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Review

Bromelain: biochemistry, pharmacology and medical use

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Abstract. Bromelain is a crude extract from the pineapple that contains, among other components, various closely related proteinases, demonstrating, in vitro and in vivo, antiedematous, antiinflammatory, antithrombotic and fibrinolytic activities. The active factors involved are biochemically characterized only in part. Due to its efficacy after oral administration, its safety and lack of undesired side effects, bromelain has earned growing acceptance and compliance among patients as a phytotherapeutical drug. A wide range of therapeutic benefits has been claimed for bromelain, such as reversible inhibition of platelet aggregation, angina pectoris, bronchitis, sinusitis, surgical traumas, thrombophlebitis, pyelonephritis and enhanced absorption of drugs, particularly of antibiotics. Biochemical experiments indicate that these pharmacological properties depend on the proteolytic activity only partly, suggesting the presence of nonprotein factors in bromelain. Recent results from preclinical and pharmacological studies recommend bromelain as an orally given drug for complementary tumor therapy: bromelain acts as an immunomodulator by raising the impaired immunocytotoxicity of monocytes against tumor cells from patients and by inducing the production of distinct cytokines such as tumor necrosis factor-α, interleukin (Il)-1β, Il-6, and Il-8. In a recent clinical study with mammary tumor patients, these findings could be partially confirmed. Especially promising are reports on animal experiments claiming an antimetastatic efficacy and inhibition of metastasis-associated platelet aggregation as well as inhibition of growth and invasiveness of tumor cells. Apparently, the antiinvasive activity does not depend on the proteolytic activity. This is also true for bromelain effects on the modulation of immune functions, its potential to eliminate burn debris and to accelerate wound healing. Whether bromelain will gain wide acceptance as a drug that inhibits platelet aggregation, is antimetastatic and facilitates skin debridement, among other indications, will be determined by further clinical trials. The claim that bromelain cannot be effective after oral administration is definitely refuted at this time.

Key words. Bromelain; proteinases; antiinflammation; edema prevention and destruction; fibrinolysis; thrombosis and metastasis prophylaxis; immunomodulation; skin debridement; complementary tumor therapy.

Introduction

The role of proteolytic enzymes for therapeutic use

Bromelain belongs to a group of proteolytic enzymes which are used as drugs for the oral systemic treatment of inflammatory, blood-coagulation-related and malignant diseases. Apart from the plant cysteine-proteinases bromelain and papain, the group comprises proteinases from animal organs such as trypsin and chymotrypsin. These enzymes offer a wide spectrum of therapeutic efficacies: they demonstrate, in vitro and in vivo, antiedemateous, antiinflammatory, antithrombotic and fibrinolytic activities. They modulate the functions of adhesion molecules on blood and endothelial cells, and also regulate and activate various immune cells and their cytokine production. Indeed, these enzymes are used in the United States and Europe as an alternative or complementary

medication to glucocorticoids, nonsteroidal antirheumatics and immunomodulatory agents. Their very low toxicity makes them suitable tools for controlling chronic inflammatory diseases.

For the therapy of inflammatory and malignant disorders, these proteinases are employed as

- additives for chemotherapy (to reduce side effects of drugs and to improve quality of life);
- additives for radiotherapy (to reduce inflammation and edema);
- additives in surgery (to reduce edema and to improve wound healing);
- additives to prevent lymphedema by reducing lymphocongestion, detritus, viscosity of the exsudate and stimulation of phagocytosis of associated leukocytes.

It should be added that clinical studies support these recommended indications only to a limited extent. Yet the large body of preclinical, pharmacological and daily experience offers an important and worthwhile field for well-designed clinical studies in order to evaluate evidence-based medical indications.

Biochemistry of bromelain

Bromelain is a crude, aqueous extract from the stems and immature fruits of pineapples (*Ananas comosus* Merr., mainly var. Cayenne from the family of bromeliaceae), constituting an unusually complex mixture of different thiol-endopeptidases and other not yet completely characterized components such as phosphatases, glucosidases, peroxidases, cellulases, glycoproteins and carbohydrates, among others [1, 2]. In addition, bromelain contains several proteinase inhibitors [3, 4]. Stem-bromelain (EC. 3.4.22.32) is distinguished from fruit-bromelain (EC. 3.4.22.33), previously called bromelin [2]. Today bromelain is prepared from cooled pineapple juice by centrifugation, ultrafiltration and lyophilization. The process yields a yellowish powder, the enzyme activity of which is determined with different substrates such as casein (FIP units), gelatine (gelatine digestion units) or chromogenic tripeptides [1, 5, 6]. In aqueous solution, bromelain rapidly deteriorates through self-digestion. The addition of serum containing α_2-macroglobulin will prevent self-digestion (see below). By high-resolution fast protein liquid chromatography (FPLC) and other biochemical methods, basic (stem bromelain, ananain, comosain) and acidic thiol-proteinases have been isolated from crude bromelain, partially or fully sequenced and characterized in more detail [6–8]. They mainly comprise glycosylated multiple enzyme species of the papain superfamily with different proteolytic activities, molecular masses between 20 and 31 kDa, and isoelectric points between >10 and 4.8. Two major basic proteinases, F4 and F5, were further characterized and showed molecular masses of 24,397 and 24,472 Da, respectively [6] (Table 1). In addition, numerous, different protein fractions were obtained by means of various biochemical methods [SDS-polyacrylamide gel electrophoresis (PAGE), isoelectric focusing (IEF), multicathodal-PAGE]. Among the basic proteinases, one fraction (F9, ananain) reveals the highest specific proteinase activity, is not glycosylated and has a molecular mass of 23,427 Da [6, 8, 9]. The enzymatic activities comprise a wide spectrum with pH optima between 5.5 and 8.0 [10]. The substrate spectrum is similarly broad, extending from synthetic low molecular mass amides and dipeptides up to high molecular substrates such as fibrin, albumin, casein, angiotensin II, bradykinin. Bromelain preferentially cleaves glycyl, alanyl and leucyl bonds.

Commercial bromelain preparations are evaluated according to their proteolytic activity. The platelet aggrega-

Table 1. Cysteine proteinases (bromelains) from pineapples (*Ananas comosus*).

Name (EC number) accordimg to [2, 8]	Abbreviation according to [6, 7]	Molecular mass (Dalton)	Isoelectric point	Sequences	Glycosylation	Reference
From pineapple stems:						
Stem bromelain (EC 3.4.22.32)	F4 and F5	23,800 (sequence + sugar)	> 10	completely sequenced (212 amino acids)	glycosylated	6
Ananain (EC 3.4.22.31)	F9	23,464 (sequence)	> 10	completely sequenced (216 amino acids)	not glycosylated	6, 9
Comosain	F9/b	24,509 and 23,569 (esms)	> 10	N-term. sequence	glycosylated	6, 8
	SBA/a and SBA/b	23,550 and 23,560 (esms)	4.8 and 4.9	N-term. sequence	highly glycosylated	7
From pineapple fruits:						
Fruit bromelain (EC 3.4.22.33)		23,000	4,6	N-term. sequence	not glycosylated	1

tion inhibitory and antiinflammatory action seem to be related to the protease activity. However, other effects such as inhibition of tumor cell growth and metastasis as well as debridement of burns are associated with other nonproteinolytic components contained in bromelain. Thus, the determination of the proteolytic activity alone may not be sufficient to completely characterize the pharmacological properties of bromelain [11].

Pharmacology of bromelain: preclinical studies

Pharmacodynamics of bromelain

From a variety of in vitro and animal experiments, mainly with rodents, as well as from clinical observations, based on uncontrolled and controlled studies, the general properties of bromelain may be summarized as follows [11–14] Bromelain

- prevents edema formation and reduces existing edemas
- reduces the blood level of fibrinogen
- supports fibrinolysis
- activates plasmin
- prolongs the prothrombin and partial thromboplastin time (after relatively high doses)
- prevents aggregation of blood platelets
- prevents adhesion of platelets to endothelial cells of blood vessels
- reduces the blood level of plasmakinins
- reduces the level of prostaglandine E_2 and of thromboxane A_2 in exsudates during acute inflammation
- acts as an antiinflammatory agent
- induces the secretion of interleukin (Il)-1, Il-6, Il-8 and tumor necrosis factor (TNF)-α from blood monocytes and granulocytes
- supports the oxidative burst and the cytotoxicity of granulocytes against tumor cells
- increases the tissue permeability of antibiotic drugs
- prevents metastases in a mouse model
- supports skin debridement of burns

Some effects of bromelain may result from its capacity to alter and modulate distinct cell surface structures by cleaving off peptides [15]. Thus, the bromelain-mediated modification of adhesion molecules on platelets and on other normal and malignant tumor cells may inhibit their aggregation. The dissolution of cell membrane constituents and the effects on components of hemostatic processes may explain antiedemateous and fibrinolytic phenomena.

Bromelain prevents edema formation and reduces existing edema

Several groups have provided significant evidence for both the edema-protective and edema-reducing efficacy of bromelain in a variety of classical animal experiments [16–20]. Among them, the data by Netti et al. [17] are particularly interesting, since papain, another cysteine proteinase, was ineffective in all experimental models, whereas bromelain induced 41% (carrageenin) and 45% (dextran) inhibition of edema formation. In addition, bromelain showed the strongest edema-protective efficacy of all drugs tested, such as indometacin, acetylsalicylic acid, aescin, oxyphenbutazon, and so on [18]. Moreover, both intraperitoneal (i.p.) and orally applied bromelain proved significantly capable of reducing edema (induced by cotton tissue, carrageenin, croton oil) in various animal models [20]. It was concluded that bromelain increases tissue permeability by fibrinolysis and promotes reabsorption of edema fluid into blood circulation. Uhlig and Seifert [18] compared enteral and i.p. application of bromelain and demonstrated a highly significant edema reduction (by 50%) 12 h after oral application, whereas i.p. administration was effective during the first hours only.

Bromelain promotes the absorption of antibiotic drugs

It has been known for a number of years that bromelain is capable of enhancing the tissue permeability of penicillins and tetracyclins after oral administration. This increases absorption and leads to an improved diffusion after subcutaneous and intramuscular application of the antibiotics. Higher serum and tissue levels are obtained, and side effects are reduced [21–23].

Among others, Neubauer evaluated the combined bromelain and antibiotic therapy of 53 hospitalized patients with pneumonia, bronchitis, staphylococcus infections, thrombophlebitis, pyelonephritis and rectal abscesses [24]. Twenty-three of the patients had been on antibiotic therapy without success. Twenty-two of these patients responded favorably to the combined treatment of bromelain and antibiotics. In every disease state significant reduction in morbidity was noted as opposed to antibiotics alone. Similarly, Ryan concluded from his double-blind clinical study on acute sinusitis that of the patients receiving bromelain, 83% showed complete resolution of nasal mucosal inflammation versus 52% in the placebo group [25].

Bromelain affects blood coagulation and fibrinolysis

The data on the edema-protective and -reducing efficacy of bromelain suggest that hemostatic processes are involved, such as prolongation of the prothrombin time, partial thromboplastin time and decrease of the fibrinogen blood level.

In the inflammatory animal models of Pirotta et al. [26], bromelain increased the fibrinolytic activity in a dose-dependent manner. Livio et al. [27] found an increase of prothrombin and partial thromboplastin time as well as a decrease of ADP-induced platelet aggregation. All these effects were clearly dose dependent and related to the

proteolytic activity of bromelain, since inactivation of the enzyme abolished the effects [28].

Bromelain prevents platelet aggregation

In 1972 Heinicke et al. [29] observed that oral administration of bromelain to healthy persons, particularly those with high platelet counts, significantly lowers the ADP-induced aggregation of platelets. Morita et al. [28] attempted to isolate and characterize platelet aggregation inhibitory factors from bromelain. At about the same time, other authors reported on fibrinolytic activities of bromelain and experiments to isolate fractions by means of biochemical methods [26, 30].

Aggregation and adhesion of platelets to endothelial cells have recently been studied in more detail [31]. When the platelets were incubated with bromelain prior to activation with thrombin, aggregation was completely prevented. Papain was less effective. Bromelain reduced, in vitro, the adhesion of thrombin-activated, fluorescent-labelled platelets onto bovine aorta endothelial cells. Using an in vivo laser thrombosis model, oral administration of bromelain to rats could significantly decrease the thrombus formation in mesenterial arterioles by 11%, in venols by 6%.

Bromelain effects on plasminogen, Quick- and partial thromboplastin time

In vitro, bromelain was able to activate plasminogen to yield plasmin, which is known to cleave fibrin [32]. This property is shared with streptokinase. In addition, bromelain inhibited the thrombin-induced formation of blood plasma fibrin in vitro; the other cysteineproteinase papain was less effective in this respect. In contrast to streptokinase, bromelain was not able to dissolve fibrin aggregates. After oral administration [3000 Fédération International Pharmaceutique (FIP) units daily for 10 days] to healthy persons, no significant influence was determined as to thromboplastin (Quick) time and plasmin formation, yet a moderate increase of the partial thromboplastin time (a PTT) still within normal range [33].

Table 2 summarizes bromelain effects on blood coagulation, fibrinolysis and platelet functions.

Mechanism of action of bromelain, apparently due to its proteolytic activity

The antiedematous, antiinflammatory, antithrombotic and fibrinolytic efficacy of bromelain reported so far suggests that several mechanisms of action are involved on different levels: The blood clotting, complement and kinin systems interconnect and mutually influence each other. Generally, they regulate cascade systems of proteinase-mediated reactions.

Besides interactions with plasmakinins, prostaglandins and the fibrinogen/fibrin system the reaction with the plasmaprotein α_2-macroglobulin plays a significant role (see below). Like most endoproteinases, bromelain circulates in blood, bound to this high molecular mass proteinase inhibitor [35]. However, binding does not completely inactivate the enzyme; the capacity to hydrolyze small substrates is still preserved [36].

Bromelain is an effective fibrinolytic agent in vitro and in vivo. However, this property is more evident in purified fibrinogen solutions than in plasma, probably due to proteinase inhibitors present in plasma. Despite this limitation, an increase of the fibrinolytic activity was observed

Table 2. Bromelain effects, in vitro (BP, F4, F9) and in vivo (POS), on blood coagulation, fibrinolysis and platelet functions.

Protease	Parameter	Effect	Significance	Ref.
Bromelain POS	extrinsic blood coagulation	=	blood coagulation, dependent on factor II, V	31
Bromelain F9	(Quick-test)	=	VII, X and fibrinogen	33
Bromelain POS	intrinsic blood coagulation	↓	blood coagulation, dependent on plasmatic	31
Bromelain F9	(PTT-Test)	=	factors except factor VII	33
Bromelain POS	activation of plasminogen	=	activation causes fibrin degradation	31
Bromelain BP	to plasmin	↑	(fibrinolysis)	33
Bromelain F9				26, 27
Bromelain BP	thrombin-dependent fibrin	↓	reduction of blood clotting	34
Bromelain F4, F9	formation			
Bromelain BP	thrombin-stimulated platelet aggregation	↓	reduction of thrombus formation	31
Bromelain BP	thrombin-stimulated platelet adhesion onto endothelial cells	↓	reduction of thrombus formation	31
Bromelain BP	in vivo thrombus formation in arterioles and venoles after oral administration	↓	reduction of thrombus formation	31

Bromelain BP (base powder), commercial crude extract; POS, BP given orally; F4, F9, bromelain fractions.

after bromelain administration [26]. Besides direct cleavage of fibrin, activation of fibrinolytic factors was discussed [37], e.g. by increasing the plasmin concentration [20]. The fibrinolytic activity of bromelain has been attributed to the enhanced conversion of plasminogen to plasmin, which limits the spread of the coagulation reaction by degrading fibrin [38].

By means of these reactions the vascular permeability may be enhanced and edematous fluid may again be absorbed by tissues. This is in agreemant with clinical findings in that bromelain administration may lead to an increased concentration of concomitantly given antibiotics in body fluids as well as in tissues (see above).

Plasmakinins and prostaglandins play important roles as mediators of pain and vascular phenomena associated with acute inflammation. Animal experiments demonstrated that bromelain lowers the plasmakinin level [39]. Similarly, bromelain injections caused a dose- dependent decrease of bradykinin levels at inflammatory sites and a parallel decrease of the prekallikrein levels in sera [40]. Studies of prostaglandin metabolism during acute inflammation showed that orally administered bromelain reduces the level of both PGE_2 and of thromboxane B_2 dose-dependently [41].

Nonsteroidal antiinflammatory drugs inhibit the enzyme cyclooxigenase, resulting in a decrease of both pro- and antiinflammatory prostaglandins. In contrast, bromelain may, according to Taussig, selectively inhibit the proinflammatory thromboxane generation and shift the ratio of thromboxane/prostacyclin (PGI_2) in favor of the antiinflammatory prostacyclin. The mechanism of action of the recently introduced 'superaspirins' and of bromelain were suggested to be identical [11].

Effects of bromelain on malignant growth

First observations on the effects of bromelain on cancer patients

Gerard in 1972 [42] and Nieper in 1976 [43] reported on beneficial effects following oral administration of bromelain to cancer patients. After treatments with relatively high doses for several weeks and months, respectively, they noted remarkable remissions of malignant tumors with negligible side effects. However, these reports must be considered to be anectodal by and large.

Bromelain inhibits tumor cell growth in vitro

Bromelain inhibits the proliferation of different tumor cells in vitro. The inhibitory activity can be traced neither to the proteolytic nor to the peroxidase activity or to the platelet aggregation-inhibitory activity [44]. Later, Garbin et al. [45] as well as Grabowska et al. [46] confirmed the concentration-dependent inhibitory activity of bromelain crude extract and bromelain fractions on various tumor cells in vitro. Maurer et al. [47] found that bromelain may induce differentiation of leukemic cells in vitro and proposed this phenomenon as a possible mechanism of action. Apoptosis of tumor cells may result from induction of differentiation, a process by which many cytostatic drugs may eliminate tumor cells.

Relationships between risk of thrombosis and risk of metastases

An association between venous thromboembolism (VTE) and cancer has been recognized since at least 1865 [48]. Patients with clinically evident malignant diseases or occult cancer have an increased risk of VTE, and necropsy studies document an increased prevalence of thrombosis among patients with visceral cancer. Conversely, two recent studies have shown that the risk of revealing malignancy one year after a VTE is increased three to four times [49, 50]. We know by now that upon contact with platelets, tumor cells release a variety of factors (growth factors such as platelet-derived growth factor (PDGF), transforming growth factor-β (TGF-β), thrombin, thrombospondin, prostaglandins, cathepsins, among others) that promote platelet aggregate formation. They are also capable of damaging the vascular wall, thereby inducing congestion, which in turn causes coagulation. Platelets may form aggregates with tumor cells that can adhere to the endothelium and stimulate initial processes of metastases [51–55].

Taussig et al. [11] deserve the credit for recognizing the significance of bromelain as an anticoagulant and as a potential antimetastatic drug. In 1988, he and co-workers [56] reported that bromelain fed to C57bl/6 mice would dramatically lower the take of subcutaneous (s.c.)-injected Lewis lung tumor cells, leading to 77–98% reduction, and that this effect was due neither to the proteolytical anticoagulant nor to the peroxidase activity of bromelain. Similarly, Grabowska et al. [46] found that B16F10 mouse melanoma cells, preincubated in vitro with bromelain, significantly reduced lung metastatic tumor weight about three times. However, no survival benefit was seen. Furthermore, bromelain diminished the capacity of these cells to migrate through an extracellular matrix layer in an in vitro invasion assay and inhibited the growth of the tumor cells in a concentration-dependent manner, whereas the antiproliferation effect did not correlate with the proteolytic activity. Finally, human platelets pretreated in vitro with bromelain lost their capacity to stimulate the invasiveness of several metastatic tumor cells in the in vitro invasion assay.

Table 3 summarizes the effects of bromelain on tumor cell growth and metastasis, whereas figure 1 depicts, in more detail, a number of reactions and targets with which bromelain may interfere when it meets interacting plate-

Table 3. Bromelain effects, in vitro (BP, F4, F5, F9) and in vivo (POS), on tumor cell growth and metastasis.

Protease	Parameter	Effect	Significance	Ref.
Bromelain BP Bromelain F9	tumor cell proliferation	↓	reduction of tumor cell growth	44, 45 46
Bromelain BP Bromelain F4, F9 Bromelain F5	tumor cell invasion through extracellular matrix	↓ =	reduction of the invasive potential of tumor cells	46
Bromelain POS	growth of lung metastases in mice	↓	reduction of the metastatic potential of tumor cells	44
Bromelain BP Bromelain F9	CD44 expression on metastatic cells	↓	reduction of tumor cell adhesion to endothelial cells	46, 57 58
Bromelain BP	growth of lung metastasis in mice	↓	reduction of the metastatic potential of tumor cells	46
Bromelain BP	survival time of mice bearing lung metastases (in vitro → in vivo)	=	mouse survival time	46

Bromelain BP (base powder), commercial crude extract; POS, BP given orally; F4, F9, Bromelain fractions.

Figure 1. Young pineapple fruits between leaves suggested to secrete bromelain.

lets, tumor and endothelial cells in blood vessels. Metastasized tumor cells, while migrating through the vessels, carry CD44 adhesion molecules on their surface, by which they adhere to endothelial cells via the ligand hyaluron. Bromelain preferentially cleaves off CD44 molecules by virtue of its proteolytic activity, thus inhibiting one of the first steps of the metastatic process (shown at the upper right).

In addition, metastasized tumor cells carry the receptor (uPAR) for the urokinase plasminogen activator (uPA), which generates plasmin from plasminogen. Plasmin degrades the extracellular matrix (ECM), composed of collagen type IV, laminin and fibronectin. Tumor cells also secrete matrix metalloproteinases (MMPs), enabling the malignant cells to invade through the ECM. Bromelain diminishes uPAR expression and uPA activity, thus inhibiting the invasion step of metastasis (shown at the upper left).

Recently, relevant interactions between tumor cells and platelets were elucidated in more detail. They take place on different levels: intravasal distribution, adhesion on endothelial cells, invasion and extravasation. Platelets directly bind to tumor cells, a process promoted by the release of factors such as platelet factor 4, thrombospondin, thrombin and gelatinase A from platelets, which facilitate thrombus formation.

TGF-β, produced by both platelets and tumor cells, plays an important role: it induces the synthesis of ECM proteins and stimulates the activity of uPA, MMPs and angiogenesis. Thus, disturbance of the blood coagulation system may lead to the formation of thrombi by aggregating platelets and tumor cells. Bromelain is capable of inhibiting both platelet aggregation in vitro and in vivo, as well as platelet-stimulated invasiveness of tumor cells (shown at the lower endothelium).

Bromelain modulates the function of cell adhesion molecules

In 1992, Hale and Haynes [15] reported that in vitro treatment of T lymphocytes with bromelain removes distinct surface molecules, thereby enhancing CD2-mediated T cell stimulation. The adhesion molecule CD44 has attracted particular interest since it was recognized as a marker for circulating cells, especially metastasizing tumor cells [59]. Harrach et al. [57] showed that bromelain diminished CD44 expression on Molt 4/8 leukemia and SK-Mel 28 melanoma cells, the effect being most pronounced with bromelain fraction F9, with its high proteolytical activity. Yet the proteinase activity of the bromelain fractions tested did not correlate with the manner in which CD44 expression could be modulated. Bromelain was more effective than chymotrypsin, papain and trypsin. Treatment of human lymphocytes with bromelain F9 reduced the expression of CD44, yet did not affect CD11a (LFA-1) molecules; the adhesion of lymphocytes onto umbilical vein endothelial cells was also lowered [60].
In addition, selective influences of bromelain on the expression of other surface molecules on lymphocytes were observed by Kleef et al. [61]. It is still an open question whether the modulation of CD44 molecules is a prerequisite for the inhibition of metastasis by bromelain. Oral administration of bromelain (3000 FIP units daily for 10 days) did cause a moderate reduction of CD44 expression on the lymphocytes from mammary tumor patients. Conversely, the expression of CD11a and CD62L molecules was weakly increased; CD16 molecules remained unchanged [33].

Bromelain modulates functions of immune cells

Bromelain and papain stimulate, in vitro, mononuclear blood leukocytes to produce considerable quantities of TNF-α, Il-1β and Il-6, particularly in monocytes [62, 63]. The production of these cytokines by leukocytes could also be demonstrated after oral administration of a polyenzyme drug containing bromelain [64]. Moreover, granulocytes reacted to the same drug by forming reactive oxygen radicals known to exert antimicrobial and antitumor inhibitory effects [65]. Garbin et al. [45] found that purified bromelain fraction F9 augments, in vitro, at suboptimal concentrations of Il-2, the lymphocyte-mediated inhibition of proliferation of various tumor cells. It remains to be shown in clinical studies whether bromelain can be used for unspecific immunostimulation to treat distinct disorders.
Mynott et al. recently suggested that bromelain may be a novel inhibitor of T cell signal transduction [66]. However, since bromelain contains different biological activities, these findings need confirmation with purified factors.
In a study with 15 healthy donors and 15 mammary tumor patients, orally given bromelain (3000 FIP units daily for 10 days) doubled the capacity of patient's blood monocytes to kill tumor cells in vitro [33]. Among both patients and healthy donors, individual monocyte responses varied to a great extent. Bromelain responders (>10% increase in immunocytotoxicity) showed a significantly weaker cytotoxicity than those grouped as nonresponders. This weakness could be overcome by bromelain. The effects were reversible. NK- and LAK-cell activities dropped during bromelain administration, but normalized again afterwards.
Table 4 summarizes immune-cell-mediated effects of bromelain against tumor cells.

Bromelain achieves debridement of burns

Rapid debridement of third-degree burns considerably reduces the morbidity and mortality of severely burned patients. It permits early skin grafting and lessens the problem of sepsis, thus abbreviating the convalescence period. Chemical debridement as opposed to surgical debridement selectively removes only the burned dena-

Table 4. Immune cell mediated bromelain effects, in vitro (by bromelain F9) and in vivo (by bromelain POS), against tumor cells.

Protease	Parameter	Effect	Significance	Ref.
Bromelain F9	immunocytotoxicity of lymphocytes against tumor cells	↑	increase of cellular immune response	45
Bromelain F9	secretion of TNFα, Il-2	↑	stimulation of lymphocytes	45
Bromelain POS	immunocytotoxicity of lymphocytes, monocytes from tumor patients against tumor cells in vivo	= ↑	cellular immune response in vivo	39
Bromelain POS	secretion of Il-1β from human monocytes	↑	reduction of tumor cell adhesion to endothelial cells	33
Bromelain POS	expression of cell surface markers on lymphocytes from tumor patients CD44	 ↓	regulation of cell adhesion and signal transduction of lymphocytes	33

tured skin. Topical bromelain (35% in a lipid base) has achieved complete debridement on experimental burns in rats in about 2 days, as compared with collagenase, which required about 10 days, with no side effects or damage to adjacent burned tissue [67]. A debridement agent apparently free of proteolytic activity was extracted from commercial bromelain; it was called escharase [68]. Morever, the use of topical bromelain for frostbite eschar removal was investigated: no debridement other than that of the superficial eschar layers was noted; after two topical applications of bromelain, frostbite injuries remained unaffected [69].

Pharmacokinetics of bromelain

Is bromelain absorbed following oral application?

This frequently asked question can now be answered in the affirmative. In 1992, Smyth et al. [20] showed that bromelain given orally to rabbits increases the plasmin serum level and prolongs the prothrombin and antithrombin times. Seifert et al. [70] found that up to 40% of ^{125}J-labelled bromelain is absorbed from the intestine in high molecular form. Later, by means of different methods, a large body of direct and indirect evidence supported the conclusion that bromelain is absorbed from the intestine [36,71]. Similarly, other enzymes, such as kallikrein [72], known to lower arterial blood pressure, and several cytokines such as, IL-2, -5 and -6 [73] reveal pharmacological effects following oral application, thus strongly suggesting intestinal absorption of these proteins. However, one question still remains to be answered: How much bromelain is absorbed and in which form does it circulate in the blood? Proteinases similar to bromelain are rapidly complexed with antiproteinases, mainly with α_2-macroglobulin (AMG) and α_1-antitrypsin. This fact creates difficulties for the quantitative determination of bromelain in serum. Consequently, the recovery of bromelain considerably varied depending on the analytical method used. In any case, the protective AMG molecule leaves the proteolytical activity of bromelain intact but reduced [74]. Three days after oral administration of 8.6 g of bromelain, Castell [71] determined a mean half-life of 6–9 h and a plasma concentration (AUC) of 2.5–4 ng/ml.

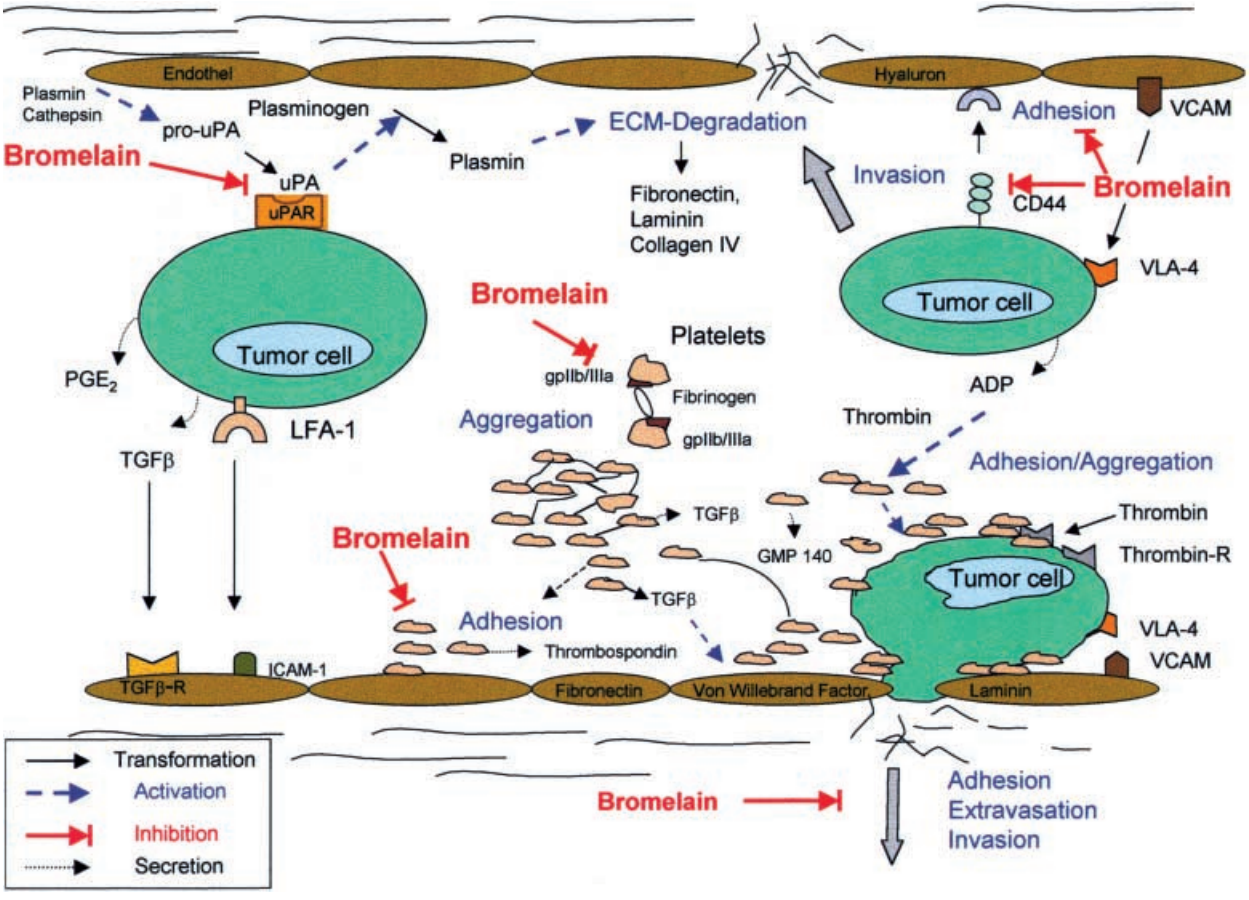

Figure 2. Effects of bromelain on the interactions of platelets, tumor and endothelial cells inside a blood vessel. ECM, extracellular matrix; Il-1β, interleukin-1β; PGE_2, prostaglandin E_2; TGFβ, transforming growth factor β; TGFβ-R, transforming growth factor β receptor; uPA, urokinase plasminogen activator; uPAR, urokinase plasminogen activator receptor; LFA-1, ICAM-1, VCAM, VLA-4 are cell surface adhesion molecules and their corresponding ligands; GMP 140 and Gp IIb/IIIa (fibrinogen receptor) are platelet products.

AMG, the main complexing agent for bromelain in blood, is secreted by macrophages and consists of four identical subunits (or two identical halves) in the so-called slow form [36]. After entrapping the bromelain molecule, the slow form will undergo a conformational change in order to yield the AMG fast form, causing an increased affinity to LDL or other AMG receptors, yet retaining proteolytic activity. The plasma half-life of the AMG slow form is reduced from its original 8 days to 10–30 min in the fast form. The latter can still interact with additional cytokines (Il-1β, Il-6, IFNγ, TNFα, TGFβ, PDGF etc.) and hormones and is known as activated AMG. The immunogenic determinants of the entrapped enzyme molecules are covered by AMG, which prevents determination using antibodies.

In a remarkable clinical study meeting all requirements of good clinical practice (randomized, double-blind, cross-over-design), the bioavailability of a polyenzyme drug (combining bromelain, trypsin, and rutosid) was examined in 21 healthy males [75–78]. Following oral administration of 400-mg and 800-mg tablets (corresponding to 1.94 and 3.88 × 10^4 FIP units) four times daily up to 4 days, the specific activities of bromelain and trypsin were determined in plasma. The activities and AUC values proportionally correlated with the respective dosage. In addition, quantitative studies by means of enzyme immunoassays and Western blot analyses confirmed these findings. Moreover, plasma concentrations of trypsin and specific proteinase activities correlated as well. These results support the notion that the enzymes are absorbed from the gastrointestinal tract in a functionally intact form. The relatively high doses were well tolerated, with few side effects such as pasty faeces, flatulence, and fullness. This is consistent with observations in athletes taking as much as 1.2 × 10^4 FIP units of bromelain daily.

Toxicology of bromelain

Acute toxicology

According to Moss et al. [16] no LD_{50} could be determined with oral doses up to 10 g/kg in mice, rats or rabbits. Lethal doses (LD_{50}) after i.p. administration: mice 37 mg/kg, rats 85 mg/kg; after intravenous (i.v.) administration: mice 30 mg/ kg, rabbits 20 mg/kg. No immediate toxic reactions were seen. These relatively high doses exceed those normally given to human by far.

Chronic toxicology

Five hundred milligrams of bromelain per kilogram per day given orally to rats did not provoke any alteration in food intake, growth, histology of the heart, kidney and spleen, or hematological parameters [16]. Normal doses of 3000 FIP units/day given to human over a period of 10 days did not significantly affect blood coagulation parameters [33].

Drug safety

In 12 placebo-controlled studies, very few side effects were observed; one study noted a 1.8% incidence of diarrhea, nausea, occasional gastric disorders and allergic reactions. One company registered only eight cases of side effects such as exanthema and urticaria out of >3.5 million bromelain tablets (500 FIP units each) sold over 7 years [79]. Bromelain is considered to be nontoxic and without side effects; therefore it can be used without concern in daily doses from 200 up to 2000 mg (500–5000 FIP units) for prolonged periods of time [13]. Bromelain has shown therapeutic benefit in doses as small as 160 mg/day, but the best results occur when starting at a dose of 750 mg/day. It is generally recommended that bromelain be taken at least 1 h before meals. To minimize trauma from sporting activities or tooth extractions, administration should begin 48 h prior to event. Tablets must be coated in such a way that they resist stomach digestion.

Clinical efficacy of bromelain

Table 5 presents a selection of controlled clinical studies, demonstrating, in human, several of the pharmacological effects reported above from in vitro and animal experiments.

It should be noted that the medical use of drugs containing bromelain and other proteinases such as papain, trypsin and chymotrypsin (to mention the most frequently used) is a matter of dispute: Many medical doctors are still sceptical about findings that proteinases are absorbed from the gastrointestinal tract in a functionally intact form, and consequently deny any efficacy of orally applied enzymes. On the other hand, a remarkable list of clinical studies, conforming to GCP rules and mainly performed with polyenzyme drugs, clearly makes a case for evidence-based pharmacological efficacy of proteinases [85].

Arguments for the use of bromelain for medical indications

- Bromelain belongs to a group of proteolytical enzymes that have found a wide range of applications for the indications mentioned above.
- Bromelain is a phytotherapeutic drug that can be given orally and reveals few toxic side effects, thus favoring acceptance and compliance by patients.
- Bromelain is orally absorbed and generates various pharmacological systemic effects: prevention and re-

Table 5. Selection of controlled clinical studies with bromelain.

Diagnosis	Design of study	*n*	Drug, daily dosage	Critical parameters, results, observations	Ref.
Acute sinusitis	r, db, P1	V : 23 Pl : 25	4 × 40 mg Br	inflammation, secretion, breathing, disturbance, pain. V significantly better than Pl	25
Face and head trauma	db, Pl	V : 20 Pl : 21	4 × 40 mg Br	edema, ecchymoses; reduction by V highly significant	19
Trauma of lower extremity	r, b, Cd	V : 18	3 × 40 mg Br 3 × 1000 mg Cd	pain, edema, hematoma. V significantly better than oxyphenbutazone (Cd)	80
Posttraumatic inflammation and swelling	r, b, Cd	V : 60 Cd : 60	3 × 40 mg Br 3 × 1000 mg Cd	hematoma, edema, flexibility, pain; equivalence of V and oxyphenbutazone (Cd)	81
Postoperative tumefactions	r, db, PI	V : 50 Pl : 50	3 × 80 mg Br	girth of ball of forefoot, smallest girth of forefoot pain intensity; significant improvement of all parameters by V	82
Mediolateral episiotomy	r, db, Pl	V : 80 Pl : 80	4 × 40 mg Br	edema, inflammation, pain; V significantly better than Pl	83
Oral surgery (teeth extraction)	r, db, cr	16	4 × 40 mg Br	swelling, pain; less inflammation and pain by V	84

n, number of patients; r, randomized; db, double-blind; cr, cross-over; Pl, placebo; Cd, control drug; Br, bromelain; V, verum.

duction of edema antiinflammation, stimulation of monocytes to secrete cytokines such as Il-1β and TNF-α, induction of phagocytosis and cytotoxicity by granulocytes, inhibition of platelet aggregation and stimulation of fibrinolysis, immunomodulatory effects promoting antigen-unspecific tumor cytotoxicity, among others.

– In vitro and in vivo data suggest that bromelain may act as a prophylactic drug to prevent metastases. However, clinical data to support this suggestion are still lacking.
– There are satisfactory prescriptions for quality control and production of a standardized pharmaceutical drug.

These arguments may recommend bromelain as a suitable model substance for further scientific evaluation of the class of proteinases to which bromelain belongs. The reversible platelet aggregation inhibitory property of bromelain may attract interest in cardiovascular surgery. Bromelain's potential for debridement of skin burns may be beneficial for early skin grafting. For applications in oncology, further preclinical and well-designed clinical studies are undoubtedly required. Bromelain effects on lymphedema in mammary tumor patients, and the prophylaxis of metastases and antiangiogenic phenomena with respect to tumor growth should be worthwhile subjects for further, more detailed investigations.

Finally: Why do pineapple plants produce and need bromelain?

The significance of bromelain proteinases for pineapples has been a mystery for a long time. The most compelling hypothesis is based on the well-known fact that carnivorous plants derive their supply of nitrogen and phosphorus from degradation of organic material (foliage, insects, microbes) by means of highly active proteinases and other digesting enzymes. In the tropical jungle, the pineapple plant is an epiphytic bromeliad, growing on other plants which offer hardly any nutrients. The rosette-like arrangement of the pineapple plant's leaves forms funnel-type rainwater reservoirs, so-called phytotelmata, that are always filled with water, as well as with nitrogen and phosphorus suppliers. This hypothesis is supported by recent findings that leaves react to mechanical stimuli of only 2 s by producing proteinkinases [86]. Moreover, the carnivorous pitcher plant Sarracenia purpurea was shown to respond to various chemical signals (nucleic acids, proteins, ammonia) by secreting hydrolytic enzymes [87]. In order to digest as many proteins from insects and microorganisms as possible, enzyme 'families' with a broad spectrum of pH optima, such as the 'papain superfamily', have evolved.

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