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Proteolytic Enzyme Therapy in Evidence-Based Complementary Oncology: Fact or Fiction?

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Systemic enzyme therapy was recently subjected to experimental investigations and to rigorous clinical studies in cancer patients. The designs of the relevant clinical cohort studies followed the guidelines of Good Epidemiological Practice and represent level IIB in evidence-based medicine (EBM). Scientifically sound experimental in vitro and in vivo investigations are far advanced and document promising immunological, anti-inflammatory, anti-infectious, and antitumor/antimetastatic activities of proteolytic enzyme mixtures (containing trypsin, chymotrypsin, and papain) or bromelain. EBM level II clinical studies, which are accepted by the European Union to show safety and efficacy of medical treatments, were performed to evaluate the benefit of complementary systemic enzyme therapy in cancer patients suffering from breast and colorectal cancers and plasmacytoma. These studies demonstrated that systemic enzyme therapy significantly decreased tumor-induced and therapy-induced side effects and complaints such as nausea, gastrointestinal complaints, fatigue, weight loss, and restlessness and obviously stabilized the quality of life. For plasmacytoma patients, complementary systemic enzyme therapy was shown to increase the response rates, the duration of remissions, and the overall survival times. These promising data resulted in an "orphan drug status" designation for a systemic enzyme product, which should motivate further studies on this complementary treatment.

Keywords: enzyme therapy; cancer; proteolytic; in vivo; clinical studies

Basis of Evidence-Based Complementary Medicine in Oncology

Cancer diseases demand diagnostic and therapeutic measures with proven quality, safety, and efficacy. The basis for evaluation is clinical studies representing levels I or II (randomized controlled trials or epidemiological cohort studies) in accordance with recommendations of the Centre for Evidence-Based Medicine, University of Oxford, UK.[1] Regarding these claims, surgery, chemotherapy, radiotherapy, and hormone therapy have emerged as gold standards in the treatment of cancers. These therapies have demonstrated their cancer destructive potencies and their curative feasibility, depending on cancer entity and stage.[2] Complementary therapies are recommended to support and optimize the scientifically based cancer standard treatment.[3,4]

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Rationale of Systemic Enzyme Therapy

The administration of plant extracts with a high content of proteolytic enzymes (systemic enzyme therapy) had its origin in the traditional medicine of Central and South America.[5,6] It is currently being studied for a variety of indications, for example, in oncology, infectious diseases, trauma, and inflammation.[7-9] Its therapeutic use is partly based on scientific studies in agreement with evidence-based medicine; however, it is mostly empirical.[5,10]

The scientific rationale of systemic enzyme therapy was initially published in 1911, *The Enzyme Treatment of Cancer and its Scientific Basis*, by John Beard,[11] an English embryologist working in Scotland. However, this work was gradually forgotten until the 1950s when Max Wolf and Helene Benitez developed the concept of systemic enzyme therapy for oncology.[5,10] Their observation that an optimized combination of plant and animal proteinases exerted anticancer activities by restoring the reduced cytotoxic activity of patients' sera formed the basis of this therapy. In addition, it was discovered that cancer diseases were more common in elderly patients concurrent with a reduction of pancreatic enzymes as well as hydrolytic activity in the serum. This led to attempts to

restore the oncoloytic activity of the serum by oral administration of mixtures of proteolytic enzymes.

Proteolytic enzymes are large molecules that can be absorbed before they are dispersed into different compartments of the body where they can be detected in various concentrations.[12] Initial animal studies showed that the growth of experimental tumors was reduced when the hydrolytic activity of the serum was normalized. During this time, the role of proteolytic enzymes in clotting and fibrinolysis was also discovered. Metastases and their organotrophy were explained by "fibrinolytic" stickiness through which tumor cells settled in other organs and evaded immune surveillance.[12]

Proteolytic enzymes (serine endopeptidases such as trypsin or chymotrypsin and cysteine endoproteinases such as bromelain and papain or combinations of those enzymes) have long been available for diverse medical indications. However, their mechanisms of action, for example, in complementary oncology are not yet fully understood. There are a variety of mechanisms by which they are thought to contribute to efficacy. Thus, they are known to bind irreversibly to antiproteinases such as α-2-macroglobulin or α-1-antitrypsin, leading to synthesis of antiproteinases. Increased levels of antiproteinases inactivate other proteinases, for example, cathepsins, which are thought to play a role in tumor development and metastasis.[13] Cysteine proteinases of plant origin (eg, papain, bromelain) are known to influence the balance between proteinases and antiproteinases and, as a consequence, may also influence tumor metastasis.[14] Furthermore, enzymes are known to interact with the cytokine network. The binding of proteinases to α-2-macroglobulins leads to the formation of α-2-macroglobulin–proteinase complexes with high capacity for binding and clearing cytokines, for example, interleukin-1 (IL-1), IL-6, interferon-γ, and transforming growth factor-β (TGF-β). TGF-β promotes immunosuppression in the host and tumor escape, thus modulating tumor growth. Furthermore, proteolytic enzymes reduce TGF-β overproduction at the levels of RNA and protein synthesis.[15]

It was observed that tumor cells emitted factors blocking the immune system. Thus, tumor cells protect themselves from being recognized by antibodies and from the attack by cells of the immune system by shedding soluble surface antigens. Through soluble antigens, antibodies are bound, creating immune complexes that inhibit nonspecific immune cells such as monocytes/macrophages and natural killer cells. According to a postulation, those blocking factors can be reduced by systemic enzyme therapy.[16]

Proteolytic enzymes also interact with adhesion molecules that can play an important role in tumor development and metastasis. The modulation or downregulation of adhesion molecules by enzymes has been shown, among others, for CD-44, CD-49, CD-54, and CD-58, which may contribute to their antitumor activity.[17]

Finally, proteolytic enzymes influence the levels of antioxidant enzymes and reactive oxygen species.[18] Recently, a novel role for extracellular proteases as inhibitors of intracellular signal transduction pathways has been described.[5]

Experimental Investigations of Systemic Enzyme Therapy

In evidence-based medicine, proteolytic enzymes are orally administered for the reduction of side effects of chemotherapy and radiotherapy. In animal studies this empirical finding has been verified. The cytotoxic agent bleomycin induced the release of TGF-β, which regularly causes fibrosis of the lung. With concomitant administration of proteolytic enzymes, the pulmonary cytotoxicity of bleomycin was significantly reduced. Cisplatin, used in the treatment of ovarian and head and neck cancers, causes damage of spleen and kidneys. The complementary application of proteolytic enzymes reduced these side effects significantly.[5]

The influence of systemic enzyme therapy on tumor growth and metastasis was demonstrated in various experimental models. The survival time of mice with experimental melanoma B16 was significantly prolonged and was associated with histologically smaller metastases. In animals having Lewis lung cancer, the beneficial effects of proteolytic enzyme administration was even more pronounced. Animals treated with proteolytic enzymes 24 hours before tumor transplantation showed a significantly increased survival rate when compared with the control group during the period of observation.[19]

Recently, the antitumor and antimetastatic activities of bromelain were evaluated in a murine model. Syngeneic sarcoma L-1 cells were incubated with bromelain, after previous time and dosage kinetics, before being subcutaneously or intravenously inoculated into BALB/c-mice to induce local tumor growth or lung colonization. Compared with non-protease-incubated tumor cells, local tumor growth and experimental lung metastases decreased significantly ($P < .005$) after bromelain incubation of the tumor cells.[20]

Previously unpublished experimental data from our institute confirm the data in the literature. Compared with 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 ($P < .05$) after incubation of the tumor cells with a standardized proteolytic enzyme mixture (trypsin, chymotrypsin, and papain; TCP). Table 1 shows data from the optimal enzyme concentration (20 μg/mL incubation medium) taken from kinetic studies (range 0.2-80 μg TCP/mL medium).

To evaluate the antitumor and antimetastatic activity of systemic administration of a standardized proteolytic enzyme mixture (TCP), BALB/c-mice were subcutaneously

Table 1. Mean Tumor Weight and Mean Number of Lung Colonies in BALB/c-Mice[a]

BALB/c-Mice Subcutaneously Challenged With L-1 Cells Incubated With	Mean Tumor Weight, mg (SD)
Medium (control)	228.6 (71)
TCP	37.4 (21)[b]
BALB/c-Mice Intravenously Challenged With L-1 Cells Incubated With	**Mean Number of Lung Colonies (SD)**
Medium (control)	33.0 (12)
TCP	5.4 (4)[b]

NOTES: TCP = trypsin, chymotrypsin, and papain; SD = standard deviation.
[a] BALB/c-mice (n = 8 per experimental group) injected with sarcoma L-1 cells from cell culture medium (control) or standardized proteolytic enzyme mixture–containing medium (TCP; 20 µg/mL). All experiments were repeated.
[b] $P < .05$ (significantly different from control).

(local tumor) or intravenously (experimental lung metastases) inoculated with sarcoma L-1 cells. The standardized proteolytic enzyme mixture (TCP) was regularly administered subcutaneously or intraperitoneally (0.5 mg/application/mouse; dosage was chosen from limited kinetic studies, as described elsewhere[20]), and local tumor weight and lung colonization were evaluated on day 14. No obvious side effects (concerning fur, vigilance, weight) were seen in enzyme-treated mice. As shown in Table 2, mean tumor weight was significantly reduced ($P < .05$) after subcutaneous or intraperitoneal enzyme administration.

Experimental lung metastases were nonsignificantly reduced in enzyme-treated mice, as shown in Table 3.

Clinical Studies of Systemic Enzyme Therapy

For defined standardized mixtures of proteolytic enzymes as well as for bromelain, several well-documented observation studies exist that demonstrate a beneficial influence on immune functions and on quality of life in various tumor entities, for example, breast and colorectal cancers and plasmacytoma. These effects obviously were the results of reduced numbers and severity of side effects of the standard therapies. These observation studies were ultimately verified in Good Epidemiological Practice (GEP)–compliant cohort studies, which have been accepted by the European Union as demonstration of efficacy and safety.[21] Therefore, the US Food and Drug Administration evaluated a cohort study for the indication of plasmacytoma, where complementary systemic enzyme therapy was applied to optimize the standard therapy, and awarded it "orphan drug" designation. Systemic enzyme therapy, therefore, has successfully made the step into "evidence-based medicine."

Table 2. Mean Tumor Weight in BALB/c-Mice[a]

BALB/c-Mice Subcutaneously Challenged With L-1 Cells and Treated With	Mean Tumor Weight, mg (SD)
PBS (control) subcutaneously	220.4 (52)
TCP subcutaneously	72.6 (39)[b]
PBS (control) intraperitoneally	270.5 (61)
TCP intraperitoneally	82.8 (31)[b]

NOTES: PBS = phosphate-buffered saline; TCP = trypsin, chymotrypsin, and papain; SD = standard deviation.
[a] BALB/c-mice (n = 8 per experimental group) after subcutaneous inoculation of L-1 sarcoma cells and subcutaneous or intraperitoneal enzyme (TCP) treatment (0.5 mg/mouse on days 1, 4, 7, 10, and 13). All experiments were repeated and yielded reproducible results.
[b] $P < .05$ (statistically significant from control).

Table 3. Mean Number of Experimental Lung Metastases in BALB/c-Mice[a]

BALB/c-Mice Intravenously Challenged With L-1 Cells and Treated With	Mean Number of Lung Colonies (SD)
PBS (control) subcutaneously	37.2 (14)
TCP subcutaneously	14.0 (12)
PBS (control) intraperitoneally	34.4 (12)
TCP intraperitoneally	18.6 (9)

NOTES: PBS = phosphate-buffered saline; TCP = trypsin, chymotrypsin, and papain; SD = standard deviation.
[a] BALB/c-mice (n = 8 per experimental group) after intravenous inoculation of L-1 sarcoma cells and subcutaneous or intraperitoneal enzyme (TCP) treatment (0.5 mg/mouse on days 1, 4, 7, 10, and 13). All experiments were repeated and yielded reproducible results.

Systemic Enzyme Therapy in Breast Cancer Patients: A GEP-Compliant Cohort Study

The evaluation of the frequency and severity of symptoms and side effects of the disease and its treatment were the primary aims of a study to demonstrate the safety and efficacy of systemic enzyme therapy in breast cancer patients.[22] Overall, data from 2339 patients who suffered from primary nonmetastasizing breast cancer and received the recommended standard therapies (surgical operation, chemotherapy, radiotherapy, and hormone therapy) were evaluated. Further complementary treatments were also possible; however, they had to be documented. In the therapy group, 1283 patients were complementarily treated with systemic enzyme therapy (trypsin, chymotrypsin, papain) over a median duration of about 10 months. The control group consisted of 1056 patients who had not received complementary enzyme treatment. Because the data of a sufficient large number of patients without complementary therapy (n = 410) were available, a relevant subgroup was formed for analysis of the results: systemic enzyme therapy without further complementary treatment (n = 239).

The tolerability of systemic enzyme therapy was studied in the overall sample of all 1283 patients. In about 2.3% of all patients side effects were reported that were attributed to the enzyme therapy. These were mainly low to moderately pronounced gastrointestinal complaints, which were controlled, if necessary, through a reduction of the dosage. Accordingly, complementary systemic enzyme therapy basically did not cause any serious additional problems with respect to tolerability.

To demonstrate efficacy, 239 enzyme-treated patients were compared with 410 patients in the control group. The outcome showed a significant and clinically relevant superiority of the enzyme group. Systematic enzyme therapy stabilized and improved the condition of the patients, mainly because of a significant reduction in the number and severity of side effects induced by chemotherapy and radiotherapy. In the control group, 45% of patients showed side effects, whereas the incidence was only 25% in the enzyme-treated group. Enzymes had an especially beneficial effect on the disease and therapy-induced symptoms such as nausea, loss of appetite, gastrointestinal complaints, headache, fatigue, and restlessness. The total symptom score was significantly influenced to a clinically relevant degree.

In the monotherapy groups (treatment and control), an exploratory analysis was conducted for the time until appearance of events such as tumor remission, metastasis, and death for the various UICC stages. The patient numbers and period of observation were sufficient for UICC stages IIA/IIB to obtain a significant outcome trend: the time of remission was significantly longer in the enzyme group.[22]

Systemic Enzyme Therapy in Colorectal Cancer Patients: A GEP-Compliant Cohort Study

Overall, the data from 1242 patients with primary colorectal cancers of variable sizes and dissemination were collected for this study.[23] A total of 616 patients of the treatment group were complementarily treated with defined amounts of a standardized enzyme preparation (papain, trypsin, chymotrypsin) over a median time of 9 months. In the control group, 626 patients were enrolled. They all were treated with the internationally recommended tumor destructive therapy (surgical operation, chemotherapy, and radiotherapy), however, without enzymes. Both groups were divided into subgroups that had either received additional complementary therapies (combination therapy group) or did not receive complementary therapies (enzyme monotherapy group). The groups were comparable with regard to their basic data, risk factors, prognostic criteria, and conventional therapies. The tolerability of the systemic enzyme therapy was practically identical with the observations made in the breast cancer study.[22]

The evaluation of the influence of complementary enzyme treatment on symptoms of disease and frequency and severity of side effects of the tumor-destructive therapies were the primary study goals. Besides the multivariate analysis of disease symptoms and side effects of chemotherapy or radiotherapy (Mann–Whitney test), the data were evaluated in an independent propensity matched pairs analysis, which revealed the significant and clinically relevant superiority of complementary systemic enzyme therapy. A matched pairs analysis confirmed the results.

Proteolytic enzymes administered as a complementary treatment especially improved the disease-specific and therapy-specific symptoms of nausea, vomiting, loss of appetite, diarrhea, fatigue, depression, sleep disturbances, and restlessness. A notable reduction in the number and severity of chemotherapy- and radiotherapy-induced side effects was documented in the enzyme-treated group. In the control group, 24.1% of patients reported side effects, whereas the incidence of side effects was 9.4% in the enzyme-treated group.

For the overall sample size of all 1242 patients, the survival time for the various Dukes stages was evaluated with the propensity-matched pairs technique. For patients with Dukes D, prolonged survival was documented in the enzyme group.[23]

The analysis of survival time was not a primary goal of the study in both breast and colorectal cancers. The period of observation was insufficient for a comprehensive and complete Kaplan–Meier analysis. These data can, therefore, only be first hints of a possible life-prolonging effect of complementarily administered proteolytic enzymes. Within the study protocol, arrangements were made to obtain the missing observation times through a follow-up evaluation of survival times of the patients from these epidemiological cohort studies. This would be absolutely necessary to confirm the data to integrate systemic enzyme therapy into evidence-based oncology.

Systemic Enzyme Therapy in Plasmacytoma Patients: A GEP-Compliant Cohort Study

Since 1987, Sakalova et al[24] have treated patients with internationally recommended standard therapies and complementarily added standardized enzyme mixtures containing trypsin, chymotrypsin, and papain. In 1997, the data of all patients treated and documented for plasmacytoma in the context of an optimized retrospective cohort study in parallel groups were gathered. The guidelines of GEP were strictly followed.

Out of 333 identified patients, 265 fulfilled the inclusion criteria. Patients with stages I to III plasmacytoma according to Durie and Salmon, who had received an "optimized chemotherapy," were enrolled into the therapy group if they had received a minimum of 6 months of

proteolytic enzyme therapy. The patients of the control group had not received enzyme therapy. The initial decision by the examiner as to whether the patient should receive complementary enzyme therapy was made in a quasirandomized fashion, according to the availability of enzyme therapy, but without reference to any prognostic consideration. The basic "optimized chemotherapy" included methyl prednisolone, vincristine, cyclophosphamide, CCNU, and melphalan (MOCCA) or vincristine, melphalan, cyclophosphamide, and prednisone (VMCPI) in 4- to 6-week intervals until remission, followed by readministrations in 4- to 8-month intervals. From stage IIA onwards patients with bone lesions received either bisphosphonates and/or vitamin D.

The data were collected according to the current standard and subjected to an independent audit. The evaluation was carried out with various statistical testing procedures, and the primary study aim was the survival of the patients with Kaplan–Meier analysis and Cox regression analysis. Secondary study aims were the first response to therapy, the duration of the first remission, and the tolerability of the systemic enzyme therapy.

For patients with stage III plasmacytoma, the median survival time of 47 months in the control group was shorter than the 83 months observed in the enzyme-treated group ($P = .0014$). A significant prolongation of survival was also verified for the overall group of patients with all stages (I-III; $P = .0003$). Because of the insufficient time of observation, no median survival time was calculated. The significant prolongation of survival, however, was still valid when in an "intention to treat" analysis all patients who had received proteolytic enzymes at least once were allocated to the therapeutic group.

Sensitivity analysis resulted in no indications as to relevant differences between the patient groups. The covariates age, sex, and known risk factors (secretory myeloma type, paraprotein in serum, osteolysis, recurrent infection) proved to be without influence. The disease stage was of relevance, where the risk increases with the advancement of the stage. Complementary treatment with proteolytic enzymes increased the response rate and the duration of remissions. An early and long-lasting first remission was an important prognostic factor for survival.

Proteolytic enzymes were tolerated without serious problems by the patients. Only 3.6% of patients suffered from moderate gastrointestinal complaints. In the context of this study, the mixture of proteolytic enzymes (trypsin, chymotrypsin, and papain) was, therefore, awarded with an "orphan drug" designation.

There are many other studies on the efficacy of systemic enzyme therapies; however, their quality mostly does not meet scientific demands. A thesis at the University of Cologne recently presented a meta-analysis of the studies on systematic enzyme therapy in oncology and came to the following conclusion: "Systemic enzyme therapy is on its way into evidence based oncology, however, further randomized controlled trials (RCT) and confirmation studies are urgently warranted to integrate them into recommended treatment protocols."[25]

Conclusions

Complementary medicine is currently widely debated by the oncology community, because the required scientific proof of safety and effectiveness for most of the therapeutic approaches has not yet been met with definite results.[26,27] In the past years, basic research and clinical evaluation of defined complementary therapeutic concepts in oncology have been intensified in an attempt to integrate these procedures into evidence-based medicine.[3,4]

According to definition, scientifically based therapies of complementary medicine cannot replace the well-studied conventional cancer-destructive therapies such as surgery, chemotherapy, radiotherapy, or hormone therapy. Accordingly, they are by no means "alternative therapies." Complementary approaches in oncology that are recommended as addition to standard cancer-destructive therapies claim to optimize this therapy. A great body of data emerging from scientifically sound clinical trials indicates that defined complementary procedures are beneficial for patients.[28,29]

Complementary medicine should primarily be regarded as an optimization of current standard treatment options in oncology. It is to be differentiated from "alternative medicine," which postulates to have replacements for conventional toxic approaches. Although complementary and alternative medicines are grouped together in the popular acronym "CAM," they are in fact quite different in their aims. Because many alternative treatments are still poorly documented, equating the 2 types could lead to a misguided and undeserved rejection of all complementary medicine. That complementary recommendations concerning balanced nutrition, physical activity, psychooncologic support as well as defined medications, for example, proteolytic enzymes or defined trace elements and vitamins can optimize standard treatment has been proved in clinical studies that have shown an increase in quality of life as well as in overall survival.[3,4,30-32]

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