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Anti-inflammatory effect of bee venom on antigen-induced arthritis in rabbits: Influence of endogenous glucocorticoids

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Abstract

Aim of the study: This study assessed the involvement of endogenous glucocorticoids (GCs) in the anti-arthritic properties of bee venom (BV) on antigen-induced arthritis (AIA) in rabbits.

Materials and methods: BV (1.5–6 μg/kg/day) was injected for 7 days before AIA induction, whereas the control group received sterile saline. The total and differential leukocyte count, PGE2 levels in synovial fluid and synovial membrane cell infiltrate were evaluated. The contribution of GCs to BV action was assessed in rabbits treated with BV plus metyrapone, an inhibitor of GC synthesis, or RU-38 486, a steroid antagonist.

Results: Treatment with BV (1.5 μg/kg/day) reduced the leukocyte count and PGE2 level (18571 ± 1909 cells/mm3 and 0.49 ± 0.05 ng/mL, respectively) as well as the cellular infiltrate compared with the control group (40968 ± 5248 cells/mm3 and 2.92 ± 0.68 ng/mL, p < 0.05). The addition of metyrapone to BV treatment completely reversed the inhibition of AIA, whereas RU-38 486 was ineffective.

Conclusion: Our data show that bee venom treatment prevents the development of antigen-induced arthritis in rabbits through the action of GCs.

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1. Introduction

Bee venom (BV) is a complex mixture of substances that are known to induce immune/allergic responses in humans. Nevertheless, BV has been used to treat rheumatoid arthritis (RA) for centuries. Clinical trials in RA demonstrated a treatment benefit in patients treated weekly with 0.06–1.62 mg of BV (Maberly, 1910). A recent study in RA patients showed an additive effect when simultaneously applied (bee sting) with classical oral drugs such as methotrexate, sulfasalazine and meloxicam (Liu et al., 2008). However, the placebo effect has also been described in studies investigating BV anti-inflammatory properties in arthritic patients (Hollander, 1941; Steigerwaldt et al., 1966).

The controversial effects of BV have also been described with respect to the experimental model. Lee et al. (2005) reported the best results for inhibition of edema when BV was administered after arthritis induction (Hadjipetrou-Kourounakis and Yiannou, 1988). Regarding mechanism of action, BV suppresses leukocyte migration and TNFα levels in the mouse air pouch (Kwon et al., 2003) and reduces cytokine production upon uptake of the antigen by dendritic cells (Lee et al., 2008) as well as infiltration of synovial CD4+ T cells at the site of inflammation (Kim et al., 2005).

Studies have demonstrated the ability of BV and its major fraction, melittin, to induce an increase in GC levels, which may be responsible for its anti-inflammatory action (Kwon et al., 2001; Lee et al., 2001). Although high levels of GCs have been detected after BV administration, no studies have evaluated the effect of blocking GC production on the anti-inflammatory effect of BV. This study was undertaken to gain further insights on the participation of endogenous GCs in the anti-inflammatory effect of BV on AIA in rabbits.

2. Materials and methods

2.1. Induction of antigen-induced arthritis (AIA)

The Animal Ethics Committee of the Brazilian College of Experimental Animals approved the experimental procedures. Male
adult NZW rabbits were sensitized with methylated bovine serum albumin (mBSA, Sigma–Chemical Company, St. Louis, USA) as previously described (Palácios et al., 1999). AIA was induced in the knee joint by intra-articular injection of 0.5 mL of a sterile solution of mBSA (2 mg/mL). The contralateral joint was injected with saline. Twenty-four hours after the injection, the animal was sacrificed by an overdose of a mixture of xylazine (15 mg/kg, Bayer-Brazil) together with ketamine (150 mg/kg, Parke Davis, Brazil).

2.2. Bee venom treatment

Lyophilized whole BV was maintained at −20°C, dissolved in sterile saline and filtered through an ester cellulose filter (0.22 μm pore size, Millipore, Brazil). The amount of protein was spectrophotometrically estimated at 280 nm before injection.

Two schedules of treatments were employed as follows: (a) subcutaneous injection of one dose of BV (0.75–100 μg/kg/day) simultaneously with AIA induction (n = 3–4), and (b) daily injection (sc.) of seven doses of BV (1.5 g/kg (n = 9); 3.0 g/kg (n = 9); 6.0 g/kg (n = 7)) prior to the induction of arthritis. The control group received sterile saline (n = 12).

2.3. Influence of glucocorticoids

One group of animals (n = 4) was treated with BV (1.5 μg/kg/day/7 days) plus metyrapone (ME, Cyba Geigy, São Paulo, Brazil, 30 mg/kg i.p. injected twice a day for 3 days before AIA induction), which promotes GC deficiency (Sampath-Kumar et al., 1997; Farsky et al., 1995). Another group of animals (n = 6) was treated with BV (1.5 μg/kg/day for 7 days) plus RU-38486 (10 mg/kg, Sigma, Chemical Company, St. Louis, USA), a potent GC receptor antagonist (Moguliewsky and Philiber, 1984; Farsky et al., 1995) 2 h prior to AIA induction. Control groups included untreated animals with AIA and animals with AIA pre-treated with BV (1.5 μg/kg/day for 7 days).

2.4. Sampling of synovial fluid and measurement of leukocytes

After the animals were sacrificed, 2 mL of saline containing EDTA (1 mg/mL) was injected into the knee joint. The synovial fluid (SF) was aspirated, and the synovial membrane (SM) was surgically removed and immersed in 4% buffered formalin. Total and differential leukocyte counts were carried out in a Neubauer chamber and smears stained with hematoxylin and eosin (H&E), respectively.

2.5. Synovial membrane histology

Sections (5 μm) of the SM were stained with H&E for evaluation of the cell infiltrate under light microscopy.

2.6. Assay of eicosanoid

PGE2 levels in the SF were assayed (stored at −80°C) using commercial kits (Amersham, UK) as previously described (Palácios et al., 1999).

2.7. Statistical analysis

Results are expressed as the mean ± SE and were analyzed by repeated measures ANOVA followed by the Newman-Keuls test. p < 0.05 was considered as significant.

3. Results

3.1. Leukocyte influx into the articular cavity

A single dose of BV given concomitantly with AIA induction did not affect leukocyte migration or other inflammatory parameters evaluated 24 h after AIA induction (data not shown). The effect of seven BV injections prior to AIA induction on leukocyte migration to the articular cavity is depicted in Fig. 1A. Only pre-treatment with 1.5 μg/kg/day of BV over 7 days significantly reduced leukocyte influx when compared to the controls (p < 0.001). This reduction was due to a decrease in polymorphonuclear cell (PMN). The SM of arthritic joints exhibited only resident cells (dotted line). The SM of arthritic joints presented high infiltration of PMNs (Fig. 1B). The efficacy of 7 days of BV (1.5 g/kg/day) treatment in reducing cellular infiltration is illustrated in Fig. 1C.

3.2. PGE2 levels in the synovial fluid

An effect of BV treatment was also detected in the intra-articular production of PGE2. Only pre-treatment with 1.5 μg/kg/day of BV for 7 days was significantly effective in reducing PGE2 vs. control (0.49 ± 0.05 vs. 2.92 ± 0.69 ng/mL, respectively; p = 0.006). The values of intra-articular PGE2 levels in animals treated with 3.0 (2.21 ± 0.41 ng/mL) or 6.0 μg/kg/day of BV/7 days (3.13 ± 1.02 ng/kg) did not significantly differ from the control (p = 0.422 and p = 0.861, respectively). The PGE2 levels in the saline-injected joints (0.02 ± 0.01 ng/mL) were still greater than those measured in animals treated with BV (p = 0.001).
of expression of pro-inflammatory genes (Park et al., 2004), could also be explained by GC action, that directly act on this pathway (Almawi et al., 2004).

Further evidence corroborating the contribution of GCs to bee venom AIA inhibition was demonstrated by the complete reversal of the effect of BV when animals were treated with an inhibitor of GC synthesis. Interestingly, the anti-inflammatory action of BV in animals treated with BV plus RU 38 486, a competitive GC receptor inhibitor, was not abolished. The anti-inflammatory effect was probably sustained by prior binding of endogenous GCs to their receptors. Normally, animals receiving the steroid antagonist RU-38 486 behave like adrenalectomized or ME-treated animals (Farsky et al., 1995). However, as in our study, the administration of the receptor antagonist RU-38 486 plus BV did not reduce inflammation in air pouch although caused an increase of GC levels (Kwon et al., 2003). In this study, the pretreatment with beta-adrenergic antagonist reversed the BV-induced inhibitory effect on leucocyte migration, suggesting that the anti-inflammatory effect of BV is partially mediated by adrenal medullary hormone.

Another possible explanation for the maintenance of the anti-inflammatory action of BV with RU-38 486 treatment is the non-genomic mechanism, which is important in mediating some GC effects (Hinz and Hirschelmann, 2000) independent of corticosteroid type II receptor occupation (Liu et al., 2005; Long et al., 2005). However, the above-mentioned effects of insensitivity to RU-38 486 were not observable with doses of GCs detected in the plasma only under pulse therapy (Liu and Hirschelmann, 2005).

Although we are aware that other mechanisms may account for this property, the present study suggests that endogenous GCs contribute, at least in part, to the anti-inflammatory action of BV.

**4. Discussion and conclusion**

Our investigation into the anti-inflammatory properties of BV confirms and extends previous observations and also demonstrates the contribution of endogenous GCs to this effect.

Beyond the experimental model employed is very similar to RA in humans, the great advantage of the present study design is the ability to suppress the synthesis of GCs or to inhibit their effects and simultaneously evaluate the consequences in terms of inflammation. Another important aspect is the use of a small dose of the venom (1.5 μg/kg/day/7 days) that corresponds to approximately 90 μg in an adult person (approximately one bee sting, Thomsen et al., 1984). Using this specific dose, we found an anti-inflammatory effect with pre-treatment of BV but not when the venom was applied simultaneously to AIA induction. The controversial findings regarding BV anti-inflammatory action might be related to different doses and experimental models as well as the frequency and routes of administration (Kwon et al., 2003; Lee et al., 2005; Moon et al., 2007; Stuhlmeier, 2007; Chen et al., 2009).

The observation of an edematogenic response where the venom had been injected led us to hypothesize that BV induces an initial inflammatory response that might be related to an increase in endogenous GC levels (Vick et al., 1972), which in turn regulates the course of arthritis in the joint (Garcia-Leme and Schapoval, 1975). Based on these inferences, we could justify both our own and other results showing that BV does not have an effect on established arthritis (Hadjipetrou-Kourounakis and Yiango, 1988; Chang and Bliven, 1979). The contribution of GCs to BV action has been confirmed by a study showing that melittin produces an increase in GC levels that is correlated with their antiarthritic effects (Dunn and Kilion, 1988). In addition, GCs inhibit the leukocyte-endothelial interaction, which could explain the reduced cellular migration to the inflammatory site (Farsky et al., 1995). The low levels of intra-articular PGE2 also provide supporting evidence of GC involvement in the anti-arthritic effect of BV, as the activities of PLA2 and COX-2 are known to be decreased by GCs (Crofford, 1997). Of note, BV-induced inactivation of NFκB, one of the most important regulators

**References**


