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Original Research Communication

Regulation of Vascular Responses to Inflammation: Inducible Matrix Metalloproteinase-3 Expression in Human Microvascular Endothelial Cells Is Sensitive to Antiinflammatory *Boswellia*

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ABSTRACT

Endothelial cells are critical elements in the pathophysiology of inflammation. Tumor necrosis factor (TNF) α potently induces inflammatory responses in endothelial cells. Recently we have examined the genetic basis of the antiinflammatory effects of *Boswellia* extract (BE) in a system of TNFα-induced gene expression in human microvascular endothelial cells (HMECs). Of the 522 genes induced by TNFα in HMECs, 113 genes were sensitive to BE. BE prevented the TNFα-induced expression of matrix metalloproteinases (MMPs). In the current work, we sought to test the effects of BE on TNFα-inducible MMP expression in HMECs. Acetyl-11-keto-beta-boswellic acid (AKBA) is known to be an active principle in BE. To evaluate the significance of AKBA in the antiinflammatory properties of BE, effects of BE containing either 3% (BE3%) or 30% (BE30%, 5-Loxin®) were compared. Pretreatment of HMECs for 2 days with BE potently prevented TNFα-induced expression and activity of MMP-3, MMP-10, and MMP-12. *In vivo*, BE protected against experimental arthritis. In all experiments, both *in vitro* and *in vivo*, BE30% was more effective than BE3%. In sum, this work lends support to our previous report that BE has potent antiinflammatory properties both *in vitro* as well as *in vivo*. *Antioxid. Redox Signal.* 8, 653–660.

INTRODUCTION

INFLAMMATORY DISEASE REPRESENT a major problem in human health (11, 13–15, 29, 31). At present, monoclonal antibody-based therapeutics show clear promise in treating inflammatory disorders (19, 24). The central role of tumor necrosis factor (TNF) α in causing inflammation was initially provided by the demonstration that anti-TNFα antibodies added to *in vitro* cultures of a representative population of cells derived from diseased joints inhibited the spontaneous production of interleukin-1 and other proinflammatory cytokines. Systemic administration of anti-TNFα antibody or soluble TNF receptor fusion protein to mouse models of rheumatoid arthritis was shown to be antiinflammatory and protective for joints (19, 20). Clinical investigations in which the activity of TNFα in rheumatoid arthritis patients was blocked with intravenously administered infliximab, a chimeric anti-TNFα monoclonal antibody, has provided evidence that TNF regulates interleukin-6, interleukin-8, monocyte chemoattractant protein-1, and vascular endothelial growth factor production,

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recruitment of immune and inflammatory cells into joints, angiogenesis, and reduction of blood levels of matrix metalloproteinases (MMPs)-1 and -3 (19, 20). The development of anti-TNF therapy is a key step forward in rheumatology as it is the first new therapy based on investigating the molecular mechanisms of this disease. Despite such major breakthrough in investigative medicine, the fact remains that a vast population of individuals in developing countries suffering from inflammatory disorders do not benefit from monoclonal antibody-based therapy primarily because of excessive cost of acquisition and limited availability. For example, the cost associated with a single dose of infliximab is several thousand U.S. dollars (49).

The medical cost of rheumatoid arthritis averages $5,919 per case per year in the United States and approximately £2,600 per case per year in the United Kingdom. Current slow-acting antirheumatic drugs have limited efficacy and many side effects. Moreover, they do not improve the long-term prognosis of rheumatoid arthritis (11). The use of medicinal plants to treat inflammatory disorders continues to be in practice worldwide (8, 10, 12, 18, 30, 36). Medicinal plants often contain complex mixtures of phytochemicals that have additive or synergistic interactions. The antiinflammatory properties of herbal preparations have been recognized in ancient Indian and Chinese medical literature. The gum resin of *Boswellia serrata,* known in Indian Ayurvedic system of medicine as *Salai guggal,* contains boswellic acids (BAs), which have been shown to inhibit leukotriene biosynthesis. Compounds from the gum with proven antiinflammatory effects are pentacyclic triterpenes of the BA type. Recently, the tetracyclic triterpene 3-oxo-tirucallic acid has been identified as a key active principle in *Boswellia* resin (7). BAs function as specific, nonredox inhibitors of leukotriene synthesis either interacting directly with 5-lipoxygenase or blocking its translocation (2, 4, 5). Among the BAs, acetyl-11-keto-beta-BA (AKBA) potently inhibits 5-lipoxygenase product formation with a 50% inhibitory concentration of 1.5 μM. In contrast to the redox type 5-lipoxygenase inhibitor nordihydroguaiaretic acid, BAs in concentrations up to 400 μM did not impair the cyclooxygenase and 12-lipoxygenase in isolated human platelets and the peroxidation of arachidonic acid by Fe-ascorbate. These data support that BAs are specific, non–reducing-type inhibitors of 5-lipoxygenase (41). In addition to their effects on the lipoxygenase system, certain BAs inhibit elastase in leukocytes, inhibit proliferation, induce apoptosis, and inhibit topoisomerases of leukoma and glioma cell lines. A series of chronic inflammatory diseases are plausibly perpetuated by leukotrienes. In clinical trials promising results supporting the antiinflammatory effects of *Boswellia* extract (BE) were observed in patients with rheumatoid arthritis, chronic colitis, ulcerative colitis, Crohn's disease, bronchial asthma, and peritumoral brain edema (3, 22, 23). The enzymatic oxidation of arachidonic acid yields potent pathological agents by two major pathways: cycloxygenase and lipoxygenase. The lipoxygenase pathway generates a new class of arachidonic acid oxygenation products, called the leukotrienes, which mediate inflammation. Unlike the prostaglandins, some of which play important roles as biological regulators, the action of the lipoxygenase products appear to be exclusively of a pathological nature (28). Thus, the anti-lipoxygenase effects of BAs are likely to have therapeutic implications.

Endothelial cells are critical elements in the pathophysiology of inflammation. TNFα potently induces inflammatory responses in endothelial cells (37). Recently we have examined the genetic basis of the antiinflammatory effects of BE in a system of TNFα-induced gene expression in human microvascular endothelial cells (HMECs) (40). We conducted the first whole genome screen for TNFα-inducible genes in human microvascular cells. Acutely, TNFα induced 522 genes and downregulated 141 genes in nine out of nine pair-wise comparisons. Of the 522 genes induced by TNFα in HMECs, 113 genes were clearly sensitive to BE treatment (40). Such genes directly related to inflammation, cell adhesion, and proteolysis. The robust BE-sensitive candidate genes were then subjected to further processing for the identification of BE-sensitive signaling pathways. The use of resources such as GenMAPP, KEGG, and gene ontology led to the recognition of the primary BE-sensitive TNFα-inducible pathways. BE prevented the TNFα-induced expression of MMPs. In the current work, we sought to test the effects of BE on TNFα-inducible MMP expression in HMECs. The Freund's adjuvant-induced rat paw edema experimental system was utilized to test the significance of the antiinflammatory properties of BE *in vivo*.

MATERIALS AND METHODS

Materials

Standardized BEs—BE3% and BE30% (also known as 5-Loxin®)—were obtained from Laila Impex (Vijayawada, India). BE3% contained 1–3% AKBA, 4–6% keto-beta-BA, 15–20% beta-BA, and 8–10% acetyl-beta-BA. BE30% containing 30– 35% 3-AKBA was prepared according to the procedure described in U.S. patent application number 2004/0073060A1. Prednisolone (Wyeth Lederle Ltd., Mumbai, India) and carboxymethyl cellulose (CMC) (Loba Chemie, Mumbai) were purchased for *in vivo* studies. Unless otherwise stated all other chemicals and reagents were obtained from Sigma Chemical Co. (St. Louis, MO) and were of analytical grade or the highest grade available.

Cells and cell culture

HMEC-1 cells were cultured under standard culture conditions in MCDB-131 growth medium (GIBCO-BRL, Grand Island, NY) supplemented with 10% fetal bovine serum, 100 IU/ml penicillin, 0.1 mg/ml streptomycin, and 2 mmol/L L-glutamine (32).

MMP mRNA quantitation

The mRNA were quantified by real-time polymerase chain reaction (PCR) assay using double-stranded DNA binding dye SYBR Green-I as described previously (39). The primer set used for the genes were:

h_β-actin forward, 5' GTA CCA CTG GCA TCG TGA TGG ACT 3';

h_β-actin reverse, 5' CCG CTC ATT GCC AAT GGT GAT 3';

h_MMP-3 forward, 5' GAG GCT GAT ATA ATG ATC TC 3';

h_MMP-3 reverse, 5' TAA ATT GGT CCC TGT TGT AT 3';

h_MMP10 forward, 5' CCC ACT CTA CAA CTC ATT CA 3';

h_MMP10 reverse, 5' ACA AAG CAG GAT CAC ACT TG 3';
h_MMP12 forward, 5' TTC GAT GAG GAC GAA TTC TGG ACT A 3';
h_MMP12 reverse, 5' TCA TCA GCA GAG AGG CGA AA 3'.

MMP-3 enzyme-linked immunosorbent assay (ELISA)

MMP-3 ELISA is a non-isotopic immunoassay for the *in vitro* quantitation of human MMP-3 protein. Pro-MMP-3 was detected from culture media using a commercially available ELISA kit (Oncogene Research Products, San Diego, CA) following the manufacturer's instructions. This ELISA specifically detects pro-MMP-3 from protein samples.

MMP-3 activity assay

Total (pro- and active) MMP-3 activity was determined using a Biotrak MMP-3 activity assay system (Amersham Biosciences, Piscataway, NJ).

Freund's adjuvant-induced inflammation

Wistar Albino rats were supplemented with BE30% (25, 50, and 100 mg/kg of body weight orally), BE3% (100 mg/kg of body weight orally) in 1% CMC, or prednisolone (10 mg/kg of body weight orally) for 30 days. The control group received the same volume of vehicle (*i.e.,* 1% CMC) orally. On day 31, 50 μl of complete Freund's adjuvant was injected subcutaneously in the subplantar region of the left hind paw. The paw volume was measured before and after the injection of Freund's adjuvant. The difference in volume of edema on day 13 after injection and on the day of injection is calculated to estimate the inflammatory response (35).

Statistical analyses

Results were expressed as means ± standard deviation. Means were compared using analysis of variance. A value of $p < 0.05$ was interpreted as statistically significant.

RESULTS

Recently, we have conducted the first whole genome screen for TNFα-inducible genes in human microvascular cells. We had observed that out of 522 genes induced by TNFα in HMECs, 113 genes were significantly sensitive to BE30% treatment. Employing resources such as the GenMAPP, KEGG, and gene ontology, BE-sensitive TNFα-inducible pathways were identified (40). The MMP family emerged as a prominent class of TNFα-inducible genes sensitive to BE30%.

In this follow-up work, members of the MMP family of genes were subjected to hierarchical clustering. MMP-3, MMP-10, MMP-12, and MMP-19 were recognized as TNFα-inducible genes that were sensitive to BE30% (Fig. 1). A quantitative real-time PCR approach was utilized in this study to follow up on our results from the microarray study. In HMECs, TNFα caused a dose-dependent induction of MMP-3, MMP-10, and MMP-12 (Fig. 2). Pretreatment of HMECs for 2 days with BE30% potently prevented TNFα-induced expression of MMP-3, MMP-10, and MMP-12 (Fig. 3).

To test whether the AKBA content of BE influenced the observed outcome, BE30% was compared with BE3%. BE30% was observed to be significantly more potent than BE3% in preventing TNFα-induced expression of MMP-3, MMP-10, and MMP-12 (Fig. 3).

MMP-3 possesses well-defined critical biological functions (51). Thus, we sought to focus on the regulation of TNFα-inducible MMP-3 expression by BE. To test whether the observed effects of BE on TNFα-induced MMP-3 gene expression manifest at the level of protein expression, MMP-3 ELISA was performed. Consistent with the findings with quantitative gene expression, BE potently inhibited TNFα-induced MMP-3 protein expression. AKBA content of BE influenced the efficacy. BE30% was significantly more potent than BE3% in preventing TNFα-induced MMP3 protein expression (Fig. 4).

MMP-3 is also a collagen-decomposing enzyme. To investigate the effect of BE on the TNFα-induced increase in MMP-3 activity, we employed an assay that uses the pro form

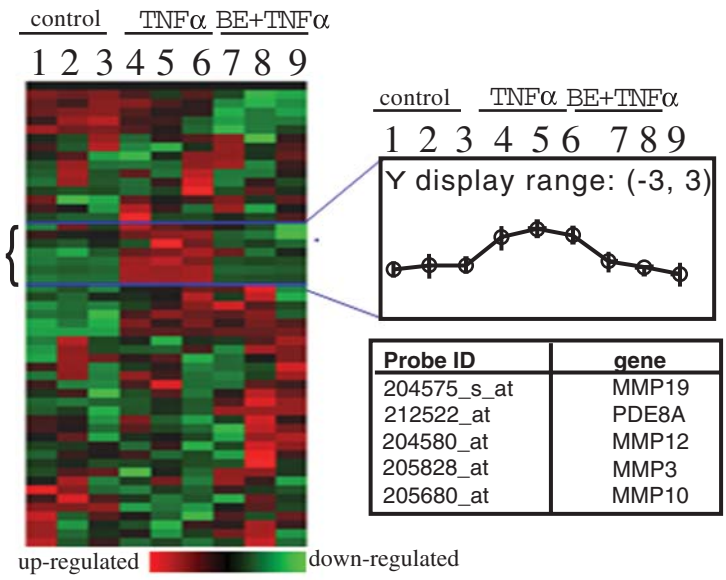

FIG. 1. *Boswellia*-sensitive genes of the MMP family in TNFα-treated HMECs. (Left panel) GeneChip data (40) were subjected to hierarchical clustering to visualize expression pattern of BE-sensitive MMP-family genes. TNFα, 50 ng/ml, 6 h; TNFα + BE, 48-h pretreatment with BE30% (25 μg/ml) followed by TNFα for 6 h. Red to green gradation in color represent higher to lower expression signal. **(Upper right panel)** The line graphs show the average pattern of gene expression in the corresponding cluster graph. **(Lower right panel)** The members [probe identification (ID) and gene abbreviations] of the cluster are shown. PDE8A, phosphodiesterase 8A. MMP-12 is also known as macrophage elastase; MMP-3, as stromelysin 1 or progelatinase; and MMP-10, as stromelysin 2.

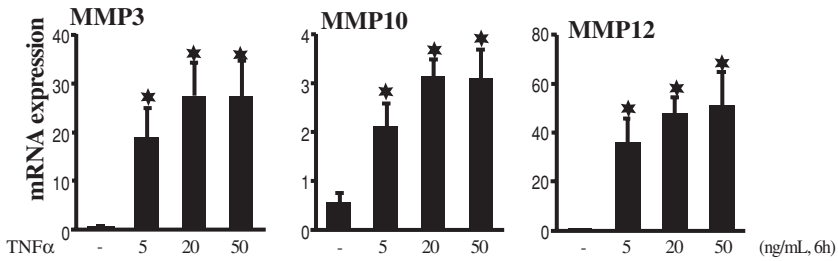

FIG. 2. TNFα-induced MMP gene expression. HMECs were activated with different dosages (5–50 ng/ml) of recombinant human TNFα for 6 h. Expression levels of MMP-3, -10, and -12 mRNA were determined using real-time PCR. The data presented are normalized for β-actin (housekeeping gene) expression. Data represent mean ± standard deviation. *$p < 0.001$, significantly different compared to control (untreated) sample.

of a detection enzyme that can be activated by captured active MMP-3, and converted into an active detection enzyme, through a single proteolytic event. MMP-activated detection enzyme is then measured using a specific chromogenic peptide substrate. Using this assay system, free active MMP-3 can be detected. In order to measure the total MMP-3 content any bound MMP-3 in its pro form was activated using p-aminophenylmercuric acetate. TNFα significantly induced MMP-3 activity in HMECs. Such inducible MMP-3 activity was significantly inhibited in the presence of BE. BE30% was significantly more potent than BE3% in restricting TNFα-induced MMP-3 activity in HMECs (Fig. 5).

Finally, we sought to test the antiinflammatory properties of the two BE preparations in a setting of experimental arthritis. Prednisolone, used as a positive control, attenuated the adjuvant-induced inflammatory response. BE protected against the induced inflammatory response. The effect was maximal at the lowest dose studied. Compared to BE3%, BE30% was significantly more effective in preventing the adjuvant-induced inflammatory response *in vivo* (Fig. 6).

DISCUSSION

MMPs are a family of zinc-containing enzymes involved in the degradation and remodeling of extracellular matrix proteins. Under normal physiological conditions, the activities of these enzymes are well regulated by endogenous tissue inhibitors of metalloproteinases (TIMPs). Chronic stimulation of MMP activities, due to an imbalance in the levels of MMPs and TIMPs, has been implicated in the pathogenesis of a variety of diseases such as cancer, osteoarthritis, and rheumatoid arthritis. Thus, MMP inhibitors are expected to be useful for the treatment of these disorders. Because of their importance in a variety of pathological conditions, several small-molecular-weight MMP inhibitors have entered clinical trials in humans. However, the results of these trials have been disappointing (47). Herbal medicines are widely used in the United States, with approximately one-quarter of adults reporting use of an herb to treat a medical illness within the past year. Of the 10 most commonly used herbs in the United States, systematic reviews have concluded that only four are likely to be effective, and there is very limited evidence to evaluate the efficacy of the approximately 20,000 other available products (6).

Stromelysin or MMP-3 production is increased in rheumatoid arthritis, and has been proposed as a marker of joint damage (33, 48). Direct evidence substantiating the implication of MMP-3 in inflammation has been reported (50). In addition to being proinflammatory, MMP-3 has been proven to be a cartilage-degrading enzyme (54). Recently, neurodegenerative properties of MMP-3 have been reported (25). The cur-

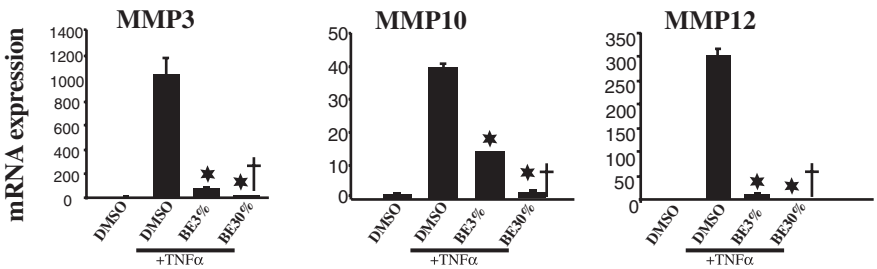

FIG. 3. *Boswellia* potently down-regulated TNFα-induced MMP mRNA expression. HMECs were treated with 25 μg/ml BEs (BE3% or BE30%) for 48 h. BEs were prepared in dimethyl sulfoxide (DMSO) at concentrations such that the final concentration of the solvent in cell suspension never exceeded 0.1% (vol/vol). Controls were treated with a matching volume of DMSO. After the treatment period, cells were activated with recombinant human TNFα (50 ng/ml) for 6 h. Expression of MMP-3, -10, and -12 mRNA was determined using real-time PCR. The data presented are normalized for β-actin (housekeeping gene) expression. Data represent mean ± standard deviation. *$p < 0.05$, significantly lower compared to TNFα-treated sample. †$p < 0.05$, significantly lower compared to BE3%-treated sample.

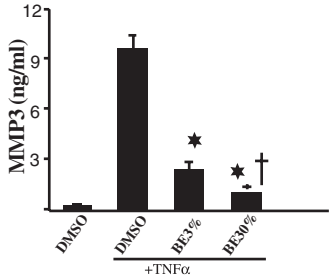

FIG. 4. *Boswellia* inhibited TNFα-induced MMP-3 protein expression. HMECs were treated with 25 μg/ml BEs (BE3% or BE 30%) for 48 h. After the treatment period, cells were activated with recombinant human TNFα (50 ng/ml) for 24 h. Pro-MMP-3 levels in culture media were determined using ELISA. Data (mean ± standard deviation) are presented as actual MMP-3 levels (ng/ml) in the culture media. *$p < 0.05$, significantly lower compared to TNFα-treated sample. †$p < 0.05$, significantly lower in compared to BE3%-treated sample. DMSO, dimethyl sulfoxide.

rent work presents the first evidence demonstrating the ability of an herbal preparation, BE, to specifically inhibit inducible MMP-3 gene and protein expression as well as catalytic activity. Plant-derived compounds represent potential molecules for the development of new drugs, especially designed for the treatment and control of several inflammatory states (9). Medicinal plants belonging to the Burseraceae family, including *Boswellia,* are especially known for their antiinflammatory properties (16).

B. serrata (frankincense) has been used in traditional medicine for treatment of inflammatory diseases since antiquity. Osteoarthritis is a common, chronic, progressive, skeletal, degenerative disorder, which commonly affects the knee joint.

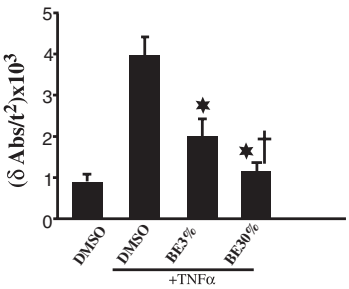

FIG. 5. *Boswellia* down-regulated TNFα-induced MMP-3 activity. HMECs were treated with 25 μg/ml BEs (BE3% or BE 30%) for 48 h. After the treatment period, cells were activated with recombinant human TNFα (50 μg/ml) for 24 h. Pro- and active MMP-3 activity was determined using a Biotrak MMP-3 activity assay system (see Materials and Methods). Data (mean ± standard deviation) are presented as actual MMP-3 activity in the culture media. The MMP-3 activity is represented by the rate of change of absorbance at 405 nm, i.e., $\delta Abs_{405}/t^2$, where t is the incubation time in hours. Data are expressed as $[(\delta Abs_{405}/t^2) \times 10^3]$. *$p < 0.05$, significantly lower compared to TNFα-treated sample. †$p < 0.05$, significantly lower compared to BE3%-treated sample. DMSO, dimethyl sulfoxide.

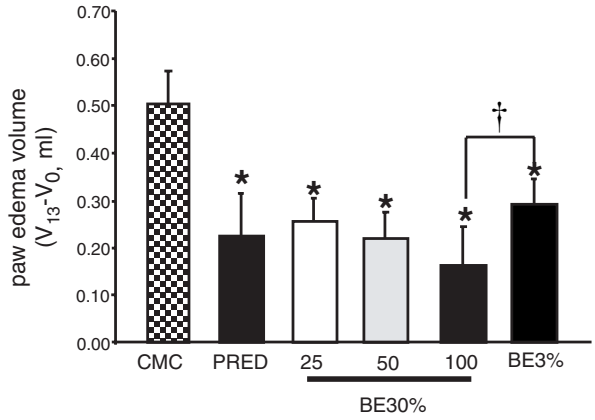

FIG. 6. BE protects against Freund's adjuvant-induced arthritis in rats. Wistar Albino rats ($n = 6$) were supplemented with BE30% (25, 50, and 100 mg/kg of body weight orally), BE3% (100 mg/kg of body weight orally) in 1% CMC, or prednisolone (PRED, 10 mg/kg of body weight orally) for 30 days. The control group (marked CMC) received the same volume of vehicle (*i.e.,* 1% CMC) orally. On day 31, 50 μl of complete Freund's adjuvant was injected subcutaneously in the subplantar region of the left hind paw. V_0 and V_{13} represent the paw volume on days 0 and 13, respectively, after adjuvant injection. *$p < 0.05$, significantly lower than CMC. †$p < 0.05$ significantly lower in BE30% compared to BE3%.

An open multicenter Swiss veterinary clinical trial, comparing conditions before and after treatment with a herbal dietary supplement consisting of a natural resin extract of *B. serrata,* led to the conclusion that a standardized preparation of BE can be recommended as a herbal dietary supplement providing symptomatic support in canine osteoarthritic disease. In dogs with manifestations of chronic joint and spinal disease, it was observed that BE (40 mg/kg of body weight, once a day, for 6 weeks) significantly reduced the severity and caused resolution of typical clinical symptoms in 71% of 24 dogs eligible for the study (38). These findings in the canine are consistent with observations in humans. A randomized double-blind placebo-controlled crossover study has been conducted to assess the efficacy, safety, and tolerability of BE in 30 patients of osteoarthritis of the knee, with 15 each receiving active drug or placebo for 8 weeks. After the first intervention, washout was given, and then the groups were crossed over to receive the opposite intervention for 8 weeks. All patients receiving drug treatment reported significant decrease in knee pain, increased knee flexion, and increased walking distance. The frequency of swelling in the knee joint was significantly decreased (26). Another inflammatory condition that has been clinically tested for its sensitivity to BE is colitis. Thirty patients (17 males and 13 females; age range 18–48 years) with chronic colitis were included in this study. Twenty patients were given a preparation of the gum resin of *B. serrata* (900 mg daily divided into three doses for 6 weeks), and 10 patients were given sulfasalazine (3 g daily divided into three doses for 6 weeks) and served as controls. Out of 20 patients treated with *Boswellia* gum resin, 18 patients showed an improvement in one or more parameters, including stool proper-

ties, histopathology as well as scanning electron microscopy, hemoglobin, serum iron, calcium, phosphorus, proteins, total leukocytes, and eosinophils. In the control group, six out of 10 patients showed similar results with the same parameters. Thus, the study showed that a gum resin preparation from *B. serrata* was effective in the treatment of chronic colitis (23). Consistent with this, BE has been observed to be beneficial in treating the inflammatory bowel disease ileitis (27).

BAs have been studied for over 30 years (17). AKBA is a naturally occurring pentacyclic triterpene isolated from the gum resin exudate from the stem of the tree *B. serrata*. AKBA has been recently identified as a novel, orally active, non-redox, noncompetitive 5-lipoxygenase inhibitor that also inhibits topisomerases I and II *in vitro* (34). This finding is consistent with our current finding that BE containing a higher concentration of AKBA is more effective as an antiinflammatory agent. In humans, orally taken BE manifests as plasma keto-BA. The peak plasma levels of BE were reached at 4.5 h. The plasma concentration attained a steady state after approximately 30 h. BE has proven to be safe and well tolerated on oral administration in humans. No adverse effects were seen with this drug when administered as a single dose of 333 mg (46).

The inflammatory process in colitis is associated with increased formation of leukotrienes causing chemotaxis, chemokinesis, synthesis of superoxide radicals, and release of lysosomal enzymes by phagocytes. The key enzyme for leukotriene biosynthesis is 5-lipoxygenase. The pentacyclic triterpenes from the 11-keto-BA series have been identified as the active principal ingredients of *Boswellia* resin, inhibiting the key enzyme of leukotriene biosynthesis, 5-lipoxygenase (43, 45). Biochemical studies have established that BAs are selective, non-redox, potent inhibitors of the biosynthesis of leukotrienes *in vitro* (21, 53). Like other non-redox 5-lipoxygenase inhibitors, BAs require glutathione peroxidase for the efficient inhibition of 5-lipoxygenase activity (52). Among the BAs, AKBA induced the most pronounced inhibition of 5-lipoxygenase product formation with a 50% inhibitory concentration of 1.5 μM. In contrast to the redox type 5-lipoxygenase inhibitor nordihydroguaiaretic acid, BA in concentrations up to 400 μM did not impair the cyclooxygenase and 12-lipoxygenase in isolated human platelets and the peroxidation of arachidonic acid by Fe-ascorbate (41). AKBA is the only leukotriene-synthesis inhibitor so far identified that inhibits 5-lipoxygenase activity as an allosteric regulator and not by a reducing or competitive mechanism. In the presence of calcium, AKBA binds to a site that is distinct from the substrate binding site of 5-lipoxygenase (44).

Leukotrienes are implicated in inflammatory disorders associated with bronchial asthma. In a double-blind, placebo-controlled study 40 patients (23 males and 17 females; age range 18–75 years; mean duration of bronchial asthma 9.58 $\pm$ 6.07 years) were treated with a preparation of BE (300 mg three times a day for a period of 6 weeks). Most (70%) patients showed improvement of disease as evident by disappearance of physical symptoms and signs such as dyspnea, rhonchi, number of attacks, increase in forced expiratory volume in 1 s, forced vital capacity, and peak expiratory flow rate as well as decrease in eosinophilic count and eosinophil sedimentation rate. In the control group, 40 patients were treated with lactose (300 mg three times a day for 6 weeks). Only 27% of patients in the control group showed improvement. These findings point towards a definite role of BE in the treatment of bronchial asthma (22). Screening for additional effects of BA on further proinflammatory pathways led to the observation that AKBA decreased the activity of human leukocyte elastase *in vitro* with a 50% inhibitory concentration of about 15 μM. Dual inhibition of 5-lipoxygenase and human leukocyte elastase is unique to BAs (42). Both leukotriene formation and human leukocyte elastase release are increased simultaneously by neutrophil stimulation in a variety of inflammation- and hypersensitivity-based human diseases.

In sum, this work lends support to our previous reporting that BE has potent antiinflammatory properties both *in vitro* and *in vivo*. Higher AKBA content improved the antiinflammatory properties of BE. Although it has been recently reported that 11-keto-BA might function as a potent activator of polymorphonuclear leukocytes by stimulation of mitogen-activated protein kinase and mobilization of intracellular Ca^{2+} (1), very little is known about BE-sensitive pathways within the cell. Further studies characterizing the BE-sensitive mechanisms involved in regulating gene expression relevant to inflammation are warranted.

ABBREVIATIONS

AKBA, alpha-11-keto-boswellic acid; BA, boswellic acid; BE, *Boswellia* extract; CMC, carboxymethyl cellulose; ELISA, enzyme-linked immunosorbent assay; HMEC, human dermal microvascular endothelial cell; MMP, matrix metalloproteinase; PCR, polymerase chain reaction; TIMP, tissue inhibitor of metalloproteinases; TNF, tumor necrosis factor.

REFERENCES

1. Altmann A, Fischer L, Schubert-Zsilavecz M, Steinhilber D, and Werz O. Boswellic acids activate p42(MAPK) and p38 MAPK and stimulate Ca^{2+} mobilization. *Biochem Biophys Res Commun* 290: 185–190, 2002.
2. Ammon HPT. *Salai Guggul–Boswellia serrata:* from a herbal medicine to a non-redox inhibitor of leukotriene biosynthesis. *Eur J Med Res* 1: 369–370, 1996.
3. Ammon HPT. Boswelliasauren (Inhaltsstoffe des Weihrauchs) als wirksame Prinzipien zur Behandlung chronisch entzundlicher Erkrankungen. *Wien Med Wochenschr* 152: 373–378, 2002.
4. Ammon HPT, Mack T, Singh GB, and Safayhi H. Inhibition of leukotriene B4 formation in rat peritoneal neutrophils by an ethanolic extract of the gum resin exudate of *Boswellia serrata. Planta Med* 57: 203–207, 1991.
5. Ammon HPT, Safayhi H, Mack T, and Sabieraj J. Mechanism of antiinflammatory actions of curcumine and boswellic acids. *J Ethnopharmacol* 38: 113–119, 1993.

6. Bent S and Ko R. Commonly used herbal medicines in the United States: a review. *Am J Med* 116: 478–485, 2004.
7. Boden SE, Schweizer S, Bertsche T, Dufer M, Drews G, and Safayhi H. Stimulation of leukotriene synthesis in intact polymorphonuclear cells by the 5-lipoxygenase inhibitor 3-oxo-tirucallic acid. *Mol Pharmacol* 60: 267–273, 2001.
8. Borchers AT, Keen CL, Stern JS, and Gershwin ME. Inflammation and Native American medicine: the role of botanicals. *Am J Clin Nutr* 72: 339–347, 2000.
9. Calixto JB, Campos MM, Otuki MF, and Santos AR. Anti-inflammatory compounds of plant origin. Part II. Modulation of pro-inflammatory cytokines, chemokines and adhesion molecules. *Planta Med* 70: 93–103, 2004.
10. Chainani-Wu N. Safety and antiinflammatory activity of curcumin: a component of tumeric (*Curcuma longa*). *J Altern Complement Med* 9: 161–168, 2003.
11. Choy EH and Panayi GS. Cytokine pathways and joint inflammation in rheumatoid arthritis. *N Engl J Med* 344: 907–916, 2001.
12. Cohen SM, Rousseau ME, and Robinson EH. Therapeutic use of selected herbs. *Holistic Nurs Pract* 14: 59–68, 2000.
13. Colten HR and Krause JE. Pulmonary inflammation—a balancing act. *N Engl J Med* 336: 1094–1096, 1997.
14. Danesh J, Wheeler JG, Hirschfield GM, Eda S, Eiriksdottir G, Rumley A, Lowe GD, Pepys MB, and Gudnason V. C-reactive protein and other circulating markers of inflammation in the prediction of coronary heart disease. *N Engl J Med* 350: 1387–1397, 2004.
15. De Caterina R and Zampolli A. From asthma to atherosclerosis—5-lipoxygenase, leukotrienes, and inflammation. *N Engl J Med* 350: 4–7, 2004.
16. Duwiejua M, Zeitlin IJ, Waterman PG, Chapman J, Mhango GJ, and Provan GJ. Antiinflammatory activity of resins from some species of the plant family Burseraceae. *Planta Med* 59: 12–16, 1993.
17. el-Khadem H, el-Shafei ZM, el-Sekeily MA, and Rahman MM. Derivatives of boswellic acids. *Planta Med* 22: 157–159, 1972.
18. Ernst E and Chrubasik S. Phyto-anti-inflammatories. A systematic review of randomized, placebo-controlled, double-blind trials. *Rheum Dis Clin North Am* 26: 13–27, 2000.
19. Feldmann M and Maini RN. Anti-TNF alpha therapy of rheumatoid arthritis: what have we learned? *Annu Rev Immunol* 19: 163–196, 2001.
20. Feldmann M, Brennan FM, Paleolog E, Cope A, Taylor P, Williams R, Woody J, and Maini RN. Anti-TNFalpha therapy of rheumatoid arthritis: what can we learn about chronic disease? *Novartis Found Symp* 256: 53–69; discussion 69–73, 2004.
21. Fischer L, Szellas D, Radmark O, Steinhilber D, and Werz O. Phosphorylation- and stimulus-dependent inhibition of cellular 5-lipoxygenase activity by nonredox-type inhibitors. *FASEB J* 17: 949–951, 2003.
22. Gupta I, Gupta V, Parihar A, Gupta S, Ludtke R, Safayhi H, and Ammon HPT. Effects of *Boswellia serrata* gum resin in patients with bronchial asthma: results of a double-blind, placebo-controlled, 6-week clinical study. *Eur J Med Res* 3: 511–514, 1998.
23. Gupta I, Parihar A, Malhotra P, Gupta S, Ludtke R, Safayhi H, and Ammon HPT. Effects of gum resin of *Boswellia serrata* in patients with chronic colitis. *Planta Med* 67: 391–395, 2001.
24. Kaplan M. Eculizumab (Alexion). *Curr Opin Investig Drugs* 3: 1017–1023, 2002.
25. Kim YS, Kim SS, Cho JJ, Choi DH, Hwang O, Shin DH, Chun HS, Beal MF, and Joh TH. Matrix metalloproteinase-3: a novel signaling proteinase from apoptotic neuronal cells that activates microglia. *J Neurosci* 25: 3701–3711, 2005.
26. Kimmatkar N, Thawani V, Hingorani L, and Khiyani R. Efficacy and tolerability of *Boswellia serrata* extract in treatment of osteoarthritis of knee—a randomized double blind placebo controlled trial. *Phytomedicine* 10: 3–7, 2003.
27. Krieglstein CF, Anthoni C, Rijcken EJ, Laukotter M, Spiegel HU, Boden SE, Schweizer S, Safayhi H, Senninger N, and Schurmann G. Acetyl-11-keto-beta-boswellic acid, a constituent of a herbal medicine from *Boswellia serrata* resin, attenuates experimental ileitis. *Int J Colorectal Dis* 16: 88–95, 2001.
28. Kuehl FA Jr and Egan RW. Prostaglandins, arachidonic acid, and inflammation. *Science* 210: 978–984, 1980.
29. Lindahl B, Toss H, Siegbahn A, Venge P, and Wallentin L. Markers of myocardial damage and inflammation in relation to long-term mortality in unstable coronary artery disease. FRISC Study Group. Fragmin during Instability in Coronary Artery Disease. *N Engl J Med* 343: 1139–1147, 2000.
30. Long L, Soeken K, and Ernst E. Herbal medicines for the treatment of osteoarthritis: a systematic review. *Rheumatology* 40: 779–793, 2001.
31. Luster AD. Chemokines—chemotactic cytokines that mediate inflammation. *N Engl J Med* 338: 436–445, 1998.
32. Mason JC, Yarwood H, Sugars KB, Morgan P, Davies KA, and Haskard DO. Induction of decay-accelerating factor by cytokines or the membrane-attack complex protects vascular endothelial cells against complement deposition. *Blood* 94: 1673–1682, 1999.
33. Panula HE, Lohmander LS, Ronkko S, Agren U, Helminen HJ, and Kiviranta I. Elevated levels of synovial fluid PLA2, stromelysin (MMP-3) and TIMP in early osteoarthrosis after tibial valgus osteotomy in young beagle dogs. *Acta Orthop Scand* 69: 152–158, 1998.
34. Park YS, Lee JH, Harwalkar JA, Bondar J, Safayhi H, and Golubic M. Acetyl-11-keto-beta-boswellic acid (AKBA) is cytotoxic for meningioma cells and inhibits phosphorylation of the extracellular-signal regulated kinase 1 and 2. *Adv Exp Med Biol* 507: 387–393, 2002.
35. Pearson CM. Rheumatic manifestations of polymyositis and dermatomyositis. *Arthritis Rheum* 2: 127–143, 1959.
36. Phillipson JD. 50 years of medicinal plant research—every progress in methodology is a progress in science. *Planta Med* 69: 491–495, 2003.
37. Pober JS. Endothelial activation: intracellular signaling pathways. *Arthritis Res* 4 Suppl 3: S109–S116, 2002.
38. Reichling J, Schmokel H, Fitzi J, Bucher S, and Saller R. Dietary support with Boswellia resin in canine inflammatory joint and spinal disease. *Schweiz Arch Tierheilkunde* 146: 71–79, 2004.

39. Roy S, Khanna S, Wallace WA, Lappalainen J, Rink C, Cardounel AJ, Zweier JL, and Sen CK. Characterization of perceived hyperoxia in isolated primary cardiac fibroblasts and in the reoxygenated heart. *J Biol Chem* 278: 47129–47135, 2003.
40. Roy S, Khanna S, Shah H, Rink C, Phillips C, Preuss H, Subbaraju GV, Trimurtulu G, Krishnaraju AV, Bagchi M, Bagchi D, and Sen CK. Human genome screen to identify the genetic basis of the antiinflammatory effects of *Boswellia* in microvascular endothelial cells. *DNA Cell Biol* 24: 244–255, 2005.
41. Safayhi H, Mack T, Sabieraj J, Anazodo MI, Subramanian LR, and Ammon HPT. Boswellic acids: novel, specific, nonredox inhibitors of 5-lipoxygenase. *J Pharmacol Exp Ther* 261: 1143–1146, 1992.
42. Safayhi H, Rall B, Sailer ER, and Ammon HPT. Inhibition by boswellic acids of human leukocyte elastase. *J Pharmacol Exp Ther* 281: 460–463, 1997.
43. Safayhi H, Boden SE, Schweizer S, and Ammon HPT. Concentration-dependent potentiating and inhibitory effects of *Boswellia* extracts on 5-lipoxygenase product formation in stimulated PMNL. *Planta Med* 66: 110–113, 2000.
44. Sailer ER, Schweizer S, Boden SE, Ammon HPT, and Safayhi H. Characterization of an acetyl-11-keto-beta-boswellic acid and arachidonate-binding regulatory site of 5-lipoxygenase using photoaffinity labeling. *Eur J Biochem* 256: 364–368, 1998.
45. Schweizer S, von Brocke AF, Boden SE, Bayer E, Ammon HPT, and Safayhi H. Workup-dependent formation of 5-lipoxygenase inhibitory boswellic acid analogues. *J Nat Prod* 63: 1058–1061, 2000.
46. Sharma S, Thawani V, Hingorani L, Shrivastava M, Bhate VR, and Khiyani R. Pharmacokinetic study of 11-keto beta-boswellic acid. *Phytomedicine* 11: 255–260, 2004.
47. Skiles JW, Gonnella NC, and Jeng AY. The design, structure, and clinical update of small molecular weight matrix metalloproteinase inhibitors. *Curr Med Chem* 11: 2911–2977, 2004.
48. So A, Chamot AM, Peclat V, and Gerster JC. Serum MMP-3 in rheumatoid arthritis: correlation with systemic inflammation but not with erosive status. *Rheumatology* 38: 407–410, 1999.
49. Valle E, Gross M, and Bickston SJ. Infliximab. *Expert Opin Pharmacother* 2: 1015–1025, 2001.
50. Warner RL, Bhagavathula N, Nerusu KC, Lateef H, Younkin E, Johnson KJ, and Varani J. Matrix metalloproteinases in acute inflammation: induction of MMP-3 and MMP-9 in fibroblasts and epithelial cells following exposure to proinflammatory mediators in vitro. *Exp Mol Pathol* 76: 189–195, 2004.
51. Wei L and Shi YB. Matrix metalloproteinase stromelysin-3 in development and pathogenesis. *Histol Histopathol* 20: 177–185, 2005.
52. Werz O, Szellas D, Henseler M, and Steinhilber D. Nonredox 5-lipoxygenase inhibitors require glutathione peroxidase for efficient inhibition of 5-lipoxygenase activity. *Mol Pharmacol* 54: 445–451, 1998.
53. Wildfeuer A, Neu IS, Safayhi H, Metzger G, Wehrmann M, Vogel U, and Ammon HPT. Effects of boswellic acids extracted from a herbal medicine on the biosynthesis of leukotrienes and the course of experimental autoimmune encephalomyelitis. *Arzneimittelforschung* 48: 668–674, 1998.
54. Zeisel MB, Druet VA, Wachsmann D, and Sibilia J. MMP-3 expression and release by rheumatoid arthritis fibroblast-like synoviocytes induced with a bacterial ligand of integrin alpha5beta1. *Arthritis Res Ther* 7: R118–R126, 2005.

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2. Piergiacomo Calzavara-Pinton, Cristina Zane, Elena Facchinetti, Rossana Capezzera, Alessandra Pedretti. 2010. Topical Boswellic acids for treatment of photoaged skin. *Dermatologic Therapy* **23**, S28-S32. [CrossRef]
3. Melainie Cameron, Joel J. Gagnier, Christine V. Little, Tessa J. Parsons, Anette BlÃ¼mle, Sigrun Chrubasik. 2009. Evidence of effectiveness of herbal medicinal products in the treatment of arthritis. *Phytotherapy Research* **23**:12, 1647-1662. [CrossRef]
4. Melainie Cameron, Joel J. Gagnier, Christine V. Little, Tessa J. Parsons, Anette Blümle, Sigrun Chrubasik. 2009. Evidence of Effectiveness of Herbal Medicinal Products in the Treatment of Arthritis. *Phytotherapy Research* **23**:11, 1497-1515. [CrossRef]
5. KRISHANU SENGUPTA, TRIMURTULU GOLAKOTI, AJIT K. MARASETTI, TEJASWI TUMMALA, SURYACHANDRA R. RAVADA, ALLURI V. KRISHNARAJU, SIBA P. RAYCHAUDHURI. 2009. INHIBITION OF TNF# PRODUCTION AND BLOCKING OF MITOGEN-ACTIVATED PROTEIN KINASE/NF#B ACTIVATION IN LIPOPOLYSACCHARIDE-INDUCED THP-1 HUMAN MONOCYTES BY 3-O-ACETYL-11-KETO-#-BOSWELLIC ACID. *Journal of Food Lipids* **16**:3, 325-344. [CrossRef]
6. Bhahwal Ali Shah, Ghulam Nabi Qazi, Subhash Chandra Taneja. 2009. Boswellic acids: a group of medicinally important compounds. *Natural Product Reports* **26**:1, 72. [CrossRef]
7. Sashwati Roy , Hiral Shah , Cameron Rink , Savita Khanna , Debasis Bagchi , Manashi Bagchi , Chandan K. Sen . 2007. Transcriptome of Primary Adipocytes from Obese Women in Response to a Novel Hydroxycitric Acid–Based Dietary SupplementTranscriptome of Primary Adipocytes from Obese Women in Response to a Novel Hydroxycitric Acid–Based Dietary Supplement. *DNA and Cell Biology* **26**:9, 627-639. [Abstract] [PDF] [PDF Plus]