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# Systemic Enzyme Therapy in Oncology
## Effect and Mode of Action

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## Abstract

Plant extracts with a high content of proteolytic enzymes have been used for a long time in traditional medicine. Besides proteolytic enzymes from plants, 'modern' enzyme therapy additionally includes pancreatic enzymes. The therapeutic use of proteolytic enzymes is partly based on scientific studies and is partly empirical. The aim of the current review is to provide an overview of clinical trials of systemic enzyme therapy in oncology, and to discuss the evidence for their possible mechanisms of action.

Clinical studies of the use of proteolytic enzymes in oncology have mostly been carried out on an enzyme preparation consisting of a combination of papain, trypsin and chymotrypsin. This review of these studies showed that enzyme therapy can reduce the adverse effects caused by radiotherapy and chemotherapy. There is also evidence that, in some types of tumours, survival may be prolonged.

The beneficial effect of systemic enzyme therapy seems to be based on its anti-inflammatory potential. However, the precise mechanism of action of systemic enzyme therapy remains unsolved. The ratio of proteinases to antiproteinases, which is increasingly being used as a prognostic marker in oncology, appears to be influenced by the oral administration of proteolytic enzymes, probably via an induction of the synthesis of antiproteinases. Furthermore, there are numerous alterations of cytokine composition during therapy with orally administered

enzymes, which might be an indication of the efficacy of enzyme therapy. Effects on adhesion molecules and on antioxidative metabolism are also reviewed.

Plant extracts with a high content of proteolytic enzymes have been used for a long time in the traditional medicine of Central and South America.[1,2] Systemic enzyme therapy is currently being studied for a variety of indications. Its therapeutic use is based partly on scientific studies and is partly empirical. The fact that systemic enzyme therapy has found a special place in oncology is, in part, explained by historical factors. The foundations of 'modern' enzyme therapy can be found in a book by the English physician John Bard, published in 1907 under the title of 'The Enzyme Treatment of Cancer and its Scientific Basis'. After Adolf Gaschler had developed the anticancer agent Cardozelan, based on chymotrypsin, Max Wolf and Helene Benitez carried out systematic research in the mid-1950s leading to the development of an 'optimised combination' of plant and animal proteinases, which in their view possessed an optimal anticancer effect. The principle of enzyme combinations, developed by Wolf and Benitez, continues to find application today, admittedly sometimes in the form of preparations that differ from the original enzyme combination preparation (WoBe). In adjuvant or palliative cancer therapy, oral enzyme therapy has generally been found to be a well tolerated form of treatment for the relief of adverse effects caused by other tumour therapies and for improving quality of life.

The aim of the current paper is to provide an overview of clinical trials of systemic enzyme therapy in oncology, and to discuss the evidence for possible mechanisms of action of this form of therapy from these clinical studies and experimental studies. The literature search was based on the Medline (1966–1999), EMBASE (1980–1999) and AMED (Allied and Alternative Medicine, 1985–1999) databases. In addition, the firm MUCOS (Geretsried, Germany) allowed us to examine data from unpublished studies.

## 1. Proteinases in Systemic Enzyme Therapy

Currently available enzyme preparations for oral enzyme therapy usually consist of a combination of the animal serine endoproteinase trypsin (EC 3.4.21.4) and chymotrypsin (EC 3.4.21.1) and the plant cysteine endoproteinases stem bromelain (EC 3.4.22.32) and papain (EC 3.4.22.2). Trypsin and chymotrypsin are currently obtained from the pancreatic juice of cattle or pigs. It is likely that genetically engineered animal proteinases will become available in the foreseeable future. Both proteinases belong to the chymotrypsin family.[3] Plant bromelain is obtained from the stem of the pineapple (*Ananas comosum L.*), and papain from the milky sap of the papaya (*Carica papaya L.*). Sequencing of the plant cysteine endoproteinases has demonstrated that both papain and stem bromelain are members of the papain family.[3] Raw stem bromelain consists of at least 3 immunologically distinct proteinases: stem bromelain, fruit bromelain and ananain.[4] Harrach et al.,[5] using high performance liquid chromatography cation exchange chromatography, were able to characterise as many as 9 proteolytically active components in raw stem bromelain.

## 2. Clinical Studies of Systemic Enzyme Therapy

### 2.1. Patients Receiving Chemotherapy

The use of enzyme therapy in patients with malignant disease is based partly on empirical experience, but increasingly also on systematically collated experience and clinical studies. Although the precise mechanism of action of systemic enzyme therapy has not been fully explained as yet, it is clinically used worldwide. Clinical studies have been conducted using an enzyme preparation consisting of a combination of papain, trypsin and chymotrypsin in a weight ratio of 5 : 2 : 2. Relief from

the adverse effects associated with chemotherapy in cancer patients (e.g. mucositis, loss of appetite, fatigue) is an indication for the use of systemic enzyme therapy with proteolytic enzymes (table I). In a prospective randomised study, 51 patients with inoperable bronchopulmonary carcinoma, who were not pretreated, received combination cytotoxic chemotherapy with fluorouracil, vinblastine, methotrexate and cyclophosphamide.[6] A subgroup of 25 patients also received papain/trypsin/chymotrypsin ($2 \times 5$g micro-enemas daily) for 1 to 4 weeks. Outpatient continuation of treatment with papain/trypsin/chymotrypsin tablets (dose not specified), extending for 1 to 11 months, could only be monitored in detail in 14 patients. Nevertheless, all 25 patients in the enzyme group were included in the evaluation. The therapeutic outcome as regards tolerability of the chemotherapy and adverse effects of the tumour-specific treatment (leucopenia, oral mucosal ulceration and increase in blood urea nitrogen levels) was better in the group of patients who received the enzyme preparation. The results of this study were not statistically evaluated, but they suggested that the use of systemic enzyme therapy could improve the patients' general clinical condition and quality of life. Patients treated with chemotherapy alone showed a mean survival of 16 months, while those receiving concomitant treatment with the enzyme preparation had a mean survival of 20 months.

Schedler et al.,[8] in a postmarketing surveillance study, investigated 58 patients with carcinomas of

**Table I.** Clinical studies of the use of systemic enzyme therapy with papain/trypsin/chymotrypsin (P/T/C) in patients receiving chemotherapy (CT)

| Diagnosis | Study design | n | Medication | Duration of treatment | Effects of enzyme therapy | Reference |
|---|---|---|---|---|---|---|
| Inoperable broncho-pulmonary carcinoma | Prospective randomised | 26 *vs* 25 | CT (fluorouracil, vinblastine, methotrexate, cyclophosphamide) *vs* CT + P/T/C 5g enemas (or coated tablets) twice daily | 1–4wk (enemas) + 1–11mo (coated tablets) | Improvement in general condition and quality of life, some improvement in life expectancy, fewer adverse effects of CT | 6 |
| Gastric carcinoma | Prospective open | 76 *vs* 80 *vs* 89 | CT (MFC, MeCCNU, 5-FU) *vs* immunochemotherapy (picibanil) *vs* picibanil + P/T/C/ $6 \times 5$g tablets/day | 6–12mo | Rise (compared with CT) or more marked rise (compared with picibanil) in ratio of T lymphocytes to total lymphocytes | 7 |
| Carcinoma of head and neck | Postmarketing surveillance | 58 | CT (bleomycin + cisplatin + vindesine) + P/T/C 1–4 tablets/day | NA | No patients showed a toxic pulmonary reaction to bleomycin | 8 |
| Ovarian carcinoma | Prospective randomised single-blind placebo-controlled | 23 *vs* 24 *vs* 12 | CT (carboplatin, epirubicin, prednimustine) + placebo *vs* CT + P/T/C 2 tablets 3 times daily *vs* CT + P/T/C 10 dragees (5g) 3 times daily | 6 mo: on days 2–7 after each monthly CT cycle | More rapid fall in AST, ALT, γ-GT, AP and LDH in the enzyme-treated groups | 9 |
| Multiple myeloma | Retrospective parallel group cohort analysis | 99 *vs* 166 | CT (VMCP, MOCCA or VAD) *vs* CT + P/T/C/ 2 tablets 3 times daily | At least 6mo | Survival of patients with stage III multiple myeloma prolonged by 36mo | 10 |
| Large bowel carcinoma | Prospective randomised double-blind placebo-controlled | 30 *vs* 30 | CT (5-FU + levamisole) *vs* CT + P/T/C 3 tablets (extended release) 3 times daily | 2–45mo; mean 16mo | Reduction in adverse effects of CT (sum score), fewer patients with metastases and more patients surviving longer than 42mo | 11 |

**5-FU** = fluorouracil; **AP** = alkaline phosphatase; **γ-GT** = γ glutamyl transferase; **LDH** = lactate dehydrogenase; **MFC** = mitomycin, fluorouracil, cytarabine; **MeCCNU** = semustine (methyl-lomustine); **MOCCA** = methylprednisolone, vincristine, cyclophosphamide, mephalan; **VAD** = vincristine, doxirubicin, dexamethasone; **VMCP** = vincristine, melphalan, cyclophosphamide, prednisone.

the head and neck (adenoid cystic carcinoma, malignant melanoma or lymphoma, all in stage III) receiving combination chemotherapy with cisplatin, bleomycin and vindesine. Adjuvant treatment was given with hydrolytic enzymes (1 to 4 tablets, 3 times daily). None of the patients showed a bleomycin-induced toxic pulmonary reaction, although this generally occurs in about 40% of bleomycin-treated patients. This was true even among patients receiving up to bleomycin 180 mg/day. Bleomycin can be hydrolysed by bleomycin hydrolase (EC 3.4.22.40) a cysteine endopeptidase of the papain family.[12] In mice, it was demonstrated that the presence of bleomycin hydrolase is a protectant against bleomycin-induced death.[13] Therefore, we assume that the reduction of bleomycin-induced adverse effects could possibly be caused by hydrolysis of bleomycin by papain.

In a prospective, randomised, single-blind, placebo-controlled pilot study, 59 female patients were treated with chemotherapy (carboplatin, epirubicin and prednimustine) after surgical excision of an ovarian carcinoma, Figo stage 1B-IV.[9] In addition, 36 of the 59 patients received papain/trypsin/chymotrypsin from day 2 to day 7 of each of the 6-monthly chemotherapy cycles; the dosages used were 2 tablets 3 times daily (24 patients) or 10 coated tablets 3 times daily (12 patients). Although tablets and coated tablets differed in their composition, both subgroups received an equal amount of pancreatic proteinases (480mg daily). Immediately before each treatment cycle, blood was taken for measurement of liver parameters. Patients receiving palliative enzyme therapy showed a clear trend towards lower values for transaminases (AST, ALT), γ-glutamyl transpeptidase, alkaline phosphatase and lactate dehydrogenase than patients receiving placebo. No clinically significant changes were seen in laboratory analysis (red and white blood cell counts, liver enzyme, electrolyte, urea and creatinine levels, or urinalysis). The subjective evaluation of efficacy and tolerability of enzyme therapy on a 5-point scale was rated as very good both by the doctor and the patients.

Sakalova et al.[10] studied the effect of enzyme therapy on survival in 265 patients with multiple myeloma (stages I to III) using standard recording of relevant patient data. The patients had been treated with chemotherapy, namely VMCP/MOCCA (vincristine, melphalan, cyclophosphamide/methylprednisolone, vincristine, cyclophosphamide, lomustine, melphalan) and VAD (vincristine, doxirubicin, dexamethasone), over a period of 12 years. In this retrospective parallel-group cohort analysis, 166 patients who had received papain/trypsin/chymotrypsin (2 tablets 3 times daily) for at least 6 months were assigned to the active treatment group. The aim of the study was to investigate any effect of enzyme therapy on survival. A statistically significant mean prolongation of survival of 36 months was seen in patients with Stage III multiple myeloma (n = 54) in the enzyme group compared with the control group (n = 36) [83 months versus 47 months].

### 2.2 Patients Receiving Radiotherapy

In the field of radiotherapy too, adjuvant enzyme therapy has been shown to reduce radiation-induced adverse effects (table II). In an open randomised study, 19 patients with carcinoma of the floor of the mouth who had undergone 5 weeks of preoperative radiotherapy (total dose 50Gy) received palliative treatment with papain/trypsin/chymotrypsin 5 tablets 3 times daily over the whole treatment period.[16] Radiation-induced mucositis occurred in both the enzyme group and in the control group of 20 patients receiving radiotherapy without enzyme therapy. In both groups, the major manifestation was mucosal oedema. This developed in 13 patients in the enzyme group and 11 in the control group. Mucosal necrosis (ulceration), however, occurred in only 2 patients in the enzyme group, while 9 patients in the control group experienced this adverse effect. Although patients in the enzyme-treated group developed mucosal oedema earlier, mucosal necrosis was seen at a much later stage in this group than in the control group. C-reactive protein (an indicator of inflammation) levels were also comparatively lower in patients receiv-

**Table II.** Clinical studies of the use of systemic enzyme therapy with papain/trypsin/chymotrypsin (P/T/C) in patients receiving radiotherapy

| Diagnosis | Study design | n | Medication | Duration of treatment | Effects of enzyme therapy | Reference |
|---|---|---|---|---|---|---|
| Bronchial carcinoma | Postmarketing surveillance | 73 | No standard treatment, but usually radiotherapy + initial treatment: P/T/C 5 × 5g tablets twice daily for 6wk; then: 3-wk break, then 3 tablets twice daily × 5 days – repeated throughout the treatment period | 2–44wk | Delay in appearance of metastases, reduction in size of initial radiological abnormalities | 14 |
| Carcinoma in the abdominal region[a] | Prospective randomised | 32 *vs* 25 | 47Gy *vs* 54Gy + P/T/C/ 3 tablets twice daily (first wk: 5 tabs twice daily) | 5wk | Shorter duration of radiotherapy-induced adverse effects | 15 |
| Carcinoma of the floor of the mouth | Open randomised | 20 *vs* 19 | 50Gy *vs* 50Gy + P/T/C 5 tablets 3 times daily | 5wk | Lower incidence of mucosal necrosis | 16 |
| Carcinoma of head and neck | Prospective randomised open 2-centre | 47 *vs* 53 | 59Gy *vs* 59Gy + P/T/C (extended release) 3 tablets 3 times daily | At least 7wk | Significant reduction in radiation-induced mucositis, dysphagia and skin reactions | 17 |
| Cervical carcinoma | Prospective randomised open | 60 *vs* 60 | 50Gy *vs* 50Gy + P/T/C (extended release) 3 tablets 3 times daily | Not more than 10wk | Significant reduction in skin reactions, subcutaneous changes, and symptoms affecting the urogenital tract | 18 |

a Mostly uterine or prostatic carcinoma.

ing the enzyme preparation throughout the period of treatment.

The effect of systemic enzyme therapy on radiotherapy-induced adverse effects was also studied in a prospective randomised trial of 57 patients with carcinoma in the abdominal region (mainly prostatic and uterine carcinoma).[15] Papain/trypsin/chymotrypsin 5 tablets 3 times daily for the first week and 3 tablets twice daily from the second week onwards was administered to 25 out of 57 patients, in addition to radiotherapy. Although patients in the enzyme group were exposed to higher radiation doses over the 5-week treatment period (53.4Gy versus 46.7Gy in the control group), the frequency and severity of adverse effects were approximately the same in both groups. However, an advantage of the enzyme therapy was seen in the mean duration of adverse effects (mainly symptoms affecting the gastrointestinal and urogenital tracts) – which was reduced from 25 days (controls) to 14 days (enzyme group). Since the adverse effects developed at approximately the same time in both groups, that is, approximately 16 days after the start of treatment, it appears that, in this study, the systemic enzyme therapy may have exerted some effect on repair mechanisms. According to the authors, a further benefit of enzyme therapy was a clear reduction in the patients' impaired general condition and skin symptoms during irradiation.

Two prospective, randomised, open studies, as yet unpublished, have demonstrated that concomitant treatment with papain/trypsin/chymotrypsin 3 tablets 3 times daily reduces the adverse effects of radiotherapy.[17,18] In the first of these 2 studies, patients with carcinoma of the mouth or pharynx who were treated with the enzyme preparation (n = 53) showed a statistically significant reduction in the number of events compared with the control group (n = 47) in mucositis, dysphagia, skin reactions and skin damage in the radiation field. The second study, carried out on 120 female patients with carcinoma of the cervix uteri, yielded comparable results.[18] In this study, concomitant administration of the enzyme preparation significantly reduced the severity of radiotherapy-induced damage to skin and subcutaneous tissue in the irradi-

ated field, and symptoms relating to the urogenital tract, compared with the control group.

### 2.3 Summary of Clinical Effects

We conclude from the clinical studies that enzyme therapy not only has an anti-inflammatory effect but also a beneficial effect on repair mechanisms. Patients with burns who were treated with proteolytic enzymes had lower levels of acute phase proteins than patients in a control group, which provides further evidence for the anti-inflammatory potential of the enzyme therapy.[19,20] Furthermore, the anti-inflammatory efficacy has been demonstrated in *in vitro* and in *in vivo* models[21] as well as in clinical studies.[22] However, the question of whether enzyme therapy has a direct anticancer effect in clinical trials remains. Until now, a direct anti-tumour effect of enzyme therapy has only been shown in experimental studies. The anticancer effect of enzyme preparations is discussed in section 3.

## 3. Mechanism of Action

The mechanism of action of enzyme therapy has not yet been fully explained. However, it is assumed that a number of effects contribute to this mechanism.

### 3.1 Interaction Between Proteinases and Antiproteinases

#### *3.1.1 Serine Proteinases*

Trypsin and chymotrypsin, the proteinases used in enzyme preparations, are serine proteinases which are irreversibly inactivated by serine antiproteinases. The principal serine antiproteinases include $\alpha_1$-antitrypsin (= $\alpha_1$-proteinase inhibitor), $\alpha_1$-antichymotrypsin, antithrombin III, $\alpha_2$-antiplasmin and C1 inhibitor.[23]

Oral administration of a combination of trypsin and chymotrypsin in a 6 : 1 ratio raised serum levels of $\alpha_2$-macroglobulin and $\alpha_1$-antitrypsin in two studies in patients.[19,24] This effect was also reflected in a raised trypsin inhibitory capacity (TIC). In one of these studies, in 29 patients undergoing hernia surgery,[24] there was a smaller rise in C-reactive protein levels among patients treated with the enzyme preparation (n = 16) compared with those in the control group (n = 13) who did not receive treatment with the trypsin/ chymotrypsin preparation. In the other study, in 30 patients with second-degree burns,[19] enzyme-treated patients (n = 15) had lower serum levels of C-reactive protein throughout compared with patients not receiving enzymes (n = 15). These findings suggest that the preparation inhibits inflammation. An elevated level of antiproteinases appears to account for the fact that the serum level of cathepsin D, an aspartic proteinase, was lower in burned patients treated with proteolytic enzymes than in the control group.[20] Cathepsin D appears to play an important role in the development and metastasis of cancer. Foekens et al.[25] showed in a study involving 2810 patients with breast cancer that a high level of cathepsin D in tumour tissue was associated with a shorter relapse-free time and survival.

Because of the increased activity of proteinases in tumour tissue, the use of protease inhibitors as anticarcinogenic agents is logical. Certain protease inhibitors, especially serine proteinase inhibitors such as the soybean-derived protease inhibitor BBI (Bowman-Birk inhibitor), have been shown to be capable of preventing carcinogenesis (for review see Kennedy[26]). Furthermore, endogenous serpins (serine proteinase inhibitors) were shown to exert an inhibitory effect on invasion and metastasis by cancer cells.[27] This may constitute one of the mechanisms of action of enzyme therapy: orally administered enzymes seem to induce the synthesis of antiproteinases which in turn inactivate proteinases such as cathepsines (fig. 1). The induction of $\alpha_1$-antitrypsin synthesis is mediated by a serpin-enzyme complex (SEC) receptor that is located on the cell surface. In human hepatoma cell lines, Joslin et al.[28] showed that several proteinase-antiproteinase complexes can bind to the SEC receptor. In this context, a decisive factor for binding to the SEC receptor is a highly conservative pentapeptide domain in the serpin. A functional catalytic centre on the serine proteinase is essential for the formation of a serpin-proteinase complex.[29] This mech-

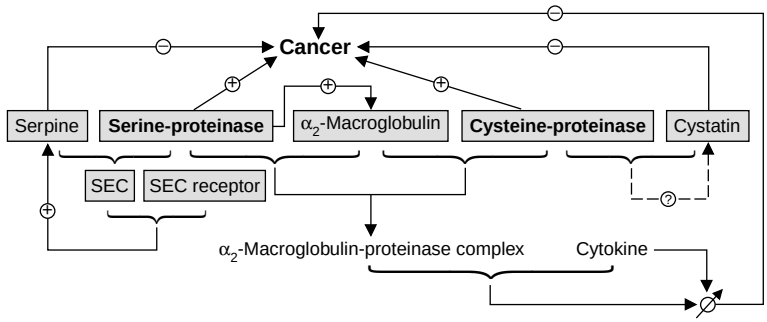


**Fig. 1.** Schematic representation of the interaction between proteinases and antiproteinases and their effects on cancer. **SEC** = serpin-enzyme complex.

anism of action – proteinase-induced synthesis of antiproteinases – would only apply to the animal proteinases trypsin and chymotrypsin.

#### *3.1.2 Plant Cysteine Proteinases*

Cysteine proteinases also appear to be involved in cancer growth. Lah and Kos[30] have suggested that an imbalance between cysteine proteinases and cysteine antiproteinases (cystatin) may have an influence on tumour metastasis. The use of proteinases as prognostic markers in oncology is a topic of ongoing discussion.[31] Lah et al.[32] have observed a marked increase in the level of cathepsin (particularly cathepsins B and L) in the cytosol of tumour tissue in patients with breast cancer. Bromelain caused inhibition of metastasis formation when fed to mice carrying implanted Lewis lung carcinoma cells.[33,34] It is interesting to note that in this experiment, bromelain exposed to 30 minutes' heating at 70°C to inactivate its proteolytic and anticoagulant activity showed comparable efficacy to that of proteolytically active bromelain. Bromelain with no proteolytic activity also inhibited tumour cell growth *in vitro*.[35] However, this did not occur if bromelain was so thoroughly denatured that it also lost its peroxidative activity. It is not clear at present how far the anti-tumour effect of bromelain depends on its peroxidative properties, nor whether bromelain or inactivated bromelain can induce the synthesis or release of antiproteinases in a similar way to serine proteinases.

Like bromelain, papain also showed anti-tumour effects in animal studies. The growth rate, tumour invasion and metastasis of $B_{16}$ melanoma and Lewis lung carcinoma was reduced in mice administered papain (0.25 mg/week) by intramuscular or intraperitoneal injection compared with control animals.[36] The mechanism of action here seems to depend on the fact that papain-immunised mice developed antibodies reacting with the cysteine proteinases cathepsin B and cathepsin H.

### 3.2 Effects on Cytokines

In a clinical trial of 156 patients with rheumatoid arthritis, 91 patients received additional treatment with pancreatin/papain/bromelain/trypsin/chymotrypsin in a weight ratio of 100 : 60 : 45 : 24 : 1.[37] Both groups were treated with methotrexate and nonsteroidal anti-inflammatory drugs (NSAIDs). Over the period of treatment, patients in the enzyme group showed significantly more marked falls in interleukin (IL)1β and tumour necrosis factor (TNF)α, and higher levels of serum interferon (IFN)α and IFNγ than those in the control group. Lackovic et al.[38] observed a more marked fall in transforming growth factor (TGF)-β1 in the plasma of healthy volunteers during several days' administration of pancreatin/papain/bromelain/trypsin/chymotrypsin. The fall in the levels of the principal mediators of inflammatory reactions, TNFα and IL-1β, appears to represent at least one of the reasons for the greater efficacy of

treatment when supplemented by the enzyme preparation. The improved efficacy was reflected in a higher Ritchie index and reduced morning stiffness at the end of treatment in actively treated patients compared with control participants.

The higher levels of IFNα caused by enzyme administration may also be related to the tumour-inhibiting effect of proteolytic enzymes, since IFNα inhibits cell proliferation. An important factor in this connection appears to be the capacity of IFNα to inhibit the expression of proto-oncogenes and oncogenes which induce cell division.[39] Evidence of a tumour-inhibiting effect of the enzyme preparations was provided by the observed rise in the level of IFNγ which (together with TNFα) is capable of activating macrophages in such a way that they become able to kill tumour cells. A schemata of the effects of proteinases on cytokines observed *in vivo* is shown in figure 2.

However, some of the results mentioned in the preceding paragraph appear to contradict those of *in vitro* experiments. These *in vitro* experiments showed that the formation of TNFα, IL-6 and IL-1β, induced *ex vivo* by IFNγ, was significantly increased in peripheral blood mononuclear cells (PBMNC) of participants receiving pancreatin/papain/bromelain/trypsin/chymotrypsin compared with PBMNC of a control group not receiving the enzyme preparation.[40] However, if the PBMNC were not stimulated by IFNγ, no effect of enzyme administration on the content of TNFα and IL-6 was seen. IL-1β was not investigated in this study.[40]

The effect of the proteinases on cytokines appears to be mediated by $\alpha_2$-macroglobulin. Both serine proteinases and cysteine proteinases can undergo irreversible noncovalent binding to $\alpha_2$-macroglobulin. The proteinases lose most of their catalytic activity in the process. Small low molecular-weight compounds can, however, still be hydrolysed.[41] The reaction of a proteinase molecule with $\alpha_2$-macroglobulin brings about a conformational change in $\alpha_2$-macroglobulin. The $\alpha_2$-macroglobulin: proteinase complex thus formed (known as the 'fast form' because of its electrophoretic mobility), undergoes rapid clearance by the reticuloendothelial system.[41] Under certain conditions, $\alpha_2$-macroglobulin even seems to bind to proteinase molecules. Proteinases may also, in part, be covalently bound to $\alpha_2$-macroglobulin (up to 8% for papain and 61% for trypsin). It is assumed that after $\alpha_2$-macroglobulin has undergone non-covalent binding with one proteinase molecule, the $\alpha_2$-macroglobulin molecule is briefly present in an activated form (half-life approximately 2 minutes). This activated $\alpha_2$-macroglobulin can undergo covalent binding (in a nucleophilic reaction) with a second proteinase molecule[41] or with a cytokine.[42] Depending on their nature, cytokines can also undergo reversible, non-covalent, relatively high affinity binding to $\alpha_2$-macroglobulin: proteinase complexes.[43] In this situation, the binding of the cytokines depends both on the stoichiometric ratio of proteinase to $\alpha_2$-macroglobulin and on the type of proteinase. It has been found that, *in vivo*, $\alpha_2$-macroglobulin is always present in excess compared with proteinases, even at foci of inflammation.[44] The level of $\alpha_2$-macroglobulin in serum is approximately 2 to 4 mg/ml.[42] Thus $\alpha_2$-macroglobulin:proteinase complexes in a

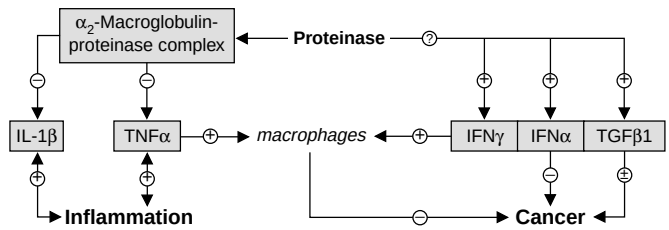


**Fig. 2.** Schematic representation of the interaction between proteinases, $\alpha_2$-macroglobulin and cytokines, and their effects on cancer and inflammation. **IFN** = interferon; **IL** = interleukin; **TGF** = transforming growth factor.

stoichiometric ratio of 1 : 1 are favoured, and these show a high cytokine binding capacity.[43] On the basis of the observation that the administration of proteolytic enzymes raises the level of $\alpha_2$-macroglobulin,[19,24] it can be supposed that the effect of the proteolytic enzymes on cytokines is regulated by the level of $\alpha_2$-macroglobulin and its cytokine-binding capacity.

### 3.3 Effects on Adhesion Molecules

Proteinases differ markedly from adhesion receptors in their capacity for down-regulation. Using the CD44 adhesion molecule, which is involved in carcinogenesis and metastasis, as an example, it has been shown that the number of CD44 epitopes can only be slightly decreased by trypsin, while bromelain causes a marked reduction in CD44 epitopes.[45,46] Grabowska et al.[47] obtained similar results in a study of the expression of CD44 on $B_{16}F_{10}$ melanoma cells *in vitro*. This study also demonstrated that raw bromelain produces a very marked reduction in CD44 expression, more marked than that observed with purified bromelain F9. Papain was found to be much less effective in reducing CD44 expression. However, it must be borne in mind that these investigations were carried out in a much (up to 1000 times) higher concentration range than that achievable in the body by oral administration of proteolytic enzymes. No down-modulation of adhesion molecules (CD4, CD44 and B7-1) was observed in mice fed with a combination of bromelain and trypsin in a weight ratio of 45 : 24.[48] However, a significant decrease in the adhesion molecules CD29, CD24 and CD58 was observed *ex vivo* on myeloma cells of patients who had received oral papain/trypsin/chymotrypsin.[49] After administration of oral pancreatin/papain/bromelain/trypsin/chymotrypsin, flow-cytometric studies demonstrated a reduction in adhesion molecules CD49, CD51 and CD58. A reduction in CD44 molecules was also observed, but this was not significant.[49] The potential value of a reduction in levels of the adhesion molecule CD44 in tumour therapy has been demonstrated by the investigations of Strobel et al.[50] and Zawadzki et al.[51] in mice. In the first of these 2 studies, administration of CD44 antibodies reduced the number of tumour implants produced by metastasis of ovarian carcinoma cells.[50] Zawadzki et al.[51] demonstrated that CD44 antibodies reduced the deposition of $B_{16}F_{10}$ melanoma cells in the lungs. CD44 receptor globulins additionally reduced the spread of $B_{16}F_{10}$ melanoma cells to a variety of organs.

### 3.4 Effects on Antioxidants and Reactive Oxygen Compounds

The formation of reactive oxygen compounds appears to be a widely distributed stress response, and has been related to a large number of different indications. The studies of Zavadová et al.[52] indicate that the polymorphonuclear leucocytes of individuals receiving pancreatin/papain/bromelain/trypsin/chymotrypsin (5 to 20 tablets as a single oral dose) produce increased quantities of reactive oxygen compounds. The authors conclude that treatment with proteolytic enzymes induces an oxidative stimulus with an immunomodulatory action. The observations of Latha et al.[53] appear to argue against the existence of such an oxidative stimulus. In this study, patients with burns who received oral treatment with trypsin/chymotrypsin showed marked increases in the activities of the antioxidative enzymes superoxide dismutase, catalase and glutathione peroxidase, within a few days. In addition to a lower level of the inflammatory indicator C-reactive protein,[19] and a reduction in lipid peroxidation was also observed.[53] Although the observations reported by Zavadová et al.[52] and Latha et al.[53] appear at first sight contradictory, their results are not necessarily incompatible. It is possible to imagine that enzyme therapy induces the synthesis of antioxidative protective mechanisms via a low degree of chronic oxidative stress, and that these mechanisms produce an ultimate beneficial effect.

## 4. Effects of Proteinase Combinations

In systemic enzyme therapy, proteinases may be used as single agent preparations, but are usually given as combinations of animal and plant protein-

ases. Combinations of proteinases represent a rational approach since proteinases differ markedly from one another at the biochemical level, so that a combined preparation can show a broader spectrum of activity. The biochemical differences between proteinases relate to their preferred sites of hydrolysis, their degree of inhibition by antiproteinases, and their pH optima. Using the induced-oedema model, it has been shown that orally administered proteinases also differ in their anti-inflammatory potential.[21,54] The fact that an enzyme combination is more effective than its individual components has been demonstrated using the model of carrageenin-induced oedema in rabbits.[55] It was found that inhibition of oedema formation was dependent on the dose of oral enzyme, and that inhibition was markedly more effective when a combination of bromelain and trypsin was given than when either was given as monotherapy.

## 5. Conclusions

Radiotherapy and chemotherapy are the most often and most successfully used therapies in oncology; however, they frequently cause serious adverse effects. Clinical studies have shown that the administration of proteolytic enzymes can reduce these adverse effects and, moreover, in some types of tumours, survival may be prolonged. Nevertheless, there are limited numbers of clinical studies on which to base a final judgement of the efficacy of systemic enzyme therapy, although this therapy is well accepted worldwide as an evidenced-based therapy.

The precise mechanism of action of systemic enzyme therapy remains unknown. The ratio of proteinases to antiproteinases, which is increasingly being used as a prognostic marker in oncology, is influenced by the oral administration of proteolytic enzymes, probably via an induction of the synthesis of antiproteinases. An effect of orally administered enzymes on the metabolism of antiproteinases seems to be obvious, but it still requires further investigation. Systemic enzyme therapy would be a variation of the successful use of proteinase inhibitors in oncology. This mode of action would explain the therapeutic efficacy of systemic enzyme therapy although only low activities of administered enzymes were detected in the plasma of patients. It also explains the influence of systemic enzyme therapy on cytokine metabolism. However, whether the induction of antiproteinases has a direct or indirect effect on cytokine metabolism is a question that cannot yet be answered. The numerous alterations of the cytokine composition, which were observed during systemic enzyme therapy, seem to be an indication of the therapeutic efficacy rather than a mode of action. Whether the effect of enzyme therapy on adhesion molecules is of genuine relevance to patients is another question that cannot yet be definitively answered. It is still uncertain why endogenous proteinases, the activity of which are usually enhanced in cancer tissue, do not also downregulate adhesion molecules. Since proteinases are neither pro-oxidants nor antioxidants, their effect on the formation or on the scavenging of reactive oxygen species seems to be indirect or an artefact rather than a mode of action of orally administrated enzymes in oncology.

## References

1. Vanhoof G, Cooreman W. Bromelain. In: Lauwers A, Scharpé S, editors. Pharmaceutical enzymes. New York: Marcel Dekker, 1997: 131-53
2. De Feo V. Medicinal and magical plants in the northern Peruvian Andes. Fitoterapia 1992; 53: 417-40
3. Rawlings ND, Barrett AJ. Evolutionary families of peptidases. Biochem J 1993; 290: 205-18
4. Rowan AD, Buttle DJ, Barrett AJ. The cysteine proteinases of the pineapple plant. Biochem J 1990; 266: 869-75
5. Harrach T, Eckert K, Schulze-Forster K, et al. Isolation and partial characterization of basic proteinases from stem bromelain. J Protein Chem 1995; 14: 41-52
6. Wrbka E, Kodras B. Unterstutzung der Chemotherapie inoperabler bronchopulmonaler Karzinome durch proteolytische Fermente. Wien Med Wochenschr 1978; 128: 153-8
7. Kim J-P, Wa WS, Kim SJ. Effect of rosette forming T-lymphocyte level in immunochemotherapy using Picibanil and Wobe-Mugos in gastric cancer patients. Leipner J [translation (data on file)]
8. Schedler M, Lind A, Schatzle W, et al. Adjuvant therapy with hydrolytic enzymes in oncology: a hopeful effort to avoid bleomycin induced pneumotoxicity [abstract]? J Cancer Res Clin Oncol 1980; 116: 697
9. Lahousen M. Modification of liver parameters by adjuvant administration of proteolytic enzymes following chemotherapy in patients with ovarian carcinoma [in German]. Wien Med Wochenschr 1995; 145: 663-8

10. Sakalova A, Dedik L, Gazova S, et al. Survival analysis of an adjuvant therapy with oral enzymes in multiple myeloma patients [abstract]. Br J Haematol 1998; 102: 353
11. Popiela T. Wobe-Mugos E® as additive therapy after surgical treatment of colon cancer patients in combination with chemotherapy. Gerestried, Germany: MUCOS Pharma GmbH & Co., 1999 (data on file)
12. Sebti SM, Mignano JE, Jani JP, et al. Bleomycin hydrolase: molecular cloning, sequencing, and biochemical studies reveal membership in the cysteine proteinase family. Biochemistry 1989; 28: 6544-8
13. Schwartz DR, Homanics GE, Hoyt DG, et al. The neutral cysteine protease bleomycin hydrolase is essential for epidermal integrity and bleomycin resistance. Proc Natl Acad Sci U S A 1999; 96: 4680-5
14. Kesztele V, Hürbe E, Wischin W. Erfahrung mit proteolytischen Enzymen beim Bronchuskarzinom. Wien Med Wochenschr 1976; 25-27: 412-4
16. Vinzenz K, Stauder U. Die therapie der radiogenen mukositis mit enzymen. In: Vinzenz K, Waclawicek HW, editors. Chirurgische therapie von kopf-hals-karzinomen. Berlin: Springer-Verlag, 1992: 300-14
15. Beaufort F. Reduzierung von Strahlennebenwirkungen durch hydrolytische enzyme. Therapeutikon 1990; 4: 577-80
17. Gujral MS, Patnaik PM. Efficacy and safety of Wobe-Mugos E® in preventing side-effects of radiation therapy in patients with head and neck cancer – Gerestried, Germany: MUCOS Pharma GmbH & Co, 1998 (data on file)
18. Dale P. Efficacy and tolerance of Wobe-Mugos E® in reducing side-effects of radiation therapy in locally advanced cervical cancer – Gerestried, Germany: MUCOS Pharma GmbH & Co, 1998 (data on file)
19. Latha B, Ramakrishnan KM, Jayaraman V, et al. Action of trypsin: chymotrypsin (Chymoral forte DS) preparation on acute-phase proteins following burn injury in humans. Burns 1997; 23 Suppl. 1: 3-7
20. Latha B, Ramakrishnan M, Jayaraman V, et al. Serum enzymatic changes modulated using trypsin: chymotrypsin preparation during burn wounds in humans. Burns 1997; 23: 560-4
21. Wood GR, Ziska T, Morgenstern E, et al. Sequential effects of an oral enzyme combination with rutoside in different *in vitro* and *in vivo* models of inflammation. Int J Immunother 1997; 13: 139-46
22. Stauder G, Pollinger W, Fruth C. Systemic enzyme therapy: an overview of new clinical studies [German]. Allgemeinmedizin 1990; 19: 188-91
23. Gettins P, Patston PA, Olson ST. Serpins: structure, function and biology. Heidelberg: Springer-Verlag, 1996
24. Fisher JD, Weeks RL, Curry WM, et al. Effects of an oral enzyme preparation, Chymoral®, upon serum proteins associated with injury (acute phase reactants) in man. J Med 1974; 5: 258-73
25. Foekens JA, Look MP, Bolt-de Vries J, et al. Cathepsin-D in primary breast cancer: prognostic evaluation involving 2810 patients. Br J Cancer 1999; 79: 300-7
26. Kennedy AR. Chemopreventive agents: protease inhibitors. Pharmacol Ther 1998; 78: 167-209
27. Pemberton PA. The role of serpin superfamily members in cancer. Cancer J 1997; 10: 24-30
28. Joslin G, Wittwer A, Adams S, et al. Cross-competition for binding of $\alpha_1$-antitrypsin ($\alpha_1$ AT)-elastase complexes to the serpin-enzyme complex receptor by other serpin-enzyme complexes and by proteolytically modified $\alpha$1 AT. J Biol Chem 1993; 268: 1886-93
29. Olson ST, Bock PE, Kvassman J, et al. Role of the catalytic serine in the interactions of serine proteinases with protein inhibitors of the serpin family. J Biol Chem 1995; 270: 30007-17
30. Lah TT, Kos J. Cyteine proteinases in cancer progression and their clinical relevance for prognosis. Biol Chem 1998; 379: 125-30
31. Verspaget HW. Proteases as prognostic markers in cancer. BMJ 1998; 316: 790-1
32. Lah TT, Kokalj-Kunovar M, Strukelj B, et al. Stefins and lysosomal cathepsins B, L and D in human breast carcinoma. Int J Cancer 1992; 50: 36-44
33. Batkin S, Taussig SJ, Szekerezes J. Antimetastatic effect of bromelain with or without its proteolytic and anticoagulant activity. J Cancer Res Clin Oncol 1988; 114: 507-8
34. Batkin S, Taussig S, Szekerczes J. Modulation of pulmonary metastasis (Lewis lung carcinoma) by bromelain, an extract of the pineapple stem (*Ananas comosus*). Cancer Invest 1988; 6: 241-2
35. Taussig SJ, Szekerczes J, Batkin S. Inhibition of tumor growth *in vitro* by bromelain, an extract of the pineapple plant (*Ananas comosus*). Planta Medica 1985; 51: 538-9
36. Bellelli A, Mattioni M, Rusconi V, et al. Inhibition of tumor growth, invasion and metastasis in Papain-immunized mice. Invasion Metastasis 1990; 10: 142-69
37. Mazourov VI, Lila AM, Klimko NN, et al. The efficacy of systemic enzyme therapy in the treatment of rheumatoid arthritis. Int J Immunother 1997; 13: 85-92
38. Lackovic V, Rovensky J, Horvathová M, et al. Interferon production in whole blood cultures from volunteers and rheumatoid arthritis patients after medication with oral enzymes. Int J Immunother 1997; 13: 159-66
39. Baenkler H-W. Medizinische Immunologie: Grundlagen, Diagnostik, Klinik, Therapie, Prophylaxe, Sonderbereiche. Landsberg am Lech, Germany: Ecomed, 1995
40. Desser L, Rehberger A, Kokron E, et al. Cytokine synthesis in human peripheral blood mononuclear cells after oral administration of polyenzyme preparations. Oncology 1993; 50: 403-7
41. Barrett AJ. $\alpha_2$-Macroglobulin. Methods Enzymol 1981; 80: 737-54
42. James K. Interactions between cytokines and $\alpha_2$-macroglobulin. Immunol Today 1990; 11: 163-6
43. Borth W. $\alpha_2$-Macroglobulin, a multifunctional binding protein with targeting characteristics. FASEB J 1992; 6: 3345-53
44. Borth W, Dunky A, Kleesiek K $\alpha_2$-Macroglobulin-proteinase complexes as correlated with $\alpha_1$-proteinase inhibitor-elastase complexes in synovial fluids of rheumatoid arthritis patients. Arthritis Rheum 1986; 29: 319-25
45. Harrach T, Gebauer F, Eckert K, et al. Bromelain proteinases modulate the CD44 expression on human Molt 4/8 leukemia and SK-Mel 28 melanoma cells *in vitro*. Int J Oncol 1994; 5: 485-8
46. Gebauer F, Micheel B, Stauder G, et al. Proteolytic enzymes modulate the adhesion molecule CD44 on malignant cells *in vitro*. Int J Immunother 1997; 13: 111-9
47. Grabowska E, Eckert K, Fichtner I, et al. Bromelain proteases suppress growth, invasion and lung metastasis of B16F10 mouse melanoma cells. Int J Oncol 1997; 11: 243-8

48. Targoni OS, Tary-Lehmann M, Lehmann PV. Prevention of murine EAE by oral hydrolytic enzyme treatment. J Autoimmun 1999; 12: 191-8
49. Sakalova A, Kunze R, Holomanova D, et al. Density of adhesive proteins after oral administration of proteolytic enzymes in multiple myeloma [in Slovak]. Vnitr Lek 1995; 41: 822-6
50. Strobel T, Swanson L, Cannistra CA. *In vivo* inhibition of CD44 limits intra-abdominal spread of a human ovarian cancer xenograft in nude mice: a novel role for CD44 in the process of peritoneal implantation. Cancer Res 1997; 57: 1228-32
51. Zawadzki V, Perschl A, Rosel M, et al. Blockade of metastasis formation by CD44-receptor globulin. Int J Cancer 1998; 75: 919-24
52. Zavadová E, Desser L, Mohr T. Stimulation of reactive oxygen species production and cytotoxicity in human neutrophils *in vitro* and after oral administration of a polyenzyme preparation. Cancer Biother 1995; 10: 147-52
53. Latha B, Ramakrishnan M, Jayaraman V, et al. The efficacy of trypsin: chymotrypsin preparation in the reduction of oxidative damage during burn injury. Burns 1998; 24: 532-8
54. Netti C, Bandi GL, Pecile A. Anti-inflammatory action of proteolytic enzymes of animal vegetable or bacterial origin administered orally compared with that of known anti-phlogistic compounds. Farmaco – Edizione Pratica 1972; 27: 453-66
55. Ito C, Yamaguchi K, Shibutani Y, et al. Anti-inflammatory actions of proteases, bromelain, trypsin and their mixed preparation. Folia Pharmacol Japan 1979; 75: 227-37

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