BEE VENOM INHIBITS SUPEROXIDE PRODUCTION BY HUMAN NEUTROPHILS

STANLEY D. SOMERFIELD, JEAN-LOUIS STACH, CHARLES MRAZ, FRANCINE GERVAIS, and EMIL SKAMENE

2Montreal General Hospital Research Institute
Montreal, Quebec, Canada
3Middlebury, Vermont

Abstract—Investigation of the antiinflammatory properties of bee venom demonstrates that it inhibits production of superoxide anion by human neutrophils in a potent, selective, nontoxic, dose-dependent fashion, both pre- and poststimulation by particulate and soluble activators of the neutrophil oxidative metabolism burst. The effect is not due to receptor competition, superoxide dismutase, and/or catalase activity, scavenging, or indicator media effects. These findings may explain the antiinflammatory effects of whole bee venom in experimental systems, its widespread use in folk medicine, and lead to the development of potent, new antiinflammatory substances for therapeutic use in man.

INTRODUCTION

Bee stings, or bee venom therapy (apitherapy), have been reported to be effective in the treatment of human rheumatic disease (1, 2) and experimental animal models of chronic inflammation (3–5). It is widely practiced throughout the world as folk medicine. Apitherapy involves direct application of bee stings to the site of chronic inflammation (1, 2) and despite medical scepticism as to its clinical effects (6), popular belief in its efficacy persists.

Chang and Bliven (4) found that bee venom (BV) (0.5 mg/rat subcutaneously) and cyclophosphamide (60 mg/kg per os) behaved similarly in suppressing adjuvant-induced arthritis in the rat and showed a distinctly different temporal pattern of activity from steroid therapy (4), countering the argument that the effects observed were due to stress-induced steroid production.

1Supported by grants from Medical Research Council of Canada (No. 6431), Arthritis Society of Canada (No. 5-261-83), Arthritis Foundation of New Zealand, and Rose Hellaby Trust.
The apparent potency of bee venom in animal models of arthritis (4), the possible immunomodulatory activity of the substance in producing its effect (4), the dissimilarity to steroid therapy (4), the apparent lack of local toxicity of bee stings, and the similarity of the effect of bee venom on disease models affected favorably by superoxide dismutase enzyme administration (7, 8) stimulated us to assess the effect of bee venom in nontoxic doses on the production of superoxide anion (O$_2^-$) by human peripheral blood polymorphonuclear leukocytes.

**MATERIALS AND METHODS**

Human neutrophils were obtained and separated from heparinized blood as described (9). Polymorphonuclear leukocytes (PMNs) were washed twice and resuspended in Hanks' buffer without phenol red (Gibco) at 2.0 x 10$^7$ cells/ml, for use in assay systems. Bee venom (BV) was obtained as described (10) and dissolved in Hanks' buffer at suitable concentrations for study. Bee venom was added to cells in reaction cuvettes either 4 min before or immediately following commencement of an assay at final concentrations between 0.5 and 2.0 µg/ml as indicated in results. The toxic level of BV for PMNs was found to be >10 µg/ml by trypan blue exclusion, following 30 min incubation at 37°C.

Superoxide anion production was measured in a continuous system (11) using phorbol myristate acetate (PMA), f-Met-Leu-Phe (FMLP), and opsonized zymosan (OZ) as production stimulants, using previously described methods for each system (12). Hydrogen peroxide (H$_2$O$_2$) production by PMN in response to PMA stimulation was measured by the method of Pick and Keisari (13), using BV-treated (1.5 µg/ml) and control nontreated cells. Receptor competition was examined at 4°C by incubating cells with tritiated FMLP for 30 min, washing twice in Hanks', resuspending in fresh buffer, and incubating with either BV 2 µg/ml or buffer alone for 5 min, then measuring radioactivity remaining on cells and that displaced into the surrounding medium. The effects of BV on in vitro O$_2^-$ production using a xanthine oxidase-hypoxanthine system as described (14) was assessed.

Each reaction contained 1 ml of cytochrome c (1 mg/ml) indicator medium and either xanthine oxidase (XO) 0.1 units/ml, hypoxanthine (HX) 1 x 10$^{-4}$ M, both with or without bee venom 2 µg/ml and/or superoxide dismutase (SOD) 50 µg as shown in Results. Each reaction system was incubated at 37°C for 15 min and read at 550 nM as described (14). To assess any catalase-like effect of BV at concentrations inhibitory to O$_2^-$ production, 3 µg BV was reacted with 1 ml 25 nM standardized H$_2$O$_2$ solution. This higher BV concentration was chosen to maximize any such possible catalase effect. Control received the equivalent amount of buffer only. Following 20 min incubation at room temperature the optical density of treated and control solutions were compared against a blank at 610 nm as described (13) using catalase (Sigma) 200 units as positive control.

**RESULTS**

The dose-dependent inhibitory effect of whole BV on PMA-mediated superoxide production from normal human PMNs is shown in Figure 1. This