

## Modulation of Murine Tumor Growth and Colonization by Bromelaine, an Extract of the Pineapple Plant (*Ananas Comosum* L.)

JOSEF BEUTH and JAN MATTHIAS BRAUN

*Institute for Scientific Evaluation of Naturopathy, University of Koeln, Robert-Koch-Str. 10, 50931 Koeln, Germany*

**Abstract.** *The antitumor and antimetastatic activities of the plant cysteine endoproteinase bromelaine were evaluated in a murine model. Syngeneic sarcoma L-1 cells were incubated with bromelaine (after preceding time and dosage kinetics) and subcutaneously; (s.c.) or intravenously; (i.v.) inoculated into BALB/c-mice (n=5 per experimental group) to induce local tumor growth or lung colonization. Compared to non-protease incubated L-1 cells, local tumor growth and experimental lung metastasis decreased significantly ( $p<0.05$ ) after bromelaine incubation of the tumor cells. Sarcoma L-1 cells induced local tumor growth after s.c. inoculation and lung colonization after i.v. injection. Intraperitoneal (i.p.) or s.c. administration of bromelaine (optimal dosage and time schedule tested in preceding kinetic studies) significantly ( $p<0.05$ ) reduced local tumor weight, however, lung colonization was non-significantly reduced. Bromelaine incubation of sarcoma L-1 cells significantly reduced their tumorigenic/metastatic capacities. Bromelaine treatment after tumor cell inoculation significantly reduced local tumor growth, experimental lung metastasis, however, to a lesser, non-significant degree.*

Plant extracts with a high content of proteolytic enzymes have been abundantly used in traditional medicine of Central and South America (1). Systemic enzyme therapy is currently being studied for defined indications, e.g. in oncology, infectiology, rheumatology and traumatology (2-4). Its therapeutic use is partly based on scientific studies in agreement with evidence-based medicine (EBM), however, it is mostly empirical (5-7).

Currently available enzyme preparations for oral therapy usually consist of a combination of the animal serine

endoproteinases trypsin and chymotrypsin and the plant cysteine endoproteinases, bromelaine and papain. Bromelaine monotherapy is empirically well established in traumatology, infectiology and rheumatology.

Plant bromelaine is obtained from the stem of the pineapple (*Ananas comosum* L.). Sequencing of the plant cysteine endoproteinases, bromelaine and papain, demonstrated that both are members of the papain family (8). Raw stem bromelaine consists of at least 3 immunologically distinct proteases: stem bromelaine, fruit bromelaine and ananain (9). High performance liquid cation exchange chromatography characterized as many as 9 proteolytically active components in raw stem bromelaine (10). Distinct scientific evaluations are currently being performed to demonstrate the medical/clinical relevance of those bromelaine components.

This preclinical investigation was performed to evaluate the antitumor/antimetastatic activity of bromelaine in a well-established murine (BALB/c-mouse/sarcoma L-1) model.

### Materials and Methods

**Animals.** Inbred male BALB/c-mice (Charles River Wiga Breeding Company, Sulzbach, Germany), 8-10 weeks, weighing about 20 g, were used for these studies. The animals were kept in plastic cages and allowed free access to food and water.

**Tumor.** Spontaneously grown syngeneic sarcoma L-1 cells were maintained in tissue culture using RPMI medium with 10% fetal calf serum (FCS), penicillin (100 U/mL), streptomycin (100 mcg/mL) and L-glutamine (2 mMol), all purchased from Sigma Chemicals Co., Heidelberg, Germany, as described elsewhere (11,12).

**Proteolytic enzyme.** Bromelaine (5 FIP units per mg) was purchased from the International Pharmacy, Koeln, Germany, and used throughout these studies.

**Experimental design.** Tumor cell suspensions (0.1 mL) of  $1 \times 10^5$ /mL sarcoma L-1 cells were i.v. inoculated into the tail veins of BALB/c-mice (n=5 per experimental group). This tumor cell density proved to be optimal with respect to the extent of lung colonization in preliminary investigations (12,13). To evaluate local tumor growth,

*Correspondence to:* Josef Beuth, Institute for Scientific Evaluation of Naturopathy, University of Koeln, Robert-Koch-Str. 10, 50931 Koeln, Germany. Tel: 0049-221-478 6414, Fax: 0049-221-478 7017, e-mail: hans.beuth@uk-koeln.de

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Table I. Mean tumor weight and mean number of lung colonies in BALB/c-mice (n=5 per experimental group) injected with sarcoma L-1 cells from cell culture medium (control) or bromelain (20 mcg/mL) containing medium. All experiments were repeated. \*  $p < 0.05$  = statistically significant from control.

BALB/c-mice s.c. challenged with L-1 cells incubated with	mean tumor weight mg (SD)
Medium (control)	228.6 (71)
Bromelain	102.0 (44) *
BALB/c-mice i.v. challenged with L-1 cells incubated with	mean number of lung colonies (SD)
Medium (control)	33.0 (12)
Bromelain	10.6 (6) *

0.1 mL of a sarcoma L-1 cell suspension ( $1 \times 10^7$  cells/mL) was s.c. inoculated into the right abdominal region of BALB/c-mice (n=5 per experimental group).

Experiment 1: Sarcoma L-1 cells were incubated for 1 h with 20 mcg/mL bromelain. The incubation time and bromelain concentration proved to be optimal from preceding kinetic studies. Following incubation, L-1 cells were washed by centrifugation (3 times), checked for vitality (trypan blue dye exclusion test), counted and i.v. or s.c. inoculated into BALB/c-mice. Control mice were inoculated with non-bromelain-treated L-1 cells from the cell culture. Lung surface tumor colonies were counted under a dissecting microscope by two independent observers. Local tumor were explanted and weighed, both at day 14 after tumor cell inoculation.

Experiment 2: After i.v. or s.c. inoculation of sarcoma L-1 cells into BALB/c-mice (day 0), 1 mg/day of bromelain (dosage proved to be optimal from preceding kinetic studies and approximately calculated from human medicine) was s.c. or i.p. administered on days 1, 4, 7, 10 and 13. Investigation was terminated on day 14 and lung colonization and local tumor weight were evaluated as described.

Statistics. Student's *t*-test was used for statistical analysis of the data. All experiments were performed twice and yielded reproducible results.

## Results

Compared to a control group of BALB/c-mice, local tumor weight and number of lung colonies on the 14th day after sarcoma L-1 cell inoculation were significantly reduced ( $p < 0.05$ ) after bromelain incubation of the tumor cells. Table I demonstrates data from the optimal bromelain concentration (20 mcg/mL incubation medium) taken from kinetic studies (range 0.02-20.0 mcg bromelain/mL medium).

To evaluate the antitumor and antimetastatic activity of systemic bromelain therapy, BALB/c-mice were s.c. (local

Table II. Mean tumor weight in BALB/c-mice (n=5 per experimental group) after s.c. inoculation of sarcoma L-1 cells and s.c. or i.p. bromelain treatment (1 mg/day/mouse on days 1, 4, 7, 10 and 13). All experiments were repeated and yielded reproducible results. \*  $p < 0.05$  = statistically significant from control.

BALB/c-mice s.c. challenged with L-1 cells and treated with	mean tumor weight mg (SD)
PBS (control) s.c.	220.4 (52)
Bromelain s.c.	103.2 (41) *
PBS (control) i.p.	270.5 (63)
Bromelain i.p.	92.6 (42) *

Table III. Mean number of experimental lung metastases in BALB/c-mice (n=5 per experimental group) after i.v. inoculation of sarcoma L-1 cells and s.c. or i.p. bromelain treatment (1 mg/day/mouse on days 1, 4, 7, 10 and 13). All experiments were repeated and yielded reproducible results.

BALB/c-mice i.v. challenged with L-1 cells treated with	mean number of lung colonies (SD)
PBS (control) s.c.	37.2 (14)
Bromelain s.c.	21.4 (8)
PBS (control) i.p.	34.4 (12)
Bromelain i.p.	18.6 (7)

tumor) or i.v. (experimental lung metastases) inoculated with sarcoma L-1 cells. Bromelain (1 mg/day/mouse) was regularly administered s.c. or i.p. and local tumor weight and lung colonization were evaluated on day 14. As shown in Table II, mean tumor weight was significantly reduced ( $p < 0.05$ ) after both s.c. or i.p. bromelain administration.

Experimental lung metastases were reduced in bromelain-treated mice, however, non-significantly, as shown in Table III.

## Discussion

The plant cysteine endoproteinase bromelain (from the stem of pineapple, *Ananas comosum* L.) has previously been shown to suppress tumorigenesis (14). It was suggested that an imbalance between cysteine proteinases and cysteine antiproteinases (e.g. cystatin) may have an influence on tumor metastasis (15). However, it is not clear at present whether the antitumor effect of bromelain depends on its peroxidative properties, nor whether bromelain can induce the synthesis or release of antiproteinases similar to serine proteinases.

Several groups have shown that proteolytic enzymes (serine endoproteinasases like trypsin or chymotrypsin and cysteine endoproteinasases like bromelaine or papain, or combinations of those enzymes) modulate blood protein, antioxidant, cytokine and chemokine levels, cellular adhesion molecules and reactive oxygen species (16). Experimental investigations in murine models suggest that a defined mixture of proteolytic enzymes reduces the formation of metastases and extends survival time of the treated mice (17). They also report a decreased expression of CD-44 and CD-54 molecules in tumors exposed to proteolytic enzymes and conclude that serine and cysteine endoproteinasases are able to inhibit organ colonization (18).

The precise mechanism of action of systemic bromelaine therapy is still unknown. The numerous alterations of the cytokine composition during/after enzyme therapy seem to be an indication of the therapeutic efficacy rather than a mode of action. Whether the effect of bromelaine on adhesion molecules is of relevance to patients remains unclear. To further study the antitumorigenic/antimetastatic capabilities of the endoproteinasase bromelaine, this investigation in the BALB/c-mouse / sarcoma L-1 tumor model was performed.

To elucidate the bromelaine activity on sarcoma L-1 cell capability to induce local or metastatic tumors in BALB/c-mice, the cells were incubated (defined dose and time kinetics) and inoculated. Both tumor weight and lung colonization decreased significantly after bromelaine incubation of the cells, suggesting a distinct effect of the protease on tumorigenicity. The precise mode of action has to be determined in further investigations, however, the modulation of membrane proteins and adhesion molecules might play a key role.

Systemic bromelaine administration (*s.c.* or *i.p.*), as applied in complementary oncology, demonstrated a relevant effect on local sarcoma L-1 growth in BALB/c-mice. Tumor weight was significantly reduced in bromelaine-treated animals as compared to non-treated control mice. Experimental lung metastases, however, were shown to decrease non-significantly after bromelaine treatment. For this set of investigations, further experiments are warranted to clarify the mode of action of the plant endoproteinasase. It might be suggested from experimental data that immunological modulations are responsible for the antitumor/antimetastatic effects. Further studies are currently being performed to investigate the molecular basis.

Systemic plant endoproteinasase (bromelaine) administration seems to be promising in complementary oncology since the side-effects of tumor destructive standard therapies could be minimized in defined tumor entities (5-7). Accordingly, a precise knowledge of the mode of action has to be obtained so as to integrate bromelaine therapy into standard oncologic treatment modalities.

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