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REVIEW

Melittin: a venom-derived peptide with promising anti-viral properties

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Abstract

Despite tremendous advances in the development of anti-viral therapeutics, viral infections remain a chief culprit accounting for ongoing morbidity and mortality worldwide. Natural products, in particular animal venoms, embody a veritable cornucopia of exotic constituents, suggesting an immensurable source of anti-infective drugs. In this context, melittin, the principal constituent in the venom of the European honeybee *Apis mellifera*, has been demonstrated to exert anti-cancer, anti-inflammatory, anti-diabetic, anti-infective, and adjuvant properties. To our knowledge, there is no review appertaining to effects of melittin against viruses, prompting us to synopsize experimental investigations on its anti-viral activity throughout the past decades. Accumulating evidence indicates that melittin curbs infectivity of a diverse array of viruses including coxsackievirus, enterovirus, influenza A viruses, human immunodeficiency virus (HIV), herpes simplex virus (HSV), Junín virus (JV), respiratory syncytial virus (RSV), vesicular stomatitis virus (VSV), and tobacco mosaic virus (TMV). However, medication safety, different routes of administrations, and molecular mechanisms behind the anti-viral activity of melittin should be scrutinized in future studies.

Keywords Venom · Bee · Melittin · Anti-viral activity · Drug

Introduction

Viruses virtually parasitize every living creature on planet earth, from animals and plants to bacteria and archaea. Human beings have been also afflicted by these non-living entities throughout history. Some viral diseases such as acquired immune deficiency syndrome (AIDS), Ebola hemorrhagic fever, hepatitis B and C, influenza, and rabies still continue to evoke inordinate fear in societies [1]. For instance, the "Spanish flu" pandemic, which swept around the globe in 1918, claimed the lives of more people than perished in World War I [2]. The World Health Organization (WHO) estimates that 35 million individuals have succumbed to AIDS-related illnesses since the beginning of the human immunodeficiency virus (HIV) epidemic in the early 1980s. As of 2017, nearly 36.9 million people are living with HIV worldwide [3].

Over the past half-century, tremendous efforts have been devoted to develop anti-viral drugs. However, this process is time-consuming, exorbitantly expensive, and tediously meticulous [4]. These problems are even further exasperated when mutations in a viral genome give rise to drug resistance [5]. All these facts have impelled researchers to discover unique biochemical compounds for the treatment of viral diseases. In this respect, natural products embody a miscellaneous array of exotic constituents, propounding an immensurable source of anti-infective drugs [6].

Some animals such as snake, scorpions, spiders, and bees produce poisonous secretions termed venoms to kill/incapacitate preys or defend against predators. Regardless of their detrimental effects, animal venoms have long held a fascination for humankind owing to their pharmacologically active components including enzymes and peptides [7, 8]. In this context, therapeutic properties of venoms for treating neurologic and cardiovascular illnesses, cancer, atopic dermatitis, diabetes, and gastrointestinal maladies have been documented since medieval times [9]. Venom-derived peptides have recently provoked great attention among newly enthused researchers, since they are not only selective and potent but also relatively innocuous as therapeutics [9, 10]. Indeed, these features together with infinite

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biodiversity of venom-derived peptides may revitalize flagging drug development programs.

Heretofore, six medications derived from venom peptides have been approved by the US Food and Drug Administration (FDA) for clinical use: captopril (from snake, *Bothrops jararaca*; 1981), eptifibatide (from snake, *Sistrurus miliarius barbouri*; 1998), tirofiban (from snake, *Echis carinatus*; 1999), bivalirudin (from medicinal leech, *Hirudo medicinalis*; 2000), ziconitide (from cone snail, *Conus magus*; 2004), and exenatide (from lizard, *Heloderma suspectum*; 2005) are used for the treatment of hypertension, acute coronary syndromes, acute coronary syndromes, coagulation during surgery, chronic pain, and diabetes mellitus type 2, respectively [9, 11–16]. At the time of writing this article, several venom-derived peptides are in clinical trials or preclinical development for curing a vast array of maladies [17].

Melittin is the principal constituent in the venom of the European honeybee *Apis mellifera* [18]. It is an amphipathic hexacosapeptide (NH_2-Gly-Ile-Gly-Ala-Val-Leu-Lys-Val-Leu-Thr-Thr-Gly-Leu-Pro-Ala-Leu-Ile-Ser-Trp-Ile-Lys-Arg-Lys-Arg-Gln-$CONH_2$) in which the N- and C-terminal regions are predominantly hydrophobic and hydrophilic, respectively [19, 20]. This uneven distribution of polar and non-polar amino acid residues gives the melittin amphipathic structure when it is folded into an α-helical configuration [21]. Melittin is composed of two α-helices connected through a flexible segment [22]. Tetrameric melittin is predominant at concentrations found in the venom sac of the honeybee, but changes in peptide concentration and ionic strength result in tetramer to monomer dissociation [23, 24]. Melittin interacts with cell membranes and induces pore formation at micromolar concentrations, thereby disturbing membrane function and triggering cell lysis [25, 26].

In spite of some concerns over cytotoxic properties of melittin, there is a mounting body of evidence on its therapeutic values. Melittin has been shown to exert anti-cancer [27], anti-inflammatory [28], anti-diabetic [29], anti-microbial [30], anti-biofilm [24], and adjuvant [31] properties. Since the late 1970s, praiseworthy endeavors have been devoted to ascertain the anti-viral action of melittin in vitro and in vivo. To the authors' knowledge, there is no review appertaining to effects of melittin against viruses, prompting us to synopsize experimental investigations on its anti-viral activity throughout the past decades.

In vitro studies

Cell culture models are convenient and cost-effective tools to study the molecular mechanisms of viral life cycles as well as preliminary toxicological screening of drug candidates. Thus far, many investigations have been conducted to measure efficacy of melittin against diverse viral species, which are recapitulated in Tables 1 and 2. For the reader's convenience, we categorized these studies based on viral families.

Arenaviridae

The family *Arenaviridae* encompasses enveloped viruses with two single stranded, ambisense RNA molecules, and is usually associated with rodent-transmitted infections in human beings [45, 46]. The family comprises three newly separated genera including *Mammarenavirus*, *Reptarenavirus*, and *Hartmanivirus.* Both *Reptarenavirus* and *Hartmanivirus* infect reptilian hosts, whereas *Mammarenavirus* infects mammalian hosts [47]. On the basis of serological cross-reactions, genetic, and geographic relationships, the genus *Mammarenavirus* is further subdivided into two major serogroups: The New World and the Old World [45, 47]. Noticeably, some Old World (Lassa and Lujo) and New World (Chapare, Guanarito, Junín, Machupo, and Sabia) arenaviruses are responsible for viral hemorrhagic fever, one of the most devastating emergent human diseases, with a fatality rate of 15–30% in untreated cases [48, 49]. For instance, Junín virus (JV) causes Argentine hemorrhagic fever, a severe viral illness endemic to the humid pampas of Argentina, with roughly five million people at risk [50]. Though ribavirin is the only approved anti-viral agent for treating arenaviruses in the USA; however, it exhibits undesirable secondary reactions [32, 51]. Thus, there is exigency to develop efficient therapeutics against arenaviruses.

Melittin has been shown to cripple JV multiplication at non-toxic concentration ranges (0.5–3 μM) in vitro [32]. Surprisingly, 3 μM of melittin was enough to achieve a 99% reduction of JV infectivity (Table 1). Melittin concentration required to decrease virus yield by 50%, known as EC_{50}, was 0.86 μM for JV (Table 2). Based on 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) assay, concentration of melittin needed to lessen cell viability by 50% (CC_{50}) was 8.51 μM. Besides, selectivity index (CC_{50}/EC_{50}) of melittin was 9.89, suggesting that it can serve as a conceivable drug for anti-viral therapy against JV [32].

Flaviviridae

The *Flaviviridae* is a family of arthropod-borne, enveloped viruses with a single-strand RNA of positive polarity, and currently has four genera, namely *Flavivirus*, *Pestivirus*, *Hepacivirus*, and *Pegivirus.* They frequently infect mammals and birds, causing wide range of diseases such as hepatitis, hemorrhagic fever, fatal mucosal disease, and neurological illnesses [52]. Some notable examples of the family are hepatitis C virus, yellow fever virus, West Nile virus, dengue virus, Japanese encephalitis virus, and Zika virus, representing

Table 1 In vitro anti-viral effects of melittin

Family/virus (strain)	Methods	Results	References
Arenaviridae			
Junín virus (IV_{4454})	Virucidal assay and viral yield inhibition	Melittin hampered multiplication of Junín virus in Vero cells infected at a multiplicity of infection (MOI) of 0.1.	[32]
Flaviviridae			
Bovine viral diarrhea virus (NADL)	Treatment of cells with melittin (before and after viral infection)	Melittin was failed to reduce viral particles, though addition of apamin potentiated its anti-viral activity.	[33]
Herpesviridae			
HSV-1 (MP, *syn20*, FFV3, *ts*B5, and *amb* 1511-7)	Phase-contrast microscopy (evaluating cell fusion and plaque morphology), viral yield inhibition, adsorption and penetration assays	Melittin (0.5 μM) impeded HSV-1-induced cell fusion in glycoprotein K mutants, but not glycoprotein B mutants. It was also effective in inhibiting HSV-1 adsorption and penetration.	[34]
HSV-1 M (ATCC VR-539) and HSV-2 G (ATCC VR-734)	Virucidal assay	Melittin completely inactivated HSV-1 M and HSV-2 G.	[35]
HSV-1 (F) and HSV-2 (G)	Virucidal assay and viral yield inhibition	Melittin (0.5–3 μM) inhibited infectivity of both HSV-1 and HSV-2.	[32]
GFP-fused HSV	Viral yield inhibition and analysis of GFP expression	Compared to untreated groups, melittin treatment (2 μg/mL) led to a 16-fold reduction in viral titers and a pronounced decrease in GFP expression in infected cells.	[36]
BoHV-1 (Los Angeles)	Treatment of cells with melittin (before and after viral infection) and virucidal kinetics	Melittin (2 μg/mL) exhibited potent anti-viral effects on BoHV-1. Melittin (25 μg/mL) required 2 and 4 h to completely wipe out BoHV-1 at 37 °C and 22 °C, respectively.	[33]
Orthomyxoviridae			
GFP-fused influenza A (PR8)	Viral yield inhibition, analysis of GFP expression, virus attachment assay, entry assay, and virucidal mechanism	Compared to untreated groups, melittin (2 μg/mL) reduced both viral titers and GFP expression in infected cells (without affecting either virus-cell attachment or virus entrance into cells).	[36]
Picornaviridae			
EV-71	Viral yield inhibition, analysis of GFP expression, and real-time polymerase chain reaction	Melittin reduced EV-71 infectivity and cytopathic effects as well as mRNA expression levels of VP1 (4-fold) compared to untreated groups.	[36]
GFP-fused coxsackievirus (H3)	Viral yield inhibition and analysis of GFP expression	Melittin (2 μg/mL) diminished both GFP expression (1.5-fold) in infected cells and virus titers (5-fold) compared to untreated groups.	[36]
Pneumoviridae			
GFP-fused RSV	Viral yield inhibition and analysis of GFP expression	Melittin (2 μg/mL) markedly reduced not only virus titers but also GFP expression in infected cells compared to untreated groups.	[36]
Rhabdoviridae			
GFP-fused VSV	Viral yield inhibition, analysis of GFP expression, and virucidal kinetics	Melittin (2 μg/mL) rapidly (5–30 min) suppressed VSV infectivity, and caused substantial reduction in both virus titer and GFP expression in infected cells compared to untreated groups.	[36]
VHSV	Immunostaining focus assay	Melittin-loaded liposomes and immunoliposomes inhibited VHSV-infected cell foci formation and reduced the VHSV spread in cell culture.	[37]

Table 1 (continued)

Family/virus (strain)	Methods	Results	References
Retroviridae			
MuLV (ATS-124)	Direct virolysis and electron microscopy	Melittin (50 μg) disintegrated the viral membrane, resulting in complete release of reverse transcriptase after 30 min of incubation at 20 °C.	[38]
RAV-2	Direct virolysis (permeabilization of viral envelope)	Melittin made the viral envelope permeable. Compared to NP-40, melittin caused less damage to viral structure, permitting synthesis of full-length cDNA.	[39]
HIV-1 (SF2)	Direct virolysis (permeabilization of viral envelope)	Melittin (20–100 μg/mL) was exploited to permeabilize HIV-1 envelope. Melittin treatment led to a 30% higher endogenous cDNA yield compared to Triton X-100.	[40]
HIV-1 (IIIB)	Viral yield inhibition, treatment of HIV-1-infected cells with melittin, and western blot analysis	Melittin at 0.5 and 2.5 μg/mL reduced HIV infectivity in supernatants of KE37/1 T lymphoma cells by ≤ 40% and 100%, respectively. Compared to untreated cells, expression of a 31 kDa protein was reduced in melittin-treated cell extracts.	[41]
HIV-1 (IIIB) and HIV-1 (RF)	Treatment of infected cells with melittin, quantitative RT–PCR analysis, assessment of HIV LTR activity, and western blot analysis	Melittin dose-dependently inhibited virus production in T lymphoma or fibroblastoid cells infected with HIV-1. Melittin treatment of T cells diminished levels of Gag antigen, viral mRNA, and HIV LTR activity.	[42]
HIV-1 (NLHX) and HIV-1 (NLYU2)	Virucidal assay (measuring luciferase activity) and HIV-1 capture assay (measuring total amount of viral protein p24 by ELISA)	Both free melittin and melittin-loaded nanoparticles reduced HIV-1 infectivity. Melittin-loaded nanoparticles captured more HIV-1 compared to blank nanoparticles.	[43]
Virgaviridae			
TMV (U1)	Virucidal assay (determining percentage of local lesions on tobacco leaves), bond-shift assay, and circular dichroism measurements	Melittin diminished infectivity of TMV and induced conformational changes in TMV RNA.	[44]

BoHV-1 bovine herpesvirus type 1, *ELISA* enzyme-linked immunosorbent assay, *EV-71* enterovirus 71, *GFP-fused influenza A* green fluorescent protein-fused influenza A (A/PuertoRico/8/34) (H1N1), *HIV-1* human immunodeficiency virus-1, *HSV-1* herpes simplex virus 1, *LTR* long terminal repeat, *MuLV* Rauscher murine leukemia virus, *RAV-2* Rous associated virus-2, *RSV* respiratory syncytial virus, *RT-PCR* quantitative reverse transcriptase-polymerase chain reaction, *TMV* tobacco mosaic virus, *VHSV* fish viral hemorrhagic septicemia rhabdovirus, *VSV* vesicular stomatitis virus

a severe global public health problem with major socioeconomic consequences [53].

Very recently, Picoli et al. investigated anti-viral effects of melittin on bovine viral diarrhea virus (BVDV) [33], the causative agent of bovine viral diarrhea which leads to considerable financial losses in many beef-exporting countries [54]. Melittin had no satisfactory anti-viral activity against BVDV, before and after infection of Madin–Darby bovine kidney cells with the virus (multiplicity of infection; MOI = 0.1). Intriguingly, combinations of melittin with bee venom-derived apamin were superior against BVDV than each agent alone, highlighting that apamin potentiates anti-BVDV efficacy of melittin [33]. Based on these findings, it is sensible to combine melittin with other available anti-viral drugs to ascertain whether the new combinations can abolish *Flavivirus* infectivity.

Herpesviridae

Viruses forming the family *Herpesviridae* contain double-stranded linear DNA encased within an icosapentahedral capsid, which is wrapped in a tegument and a lipid envelope [55]. Among more than hundred known herpes viruses, nine infect humans including herpes simplex virus 1 (HSV-1), HSV-2, varicella zoster virus (VZV), cytomegalovirus (CMV), human herpes virus (HHV)-6A, HHV-6B, HHV-7, Epstein-Barr virus (EBV), and Kaposi's sarcoma-associated herpesvirus (KSHV/HHV-8) [56]. Unquestionably, herpes simplex viruses are one of the most pervasive pathogens among humans, afflicting up to 95% of the adult population worldwide [57, 58]. Clinical manifestations range from benign and generally self-limiting forms including cold sores and genital herpes to the rare but severe and sometimes even life-menacing infections such as herpes encephalitis. Acyclovir (ACV) and

Table 2 Anti-viral activities, cytotoxicity effects, and selectivity indices of melittin

Family/virus (strain)	$EC_{50} \pm SD$	Cells	$CC_{50} \pm SD$	SI	References
Arenaviridae					
Junín virus (IV_{4454})	0.86 μM	Vero	8.51 μM	9.89	[32]
Flaviviridae					
Bovine viral diarrhea virus (NADL)	ND	MDCK	2.32 μg/mL	ND	[33]
Herpesviridae					
HSV-1 (F)	1.35 μM	Vero	8.51 μM	6.30	[32]
HSV-2 (G)	2.05 μM	Vero	8.51 μM	4.15	[32]
GFP-fused HSV	0.94 ± 0.07 μg/mL	Vero	6.23 ± 0.07 μg/mL	6.62	[36]
Orthomyxoviridae					
GFP-fused influenza A (PR8)	1.15 ± 0.09 μg/mL	MDCK	7.66 ± 0.94 μg/mL	6.66	[36]
Picornaviridae					
EV-71	0.76 ± 0.03 μg/mL	HeLa	4.36 ± 0.54 μg/mL	5.73	[36]
GFP-fused coxsakievirus (H3)	0.99 ± 0.09 μg/mL	HeLa	4.36 ± 0.54 μg/mL	4.40	[36]
Pneumoviridae					
GFP-fused RSV	0.35 ± 0.08 μg/mL	HEp2	5.02 ± 0.17 μg/mL	14.34	[36]
Rhabdoviridae					
GFP-fused VSV	1.18 ± 0.09 μg/mL	Vero	6.23 ± 0.07 μg/mL	5.27	[36]
Retroviridae					
HIV-1 (NLHX)	2.4 μM	Vaginal epithelial cells (VK2)	ND	ND	[43]
HIV-1 (NLYU2)	3.6 μM	Vaginal epithelial cells (VK2)	ND	ND	[43]

BoHV-1 bovine herpesvirus type 1, CC_{50} melittin concentration needed to lessen cell viability by 50%, EC_{50} melittin concentration required to decrease virus yield by 50%, *EV-71* enterovirus 71, *GFP-fused influenza A* green fluorescent protein-fused influenza A (A/PuertoRico/8/34) (H1N1), *HIV-1* human immunodeficiency virus-1, *HSV-1* herpes simplex virus 1, *ND* not determined, *MDCK* Madin–Darby canine kidney, *RSV* respiratory syncytial virus, *SD* standard deviation, *SI* selectivity index (CC_{50}/EC_{50})

related nucleoside analogues have been successfully employed in treating HSV infections, but the treatment should be commenced as soon as possible after onset of symptoms. Furthermore, efficiency of the current anti-HSV drugs is generally limited and gives rise to only marginal improvements in lesion healing time or episode duration [59]. For this reason, there is room for more efficacious therapies.

HSV entrance into cells occurs following fusion of viral envelope with host cell membrane. Several glycoproteins are involved in HSV-induced cell fusion [60]. It is worth mentioning that wild-type HSV-1 strains usually induce a limited amount of cell fusion, while certain HSV mutants known as *syn* mutants lead to extensive syncytium formation [61]. Disturbance of trans-membrane ion gradients impedes HSV-1-induced cell fusion [62]. This fact together with perturbation effects of melittin on Na^+, K^+ pump [63] propelled researchers to explore whether melittin influences HSV-1-induced cell fusion [34]. Fusion of Vero cells infected with HSV strains (MP, *syn20*, and FFV3) harboring the *syn*1 mutation in glycoprotein K was inhibited in the presence of melittin (0.5 μM), with no evidence of cytotoxicity toward Vero cells (Table 1). By contrast, melittin (0.5 μM) failed to affect cell fusion induced by HSV strains containing mutations in glycoprotein B (*ts*B5 and *amb* 1511–7). In presence of melittin, binding of ouabain (a specific inhibitor of the Na^+, K^+ ATPase) to the Na^+, K^+ pump of HSV-1-infected Vero cells was drastically diminished. The peptide also reduced HSV-1 yield in Vero cells compared to untreated control. In addition, the authors found that melittin is able to obstruct HSV-1 attachment onto Vero cells in a dose-dependent manner and to hinder HSV-1 penetration into cells [34].

Melittin has been demonstrated to exert marked antiherpetic activity against HSV-1 M and HSV-2 G [35]. However, melittin at concentration of 100 μg/mL displayed 99.9 ± 0.2% cytotoxicity towards ME-180 human cervical carcinoma cells. Similarly, an extensive hemolysis (94.6%) occurred at concentration of 80 μg/mL [35]. It has been also evinced that 3 μM of melittin curbed in vitro infectivity of both HSV-1 and HSV-2 by 80%. Incubation of Vero cell with melittin (> 5 μM) at 37 °C for 24 h resulted in cell rounding and monolayer detachment, as manifested by light microscopy [32]. Selectivity index of melittin was calculated to be 6.30 and 4.15 for HSV-1 and HSV-2, respectively (Table 2). In another major study, Uddin et al. found that melittin directly inhibits Green Fluorescent Protein (GFP)-fused HSV (EC_{50} of

0.94 ± 0.07 μg/mL) [36], which corroborates the findings of the earlier investigations [32, 34, 35]. Compared to untreated viruses, melittin treatment of GFP-HSV minimized not only GFP expression in infected cells but also viral titers (16-fold).

A new investigation [33] revealed the potential anti-viral effects of melittin on bovine herpesvirus type 1 (BoHV-1, Los Angeles strain). Administration of melittin (2 μg/mL) on Madin–Darby bovine kidney cells before and after infection with BoHV-1 (MOI = 0.1) resulted in marked reduction of viral titers. In light of virucidal kinetics, complete obliteration of BoHV-1 was achieved after a 2-h incubation of the virus with 25 μg/mL of melittin at 37 °C [33], implying rapid anti-viral effects of melittin. Given that melittin curtails the infectivity of HSV in several ways, it is imperative to evaluate its anti-viral effectiveness against other members of *Herpesviridae* as well.

Orthomyxoviridae

The family *Orthomyxoviridae* comprises enveloped viruses with negative sense, segmented, single-stranded RNA, and includes seven genera: *Influenzavirus A*, *Influenzavirus B*, *Influenzavirus C*, *Influenzavirus D*, *Isavirus*, *Quaranjavirus*, and *Thogotovirus* [46, 64]. Influenza viruses are the most prominent member of this family [65]. WHO has been estimated that influenza-mediated debilitating respiratory ailments occur in 3 to 5 million people annually, of whom roughly 290,000 to 650,000 succumb to influenza-related illnesses [66]. Influenza A viruses are further subtyped on the basis of two main antigenic determinants named hemagglutinin (HA; H1–H16) and neuraminidase (NA; N1–N9) [67]. High genetic variation rates of influenza viruses due to mutation, reassortment, and/or recombination together with the lack of effective anti-influenza agents underscore the necessity of developing novel anti-viral drugs [68].

Melittin is able to mitigate infectivity of influenza A virus [36]. In this regard, 1.15 ± 0.09 μg/mL of melittin was sufficient for 50% reduction in plaque-forming units (PFUs) of GFP-fused influenza A (H1N1, PR8-GFP). Furthermore, CC_{50} of melittin was 7.66 ± 0.94 μg/mL for Madin–Darby canine kidney cells. Considering both anti-viral and cytotoxic activities, selectivity index of melittin was 6.66 (Table 2), inferring that anti-influenza activity of melittin does not emanate from cytotoxic effect of the peptide [36]. In initial stages of infection, melittin (2 μg/mL) did not interfere with both cell attachment and entry of PR8-GFP strain. When PR8-GFP strain was co-incubated with melittin (for 30 min at 4 °C), waning in viral mass was observed, as evaluated by velocity sedimentation ultracentrifugation and subsequent immunoblotting [36]. At 24 h post-infection, melittin treatment (2 μg/mL, 30 min) of PR8-GFP led to significant reduction in viral titers (5-fold, $P < 0.01$) and GFP expression compared to untreated PR8-GFP. These data suggest direct effect of melittin on PR8-GFP surface, prior to virus-cell attachment. Li et al. postulated that surface charge interactions between a cationic peptide from scorpion venom named Mucroporin-M1 and influenza H5N1 can diminish viral infectivity [68]. Thus, melittin may interact with phospholipid bilayer of viral envelope through electrostatic interactions and destabilize viral particles, eventually leading to virolysis.

Picornaviridae

All of the *Picornaviridae* members have single-stranded positive sense RNA genome with a non-enveloped icosahedral capsid [65]. As one of the largest viral families, it currently has 35 genera including 80 species. These viruses cause a wide variety of maladies involving respiratory and gastrointestinal tracts, central nervous system, heart, liver, skin, and eye [69].

One study [36] demonstrated the anti-viral effects of melittin against enterovirus 71 (EV-71), one of the chief culprits behind the hand, foot, and mouth disease, which can lead to neurological, cardiac, and respiratory complications in young children [70]. Melittin/EV-71-treated cells exhibited lower cytopathic effects (CPEs) and higher cellular viability than those of EV-71-infected cells. Furthermore, mRNA expression levels of capsid protein VP1 in melittin/EV-71-treated cells displayed a 4-fold decrement compared to EV-71-infected cells (Table 1). As evidenced in Table 2, EC_{50} and CC_{50} of melittin for EV-71 and HeLa were 0.76 ± 0.03 and 4.36 ± 0.54 μg/mL, respectively, resulting in selectivity index of 5.73. These observations confirmed the inhibitory effects of melittin on either EV-71 replication or CPE induction, making the peptide an attractive candidate for prophylactic or therapeutic use against enterovirus infections [36].

Uddin et al. [36] also found that melittin suppresses infectivity of GFP-fused coxsackievirus H3 (cardiopathogenic H3 strain of coxsackievirus B3) with EC_{50} of 0.99 ± 0.09 μg/mL. Moreover, CC_{50} of melittin for HEp-2 cells was 4.36 ± 0.54 μg/mL. Selectivity index of melittin was also calculated to be 4.40. Co-incubation of H3-GFP (MOI = 2) with 2 μg/mL of melittin for 30 min at 4 °C and subsequent inoculation of the mixture to HeLa cells resulted in 5-fold ($P < 0.05$) and 1.5-fold ($P < 0.05$) reduction in viral titers and GFP expression, respectively, compared to H3-GFP-infected cells not subjected to melittin treatment. Indeed, these findings imply that melittin has pronounced virucidal activity against coxsackievirus at non-cytotoxic concentrations.

Pneumoviridae

The family *Pneumoviridae* contains enveloped viruses with single-stranded, negative-sense RNA, and has two genera, *Orthopneumovirus* and *Metapneumovirus* [65]. The genus

Metapneumovirus has two species (*Avian metapneumovirus* and *Human metapneumovirus*), while *Orthopneumovirus* contains three species (*Bovine respiratory syncytial virus*, *Human respiratory syncytial virus*, and *Murine pneumonia virus*) [71]. Human respiratory syncytial virus (RSV) is a major etiological agent of respiratory diseases such as pneumonia and bronchiolitis, particularly in children, elderly, and immunocompromized patients [72]. Worldwide, around 33.8 million new cases of RSV-associated acute lower respiratory infection are estimated to occur in children under the ages of 5 years annually, of whom at least 3.4 million required hospitalizations [73, 74]. Despite the magnitude of RSV disease, treatment has been limited to supportive measures, bronchodilators, epinephrine, and ribavirin [75].

Melittin has the ability to extinguish RSV infectivity [36]. Compared to RSV-infected HEp-2 cells without melittin treatment, incubation of GFP-RSV with 2 μg/mL of melittin for 30 min at 4 °C and subsequent inoculation of the mixture to HEp-2 cells (MOI of 1) caused significant decrements in GFP expression ($P < 0.01$) and viral supernatant titers (82-fold, $P < 0.01$) at 24 h post-infection (Table 1). EC_{50} and CC_{50} toward RSV-GFP and HEp-2 cells were 0.35 ± 0.08 and 5.02 ± 0.17 μg/mL, respectively (Table 2). Given that melittin displayed higher level of selectivity toward RSV over HEp-2 cells (selectivity index of 14.34), the peptide can be considered as an auspicious agent for anti-RSV therapy.

Retroviridae

Retroviruses are enveloped viruses with two copies of positive-sense RNA which use their own reverse transcriptase (RT) to generate DNA from its RNA genome [65]. Viruses belonging to *Retroviridae* are responsible for economically devastating diseases ranging from malignancies to immune deficiencies and neurologic disorders. HIV, which is historically related to the AIDS pandemic, is categorized under the genus *Lentivirus* within the family of *Retroviridae*. Thus far, six therapeutic classes of antiretroviral drugs are available for the management of HIV infection including entry or fusion inhibitors, nucleoside/nucleotide analogue reverse-transcriptase inhibitors (NRTIs/NtRIs), non-nucleoside reverse transcriptase inhibitors (NNRTIs), integrase inhibitors, and protease inhibitors [76]. Although anti-retroviral combination therapy enhances life expectancy substantially, there is still no cure for AIDS. In fact, all HIV cure approaches are generally in their infancy [65].

There are several lines of evidence concerning antiretroviral activities of melittin against different retroviruses (Table 1). The first investigation on anti-viral efficacy of melittin dates back to the late 1970s, when Esser et al. appraised direct virolytic effect of melittin toward Rauscher murine leukemia virus (MuLV). The authors demonstrated that 50 μg of melittin is enough to "peel off" the viral envelope [38]. As an alternative to non-ionic detergent NP-40, melittin can permeabilize avian retrovirus envelope for cDNA synthesis [39], confirming an earlier finding reported by Esser et al. [38]. Permeabilization of HIV-1 envelope for synthesis of cDNA is further exemplified in a study conducted by Yong et al. [40].

Melittin can also minimize production of HIV-1 in persistently HIV-1-infected KE37/1 T lymphoma cells [41]. In this context, complete reduction of viral particles in supernatants of HIV-1-infected cells was observed after applying of melittin at a non-cytotoxic concentration of 2.5 μg/mL. Western blot analysis demonstrated the reduction of a 31 kDa protein in melittin-treated cell extracts [41]. This protein could relate to some fragments of processed Gag/Pol precursor polyprotein or p31 integrase. Furthermore, data retrieved from C-terminal and truncated derivatives of melittin suggest that both amphipathic alpha-helical part (residues 1–20) and cationic amino acid residues in the C-terminal end of melittin are accounted for its anti-viral properties against HIV-1, resulting in intracellular impairment of viral protein production rather than a direct disruption of viral envelope [41].

Another survey proved the anti-HIV effectiveness of melittin at non-cytotoxic concentrations [42]. In this respect, melittin attenuated HIV-1 production in HUT78-RF (chronically HIV-1-infected T cells), HUT78 (acutely HIV-1-infected T cells), and LC5-CD4 (acutely HIV-1-infected fibroblasts) in a dose-dependent manner. In the case of melittin-treated cells, metabolic activity at the 50% infectious dose (ID_{50}) was higher than 85% of control cultures. Furthermore, western blot analysis indicated that levels of Gag antigen declined in KE37/1 (acutely HIV-1-infected T lymphoma cells) lysates following 9 days treatment with melittin (1.05 and 1.4 μM) compared to controls [42]. Quantitative reverse transcriptase-polymerase chain reaction (RT-PCR) results also demonstrated that melittin does not suppress expression of porphobilinogen deaminase, a cellular housekeeping gene. Interestingly, melittin suppresses HIV long terminal repeat (LTR) activity in a Tat-independent manner, indicating that melittin interferes with host cell-directed viral gene expression [42]. All the cumulative evidence indicates that dose-dependent anti-HIV effect of melittin is mediated by suppressing HIV transcription and decreasing overall levels of viral gene products rather than the lysis of cellular or viral membranes.

Hood et al. reported the first proof-of-concept investigation concerning inhibition of HIV-1 infectivity by melittin-loaded nanocarriers [43]. In order to quantify HIV-1 infectivity, the authors applied TZM-bl cell line, which is HeLa-derived cells capable of expressing CD4, CCR5, and CXCR4. The cell line also harbors luciferase reporter gene under the control of an HIV-1 promoter [77]. After incubation of 50 ng HIV-1 NLHX (CXCR4 tropic) or HIV-1

NLYU2 (CCR5 tropic) strains with soluble CD4 (served as a positive control), nanoparticles, and free melittin at 37 °C, treated viruses were exploited to infect TZM-bl reporter cells for 48 h at 37 °C. This is followed by lysing the cells and gauging luciferase activity (as a measurement of HIV-1 infectivity). Remarkably, free melittin at concentrations greater than 6 μM was able to extirpate infectivity of both NLHX and NLYU2 strains. Although 2 μM of free melittin did not influenced viability of TZM-bl reporter cells, concentrations above 2 μM rapidly diminished cellular viability, indicating a narrow therapeutic range of melittin. Contrary to free melittin, melittin-loaded nanoparticles had no toxicity toward vaginal keratinocytes in vitro [43]. Besides, 50% inhibitory concentration of melittin-loaded nanoparticles were 2.4 and 3.6 μM against NLHX and NLYU2 strains, respectively, with no adverse effects on reporter cell viability. Lipid-to-lipid membrane hemifusion events may facilitate melittin transportation from nanoparticle lipid monolayers to HIV-1 envelope bilayers, subsequently resulting in melittin aggregation, pore formation, and deactivation of viral packaging [43]. Simplicity of nanoparticle production, lack of melittin nanoparticles toxicity against vaginal keratinocytes, and their potential in reducing HIV-1 infectivity are striking properties of this approach for intra-vaginal prevention of HIV transmission.

Rhabdoviridae

Members of *Rhabdoviridae* have characteristic bullet-shaped or bacilliform membrane-enveloped particles with single-stranded, negative-sense RNA. Viruses belonging to *Rhabdoviridae* afflict an extremely broad range of hosts including plants, fish, mammals, reptiles, and even invertebrates [65]. Vesicular stomatitis virus (VSV) is an arthropod-borne *Rhabdovirus* that cause vesicular disease in cattle, horses, and swine, leading to negative economic impacts on animal husbandry [78, 79].

One study was performed in an attempt to appraise antiviral effects of melittin on VSV in vitro [36]. In this respect, melittin (0.5–10 μg/mL) was co-incubated with VSV-GFP for 30 min at 4 °C, after which viral suspensions with MOI of 0.2 were inoculated to Vero cells. Melittin displayed EC_{50} value of 1.18 ± 0.09 μg/mL against VSV-GFP, while it showed CC_{50} of 6.23 ± 0.07 μg/mL toward Vero cells, resulting in selectivity index of 5.27 [36]. Incubation of melittin (2 μg/mL) with VSV-GFP at 4 °C for 30 min and subsequent inoculation to Vero cells caused a discernible depletion of GFP expression at 24 h post-infection, while high levels of GFP expression was observed in virus-infected groups without melittin treatment, as disclosed through fluorescence microscopy. In comparison to virus-infected groups, a pronounced decline in viral titer of VSV-GFP (1598-fold, $P < 0.01$) was observed following a 30 min of exposure to melittin. Moreover, VSV-GFP infectivity to HEK293T cells began to reduce after a 5-min incubation with melittin (2 μg/mL) and continued to wane during 10, 20, and 30 min, indicating rapid virucidal kinetics of melittin [36].

Delivery of biochemical compounds by immunoliposomes encompassing complete or fragmented antibodies represents an optimistic strategy for coping with cancers and viral infections [80]. In an effort to construct and to evaluate antimicrobial peptide (AMP)-loaded immunoliposome system, Falco et al. incorporated melittin into immunoliposomes containing antibodies against glycoprotein G of fish viral hemorrhagic septicemia rhabdovirus (VHSV), a rhabdovirus infecting cold-blooded aquatic creatures [37]. At concentrations equivalent to 25 and 50 μM, both melittin-loaded liposomes and immunoliposomes were capable of inhibiting VHSV-infected cell foci formation in a dose- and time-dependent manner. For instance, inhibition rates of VHSV infectivity were 89.9% and 95.2% in the presence of melittin-loaded liposomes (50 μM) and immunoliposomes (50 μM), respectively. Both melittin-loaded liposomes and immunoliposomes interdicted the infectivity of VHSV after virus adsorption to fish cell line *epithelioma papulosum cyprini* (EPC) at time point 0 and 4 h post-infection [37]. In addition, EPC cell monolayers exhibited > 80% viability after a 24-h exposure to melittin-loaded liposomes (25 μM) and immunoliposomes (50 μM) at 14 °C. These findings suggest that AMP-loaded immunoliposomes might have an enormous potential to prevent or treat viral infections as the configuration of their constituents (i.e., AMP type, antibody fragments, and/or phospholipid composition) can be optimized.

Virgaviridae

Virgaviridae is a family of plant-associated viruses with rod-shaped virions and single-stranded, positive-sense RNA genome [81]. As a typical member of *Virgaviridae*, tobacco mosaic virus (TMV) had a long and illustrious history since the late nineteenth century. The virus invades a wide spectrum of plants, in particular genera belonging to *Solanaceae* [82].

Amino acid sequences of melittin and coat protein of tobacco mosaic virus (TMV) at positions 71–94, which are known to be pivotal for protein-RNA and protein-protein interactions, exhibit partial resemblance. Based on this similarity, an investigation was conducted by Marcos et al. to decipher whether melittin abrogates TMV infectivity and interacts with the viral particles and their RNA genomes [44]. Addition of melittin (5 μM) into a solution containing TMV led to reduction (10%) in number of necrotic local lesions on tobacco leaves compared to non-treated samples. As inferred from far-ultraviolet circular dichroism (CD) spectroscopy, melittin adopted a random coil and alpha-helical conformations in

the absence and presence of TMV RNA, respectively. When combined with 5 μM of melittin, TMV RNA showed not only a significant enhancement in electrophoretic mobility but also shifts in CD spectrum, suggesting RNA conformational changes are induced by melittin [44]. In general, these findings open up a range of new applications for melittin in the field of plant and agricultural virology.

In vivo studies

Apart from in vitro investigations, some empirical evidences exist with regard to anti-viral efficiency of melittin in animal models. For instance, co-incubation of melittin (100 ng) with $5MLD_{50}$ (dose lethal to 50% of mice) of influenza A virus subtype H1N1 for 30 min and subsequent intranasal administration of the mixture resulted in 100% survivability of six-week-old C57BL/6 female mice up to 8 days post-infection (dpi), whereas all phosphate-buffered saline (PBS)/H1N1-treated mice displayed several respiratory disease symptoms and perished at 8 dpi. Unlike PBS/H1N1-treated mice, melittin/H1N1-treated mice were protected from body weight loss. Since melittin/H1N1-treated mice exhibited considerably lower lung viral titer in comparison to PBS/H1N1 treated mice at 5 dpi, melittin rescued them from lethal infections of influenza A [36].

The effectiveness of melittin for the treatment of influenza-infected chicken embryos has been exemplified in a report by Michálek et al. [83]. In this regard, influenza A virus subtype H7N7 was inoculated into embryo's allantois of 9-day-old specific pathogen-free (SPF) embryonated chicken eggs and incubated for 24 h, after which different concentrations of melittin was injected into allantoic fluid. Chicken embryos received only influenza A virus showed survival rates of 40%, implicating high pathogenicity of the virus against embryos. By contrast, influenza-infected embryos which were inoculated with melittin (0.05, 0.5, and 1 μM) exhibited 80% viability. However, higher concentrations of melittin (2 and 4 μM) were toxic for influenza-infected embryos, resulting in survival rates of 40%. These experimental data suggest that melittin is well tolerated by chicken embryos for up to 1 μM [83]. On the whole, melittin holds promise for a new avenue of anti-influenza therapy, from medicine to husbandry.

A prospective, placebo-controlled double-blinded trial was conducted to evaluate the effects of subcutaneously administrated melittin (500 μg per kg body weight) on the general health status of feline immunodeficiency virus (FIV)-infected cats and the severity of clinical symptoms during a 6-week treatment period [84]. In contrast to the placebo group receiving PBS, treatment with melittin led to a constant improvement in cats' general health status, expressed as Karnofsky's score. Statistically, a significant difference ($P = 0.015$) in improvement of conjunctivitis was observed between melittin-treated and placebo-treated cats. Although both groups exhibited amelioration of stomatitis, however, this was not significant. Moreover, no adverse effects including hemolysis and irritation at the injection site in FIV-infected cats were noted [84]. In the case of laboratory parameters (e.g., packed cell volume, hemoglobin, and white blood cells), there were no statistically significant differences between both groups. As for immunologic parameters including $CD4^+$ lymphocytes, $CD8^+$ lymphocytes, and CD4/CD8 ratio, no significant differences between both groups were evident. Similar results were also observed with regard to surrogate parameters (biopterin and 7-xanthopterin in serum and urine) in both groups. Authors stated that lack of significant changes could be attributable to various reasons including inability of melittin to yield strong anti-viral activity in vivo, administration of low dosage of melittin, long treatment intervals, short length of treatment period, and development of antibodies against melittin [84]. Overall, assessment of changes in FIV load together with increasing the total number of cats should be considered for future investigations to provide more trustworthy statistical findings.

Plausible anti-viral mechanisms

A better understanding of anti-microbial mechanisms of melittin will definitely help us to optimize anti-viral strategies. Many AMPs act primarily through membrane disruption [85]. In this context, direct interaction of melittin with viral envelopes or capsid proteins interferes with binding or uptake of viruses by cells [34, 42, 43]. Besides, other plausible anti-viral mechanisms of action such as impediment of viral multiplication [36], decreasing expression levels of viral mRNAs [36, 42], inducing conformational changes in viral genome [44], deactivation of viral packaging [43], attenuation of viral cytopathic effects [36], and inhibition of viral-induced cell fusion [34] have been documented in the literature, as depicted in Fig. 1.

Future prospects

As hinted above, melittin exerts broad spectrum of anti-viral activities, albeit being relatively cytotoxic at higher doses. Multiple approaches can be propounded to diminish cytotoxicity of melittin while augmenting its anti-viral effects, thereby heightening therapeutic indices of the peptide. In this regard, targeted in vivo delivery of AMPs like melittin through a

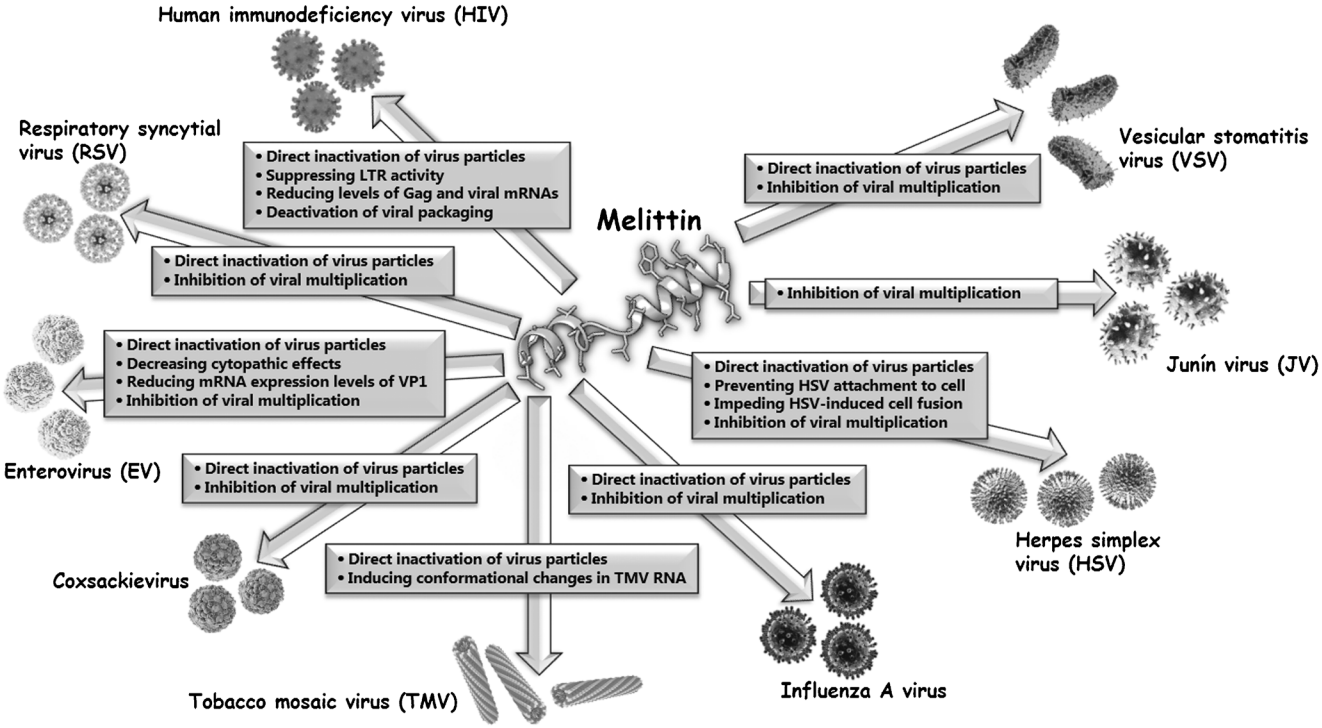

Fig. 1 Possible inhibitory mechanisms of melittin toward different viruses

nanocarrier exemplifies a safe solution with desirable pharmacokinetics for both anti-viral and anti-cancer therapies [86, 87]. An alternate novel enticing strategy is conjugation of melittin with aptamers, which are oligonucleotide or peptide molecules capable of binding to their targets with high affinity and specificity [88]. Designing hydrogels embedded with melittin for topical treatment of herpes blisters and papilloma virus-related warts is the other practicable approach which has not been reported hitherto. Last but not least, combination of melittin and current anti-viral drugs may reduce both concerns associated with cytotoxicity of melittin and probability of developing drug-resistant viruses.

Conclusions

Several decades of endeavor have allowed researchers to partially disclose anti-viral effects of melittin against both RNA and DNA viruses that fall within diverse viral families. However, tangible challenges such as medication safety lie ahead in the path toward clinical application of melittin as an anti-viral drug. As a consequence, future investigations may need to focus on deciphering mechanisms behind the anti-viral activity of melittin, examining various routes of administrations, and scrutinizing the effectiveness of melittin in primate models to retrieve additional pre-clinical data. Undoubtedly, anti-infective properties of melittin will provide new avenues in all fields of clinical researches, particularly medical virology.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflicts of interest.

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