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Selective anti-tumor activities of venom peptides from the lesser paper wasp *Parapolybia varia*

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ABSTRACT

We identified vespid chemotactic peptide (VCP) and vespakinin (Vespk) from the lesser paper wasp, *Parapolybia varia*. The cDNA, genomic DNA, and mature peptide sequences of *P. varia* VCP (*Pv*VCP) and Vespk (*Pv*Vespk) were determined. To investigate the pharmacological and toxicological properties of *Pv*VCP and *Pv*Vespk, their hemolytic, anti-microbial, anti-fungal, and anti-tumor activities were evaluated and compared with those of *Vespa mandarina* VCP (*Vm*VCP) and Vespk (*Vm*Vespk). *Pv*VCP, *Pv*Vespk, and *Vm*Vespk showed little to low hemolytic activities. Only *Vm*VCP showed hemolytic activity at a high concentration. Among the four peptides tested, *Vm*VCP showed both anti-microbial and anti-fungal activities, whereas *Pv*VCP showed only anti-fungal activity to *Candida albicans*. Interestingly, *Pv*VCP showed significantly stronger anti-tumor activities to two ovarian cancer cell lines compared with *Vm*VCP. Vespks only showed anti-tumor activity to SK-OV-3 cells but not to NIH-OVCAR-3 cells. These differences in anti-tumor activity might have been caused by the differences in secondary structure among peptides. A circular dichroism spectrometry analysis revealed that VCPs have more amphiphilic α-helix structures than Vespks. Taken together, the low hemolytic but strong anti-tumor activities of *Pv*VCP suggest that this peptide could be a candidate for developing a new anti-tumor peptide drug or drug carrier in the future.

Introduction

Animal venoms enriched with various functional materials include amines, lipids, proteins, peptides, and other small molecules. Among them, peptides have been considered important resources for developing drugs because they have high specificity to their targets and low non-specific toxicity (Kaas and Craik, 2015). Thus, one important goal in drug discovery studies is to develop peptide drugs for human diseases such as cancers, diabetes, cardiovascular diseases, pains, epilepsy, etc., using their advantages (Craik et al., 2013; Fosgerau and Hoffmann, 2015). The global peptide therapeutic market has been expected to increase from US$14.1 billion in 2011 to an estimated US$25.4 billion in 2018 (Fosgerau and Hoffmann, 2015). Approximately 140 peptide drugs were in clinical trials, and >500 therapeutic peptides were in preclinical development in 2015 (Bogin, 2005; Craik et al., 2013; Fosgerau and Hoffmann, 2015).

Recent studies have reported that the venom of a single species could contain more than one hundred functional peptides (Escoubas et al., 2008), and arthropod venoms are known to provide novel peptides which might have various clinical benefits in treating diverse diseases such as inflammation, arthritis, multiple sclerosis, pain, etc. (Cherniack, 2010). Most of the studies on functional peptides in venoms have focused on those from relatively large animals such as frogs, snakes, scorpions, spiders, and snails because their venom can threaten human health and life if the envenomed people were not appropriately treated (Lee and Vadas, 2011). In addition, those animals produce a sufficient amount of venom for their components to be analyzed by biochemical methods.

Ants, bees, hornets, and wasps in the order Hymenoptera are known to produce venom for various purposes, such as protecting themselves from predators, hunting prey for foods, etc. (Lee et al., 2016). The major components of Hymenopteran venom are a variety of peptides, including mastoparans, chemotactic peptides, and kinins, which share a

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common amphipathic α-helical structure (Lee et al., 2016). Mastoparan is the most abundant peptide component of Vespidae wasps. The genes encoding premastoparans were the most and second most abundantly transcribed in the venom glands of *Vespa crabro* and *V. analis*, respectively (Yoon et al., 2015a,b). They showed anti-microbial, anti-fungal, anti-tumor, and hemolytic activities (Yoon et al., 2015b). Along with premastoparan, vespid chemotactic precursor (VCP) and vespakinin (Vespk) have been identified as the top three genes most prevalently transcribed in the venom glands of *V. crabro* and *V. analis*.

The lesser paper wasp *Parapolybia varia* is a mid-sized social wasp species widely distributed in the Middle East, the Indo-Papuan region, and East Asia (Katada et al., 2007; Saito-Morooka et al., 2015). Despite its abundance, little information is available on its venom composition and functional venom peptides. In particular, no information regarding Vespks from the genus *Parapolybia* has been reported yet. The amino acid sequences of venom peptides even in closely related species show variances, and changes of only a few amino acid residues in peptides can significantly alter the bio-physical and chemical properties of peptides (Kaas and Craik, 2015). In this study, we identified genomic structures, cDNA sequences, and functional peptide sequences of *P. varia* VCP (*Pv*VCP) and Vespk (*Pv*Vespk). Then, we examined and compared their hemolytic, anti-microbial, anti-fungal, and anti-tumor activities with those of *Vespa mandarina* VCP (*Vm*VCP) and Vespk (*Vm*Vespk).

Materials and methods

Wasp collection, venom isolation, and total RNA extraction

P. varia was collected from Taehwa Mountain in Gwangju, Gyeonggi-do, Korea. The collected wasps were anesthetized using lower-pressure carbon dioxide, and their abdominal organs were extracted by pulling out the stinger with forceps in 1 × phosphate-buffered saline (PBS) on a slide glass. The venom glands of 30 wasps were separated and used for the next step. The total RNA was extracted with 200 μl of TRI reagent following the manufacturer's protocol (Molecular Research Center, Cincinnati, OH, USA).

cDNA sequences and genomic DNA structure characterization

Genomic DNA (gDNA) was extracted from the whole body of *P. varia* female with the DNeasy® Blood & Tissue Kit (Qiagen, Valencia, CA, USA) following the manufacturer's protocol. The cDNA sequences of *Pv*VCP and *Pv*Vespk were obtained from the whole transcriptome analysis results of *P. varia* (K.J.Y., Y.H.K., and S.H.L., unpublished data). The total RNA was treated with DNase I (Ambion, Austin, TA, USA) to eliminate contaminated gDNA, and cDNA was synthesized using SuperScriptIII reverse transcriptase (Invitrogen, Carlsbad, CA, USA). The gDNA and cDNA of *P. varia* were used as templates for PCR amplification with the following primers: *Pv*VCP forward, 5′-ATGAAGTTCAATATCG TAGC-3′; *Pv*VCP reverse, 5′-TCACTTTCCAAGGGAGC-3′; *Pv*Vespk forward, 5′-ATGAAAAATATATCCCTTTT-3′; and *Pv*Vespk reverse, 5′-TTAGCCACTTCTGAATG-3′. A thermal cycling program was started with pre-incubation at 95 °C for 1 min followed by 35 cycles at 95 °C for 30 s, 50 °C (for *Pv*VCP) or 45 °C (for *Pv*Vespk) for 20 s, and 68 °C for 1 min using Advantage® 2 Polymerase Mix (Clontech, Palo Alto, CA, USA). The PCR products were separated on the gel and then extracted using the QIAquick® Gel Extraction Kit (Qiagen) for direct sequencing with amplifying primer sets.

Peptide synthesis

Mature peptides of *Pv*VCP, *Pv*Vespk, *Vm*VCP, and *Vm*Vespk were synthesized through GenScript (GenScript, Piscataway, NJ, USA) and stored at −80 °C until use for biological activity assays. The peptides were dissolved in a 1% dimethyl sulfoxide (DMSO) solution with various concentrations.

Hemolytic activity assay

The hemolytic activities of VCPs and Vespks were determined using human red blood cells (RBCs) purchased from Korean Red Cross Blood Services. The RBCs were washed with the same volume of PBS and centrifuged at 960 rpm for 15 min for recovery. After washing three times, the RBCs were re-suspended with PBS to obtain a final concentration at 2% (V/V). Ten microliters of synthetic peptides with various concentrations (10–200 μM) were incubated with the washed RBCs for 30 min at 37 °C. The incubated mixture was centrifuged for 15 min at 960 rpm, and the optical density (OD) values of the supernatants were measured at 540 nm using a microplate reader (VersaMax, Molecular Devices, Sunnyvale, CA, USA). The relative hemolytic activity was determined by establishing the activity of 1% DMSO solution as a blank and that of the 0.1% Triton X-100 solution as 100%. Hemolytic activity assays were conducted with three replications. The protocol was approved by the Institutional Review Board (IRB# E1508/001–005, Seoul National University IRB).

Anti-microbial activity assay

Gram-negative *Escherichia coli* (ATCC 11775), Gram-positive *Staphylococcus aureus* (ATCC 12600), Gram-positive yeast *Candida albicans* (ATCC 10231), and gray mold *Botrytis cinerea* were used for examining the anti-microbial activities of peptides. Luria–Bertani (LB) broth, brain heart infusion (BHI) broth, and potato-dextrose (PD) broth were used to culture *E. coli*, *S. aureus*, and *C. albicans*, respectively. The bacterial and fungal cultures were incubated with various concentrations of synthetic peptides (0.05–0.1 mM) in a shaking incubator (150 rpm) overnight at 37 °C, and then the OD of each culture was measured using a microplate reader (VersaMax) to identify the minimal inhibition concentration (MIC). *B. cinerea* was incubated for 5 days at 25 °C on PD agar, and 20 μl of synthesized venom peptides were applied in various concentrations (0.1–2 mM) overnight. The MICs were determined by measuring the size of the inhibition zone on the fungi-incubated agar. A top agar assay was conducted with the same bacteria and yeast used in the broth inhibition assay. After incubating *E. coli* in LB broth to 0.5 OD_{600}, 1 ml of liquid culture was mixed with 10 ml of 0.7% agar-containing LB and then poured into a plate. Various concentrations (0.05–1 mM) of synthesized venom peptides were applied onto hardened agar plates, and the inhibition zone was measured.

Anti-tumor activity assay

SK-OV-3 (ATCC HTB-77) and NIH-OVCAR-3 (ATCC HTB-161) ovarian cancer cell lines were used for the anti-tumor activity assays. After culturing the ovarian tumor cells (1×10^4 cells/plate) for 1 day, various concentrations (2.5–100 μM) of synthesized peptides were added into the cell plates. Non-treated and 0.001% DMSO-treated plates were used as controls. The survivorship of tumor cells was measured by an MTT assay. After incubating with various concentrations of peptides for 24 h and 72 h, the cells were additionally incubated for 4 h at 37 °C in a CO_2 incubator, and then OD was measured at 540 nm.

Circular dichroism spectrum characterization

The circular dichroism (CD) spectra of *Pv*VCP, *Pv*Vespk, *Vm*VCP, and *Vm*Vespk were measured in 10 mM sodium phosphate buffer at pH 7.2 (SPB), 30 mM sodium dodecyl sulfate (SDS), and 50% trifluoroethanol (TFE) to identify the secondary structures. The spectra were recorded from 180 to 260 nm on a JASCO J-1500 spectropolarimeter (JASCO, Easton, MD, USA) at 298 K using a quartz cuvette of 0.1 and averaged by three scans. The protein sample concentration was 0.1 mg/ml. The data were recorded at a scan speed of 200 nm/min, bandwidth of 1.0 nm with 1 s response and 0.1 nm resolution. The percentage of α-helical, β-sheet, turn, and random structures was calculated.