyllysine (OAK) tetramer designed to mimic the characteristics of dermaseptin was synthesized and assessed for its antimalarial activity in cultures of *P. falciparum*. The tetramer inhibited the growth of different plasmodial strains at low micromolar concentrations (mean 50% inhibitory concentration [IC_{50}], 1.8 μM). A structure-activity relationship study involving eight derivatives unraveled smaller, more potent OAK analogs (IC_{50}s, 0.08 to 0.14 μM). The most potent analogs were the most selective, with selectivity ratios of 3 orders of magnitude. Selectivity was strongly influenced by the self-assembly properties resulting from interactions between hydrophobic OAKs, as has been observed with conventional antimicrobial peptides. Further investigations performed with a representative OAK revealed that the ring and trophozoite stages of the parasite developmental cycle were equally sensitive to the compound. A shortcoming of the tested compound was the need for long incubation times in order for it to exert its full effect. Nevertheless, the encouraging results obtained in this study regarding the efficiency and selectivity of some compounds establish them as leads for further development.

Malaria constitutes the most widespread infectious disease, affecting hundreds of million people and causing the deaths of 1 million children every year in Africa alone (44). This dreadful situation could worsen due to the increasing resistance of parasites to the available antiplasmodial drugs; therefore, new drugs must be continuously developed. The principal rationale for the development of new antimalarial drugs is the targeting of specific processes, enzymes, or a structure(s) in the malaria parasite-infected cell with the affecting molecules. Antimicrobial peptides (AMPs) have recently emerged as interesting tools for use in the exploration of new antimalarial targets (8, 21, 23, 47, 48). These ubiquitous peptides vary considerably in their structures, sizes, amino acid sequences, and spectra of action (3, 31, 32, 37, 52); typical peptides always have pronounced amphipathic and distinctly basic characters (46, 51). Antimicrobial action is rarely mediated by interaction with stereospecific targets, such as receptors or enzymes (10, 48). Apparently, their charges and hydrophobicities are the main features affecting their cytotoxicities (5, 10, 13). Some antimicrobial peptides are believed to target intracellular components (6); others were stipulated to disrupt membrane functions (2, 14, 33). Various basic models for a mechanism of action were proposed, ranging from pore formation to induction of structural defects (34, 43, 50), both of which lead to membrane permeabilization. Consequently, essential ions and metabolites are free to leak in and out and to dissipate the electric potential across the membrane, eventually leading to cell death.

Antimicrobial peptides often display a broad spectrum of activity affecting gram-negative and gram-positive bacteria, yeast and filamentous fungi, some enveloped viruses, and many types of cancer cells. Yet many are relatively inactive on erythrocytes (RBCs) and other normal eukaryotic cells (25, 36, 40). Although the basis for this discrimination is not fully understood, it appears to be related to the composition of the target membrane (i.e., fluidity, negative charge density, and the absence or presence of cholesterol) and the presence in the peptide-susceptible organisms of a large negative transmembrane electrical potential (35, 51). Such a peptide-based antimicrobial system has attractive advantages over classical antibiotics and makes it extremely difficult for microbial targets to develop resistance (38, 39, 51). Nevertheless, a major drawback is reflected in their unselective activity over a wide range of cell types, which could be problematic, for instance, with systemic routes of administration (17, 20, 22).

Dermaseptins are a large family of linear AMPs identified in frogs (37). In recent years, we assessed the antimalarial properties in cultures of *Plasmodium falciparum* of dermaseptins S3 and S4 (15) as well as of various derivatives of those peptides (7, 27). Namely, we have shown that native dermaseptins and truncated derivatives, such as S4(1-13), kill intraerythrocytic malaria parasites through the lysis of the host cells (27). Further modification of S4(1-13) indicated that increased hydrophobicity results in an amplified antiplasmodial effect, irrespective of the linearity or bulkiness of the additive (7). Although increased hydrophobicity was generally associated with increased hemolysis, we found various derivatives, such as isobutyryl (7) and aminoheptanoyl (8) analogs, that were less

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▽ Published ahead of print on 16 February 2007.

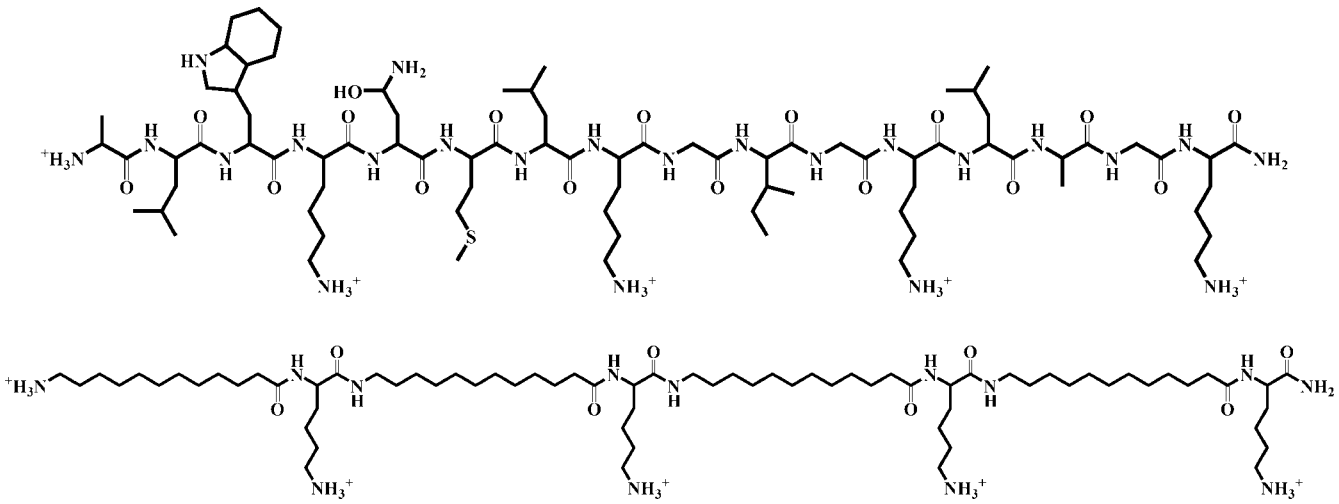

FIG. 1. Peptidomimetic design. Shown are the primary structures of the first 16 amino acids from the N-terminal sequence of dermaseptin S3 (ALWKNMLKGIGKLAGK) (upper structure) and of its mimetic compound (lower structure), which is composed of tandem repeats of four aminolauryl-lysines (*al*K) linked by amide bonds.

hemolytic and more effective. The antiparasitic effect was time dependent and irreversible, implying a cytotoxic effect. The acyl derivatives, but not the parent compound, were able to dissipate the parasite plasma membrane potential and cause the depletion of intraparasite potassium under nonhemolytic conditions. These results clearly demonstrate that the acyl peptides can affect parasite viability in a manner that is dissociated from lysis of the host cell. These findings also established that membrane-active peptides could be engineered to act specifically on the membrane of the intracellular parasite to perturb its functions. Here, we report on the antiplasmodial effects of a new family of synthetic compounds designed to mimic the main characteristics of conventional AMPs.

MATERIALS AND METHODS

Peptide synthesis. The oligoacyllysines (OAKs) were synthesized by the solid-phase method by applying the 9-fluorenylmethyloxy carbonyl (Fmoc) active ester chemistry (model 433A; Applied Biosystems) essentially as described previously (11). 4-Methylbenzhydrylamine resin was used to obtain amidated compounds (41). 12-Aminolauroic acid was protected with an Fmoc group at the N terminus prior to synthesis (4). The crude compounds were purified to chromatographic homogeneity in the range of >95% by reverse-phase high-performance liquid chromatography (HPLC) on a chromatograph equipped with a mass spectrometer (Alliance-ZQ Waters). HPLC runs were performed on a C_{18} column (Vydac) with a linear gradient of acetonitrile in water (1%/min); both solvents contained 0.1% trifluoroacetic acid. The purified compounds were subjected to mass spectrometry analysis in order to confirm their compositions and were stocked as lyophilized powders at −20°C. Before the compounds were tested, fresh solutions were prepared in water (MilliQ; Millipore), briefly vortexed, sonicated, centrifuged, and then diluted in the appropriate medium.

Parasite cultivation. The W2, FCR3, and NF54 strains of *P. falciparum* were cultivated as described previously (29) with human RBCs (hRBCs). The culture was synchronized by the sorbitol method (30).

Determination of IC_{50}. Synchronized cultures at the ring stage were cultured at 1% hematocrit and 2% parasitemia in the presence of increasing concentrations of the test compounds. After 18 h of incubation, parasite viability was determined by measurement of the uptake of [^{3}H]hypoxanthine (final concentration, 2 μCi/ml) for 6 h, and the amount of [^{3}H]hypoxanthine taken up was compared to the amount taken up by the controls (without OAK). The 50% inhibitory concentration (IC_{50}) was determined by nonlinear regression fitting of the data by using Sigmaplot software. Statistical data for each experiment were obtained from at least two independent assays performed in duplicate.

Time and stage dependence of action. Cultures at the ring stage were seeded in 24-well plates at 1% hematocrit and 7% parasitemia in plate medium (growth medium without hypoxanthine, 10 mM $NaHCO_3$, and 7% heat-inactivated human plasma). The test compounds were immediately added at different concentrations and were removed after 2, 5, 24, and 48 h. Cultures without an OAK were left to mature to the trophozoite stage and were incubated with the compounds for 2, 5, and 24 h. A total of 2 μCi of [^{3}H]hypoxanthine/well was added to all cells after 30 h from the onset of the experiment, and the cells were harvested after 24 h. Two independent experiments were performed in duplicate.

Effects on mammalian cells in culture. MDCK epithelial cells and HepG2 hepatoma cells were grown to confluence (~3 days in culture). Parallel cultures were grown with different concentrations of the derivatives. Then 10 μl of Alamar blue was added and the fluorescence was tested after 3.5 h. As a positive control, 10 μM cycloheximide was added at the beginning of cultivation (12). Statistical data were obtained from at least two independent experiments performed in duplicate.

Testing of hemolytic effect. (i) Hemolytic assay with normal (uninfected) RBCs. The hemolytic potentials of the derivatives were assessed after incubation with hRBCs in phosphate-buffered saline (PBS). Heparinated fresh blood was rinsed three times in PBS (by centrifugation at 200 × *g* for 2 min) and resuspended in PBS at 8% hematocrit. A 150-μl suspension was added to test tubes containing 150 μl of the derivative solutions (serial twofold dilutions in PBS), PBS alone (for baseline values), or 0.4% Triton X-100 (for 100% hemolysis). After incubation at 37°C with stirring, the samples were centrifuged and the hemolytic activity was determined by measuring the absorbance at 460 nm in 200 μl of the supernatant.

(ii) Hemolysis of infected cells. To assess the hemolysis of infected cells, cultures were exposed to increasing concentrations of the test compounds for 24 h. The optical density in the supernatant was determined after centrifugation, and the percent lysis compared to the amount of full lysis (by water) of the cells present in the culture was calculated. Hemolytic activity data were obtained from at least two independent experiments.

OAK organization in solution. Self-assembly in solution was investigated by obtaining static light-scattering measurements, as described previously (28), by using a Cary Eclipse fluorescence spectrophotometer (Varian Inc.). Briefly, twofold dilutions of the OAKs were prepared in PBS (50 mM sodium phosphate, 150 mM NaCl, pH 7.3), and the light scattering of each dilution was measured by holding both the excitation and the emission at 400 nm (slit width, 5 nm). The data represent the averages of two separate recordings.

RESULTS

Design of the peptide mimetics. Figure 1 depicts the primary structures of the 16-mer dermaseptin S3 derivative and its mimetic compound, an OAK composed of tandem repeats of

TABLE 1. Effects of OAKs on parasite growth and on mammalian cells

OAK no.	OAK sequence[a]	Q^b	% AcN[c]	CAC[d] (μM)	IC_{50}[e] (μM) for strain W2	% Hemolysis[f]	IC_{50}[g] (μM) for MDCK cells	Estimated LC_{50} for RBCs/IC_{50} for strain W2 ratio[h]	Calculated IC_{50} for MDCK cell/IC_{50} for strain W2 ratio
1	*al*K-*l*K-*l*K-*l*K	5	46.8	0.9	1.59	24.6 ± 1.2	>75	126	>47
2	*l*K-*l*K-*l*K-*l*K	4	53.5	0.2	0.08	26.1 ± 4.5	37	2,317	1,052
3	K-*l*K-*l*K-*l*K	5	44.0	>50	1.72	2.3 ± 1.5	>270	1,263	>157
4	*al*K-*l*K-*l*K	4	44.3	>50	0.14	3.4 ± 0.8	91	10,734	650
5	*l*K-*l*K-*l*K	3	52.9	<3	0.85	13.3 ± 4.3	75	442	88
6	K-*l*K-*l*K	4	40.0	>50	7.85	<0.1	>400	>6,300	>51
7	*al*K-*l*K	3	38.8	>50	4.63	0.14 ± 0.3	>150	7,714	>32
8	*l*K-*l*K	2	54.8	1.3	3.54	4.9 ± 1.7	>100	288	>28
9	K-*l*K	3	32.0	>50	68.2	<0.1	>800	>700	>12

[a] *a*, amino; *l*, lauric acid; K, lysine.
[b] *Q*, molecular charge at physiological pH.
[c] Estimated hydrophobicity (percent of acetonitrile [AcN] eluent), as determined by reversed-phase HPLC.
[d] The CAC was evaluated by linear extrapolation of the curves (e.g., in Fig. 2) to the intercept with the *x* axis at the concentration range close to the monomer-micelle transition zone.
[e] The IC_{50} represents the peptide concentration that produced 50% inhibition of hypoxanthine uptake of the W2 strain of *P. falciparum* after 18 h in culture.
[f] Percent hemolysis of hRBC induced by OAKs at 100 μM after 1 h of incubation with 4% hematocrit.
[g] The IC_{50} represents the peptide concentration that decreases the viability of 50% of the cells after 3.5 h incubation (Alamar blue assay).
[h] The OAK LC_{50}s (for hRBCs) were estimated by assuming a linear correlation between hemolysis (at 4% hematocrit, 1 h of incubation) and the OAK concentration.

four aminolauryl-lysines (*al*K) linked by amide bonds. The lysine residues preserve the molecular charge, while the fatty acid residues (each of which replaces three amino acids) contribute the hydrophobicity. Eight additional OAKs were designed and produced to assess the importance of the backbone length and its associated charge and hydrophobicity for antiparasitic potency. These include two shorter versions, a trimer (*al*K-*l*K-*l*K) and a dimer (*al*K-*l*K), as well as truncated versions of each oligomer representing either the removal of an amino group (which increases the hydrophobicity and reduces the charge) and a further removal of a lauryl group (which reduces the hydrophobicity and restores the charge). The sequences of all nine compounds are shown in Table 1, where they are presented as a continuous series of gradual truncations of the parent tetramer.

Self-assembly in solution. Figure 2 shows the aggregation properties of representative OAKs, as determined by light scattering. The critical aggregation concentration (CAC) was estimated from extrapolation of the concentration axis. Self-assembly was spontaneous and occurred instantaneously upon solubilization (data not shown). As shown in Table 1, some compounds were highly aggregative and a few displayed a CAC in the submicromolar range (namely, OAKs 1 and 2). The CAC data suggest that self-assembly is mediated by hydrophobic interactions between acyl moieties at the N terminus (e.g., OAKs 2, 5, and 8). These interactions were readily reduced by the amino group of the shorter OAKs (compare OAK 5 with OAK 4 and OAK 8 with OAK 7), but this was less so for the tetramer (OAK 1), which remained rather hydrophobic, despite the presence of an amine group (compare OAK 1 with OAK 2). It seems that in aggregate formation the hydrophobic interaction is stronger than charge repulsion.

Antiplasmodial effects. The dose dependence of the antiplasmodial effect is shown in Fig. 3 for representative OAKs

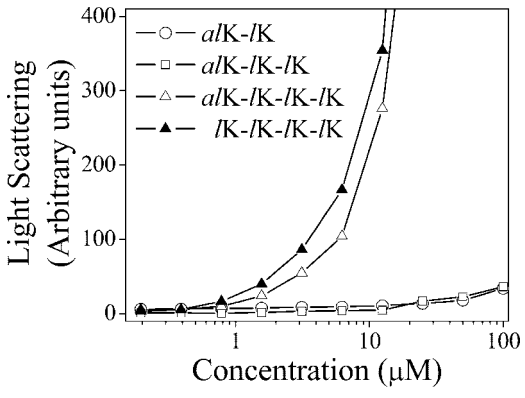

FIG. 2. Self-assembly properties of representative OAKs in phosphate buffer. The scattered light intensity of four representative OAKs is plotted against total peptide concentrations. The critical micelle concentration is evaluated by extrapolating the curve to the intercept with the *x* axis.

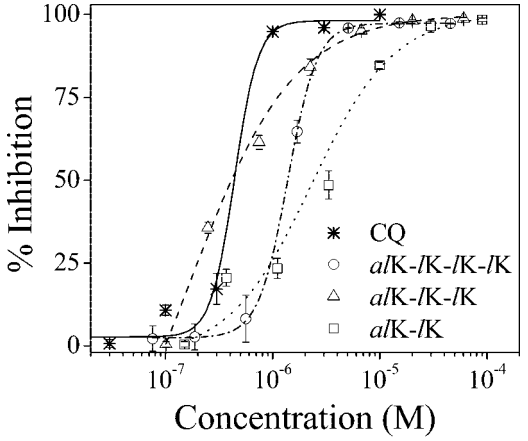

FIG. 3. Representative growth inhibition curves. Cultures were exposed to increasing concentrations of various OAKs and chloroquine (CQ) as a reference inhibitor. The inhibitory effects of the drugs on parasite viability were determined as described in Materials and Methods. The lines connecting the experimental points were drawn according to nonlinear regression analysis of the experimental results converted into percent values.

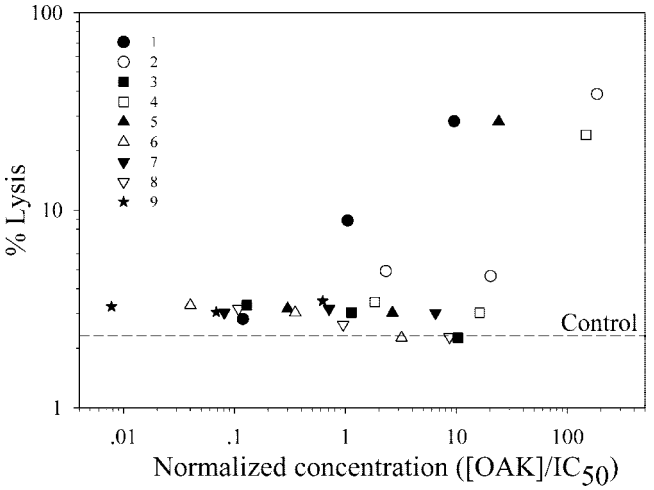

FIG. 4. Lysis in cultures exposed to increasing concentrations of the various derivatives. Cultures of the W2 strain (2% parasitemia) were exposed to three different concentrations of OAKs below and above their IC_{50}s. After 24 h in culture conditions, the cells were spun and the absorbance at 415 nm was recorded. Lysis was calculated as the percentage of total lysis of the culture, i.e., the lysis of both infected and uninfected cells. Lysis was plotted against the normalized concentrations; OAK concentrations were divided by the respective IC_{50} values for strain W2.

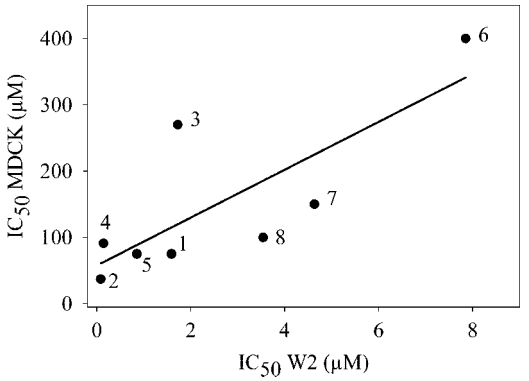

FIG. 5. Relationship between toxicity to MDCK cells and antiplasmodial potency. Data for MDCK cell toxicity and antiplasmodial effects were taken from Table 1 and plotted against each other. The correlation coefficient of the regression line is 0.804.

and for the classical antimalarial drug chloroquine. The inhibitory effects of all compounds on the W2 strain are shown in Table 1. Very similar results were obtained for the FCR3 and the NF54 strains (data not shown).

As shown in Table 1, some of the compounds were highly active, with the IC_{50}s of some compounds being in the submicromolar range (namely, OAKs 2 and 4). The structure-activity relationship (SAR) that emerges from this series is that the presence of a hydrophobic acyl moiety at the N terminus invariably increased the antiplasmodial activity (compare OAK 2 with OAK 3, OAK 5 with OAK 6, and OAK 8 with OAK 9). Shortening of the molecule (compare, for example, OAK 3 with OAK 6, OAK 9 with OAK 2, and OAK 5 with OAK 8) reduced the potency; the results were less consistent, however, with shortening of the aminolauryl group (compare OAKs 1, 4, and 7). The data therefore indicate that the antiplasmodial potencies of the OAKs depend on an optimal combination of a set of properties including length, charge, and hydrophobicity, as is observed with conventional AMPs (46, 49).

Toxicity to mammalian cells. Selective cytotoxicity was assessed by using cultures of hRBCs (infected and uninfected) and MDCK and HepG2 cells. The results shown in Table 1 indicate that, overall, hemolysis increases with hydrophobicity and aggregation (a reduction in CAC), as is usually the case with AMPs (10, 28). As far as MDCK cells are concerned, although it was not possible to obtain precise IC_{50} values in these experiments because excessively high concentrations were needed, the same dependence on hydrophobicity and aggregation as on hemolysis was observed. These trends in cytotoxicity, which were also observed with HepG2 cells (data not shown), are obviously different from those seen in the antiplasmodial activities of OAKs.

Hemolysis of infected and uninfected RBCs. Since we observed in our previous work that some dermaseptin derivatives acted through lysis of infected cells while others caused lysis at concentrations that were not much higher than their respective IC_{50}s (7, 8), it was important to test the effects of the OAKs on hemolysis. In order to verify if inhibition of parasite growth is due to the hemolysis of the infected cells, the hemolysis was tested under the same conditions used for growth inhibition, i.e., after 24 h of incubation with the different OAKs at different concentrations. The observed hemolysis was plotted against the normalized OAK concentration, i.e., the compound concentration divided by the respective IC_{50}. As shown in Fig. 4, appreciable hemolysis could usually be observed at concentrations much higher than those needed to inhibit parasite growth. OAKs 1 and 5 were relatively highly hemolytic, and it is plausible that they act by lysis of the host cell. In contrast, the high degree of lysis caused by OAKs 2 and 4 were obtained at concentrations that were 2 orders of magnitude higher than their respective IC_{50}s. This suggests that the mode of parasite growth inhibition is unlikely mediated by destruction of the host cell.

Hemolysis of normal (uninfected) RBCs was assessed at a single very high concentration (100 μM). As none of the OAKs attained 50% hemolysis, their 50% lethal concentrations (LC_{50}s) were estimated by assuming a linear correlation between hemolysis and the OAK concentration. As shown in Table 1, all monomeric OAKs displayed little if any hemolytic activity. In fact, hemolysis correlated with self-assembly, suggesting that hydrophobic OAKs can damage the RBC membrane only as a consequence of monomer organization into a polymeric form. The ratio of the LC_{50} for RBCs to the IC_{50} for strain W2 shown in Table 1 is an indicator of drug selectivity; i.e., the higher that it is, the more selective the compound is to the parasite and the less likely it is to cause the lysis of RBCs. For the most active compounds, OAKs 2 and 4, the ratios were 2,317 and 10,734, respectively, implying that at the relevant antiplasmodial concentrations these compounds should not be hemolytic. The relationship between toxicity to MDCK cells and antiplasmodial potency is summarized in Fig. 5 and is discussed below.

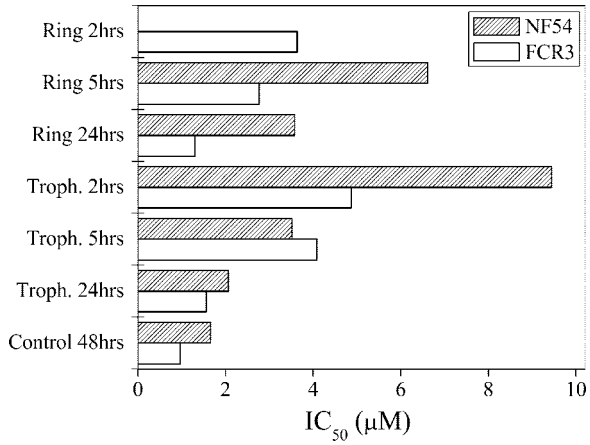

FIG. 6. Effect of OAK 2 (*l*K-*l*K-*l*K-*l*K) on parasite viability as a function of developmental stage and time of exposure. Parasites at the ring or trophozoite (Troph.) stage were exposed to OAK 2 at different concentrations for the indicated time periods. At the end of the incubation, the peptide was washed off and the parasites were returned to the culture conditions. [^{3}H]hypoxanthine was added to all cultures at 30 h from the onset of the experiment. The parasite-associated radioactivity was measured 6 h later and compared to that for the control. The IC_{50} values against the NF54 and FCR3 *P. falciparum* strains are presented.

Time and stage dependence of OAK action. The pharmacodynamics of drugs are essential aspects of chemotherapy. It is therefore imperative to know how long the pathogen must be exposed to the drug in order to elicit a maximal irreversible toxic effect and what is the developmental stage that is the most susceptible to the drug. The effect of the most potent derivative (OAK 2) was tested by exposing parasite cultures at the ring and the trophozoite stages for various lengths of time and to different drug concentrations. The drug was then removed, and after 30 h from the onset of the experiment, all cultures that were subjected to the different treatments were tested for parasite viability by using the hypoxanthine incorporation test. The IC_{50} for each treatment was calculated, and results for two different strains are shown in Fig. 6. Although the 2-h time point for NF54 is missing, we conclude that for each strain the ring stage was as sensitive as the trophozoite stage. It is obvious that the effect was cumulative, in that the IC_{50} values decreased with the incubation time for each strain and stage. The effect was also irreversible, in that removal of the drug after incubation did not allow the parasite to recover.

DISCUSSION

In this investigation we have extended our previous studies on the antiplasmodial effects of acyl derivatives of dermaseptin. The peptide sequence of dermaseptin has been reduced to a mere acyl-lysine copolymer (Fig. 1), while different combinations of length, charge and hydrophobicity were investigated.

In the series of compounds that were tested, we have identified important lead compounds that are worthy of use for further development. We found that some of the OAKs have a tendency to aggregate (as evidenced by the CAC values displayed in Table 1). The tendency to aggregate increased with augmented hydrophobicity, and it seems that both (or either) properties underlied the lytic effect on uninfected cells. This SAR thus provides a guideline for the further improvement of OAKs or similar compounds, as hemolysis is obviously harmful in the treatment of the infected host. This is further exacerbated by the apparent dependence of the toxicity of the OAKs to mammalian cells on their propensity to aggregate. No such clear tendency could be identified for the antiplasmodial effect. These approximate relationships may imply that the antiplasmodial effect is mechanistically different from the effects of OAKs on RBC lysis or on toxicity to mammalian cells. However, a direct inspection of the data shows a general relationship between the IC_{50}s of the drugs for both types of cells (Fig. 5). Such a relationship may suggest, although by no means confirms, that OAKs do permeabilize both types of cells, although over significantly different concentration ranges.

Some of the compounds displayed a very high selectivity for parasites (3 orders of magnitude) compared to their effect on mammalian cells (Table 1). The outstanding feature of some of the compounds is their lack of hemolytic effect within the range of relevant antiplasmodial concentrations: the ratios of the half hemolytic concentration to the IC_{50} were >2,000 and >10,000 for the two most active derivatives (OAKs 2 and 4, respectively). In achieving this selectivity, we have circumvented the major handicap of dermaseptins and their derivatives observed before, i.e., their hemolytic activity (7, 8, 15, 27). The most active compound was *l*K-*l*K-*l*K-*l*K, with an IC_{50} of 0.08 μM and a selectivity ratio of >1,000 (Table 1). Although at 100 μM it caused the lysis of 26% of normal RBCs, this is 1,200-fold higher than the IC_{50} and will not need to be reached to kill the parasites. A similar result was obtained when hemolysis was measured in parasite cultures (Fig. 4). Although it was not possible to discriminate between the lysis of infected cells and the lysis of the uninfected cohort, the overall lysis was very small at the relevant antiplasmodial concentrations. These results also indicate that OAKs do not exert their antimalarial action by lysing the infected RBC, as was the cases with the parent dermaseptins (15, 27).

On the basis of the finding of our previous investigations (7, 8), the mechanism of action of OAKs is likely to be similar to that of the acyl-dermaseptin derivatives, e.g., the permeabilization of the parasite membrane, with the ensuing dissipation of the membrane potential and the K^+ gradient. Although we have previously tested only the effects of dermaseptin derivatives on the parasite membrane potential and potassium leakage, it is very likely that outright permeabilization could also dissipate the pH gradient, which is responsible for the maintenance of intracellular [Ca^{2+}] (26) and lactate (9) and for the uptake of pantothenic acid (a precursor of coenzyme A) (42). The decrease in membrane potential could affect the parasite [K^+] (1) and the export of ATP from the parasite to the host cell, which is mediated by a potential-dependent ATP:ADP translocator (24). The inhibition of all these H^+ gradient- and membrane potential-dependent processes is expected to inhibit parasite growth at different targets.

Our previous investigations have established the permeability of the parasite membrane as a drug target (7, 8). A similar approach that aimed ionophores at the membrane of the infected RBC was attempted (16, 18, 19), and although that approach passed the proof-of-principle hurdle by obtaining a

demonstrable cure of disease in murine models, it never materialized into therapeutic feasibility. We should underscore the notion that the targeting of general permeabilization of a membrane is mechanistically different from the targeting of an enzyme or protein, since it involves a cellular structure that is composed of a multitude of different factors and not a single entity. Hence, the probability that the target cell will develop a drug resistance-conferring mutation (as occurs in enzymes or membrane transporters) is very unlikely.

A somewhat disconcerting result of this investigation is the time needed to expose the parasite to the compounds in order to get an irreversible killing effect (as is the case for some of the ionophores). The long time of exposure required is reminiscent of the actions of the ionophores and of several drugs that act on targets inside the apicoplast: there the effects are sometimes seen only in the next generation of the parasite (45). This similarity, however, is insufficient for suggesting that the target of OAKs is inside the apicoplast (or any other organelle, for that matter).

In conclusion, we have demonstrated that simple OAK compounds and their derivatives can inhibit *P. falciparum* in culture with IC_{50}s in the submicromolar concentration. The SAR analysis may provide clues for further improvement of drug activity and selectivity. Overall, the data indicate the potential usefulness of this strategy for the development of selective peptides as investigative tools and, eventually, as antimalarial agents.

ACKNOWLEDGMENT

This research was supported by The Israel Science Foundation (grant 387/03).

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