Linear Peptides Related to Angiotensin II with Antiplasmodial Activity

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Introduction

The antiplasmodial activities of angiotensin II and its analogs have been extensively investigated in *Plasmodium gallinaceum* and *Plasmodium falciparum* parasite species. Angiotensin II cannot be used as an antimalarial drug due to its vasoconstrictor effect [1,2]. Ten peptides related to it were synthesized by Fmoc solid phase strategy, purified by RP-HPLC, characterized by LC/ESI-MS and tested against mature *Plasmodium gallinaceum* sporozoites and *Plasmodium falciparum* early trophozoites.

Results and Discussion

From the Ang II primary sequence (DRVYIHPF), we synthesized ten peptides (Table 1). Peptide 1 (DRVY**H**IPF) and peptide 2 (DRVYPF) were designed to verify if the hydrophobic cluster exerts some influence on the parasite membrane. We inverted the position of the His and Ile amino acid residues in peptide 1. We observed that this change promotes an increase in the antiplasmodial activity (94%), about 7% that presented by native Ang II (Figure 1a). Instead, in the peptide 2 which had deleted His and Ile amino acid residues, this modification decreased the antiplasmodial activity (64%), about 15% of native Ang II and about 26% of the peptide 1.

Peptide 3 (RYPF) was designed to verify if the R, Y, P and F residues are relevant in native Ang II [3,4] and peptide 4 (RY**H**IPF) was designed to verify if the hydrophobic cluster exerts some influence on the peptide-parasite interaction (Figure 1a). Peptide 4 did not have more activity than peptide 3 (77% and 76% antiplasmodial activity, respectively). This was probably due to the influence of the cation- π interactions between Tyr and Arg residues providing greater stability [5], i.e., the effect of the cluster (Ile and His inverted residues) was not significant on biological activity because the interactions between Arg-Tyr residues are more effective than the interactions between Tyr-His residues [6].

Silva and colleagues suggested that the aromatic or hydrophobic residues can promote peptide-parasite interactions [4]. Thus, peptide 5 (YHPF) and peptide 6 (VIPF) were designed based on this information. We observed that both peptides could maintain the Ang II and peptide 1 antiplasmodial activity (89% and 94%, respectively). We studied the effects of each amino acid residue on the VIPF (peptide 6). The unusual amino acid 2-naphthylalanine (2-Nal) scan libraries of four VIPF analogs were synthesized (Peptides 7 to 10 - Table 1). Their activities against mature *P. gallinaceum* sporozoites were performed (Figure 1a) as well as CD studies (data not shown). The unusual amino acid (2-Nal) was chosen because it is similar to quinine, a potent antimalarial drug.

Table 1. Purity of peptides determined by LC/MS.

Entry	Name	Sequence	HPLC Purity ^a (%)	Calcd. Mass ^b (g.mol ⁻¹)	Obsd. Mass ^b (g.mol ⁻¹)
1	[Ile ⁶ ,His ⁵]-AII	DRVYHIPF	98	1045.5	1046
2	des-Ile ⁵ ,His ⁶ -AII	DRVYPF	98	795.4	796
3	des-Asp ¹ ,Val ³ ,Ile ⁵ ,His ⁶ -AII	RYPF	99	581.3	582
4	[Ile ⁴ ,His ³]-des-Asp ¹ ,Val ³ -AII	RYHIPF	98	831.4	833
5	des-Asp ¹ ,Arg ² ,Val ³ ,Ile ⁵ -AII	YHPF	99	562.2	563
6	des-Asp ¹ ,Arg ² ,Tyr ⁴ ,His ⁶ -AII	VIPF	99	474.3	475
7	[2-Nal ¹]- des-Asp ¹ ,Arg ² ,Tyr ⁴ ,His ⁶ -AII	(2-Nal)-IPF	97	572.3	574
8	[2-Nal ²]- des-Asp ¹ ,Arg ² ,Tyr ⁴ ,His ⁶ -AII	V-(2-Nal)-PF	99	558.3	559
9	[2-Nal ³]- des-Asp ¹ ,Arg ² ,Tyr ⁴ ,His ⁶ -AII	VI-(2-Nal)-F	97	574.3	575
10	[2-Nal ⁴]- des-Asp ¹ ,Arg ² ,Tyr ⁴ ,His ⁶ -AII	VIP-(2-Nal)	98	523.3	524

^aHPLC profiles were obtained under the following conditions: Column Supelcosil C18 (4.6 x 150 mm), 60 Å, 5 µm; Solvent System: A (0.1% TFA/H₂O) and B (0.1% TFA in 60% ACN/H₂O); Gradient: 5-95% B in 30 minutes; Flow: 1.0 mL/min; λ=220 nm; Injection Volume: 50 µL and Sample Concentration: 1.0 mg/mL. ^bThe masses were determined by LC/ESI-MS using a Micromass instrument (model ZMD) coupled to a Waters Alliance (model 2690) system. Mass measurements were performed in a positive mode with the following parameters: mass range between 300 and 2000 m/z; nitrogen gas flow: 4.1 L/h; capillary: 2.3 kV; cone voltage: 32 V; extractor: 8 V; source heater: 100 °C; solvent heater: 400 °C; ion energy: 1.0 V and multiplier: 800 V.

We observed that all replacements affected peptide 6 integrity. The substitution in a C-terminal extremity decreased peptide-parasite interaction (peptide 9 and peptide 10-70% and 73%, respectively). This behavior was observed by Chamlian, et al [7]. The antiplasmodial activity decreased in comparison to VIPF (analog 6), analog 7 and 8 had activity ≥ 75%.

Fig. 1. Peptides activities on *Plasmodium gallinaceum* (a) and *Plasmodium falciparum* (b). (a) Effects of AII analogues on membrane permeability of the mature sporozoites. Data were expressed in mean ± standard deviation of fluorescent sporozoites percentage/blade (n=9). Different letters and colors indicate significant difference between the groups treated with AII. Peptide analogues and positive control group (P, treated with digitonin and PBS) and negative (N, treated with PBS), respectively (ANOVA followed by Tuckey test, $p < 0.05$). (b) The percentage of rings was determined after 24 hours incubation of erythrocyte cultures infected with 2-3% schizonts in the absence (control) or in the presence of 10-8 mol L⁻¹ peptides. * Statistically significant compared with control value $p < 0.05$.

***Statistically significant compared with control value $p < 0.001$. Dark grey shading indicates that the result is statistically significant compared with control. (mean $\pm$ standard deviation, $n=2$).

From the CD studies, peptides that tend to form β -turn conformations present higher interactions with *P. gallinaceum* sporozoites membrane ($>75\%$) [3,4,8,9]. Our results indicate the same characteristics in the peptides studied here.

To verify if some peptides influence the same stage, all peptides were tested *in vitro* on red blood cells infected with *P. falciparum* species. Four peptides presented some activity in the parasite rings form reduction between 27% and 53% (Figure 1b), and this contribution was seen only by ultra-short peptides containing Val, Ile, Pro, Phe, Tyr, Arg and His amino acid residues in their primary sequences. Our results also concur with the Perez-Picaso observations [10].

The results indicate two peptides with antiplasmodial activity in both models: peptide 5 (YHPF), with 89% biological activity against *Plasmodium gallinaceum* sporozoite and 50% against *Plasmodium falciparum* early throphozoite and peptide 6 (VIPF), with 94% against *Plasmodium gallinaceum* and 53% against *Plasmodium falciparum*.

The conformational studies by Circular Dichroism spectra suggested that all the peptides presented β -turn conformation tendency in different solutions except peptide 2 (DRVYPF), which presented an α -helix conformation tendency and presented lower biological activity (63% in *Plasmodium gallinaceum* only – data not shown).

The IC₅₀ values of antiplasmodial activity for peptides 5 and 6 were also obtained and the absence of hemolysis or contractile response was observed (data not shown). In conclusion, when the hydrophobic portion and/or the Arg, Tyr, Pro or Phe residues are present on peptide primary sequence, it increases the antiplasmodial activity. These peptides are promising candidates for *in vivo* testing and offer a new direction for the design of potential antimalarial drugs.

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