Hindawi
Journal of Chemistry
Volume 2017, Article ID 4035626, 7 pages
https://doi.org/10.1155/2017/4035626

Review Article

Melittin, a Potential Natural Toxin of Crude Bee Venom: Probable Future Arsenal in the Treatment of Diabetes Mellitus

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Received 29 March 2017; Revised 8 June 2017; Accepted 14 June 2017; Published 12 July 2017

Academic Editor: Sevgi Kolaylı

Since diabetes mellitus (DM) is one of the most common and serious endocrine metabolic disorders, it is important to elucidate novel antidiabetic therapeutic agents from various sources, including natural products. Bee venom (BV) is a complex mixture of proteins, peptides, and low molecular components, and melittin is the main constituent. Melittin is a peptide consisting of 26 amino acids with the sequence GIGAVLKVLTTGLPALISWIKRKRQQ. It has several important biological effects and has a relatively low toxicity. Recent studies using animal models have confirmed that melittin has significant glucose and lipid lowering activities by acting on several mechanistic pathways. The main antidiabetic activity of melittin is increasing insulin secretion via depolarization of pancreatic β-cells. Other possible mechanisms may involve stimulation of phospholipase A_2, increase of glucose uptake, improving lipid profile, and/or reduction of inflammation. This review summarizes the various sources, proteomics, biological roles, adverse effects, and medical applications of melittin and its mechanism of action in combating DM.

1. Introduction

Diabetes mellitus (DM) is a serious disease in which the body cannot control the amount of sugar in circulation due to either a deficiency of insulin secretion or a decreased sensitivity of the tissues to insulin. There are two main types of diabetes as follows: Type 1 and Type 2 [1]. Both types can cause serious health complications, including kidney failure, heart disease, blurred vision, ketoacidosis, peripheral neuropathy, itchiness, fatigue, and even coma [2]. An insulin deficiency leads to elevations of cholesterol, phospholipids, and free fatty acids [3]. Therefore, it is important that an ideal DM therapy should not only involve maintaining blood glucose levels but also involve the regulation of the lipid profile.

To treat DM, several antidiabetic drugs are used. However, these drugs are not without side effects and pose an economic burden to the patient. Therefore, scientists have turned to natural remedies, including honey and bee products, such as bee venom (BV). BV is a complex mixture of proteins, peptides, and low molecular components secreted by the worker and the queen bees. The main active constituent of BV (apitoxin) is melittin, which has a relatively low toxicity [4]. It exerts important effects on cells, such as hemolysis, membrane depolarization and muscle contraction, cytotoxicity, and phospholipase C and arachidonic acid following phospholipase A2 activation. In addition, it is also responsible for various allergic reactions in the body and can suppress the signal pathways of toll-like receptors (TLR2, TLR4), cluster of differentiation (CD14), nuclear factor kappa-B essential modulator (NEMO), and platelet-derived growth factor beta (PDGFRβ). By suppressing these pathways, melittin diminishes the activation of p38, ERK1/2, AKT, and PLCγ1 and the translocation of NF-κB into the nucleus, consequently leading to reduced inflammation in the skin, aorta, joints, liver, and neuronal tissues [5].

BV proteins, mainly melittin, are also widely used in the treatment of arthritis, frozen shoulder, diseases of the central and peripheral nervous system (CNS, PNS), skin diseases, heart and blood system related diseases, cancer, ulcer, colitis,

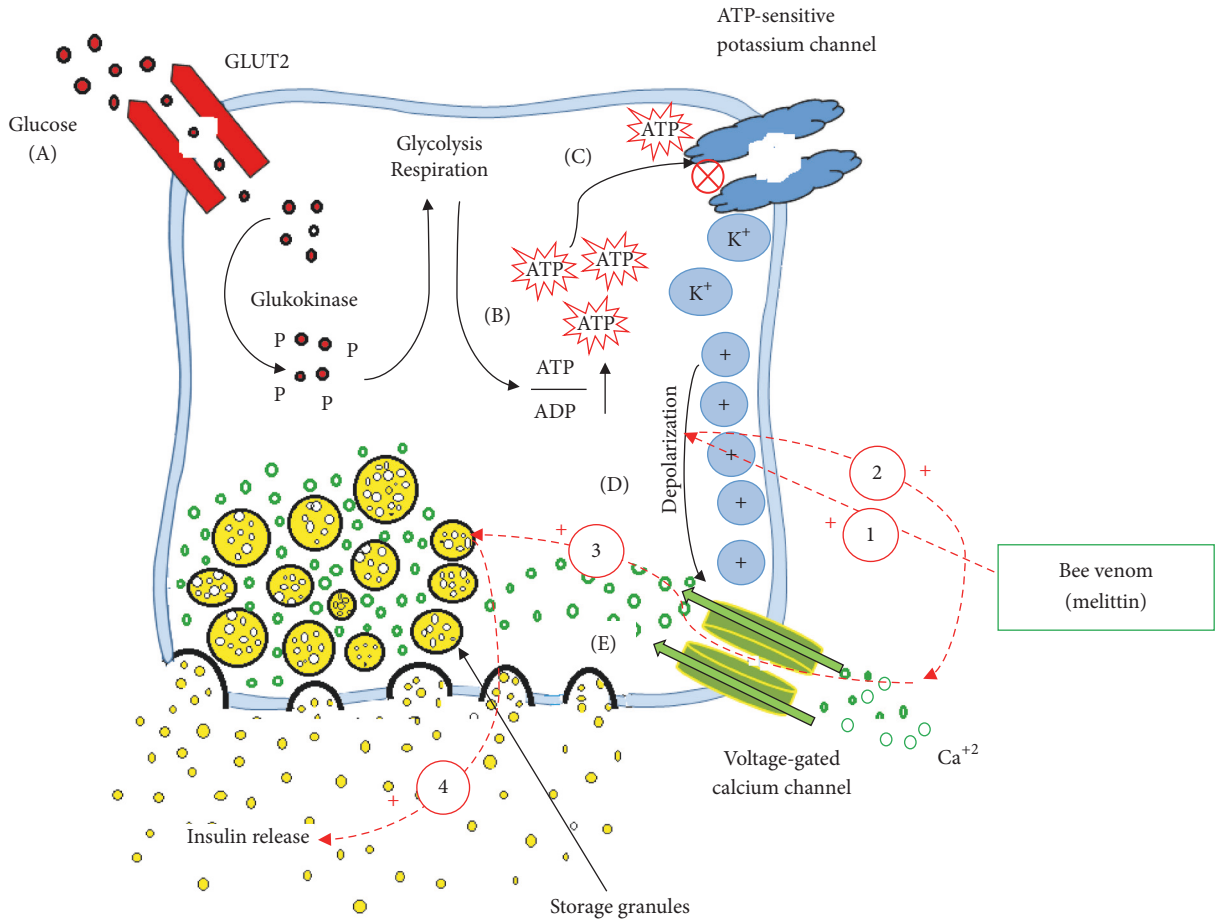

FIGURE 1: Basic mechanism of insulin secretion and sequential mechanistic pathway of BV (melittin) leading to an increase in insulin secretion in pancreatic β-cells. (A) Blood glucose (>5 mM in concentration) enters the β-cell through the glucose transporter (GLUT2). (B) ATP production rapidly occurs as a result of glycolysis and respiration. (C) Then, ATP-sensitive K^+ channels are closed, and K^+ efflux is inhibited. (D) Consequently, plasma membrane depolarizes, and (E) voltage-gated Ca^{2+} channels open. Finally, Ca^{2+} influx increases, which triggers exocytosis of insulin granules. ① Melittin directly depolarizes the cell membrane, ② it increases Ca^{2+} inflow into the cell, ③ it promotes more granule secretion, and ④ it increases insulin secretion into the blood stream. Note: phosphorus: P; calcium: Ca^{2+}. Adapted from https://www.dolcera.com/wiki/index.php?title=Diabetes_products_and_services.

and neuritis [14, 15, 17, 19, 24–28]. Recent studies also confirmed that melittin can significantly reduce blood glucose via insulin secretion and glucose uptake in animal models [21–23]. These studies also reported that melittin has significant lipid regulating activities by activating phospholipase A_2. Thus, melittin could be a potential therapeutic agent against DM.

A number of reviews are available in the literature, which focused on the miscellaneous pharmacological effects of BV proteins [17, 29–37]. However, to date, reviews of the mechanisms by which BV contributes to the antidiabetic activity are currently not available. In this review, we discussed the principal activity of melittin (Table 1) and its antidiabetic mechanism of actions (Figure 1).

2. Composition of Honeybee Venom

BV is a bitter colorless liquid (pH 4.5–5.5) that dries up easily even at room temperature and is soluble in water but insoluble in alcohol and ammonium sulfate [5, 38, 39]. A bee can inject up to 0.1 mg venom via its stinger. It has been reported to be similar to the toxin of the sea nettle [40]. The venom is mainly produced in the abdomen of the worker bees and originates from a mixture of acidic and basic secretions. It is a complex mixture of proteins, in which some enzymes (catalyzing specific reactions), some peptides (which consist of two or more amino acids), and others, including a variety of low molecular components, such as carbohydrates (2% of venom dry weight), phospholipids (5% of venom dry weight), amino acids (1% of venom dry weight), minerals (3-4% of venom dry weight), and volatile compounds (5–8% of venom dry weight) [15, 41], are present. Among these, the main component comprises proteins and peptides, the combination of which is commonly known as venom toxins.

3. Melittin

In a previous study, Danneels et al. determined the constituents of 34 samples of venom toxins by using mass spectrometry [42]. Among all of the honey proteins, the

Table 1: Principal honeybee protein (melittin).

Bee venom protein name	Melittin
Alternative names	Allergen Api m 3, Allergen Api m III, Allergen Api m 4
Percentage of dry weight (%)	40–50
Sources	
Honeybee	*Apis mellifera* (honeybee), *Apis mellifera carnica* (Carniolan honeybee), *Apis dorsata* (giant honeybee), *Apis florea* (dwarf honeybee), *Apis cerana* (Indian honeybee)
Bee	*Osmia rufa* (red mason bee)
Insect	*Vespa magnifica* (hornet), *Polistes hebraeus* (paper wasp), *Polistes* sp. (golden paper wasp), *Vespula maculifrons* (eastern yellow jacket), *Vespa velutina nigrithorax* (hornet)
Bacteria	*Salmonella typhi, Pseudomonas fluorescens, Synechocystis* sp., *Aphanocapsa* sp., *Escherichia coli, Stenotrophomonas maltophilia, Pseudomonas maltophilia, Xanthomonas maltophilia, Moellerella wisconsensis, Pseudomonas batumici, Ralstonia solanacearum, Thioalkalivibrio nitratireducens, Klebsiella pneumonia, Francisella* sp., *Brucella melitensis biotype 1, Ralstonia solanacearum, Bacteroides xylanisolvens, Acinetobacter baumannii, Acinetobacter bereziniae, Sporomusa* sp., *Zunongwangia profunda, Leeuwenhoekiella blandensis, Cyclobacterium qasimii M12-11b*
Others	*Rana arvalis* (moor frog), *Rana temporaria* (European common frog), *Rana tagoi* (Tago frog), *Rana tagoi okiensis* (Oki brown frog), *Rana sakuraii* (Japanese brown frog), *Naja oxiana* (Central Asian Cobra) (Oxus cobra), *Pardachirus pavoninus* (peacock sole)
Tissue specificity	Expressed by the venom gland
Gene name	MELT
Formula	$C_{350}H_{552}N_{84}0_{99}S_2$
Total number of atoms	1087
Molecular weight (Dalton)	7584.8
Total length (amino acids)	70
Peptide length (amino acids)	26
Total sequence	10: MKFLVNVALV, 20: FMVVYISYIY, 30: AAPEPEPAPE, 40: PEAEADAEAD, 50: PEAGIGAVLK, 60: VLTTGLPALI, 70: SWIKRKRQQG
Peptide sequence	44–69: GIGAVLKVLTTGLPALISWIKRKRQQ
Theoretical isoelectric point (pI)	4.69
Subunit	Monomer and homotetramer
Total number of negatively charged residues (Asp + Glu)	9
Total number of positively charged residues (Arg + Lys)	6
Extinction coefficient ($M^{-1}cm^{-1}$)	9970, at 280 nm measured in water
Estimated half-life	(i) 30 hours (mammalian reticulocytes, in vitro) (ii) >20 hours (yeast, in vivo) (iii) >10 hours (*Escherichia coli*, in vivo)
Grand average of hydropathicity (GRAVY)	0.239
Toxic dose	LC (50) is 2.7 μg/mL against killifish
Investigated biological functions	
Major	(i) Hemolytic activity (ii) Anti-inflammatory activity (iii) Anticancer, antibacterial, antifungal, antiviral activities

Table 1: Continued.

Others	(i) Inhibits well-known transport pumps (such as Na^+-K^+-ATPase, H^+-K^+-ATPase) (ii) Activates phospholipase A_2 (iii) Diminishes membrane surface tension (iv) Stimulates smooth muscle (v) Lowers blood coagulation (vi) Influences central nervous system (CNS) (vii) Increases capillary permeability
Adverse effects	(i) Initiates various allergic reactions (ii) Lyses erythrocytes (iii) Creates cytotoxicity in human peripheral blood lymphocytes (iv) Modulates gene expression related to apoptosis, DNA damage response, and oxidative stress
Medical applications	(i) Arthritis (ii) Cancer (iii) Diseases of central and peripheral nervous systems (iv) Skin diseases (v) Heart and blood system related diseases (vi) Frozen shoulder (vii) Asthma, bronchitis (viii) Colitis, ulcers (xi) Others: ophthalmology, endocrinology, urology, gynecology, otorhinolaryngology
Data sources and references	(i) Research Collaboratory for Structural Bioinformatics, Protein Data Bank (RCSB PDB) (ii) Protein Information Resource (PIR) (iii) UniprotKB (iv) [5–19]

Note. Lethal concentration: LC, three-dimensional: 3D, and nuclear magnetic resonance: NMR.

principal protein is confirmed to be melittin, which constitutes approximately 40–50% of the venom dry weight [15]. Melittin consists mainly of a 26-amino-acid peptide with no disulfide bridge in which the N-terminal part of the molecule is predominantly hydrophobic while the C-terminal is hydrophilic and is strongly basic [PMID: 10692322]. The characteristics of melittin, including the sources, proteomics, biological functions, adverse effects, and medical applications, are summarized in Table 1.

4. Previous Breakthroughs of Bee Venom's Role in Diabetes

Several epidemiological studies using animal models have shown that the use of BV is associated with a lower DM risk (Table 2). Kim et al. investigated whether BV can prevent insulitis and the development of diabetes in nonobese diabetic (NOD) female mice that have been subdivided into control ($n = 24$) and BV ($n = 24$) treated groups [20]. BV (purchased from Sigma, MO, USA) was injected at 0.5 mg/kg into the hind limb twice per week when the mice were between 4 and 10 weeks of age. The cumulative incidences of diabetes in the control and BV treated NOD mice were 58% and 21%, respectively, at 25 weeks of age. Therefore, it was postulated that BV treatment in NOD mice inhibits insulitis and the onset and overall incidence of diabetes at an early age.

Another in vivo study conducted by Mousavi et al. reported that Iranian BV (*Apis mellifera*) can lower blood glucose and lipid levels in diabetic rats [21]. Adult male rats ($n = 18$) weighing 200 ± 20 g were placed into the following three random groups: control rats, alloxan monohydrated-induced diabetic rats (150 mg/kg intraperitoneally), and BV treated rats (0.5 mg/kg). In their experiment, BV was collected from beehives using an electric shocker.

In addition to Apis *mellifera* BV, BV from *Apis cerana* has also been reported to have antidiabetic activity as reported by Prakash and Bhargava [22]. In their study, the samples were divided into test and control groups in which the test group was categorized as "before" and "after" being stung by *Apis cerana* bees. Their study also reported that *Apis cerana* BV reduced blood cholesterol and triglyceride levels. Then, Khulan AM and Chimedragcha [23] investigated the effects of Mongolian BV on hyperglycemia and hyperlipidemia in alloxan-induced diabetic rabbits [23]. For this study, chinchilla rabbits ($n = 22$) were divided into the following three groups: control ($n = 6$), diabetic ($n = 8$), and BV treated ($n = 8$) groups. To induce diabetes, an alloxan monohydrated solution (5%) was administered at 100 mg/kg intravenously via the marginal vein behind the ear over two minutes. Meanwhile, the BV treated group received a bee sting on their hind paw every other day following the confirmation of diabetes. Their results also supported that BV significantly lowers serum glucose levels and improves the lipid profile. All of the researchers confirmed that the principal protein in BV, melittin, is mainly responsible for the antidiabetic actions, which was also confirmed by an in vitro test that indicated

Table 2: In vivo studies of DM based on BV.

Venom source	Animal model	Duration	Dose	Outcomes	Year, reference
BV from Sigma, MO, USA	Mice	25 weeks	0.5 mg/kg	(i) Inhibited insulitis (ii) Inhibited onset and cumulative incidence of diabetes	1999 [20]
Iranian Honey BV *(Apis mellifera)*	Lewis rats	4 months	0.5 mg/kg	(i) Increased serum insulin level (ii) Decreased serum triglyceride contents (iii) Decreased serum total cholesterol	2012 [21]
Apis cerana BV	Human	≈30 minutes	*Apis cerana* bees ($n = 8$) sting the groups individually	(i) Decreased blood sugar (ii) Decreased blood cholesterol (iii) Decreased blood triglyceride contents (iv) Increased eosinophil count	2014 [22]
Mongolian BV *(Apis mellifera)*	Rabbits	14 days	A bee sting (contains 0.2–0.5 mL of BV)	(i) Decreased blood glucose levels (ii) Decreased blood cholesterol levels (iii) Decreased low density lipoproteins (iv) Increased high density lipoproteins	2015 [23]

that melittin can increase insulin secretion from pancreatic β-cells.

5. Antidiabetic Mechanism of Actions of Melittin

DM is a syndrome of impaired carbohydrate, fat, and protein metabolisms due to a combination of both hereditary and environmental causes and is characterized by increased blood glucose levels. In DM therapy, great attention is paid to lowering blood glucose levels by increasing insulin secretion and lipid regulating mechanisms of various medical agents, including those from animal toxins. Melittin reduces blood glucose levels by increasing insulin secretion from the β-cells of the pancreas and facilitating glucose uptake [43]. It also alleviates complications of DM by ameliorating lipid profiles as verified by several studies [21–23].

Recent studies reported that various types of BV significantly reduce not only blood glucose levels but also total cholesterol (Table 2). Melittin, which is present in BV, is mainly responsible for the antidiabetic role as confirmed by an in vitro test conducted by Morgan and Montague [43]. Melittin reduces blood glucose levels by several different mechanisms, including depolarization of β-cell membranes, increasing the extracellular calcium and calcium channels [21, 44], activating cytosolic phospholipase A2 [23, 45], increasing glucose transporter lipid uptake into adipose tissues [23], and suppression of β-cell inflammation [46].

Melittin initiates membrane depolarization to cause closure of the ATP-sensitive K^+ channels. Thus, voltage-gated Ca^{2+} channels are opened to promote increased insulin secretion into the blood stream [43] (Figure 1). Melittin also promotes insulin secretion by activating phospholipase A_2 [44]. The activated phospholipase A_2 promotes arachidonic acid production, which, in turn, acts as a calcium transporter into the β-cells to stimulate insulin secretion [47].

It is also reported that phospholipase A_2 has an alternative antidiabetic mode of action by increasing glucose uptake into the adipose tissues rather than increasing insulin secretion. Due to its enzymatic action, it can partially break down the cell membrane of plasmatic lipoproteins [48]. This activity leads to increased glucose transport and lipid uptake into the adipose tissues through partial lyses of adipocyte membranes and binding of a higher number of insulin molecules [49]. Meanwhile, phospholipase A_2 also has a higher affinity for the plasmatic lipoproteins, which leads to cytotoxic effects and generation of free fatty acids and lysophospholipids. Thus, circulating cholesterol in HDL is esterified and significantly regulates lipid profiles [50].

Another in vivo study conducted by Kim et al. reported that BV can prevent insulitis and the development of diabetes in NOD mice due to its immune-modulating activities, often attributed to an autoimmune process that damages the pancreatic cells [20]. Their investigations also confirmed that BV injections did not yield any side effects during the experimental period, indicating its safety.

6. Future Directions

Since melittin is a small peptide of 26 amino acids, it is often administered subcutaneously, which can lead to several adverse reactions, including local erythema, swelling, tenderness, itching, edema, malaise, flu-like symptoms, and urticaria [15]. Although melittin significantly reduces glucose and cholesterol levels in the bloodstream, its toxicity must be reduced before it can be used as an antidiabetic agent.

Computational bioinformatics and recombinant technology may be used to produce various modified melittin with high therapeutic effects and minimal toxicity. Further investigation should be carried out to (1) improve the procedure of BV collections without killing the bees, (2) develop melittin extraction methods to allow high yields of BV, and (3) improve the preservation methods of BV to maintain its efficacy.

7. Conclusion

This review article was prepared as a preliminary resource to gather information on the potential of BV, specifically its major active constituent melittin against diabetes mellitus. In diabetes treatment, great consideration is given to lowering blood glucose with various in vitro studies showing that bee venom significantly reduces blood glucose levels via several mechanisms. Nevertheless, there is still lack of adequate in vivo studies done especially using pure melittin from BV. Finally, due to the potential of melittin for reducing blood glucose levels and total cholesterol by several different mechanisms, it is concluded that melittin can have a great impact in the near future in the treatment of diabetes mellitus.

Conflicts of Interest

The authors confirm that the content of this article has no conflicts of interest.

Authors' Contributions

Md. Sakib Hossen conceived the ideas for this manuscript and also wrote the manuscript. Dr. Md. Ibrahim Khalil and Dr. Siew Hua Gan reviewed and approved this manuscript.

Acknowledgments

The authors acknowledge the National Science and Technology (NST) Fellowship (2014/2015) for providing financial support to Mr. Md. Sakib Hossen to pursue his M.S.

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