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Anti-inflammatory Effect of Bee Venom on Type II Collagen-Induced Arthritis

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Abstract: Bee venom (BV) has been used to relieve pain and reduce inflammation in traditional Oriental medicine, especially in chronic inflammatory diseases such as rheumatoid arthritis (RA). We previously reported that the BV injection into a traditional acupuncture point (Zusanli) reduced arthritis-associated edema and nociceptive responses in Freund's adjuvant-induced arthritis in rats (Kwon *et al*., 2001). This study was designed to evaluate the anti-inflammatory and anti-cytokine effect of BV on a murine type-II collagen-induced arthritis (CIA) model. Male mice were immunized by spontaneous injection of 100 μg of an emulsion of bovine type-II collagen and complete Freund's adjuvant (CFA), with a booster injection after 2 weeks. In the experimental group, 0.1 ml BV was injected at acupuncture point (Zusanli) near both knees twice a week for a total of 5 times. In the control group, normal saline was injected at the same frequencies. These injections began 5 weeks after the first collagen injection. Starting the 3rd week after the first collagen injection, we examined limb swelling and severity of arthritis twice a week. At 8 weeks, mice were sacrificed and synovial tissue was examined with the light microscope and serum cytokines (IL-1β and TNF-α) were measured by ELISA.

The incidence of arthritis, the mean arthritis index and the number of arthritic limbs were significantly lower in the treatment compared to the control group (63% versus 75%, 3.4% versus 8.5%, 23% versus 75%, respectively). Among the serum proinflammatory cytokines, the production of TNF-α in the BV group was suppressed compared to the control group (59+/– 4.5 versus 99.5+/– 6.5, $p < 0.05$), but IL-1β was not suppressed. The examination of the histopathology of the joints of murine CIA showed decreased inflammation signs and less lymphocyte infiltration after BV acupuncture therapy. Acupuncture therapy with BV suppressed the development of arthritis and caused inhibition of the immune responses in type-II collagen-induced arthritis.

Keywords: Bee Venom; IL-1β; Rheumatoid Arthritis; TNF-α; Type-II Collagen; Zusanli.

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Introduction

Bee venom (BV) treatment as an Oriental medicine has been used for many years to treat inflammatory diseases such as rheumatoid arthritis (RA), an autoimmune disorder of uncertain etiology (Billingham *et al.*, 1973; Kwon *et al.*, 2001). Several investigators assessed the anti-inflammatory effect of BV treatment on RA, the effect of the components of BV and allergic reactions to BV in both experimental animals and humans (Kwon *et al.*, 2001; Kang *et al.*, 2002). Recently, Kwon *et al.* (2001) and Kang *et al.* (2002) reported that the induction of arthritis by Freund's adjuvant injection in rats was suppressed by BV treatment. It was also reported that BV contained several different peptides including melittin, its major component, which binded to secretory phospholipase A2 (sPLA2) and inhibited its enzymatic activity (Ranges *et al.*, 1985; Saini *et al.*, 1997). Since the phospholipases could release arachidonic acid that is ultimately converted to prostaglandin, it was possible that the inhibition of PLA2 activity by BV played an important role in suppressing the progression of RA. Potential mechanisms of BV anti-inflammatory effects, however, remain unclear.

Type-II collagen (CII)-induced arthritis (CIA) in rats and mice was known to be a useful animal model for human RA, because of its clinical and histological resemblance to human RA (Trentham *et al.*, 1977; Courtenay *et al.*, 1980). The excessive production of several types of cytokines including IL-1β and TNF-α in affected joints was observed in both RA and CIA (Mauri *et al.*, 1996; Gattorno *et al.*, 1997; Chu *et al.*, 1991), and IL-1β and TNF-α were thought to be involved in cartilage destruction by stimulating the synthesis of metalloproteinase and by inhibiting proteoglycan synthesis (Dayer *et al.*, 1985; Schnyder *et al.*, 1987; Tyler, 1985).

In the present study, this CII-induced arthritis model was used to evaluate the anti-inflammatory effect of BV treatment on RA. The results were evaluated in two ways. One was assessment through clinical evaluations of arthritis (the incidence of arthritis, the mean arthritis index, and the number of the arthritic limbs) and the other was the serum inflammatory response (serum inflammatory cytokines: IL-1β and TNF-α).

Materials and Methods

Animals

Twenty-seven male DBA/1 mice (obtained from the Jackson Lab, Westgrove, PN, USA) were used. All mice were between 8 and 12 weeks old, weighing 20 g to 25 g, and were brought up in specific pathogen free (SPF) conditions to prevent inflammation from outside pathogens (22 ± 1°C, 55 ± 5% humidity, hepafiltrated air supply).

Injection

Type II collagen (CII) extracted from bovine articular cartilage (BnCII) obtained from Dr. Andrew H. Kang (University of Tennessee, Memphis, TN), was dissolved in 10 mM acetic acid (4 mg/ml), after which the solution was emulsified in an equal volume of complete

Freund adjuvant (CFA) (Difco Laboratory, Detroit, MI, USA), and mixed for 30 minutes at 1000 rpm with a homogenizer.

Arthritis was induced by an intradermal injection of 50 μℓ of the emulsion (BnCII 100 μg + CFA 100 μg) into the base of the tail. At day 14 after the first adjuvant injection, mice were boosted subcutaneously into the plantar surface with the same volume (50 μℓ) of the emulsion (BnCII 50 μg + CFA 50 μg).

The experimental group (n = 20) was treated with 0.1 ml BV (Sigma, St. Louis, MO; 1 mg/kg) suspended in saline at the Zusanli point (ST36) 5 times twice a week starting 5 weeks after the first BnCII injection. The control group (n = 7) was treated with saline. The Zusanli point was located 5 mm distal and lateral to the anterior tubercle of the tibia.

Measurements

The clinical symptoms of arthritis in all three limbs except the injected foot were observed in the BV treated group and the control group. Arthritis incidence, mean arthritis index, and the number of arthritic limbs were calculated a score with a range of 0 to 4 (score: 0 = no change, 1 = mild swelling and erythema of the mid foot or tarsal bone or the ankle joint, 2 = mild swelling and erythema of the mid foot or tarsal bone through the ankle joint, 3 = moderate swelling and erythema of the ankle joint through the metatarsal bone, and 4 = gross swelling and erythema of the ankle joint through the digit).

The level of serum cytokines IL-1β and TNF-α was measured by ELISA methods.

Data Analysis

All the data were analyzed using a one-way analysis of variance (ANOVA). Significance of differences between the groups was tested with a Mann-Whitney U-test. The criterion for significance was $p < 0.05$.

Results

This study was done to analyze the anti-inflammatory activity of BV in mice with arthritis induced by bovine type-II collagen. The clinical evaluation of type-II collagen-induced arthritis in mice after BV treatment at the Zusanli point is shown in Table 1.

While the incidence of arthritis in the control increased progressively during the whole period of treatment, that of the BV-treated group increased for 2 weeks after the beginning of BV treatment, but gradually decreased to 31.57% after that period, which was significantly lower than that of the control ($p < 0.05$).

The mean arthritis index in the control group increased throughout the experiment. In contrast, that of the group treated with BV increased for 3 weeks after the beginning of the BV treatment, but decreased from that period to the last day of the experiment. The mean arthritis index of the BV-treated group was significantly lower than that of the control group at the 8th week after the first injection of BV ($p < 0.05$).

Table 1. Clinical Assessment of Type-II Collagen-Induced Arthritis in Mice after Bee Venom Treatment at the Zusanli Point According to Arthritis Incidence, Mean Arthritis Index and Number of Arthritic Limbs

Group	After First Injection of BnCII			
	5 Weeks	6 Weeks	7 Weeks	8 Weeks
Arthritis incidence				
Control (n = 7)	62.5	87.5	100	100
Bee venom (n = 20)	36.84	70	63.15	31.57*
Mean arthritis index				
Control (n = 7)	4	5.375	8.5	8.25
Bee venom (n = 20)	1.5	2	6.2	3.385*
Number of arthritic limbs				
Control (n = 7)	25	34.3	87.5	75
Bee venom (n = 20)	12.5	37.5	40.25*	26.62*

BnCII: Native Bovine type-II collagen.
Arthritis incidence is the percent of the number of mice with score ≥2 divided by the total number of mice.
Mean arthritis index is the sum of the score of all mice divided by the total number of mice.
Number of arthritic limbs is the percent of the number of mice with score ≥2 divided by the total number of the limbs.
*$p < 0.05$, compared with control, Mann-Whitney U-test.

In both BV-treated and the control groups, the pattern of the change of the number of the arthritic limbs was similar to that of the mean arthritis index. The number of the arthritic limbs in the BV group was significantly lower than that of the control group at 7th and 8th weeks after the first BnCII injection ($p < 0.05$). Above results suggest that treatment with BV results in inhibition of development of arthritis.

The effect of BV on the production of TNF-α and IL-1β in mice with type-II collagen-induced arthritis using ELISA is shown in Fig. 1. Serum of both groups were collected and proinflammatory cytokines (IL-1β, TNF-α) were measured 1 week after the last BV or saline injection. The production of TNF-α in the BV-treated group was significantly lower than that of the control ($p < 0.05$), but IL-1β was not different between the two groups ($p > 0.05$).

Discussion

Immunization with type-II collagen (CII) is well known to be able to induce inflammatory polyarthritis in rats and susceptible strains of mice (Trentham *et al.*, 1977; Courtenay *et al.*,

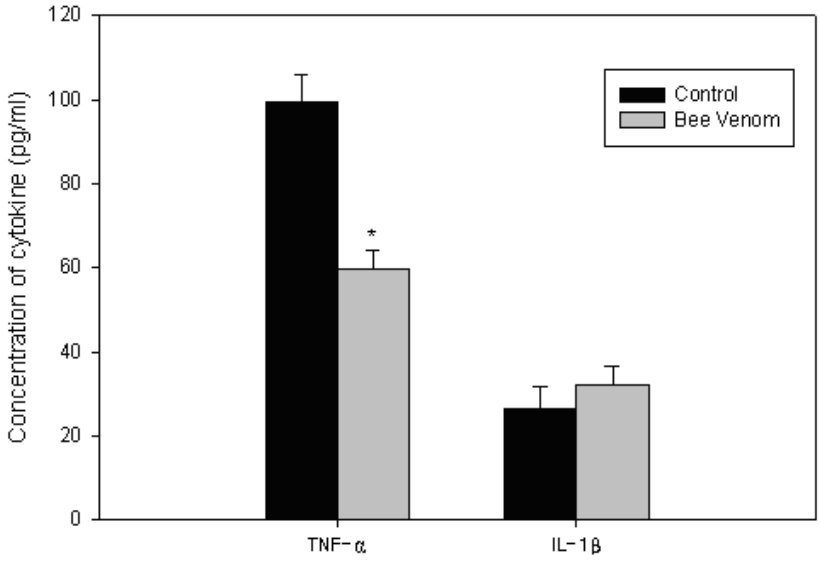

Figure 1. Effect of bee venom on the production of TNF-α and IL-1β in mice with type-II collagen-induced arthritis using ELISA. The bar indicates the standard deviation of the mean (*$p < 0.05$, as compared to the control, Mann-Whitney U-test).

1980). Although immune mechanisms that include both humoral and cellular immunity to CII have been implicated in the pathogenesis of the disease, there is much evidence that anti-CII antibodies play an important role in the initiation of the diseases (Kaibara *et al.*, 1985; Takagishi *et al.*, 1990). Since CII-induced arthritis (CIA) in rats and mice is well known to have both clinical and histological similarities to human RA, these models have been widely used to evaluate anti-arthritic drugs (Kaibara *et al.*, 1985; Takagishi *et al.*, 1990, Paska *et al.*, 1986; Takagishi *et al.*, 1986a). Bee venom (BV) as an anti-arthritic drug has been used in Oriental medicine to treat inflammatory disease such as rheumatoid arthritis (RA) (Billingham *et al.*, 1973; Kwon *et al.* 2001). Yet the potential mechanisms of the anti-arthritic BV effects, including anti-inflammation, are still uncertain.

In the present study, this CII-induced arthritis model was used to evaluate the anti-inflammatory effect of BV for treatment of RA. In this study, the incidence of arthritis increased progressively up to 100% in the control, but the group treated with BV increased to 70% and then decreased back to 31.57% at 8 weeks after first injection of native bovine type-II collagen. The mean arthritis index and number of arthritic limbs in the control were increased. Those of the group treated with BV were increased 21 days after the treatment of BV, but then decreased to 3.385 and 26.62, respectively.

B-cells in patients with RA are usually pronounced and secrete large amounts of autoantibodies which can enhance tissue destruction and the release of autoantigens, indicating that B-cells are also critically important in the severity and length of the disease (Holmdahl *et al.*, 1990). Similarities between RA and CIA include the fact that susceptibility is linked to specific Major Histocompatibility Complex (MHC) class II genes (Staines and Wooley,

1994; Griffoths and Dewitt, 1984). The excessive production of several types of cytokines including IL-1β and TNF-α in the local of affected joints is observed in these two diseases (Mauri *et al.*, 1996; Gattorno *et al.*, 1997; Chu *et al.*, 1991). IL-1β and TNF-α are thought to be involved in cartilage destruction by stimulating the synthesis of metalloproteinase and by inhibiting proteoglycan synthesis (Dayer *et al.*, 1985; Schnyder *et al.*, 1987; Tyler, 1985).

It seems that RA is an autoimmune disease of the cartilage and synovial membranes. In the initiation and development of this disease, immunological and inflammatory pathways are critical, and the antigen-specific T-cell responses to CII are especially important. Immunosuppressive agents targeting T-lymphocytes, such as cyclosporin and the anti-Crossed Diffusrater 4 (anti-CD4) antibody, are used in patients and experimental animals as therapeutic drugs (Takagishi *et al.*, 1986b; Williams and Whyte, 1996). Many investigators have tested the hypothesis that the modulation of immune responses to CII, especially the T-cell-mediated response, can depress the incidence and the severity of arthritis. Treatments using cytokines and anti-cytokine antibodies have been shown to up- and down-regulate the development of arthritis induced by CII and complete Freund's adjuvant (CFA) in rodents (Williams and Whyte, 1996; Taylor *et al.*, 1996).

In the present study, the level of the proinflammatory cytokine TNF-α was significantly lower in the BV group than the control group, but not IL-1β. These results indicate that treatment with BV might be used to inhibit development of human RA.

Acknowledgments

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