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Vasodilator and hypotensive effects of the spider peptide Lycosin-I in vitro and in vivo

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ABSTRACT

Lycosin-I, a spider peptide isolated from the venom of the spider *Lycosa singoriensis*, has anti-bacteria and anti-cancer properties in organisms. However, cardiovascular effects of Lycosin-I have not been studied. In this study, we investigated for the first time the vasodilator and hypotensive effects of Lycosin-I and the possible mechanisms, in order to develop a promising treatment for hypertension-related diseases. For in vitro experiments, thoracic aortas were isolated, and divided into two groups, endothelium-intact and endothelium-denuded aortic rings. Lycosin-I induced a remarkable dose-dependent relaxation in endothelium-intact aortic rings pre-treated with phenylephrine ($p < 0.05$), while it showed no obvious vasodilator effects in endothelium-denuded aortic rings ($p > 0.05$). The vasodilator effects of Lycosin-I were significantly weakened by a nitric oxide synthase (NOS) inhibitor, L-NAME ($p < 0.001$) and a selective inhibitor of nitric oxide (NO)-sensitive soluble guanylate cyclase (sGC), ODQ ($p < 0.05$), respectively. The levels of endothelial nitric oxide synthase (eNOS) phosphorylation and the NO production were significantly higher in human umbilical vascular endothelial cells pre-cultured with Lycosin-I than the control ($p < 0.001$), determined via western blot analysis and ozone-chemiluminescence technology. For in vivo experiments, arterial and venous catheters were inserted for mean arterial pressure (MAP) recording and drug administration in anaesthetized spontaneously hypertensive rats. Lycosin-I caused a transient drop of MAP 2 min after the administration compared with the control ($p < 0.001$). In conclusion, Lycosin-I has the potential to be an anti-hypertensive drug by endothelium-dependent vasodilatation, in which eNOS and NO-sensitive sGC are two main involved factors.

1. Introduction

The rapid development of the technology in separation and identification of proteins lays a solid foundation for the investigation of biologically active polypeptides. Therefore, the application of spider bioactive peptides in medicine is vigorously developing [[1–4],[1–4]]. Peptides of spider venoms have high affinity to ion channel receptors, including voltage-gated channels as well as ligand-gated channels, which implies that bioactive peptides may have medicinal benefits in many diseases [1–4].

In recent years, it has been shown that spider venoms have biological effects on cardiovascular system, such as toxin of *Loxosceles deserta*, acting on the endothelial cells (ECs) [5–8]. Likewise, BPP-BrachyNH2, a novel bradykinin-potentiating peptides (BPPs) isolated from the skin secretion of the frog *Brachycephalus ephippium*, induces potent endothelium-dependent vasodilatation with similar magnitude as captopril [9]. Bj-BPP-10c, a bioactive proline-rich decapeptide expressed in the brain and the venom gland of *Bothrops jararaca*, increases nitric oxide (NO) metabolite production in human umbilical vascular endothelial cells (HUVECs) [10]. Nitric oxide releasing fraction (NORF) derived from *Phoneutria nigriventer* spider venom has been proved to increase liberation of NO from the endothelium of rat mesenteric

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arterial rings [11]. Furthermore, Malayan Krait (*Bungarus candidus*) envenoming causes cardiovascular disturbance involving autonomic reflex and vascular NO mechanisms [12].

In living bodies, vasodilator actions occur mainly via two pathways. One is the endothelium-dependent way, in which ECs and smooth muscle cells (SMCs) work together. The other is the endothelium-independent way, in which only SMCs are involved. Many investigations indicate that NO and prostacyclin I2 (PGI2) are the vital endothelium-derived relaxing factors [13]. In the endothelium, NO synthesis is catalyzed by nitric oxide synthase (NOS). The process is either calcium-dependent or calcium-independent. The former mainly depends on the activation of kinases, such as protein kinase B (PKB). After the phosphorylation of Ser1177 or Ser1179 site, NO is produced. As a crucial signaling molecule, NO regulates the structure and function of vascular smooth muscle cells (VSMCs) by activating soluble guanylate cyclase (sGC) [14], which increases the levels of cyclic guanosine monophosphate (cGMP) effectively, leading to the relaxation of vascular smooth muscle. During the process, Ca^{2+}, K^{+} and calmodulin (CaM) play important roles in signal transduction in the constriction of VSMCs [15]. Accordingly, these factors have close relationship with cardiovascular diseases such as hypertension and coronary heart disease [16,17].

Lycosin-I is a 24-residue peptide with 8 net positive charges and an amidated carboxyl terminus. It shows a linear amphipathic alpha-helical conformation which is common to alpha-helical cationic antimicrobial peptides [18]. Lycosin-I is isolated from the venom of the spider *Lycosa singorensis* and demonstrates an anti-cancer effect in in vitro and in vivo studies [[18],19]. This peptide has rapid, selective and broad-spectrum antimicrobial activities, as well as synergistic effects with traditional antibiotics [20]. Moreover, Lycosin-I displays high antibacterial activities and rapid bactericidal effects against multidrug-resistant *Acinetobacter baumannii* (MDRAB), which is resistant to most traditional antibiotics [21]. Taken together, Lycosin-I is a promising peptide with the potential for the development of novel drugs.

The influence of Lycosin-I on the cardiovascular system in organisms remains unknown. It is known that elevated blood pressure is still the remediable risk factor for cardiovascular diseases [22]. Control of hypertension among hypertensive adults remains unsatisfying [23]. In other words, the development of antihypertensive drugs is still necessary. Our study is the first report to reveal the anti-hypertensive effects of Lycosin-I and investigate the possible involved mechanisms. The findings suggest that this peptide is a promising candidate for the treatment of hypertension-related diseases in clinic.

2. Materials and methods

2.1. Lycosin-I synthesis

Lycosin-I was synthesized by Pepmic Co., Ltd (Suzhou, China). The purity of Lycosin-I was more than 98%, detected by high performance liquid chromatography and mass spectrum analysis.

2.2. Chemicals and drugs

Dulbecco's Modified Eagle Medium (DMEM) and fetal bovine serum (FBS) were purchased from GIBCO-BRL (USA). The antibodies against phospho-NOS and β-actin were purchased from Cell Signaling Technology (USA). A NOS inhibitor, L-NAME (Nω-Nitro-L-arginine methyl ester hydrochloride, CAS number: 51298-62-5), a selective inhibitor of NO-sensitive sGC, ODQ (1H-[1,2,4]Oxadiazolo[4,3-a]quinoxalin-1-one, CAS number: 41443-28-1) and other chemicals were purchased from Sigma Chemical Co., Ltd (St. Louis, MO, USA).

2.3. Animals

Eleven week-old male 210–250 g Sprague-Dawley rats and 220–260 g spontaneously hypertensive rats (SHRs) were purchased from Hunan SJA Laboratory Animal Co., Ltd, Changsha, China. The animals were kept in cages with dry and clean beddings in a room on a 12/12 h light-dark cycle for 1 week before the start of the experiment. All the rats received standard care and had free access to a standard diet in Department of Laboratory Animals in Central South University, in compliance with the Guide for the Care and Use of Laboratory Animals, published by the US National Institutes of Health. All of the animal experiments were approved by the Institutional Animals Care and Use Committee of Central South University.

2.4. Aortic rings protocol

2.4.1. Rat aortic rings preparation

This protocol was performed as described previously [24]. Under general anesthesia, the thoracic aorta descending segments of Sprague-Dawley rats were excised, and cut into rings about 3–4 mm in length after removing attached fat and connective tissue. The aortic rings were grouped into two groups, endothelium-intact rings and endothelium-denuded rings following the protocol described by Wang G J [25]. Each ring was mounted vertically between two stainless steel hooks. The upper one was connected to force transducer and its mechanical activity was recorded by computerized recorder (BL-New Century 420, Taimeng, Chengdu, China). Each aortic ring was mounted in an organ chamber containing Krebs-Henseleit solution ((mM): NaCl, 110.8; KCl, 5.9; $NaHCO_3$, 25.0; $MgSO_4$, 1.07; $CaCl_2$, 2.49; NaH_2PO_4, 2.33; glucose, 11.51) constantly, maintaining at 37 °C under a basal tension of 2 g, and was equilibrated for 1 h before starting experiment [26].

2.4.2. Vasodilator activity in rat aortic rings

The endothelium-intact rings were pre-contracted with phenylephrine (1 μM). Then Lycosin-I was added with increasing cumulative concentration (10–60 μg/ml). Data were recorded when the maximum relaxation was detected and the next concentration of Lycosin-I was added at the same time. A NOS inhibitor, L-NAME (1 μM), or a selective inhibitor of NO-sensitive sGC, ODQ (1 μM) was added to the bath 20 min prior to the addition of phenylephrine [27].

2.5. Cell culture

The HUVECs (provided by the Third Xiangya Hospital of Central South University, China.) were grown in a six-well culture plate with DMEM containing 1.0 g/L glucose, 10% FBS, streptomycin (100 μg/ml) and penicillin (100 U/ml). The cells were cultured at 37 °C under 5% CO_2 [28].

2.6. Detection of eNOS phosphorylation

The HUVECs with good growth were inoculated into 6-well culture plates with 2×10^5 cells per well. Then the HUVECs were treated with Lycosin-I (10 μM), and incubated at 37 °C in a 5% CO_2 incubator for 48 h [20]. After the cultured medium was discarded, cells were washed by saline for 3 times. The total protein was extracted by RIPA buffer mixed with phosphatase and protease inhibitors. After SDS-PAGE with 10% polyacrylamide gel, the protein was transferred to polyvinylidene fluoride membranes. The membranes were blocked with 5% Bio-Rad nonfat dry milk in 1× TBS-T buffer (0.1% Tween-20) for 2 h and then incubated with the primary antibody in 5% non-fat dry milk/TBS-T buffer at 4 °C overnight. The membranes were subsequently washed by 1× TBS-T and treated with the secondary antibody for 1 h at room temperature. Enhanced chemiluminescence detection system was used to detect the membranes and analyze the optical density (OD) of the stripes. The OD ratio of each protein band to the internal reference β-actin band was calculated and corrected with the control [29].