Biological Studies of Angiotensin II Analogs Using Nanostructured Micelles

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Introduction

Malaria is an acute infectious disease caused by the protozoa of the genus *Plasmodium*. In humans, five species are responsible for malaria: *P. falciparum*, *P. vivax*, *P. ovale*, *P. malariae* and *P. knowlesi*. Due to resistance to antimalarial drugs, it is important to develop new treatments including peptides. The antiplasmodial action of angiotensin II ($\text{H}_3\text{N}^+\text{-Asp-Arg-Val-Tyr-Ile-His-Pro-Phe-COO}^-$) and its analogs has been reported. It inhibits the development of *Plasmodium gallinaceum* in *Aedes aegypti* mosquitoes [1,2].

To protect the peptides from protease action and promote sustained release, thermoreversible synthetic copolymers can be used as a drug delivery system. A drug delivery system is defined as a formulation or device that allows the administration of a therapeutic substance and provides better efficacy and safety by controlling the speed, time and place of release of the drug in the body [3]. This study describes the encapsulation of the antiplasmodial analogs of angiotensin II *via* preparation of formulations for slow release of peptides aiming for intramuscular administration.

Results and Discussion

The peptides were manually synthesized in the solid-phase method using Fmoc strategy and Wang and Rink Amide resins. The couplings are successful and used the coupling agents *N,N'*-diisopropylcarbodiimide (DIC) and *N*-hydroxybenzotriazole (HOBt). The syntheses were satisfactory with the peptides resulting a chromatographic purity higher than 95%; mass characterization by LC/MS-ESI confirmed that the purified peptides agreed well the expected theoretical values (Table 1).

Thermoreversible hydrogels from Poloxamer (PL) are efficient systems for the prolonged release of drugs. These were prepared at concentrations of PL 407 25%, PL 407 23% + PL 403 2% and PL 407 23% + PL 188 2%. Mixed micellar systems are formed by mixing two binary copolymers and exhibit synergistic properties including increased stability of the micelles and efficient drug encapsulation versus systems with only one copolymer [4,5].

The peptide-poloxamer characterization was performed by photon correlation spectroscopy. At 25°C, the formulation showed an average diameter ranging from 33 to 225 nm. At 37°C, the diameter decreased to 21- 40 nm (Table 2) proving the aggregation and micelle formation at body temperature. With the incorporation of peptides, the average micelle diameter was not changed at either temperature. The polydispersity index ranged from 0.1 to 0.4 indicating a homogeneous particle population.

The *in vitro* permeation assays were carried out with a vertical diffusion Franz-type cell. The receptor medium was Hepes buffer, and the membranes were cellulose acetate. The peptide $\text{H}_3\text{N}^+\text{-Val-Tyr-Ile-His-Pro-Phe-COO}^-$ had a release profile that was similar to the three formulations. The 24 h release concentrations of PL 407, PL 407 + PL 403 and PL 407 + PL 188 were $61.5 \pm 1.8 \mu\text{g/mL}$, $67.0 \pm 10.6 \mu\text{g/mL}$ and $50.3 \pm 4.8 \mu\text{g/mL}$, respectively.

The release results showed that release flow for poloxamer formulations were lower than that obtained for free peptide. The sequence VYIHPF in the poloxamer systems formed by PL 407, PL 407 + PL 403 and PL 407 + PL 188 released 51, 56 and 42%, respectively, relative to free peptide. This indicates that these systems are effective models for this class of compound.

Table 1. Analogs characterization.

	Peptide	Purity HPLC ^a	Molecular weight (MW)	(<i>m</i> + <i>H</i> ⁺) ^b
1	H ₃ N ⁺ -Asp-Arg-Val-Tyr-Ile-His-Pro-Phe-CONH ₂	99%	1045.5	1047
2	H ₃ N ⁺ -Asp-Arg-Val-Tyr-His-Ile-Pro-Phe- CONH ₂	99%	1045.5	1047
3	H ₃ N ⁺ -Tyr-His-Pro-Phe- CONH ₂	99%	562.2	563
4	H ₃ N ⁺ -Ile-His-Pro-Phe- CONH ₂	98%	512.3	513
5	H ₃ N ⁺ -Val-Tyr-Ile-His-Pro- CONH ₂	99%	627.3	629
6	H ₃ N ⁺ -Val-Tyr-Ile-His-Pro-Phe-COO ⁻	98%	774.4	775
7	H ₃ N ⁺ -Asp-Arg-Val-Tyr-Ile-His-Pro-Phe-COO ⁻	99%	979.5	980

^aHPLC profiles were obtained under the following conditions: Column Supelcosil C₁₈ (4.6 x 150 mm), 60Å, 5 µm; Solvent System: A: 0.1% TFA/H₂O, 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⁻¹; ^bMass measurements were performed in a positive mode with the following parameters: Column Waters Nova-Pak C₁₈ (2.1 x 150 mm), 60 Å, 3.5 µm; Solvent System: A: 0.1% TFA/H₂O, B: 0.1% TFA in 60%ACN/H₂O; Gradient: 5-95% B in 30 minutes; Flow:0.4 mL/min; λ = 220 nm; Injection volume: 30 µL; Sample concentration: 1.0 mg.mL⁻¹; mass range between 200-2000 Daltons.

Fig. 1. Release profile of the formulations and peptide H₃N⁺-Val-Tyr-Ile-His-Pro-Phe-COO⁻.

Table 2. Diameter and size distribution percentage obtained at 25 and 37°C.

	25°C		37°C	
	Diameter	% Intensity	Diameter	% Intensity
PL 407	43 ± 0.9	86 ± 0.7	21 ± 0.1	100 ± 0.0
PL 407 + PL 403	39 ± 0.4	96 ± 3.5	37 ± 1.1	97 ± 0.9
PL 407 + PL 188	45 ± 0.5	84 ± 0.3	40 ± 0.7	97 ± 2.5
PL 407 + Peptide 2	43 ± 2.0	44 ± 0.6	27 ± 0.2	74 ± 0.6
PL 407 + PL 403 + Peptide 2	39 ± 0.8	93 ± 0.7	36 ± 0.5	91 ± 2.3
PL 407 + PL 188 + Peptide 2	41 ± 1.3	84 ± 0.6	25 ± 2.0	100 ± 0.2
PL 407 + Peptide 3	52 ± 4.1	81 ± 4.1	24 ± 2.1	100 ± 0.4
PL 407 + PL 403 + Peptide 3	54 ± 2.2	91 ± 0.2	35 ± 2.0	98 ± 0.5
PL 407 + PL 188 + Peptide 3	62 ± 3.9	76 ± 2.5	25 ± 1.5	100 ± 0.0
PL 407 + Peptide 6	188 ± 20	68 ± 2.2	27 ± 0.5	99 ± 0.7
PL 407 + PL 403 + Peptide 6	33 ± 1.0	68 ± 1.2	27 ± 1.4	99 ± 1.1
PL 407 + PL 188 + Peptide 6	197 ± 31	74 ± 5.0	28 ± 0.9	97 ± 0.6
PL 407 + Peptide 7	170 ± 14	77 ± 2.9	28 ± 0.5	94 ± 3.2
PL 407 + PL 403 + Peptide 7	34 ± 1.5	68 ± 2.5	32 ± 0.7	86 ± 0.8
PL 407 + PL 188 + Peptide 7	225 ± 69	74 ± 25	35 ± 1.8	82 ± 1.2

Data presented as (mean ± SD, n=3). The measurements of the hydrodynamic diameter was performed by Zetasizer particle analyzer ZS (Malvern® Instruments) at an angle of 173° at temperatures of 25 and 37°C.

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