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Original Article

Effect of Honey Bee Venom on Experimental Autoimmune Encephalomyelitis (EAE) as a Model for Multiple Sclerosis (MS)

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Abstract

Experimental autoimmune encephalomyelitis (EAE) has been widely employed as a model to study multiple sclerosis (MS). Interleukin-27 (IL-27) inhibits Th17 activity and breaks the normal activity of effector T cells which cause autoimmunity. Bee venom (BV) has been used as a form of medicine from the time of ancient Greece and China. BV and BV-derived active components might have potent therapeutic effects on refractory immunological and neurodegenerative diseases, such as MS. This study aimed to investigate the effect of Iranian honey bee venom on the progression of EAE in mice. Initially, EAE was induced in 12 female C57BL/6 mice through immunization with an emulsion of myelin oligodendrocyte glycoprotein 35-55 (MOG_{35-55}) in Complete Freund's adjuvant (CFA), followed by administration of pertussis toxin (PTx) in phosphate buffer. Following the appearance of clinical signs, the mice were treated intraperitoneally with BV. Histopathological and immunological studies were investigated, and EAE was induced in animals within 9-14 days. Results revealed a significant reduction in IL-27 levels following EAE induction in mice. However, BV-treated mice showed a significant increase in IL-27, compared to controls. Histopathology results revealed that the number of inflammatory cells was reduced in the brain parenchyma following BV treatment. Based on the results obtained in the present study, BV may be a suitable candidate for the treatment of inflammatory diseases, such as MS.

Keywords: Bee venom, Experimental autoimmune encephalomyelitis, Interleukin-27, MOG, C57BL/6 mice, Multiple sclerosis

1. Introduction

More than 2.5 million people live with multiple sclerosis (MS) worldwide, and women are estimated to be afflicted twice to thrice as much as men (1). In addition, MS is an autoimmune disease associated with chronic inflammatory demyelination of the central nervous system (CNS), and its clinical symptoms include ataxia, loss of coordination, sensory impairment, cognitive dysfunction, and fatigue (2). Effective treatments have not been developed due to disease complexity and heterogeneity, and almost all the therapeutics tested in MS patients are based on concepts derived from experimental autoimmune encephalomyelitis (EAE) data (3). It is established that T self-reactive cells have the potential to differentiate into effector subsets of Th1 and Th17 (4). Effector CD4+T cell subsets play

an important role in Multiple Sclerosis (MS) (5). Interleukin-27 (IL-27) suppresses Th (Th1, Th2, and Th17) cells and dampens autoimmunity and tissue inflammation by promoting the generation of Type 1 regulatory T cells (Tr1) (6).

The search for alternative therapeutic strategies for the treatment of MS continues and conflicting evidence in the literature indicates that bee venom (BV) has anti-inflammatory and antinociceptive effects on inflammatory reactions (7). Although research on BV started almost a hundred years ago, its mechanism of action is still uncertain. Some researchers found that immunotherapy using BV could lead to the production of some interleukins including IL10, while others reported the decreased amount of IL-4 and IL-5 (8). This study aimed to analyze the effect of BV on the progress cessation of EAE-induced mice.

2. Materials and Methods

2.1. Animal Model

The experiments were carried out on 8-10 weeks old female C57BL/6 mice (weighing 18-20 g) purchased from Razi Vaccine and Serum Research Institute, Karaj, Iran. Mice were housed according to institutional guidelines and had access to food and water. They were randomly divided into three groups. Group 1 included six healthy mice, as the group of control. Group 2 included six EAE Induced mice using myelin oligodendrocyte glycoprotein (MOG). Group 3 included six BV-treated mice following EAE induced by MOG_{35-55} .

2.2. EAE Induction and Treatment

Mice were injected subcutaneously with 50 µg MOG_{35-55} peptide (Sigma-Aldrich, USA) in Complete Freund's Adjuvant (CFA) containing 1 mg of killed Mycobacterium tuberculosis emulsion (Sigma-Aldrich, USA). This was followed by two intraperitoneal injections of 200 ng of pertussis toxin (Sigma-Aldrich, USA) on day 0 and the second day (9). The BV was dissolved in normal saline and was provided by Razi Vaccine and Serum Research Institute, Iran. The pharmacological dose of BV was calculated based on its effective dose in animal studies (9). The BV (3µg/0.1ml per mouse) was injected intraperitoneally in group 3 of animals (10). The BV treatment was performed two times (with 3 days interval) in the first week of the experiment. The clinical signs and weight of each mouse were monitored daily. The motor deficit was evaluated using a ten-point standardized rating scale, in which 0=no clinical signs; 1=partially limp tail; 2=paralyzed tail; 3=hind limb paresis and uncoordinated movements; 4=one hind limb paralysis; 5=both hind limbs paralysis; 6=hind limbs paralysis, weakened forelimbs; 7=hind limbs paralysis, one forelimb paralysis; 8=hind limbs paralysis, both forelimbs paralysis; 9=moribund; 10=death.

2.3. Cytokine and Histological Analysis

The blood was collected from the mice heart at the end of the experiment on day 26^{th}. Serum was separated by centrifuge and preserved at -80 $^{\circ}$c for future analysis. The serum IL-27 level was determined using a mouse IL-27 ELISA Kit [CUSABIO, Cat, No. CSB- E08466m]. Reading of Optical Density (OD) was performed at 450 nm within 5 min and the obtained data were analyzed according to the standard curve.

Mice in all three groups were euthanized on day 26^{th} of the experiment. They were perfused with PBS followed by 10% paraformaldehyde for tissue fixation. The fixed brain tissues were embedded in paraffin. Paraffin-embedded sections were cut at 6 µm thickness and mounted on silane-coated standard glass microscope slides. Histological evaluation was performed by staining with hematoxylin and eosin (H&E) for inflammatory cell infiltration analyses. The intensity of inflammatory cell infiltration was assessed according to the protocol (11).

2.4. Statistical Analysis

Data were expressed as mean±SEM, and comparisons between groups were made through one-way ANOVA with Tukey's test. A p-value less than 0.05 ($P<0.05$) was considered statistically significant.

Statistical analysis was performed using the SPSS software (Version 16).

3. Results

No change in signs and symptoms of clinical disorders was observed in the negative control group (group 1) during the experiment. The first EAE signs appeared eight days following the immunization of mice (groups 2 and 3) with MOG+CFA and PTx. The maximal degree of paralysis, indicating an acute phase, occurred around day 14 when the average clinical score reached 2.5. From this time on the animals showed a decrease in the clinical score and were partially recovered from paralysis. However, the recovery was not complete and the disease stabilized around day 20 with an average score of 1.25 (Figure 1). Variation in body weight showed an expected course characterized by a significant weight drop during the acute phase. However, weight recovery was observed in the chronic disease phase (Figure 2). Mice in groups 2 and 3 showed signs of decreased activity, nutritional behavior, and weight loss from day eight post-immunization. Clinical analysis of BV-treated mice subjected to EAE revealed no significant differences between the two groups from the onset until the peak of the disease (about 14 days post-immunization), and both groups showed higher clinical scores (ranging between 2.0 and 2.5). However, the reduction of clinical score started in BV-treated mice (group 3) from day 20 and reached score 1 on day 25, while the clinical score remained at 1.5 in non-treated mice (group 2).

3.1. Brain Parenchymal Lesions

In an attempt to establish a correlation between clinical signs and morphological changes in the mice brain, histological analysis of mice brain sections was conducted on day 26 after mice scarification, and the sections were analyzed using light microscopy. Typical mark lesions characterized by an intense perivascular inflammatory infiltrate were observed in the brain of all the mice in group 2 (Figure 3b). However, a visual inspection indicated that both the groups, EAE-induced mice and EAE-induced mice treated with BV presented comparable degrees of inflammation. The brain parenchyma of the control group showed no penetration of inflammatory cells (Figure 3a). However, an inflammatory infiltration mainly composed of mononuclear leukocytes which was noticed in the parenchyma, and inflammatory cells existed around the blood vessels. Likewise, EAE-induced mice treated with BV showed only mild inflammation around the meninges (Figure 3c). Therefore, the histological analysis indicated a significantly decreased intensity of pathological changes in the EAE-induced mice treated with BV (Figure 3).

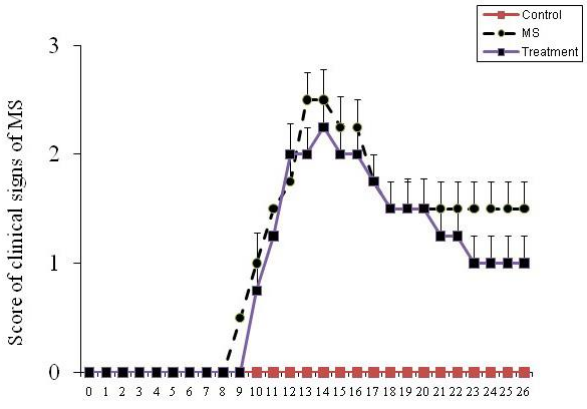

Figure 1. Effect of BV treatment on EAE development. The EAE-inducted C57BL/6 mice were injected with BV. Clinical score variations were evaluated daily. Data were presented by mean±SEM of six mice and representative of three independent experiments.

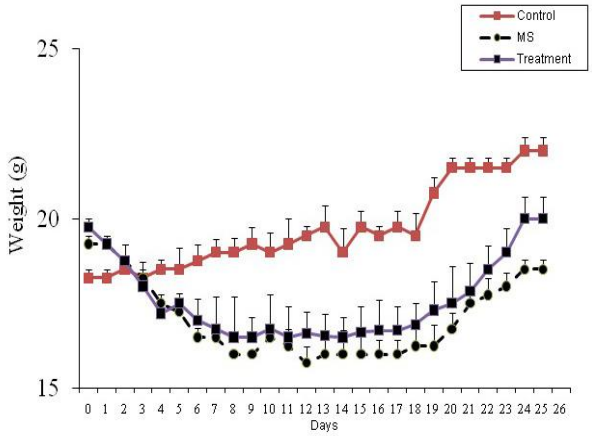

Figure 2. Effect of treatment with BV on EAE development. EAE-induced C57BL/6 mice were injected with BV. Weight variations were evaluated daily. Data were presented by mean±SEM of six mice and representative of three independent experiments.

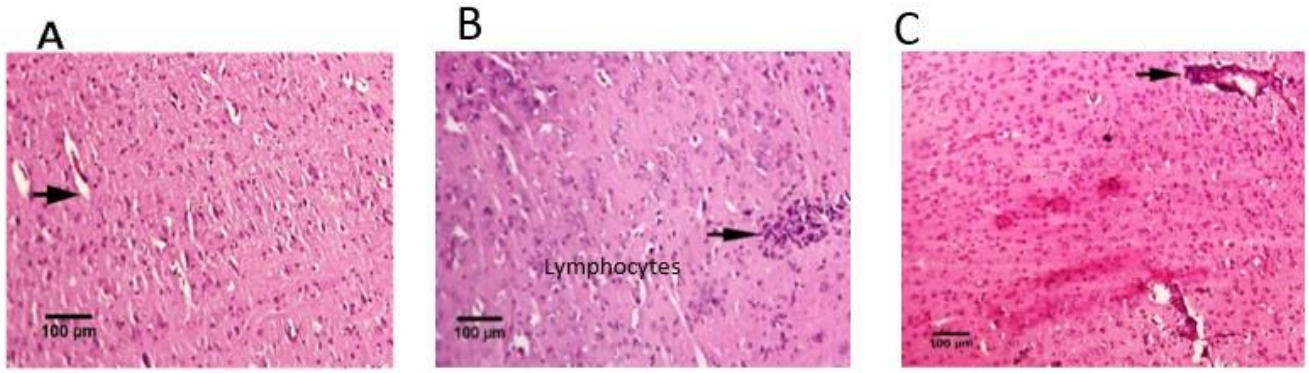

Figure 3. Effect of BV treatment on CNS inflammation. EAE-induced C57BL/6 mice were injected with BV. Brain inflammatory infiltrates in control mice without EAE (a). Control group of mice with EAE (b) and EAE-induced mice treated with BV (c). Mice were sacrificed on the 26th day. The panel is representative of six animals/groups.

3.2. Serum Cytokine Expression (IL-27)

In the present study, serum levels of IL–27 were evaluated in all 3 groups of mice on the last day of the experiment. The normal level of IL-27 in serum was found to be 127.26±35.72 pg/mL in the negative control group of mice. The results revealed a significant decrease (P<0.05) in the serum levels of IL-27 in the positive control group (EAE group) which was found to be 73±22.72 pg/mL. However, a highly significant (P<0.001) rise in IL-27 serum level was observed following BV treatment in group 3 of animals, which was found to be 232.23±38.3 pg/mL (Figure 4).

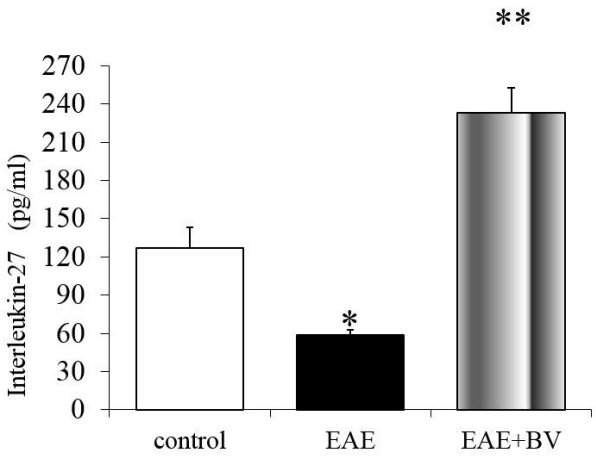

Figure 4. Interleukin-27 levels in the serum. The EAE-induced mice were injected with BV. IL-27 was tested 26 days after scarifying mice. ELISA was used to determine serum IL-27. The level of IL-27 in different groups was compared to controls. $^{*}P$< 0.05 and $^{**}P$< 0.01 ^{+++}P< 0.001.

4. Discussion

As mentioned previously, MS is an autoimmune disease of the NCS with penetration of macrophages and lymphocytes into CNS, which leads to demyelination. Although the etiology of this disease is still unknown, it seems that T cells and B cells play a significant role in attacking myelinated parts of the nervous system. This study has been conducted on C57BL/6 mice, and it is a known fact that these mice are susceptible to EAE induction using MOG- CFA-PTX. MOG along with CFA acts as an antigenic component. The PTx we used to enhance EAE in mice (12).

The results of the present study showed a marked change in the physical activity of mice that received MOG- CFA- PTx. It is reported that PTx is an immune-adjuvant utilized to effectively promote an inflammatory response in animal models with multiple autoimmune diseases and is critically involved in the pathogenesis of EAE (13). Administration of PTx is reported to be a pro-inflammatory cascade of IL-6, TGF-β, and Th17, in the CNS which have been essential in the development of EAE (14). The first EAE signs appear eight days after the disease induction. The maximal degree of paralysis, indicating an acute phase on day 14±1 when the average clinical score reached 2.5. The positive control group of mice, as well as BV, treated EAE induced mice were

sacrificed on day 26 for histological evaluation. These findings were in line with the results obtained by Peon, Ledesma-Soto (14). According to some researchers, the first signs of neurological disease generally include weight loss and loss of activity between days 10 and 17 following EAE induction. However, based on the evidence, signs are observed somewhat earlier (starting from 5 to 7 days after cell transfer) using the adoptive transfer method (15).

Based on the obtained results in this study, serum level of IL-27 was lower in the EAE group, compared to healthy mice in the group of control, suggesting that IL-27 has the potential to be an effective response modifier for immunotherapy of autoimmune disease. These results are in line with those of other studies that reported a reduction in serum IL-27, following EAE induction (16). However, the IL-27 serum level increased in BV-treated mice, compared to healthy ones and EAE group, which suggested the effectiveness of BV in interfering with the disease progress. On the other hand, the effect of BV on EAE-induced mice was investigated considering inflammatory cells and pathological changes in CNS in the present study. Based on the evidence, EAE is a disease mediated primarily by the action of Th1 and Th17 lymphocytes, which release several pro-inflammatory cytokines and chemokines with consequent mobilization and activation of peripheral leukocytes to the CNS parenchyma (17). The peak of the disease is characterized by the prevalence of effector T cells (Th1 and Th17). In fact, recent studies indicate a positive correlation between the presence of regulatory T cells and the regression of EAE symptoms, and IL-27 potently suppressed the expansion of encephalitogenic Th17 cells in culture (18). Recent studies also identified different types of inflammatory infiltrates in CNS, and Th1 cells have been shown to correlate with a predominant infiltration of monocyte into the CNS, while Th17 cells were associated with a higher proportion of neutrophil infiltration into the CBS (19). Additionally, the clinical symptoms of Th1- and Th17-mediated EAE were found to be different: Th1 cells induced classic EAE, whereas Th17 cells induced an EAE with a more sever clinical phonotype (20).

Medicinal properties of bee products have been known from ancient times, and today the BV is used extensively for the treatment of arthritis and other inflammatory, autoimmune, and destructive diseases. The BV includes some kinds of peptides, enzymes, active amines, and other components, which can be effective in the treatment of various diseases. Melittin is one of the most effective and well-known anti-inflammatory factors in the BV (21). Adolapin is another effective anti-inflammatory substance that suppresses the activity of the cyclooxygenase enzyme (22). It seems that BV plays a role in maintaining homeostasis in the human body's immune system and nervous system, since BV therapy can regulate two immunologically opposite conditions, including allergic disorders (Th2 dominant) and autoimmune diseases (Th1 dominant) (23). Other T cell populations, such as Th17 cells and Tregs have emerged as a key player in BV-induced modulation of the immune and nervous system (24). Several recent studies reported that BV or bvPLPA2 could upregulate peripheral Tregs and/or suppress Th17 responses in various animal models (25).

In conclusion, based on the results obtained in the present study, the treatment of EAE-induced mice with BV resulted in a decrease in the disease symptoms and pathological changes and an increase in IL-27serum level. This activity of BV is due to its anti-inflammatory and immune-modulatory effects.

Conclusion

Based on results obtained in the present study, BV may be a suitable candidate for the treatment of inflammatory diseases, such as MS.

Authors' Contribution

Study concept and design: Sh. J. and A. Z. M.
Acquisition of data: Sh. J.

Analysis and interpretation of data: A. Z. M.
Drafting of the manuscript: Sh. O.
Critical revision of the manuscript for important intellectual content: A. Z. M.
Statistical analysis: L. M.
Administrative, technical, and material support: Sh. J. and A. Z. M.

Ethics

All the animal experiments were carried out in accordance with the recommendations of the declaration of Helsinki and the internationally accepted principles for the use of experimental animals.

Conflict of Interest

The authors declare that they have no conflict of interest.

Acknowledgment

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References

1. Bove R, Chitnis T. The role of gender and sex hormones in determining the onset and outcome of multiple sclerosis. Mult Scler. 2014;20(5):520-6.
2. McFarland HF, Martin R. Multiple sclerosis: a complicated picture of autoimmunity. Nat Immunol. 2007;8(9):913-9.
3. Croxford AL, Kurschus FC, Waisman A. Mouse models for multiple sclerosis: historical facts and future implications. Biochim Biophys Acta. 2011;1812(2):177-83.
4. Zorzella-Pezavento SF, Chiuso-Minicucci F, Franca TG, Ishikawa LL, da Rosa LC, Colavite PM, et al. Downmodulation of peripheral MOG-specific immunity by pVAXhsp65 treatment during EAE does not reach the CNS. J Neuroimmunol. 2014;268(1-2):35-42.
5. Legroux L, Arbour N. Multiple Sclerosis and T Lymphocytes: An Entangled Story. J Neuroimmune Pharmacol. 2015;10(4):528-46.
6. Babaloo Z, Yeganeh RK, Farhoodi M, Baradaran B, Bonyadi M, Aghebati L. Increased IL-17A but decreased IL-27 serum levels in patients with multiple sclerosis. Iran J Immunol. 2013;10(1):47-54.
7. Lee JH, Kwon YB, Han HJ, Mar WC, Lee HJ, Yang IS, et al. Bee venom pretreatment has both an antinociceptive and anti-inflammatory effect on carrageenan-induced inflammation. J Vet Med Sci. 2001;63(3):251-9.
8. Bellinghausen I, Metz G, Enk AH, Christmann S, Knop J, Saloga J. Insect venom immunotherapy induces interleukin-10 production and a Th2-to-Th1 shift, and changes surface marker expression in venom-allergic subjects. Eur J Immunol. 1997;27(5):1131-9.
9. Miller SD, Karpus WJ. Experimental autoimmune encephalomyelitis in the mouse. Curr Protoc Immunol. 2007;15(1).
10. Dantas CG, Nunes TLGM, Nunes TLGM, da Paixão AO, Reis FP, Júnior WdL, et al. Pharmacological evaluation of bee venom and melittin. Rev Bras Farmacogn. 2014;24(1):67-72.
11. Evonuk KS, Moseley CE, Doyle RE, Weaver CT, DeSilva TM. Determining Immune System Suppression versus CNS Protection for Pharmacological Interventions in Autoimmune Demyelination. J Vis Exp. 2016;(115).
12. Tompkins SM, Padilla J, Dal Canto MC, Ting JP, Van Kaer L, Miller SD. De novo central nervous system processing of myelin antigen is required for the initiation of experimental autoimmune encephalomyelitis. J Immunol. 2002;168(8):4173-83.
13. Zhao CB, Coons SW, Cui M, Shi FD, Vollmer TL, Ma CY, et al. A new EAE model of brain demyelination induced by intracerebroventricular pertussis toxin. Biochem Biophys Res Commun. 2008;370(1):16-21.
14. Peon AN, Ledesma-Soto Y, Olguin JE, Bautista-Donis M, Sciutto E, Terrazas LI. Helminth Products Potently Modulate Experimental Autoimmune Encephalomyelitis by Downregulating Neuroinflammation and Promoting a Suppressive Microenvironment. Mediators Inflamm. 2017;2017:8494572.
15. Kostic M, Dzopalic T, Zivanovic S, Zivkovic N, Cvetanovic A, Stojanovic I, et al. IL-17 and glutamate excitotoxicity in the pathogenesis of multiple sclerosis. Scand J Immunol. 2014;79(3):181-6.
16. Fitzgerald DC, Ciric B, Touil T, Harle H, Grammatikopolou J, Das Sarma J, et al. Suppressive effect of IL-27 on encephalitogenic Th17 cells and the effector

phase of experimental autoimmune encephalomyelitis. J Immunol. 2007;179(5):3268-75.
17. Becher B, Segal BM. T(H)17 cytokines in autoimmune neuro-inflammation. Curr Opin Immunol. 2011;23(6):707-12.
18. Langrish CL, Chen Y, Blumenschein WM, Mattson J, Basham B, Sedgwick JD, et al. IL-23 drives a pathogenic T cell population that induces autoimmune inflammation. J Exp Med. 2005;201(2):233-40.
19. Kroenke MA, Carlson TJ, Andjelkovic AV, Segal BM. IL-12- and IL-23-modulated T cells induce distinct types of EAE based on histology, CNS chemokine profile, and response to cytokine inhibition. J Exp Med. 2008;205(7):1535-41.
20. Jager A, Dardalhon V, Sobel RA, Bettelli E, Kuchroo VK. Th1, Th17, and Th9 effector cells induce experimental autoimmune encephalomyelitis with different pathological phenotypes. J Immunol. 2009;183(11):7169-77.
21. Bkaily G, Simaan M, Jaalouk D, Pothier P. Effect of Apamin and Melittin on Ion Channels and Intracellular Calcium of Heart Cells. In: Mizrahi A, Lensky Y, editors. Bee Products: Properties, Applications, and Apitherapy. Boston, MA: Springer US; 1997. p. 203-11.
22. Habermann E, Reiz KG. [On the biochemistry of bee venom peptides, melittin and apamin]. Biochemische Zeitschrift. 1965;343(2):192-203.
23. Kim SK, Bae H. Acupuncture and immune modulation. Auton Neurosci. 2010;157(1-2):38-41.
24. Maddur MS, Miossec P, Kaveri SV, Bayry J. Th17 cells: biology, pathogenesis of autoimmune and inflammatory diseases, and therapeutic strategies. Am J Pathol. 2012;181(1):8-18.
25. Lee MJ, Jang M, Choi J, Lee G, Min HJ, Chung WS, et al. Bee Venom Acupuncture Alleviates Experimental Autoimmune Encephalomyelitis by Upregulating Regulatory T Cells and Suppressing Th1 and Th17 Responses. Mol Neurobiol. 2016;53(3):1419-45.