ORIGINAL ARTICLES

In Vitro Assessment of Bee Venom Effects on Matrix Metalloproteinase Activity and Interferon Production

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ABSTRACT

Controversial immunomodulatory properties of bee venom (BV) have provided an appropriate field for more investigation. The aim of present research was to verify the effects of honeybee venom on matrix metalloproteinase activity and interferon production as well as cell proliferation in monocyte and fibroblast cell lines.

The monocyte and fibroblast cell lines (K562, HT-1080, WEHI-164) were used in order to assess proliferative response, interferon-1 production and matrix metalloproteinase-2 (MMP-2) activity. Australian BV (ABV) and Iranian BV (IBV) preparations at concentrations of 0.025, 0.05, 0.1, 0.2, 0.3, 0.4, 0.5, and 1μg/ml were added to each overnight cultured cells. In time course study, cells were treated each with ABV and IBV. In all cases supernatants were collected 24 hours after treatment. A sample of the each medium was used for zymography and interferons assay. Non-treated cells were used as controls.

The production of IFN-α and IFN-β in supernatant of cell cultures was assessed using enzyme linked immunoassay procedure. MMP-2 activity, as an inflammatory index, was evaluated using zymoanalysis method.

The results of this study showed that, there were no significant difference between two sources of honey bee venoms when they were added to an identical cell line, whereas, the responses of various cell lines against bee venom were different. The increasing amounts of bee venom to human monocyte cell line (K562) revealed a significant increase in proliferative response. Our findings showed that the bee venom had no influence on IFN-α production in cell culture media, whereas, adding the BV to K562 cell line could significantly increase the production level of IFN-β only on day 8 post-treatment. In addition the effect of bee venom on MMP-2 activity in both cell culture media, WEHI-164 and K562 was similar. The stimulatory effect of bee venom on MMP-2 activity occurred at low doses. In contrast, its inhibitory effect was seen at high concentrations.

It is concluded that, honeybee venom affects on MMP-2 activity and interferon beta production as well as cell proliferation in a time and dose-dependent manner.

Keywords: Bee Venom; Fibrosarcoma; Interferon; Matrix Metalloproteinase; Monocyte

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INTRODUCTION

The composition of honeybee venom (BV) consists of melittin, phospholipase A2, Apamin, mast cell degranulating peptide and several bioactive amines

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such as histamine and epinephrine. Melittin and phospholipase A2 are two major components of bee venom, in quantities of about 40-60% and 15-20% respectively,[1-4] which are generally thought to play an important role in the induction of the irritation and allergic reaction associated with the bee stings.[5] Although the injection of BV has been reported to evoke hyperalgesia and sharp prick which is followed by tonic pain lasting for a few minutes up to 1-2 h,[4] there is conflicting evidence in the literature indicating that BV could also exert an anti-inflammatory and antinociceptive effects on inflammation.[6-8] In this regard, BV has been traditionally used in oriental medicine to relieve pain and to treat chronic inflammatory diseases such as rheumatoid arthritis.[6] Moreover, the anti-inflammatory property of whole venom of honeybee has been examined in various experimental models.[2,7-9] Moreover recent reports indicate that BV is also able to inhibit tumor growth and exhibit antitumor activity *in vitro* and *in vivo*.[10,11] Although investigations on the Immunomodulatory effects of BV started almost a hundred years ago but its mechanism of action is still uncertain.[12] It has been reported that BV immunotherapy could lead to induction of the interleukin (IL)-10 and resulted in decreased IL-4 and IL-5 and increased IFN-γ secretion. However, treatment with BV reversed the LPS-induced up regulation of such genes as IL-6 receptor, matrix metalloproteinase 15 (MMP-15), tumor necrosis factor (ligand) superfamily-10, caspase-6 and tissue inhibitor of metalloproteinase-1.[13,15] The goal of current study was to reveal the bee venom effects on matrix metalloproteinase activity and interferon production under *in vitro* conditions.

MATERIALS AND METHODS

Materials

The Iranian Honey Bee (Apis mellifera) Venom (IBV) was prepared by placing bee on a 6-mm wire grid, which was electrically pulsed. The bees then produced venom that dropped to a glass plate beneath, which was collected from the glass and freeze-dried, according to the method of Lariviere.[4] The Australian Honey Bee (Apis mellifera) Venom (ABV), was purchased from venom supplies Pty Ltd, PO Box 547, Tanuda South Australia. The mouse Fibrosarcoma (WEHI-164), human Fibrosarcoma (HT-1080) and human Monocyte (K562) cell lines were obtained from Iran Pasteur Institute, Tehran, Iran.

Human Interferon Alpha ELISA Kit (Lot No. 2273) and Human Interferon Beta ELISA Kit (Lot No. 2281), were purchased from PBL Biomedical laboratories, NJ 08854.

Cell Culture

The monocyte (K562) and Fibrosarcoma (WEHI-164 and HT-1080), cell lines were seeded at an initial density of $2x10^4$ cells/well in 96-well tissue culture plates. Cells were maintained in RPMI-1640 medium supplemented with 5% fetal calf serum, penicillin at 100 units/ml, and streptomycin at 100 µg/ml, with 5% CO_2, 37°C and saturated humidity.

Dose-Response Analysis

Triplicate, ABV and IBV preparations at concentrations of 0.025, 0.05, 0.1, 0.2, 0.3, 0.4, 0.5, and 1 µg/ml were added to each overnight cultured cells. Non-treated cells were used as controls. Cells were cultured overnight and then subjected to colorimetric assay. In time course study, cell lines were treated with ABV and IBV every other day until day 8. A sample of the each media was used for zymography and interferons assay

Colorimetric Assay

After each experiment, cells were washed three times with ice-cold phosphate-buffered saline (PBS), followed by fixation in a 5% formaldehyde solution. Fixed cells were washed three times and stained with 1% crystal violet. Stained cells were washed and remainder dye solubilized with 33.3% acetic acid solution. The density of developed purple color was read at 580 nm.

Measurement of Interferons

Time course and dose-dependent assessments of production levels of interferons alpha and beta (IFN-α and IFN-β) in cell culture supernatant of HT-1080 and K562 cell lines challenged with various concentrations of ABV and IBV were carried out using enzyme immunoassay kits (R&D Co., UK).

Zymography

This technique was used for determining MMP-2 (collagenase type IV or gelatinase A) and MMP-9 (collagenase type V or gelatinase B) activity, in conditioned media according to the modified Heussen and Dowdle method.[16]

Statistical Analysis

The differences in cell proliferation, gelatinase activity, and interferon production levels were analyzed using analysis of variance (ANOVA). *P* values <0.05 were considered significant.

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RESULTS

Effect of BV on Cell Proliferation

Figure 1 shows the proliferative response of fibrosarcoma (WEHI-164) and human monocyte (K562) cell lines to ABV and IBV at different doses (0-1μg/ml). As it is apparent in figure 1, there is no significant difference (*P*<0.05) between two sources of honeybee venoms (ABV and IBV) when they were

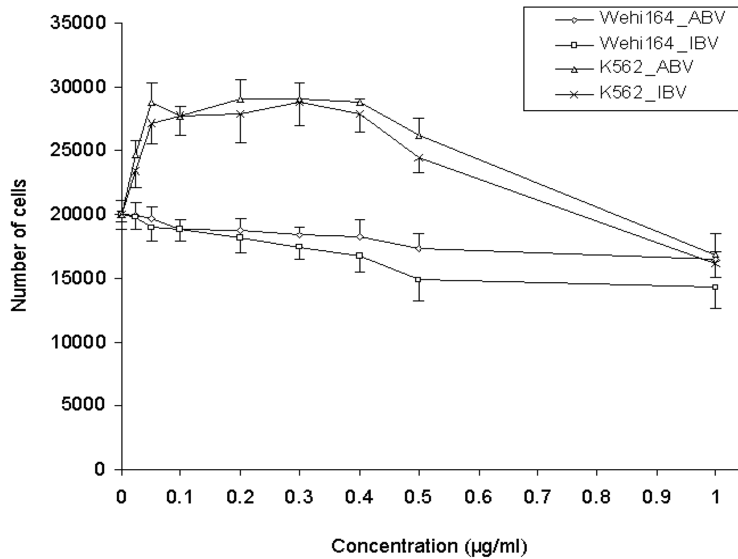

Figure 1. Proliferative response of human monocyte (K562) cell line to ABV and IBV at different doses (0-1μg/ml) compared with fibrosarcoma (WEHI-164) cell line. As data show, there is no significant difference (P<0.05) between two sources of honeybee venom (ABV and IBV) when they are added to an identical cell line, whereas, the response of WEHI-164 and K562 cell lines against bee venom could be different. The increasing amounts of ABV and/or IBV to K562 cell culture medium results in a significant (P<0.05) increase in proliferation intensity compared with the effect of BV on WEHI-164 cell line. Assays were carried out in triplicate. Each bar represents mean± SD. P<0.05 was considered significant.

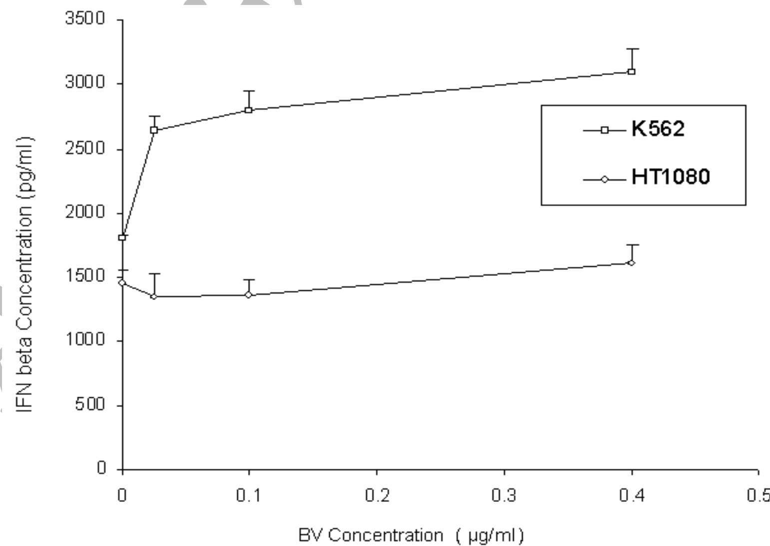

Figure 2. Comparison of production levels of IFN-beta in BV-treated cell cultures supernatant of K562 and HT-1080 cell lines. Adding the BV (ABV and/or IBV) to K562 (Human monocyte) cell culture only on day 8 post-treatment resulted in a significant increase in IFN-beta production compared with HT-1080 cell culture supernatant, which show bee venom has no effect on this cell line. Assays were carried out in triplicate. Each bar represents mean± SD. P<0.05 was considered significant.

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added to an identical cell line, whereas, the response of various cell lines (WEHI-164 and K562) against bee venom (ABV and/or IBV) were different. So that, the increasing amounts of ABV and/or IBV (between doses 0.025-0.5 µg/ml) to K562 cell culture medium resulted in a significant (*P*<0.05) increase in proliferation intensity compared with the effect of ABV and/or IBV on WEHI-164 cell line. Moreover, the data in figure 1 is indicating the tolerability and non-toxic effect of ABV and/or IBV using a very sensitive cell line (WEHI-164).

Effect of BV on Interferons Production

Time course analysis and dose response assessment of ABV and/or IBV-treated cell cultures supernatant of K562 and HT-1080 cell lines showed that bee venom had no influence on expression of IFN-alpha (data not shown). Whereas, adding the BV (ABV and/or IBV) to K562 (Human monocyte) cell culture only on day 8 post-treatment resulted in a significant increase in IFN-beta production compared with HT-1080 cell culture supernatant which showed bee venom had no effect on this cell line (Figure 2).

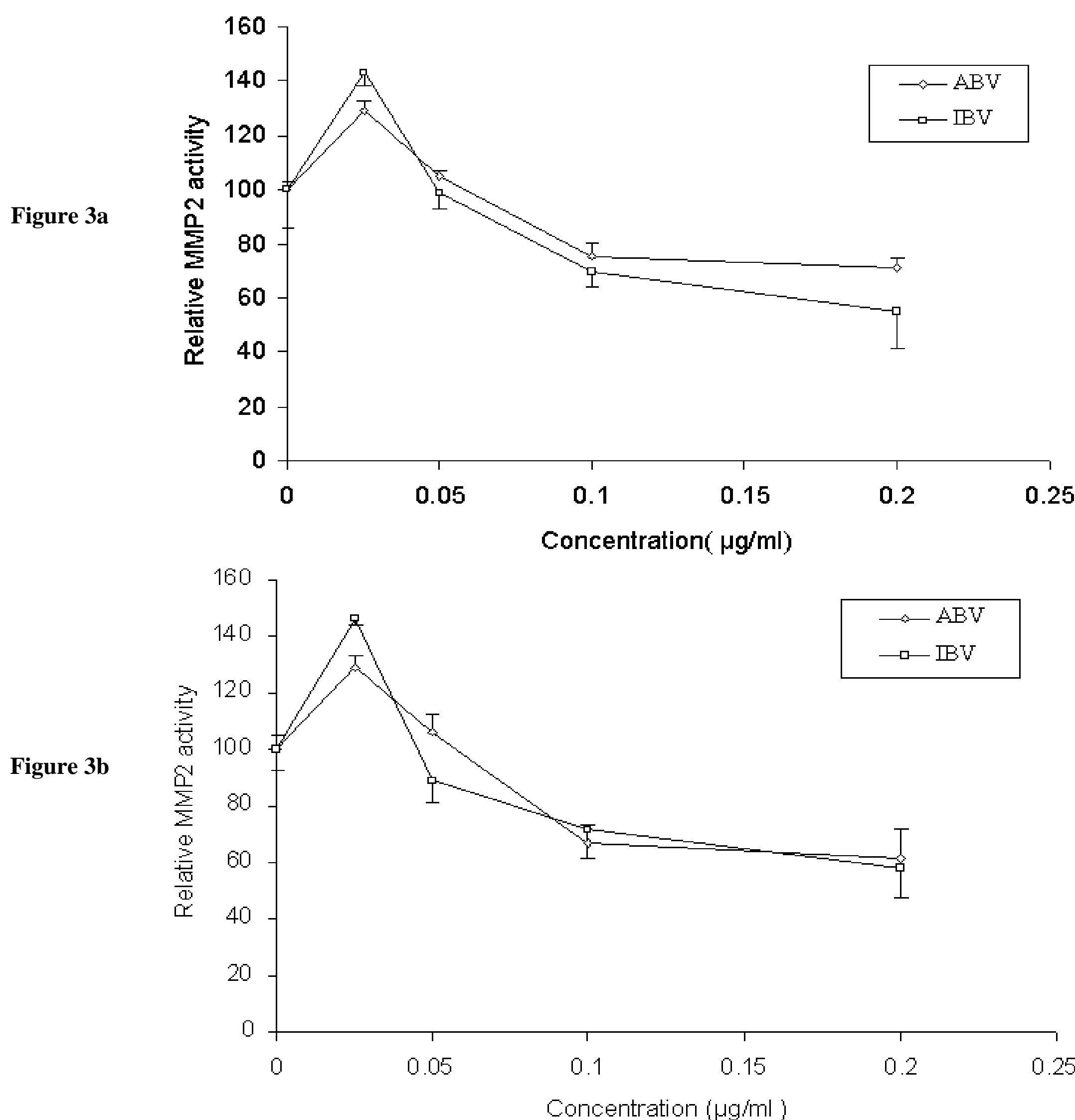

Figure 3. Effect of BV on MMP-2. The stimulatory effect of ABV and/or IBV on MMP-2 activity occurred between doses 0-0.05µg/ml in both, WEHI-164 (Figure 3a) and K562 (Figure 3b) cell culture media. In contrast, the inhibitory effect of ABV and/or IBV on MMP-2 activity is seen at concentrations of 0.05-0.2 µg/ml. Moreover there is no significant difference (P<0.05) in intensity of MMP-2 activity between ABV and IBV following their addition to both cell culture media. All experiments were performed in triplicate. Each bar is the mean± SD. P<0.05 was considered significant.

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Effect of ABV and IBV on MMP-2

Dose response analysis of ABV and IBV on MMP-2 activity is presented in figure 3. The stimulatory effect of ABV and/or IBV on MMP-2 activity was occurred between doses 0-0.05μg/ml in both cell culture media, WEHI-164 (Figure 3a) and K562 (Figure 3b). In contrast, the inhibitory effect of ABV and/or IBV on MMP-2 activity was seen at concentrations of 0.05-0.2 μg/ml. Furthermore Figure 3 shows that, there is no significant difference ($P<0.05$) in intensity of MMP-2 activity between ABV and IBV following their addition to both cell culture media.

DISCUSSION

This investigation was designed to determine the effects of honeybee venom on production of Interferon-1 and MMPs activities in Monocyte and Fibroblast Cell Lines. The whole BV of Apis mellifera was used for conducting this study, since when one is envenomated it is the intact venom, rather than individual components, which enter into the body.

The scientific literature has exhibited conflicting results in connection with BV using invivo and/or invitro models.[4-10,18,19] In the present study we demonstrated new immunological effects of BV. Our findings using human monocyte cell line (K562) indicated that the proliferation process could significantly increase when ABV and/or IBV was added to cell culture media. This effect may be attributed to the binding of PLA2 to a variety of very high affinity receptors,[20] and/or the property of components such as phospholipase A2 that represents the major antigen/allergen of honeybee venom in both allergic and non-allergic individuals.[21] The venoms of the different sources of honeybees had remarkably similar effects on an identical cell line. This finding highlights the close phylogenetic relatedness of the honeybee species.

In this study we have also shown the elevation of IFN beta in a time course manner in supernatant of monocyte cell culture. This cytokine has multiple regulatory functions on innate and adoptive immunity. Under normal physiological conditions, type 1 IFNs are secreted endogenously by most human cells at low levels.[22] Deficiency of IFN beta in the body can result in immunopathologies.[23,24] So induction of IFN beta synthesis could be effective in this manner. Interestingly, increasing the IFN-beta and also proliferation response that was seen only in this cell line and not fibrosarcoma cell line revealed the influence of BV in cells of the immune system.

A biphasic (stimulatory/inhibitory) behavior of MMP-2 against BV, in a dose dependent manner indicated the roll of BV in different concentrations. In spite of the differences of MMP-2 sources, their responses to BV were similar. According to our data, lower concentration of BV (<0.05μg/ml) had stimulatory effect whereas, higher doses (>0.05μg/ml) had an inhibitory effect on MMP-2 activity. The matrix degrading metalloproteinases have been implicated in the matrix remodelling process of a number of physiological processes including angiogenesis and wound healing as well as the pathological development of a wide variety of proliferative inflammatory conditions such as rheumatoid arthritis. Thus the inhibition and/or the stimulation of these enzymes, regarding the type of disorder could be beneficial in preventing some of them.[29,30] It seems that using of BV in the scarless wound healing is based on the stimulation of matrix metalloproteinases, which is in agreement with other findings.[25-27]

Base on our data, the intensity of proliferative response of monocytes to BV therapy, increase of IFN-beta production and the inhibitory effect of ABV and/or IBV (>0.05μg/ml) on MMP-2 activity, may recommend the anti-tumorogenic property of BV in treatment process of cancer which was paralleled with findings of magnan et al.[9,28] The results of present study revealed that, honeybee venom affects cell proliferation, MMP-2 activity and interferon beta production in a time and dose-dependent manner.

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