REVIEW ARTICLE

Systemic therapy with bromelain-trypsin-rutoside combination in inflammation: A narrative review of the pharmacodynamics

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ABSTRACT

Inflammation and wound healing are attended by interactions of numerous cell types, mediators, cytokines, and the vascular system. Any imbalance in these interactions can set off a cascade of processes, often working in different directions, and which continue till homeostasis is restored. Systemic enzyme therapy with trypsin-bromelain-rutoside combination has long been used as dietary supplements or pharmaceuticals commercially to counter various inflammatory conditions. The clinical benefits are believed to be due to the multipronged action of the combination, which include anti-kinin, antioxidant, antiplatelet, anti-chemokine, pro-fibrinolytic, and pro-fibrotic activities; with more recent evidence indicating inhibition of pro-inflammatory signal transduction. The primary modes of action of the individual agents on different components and processes involved in inflammation have been evaluated and elucidated, using various in vitro, animal model, and clinical studies. A narrative review of the evidence collected over the years has been provided.

KEY WORDS: Inflammation; Enzymes; Trypsin; Bromelain; Rutin

INTRODUCTION

Inflammation is a natural and essential response to cellular injury and almost every injury or illness will have an inflammatory component. It is a restorative and protective response in which the body disposes of pathogenic or cellular debris, ensures timely repair of injured tissues, and protects nearby cells from further injury. However, when it becomes excessive or uncontrolled, we begin to see delayed healing or chronic inflammatory conditions. Dolor, calor, rubor, tumor (corresponding to pain, heat, redness, and swelling) and functio laesa (loss of function – stiffness and immobility) are the classic signs and symptoms of local inflammation. These manifestations along with their underlying inflammatory events are induced and regulated by different types of cells (mainly platelets, keratinocytes, neutrophils, macrophages, microvascular cells, and fibroblasts) and chemical mediators.[1] It is also important to bear in mind that the inflammatory response is inseparable from the coagulation process. Normally, a fine balance between coagulation and fibrinolysis exists. This balance is disrupted by inflammation, and a state of procoagulant, antifibrinolytic environment ensues.[2]

The process of wound repair can be described as a continuum of four overlapping temporal phases.[3,4] Vasocostriction of blood vessels and platelet aggregation is the initial response implemented to stop bleeding. Multiple growth factors, survival or apoptosis-inducing agents, and cytokines are released by the platelets. Polyphosphate, released by
platelets, binds to factor XII (FXII) activating the FXII-driven contact activation system and results in the release of the inflammatory mediator bradykinin. Platelet-derived growth factor and transforming growth factors A1 and 2 (TGF-A1 and TGF-2) which act as chemoattractants are the other key components of the platelet release reaction. This is followed by influx of a variety of inflammatory cells, starting with the neutrophils, in the next phase. A variety of chemicals which promote angiogenesis, thrombosis, and re-epithelialization are released by these inflammatory cells. The cells are also phagocytic and release proteases that clear the wound of foreign bodies and debris and reactive oxygen species (ROS) and are antimicrobial. This is then followed by the release of growth factors by the inflammatory cells and migration of dermal and epidermal cells for granulation tissue formation, along with a robust angiogenic response in the proliferative phase. The fibroblasts lay down a framework composed of extracellular components in the final maturation and remodeling phase. The wound achieves its maximum strength in this phase. Pro-inflammatory agents like the hormone-like eicosanoids (prostaglandins [PGs], thromboxanes, leukotrienes [LTs], and lipoxins) play a key role in the inflammatory response.\(^\text{[3,4]}\)

Systemic enzyme therapy has been recommended for many years for the treatment of pain and inflammation, in which proteolytic enzymes, with or without flavonoids, are delivered orally. In general, the enzyme combination is provided as enteric coated tablets for oral administration which protects it from stomach acid degradation. Different processes are intervened by the systemic enzyme therapy:

The release of inflammatory mediators, the modulation of adhesion molecules, and the activation of fibrinolysis with consequent improved healing. The proteolytic enzymes, also called proteases, are naturally occurring substances derived from animal or plant sources. Combined with the bioflavonoid rutin (rutoside), the proteases, trypsin and bromelain, have been extensively investigated as alternatives to conventional pharmaceutical treatments for pain and swelling associated with several common conditions including ankle injuries,\(^\text{[5]}\) knee arthritis,\(^\text{[6-9]}\) hip arthritis,\(^\text{[10]}\) and post-surgical management\(^\text{[11-13]}\) of general surgery, orthopedic, and dental procedures. Different formulations of this combination are available in India, including enteric-coated oral tablets (e.g., Phlogam) and dispersible tablets (e.g., Disperzyme).

There are multiple known biomarkers of inflammation—major immune cell types, cytokines and chemokines, acute-phase proteins (e.g., C-reactive protein – CRP), reactive oxygen and reactive nitrogen oxide species (ROS and RNOS), PGs, cyclooxygenase-related metabolites, inflammation-related growth factors, and transcription factors (e.g., nuclear factor-kappa B – NF-κB).\(^\text{[11]}\) The constituents of trypsin-bromelain-rutoside combination have shown beneficial impact on all of these, in various experiments. This literature review aims at discussing the pharmacodynamics of the individual components and how they provide a multipronged approach at tackling inflammatory processes. Plenty of research has been conducted elucidating the mechanisms of these agents through measurement of various biological markers. Some of these have been discussed further in this review.

**BROMELAIN**

Bromelain is a group of proteolytic enzymes. These contain sulphhydryl obtained from Ananas comosus, the pineapple plant.\(^\text{[14]}\) Substrates susceptible to bromelain include many common protein materials, such as gelatin, casein, collagen, globulins, and muscle fiber.\(^\text{[15]}\) It remains biologically active with a half-life of ~6–9 h and is absorbed in the intestines. The highest concentration in the blood was seen 1 h after administration of bromelain.\(^\text{[16]}\)

Bromelain has fibrinolytic, antithrombotic, and anti-inflammatory properties; well documented in studies involving experimentally induced inflammation-edema, kininogen-induced capillary permeability, prostaglandin synthesis, coagulation parameters, and platelet aggregation.\(^\text{[15]}\)

These have been discussed further.

**Effect on Kinins and PGs**

Plasma and tissue kinogens release mediators such as bradykinin during inflammation which enhance the inflammatory process. The inflammatory changes by bradykinin are mediated by the direct activation of its receptors as well as stimulation of synthesis of other pro-inflammatory agents – PGs, LTs, histamine, endothelium-derived relaxing factor, PGl2, and platelet activating factor (PAF). Bromelain has been reported to lower bradykinin and kininogen levels by up to 60% in the serum along with symptoms of inflammation.\(^\text{[17]}\) Marked reduction in plasma pre-kallikrein and high-molecular-weight (HMW) kinogen is seen 15 min after the intravenous injection of bromelain with gradual reappearance in 72 h. In vitro experiments reported that the depleting effect of bromelain on HMW kinogen is by the activation of Hageman factor (factor XII)\(^\text{[18]}\). In a carrageenan-induced, kininogen potentiated, rat paw edema model, intravenous injection of bromelain produced reduction of plasma kininogen and suppression of edema.\(^\text{[18]}\)

The effect of bromelain on the arachidonic acid metabolism has been confirmed in multiple studies. Decrease of prostaglandin E2 levels and thromboxane B2 levels in a dose-dependent manner has been shown in experimentally induced inflammations with rats.\(^\text{[15]}\) Gaspani et al. evaluated the effects of bromelain in in vitro and in vivo settings in rats with subcutaneous carrageenin-induced inflammation. Bromelain induced a significant decrease of both PGE2 and substance P concentrations in the exudate after oral in vivo administration.\(^\text{[19]}\) An investigation through a combination of...
**Effect on Fibrinolysis and Platelets**

Bromelain is also an effective fibrinolytic agent, resulting in increased fibrinolysis by stimulating the conversion of plasminogen to plasmin, as demonstrated in vitro and in vivo.[14] Bromelain led to 80–250% increase in the prothrombin time and antithrombin time, with elevation of serum plasmin levels after administration to rabbits in an oral form at different dosage regimens.[15] In rats, the serum fibrinolytic activity of bromelain was significantly increased and was proportional to the log of the dose administered on enteral administration of bromelain.[15] The effect of bromelain on coagulation is accompanied by its inhibitory effect on platelet aggregation and was demonstrated on isolated human platelets in vitro by Coulter counter measurements. Thrombin-induced platelet aggregation was prevented by pre-incubation of platelets with bromelain. The bovine aorta endothelial cells also had less adhesion of thrombin-stimulated platelets. This suggests that its antiplatelet effects might be through anti-adhesion mechanisms. Using a laser thrombosis model, orally and intravenously applied bromelain was found to inhibit thrombus formation in rat mesenteric vessels in a time-dependent manner.[21]

**Effect on Leukocyte Migration**

Migration of leukocytes, under the influence of chemokines, like interleukin (IL)-8, and facilitated by cell surface adhesion molecules, followed by the release of proteases (mainly from neutrophils) and pro-inflammatory cytokines (by the macrophages), constitutes an important part of the inflammatory process. Fitzhugh et al. performed in vitro and in vivo leukocyte migration assays. A reduction of 40% in migration of bromelain-versus sham-treated human neutrophils in response to rhlL-8 was demonstrated by an in vitro chemotaxis assay. A 50–85% reduction in neutrophil migration into the inflamed peritoneal cavity in three different murine models of leukocyte migration was observed using in vivo bromelain treatment. In vivo bromelain treatment decreased leukocyte rolling, but reversal of firm adhesion of leukocytes to blood vessels at the inflammation site was its primary long-term effect, which is demonstrated by intravital microscopy.[22] In an in vitro study on whole blood, bromelain treatment proteolytically altered 14 of 59 leukocyte cell surface markers. The bromelain-altered cell surface molecules were those which are responsible for leukocyte homing, cellular adhesion, and activation.[23]

**Other Effects**

Few other studies further identified bromelain anti-inflammatory and/or immunomodulating properties. Bromelain was found to inhibit T-cell signal transduction in TH0 cells by blocking the activation of extracellular regulated kinase-2 (involved in mitogenesis, cytokine production, and apoptosis), shown in an in vitro study on T cells and also confirmed that this action is not simply due to a toxic effect, as there was no effect on T-cell viability. A more marked effect on IL-4 production was shown by bromelain than on IL-2 and interferon (IFN)-γ production. TH2 cells produce IL-4, and its expression was inhibited by 94%. On the other hand, IL-2 and IFN-γ produced by Th1 cells were reduced by 68% and 56%, respectively.[24] Endoscopic colon biopsies from patients with Crohn’s disease, ulcerative colitis, and non-IBD controls showed decreased secretion of pro-inflammatory cytokines and chemokines including granulocyte-macrophage colony-stimulating factor, granulocyte colony-stimulating factor, tumor necrosis factor (TNF), CCL4/macrophage inhibitory protein–1β, and IFN-γ, when treated with bromelain and cultured, which were confirmed and extended in another study.[25] Using cytokine circadian profiles, a placebo-controlled crossover randomized clinical trial was conducted to investigate the effect of bromelain on the human immune system, which confirmed its effects on stimulated whole-blood leukocytes. A significant shift in the circadian profiles of the immunosuppressive cytokine IL-10, Th1 cell mediator interferon gamma, and trends in those of the Th2-type cytokine IL-5 were demonstrated by the study, suggesting a general effect on the antigen-specific (T cell) compartment of the human immune system.[26]

**TRYPSIN**

Trypsin, a digestive enzyme, belonging to the group of serine proteases, is produced and secreted by pancreatic acinar cells. The enzyme is known to specifically hydrolyze peptides containing lysine (Lys) and arginine (Arg). The regulatory actions of trypsin are mediated through the activation of other proteins, through proteolysis at specific Lys or Arg bonds. The trypsin used in oral enzyme therapy is usually obtained from bovine or porcine pancreas. Experimental demonstration shows that when trypsin is administered orally, it is effectively absorbed into the bloodstream. This has been demonstrated based on specific esterase activity changes.[27] Enteropancreatic recirculation of trypsin leads to ~10% of orally administered trypsin dose ending up in the blood.[28] It exists in bound form, in the blood, to specific (e.g., α2-antitrypsin) or unspecific (e.g., α2-macroglobulin) antiproteinas,.[29] Although trypsin is protected from autodigestion and from degradation by other serum proteases in this complex, its enzymatic activity is retained.[28] Trypsin is known to have immunomodulating actions, to enhance fibrinolysis and to affect coagulation factors, and together with bromelain lower the pro-inflammatory cytokines.
Substantial evidence shows that trypsin can potentially enhance wound healing by regulating target cells to cleave and activate a growing family of G-protein-coupled protease-activated receptors (PARs).\textsuperscript{[30]}

**Effect on Fibrinolysis**

Plasmin, an important proteolytic enzyme present in blood, is involved in fibrinolysis, the breakdown of fibrin in clots. It has enzyme specificity similar to trypsin. Most inhibitors of trypsin also help in inhibition of plasmin, similar to serine protease inhibitors.\textsuperscript{[31]} Alpha-1 antitrypsin ($\alpha$1-AT) in blood is known to inhibit plasmin activity by forming a 1:1 stoichiometric complex with the enzyme.\textsuperscript{[32]} Moreover, the levels of $\alpha$1-AT increase in injury and inflammation.\textsuperscript{[33]} Since, trypsin circulates in blood-stream bound to $\alpha$1-AT and has more affinity to it than plasmin, it is expected to compete with plasmin and allows more plasmin to be available for fibrinolysis. The lytic effects of trypsin on experimentally produced thrombi in rabbits and dogs have been demonstrated in the early 1950s. Diminution and/or disappearance of the in situ thrombus, local circulation restoration, and restoration of vessel wall compressibility in rabbit ear vein were reported in these studies. These changes were accompanied by microscopic findings of diminution of cellular and platelet masses and fibrin diminution. The fibrinolytic effects extended into the laminations and interstices of the thrombus.\textsuperscript{[34]} In vitro studies have demonstrated that exposure of fibrinogen to trypsin immediately impaired its clottability by thrombin.\textsuperscript{[35]}

**Effect on Fibrocytes and Tissue Repair**

Fibrocytes are mesenchymal cells that arise from monocyte precursors, similar to macrophages. The inflammation driven by macrophage/fibrocyte contributes to both tissue injury and repair. Trypsin has been demonstrated to promote differentiation of these cells to a more healing/repairing profile. White et al. demonstrated this in peripheral blood mononuclear cells (PBMCs) and monocytes. These cells were isolated, cultured, and incubated for 5 days with trypsin in a defined serum-free medium. The differentiation of human monocytes to fibrocytes in cell culture was found to be potentiated by trypsin. The amount of trypsin needed to potentiate fibrocyte differentiation was increased by adding trypsin inhibitors, suggesting that the potentiating effect is dependent on trypsin proteolytic activity.\textsuperscript{[36]} PBMCs were isolated and cultured to differentiate fibrocytes and macrophages in serum-free media to polarize macrophages toward M1 and M2 phenotypes for a week, after which the cells were treated with trypsin for 2 days. Trypsin, through its profibrotic activity, mediated through PAR1 and PAR2 receptors, altered the macrophages toward an M2a phenotype, both in terms of their surface marker expression and secretion profile. Such M2a phenotype cells are involved in wound healing and fibrosis.\textsuperscript{[27]}

**Other Immunomodulating Effects**

Trypsin has also shown additional immunomodulating effects. T-cell activation threshold for recognition of nominal peptides is dramatically increased by trypsin treatment of antigen-presenting cells (APCs) in vitro. This immunomodulatory effect was also seen in mice, in vivo, with an orally administered trypsin preparation. A reduced frequency of encephalitogenic T cells was shown, which protected from experimental autoimmune encephalomyelitis. Accessory molecules on APC were selectively cleaved by trypsin, thereby modulating the activation threshold for T cells.\textsuperscript{[39]} Only three cell surface molecules out of 21 studied, were found to be sensitive to cleavage, while even at very high concentrations of the enzyme, cell viability and the 18 other molecules were completely unaffected when T lymphocytes or macrophages are cultured, in vitro, in the presence of trypsin. All the three are central for regulating T-cell activation threshold. The dose-response curve was significantly right shifted, that is, their activation threshold is increased, when T cells were isolated from enzyme-treated mice and tested freshly ex vivo for their antigen-specific recall response.\textsuperscript{[28]}

**RUTOSIDE**

Rutoside, also called by other names such as sophorin, rutin, and quercetin-3-rutinoside, is a polyphenolic bioflavonoid, extracted largely from natural sources such as lemons, oranges, grapes, berries, limes, and peaches. Its name is originated from the plant *Ruta graveolens*, which also contains rutin. It is a vital nutritional component of plants and is chemically, a glycoside comprising flavonol aglycone quercetin along with disaccharide rutinose.\textsuperscript{[39]} Homovanillic acid and 3,4-dihydroxy-phenyl-acetic-acid are its active metabolites. Several pharmacological activities have been demonstrated in a large number of studies, including antioxidant, vasoprotective, cytoprotective, neuroprotective, cardioprotective, and anticarcinogenic activities.\textsuperscript{[40]} Studies pertaining to its potential and mechanisms in local inflammatory processes, specifically its effects on inflammatory and oxidative stress markers, platelets, and capillary permeability, are discussed further.

**Antioxidant and Anti-inflammatory Effect**

The potent antioxidant effect of rutoside has been demonstrated in multiple experimental studies. The free radical scavenging and chelating activity of rutoside were studied in rat liver microsomes. The formation of superoxide ion, the generation of hydroxyl radicals, and the formation of lipid peroxy radicals were found to be markedly reduced by rutoside.\textsuperscript{[41]} The effect of rutoside on pro-inflammatory mediators release from human activated macrophages and adjuvant-induced arthritic rats in vitro was investigated by Kauss et al. This study presented substantial data on
the effects of rutoside on macrophages. On analyzing in vitro data on the transcription of inflammatory genes, rutoside was shown to have inhibitory effect on the transcription of more than 20 genes encoding critical pro-inflammatory factors, including IL-1, IL-8, TNF-α, macrophage migration inhibitory factor, and chemotactic factors. This was confirmed by decreased concentrations of IL-1β, TNF-α, and IL-6 in cell supernatants. Inducible nitric oxide synthase (iNOS)-mediated NO production by human macrophages was also decreased in a dose-dependent manner. In rat activated macrophages, rutoside significantly reduced the levels of IL-1β, TNF-α, and monocyte chemoattractant protein-1. It also inhibited clinical signs of chronic arthritis in rats, which correlated with the post-treatment levels of inflammatory cytokines and nitrite, detected in macrophage supernatants and rat sera.[42]

Rutoside exhibited anti-inflammatory effects by modulating apoptosis and cell cycle, the ROS level, based on analysis of blood and pleural exudate in rat models of acute inflammation induced by carrageenan.[43] Rutoside reduced the ROS, calpain, and ceramide levels in mouse kidneys in a kidney injury model of mice exposed to carbon tetrachloride; decreased significantly, TNF-α, IL-1β, the p53 activities, and mitogen-activated protein kinase phosphorylation in the kidneys, reduced levels protein of Bax, and increased the levels of Bcl-2 protein. It also helps in inhibition of the release of cytochrome C from mitochondria in kidneys.[44] The effects of rutoside on lipopolysaccharide (LPS)-induced inflammatory responses were evaluated in an in vitro inflammation model using mouse muscle cells (C2C12). NF-kB activation and LPS-induced ROS production were significantly blocked. Rutoside treatment led to attenuation of LPS-induced TNF-α and iNOS gene expression, as also the IL-6 mRNA abundance, in a dose-dependent manner.[45] In a mice model of LPS-induced acute kidney injury, the modulatory effect of rutoside on inflammation, oxidative stress, and apoptosis was also evaluated. Renal oxidative stress-related indices such as glutathione (GSH), malondialdehyde (MDA), and activity of superoxide dismutase and catalase were restored to some extent by rutoside. Rutoside also brought back toll-like receptor 4, renal NF-κB, cyclooxygenase-2 (COX2), TNF-α, IL-6, sirtuin 1 (SIRT1), and caspase 3 activity to their control levels.[46]

The mechanisms of rutoside antioxidant effects were further studied against hydrogen peroxide-induced apoptosis of human umbilical vein endothelial cells (HUVECs). Attenuation of excessive ROS and apoptosis in HUVECs, prevention of increased DNA fragment formation, depletion of GSH, and inhibition of the collapse of mitochondrial membrane potentials were seen with rutoside pretreatment. Thus, rutoside prevents peroxide-induced apoptosis of HUVECs through regulating ROS-mediated mitochondrial dysfunction pathway and protects the intracellular GSH antioxidant system.[67]

**Effect on Microvascular Permeability and Platelets**

In a rat skin model, O-(beta-hydroxyethyl)-rutoside was found to attenuate the permeability increase due to histamine, bradykinin, and fibrin degradation products, in a dose-related manner.[48] Similarly, hydroxyethyl rutosides also reduced microvascular permeability both in vessels showing signs of inflammation and healthy vessels, in the walls of venules and single capillaries of the frog mesentery.[49] In human platelets stimulated by agonists such as collagen, platelet aggregation, intracellular Ca²⁺ mobilization, and thromboxane A₂ formation was found to be inhibited by rutoside in a concentration-dependent manner.[50] In vitro, rutoside was found to inhibit serotonin (5-HT) release, washed rabbit platelet aggregation induced by PAF, and the intraplatelet concentration of free calcium in a concentration-dependent manner.[51]

**Other Evidence**

The reduction in inflammatory markers by rutoside has been demonstrated in other models as well. Rutoside treatment significantly decreased TNF-α and IL-1β levels and the lung wet/dry ratio in the bronchoalveolar lavage fluid and increased protein and expressions of α-ENaC (epithelial sodium channel) mRNA, in a murine LPS-induced acute lung injury model.[52] In a murine acute pancreatitis model, rutoside treatment attenuated abdominal hyperalgesia. This was accompanied by reduction in serum lipase, amylase, IL-6, and CRP concentrations. Oxidative stress markers, including edema index, pancreatic myeloperoxidase activity, MDA and 3-nitrotyrosine contents, were also reduced.[53]

Additional evidence also points to the possibility of complexing of the protease enzymes with endogenous antiproteases, to complex further with cytokines and promote their clearance from inflamed tissue through phagocytic and endocytotic routes.[54] Studies demonstrating this have been performed in murine models of collagen-induced arthritis.[55,56] The clinical benefits of systemic enzyme therapy in inflammatory conditions were demonstrated by animal and human trials, including comparative trials that demonstrated non-inferiority to nonsteroidal anti-inflammatory drugs and also in reduction of commonly used biomarkers of inflammation like CRP.[55] The primary modes of the action of trypsin-bromelain-rutoside at different components and processes involved in local inflammation are been illustrated in Figure 1.
CONCLUSION

Multiple studies, including in vitro, animal model, and clinical evidence studies, have identified various pharmacodynamic modalities by which bromelain, trypsin, and rutoside exert their beneficial action in inflammation-related conditions. As our knowledge of the myriad pathways involved in inflammation and immunity responses expand, it is likely that we will have more understanding of the exact and hitherto unknown mechanisms that these ingredients exert. It is imperative that we continue to explore their effects and mechanisms on various physiological and pathological states.

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