

Melittin: A Key Composition of Honey Bee Venom with Diverse Pharmaceutical Function

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Abstract—Melittin is the main toxic component in the the honey bee venom. It is a cationic, lytic peptide constituted by 26 amino acid residues. This peptide is an amphoteric molecule which the carboxy-terminal region is hydrophilic and the amino-terminal region is hydrophobic due to the presence of a series of positively charged amino acids. Melittin shows the interaction with biological membrane or enzyme to amphiphilic property. Based on these properties, melittin is an important candidate use for antibiotic-resistant bacteria, cancer and tumor and pathogenicity virus treatment. This review introduces the possible active mechanism of melittin and recent research progress in medical.

Keywords-melittin; active mechanism; anticancer; anti-viral; medical function

I. INTRODUCTION

Melittin is the main constituent in the honey bee venom which constitutes approximately 40-50% of the dry powder

weight of the venom[1]. It is a small linear peptide with the chemical formula $C_{131}H_{228}N_{38}O_{32}$ composed of 26 amino acid residues[2]. Melittin has various biological, toxicological, pharmacodynamics and pharmacological properties, including strong interaction with cell lipid membranes, antifungal, antibacterial, antiviral and potential anti-tumor activities[3, 4]. Melittin is a potential pore forming peptide that can form four polymers and insert in the phospholipid bilayer, Therefore, it is able to study the interaction relationship between bioactive membrane and peptide by the biological activity[5-7]. Melittin was used to be a phospholipase A2 (PLA2) activator due to the discovery that it has an enhancing effect on PLA2 activity [8, 9]. The interest of the medicinal properties of melittin increased greatly in the recent decades; therefore this paper brings an overview of recent pharmaceutical function researches of this peptide.

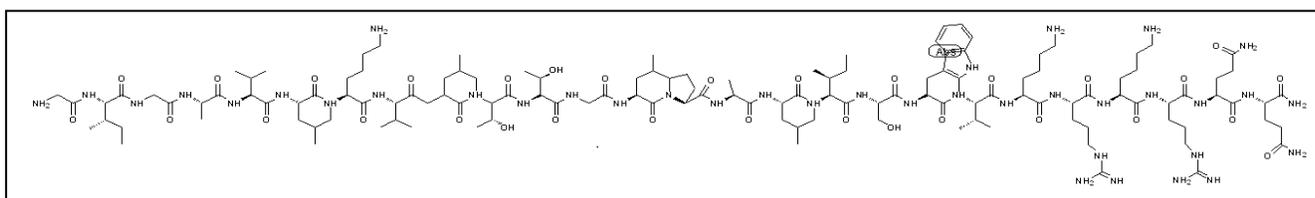


Figure 1. Structure of melittin

II. PHYSICOCHEMICAL PROPERTY AND ACTIVE MECHANISM OF MELITTIN

A. Physicochemical Property of Melittin

Melittin is an amphiphilic polypeptide. The net charge of this peptide is 6+ at physiological pH. One of the four positive charges is in the C-terminal region and the rest of two charges in the Lys-7 and the N-terminal group. The polar and non-polar amino acids asymmetric distribution

lead melittin to be an amphipathic peptide when the peptide is develop to an α -helical conformation[10]. Although, non-polar amino acids accounted for a large proportion in the structure of melittin, this peptide is marginally soluble in methanol but highly soluble in water. At low concentration, melittin is a monomer and forms a randomly coiled conformation in aqueous solution and self-assembly into tetramers in high-pH or ionic solution[11].

The melittin tetramer's three-dimensional structure in

aqueous solution determined by X-ray crystallographic analysis at 2 Å resolution which the crystals grown in high ionic strength solution. In the tetramer crystal, each peptide chain is form of two α -helical portions and the overall structure presents a curved shape. In another research[12], through NMR methods, with the change of temperature, melittin presents a conformational transition between the monomer and tetramer in aqueous solution and this change great relevance with proline residues isomers in melittin structure. In aqueous solution, the monomeric melittin self-assembly to a tetramer is increased with elevated ionic, melittin concentration, and high pH. The fluorescence experiments shown that melittin for the most part was monomeric at very low ionic resolution[13]. On the contrary, high ionic strength and high concentration of the peptide promoted the aggregation at neutral pH solution.

B. Membrane-Active Mechanism of Melittin

Melittin generally used for membrane studies as it strong lipid membrane disrupt and lysis ability. This principle is an α -helical structure melittin bind to lipid membrane in possible parallel or perpendicular way. In the perpendicular model, it is might be participate in pore formation by form a tetramer structure in lipid membrane [14, 15]. In 2010, Lee[16] found a new antimicrobial mechanism of melittin. The antifungal effect of melittin in *C. albicans* was detected by using fluorescence microscopy with FITC-annexin V, DAPI (4'-6-diamidino-2-phenyl-indole), and TUNEL (TdT-mediated dUTP Nick-End Labeling) staining. In addition, melittin increased the reactive oxygen species (ROS) production. The depth mechanism of melittin antimicrobial activity in *C. albicans* was further detected[17]. Experimental results revealed that melittin emerged highly reactive hydroxyl radicals ($\cdot\text{OH}$) that resulted in cell apoptosis.

III. BIOLOGICAL ACTIVITY OF MELITTIN

A. Anticancer Active of Melittin

The possible anti-cancer, anti-tumor mechanism of melittin is: inhibited cell growth by disturbed cell growth cycle, then induces cell apoptosis and necrosis. *In vitro* experiment, it reveals a growth inhibition and lethality in human hepatoma and glioma cell[18]. In human hepatocellular carcinoma cell(HCC), a recombinant adenovirus that fusing an melittin gene (Ad-rAFP-Mel) used in the treatment of liver cancer [19]. The result showed that Ad-rAFP-Mel infection had an inhibitory effect on the proliferation of HCC cells.

Many research results showed that melittin can significantly inhibits the growth and induces cell apoptosis of tumor cells [20-23]. An article Recently published on melittin detailedly reviewed the main mechanism of melittin antitumor activity [24]. This review show that the interaction and treatment of melittin with several types of cancer, such as renal, lung, liver, prostate, bladder, breast. Melittin studied *in vivo* animal models have also demonstrated its tumor inhibitory and anti-proliferative activity[25]. However, some side effects like hemolysis and

injury of liver and kidney were detected in the treatment process of melittin. To minimize these body damage effects, some modified strategies of melittin should be processed. A recombinant immunotoxin was obtained that an antigen binding to melittin[26]. This system was obtained by fusing genes which encoded a murine monoclonal antibody derivative with an oligonucleotide encoding melittin. This recombinant immunotoxin showed significantly binding and killing tumor cell properties *in vitro*. In Cao's study[27], a recombinant immunotoxin was obtained by fused melittin to an anti-asialoglycoprotein receptor (ASGPR) antibody (Ca). Moreover, the lysis efficacy and targeting ability of the fusion antibody were studied, results showed that the recombinant protein still kept the hemolytic activity of melittin but the lysis ability to HepG2 cells was improved. In order to reduce the lytic ability of melittin, a tumor matrix metalloproteinase2 (MMP2) cleavable melittin/avidin conjugated protein was built. It was inactive when melittin coupled to avidin, but once released from the conjugate content it induces immediate cell cytolytic [28].

In order to reduce the side effects, some researchers designed and synthesized a transporting vehicle of melittin. Through emulsion solvent diffusion method, nanoparticles were obtained by mixed melittin with the anionic detergent sodium dodecyl sulfate form into poly (D, L-lactide-co-glycolide acid) nanoparticles. Moreover, the *in vitro* studies showed that the growth inhibitory activity effects of melittin-loaded nanoparticles in breast cancer MCF-7 cells were significantly improved [29]. But this nanoparticles could not directly administrated as melittin could be released from the nanoparticles into blood vessels and induced lysis of red blood cells [30]. Further, a stable perfluorocarbon nanoparticles was designed to solve the disadvantages[31]. This kind of nanoparticles presented perfect pharmacokinetics, transported melittin to tumor cell and reduced tumor growth but no significant toxicity [32, 33].

B. Antimicrobial Active of Melittin

Melittin has showed powerfully antimicrobial activities but also has strong hemolytic and significant allergic properties[34, 35]. Merrifield worked on shortening the sequence, improving this antimicrobial peptide biological activity. Four cecropin A and melittin chimeric peptide derivatives were designed and synthesized, *in vitro* activity test showed good antibacterial activity against Gram-negative and Gram-positive bacteria but low cytotoxicity[36]. In addition, it is well known that methicillin-resistant *Staphylococcus aureus* (MRSA) was difficult to treat by existing antibiotics. A recent study showed[37] that MRSA-infected rats were treated by melittin, survived in MRSA-induced bacteraemia and restored from skin wounds. There result suggested that melittin could most likely be developed into new antibiotics or replacement.

C. Anti-Viral Active of Melittin

Although disease caused by the virus is popular all over the world, but the effective drugs of treating viral infection is very shortage. The antiviral activities of melittin and its analogs were confirmed that they could inhibit the synthesis of viral protein particles like melittin-reduced the formation of HSV-1 viral proteins [38]. In addition, melittin could inhibit replication of HIV-1 by suppressing viral gene expression[39]. Another study showed that a melittin loaded-immunoliposome was utilized to show the antiviral properties against fish viral haemorrhagic septicemia rhabdovirus (VHSV)[40]. Recently, a similar idea has been published that melittin could inhibit HIV-1 invasion by carried in a nanoparticle that might be used as a topical vaginal HIV-1 virucide[41].

IV. CONCLUSION

Nowadays, the need for new effective antimicrobial, anticancer, antiviral drugs has become a priority and new strategies should be introduced to alternative current therapeutic regimen. Melittin is probably the most studied antimicrobial peptide with potential actions against bacteria, viruses and cancer cells, it has been extensively examined *in vitro* and *in vivo* experiment, although many bioactive mechanism remains to be illuminated.

Because of the strong hemolysis and the fast degradation by biological properties, there are many challenges should be over come in the way to develop melittin to novel and variety of actives drug, therefore reduce adverse effects and improve its bioavailability will become melittin follow-up research hot spot. Based on the above elaboration, we are looking for subsequent research. Firstly, melittin mainly extract from bee venom and a variety of structural analogues will generate during the period of melittin precursor convert into melittin[42]. Thus, to obtain high purity and yield of melittin will be the key and difficult point for future research. Some scholars expected to obtain high yield and purity of melittin by biological fermentation engineering technology, their work suggested that more engineered expression systems should be develop to reduce the cost of acquiring melittin[43-46]. Secondly, Chemical modification of structure of melittin or combined with other protein could enhance the biological stability. At the same time, the fusion protein of biological activity protein and melittin might generate synergy effect in organisms. So exploring different chemical modification or design and synthesis of different fusion protein will also become an important research direction in the future. Finally, although cytotoxic to the pathogenic microorganism, tumor and cancer cells, melittin is also toxic to normal tissues or organs. Therefore, without a suitable delivery system, its therapeutic potential cannot be achieved. This could be overcome by many kinds of melittin-loading nanoparticles that can safely transmit melittin to lesions by intravenous infusion, other like oral administration, mucosal drug delivery, controlled release and targeted nanoparticles should be develop to expand the range of applications of melittin and enhance its activity

effect. With the development of above three areas, melittin will be the strongest potential to become the next generation of biological drugs.

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