Enzimas sistêmicas no tratamento de doenças – Mini revisão

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Systemic Enzyme Support - An Overview

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Introduction

A large number of conditions are primarily inflammatory in nature and may be significantly complicated by the presence of secondary forms of inflammation. Regardless of whether the cause of the problem is due to bacterial, viral or auto-immune influences, the result may be an ongoing situation with significant clinical and laboratory manifestations of the inflammatory process.

Dietary supplements designed to provide Systemic Enzyme Support (SES) can play an important role in helping to maintain normal inflammatory processes within the body and thereby help support and speed healing. This is not only beneficial for the patient, but for healthcare in general as ultimately it may help to reduce the costs associated with maintaining health.

Most healthcare professionals select treatments based on what they believe will be effective over a long period of time as well as what will bring a specific patient the fewest risks in connection with treatment. One of the major benefits of using systemic enzyme support is the relatively small amount of undesirable effects combined with good tolerance and efficacy.

Systemic enzyme support was for a long time regarded as a purely empirical treatment method. Due to the rapid development of immunology, biochemistry and molecular biology in the last few decades, systemic enzyme support has undergone significant development, as it has been shown that behind the empirically supported clinical results are a complex set of regulatory processes, which previously were unknown. Today, scientists have a better understanding with respect to the mechanisms by which Systemic Enzyme Support may be exerting it's desired effects. Specifically, the effect of proteolytic enzymes (proteases) on the cytokine network and their action at the level of the cell membrane both in terms of cellular adhesion as well as modulation of cellular receptors has been described. One of the main pioneers in the clinical use of the systemic proteases was Professor Max Wolf, who worked in New York in the 1930s not only as a sought-after physician but also as a researcher at Fordham University. At the present time, with regard to the historically best known pharmacological and clinical effects, proteases are placed in the international ATC classification in the M09AB group – anti-inflammatory enzymes.

Systemic Enzyme Support – definition

Hydrolytic enzymes have been used widely for decades and a range of scientific publications have recently demonstrated their importance in supporting numerous areas of health. At the present time proteases are indicated for parenteral application in malfunctions of blood coagulation (urokinase), to affect fibrotic processes (hyaluronidase) or in treatment of malignant hemotological conditions (asparaginase). The aim of oral application of enzymes may be either substitution of digestive enzymes in external secretory insufficiency of the pancreas (see accompanying article: "The Importance of Good Digestion") or use of their systemic effects (proteases). So, Systemic Enzyme Support can be defined as a modality which uses oral administration of exogenous hydrolytic (mainly proteolytic) enzymes of animal origin (trypsin, chymotrypsin) and plant origin (bromelain, papain) in the form of enteric-coated tablets for supporting healthy and normal inflammatory processes in the body. As a result, systemic enzyme support can help maintain a healthy immune system, healthy blood flow and circulation, healthy joint function, as well as help to reduce muscles pain after exercising. Systemic enzymes can exert a positive effect on rheological properties of blood as a result of their fibrinolytic properties. Data have also shown that administering systemic enzymes together with certain antibiotics is able to improve the tissue availability of the antibiotics.

Proteases

The main component of products designed for systemic enzyme support are proteolytic (i.e. protein splitting) enzymes of animal or plant origin. These are endopeptidases which hydrolyze peptide bonds in certain protein (peptide) chain locations on the basis of a more or less specific affinity to particular amino acid elements of these chains.

Trypsin is a pancreatic endopeptidase, which splits peptide bonds formed by the carboxylic group of the amino acids such as lysine or arginine. It is obtained from the pancreas of pigs by repeated refining and subsequent activation of the proenzyme trypsinogen.

Chymotrypsin is a pancreatic endopeptidase, which hydro-lytically splits peptide bonds formed by carboxylic groups of the amino acids tyrosine, phenylalanine and tryptophan. Chymotrypsin is obtained by extraction and chromatographic purification from the pancreas of cattle and subsequent activation of the proenzyme chymotrypsinogen.

Bromelain is an endopeptidase obtained from pineapples. Bromelain hydrolytically splits peptide bonds formed by the amino acids lysine, alanine, tyrosine and glycine. Bromelain is a family of individual macromolecules and is not a single enzyme.

Papain is a mixture of proteolytic enzymes separated from the fruit of the tropical *Carica papaya*, which is a member of the melon family. Papain splits polypeptides, particularly between the bonds of arginine, phenylalanine and lysine.

These proteases are typically combined in preparations for oral administration. The reason for these combinations is an assumption that the effects of individual enzymes will complement each other resulting in the multiplication of the final therapeutic efficacy. Another reason for these combinations is the assumption of an increase in the resorption of individual proteases by the intestinal mucous membrane when administered together with other proteases.

Most of the combined systemic enzyme support preparations currently used usually contain rutin (rutoside) in addition to two or more proteases. Rutin belongs to the group of bioflavonoids and can help to reduce the permeability of veins and capillaries.

Resorption of enzymatically active macromolecules and their pharmacokinetics

The basic condition of the systemic effect of proteases administered orally is their absorption in an enzymatically active form. The coated tablet ensures that the content will resist the acid gastric juices and not break down until it has reached the mucosa of the small intestine with a pH of about 7. After absorption, certain parts of the proteolytic enzymes pass into the blood stream and the lymph where their enzymatic activity allows them to bind to natural antiproteases of which the most important are alpha-2-macroglobulin (a-2-M) and alpha-1-antitrypsin (a-1-AT).

Many effects of SES are based on a-2-M-protease complex. The complex formation starts with the hydrolysis of the specific peptide bond in a-2-M by a protease. It causes a very deep conformational change of the entire a-2-M molecule. The protease becomes trapped in the a-2-M molecule in a way that prohibits many of its potential proteolytic abilities, however, some smaller or less protected substrates can still reach the reaction center and thus it does retain some catalytic activity. In the complex, a-2-M masks the protease macromolecule's antigenic determinants, so the enzyme has no allergenic effect on the organism. By its interaction with a protease, a-2-M is transformed into an "active" form (so called "fast form") which has new properties in relation to many physiologically active molecules, especially, to a broad spectrum of substances which participate in the immune response. Protease-antiprotease complexes are transported into tissues, where the proteases can be released (from a-1-AT, but not from a-2-M) and operate for a short time as free enzymes or have a relatively long-term effect as entire complexes. In these complexes, the proteases are captured by the liver and the pancreas where 90 % of them are eliminated in bile and excreted in stools. The biological half-life for elimination of enzymes after their resorption is relatively long (6 hours for bromelain and 12-20 hours for trypsin). The biological availability of enzymes in terms of systemic effects is relatively low after oral administration, i.e. around 1% of the total dose administered. This explains the necessity to administer proteolytic enzymes in large doses.

Bromelain and trypsin and similarly other proteases that are administered for systemic effects are resorbed from the intestine as active molecules. Penetration by the enzyme through the wall of the intestine in an active state has also been demonstrated for other enzymes (horse-radish peroxydase, 40 kDa; botulotoxin, 150 kDa). At the present time the generally accepted opinion is that even molecules with a weight of more than 1000 kDa can also penetrate the intestinal barrier to a limited extent. Currently a number of mechanisms for the transfer of macromolecules through the intestine wall are described. In the upper part of the small intestine, persorption is regarded as the main mechanism. This is linked with continuous desquamation of dying enterocytes, which causes the short-term increase in

permeability of the intestinal barrier. In addition, absorption by M-cells (microfold cells) accumulated in the intestinal mucosa over the Payer's plaques takes part in the transfer in the ileum. Another mechanism is the receptor-mediated endocytosis linked with internalisation and recycling of the receptor. In addition to transcellular paths, paracellular transfer through tight junctions also appears to be another possibility.[18]

Mechanisms of the effect of proteases after oral application

The systemic effect of proteases is realized in the organism either by way of direct proteolysis of physiologically important molecules of a protein nature or indirectly by affecting the properties of important regulatory molecules (e.g. a-2-M or proteinase-activated receptors, PARs).

1. Direct proteolytic effects

In blood plasma, equilibrium is established under physiological conditions between the body's own free proteases and those bound to antiproteases (a-2-M, a-1-AT). After oral application of exogenous proteases and their absorption in the intestine, there is a shift in this equilibrium state in terms of an increase in what is termed proteolytic activity of the blood. Proteases bound to a-2-M, which preserve part of their proteolytic activity ("limited proteolysis"), also take part in this.

Proteases take part in specific activation, regulation and degradation of a whole range of factors connected with an inflammatory response. For example, by means of revealing antigenous epitopes, specific proteolysis of a range of cytokines, degradation of regulatory factors of a protein nature or activation of receptors. In addition to this, proteases degrade proteins and peptides damaged by inflammation and thereby allow for easier phagocytosis and removal by means of the venous and lymphatic systems.

2. Effect on adhesion molecules

Adhesion molecules (AM) – are structures on the surface of cells which play an important role in intercellular communication, particularly in the case of immune cells. The degree of their expression is determined by the state of activation of the cell and has an important influence on its properties. For example, increased expression of certain AMs on endothelial cells and thrombocytes and also leukocytes, accompanies an inflammatory response of the organism in all phases. In vitro and in vivo experiments show that enzymes contained in systemic enzyme support products selectively reduce the density of certain adhesion molecules on endothelial cells, in damaged tissues and also on cell membranes of certain inflammatory cells. By reducing the density of these molecules, there occurs an increase in the activation threshold of elements which take part in an inflammatory reaction.

In terms of immunomodulation, the ability of trypsin to increase the activation threshold of T-lymphocytes, owing to reduction in the number of CD4, CD44 and B7-1 adhesion molecules on their surface, appears to be very important. Increased expression of CD4, CD44 and B7-1 and the reduction of the activation threshold of T-lymphocytes connected with this is regularly observed in the focus of inflammation. This is produced by stimulation of INFy and targeted to increasing the reaction capability of T-lymphocytes. In view of the fact that an activated T-lymphocyte produces additional INFy, the whole process is amplified. The action of trypsin on the above mentioned adhesion molecules (also with increased elimination of INFy through the binding to the complex protease – a-2-M, see below) returns T-lymphocytes to an inactive state and thereby helps to maintain normal inflammatory processes.

3. Effect on cytokines operating locally and systemically

In normal inflammatory processes, a whole range of cytokines come into play (e.g. TNF-a, TGF-β, IFNy, IL-1, IL-6). Some of these cytokines can contribute to the development of imbalances in the inflammatory process. Lately, attention has been focused mainly on the autocrine cytokine TGF-β, whose excessive formation plays a part in various immuno-pathological processes. Cytokines in plasma are bound (like proteases) to antiproteases, particularly to a-2-M. The bond of a cytokine to the antiprotease itself is reversible and a cytokine may manifest its own activity again after being liberated. However, when a cytokine is bound to a-2-M that contains a linked protease, a stable bond is formed, which in turn inactivates the cytokine. Consequently, the whole complex (protease-antiprotease-cytokine) is quickly

eliminated by phagocytosis in the liver and the spleen. Therefore, systemic proteases can help to accelerate the clearance of increased levels of certain cytokines.
A similar elimination mechanism has also been demonstrated for immune complexes and even for amyloid polymer which play a part in the development of certain chronic conditions.

4. Effect by means of protease-activated receptors

Protease-activated receptors – PARs (e.g. PAR-2 is a trypsin-activated receptor) - present on the surface of most of the body cells. They have a physiological importance, for instance in regulating the exchange of substances between the lumen of the blood vessels and the interstitial space. Through the protease-PAR interaction, proteases are considered as key modulators of immune and inflammatory responses. PAR activation by systemic enzymes can contribute to changes in hydrodynamics, and oncotic pressure and thereby can help to maintain normal inflammatory processes and blood flow. Overall, this can help to improve microcirculation and to remove cellular detritus.

5. Effect on AGEs by exogenous proteases

Advance glycation end-products (AGEs), which are formed by non-enzymatic reaction of sugars, ketones or aldehyde groups with a free amino group of proteins, lipids or amino acids, induce chemical modification of proteins and lipids, including LDL particles. This modification is the basis for changes of the structural and functional properties of plasma proteins and extracellular matrices. There is, for example, cross-linking and thickening of basal membranes. The result of AGE-induced modification of lipoproteins (apoprotein-B, LDL) is their delayed clearance through LDL receptors. The interaction of AGEs with their receptors (RAGEs) and/or binding proteins on the surface of cells may induce cell activation and increased formation of oxygen radicals with subsequent activation of the nuclear factor kB (NF-kB) and increased synthesis of cytokines, growth factors and adhesion molecules. Similarly, depending on what type of cell that AGEs are interacting with, they may impact cell proliferation as well as programmed cell death. These effects of AGEs explain the critical role they may play in the pathogenesis of vascular complications of certain chronic conditions. Additionally, aging in general is also thought to be associated with increased AGEs.
The effects of AGEs on the endothelial function have also been well characterized. In in vitro and in vivo experiments, AGEs alter the effect of nitric oxide, which results in changes in vasodilatation. Transendothelial chemotaxis of monocytes and PDGF (platelet derived growth factor) secretion are increased, as is the expression of certain adhesion molecules, such as VCAM-1 (vascular cell adhesion molecule-1) and ICAM-1 (intercellular adhesion molecule-1). When extracellular matrix glycation and inflammatory stimuli are combined, the intensity of the endothelial adhesion can be amplified.
Proteases (trypsin and bromelain) significantly reduce the concentration of AGEs and lipid oxidation products, both in vitro and and in vivo. After application of proteases, reduction in the number of over-expressed RAGEs on the surface of cells accompanied by an increase of their concentration in the intercellular area was observed. This both reduces the probability of interaction of AGEs with their receptors and also enables “inactivation” of AGEs through AGE-soluble RAGE complexes. In connection with a reduction of AGE level, a reduction in the concentration of TGF-β and a lower occurrence of DNA “damage” has also been observed.
These findings also corroborate the idea that AGEs-induced genotoxicity is mediated via the binding of receptors and that trypsin and bromelain may inactivate the extracellular domain of this receptor.

6. Immunomodulation by means of intestinal bacteria

The effect of systemic enzymes on immune function may also be mediated at the level of the intestine. While only a hypothesis, the thought that systemic effects may in part be related to local actions at the level of the gut arises from the observation that certain proteases (e.g. trypsin) can strengthen the bacteriocidal effect of intraluminal intestinal enzymes (e.g. lysozyme). This may result in the induction of immunocompetent cells occurring directly or in immediate contact with the intestinal epithelium.[1]

Pharmacodynamic effects of SES

The effects of proteases administered orally are highly interconnected and can be derived from the mechanisms stated above.

The ability of systemic enzymes to support normal inflammatory processes is a crucial and a highly complex one. The action of proteases on normal inflammatory processes works in a number of ways,[46] which helps to explain the wide spectrum of potential health issues for which systemic enzymes can help to support.

In instances involving occurrences such trauma, burns, haematoma, etc., a combination of proteolytic enzymes works mainly by improving blood rheology and by breakdown of tissue detritus. Specifically, deposits of proteins escaped from the arterial or venous lumen are cleaved and degraded by proteolytic enzymes. Small thrombi created in the periphery of the “vascular bed” can be reduced which promotes the supply of immunocompetent cells and oxygen necessary to rebalance normal inflammatory processes.

In addition to the aforementioned, in situations of ongoing imbalances of the inflammatory system, proteases can help to eliminate immunocomplexes, alter the expression of adhesion molecules, and normalize the cytokine network, and overall haemostasis.

The extent of interaction of proteolytic enzymes with key inflammation reaction mechanisms ranges from supporting the bodies normal inflammatory reaction to helping decrease on overactive system. In contrast to conventional medical products, proteases therefore optimize the physiological course of inflammation and help maintain a balanced process.

The effect on rheological blood and lymph properties, which leads to their decreased viscosity and improved fluidity, is caused by interactions with the fibrinogen/fibrin system and the ability to activate plasminogen into plasmin and increase the levels of anti-thrombine III. Restriction of aggregation and adhesion of thrombocytes and reduction in aggregation and improvement of the flexibility of erythrocytes has also been described.

Improvement of microcirculation by affecting the rheological properties of body fluids is also regarded as one of the factors contributing to the beneficial effects of systemic enzymes. Other important factors which play a part in this effect are all the mechanisms which lead to normalizing an immune response reaction and minimizing secondary damage.

The immunomodulatory effect of systemic enzymes is mediated through affecting the expression of adhesion molecules, interventions in the cytokine network and impact on protease-activated receptors. The effect on various cellular components of the immune system (macrophages, granulocytes, NK cells, T lymphocytes) and the impact on production and elimination of immunocomplexes have also been demonstrated.

It has been shown that some individual proteases and also combined preparations increase the concentration of antibiotics, chemotherapeutic drugs and certain other medical products in the blood and tissues.

Certain relatively recent papers refer to the ability of proteases to reduce the level of LDL–cholesterol. The mechanism underlying the increased elimination of LDL-cholesterol may be the ability of a-2-M-protease complexes to activate common receptors specific for LDL and a-2-M on the membranes of phagocytic cells, in particular the phagocyte system of the liver and the spleen.

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