LmrBPP9: A synthetic bradykinin-potentiating peptide from *Lachesis muta rhombeata* venom that inhibits the angiotensin-converting enzyme activity in vitro and reduces the blood pressure of hypertensive rats

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ARTICLE INFO

ABSTRACT

Bradykinin-potentiating peptides (BPPs) are an important group of toxins present in *Lachesis muta rhombeata* venom. They act directly at renin-angiotensin-aldosterone system, through the inhibition of angiotensin-converting enzyme (ACE). This action may contribute to the hypotensive shock observed during the envenoming by this species. Thus, the main goal of this study was the solid-phase synthesis of a BPP found in *L. m. rhombeata* venom and its in vitro and in vivo characterization in relation to ACE inhibition and hypotensive activity, respectively. The LmrBPP9 peptide was synthesized using an automated solid-phase peptide synthesizer and purified by reversed-phase fast protein liquid chromatography (FPLC). The in vitro IC_{50} of the synthetic peptide is $4.25 \pm 0.10\,\mu M$, showing a great capacity of ACE inhibition. The in vivo studies showed that LmrBPP9 induces blood pressure reduction, both in normotensive and hypertensive rats, being more pronounced in the last ones. These results agree with the in vitro results, showing that the synthetic peptide LmrBPP9 is a potential molecule to the development of a new antihypertensive drug.

1. Introduction

Snake venoms present a vast repertoire of bioactive compounds, many of them resembling mammal molecules, both functionally and structurally. They act similarly to endogenous components, not being, however, controlled by regulatory mechanisms. Due to that, they may cause disturbances in the physiological equilibrium of the victim [1,2].

The presence of different classes of toxins, as well as their percentage in the venom, may vary among snakes' species. For this reason, snakebite victims deal with a variety of life-threatening pathologies, which may be related to neurotoxic, cytotoxic, and hemotoxic effects. About 1.8 million people are envenomed by snakes every year worldwide, with approximately 125,000 deaths. Additionally, the survivors may have to deal with life-changing conditions, directly affecting their lives in many ways. Consequently, snakebites are considered one of the world's most severe neglected tropical diseases [3].

The venom proteome of the Brazilian snake *L. muta rhombeata* revealed a remarkable presence of bradykinin-potentiating peptides (BPPs), corresponding to approximately 28% of the whole venom.

Additionally, metalloproteases, serine proteases, phospholipases A_2 of the D49 subfamily, L-amino acid oxidases, C-type lectin-like proteins, among other components were also detected in this venom [4].

Historically, studies regarding BPPs had a great advance after the discovery of bradykinin by Mauricio Rocha e Silva et al., while studying the pathophysiology of *Bothrops jararaca* envenoming [5]. In this context, BPPs are considered one of the key physiologically active molecules present in snake venoms [6]. These peptides act unbalancing the control of arterial pressure, mainly by the inhibition of angiotensin-converting enzyme (ACE). Through this mechanism, they can decrease the production of angiotensin II, a potent hypertensive peptide, as well as the degradation of bradykinin (BK), a powerful vasodilator [7,8]. The hypotension and vasodilation caused by these substances actively contribute to the rapid diffusion of toxins throughout the organism, which may lead to a hypotensive shock, one of the main causes of death by venomous snakes [9].

BPPs were originally described in the venom of *Bothrops jararaca* as proline-rich oligopeptides, containing 5–13 amino acid residues [10]. Structurally, many of them display a pyroglutamic acid at the N-

terminal and a characteristic C-terminus sequence ending on IPP [11], which is essential for binding of the peptide on ACE catalytic site [12,13].

Several peptides found in snake venoms display cardiovascular activity [14,15], being a great source of models for the development of new drugs. A great example is the use of the structure-function relationship of BPPs to develop captopril, the first drug designed targeting ACE inhibition, to treat hypertension [16]. Globally, this disease affects approximately 31% of the adult population [17], being an important health challenge since its complications may also cause cardiovascular and chronic kidney disturbances [18].

Therefore, studies regarding the physicochemical, structural and functional characterization of proteins and peptides present in snake venoms are of great interest in research nowadays. Because of their highly specific targets on the prey's organism, these compounds turned out to be great models to be used as lead compounds, aiming the development of new drugs [19,20]. On the other hand, understanding the venom and its components, as well as their mechanisms of action, is essential to better manage the therapeutics of the accidents caused by venomous animals [21,22]. In this work, we have screened the *L. muta rhombeata* venom for peptides belonging to the BPP family. One peptide was successfully identified, and its synthetic analogue was analyzed regarding its ACE inhibition in vitro and its in vivo vasoactive effects.

2. Material and methods

2.1. Venom and reagents

Lachesis muta rhombeata venom was kindly donated by Serpentarium Bosque da Saúde, located at Americana, São Paulo, Brazil, under IBAMA authorization 647.998. All chemicals and solvents used were of analytical grade.

2.2. Fractionation of L. muta rhombeata venom

The desiccated crude venom (25 mg) was dissolved in 250 μL of 0.05 M sodium acetate buffer, pH 6.0 and centrifuged at 13,000 × *g*, at 4 °C, for 10 min, to remove insoluble components. The supernatant was collected and applied in a Sephacryl S-100® (Hiprep 16/60, GE Healthcare, UK) column pre-equilibrated with 0.05 M sodium acetate buffer, pH 6.0. Fractions were eluted using the same buffer (isocratic elution), at a flow rate of 0.5 mL/min. Subsequently, fraction S11 was submitted to a reversed-phase chromatography, using a C18 column (218MS C18 5 μm 250 mm × 2.1 mm, Vydac, UK). Elution was performed by an acetonitrile (ACN) segmented gradient (0–100%) using 0.1% (v/v) trifluoroacetic acid (TFA) (solution A) and 80% (v/v) ACN in 0.1% (v/v) TFA (solution B), at a flow rate of 0.3 mL/min. Absorbance was monitored at 214 nm.

2.3. ACE inhibitory activity in vitro

ACE inhibitory activity in vitro was evaluated following the procedures described by Li et al. [23]. Some modifications were made to miniaturize the methodology, allowing the assay to be performed in 2 mL micro tubes.

Initially, 30 μL of 5 mM Hippuryl-His-Leu (HHL, Sigma-Aldrich Co., USA) in 100 mM borate buffer, pH 8.3 (Sigma-Aldrich Co., USA) containing 300 mM NaCl was incubated for 5 min, at 37 °C, with 20 μL of the ACE inhibitor solution. As negative control of inhibition (100% ACE activity), 20 μL of the same buffer was used. ACE (20 μL of 100 mU/mL solution; Sigma-Aldrich Co., USA) was later added to the mixture, which was then incubated for 2 h, at 37 °C. The catalytic reaction was stopped by adding 20 μL of HCl 2 N. The hippuric acid (HA), formed during ACE catalysis, was quantified by the incubation with 120 μL of quinoline (Sigma-Aldrich Co., USA) and 40 μL of benzenesulfonyl chloride (BSC, Sigma-Aldrich Co., USA) for 30 min, at 30 °C. The

chromogen HA-quinoline-BSC was diluted with 250 μL of ethanol, followed by another incubation, under the same conditions. Then, 290 μL of the mixture was transferred to a 96-well plate and the absorbance was measured at 492 nm in a microplate absorbance reader (Sunrise Basic TECAN, Austria). ACE inhibitory activity was evaluated by plotting the residual ACE activity versus the concentration of ACE inhibitor. Prism 6.0 software (GraphPad Software Inc., USA) was used to create the graphs. All the experiments were performed in triplicate.

2.4. Mass spectrometry assays

Molecular mass analysis were performed on a MALDI-TOF/TOF UltraFlex II mass spectrometer (Bruker Daltonics − DE), under positive ionization mode, using α-cyano-4-hydroxy-cinnamic acid as matrix. 1 μL of aqueous solution, containing the samples, was cosedimented with the matrix (1:1). Experiments were performed by scanning from a mass-to-charge ratio (*m/z*) of 50–1800. De novo peptide sequencing was carried out in positive ionization mode with selected ions that were submitted to collision induced dissociation (CID), using the equipment LIFT™ cell. The precursor ion and its fragments were selected and re-accelerated to a kinetic power of 19 keV. The laser potency was set to 60% with an incidence of more than a thousand shots, allowing the obtainment of a satisfactory signal/noise ratio. The resulting spectra were analyzed using BioTools 3.1 and FlexAnalysis 3.0 softwares (Bruker Daltonics software, DE) to deduce the amino acid sequence of the peptides.

2.5. Peptide synthesis

LmrBPP9 was synthesized manually through the Fmoc (N-(9-fluor-enyl)methoxylcarbonyl) chemistry using the Fmoc-Pro-Wang resin (AAPPTEC, USA). The amino acid side chains were protected using 2,4,6,7-pentamethyldihydrobenzofuran-5-sulfonyl and trityl. Couplings were performed with N,N-diisopropylcarbodiimide and N-hydro-xybenzotriazole under stirring for 2 h, employing three-fold excess over the amino component in the resin. To deprotect Fmoc group, 20% 4-methyl-piperidine/dimethylformamide (DMF) was used for 20 min. The success of each coupling was monitored by ninhydrin reaction. Cleavage of the synthesized peptide from the resin was performed with 90% TFA, 5% triisopropylsilane and 5% water. The peptide was precipitated with ethyl ether anhydrous and separated from non-peptide material by centrifugation. Finally, the peptide was dissolved in 0.045% TFA and the mixture was centrifuged to eliminate the resin. The product was purified in a FPLC system, using a C18 column (218MS C18 5 μ 250 mm × 2.1 mm, Vydac, UK), equilibrated with 0.1% TFA. Elution was monitored at 214 nm, using a segmented gradient of 80% ACN and 0.1% TFA. After the purification procedure, the synthetic peptide was lyophilized and stored at −20 °C.

2.6. In vivo ACE inhibitory activity assay

The in vivo experiments were approved by the Animal Care and Use committee of the School of Pharmaceutical Sciences of Ribeirão Preto (FCFRP-USP), under the protocol number 14.1.268.53.0.

2.6.1. Experimental animals

In order to induce renovascular hypertension in rats, the 2 kidney – 1 clip (2K-1C) Goldblatt model was employed [24]. Shortly, male Wistar rats, weighting 180–200 g were anesthetized for a midline laparotomy. Subsequently, a silver clip, with an internal diameter of 0.2 mm, was placed around the left renal artery. The control group, 2 K, was only submitted to laparotomy. After the surgery, the rats were maintained on standard rat chow, with free access to food and water, on a 12-h light-dark cycle. Six weeks after the surgery, the systolic arterial pressure was measured using the tail cuff method. The animals with a systolic pressure higher than 160 mmHg were considered hypertensive.

Each group was composed by four animals.

2.6.2. Surgery for cannula implantation

A polyethylene cannula, filled with heparinized saline, was implanted into the abdominal aorta, through the femoral artery, to record the blood pressure. Another cannula was implanted into the iliac vein, for administration of the synthetic BPP or vehicle. After the procedure, the animals were kept in individual home cages under a 12 h light/dark cycle, receiving standard rat chow and water ad libitum.

2.6.3. Measurement of the mean arterial pressure

Conscious rats had their blood pressure continuously recorded using a pressure transducer and an amplifier (AD Instruments, Australia) attached to the intra-arterial cannula. The software 4 Chart (AD Instruments, Australia) was used to determine the mean arterial pressure (MAP) under basal conditions and after the administration of the synthetic BPP or vehicle, to obtain the variation of the mean arterial pressure (ΔMAP).

2.6.4. Drug administration

Prior to drug administration, a control blood pressure was recorded for 30 min for arterial pressure stabilization. Later, the synthetic BPP, captopril and saline were administrated in the corresponding groups.

2.7. Statistical analysis

All values were expressed as mean ± SEM. Differences between the groups in the MAP(s) were compared by one-way ANOVA, followed by Dunnett's Multiple Comparisons Test. Differences were considered statistically significant when $P < 0.05$.

3. Results

3.1. Fractionation of L. m. rhombeata crude venom

The desiccated *L. m. rhombeata* crude venom was submitted to the first chromatographic step, on a size exclusion column. The chromatographic profile (Fig. 1) reveals the twelve fractions obtained, named from S1 to S12. The arrow indicates the fraction that was applied in a C18 column, as second chromatographic step, which displayed ACE-inhibitory activity (Fig. 1, Insert).
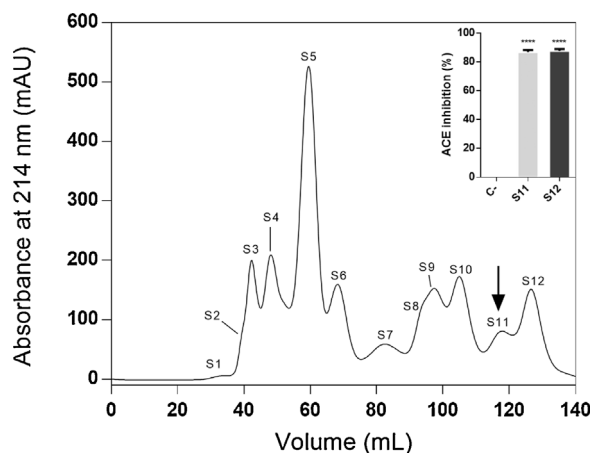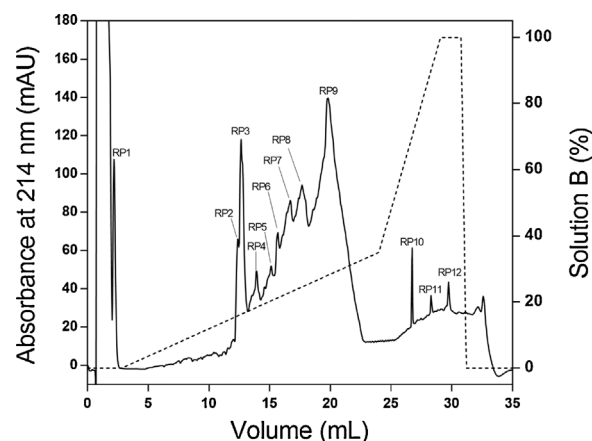

Fig. 2. Chromatographic profile of fraction S11 on a C18 column, previously equilibrated with 0.1% TFA. Fractions of 0.3 mL per tube were eluted at a flow rate of 0.3 mL/min using a step concentration from 0 to 100% of solution B (80% ACN, 0.1% TFA). Absorbance was monitored at 214 nm at 25 °C.

Fractions S11 and S12 inhibited, respectively, 90% and 91.5% of ACE catalytic activity, indicating the presence of BPPs. After the first chromatographic step, fraction S11 was submitted to a reversed-phase chromatography, using a C18 column suitable for the fractionation of peptides (Fig. 2). The obtained fractions were named from RP1 to RP12.

3.2. Screening of ACE inhibitory activity and de novo peptide sequencing

The inhibition of ACE activity by the fractions RP1 to RP12 was performed aiming to identify which fractions contained BPPs. Fractions RP5 and RP7 to RP11 strongly inhibited ACE (Fig. 3), with ACE inhibition higher than 85%. Fractions RP3, RP4, RP6 and RP12 also inhibited ACE activity, but with less intensity. These results indicate that fraction S11 presents different subfractions capable of inhibiting ACE activity. Eight fractions (RP5-RP12) were selected and submitted to mass spectrometry (data not shown), aiming to determine the molecular mass of the components in each one.

In fraction RP10, one peptide with *m/z* of 1087 was the major component, as shown in Fig. 4A. According to the ACE inhibition of fractions RP1-12, displayed in Fig. 3, this fraction presented an inhibitory effect on ACE activity higher than 90%. For this reason, de novo peptide sequencing was performed with this ion, to deduce its amino acid sequence, shown in Fig. 4B.

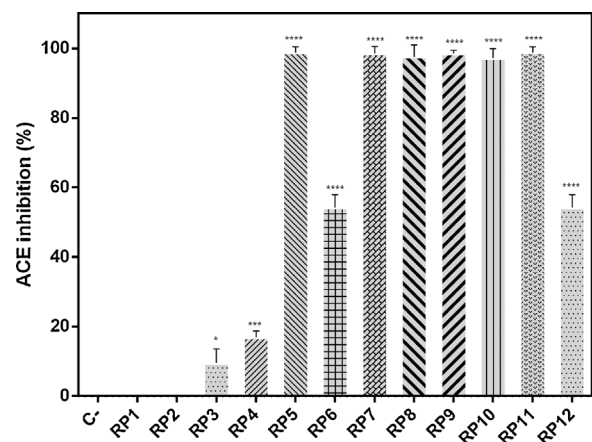The sequence obtained matches a bradykinin-potentiating peptide

Fig. 1. Chromatographic profile of *L. m. rhombeata* crude venom (LmrV) on Sephacryl® S-100 (1.6 × 60 cm) size exclusion column. Absorbance was monitored at 280 nm at 25 °C, using a FPLC ÄKTA Purifier system. LmrV (25 mg) was dispersed in 250 μL of 0.05 M sodium acetate buffer, pH 6.0 and centrifuged. The supernatant was applied on the column previously equilibrated with 0.05 M sodium acetate buffer, pH 6.0. Fractions of 1.5 mL per tube were eluted at a flow rate of 0.5 mL/min. Insert: ACE-inhibitory activity of S11 and S12 fractions. Values were calculated based on ACE catalytic activity without the presence of inhibitors (C-). (***p < 0.0001).

Fig. 3. In vitro ACE inhibition activity by RP-FPLC fractions. Values were calculated based on ACE catalytic activity without the presence of inhibitors (C-). Values are expressed as mean ± SD. (*p = 0.0205; ***p < 0.0002; ****p < 0.00001 vs negative control).
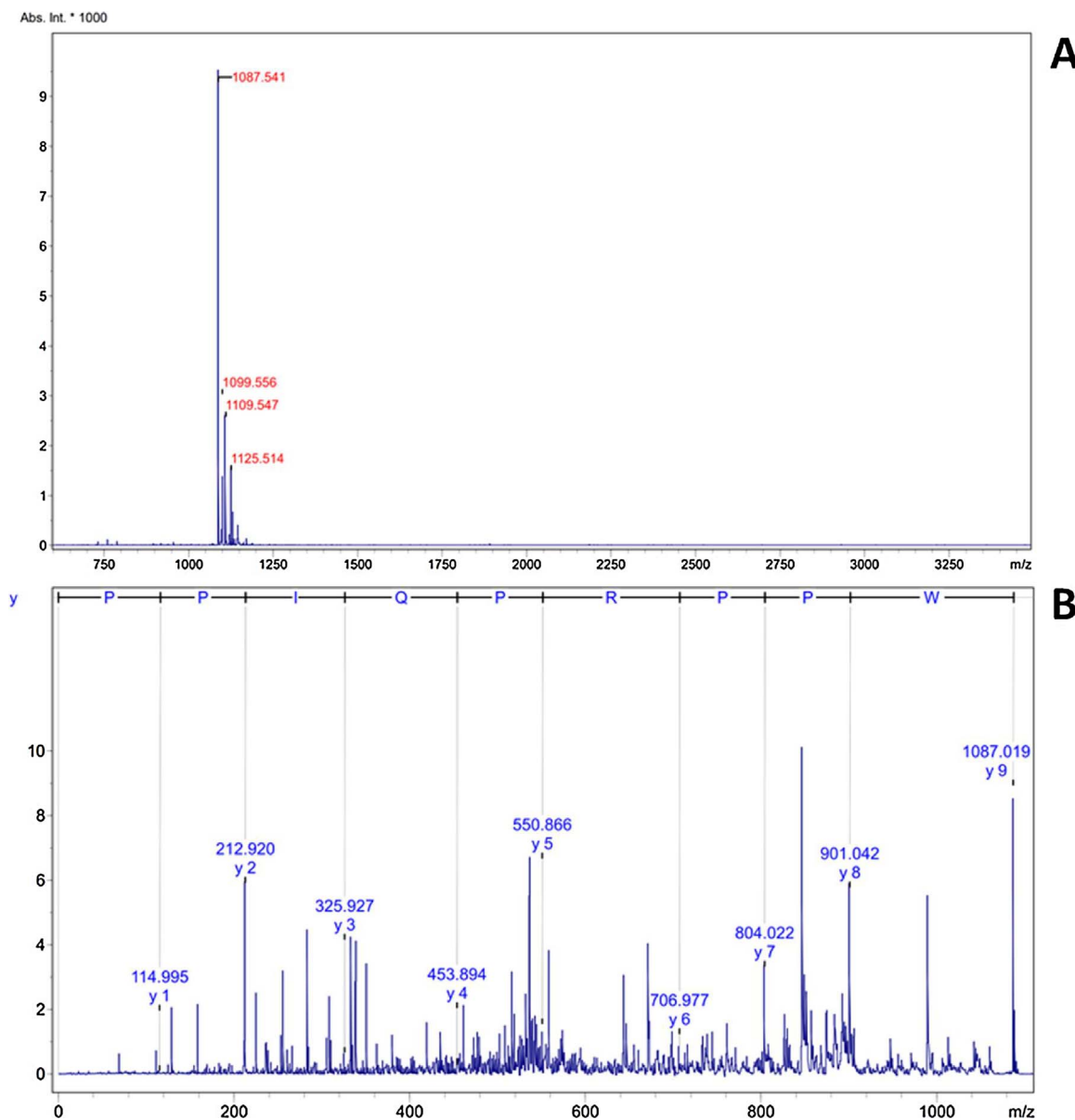
Fig. 4. A: MALDI-TOF analysis of fraction RP10. B: Representative MS/MS analysis of the peptide with *m/z* of 1087.019. Sequence was defined after manual de novo sequencing. This figure illustrates the y series.

Fig. 5. Multiple alignment of BPPs found in the venom of different species (sequences 1–4) with LmrBPP9 (sequence 5). LmrBPP9 sequence were obtained by MS/MS analysis. The highlighted box represents the consensus sequence among the aligned peptides. Z represents pyroglutamic acid. Each sequence is represented with its UniProt accession number and its species in parentheses.

identified by Soares and colleagues [25], with nine amino acid residues (WPPRPQIPP), so-called LmrBPP9. Fig. 5 shows the multiple alignment of LmrBPP9 amino acid sequence with BPPs found in different snake venoms. Multiple sequence alignment was performed by MultAlin algorithm [26]. Subsequently, the synthetic BPP was obtained using solid-phase peptide synthesis, aiming to obtain enough sample to perform its functional characterization.

3.3. Functional characterization of LmrBPP9

3.3.1. In vitro ACE inhibition assay

Although LmrBPP9 had already been identified on *L. muta* venom, the effects of this peptide on ACE activity was not studied thus far. In this way, the IC_{50} of the synthetic peptide was determined in vitro using the same methodology employed on item 3.2, and compared with IC_{50} of captopril.

According to the residual ACE activity versus different concentrations of LmrBPP9, the synthetic peptide maintained the capability of
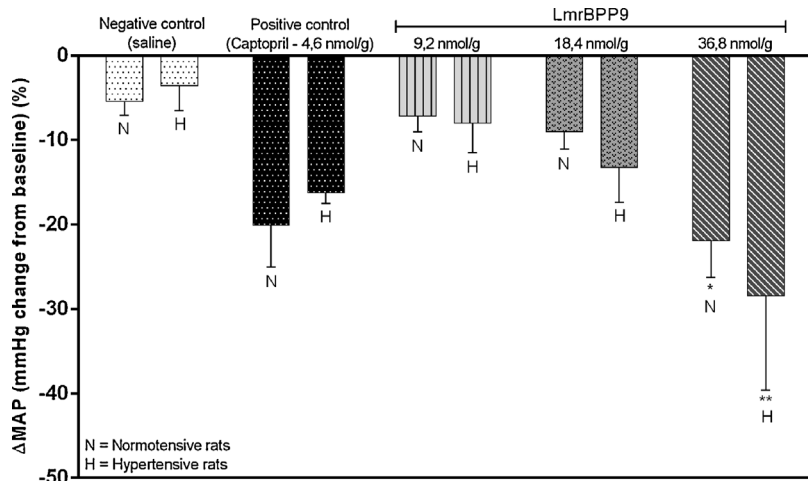
Fig. 6. Changes in MAP induced by intravenous bolus injections of different doses of LmrBPP9. A standard dose of captopril was used as positive control and saline as negative control. Drugs were injected on 6-week hypertensive 2K-1C and normotensive rats. Data are expressed as mean ± SEM. (One-way ANOVA followed by Dunnett's Multiple Comparisons Test, $*p < 0.05$; $**p < 0.005$ vs negative control; n = 4).

the native molecule to inhibit ACE, with an IC_{50} of $4.25 \pm 0.10\,\mu M$. Comparatively, the calculated IC_{50} for captopril, employed as positive control, is $8.26 \pm 0.05\,nM$. Although the difference between the synthetic peptide and Captorpil is considerable, the BPP presented similar IC_{50} values when compared to other BPPs found on *Agkistrodon bilineatus* venom (640 nM, 760 nM, 3630 nM and 30,000 nM) [27].

3.3.2. ΔMAP response to LmrBPP9 and captopril in hypertensive and normotensive rats

The difference in mean arterial pressure after peripheral injection of LmrBPP9 and captopril was evaluated in normotensive and 2K-1C renal hypertensive rats, as shown in Fig. 6. Acute injections of LmrBPP9, as well as captopril, induced hypotensive responses, when administered intravenously. The dose of 36.8 nmol of LmrBPP9 per gram of animal induced a statistically relevant change in ΔMAP (mean arterial pressure) in both normotensive and hypertensive rats. At this dose, the calculated mean difference on MAP was $-21.9 \pm 4.3\,mmHg$ for normotensive rats and $-28.5 \pm 11.1\,mmHg$ for hypertensive rats. Conversely, the doses of 9.2 nmol/g (ΔMAP $-7.1 \pm 1.9\,mmHg$ and $-8.0 \pm 3.5\,mmHg$) and 18.4 nmol/g ($-9.0 \pm 2.0\,mmHg$ and $-13.2 \pm 4.1\,mmHg$) did not significantly change the mean arterial pressure in normotensive and hypertensive animals, respectively.

Furthermore, the dose of 4.6 nmol/g of captopril, which is the maximum recommended dose to manage hypertension in humans [28], did not significantly decreased the value of ΔMAP, with a difference of $-7.1 \pm 1.8\,mmHg$ and $-8.0 \pm 3.5\,mmHg$ for normotensive and hypertensive animals, respectively (Fig. 6).

4. Discussion

Cases of human envenoming by *Lachesis* genus are not recurrent when compared to accidents caused by other snakes' species in Brazil, such as *Bothrops* ssp. and *Crotalus* ssp. [29]. However, it may cause severe complications, including intense local pain, hemorrhage, edema, necrosis in the bite site, coagulopathies, abdominal pain, nausea, bradycardia, renal failure and, specially, an accentuated hypotension [30,31]. In this context, the characterization of toxins responsible for causing these symptoms are essential to better manage the therapeutic of these envenomings.

Regarding the size exclusion chromatography (Fig. 1), fractions S11 and S12 exhibited a potent inhibitory effect on ACE (90% and 91.5%, respectively), indicating that the BPPs present in *L. m. rhombeata* venom could be present in the last fractions of the first chromatographic step. Then, fraction S11 was submitted to a second chromatographic step, on a reversed-phase C18 column, specific for fractionating peptides. This step revealed twelve fractions, named from RP1 to RP12 (Fig. 2).

Most BPPs found in snake venoms display a general structure

pattern that is represented by $< E\text{-}X_n\text{-}P\text{-}X_n\text{-}P\text{-}X_n\text{-}I\text{-}P\text{-}P$, with $< E$ being a pyroglutamic acid and X any amino acid, but Cysteine [11]. According to previous studies [25] that report the cDNA library of the venom gland of *L. muta*, the sequences of BPPs in this species' venom display great similarity among each other, which may explain the similar retention time for some components.

The screening for ACE inhibitory activity (Fig. 3) of fractions RP1 to RP12 revealed that almost all the fractions (except RP1 and RP2) were capable of inhibiting ACE activity. These results agree with the venom proteome of *L. m. rhombeata* species, performed by Pla and colleagues [4], which showed a great relative abundance of BPPs, constituting approximately 28% of the whole venom proteins.

On mass spectrometry analysis, a peptide with m/z of 1087 was the major component detected on fraction RP10 (Fig. 4A), which presented a high ACE inhibitory activity ($97.2 \pm 2.7\%$). For this reason, this peptide was analyzed by MS/MS and its amino acid sequence was deduced by de novo sequencing (Fig. 4B). The obtained sequence (WPPRPQIPP) matches with a BPP already identified by Soares et al. [25], but not yet characterized regarding its ACE inhibitory activity. In this way, this peptide, so-called LmrBPP9, was synthesized for further investigations.

Two classical methods were selected to evaluate the hypotensive effect of the synthetic peptide LmrBPP9. Firstly, the in vitro ACE inhibitory activity of LmrBPP9 was evaluated. Once stablished its capability to inhibit ACE, in vivo assays were performed to determine if this peptide affects arterial pressure of normotensive and 2K1C hypertensive rats.

Differently from the typical BPPs, which present a pyroglutamate at their N-terminus [12,13], LmrBPP9 has a tryptophan (Fig. 5). The synthetic peptide showed high ACE inhibitory activity in vitro, with an IC_{50} of $4.25 \pm 0.10\,\mu M$, indicating that the pyroglutamate is not crucial for the binding of the BPP on ACE catalytic site. Another atypical peptide, from *Crotalus durissus cascavella*, without the pyroglutamate at the N-terminus, also presented ACE inhibitory activity [32], which also supports this hypothesis.

On the other hand, LmrBPP9 presents the characteristic PP sequence at the C-terminus, which confers resistance to hydrolysis by endogenous peptidases [33]. The study of crystal complex of somatic ACE (sACE) C-domain and a BPP ($< EGLPPRPKIPP$) reported that the C-terminal prolines of the peptide strongly interacts with ACE catalytic site, creating hydrogen bonds with multiple residues of the enzyme [34]. Additionally, the presence of several proline residues in BPP sequences has armed these molecules with an intrinsic resistance to hydrolysis. In agreement with this fact, LmrBPP9 has five prolines in a primary structure of nine amino acids. Furthermore, in the same way as other BPPs already described [12,32,35–39], LmrBPP9 has an isoleucine residue next to the C-terminus prolines, which also influences the

interaction of these peptides with ACE.

In vivo assays demonstrated that LmrBPP9 significantly decrease the arterial pressure at a dose of 36.8 nmol/g, causing a ΔMAP of -21.9 ± 4.3 mmHg and -28.5 ± 11.1 mmHg for normotensive and hypertensive rats, respectively (Fig. 6), when compared to negative control (saline). The peak of hypotensive response to intravenous bolus injection of the synthetic peptide in this dosage was greater than the standard dose of captopril, which did not present a statistically significant decrease in arterial pressure, with a ΔMAP of -7.1 ± 1.8 mmHg and -8.0 ± 3.5 mmHg for normotensive and hypertensive rats, respectively.

Conversely, in a study employing a pool of BPPs from *L. muta* venom, no significant changes were observed in the mean arterial pressure of normotensive mice, when employed a dose of 200 µg of the pooled peptides intraperitonially [4]. According to the authors, this dose corresponds to approximately 10 $LD_{50}s$. They suggest that, despite BPPs represent a major venom component, with approximately 28% of its composition, they do not represent a serious clinical concern in the treatment of *Lachesis* envenomings.

In addition to our findings, several studies have demonstrated that ACE inhibition is not the only mechanism by which these peptides may potentiate the action of bradykinin (BK). ACE inhibitors may also increase the total number of BK binding sites, as well as preserving the high-affinity state of B_2 receptors, and stopping the desensitization of the receptor by ligand, as well as decelerating the receptor endocytosis [40–43]. These evidences emphasize the importance of BPPs in the venom of this species, since one of the most remarkable symptoms of the envenoming by *L. muta rhombeata* is the strong hypotension in the victims [44].

5. Conclusion

Snake venoms are considered a great source of biologically active compounds. *L. m. rhombeata* venom presents numerous compounds able to inhibit the activity of ACE. One of the peptides identified in the venom, so-called LmrBPP9, corresponds to a bradykinin-potentiating peptide with a remarkable capability to inhibit ACE activity in vitro. Accordingly, our results demonstrated that the synthetic peptide also presents a prominent hypotensive effect on both normotensive and 2K1C hypertensive animals. Furthermore, our data supports the evidence that the presence of BPPs may play a key role in the envenoming by this species, assisting the spread of other toxins present in the venom throughout the prey's body, due to the vasodilator effect caused by bradykinin. However, further studies are essential to better understand the mechanisms by which this peptide acts on the victim's homeostasis.

Funding

Conflict of interest

The authors declare that this article content has no conflict of interest.

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