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Review

Bee venom: A medical perspective

Arı zehri: Tıbbi bakış

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Abstract

Apitherapy is a complementary medical technique that has an old history and is applied in various diseases worldwide. Apitherapeutical applications are not treatment methods by themself, but they can be substantial parts of multidisciplinary approaches. One of them, bee venom therapy (BVT), is a currently-applied method worldwide. Bee venom (BV) includes several substances such as peptides, phospholipids, bioactive amines, amino acids, sugars, pheromones, enzymes and minerals.Studies on whole BV and its singular components indicated that they have a huge potential in anti-inflammatory, anti-arthritis, anti-nociceptive, neuroprotective, anti-tumoral, anti-microbial, anti-diabetic and anti-rheumatic activities. Results of in vivo studies against arthritis, Parkinson's and Alzheimer's disease and cancer are very promising, and also in vitro results indicating other activities such as antimicrobial effect are observed. Although mechanisms of action and many bioactive substances still remain unclear, beneficial effects and potential utilities in certain medical conditions are obvious. It seems bioactive components of BV may open new doors in treatment of various diseases.

Keywords: apitherapy; apitoxin; bee venom therapy; bee venom acupuncture

Öz

Apiterapi, dünya çapında çok sayıda hastalığın tedavisinde kullanılan ve kökeni çok eski tıbbi kayıtlara dayanan bir tamamlayıcı tıp uygulamasıdır. Apiterapötik teknikler kendi başlarına tedavi yöntemleri değil, aslında, çok disiplinli tıbbi yaklaşımın parçasıdırlar. Bu yöntemlerden biri, arı zehri tedavisi, dünyada şu anda uygulanan bir tekniktir. Arı zehri, peptitler, fosfolipitler, biyoaktif aminler, amino asitler, şekerler, feromonlar, enzimler ve mineraller gibi çok sayıda madde içermektedir. Arı zehrinin tümü ve içerdiği materyallere ayrı ayrı yapılan çalışmalarda, bunların, antiinflamatuvar, antiartrit, antinosiseptif, nöroprotektif, antitümöral, antimikrobiyal, antidiyabetik ve antiromatizmal etki potantisiyeli açıkça gösterilmiştir. Artrit, Parkinson ve Alzheimer hastalığı ile kansere yönelik in vivo çalışmalar ile antimikrobiyal etkinlik gibi in vitro çalışmalarda son derece umut verici sonuçlar gözlenmiştir. Her ne kadar etki mekanizması ve birçok biyoaktif içeriği henüz aydınlatılmamış olsa da, belirli tıbbi durumlarda etkinliği açıkça görülmüştür. Arı zehrinin biyoaktif komponentlerinin diğer başka hastalıklar için de yeni kapılar açacağı düşünülmektedir.

Anahtar Kelimeler: apiterapi; apitoksin; arı zehri tedavisi; arı zehri akupunkturu

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Introduction

Bee products have a wide space among complementary medicinal methods. The use of bee products in medicine, called apitherapy, includes bee venom (BV) (apitoxin), royal jelly, honey, pollen, propolis and beeswax, and each of them has recently become topics of studies worldwide [1]. Apitherapy usage has an very old history and even from the times of Hippocrates there are records of different usages. Ancient Roman and Middle-age physicians were frequently applied BV, but scientific reports mainly depend studies at the late 1800s. In 20th century, researchers were interested in potential medical components in BV. Today, bee venom therapy (BVT) itself and some components of BV are in medical use worldwide [2,3]. BVT, bee sting therapy or acupuncture is a complementary and integrative medicinal technique by application BV via bee sting to the particular points of the patient's body. Recently, this method is widely used in treatment of arthritis, some rheumatoid and chronic inflammatory diseases. Numerous studies were published about its modes of actions and various substances were identified in BV, but the actual chain of action is still unclear[4,5].

To date, over than 20 bioactive substances were isolated and identified in BV, which are acting as anti-inflammatory, anti-arthritis, anti-nociceptive, neuroprotective, anti-tumoral, anti-microbial, anti-diabetic and anti-rheumatic [3-8]. Some of these substances are peptides (melittin, apamin, adolapin, mast cell degranulating peptide-MCD), phospholipids, bioactive amines (histamine, dopamine, norepinephrine), amino acids (gamma-aminobutyric acid), sugars (glucose, fructose), pheromones, enzymes (phospholipase A2 and B, acid amino-esterase, hyaluronidase, phosphatase, glucosidase, lysophospholipase) and minerals (phosphor, calcium, magnesium) [2,3,7,9-12].

Regarding these substances and detected modes of actions, with further studies, more indications and application areas may come forward. In this article, it is aimed to consolidate the information about BV and BVT, provide an overall vision and to take a broad look to modes of actions.

Major Bioactive Substances

In overall, the studies about BV mainly focus on a few substances. These molecules seem to be the actual bioactive components, but it should be noted that researches are just in preliminary phase and there is a huge lack of in vivo studies. Major bioactive substances in BV and their potential effects were summarized in Table 1.

Table 1: Major bioactive substances in BV and their potential effects

Substance	Effect	Reference
Melittin	Anti-inflammatory, anti-arthritis, anti-tumor, antimicrobial activity	1,9,10,13-32
Apamin	Anti-neurodegenerative, anti-inflammatory, anti-tumor,	1,13,33-35
Phospholipase A2	Anti-tumor, neuroprotectiveeffect (dopaminergic cells), immunomodulatory	1,13,36-38
Adolapine	Anti-inflammatory, anti-rheumatic, antipyretic, analgesic, anti-coagulant	1
MCD	Histaminergic or Anti-histaminergic[a]	1,13
Secapin	Antimicrobial activity	1,13
Tertiapin	Parasympathomimetic	1,13
Cardiopep	Antiarrythmic	1,13
Lasiocepsin, macropin, melectin, bombolitin, halictine 1, panurgines, mastoparans, codesanes	Antimicrobial activity	39,40
Minimine, procamine, dopamine, noradrenaline, histamine, pamine, hyaluronidase	Insufficient data	1,13,14

[a]The activity varies dose-dependently

Melittin is a short, cationic, hemolytic protein consisting of 26 linear amino acid sequence. It forms the main part of the venom consisting ofboth hydrophobic and hydrophilic components, thus it provides a protein-lipid association for cell membrane penetration by opening channels that results voltage-dependent changes and membrane destruction. This peptide also supresses cyclooxygenase (COX) activity, proinflammatory cytokines such as TNF-α, IL-1, IL-6 and release of free radicals. It is believed that by this way, mellittin has a major role in anti-inflammatory and anti-arthritis actions [1,9,10,13,14].

Melittin seems to have a promising anti-tumoral effect. A recently published review article investigated anti-cancer activities of BV by focusing on melittin and it was very obvious that for several types of cancers, melittin and its derivations particularly show anti-tumoral actions via multiple pathways [14]. In vitro studies with cell culture indicated such a "selective action" that the peptide particularly chooses cells which have an active ras ongogene and are expressing oncoproteins. As previously stated, melittin destruct the

cancer cells by membrane disruption, but it also shows effects against transformation [10]. Interestingly, melittin found to be showing a cell-cycle stopper effect in hepatoma and glioma cells [15,16]. Apopytosis induction was also observed in hepatocarcinoma, osteosarkoma and leukemia cells [10]. Cell death was provided via induction of caspase and Bax, inhibiton of JAK-STAT and Bcl-2. Furthermore, matrixmetalloproteinase-9 (MMP-9), an enzyme which is crucial for malign invasion, was successfully inhibited by melittin [17-19]. Similarly, Wang et al [20] reported that melittin significantly inhibited invasion of human breast adenocarcinoma line (MCF-7) cells. In a recent study, Lee et al [21] reported a significant reduce with melittin treatment in tumor-associated macrophages, which are strongly related tumor growth. They also stated particular decrease in angiogenesis. Results of other studies supported this information. Xhang et al [22] claiming that dwindling expression of vascular endothelial growth factor (VEGF) and hypoxia-inducable factor 1-α may be an explanation about reduced tumor growth. In another study, Zhang et al [23] reported that melittin inhibited complex pathways of invasion and angiogenesis. Wang et al [24] created a hybrid peptide with melittin and VEGF, and they studied this peptide on hepatocellular carcinoma both in vitro and in vivo. Combination treatments of melittin with current agents are also showed very promising results. Wang et al [25] reported enhanced inhibition on growth of pancreatic ductal carcinoma when melittin was applied with gemcitabine. Similarly, Jin et al [26] studied melittin containing hydrogel in combination with cancer phototermal therapy for glioblastoma treatment, and the results showed enhanced success of treatment regarding to tumor size, since they observed a significant inhibiton on tumor growth. Actually it seems inhibitory effect on growth, angiogenesis and metastasis of various tumors indicates that melittin might be used as a chemotherapeutic agent.

Antimicrobial activity of melittin is another topic. Even as singular or a hybrid peptide, it was studied against *Leishmania* spp. and *Trypanosoma* spp., and researchers found direct antimicrobial and indirect immunomodulatory results [27-30].Another promising result was found about HIV. Dose-dependently, melittin was found to prevent synthesis of group-specific antigen (gag)/DNA polymerase (pol) and intracellularly reduce Gag antigen and HIV-1 mRNA levels. In addition, it has a direct inhibitory effect on the "HIV long terminal repeat". In overall, it is believed that this peptide may be an agent to inhibit viral expression of HIV [9,31,32].

One other major substance is Apamin, which is the smallest neurotoxin in venom. It has a specific inhibitory act on Ca+2 dependent K+ channels, resulting hyperpolarization in red muscle that causes hyperexcitability in neurons. Interestingly, this toxin was showed to have protective effect on dopaminergic neurons and in animal models with Parkinson's disease, partial anti-neurodegeneration was observed [1,13]. Studies also indicated that apamin caused improvement on cognitive functions in animal models as a potential agent against Alzheimer's disease [1,33]. Furthermore, this toxin also promising for anti-tumoral effect [34,35]. Apamin also have become a target molecule for immunopathologic studies. Kim et al [34] reported that apamin supressed proinflammatory and Th-2 related inflammatory pathways and this makes the molecule as a candidate in treatment of atopic dermatitis. Interestingly, Kim et al [35] studied apamin for possible effects in biliary fibrosis, and they stated that it supressed activated hepatic cells, which are major reason of arised collagen deposition causing biliary fibrosis. They also noted that apamin decreased the proinflammatory cytokine levels in hepatic tissue. It seems there is an uncharted map of ocean to explore about apamin, that it does not just affect as channel blocker, but it may have many immunologic activities.

Phospholipase A2 is the most allergic substance in BV and as known, this enzyme is actually works in inflammatory processes. The enzyme has cytotoxic ability and so it is claimed that it is one of the anti-tumoral substances in BV [1,13]. Phospholipase A2 also found to be protective for dopaminergic cells in Parkinson's disease and show alleviating effect on neuropathic pain [13,36]. In an interesting study, Bae et al [37] found that phospholipase A2 immunologically protected the fetus from abortion in mice models by regulating Treg cells. Similarly, Jung et al [38] found interactions of phospholipase A2 with Treg in treatment of atopic dermatitis. The substance seems to carry huge potential as a therapeutic against various diseases.

Adolapine, MCD, phospholipase B, secapin, tertiapin, cardiopep, minimine, procamine, dopamine, noradrenaline, histamine, pamine and hyaluronidase are other main substances in BV [1,13,14]. Adolapine inhibits COX and lipooxygenase activity resulting anti-inflammatory, anti-rheumatic, antipyretic, analgesic and anti-coagulant [1]. MCD is a 22 amino-acid long peptide associated with allergies. It has opposed actions dose-dependently, that it induces histamine release in low doses and inhibits in high doses. There are very few data about the other components, but secapin is thought

to be an antimicrobial, tertiapin seems to be a potential parasympathomimetic and cardiopep may have antiarrythmic function [1,13]. Furthermore, some of these substances are claimed to have radioprotective effect. There are more bioactive molecules isolated from different bee species such as lasiocepsin, macropin, melectin, bombolitin, halictine 1, panurgines, mastoparans and codesanes. These substances actually show antimicrobial activity with different potencies [39,40]. Finally, hyaluronidase is the enzyme that facilitates the spread of BV to the tissues. It destructs local connective tissue and arises vascular permeability [1].

Potential Effects

BV have the potential of containing various molecules that may provide dfferent effects. These substances may have not only one action, but affect on multiple chain reactions resulting various outcomes. In overall, these actions are; anti-inflammatory, anti-arthritis, anti-nociceptive, neuroprotective, anti-tumoral, anti-microbial and anti-rheumatic. Furthermore, a few studies indicated that BV has a potential of having anti-diabetic effect.

Anti-inflammatory, anti-arthritic and anti-rheumatoid effects mainly depends on the same mechanisms. As previousy stated, some substances such as melittin act through the suppression of inflammatory processes. Inhibiton of COX, supression on release of proinflammatory cytokines and nitric oxide(NO) seems to be the major routes. Lee et al. [41] compared the control group with the BVT group in mice with type 2 collagen arthritis, and they found a significant difference on TNF-α levels (decreased in bee-venom group) which is supported by histopathological and symptomatic changes. However, they reported that there was no alteration on IL-1β levels. BV stimulates Bcl-2 and caspase-3 expression in synovial cells which limits cell-level effects of rheumotoid arthritis such as synovial proliferation and hyperplasia by directing the cells to apopytosis [5,42]. Furthermore, as perviously stated, adolapin shows particular inhibition on inflammatory enzymes and MCD provides mast cell lysis dose-dependently, but low-dose MCD and the phospholipase A2 in venom make the evaluation controversial. The venom itself includes many allergic substances that provoke inflammation immediately, so the actual modes of action in supression of autoinflammatory reactions are still blurry and need further studies.

Cytotoxic activity of venom substances were previously mentioned. Studies indicate that possible anti-tumoral actions do not only depend on this activity, but molecular interactions may occur that strongly inhibit ongogenic biochemical reactions. Potential activity of melittin on ongogens and oncogenic proteins were mentioned before, which was a result of complex interactions [7,14]. However, it seems this "supression of oncogeny" is far more complex. BV substances somehow affect on cell-level messengers, causing directly apopytosis and cell-cycle arrest [2,7]. In addition, some substances somehow particularly reduce expression of angigenesis factors and supresses tumor growth [21,22]. A recent study of Sin et al [43] indicated that BV may have strong relations with immune response cells as a cancer chemotherapeutic. Although mellitin is under focus of these studies, BV has the potential of more substances or venom as a whole may have this kind of act more promisingly.

BVT shows a potent anti-nociceptive effect. It is known and used for a long time, but studies focusing on this topic have recently been published [4]. Kwon et al [44] reported that acupuncture to particular points provided a reducing activity on edema and an anti-nociceptive effect on rats that had experimental arthritis. In another study, Kwon et al [45] observed that water-soluble components of BV(>10kDa) directly reduced visceral pain. Interestingly, following the injection, major inflammation signs immediately occured, but in time, it reduced pain by stimulating axonal reflexes. In addition, venom stimulated decendent adrenergic system and Fos expression in catecholaminergic neurons. Reserchers stated that this may provoke desendent noradrenergic system that may result a anti-nociceptive effect [45]. In another study of Baek et al [46], researchers applied venom to two groups of rats with collgen-induced arthritis, and additionally, yohimbine (α2 receptor blocker) and naloxone (opioid receptor blocker) to each one group. They reported that they observed anti-nociceptive effect in only naloxone group, so they claimed this may be proof of venom to show its anti-nociceptive effect via α2 receptors. Similarly, in a recent study, Huh et al [47] notified possible analgesic mechanisms on BVT via δ-opioid and α2-adrenergic receptors. On the other hand, in the study of Kim et al [48], researchers applied opioid and 5-HT3 receptor antagonists following BVT and morphine application to mice with experimental oxaliplatin-induced neuropathic pain. Interestingly, they found a strong "anti-BV effect" due to serotonergic receptor blockage and they commented that serotonergic system may play more important role in treatments with BVT. Similar results were previously stated by Lee et al [49]. Of course, these mechanisms may not be

the only explanation, since anti-inflammatory and analgesic effects should also be noted.

Neuroprotective activities of BV are also under focus of researchers. Neuroprotective effects of BV and its components against Alzheimer's disease, Parkinson's disease, Huntington's disease and amyotrophic lateral sclerosis were reported [50]. Parkinson's disease and multiple sclerosis are by far the most studied diseases with BV and promising results were observed. In their study on rats with experimental Parkinson's disease, Doo et al [51] reported protective effect of BV on dopaminergic neurons. Although the substance apamin is mainly focused about this neuroprotective effect, Alvarez-Fisher et al [11] stated that there was a relative difference on "neuroprotection" between whole BV(higher) and just apamin itself, that venom may contain many other bioactive molecules working in the same way. Recently, Khalil et al [149] studied on mice with experimental Parkinson's disease and compared L-Dopa and BV. They stated a significant decrease on expression of apopytotic proteins such as Bax and caspase-3 in dopaminergic neurons in venom group. They claimed that BV supressed neuroinflammation and was obviously neuroprotective, comparing to L-dopa. Chung et al [52] focused on potential immunoregulatory effect of venom on mice with experimental Parkinson's disease. Following venom application, they confirmed neuroprotective activity in substantia nigra, microglial deactivation and reduced CD4+ T-cell infiltration, arised immigration of immunoregulatory CD4+CD25+Foxp3+Treg cells. However, they could not observe the same neuroprotective effect after blocking the Treg cells. It seems this effect does not only depend on suppression of inflammation by direct biochemical mechanisms, but complex immunomodulatory activities are also current. Han et al [54] reported inhibition on production of NO and TNF-α in lipopolysaccharide-induced microglia cells. Interestingly, Daghestani et al [55] studied on propionic acid-induced neurobehavioral status and effects of BV. They reported that venom strongly contributes to the propionic acid treatment in a positive way. This result actually indicates possible interactions of venom with neurotransmitter systems and transcriptions in neurons. Although these researches strongly support potential benefits of BVT, there is a huge gap on human studies. Wesselius et al [56] stated that BVT did not provide any improvement on neither pathological status nor life quality of patients. It is clear that there is a promising vision for especially neurodegenerative and autoinflammatory diseases, but correlation between in vitro studies and animal models to clinical outcome is controversial.

Antimicrobial activity of BV is another issue of studies. Acitivity of melittin on HIV and *Leishmania* were previously stated. Similarly, Fennel et al [57] had already reported antibacterial activity of melittin on gram negative and positive bacteria. Many studies were comfirmed this antibacterial action by testing BV against frequently-isolated bacterial pathogens [1]. Furthermore, Kawakami et al [33] identified two peptides (Xac-1 and Xac-2) from venom of Japanese carpenter bee, showing hemolytic and antimicrobial activity. Strong antimicrobial effects were observed against *E.coli, S.aureus, M.luteus* and *S.cerevisiae*. Interestingly, Yu et al [58] studied cecropins-melittin hybrid proteins and found strong antimicrobial effect against *E.coli, S.aureus, P.aeruginosa, K.pneumoniae, B.subtilis, B.thuringiensis* and *S.derby*. These results indicate that there is still a huge potential of novel antimicrobial peptides, and this potential might arise, even with synthetically derived ones.

There are a few studies claiming anti-diabetic effects of BV. It is believed that this potential mainly depends on two substances; melittin and phosholipase A2 [59]. But, studies about spesific molecules are very limited. In a recent in vitro study, Behroozi et al [6] compared two groups of solutions including hemoglobin and glucose with and without venom. They found a significant decrease on hemoglobin glycation in the venom group, so they claimed that BV might be a light to prevent long-term complications of diabetes. Potential beneficial effects of venom on wound healing also arouse curiosity. A recent study on diabetic mice indicated accelerated wound healing via decreasing oxidative stress and affecting on transcription factors [60]. Finally, BV is also a candidate as an anticoagulant agent, affecting on several coagulation factors dose dependently [61].

Conclusion

BV contains various bioactive substances. Only a few of these have become topic of studies. On the other hand, external factor such as climate and bee species may cause variations on components itself or amont rates of them. Even the venom from the same species may show alterations in amonts of substances, seasonally [62-64].

Although BV clearly have many utilities in clinical medicine, adverse effects may be observed. There are reports of hemolysis, liver injury, immune thrombocytopenia [13,65]. Melittin, hyaluronidase and phospholipase A2 are serious allergens in BV [13]. This condition forced researchers to derive

novel substances. For example, Wu et al [66] derived a peptide from melittin and they have stated that it showed stronger anti-hepatoma activity and also enhanced resistanse to proteases. But, it is really hard to make scientific interpretations due to serious limitation of studies, especially in vivo and human research.

In conclusion, BV is a valuable traditional agent with strong biochemical and immunological actions. Although mechanisms of action and bioactive substances still remain unclear, beneficial effects and potential utilities in certain medical conditions are obvious. Indications, possible complications, application frequency and dosage should be carefully evaluated according to the patient and physician's opinion. It should be noted that apitherapeutical applications are not treatment methods by themself, but they can be substantial parts of multidisciplinary approaches.

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