

Enzyme Therapy: Current Perspectives

Thiyagamoorthy UmaMaheswari¹, Thiagarajan Hemalatha¹, Palavesam Sankaranarayanan² & Rengarajulu Puvanakrishnan^{1*}

¹Department of Biotechnology, CSIR-Central Leather Research Institute, Chennai 600 020, Tamil Nadu, India

²Department of Biochemistry, Madras Medical College, Chennai 600 003, Tamil Nadu, India.

Received 02 June 2014; Revised 04 April 2015

Enzymes control all metabolic processes in human system from simple digestion of food to highly complex immune response. Physiological reactions occurring in healthy individuals are disturbed when enzymes are deficient or absent. Enzymes are administered for normalizing biological function in certain pathologies. Initially, crude proteolytic enzymes were used for the treatment of gastrointestinal disorders. Recent advances have enabled enzyme therapy as a promising tool in the treatment of cardiovascular, oncological and hereditary diseases. Now, a spectrum of other diseases are also covered under enzyme therapy. But, the available information on the use of enzymes as therapeutic agents for different diseases is scanty. This review details the enzymes which have been used to treat various diseases/disorders.

Keywords: Alginate lyase, Antioxidant enzymes, Asparaginase, Bromelain, Cardiovascular, Collagenase, DNAases, Gastrointestinal, Glutaminase, Hemocoagulase, Hyaluronidase, Lipases, Oncological, Pancreatic enzymes, RNAases, Serratiopeptidase, Streptokinase, Therapeutics, Tissue plasminogen activator, Urokinase

Introduction

Enzymes are widely used in medical diagnosis and their applications¹. Recently, Jain *et al.*² who reviewed the emerging prebiotic xylooligosaccharides (XOS) and their health benefits, discussed the enzymatic production of XOS including the role of xylanases. High dietary magnesium is known to modulate thyroid hormone synthesizing enzymes, and thereby modify histoarchitecture of thyroid gland, resembling goitre³. Biocontrol potential of *Streptomyces* sp. (NII 1006 strain) from the Western Ghats, India has been correlated with extracellular hydrolytic enzymes, chitinase in particular⁴. Maachia *et al.*⁵ who studied biological control of grapevine diseases reported production of hydrolytic enzymes such as biase (chitin-1,4-chitobiosidase) and nagase (N-acetyl glucosaminidase) by *Bacillus* strains B27 and B29. Velho-Pereira and Kamat⁶ have, in their review, tabulated enzymes from actinobacteria.

Enzymes are also used as therapeutic agents since they catalyse complex chemical reactions under appropriate physiological conditions. Initially, proteolytic enzymes have been used as supplements in gastrointestinal disorders⁷. Later, the use of enzymes

has been extended for treating cancer and a diverse spectrum of diseases⁸. As such, not much information is available on the optimal application of therapeutic enzymes. This review focuses on the use of enzymes as therapeutic agents in cancer, pancreatic disorders, heart diseases, kidney diseases, respiratory disorders, etc. The focus is on two themes *viz.*, enzymes presently used as therapeutic agents and enzymes having potential for future.

Enzymes presently used as therapeutic agents

Collagenase— Collagenase is a matrix metalloproteinase that breaks peptide bonds in collagen. Microbial collagenase hydrolyses native collagen and also other proteins and it is used in the debridement of dermal ulcers and burns⁹. Collagenase ointment is more effective than the petrolatum ointment for debridement of necrotic tissue¹⁰. Collagenase is used for enzymatic fasciotomy and it offers a potential advantage over nonspecific degradative enzymes by targeting collagen. Collagenase from *Clostridium histolyticum* is approved as a novel nonsurgical treatment for Dupuytren's contracture by the US Food and Drug Administration (USFDA). It provides a safe, effective alternative to surgery for patients with Dupuytren's contracture with the advantage of a quicker recovery with a minimal morbidity^{11,12}. *Streptomyces exfoliates*

*Correspondence:

Phone: +91 9444054875 (Mob.)

E-mail: puvanakrishnan@yahoo.com

CFS 106, an isolate of cultivated field soil, has been shown to produce collagenase economically using cheap nitrogen sources such as soybean/feather meal¹³.

Collagen-rich matrix constitutes the main barrier to chronic total occlusion (CTO) crossing. Local administration of a human-grade purified collagenase degrades collagen CTO and it effectively facilitates guide-wire crossing in CTO¹⁴. The oxygen-ozone combined collagenase injection for treatment of lumbar disc herniation shows significant reduction in pain and improvement in function and it can be considered as a viable option instead of surgery¹⁵. Intravenous injection of type I collagenase digests collagen and can be used as a strategy to improve systemic gene delivery into tumors¹⁶. Peyronies disease is an acquired connective-tissue wound-healing disorder of the tunica albuginea of corpus cavernosum and intralesional collagenase has been used for its treatment¹⁷. It has recently been approved by USFDA under the name Xiaflex™ (Auxilium Pharmaceuticals, Malvern, PA, USA).

Pancreatic enzymes— Insufficiency of pancreatic enzymes leads to indigestion and inadequate absorption of fat, protein and carbohydrate, causing steatorrhoea and creatorrhea resulting in abdominal discomfort, weight loss and nutritional deficiencies. This serious condition, known as exocrine pancreatic insufficiency caused by chronic pancreatitis, cystic fibrosis, pancreatic cancer and pancreatic surgery, can be treated by pancreatic enzyme replacement therapy¹⁸. Pancreatic enzyme replacement therapy with delayed-release of pancrelipase is now becoming a standard practice since it significantly improves the coefficients of fat and nitrogen absorption as well as clinical symptoms¹⁹. Currently used pancreatic enzyme supplements contain a mixture of protease, lipase and amylase, known as pancreatin. Bovine pancreatic enzymes appear to be a better alternative compared to porcine and human pancreatic extracts²⁰. Pancreatic enzymes in the form of enteric-coated mini microspheres are useful in treating patients with Celiac disease²¹. *Centella asiatica* (L.) extract that demonstrated higher pancreatic lipase inhibitory activity has been shown helpful in managing hypolipidemic and hypoglycemic effects²². Pancreatic enzymes can also be used as supplements in the treatment of indigestion, constipation and bloating⁸.

Recently, Somaraju and Solis-Moya²³ have evaluated the efficacy and safety of pancreatic

enzyme replacement therapy in children and adults with cystic fibrosis. The results show that enteric-coated microspheres score over enteric-coated tablets in terms of stool frequency, abdominal pain and fecal fat excretion. However, it requires more evidence on the long-term effectiveness and risks associated with pancreatic enzyme replacement therapy, relative dosages of enzymes needed for people with different levels of severity of pancreatic insufficiency, optimum time to start treatment and variations based on differences in meals and meal sizes.

Lipases— Lipases catalyse the hydrolysis of triacylglycerol and phospholipids and they are obtained from bacterial, fungal, plant and animal sources^{6,24}. *Acinetobacter haemolyticus* TA106 from healthy human skin under optimized medium has been shown to produce lipase with maximum activity of 55U/mL²⁵. A recent theoretical analysis by Foukis *et al.*²⁶, has proposed suitable equations for effective production of immobilized lipase. Lipases have earlier been used in the treatment of gastrointestinal disturbances and dyspepsia²⁴. Similase and Vitaline® are high-potency plant enzyme supplements that support digestion of protein, carbohydrates and fats²⁴. Microbial lipases show significant lipolytic activity and they are stable against proteolytic hydrolysis as well as bile salts resulting in higher lipolytic activity. Oral pancreatic enzyme preparation containing highly stable lipase constitutes a good candidate for enzyme substitution therapy²⁷. The pancrelipase formulations (Creon®, Zenpep® and Pancreaze®) are approved effective treatments for pancreatic enzyme insufficiency²⁸. They provide symptomatic relief, prevent morbidity and improve quality of life. Another futuristic development is the restoration of a patient's own bio-engineered lipase production with gene therapy for treating lipoprotein lipase deficiency²⁹.

Hyaluronidase— Hyaluronidases are glycosidases with both hydrolytic and transglycosidase activities and they catalyze the hydrolysis of hyaluronic acid, lower the viscosity and increase tissue permeability³⁰. Hyaluronidase, as an enzymatic component of snake venom, has been discussed by Gomes *et al.*³¹. In ophthalmology, hyaluronidase is most often used as an adjunct to local anaesthesia for retrobulbular, peribulbular and sub-Tenon's block. It decreases intraocular pressure, reduces distortion of the surgical site, decreases incidence of post operative strabismus and limited local anaesthetic myotoxicity³².

Hyaluronidase liquefies the vitreous haemorrhage as demonstrated in a phase III trial in diabetic patients and it can be used as an alternative or adjunct to conventional mechanical vitrectomy³³. Hyaluronidase is used therapeutically in combination with other drugs to speed up delivery and absorption and to diminish discomfort due to subcutaneous or intramuscular injection of fluid, to increase the effectiveness of local anaesthesia and as a spreading factor to improve better penetration of chemotherapeutic drug into tumors. Hyaluronidase treatment destroys the hyaluronate coat surrounding tumor cells and allows lymphocytes to approach the tumor membrane to enhance the cytotoxic action of immune response³⁰. Further, hyaluronidase disrupts intercellular adhesion and chemosensitizes tumor cells by a mechanism, independent of increased drug penetration in cancer chemotherapy. Hyaluronic acid levels are elevated in several cancers and its degradation using hyaluronidase has been shown to enhance the action of various chemotherapeutic agents in patients. Hyaluronidase facilitates penetration and decreases interstitial fluid pressure, permitting anticancer agents to reach malignant cells. Moreover, it has been proposed that hyaluronidase may itself have intrinsic anticancer activity³⁴.

Asparaginase— Asparaginase is an amino-hydrolase converting asparagine to aspartic acid and ammonia, which leads to cell death. It has been used for the treatment of acute lymphoblastic leukemia (ALL) for nearly 30 years. Three types of asparaginase are currently available *viz.*, native asparaginase; pegylated asparaginase (PEG-asparaginase) derived from *Escherichia coli*; and third one from *Erwinia chrysanthemi* (crisantaspase) and bacteria derived enzyme has been found to have the lowest toxicity among a large variety of similar enzymes with known antitumor activity³⁵. It is also used as a model enzyme for development of new drug delivery system³⁶. *Bacillus aryabhatai* ITBH02 has been reported to be a potential source for production of L-asparaginase. Genetic algorithm (GA) optimized yield has been shown to be 7.8% higher than RSM based optimization³⁷. Actinobacteria, such as *Streptomyces canus*, *S. cyaneus*, *S. exfoliates* and *S. phaeochromogenes* have been shown to be potential producers of glutaminase free L-asparaginase with better therapeutic properties^{6,38}.

Leukemic cells require a high amount of asparagine for their proliferation and depend on body

fluid asparagine. Administration of L-asparaginase results in depletion of circulating serum asparagine and kills tumor cells. Healthy cells are unaffected as they synthesize asparagine intracellularly with L-asparagine synthetase³⁹. L-asparaginase, in combination with other drugs and radiotherapy, has shown great success in the treatment of ALL. Achievement of complete remission in patients is observed with a few side-effects including pancreatitis, coagulation abnormalities and allergic reactions. Sometimes, tumor cells may develop resistance to L-asparaginase. To overcome this difficulty, the drug is modified by pegylation or immobilization, and also treatment protocols can be modified to increase the efficiency of the drug⁴⁰. PEG-asparaginase is widely used for the treatment of children with ALL. It is less immunogenic and has a longer half-life than native *E. coli* asparaginase, which makes it a potent drug with reduced number of doses³⁵.

Glutamine reduction is also necessary for optimal anti-leukemic activity of asparaginase³⁸. Indeed, both *Escherichia coli* and *Erwinia chrysanthemi* asparaginases possess glutaminase activity also and their administrations have shown to reduce serum glutamine level by deamidating glutamine to glutamate and ammonia. Emadi *et al.*⁴¹ have reported that asparaginase products deserve a second look particularly in non-ALL malignant blood disorders.

L-glutaminase— L-glutaminase is an amidohydrolase that catalyzes the hydrolysis of L-glutamine into glutamate and ammonia. Cancer cells, especially lymphatic tumor cells, cannot synthesize L-glutamine, and require a large amount of L-glutamine for their rapid growth. Hence, the use of L-glutaminase deprives the tumor cells from L-glutamine and causes selective death of L-glutamine dependant tumor cells. L-glutaminase has been used as an efficient anti-retroviral agent in treating acquired immunodeficiency syndrome (AIDS) caused by human immunodeficiency virus (HIV) as it lowers L-glutamine levels in both serum and tissues for prolonged periods. This results in substantial reduction in serum reverse transcriptase activity of the HIV with improved long-term survival benefits⁴². L-glutaminase alone or in combination with asparaginase is mainly used in treating cancer, specifically ALL. It is also a therapeutic agent for retroviral diseases⁴³. *Achromobacter glutaminasificans* glutaminase-asparaginase is chemically modified by

succinylation, and the succinylated enzyme has broader antitumor activity than *E. coli* asparaginase⁴⁴.

DNA and RNA enzymes— Deoxyribonucleic acid (DNA) and ribonucleic acid (RNA) enzymes can be used to study gene function and treat diseases in which gene products are involved. They can be used to block the expression of specific genes in cells and thereby prevent certain diseases. RNA enzymes can be delivered to the cells either endogenously as gene-encoding ribozymes or exogenously as preformed ribozymes. These enzymes can cleave mRNAs and thereby inactivate abnormal gene expression. It is applicable to any disease where a specific gene product can be linked to the initiation and/or perpetuation of the disease. Deoxyribonuclease (DNase), obtained from *Bacillus* sp. and *Nocardia* sp., degrades DNA and it has been investigated as a mucolytic agent for the treatment of chronic bronchitis⁴⁵. The increased viscosity of pleural pus in patients with pleural empyema is attributed to high concentrations of DNA resulting from the breakdown of phagocytes, bacteria, and other intrapleural cells⁴⁶. Streptodornase, a mixture of four DNase enzymes, reduces the viscosity of pus by the digestion of DNA, whereas commercial human recombinant DNase digests DNA and decreases the viscosity of pleural empyema pus without causing allergic reactions known to occur with streptodornase⁴⁶. Commercial Pulmozyme® (Dornase α), a DNase, liquefies accumulated mucus in the lung and also diminishes pulmonary tissue destruction in cystic fibrosis patients⁴⁵. Chemically modified DNA enzymes can cleave RNA in the absence of divalent metal ions and such RNA-cleaving DNA enzymes have potential therapeutic applications as antibacterial and antiviral agents⁴⁷.

Schmidts *et al.*⁴⁸ have investigated and suggested the use of DNA enzymes as potent novel drugs for the treatment of inflammatory diseases such as atopic dermatitis. The two challenges regarding the dermal application of DNA enzymes are the large molecular weight and the sensitivity to DNases as part of the natural skin flora. To overcome these limitations, they have developed a suitable nano-sized drug carrier system, promising for topical application of DNA enzyme.

Ribozymes are RNA molecules possessing specific catalytic activity and they are capable of catalyzing highly sequence-specific reactions determined by RNA-RNA interactions between the ribozyme and its

substrate molecules⁴⁹. The key to the recognition of and binding to the substrate molecule and the subsequent cleavage reaction resides in the RNA molecule. The capacity of ribozymes to specifically inactivate other RNAs has made ribozymes very promising molecular tools and potential gene suppressors with important applications⁵⁰. Ribozymes have been targeted against a myriad of genes, including oncogenes and drug resistance genes, viral diseases such as AIDS, viral hepatitis, mumps virus etc and cellular diseases *viz.*, Alzheimer's disease, cancer, diabetes, rheumatoid arthritis, etc⁵⁰.

RNA-based therapeutics are investigated for diseases ranging from genetic disorders to HIV infection to various cancers. The emerging drugs include therapeutic ribozymes, aptamers, and small interfering RNAs (siRNAs), demonstrating the unprecedented versatility of RNA. Hammerhead ribozymes are catalytic RNA molecules capable of inducing the site-specific cleavage of a phosphodiester bond within an RNA molecule⁵¹. Thus, they can be used to reduce the intracellular level of a specific mRNA coding for a protein which affects cellular metabolism or environment, causing disease. Nucleic acids are potentially immunogenic and typically require a delivery tool to be utilized as therapeutics. Hence, improved synthetic delivery carriers and chemical modifications of the RNA therapeutics are necessary^{51,52}. Hepatitis C virus genome is present exclusively in RNA form during replication. Various nucleic acid-based therapeutic approaches targeting the hepatitis C virus genome, such as ribozymes, aptamers, siRNAs, and antisense oligonucleotides, have been suggested as potential tools against hepatitis C virus⁵³.

Urokinase— Urokinase or urokinase-type plasminogen activator (uPA) is a serine protease which converts plasminogen to plasmin, thus promoting fibrinolysis. uPA, as a thrombolytic agent, is used to treat of pulmonary embolism, acute myocardial infarction, severe or massive venous thrombosis, ophthalmic clot and hemorrhage and peripheral arterial occlusion⁵⁴. It is also administered intrapleurally to dissolve fibrinous adhesions thereby improving the drainage of complicated pleural effusions and preventing pleural loculations⁵⁵. Activase is the first recombinant enzyme drug approved by the USFDA in 1987. Urokinase is presently marketed as KinlyticTM and used as a thrombolytic drug in infarction. It is a life saving,

therapeutically important fibrinolytic enzyme used in the treatment of many disorders requiring dissolution of blood clots⁵⁶. Prolonged thrombolysis with low-dose urokinase could be an alternative approach to therapy in patients with massive pulmonary embolism⁵⁷.

Streptokinase— Streptokinase (SK), a bacterial extracellular enzyme, is a single-chain polypeptide that exerts fibrinolytic action indirectly by activating the circulatory plasminogen. SK is used for intravenous administration immediately after the onset of a myocardial infarction in patients, thus reducing the amount of damage to the heart muscle. SK stimulates a cascade system responsible for the production of active plasmin, a proteolytic enzyme which digests fibrin, the main structural component of blood clots. It is usually administered only when the first heart attack occurs⁵⁸. SK, used in intrapleural fibrinolytic therapy, is known to accelerate drainage of loculated effusions in complicated empyema. Intrapleural streptokinase is an effective and safe adjunct in facilitating drainage in parapneumonic pleural effusions and pleural empyema⁵⁹. SK is used extensively as a thrombolytic agent⁶⁰. Treatment with antibiotics, tube thoracostomy and intrapleural streptokinase has led to resolution of the pneumonia and empyema without the need for surgical intervention in advanced pregnancy⁶¹.

Tissue plasminogen activator (tPA)— When more than one heart attack occurs, tPA is recommended. The fibrinolytic system comprises an inactive proenzyme, plasminogen which can be converted to the active enzyme plasmin using plasminogen activators and this, in turn, degrades fibrin into soluble fibrin degradation products⁶².

The fibrin specificity of tPA has stimulated great interest in its use for thrombolytic therapy. tPA is a poor enzyme in the absence of fibrin, but its activity is dramatically stimulated in the presence of a fibrin cofactor. This fibrin specificity has formed the basis for its development as a thrombolytic agent for treatment of thromboembolic disease. In the therapeutic setting, exogenous administration of tPA is an approved treatment for both myocardial infarction and acute ischemic stroke⁶².

Second-generation (recombinant activase) and third-generation plasmin activators (mutants or recombinant reteplase) have several advantages over streptokinase and urokinase *viz.*, i) not antigenic; ii) do not cause immunogenic reactions; iii) absorbed

in particular onto fibrin clots and display their effect only there; and iv) no side effects on hemostasis even when introduced into the circulating blood⁶³.

Serratiopeptidase— Serratiopeptidase is a proteolytic enzyme prescribed in various specialties such as surgery, orthopaedics, gynaecology and dentistry for its anti-inflammatory and analgesic effects⁶⁴. It is reported to possess antiatherosclerotic effects due to its fibrinolytic and caseinolytic properties and also can be used as a health supplement to prevent cardiovascular morbidity⁶⁴. Serratiopeptidase has been found useful in patients suffering from acute or chronic inflammatory disorders of ear, nose or throat such as laryngitis, catarrhal rhino-pharyngitis and sinusitis⁶⁵. It is orally effective equivalent to diclofenac sodium in both acute and chronic phases of inflammation⁶⁵.

Apart from the two common conventional drugs for inflammatory disorders *viz.*, corticosteroids and nonsteroidal antiinflammatory drugs, serration-peptidase derived from non-pathogenic enterobacteria *Serratia* sp E-15 has anti-inflammatory and anti-edemic activity in a number of tissues⁶⁶. Serratiopeptidase is reported to exert a beneficial effect on mucus clearance by reducing neutrophil numbers and altering the viscoelasticity of sputum in patients with chronic airway diseases⁶⁷. Al-Khateeb and Nusair⁶⁸ have investigated the ability of serratiopeptidase to reduce postoperative swelling, pain and trismus after third molar surgery and reported significant reduction in the extent of cheek swelling and pain intensity in 2nd, 3rd and 7th post-operative days. Serratiopeptidase hydrolyses bradykinin, histamine and serotonin responsible for the edematous status, reduces swelling and improves microcirculation and expectoration of sputum^{67,69}. The antibiofilm property of the enzyme may enhance antibiotic efficacy in the treatment of staphylococcal infections⁷⁰.

Lysosomal hydrolases— Lysosomal hydrolases deficiency causes lysosomal storage diseases (LSDs). It can cause impaired intracellular turnover, degradation and disposal of a variety of substrates present in lysosomes, including sphingolipids, glycosaminoglycans, glycoproteins and glycogen. The accumulation of these substrates in the endosome/lysosome elicits complex, secondary biochemical and cellular events that ultimately lead to cell and tissue damage⁷¹. The pathology of LSDs is typically characterized by the variable association of visceral,

ocular, hematologic, skeletal and neurological manifestations⁷². A major breakthrough in the treatment of LSDs is the enzyme replacement therapy (ERT). ERT refers to a group of glycoprotein enzyme products, each intended to augment or replace the activity of a specific endogenous catabolic enzyme within the cellular lysosomes. All ERT products are administered by intravenous infusion and the infused enzymes are taken up by cells and transported into lysosomes, where they catabolize the specific macromolecule that has accumulated⁷³.

ERT has been approved for six lysosomal storage diseases (LSDs) which include Gaucher disease, Fabry disease, mucopolysaccharidosis types I, II, and VI and Pompe disease. Some approved enzymes currently used are acid β -glucosidase from human placenta (alglucerase) and recombinant enzymes (imiglucérase, Cerezyme®) for the treatment of Gaucher disease. Two currently available preparations for Fabry disease are Replagal® and Fabrazyme®. Mucopolysaccharidosis type I (Hurler and Scheie diseases), type II (Hunter syndrome) and type VI (Maroteaux-Lamy disease) are treated using laronidase (Aldurazyme®), idursulfase (Elaprase®) and galsulfase (Naglazyme®), respectively^{74,75}. On the other hand, clinical experience with ERT has shown that this approach has limitations, mostly related to the bioavailability of recombinant enzymes and high cost of therapies. To circumvent these problems, different strategies are evaluated to improve delivery of the enzymes to target tissues, including central nervous system. In addition to these approaches, an invasive procedure, such as intrathecal ERT administration, has been evaluated in preclinical studies for several lysosomal disorders⁷². A synopsis of enzyme therapy is given in Table 1.

Enzymes for future

Antioxidant enzymes

Antioxidant enzymes such as superoxide dismutase, catalase, heme oxygenase, glutathione peroxidase and peroxiredoxin are the scavengers of reactive oxygen species (ROS), and used as indices/therapeutic agents for treatment of oxidative stress related disorders including neurological and neuroinflammatory diseases^{76,77}. Superoxide dismutases are a group of metal-containing enzymes that convert the superoxide anion to molecular oxygen and hydrogen peroxide⁷⁸. Glutathione peroxidases constitute a family of selenium-containing enzymes

that detoxify cellular organic peroxides and hydrogen peroxide by oxidation. Heme oxygenases have both antioxidative and antiinflammatory properties whereas peroxiredoxins play an important role in removing hydrogen peroxide⁷⁶.

Cysteine proteinase—Immunoglobulin G-degrading enzyme of *Streptococcus pyogenes* is a cysteine proteinase which cleaves immunoglobulin G (IgG) with a unique degree of specificity in the hinge region. Pathogenic IgG antibodies constitute an important clinical problem contributing to the pathogenesis of a number of autoimmune conditions and acute transplant rejection⁷⁹.

Amyloid β -protein ($A\beta$) cleaving proteases— $A\beta$ is degraded by a diverse set of proteolytic enzymes viz., $A\beta$ -degrading proteases such as zinc metalloproteases, serine proteases and cysteine proteases. $A\beta$ is the main component of amyloid plaques, which accumulates abnormally in the brains of patients with Alzheimer's disease. Leissring⁸⁰ has detailed the involvement of specific $A\beta$ cleaving proteases in the etiology and potential treatment of Alzheimer's disease. The $A\beta$ -cleaving proteases are referred as novel class of enzymes that may serve as therapeutic agents.

L-lysine α -oxidase—L-lysine α -oxidase belongs to the group of oxidases of L-amino acids. It was first isolated from *Trichoderma viride* and later from *T. harzanium* Rifai. This enzyme catalyzes mainly oxidative deamination of L-lysine resulting in a decreased level of the essential amino acid L-lysine and producing α -keto- ϵ -aminocaproic acid and hydrogen peroxide. This possibly provides the basis for the unique properties of L-lysine α -oxidase viz., cytotoxic, antitumor, antimetastatic, antiinvasive, antibacterial, and antiviral activities, as well as immunomodulating effect^{81,82}.

Chondroitinase ABC (ChABC)—ChABC (glycosaminoglycan lyases) degrades chondroitin sulphate and the closely-related glycosaminoglycan hyaluronan which can be used for functional recovery in the damaged central nervous system (CNS)⁸³. It is used for treatment of spinal injuries to promote regeneration of injured spinal cord and also acts by removing the accumulated chondroitin sulfate that inhibits axon growth. Chondroitin sulphate proteoglycans (CSPGs) are potent inhibitors of growth in the adult CNS and use of ChABC reduces the CSPG inhibition of spinal cord injury and promotes regeneration of injured axons, plasticity of

Table 1—Therapeutic applications of enzymes

Therapeutic enzymes	Diseases treated
Collagenase	Wound debridement. Treatment of skin ulcers and burns ¹⁰ ; Dupuytren's contractures ^{11,12} ; facilitation of guide-wire crossing in CTO ¹⁴ ; tumour ¹⁶ .
Pancreatic enzymes	Treatment of exocrine pancreatic insufficiency ^{18,19} ; fat malabsorption ²⁰ ; treatment of celiac and Crohn's disease ²¹ .
Lipases	Treatment of dyspepsia, digestive allergies, skin inflammation and lowering cholesterol ²⁴ ; malignant tumours ²⁴ ; Treatment of exocrine pancreatic insufficiency ²⁸ .
Hyaluronidase	Spreading factor ³⁰ ; Antitumour activity ³⁰ ; Fasciotomy & Virectomy ³³ .
Asparaginase	Treatment of tumour and lymphoproliferative disorders ^{35,36,39} .
L-glutaminase	Treatment of cancer & HIV therapy ⁴⁴ ; antitumour activity ⁴³ .
DNA & RNA Enzymes	Antibacterial and antiviral diseases ^{47,53} ; viral & cellular diseases ⁵⁰⁻⁵² .
Urokinase	Thrombolytic agent, Treatment of pulmonary embolism, acute myocardial infarction, ophthalmic clot & hemorrhage empyema ^{54,56,57} .
Streptokinase	Thrombolytic agent ⁶⁰ ; Treatment of heart attack, empyema & pneumonia ^{59,61} .
Tissue plasminogen activator	Myocardial infarction and acute ischemic stroke ⁶² .
Serratiopeptidase	Treatment of pain & inflammatory disorders, laryngitis & sinusitis ^{64,65} .
Antioxidant enzymes	Treatment of oxidative stress related neurological disorders ⁷⁶ .
Alginate lyase	Treatment of CF and increased antibiotic efficacy ^{87,88} .
Hemocoagulase	Reduces the bleeding time, hastens wound healing ^{89,90} ; used in plastic surgery, abdominal operation, and human vitrectomy due to antihemorrhagic property ^{91,93} .
Bromelain	Anti-inflammatory, analgesic, antithrombotic and fibrinolytic effects ⁹⁴ ; anticancer activity ⁹⁵ .

uninjured pathways and neuroprotection of injured projection neurons⁸⁴. Denholm *et al.*⁸⁵ have reported that removal of CSPGs by chondroitinase AC and B inhibits tumor growth, neovascularization and metastasis.

Alginate lyase— Alginate lyases catalyze degradation of alginate, a complex copolymer of alpha-L-guluronate and beta-D-mannuronate. Lyases have been isolated from a wide range of organisms, including algae, marine invertebrates, and marine and terrestrial microorganisms^{6,82}. For more than two decades, alginate lyases are promising therapeutic candidates for treating mucoid *Pseudomonas aeruginosa* infections. The mucoid *P. aeruginosa* strains frequently isolated from cystic fibrosis patients have alginate exopolysaccharide which acts as a barrier against host's immune defense and antibiotic treatment. It is reported that alginate lyase capable of degrading alginate polymer can be used in combination with antibiotics for the treatment of cystic fibrosis^{87,88}.

Hemocoagulase— Hemocoagulase is an enzyme complex isolated from snake venom which is known to possess coagulative and antihemorrhagic property. It accelerates the conversion of fibrinogen to fibrin polymer and promotes the interaction of platelets with

fibrin clot. The clot thus formed is resistant to plasmin. Thus, it reduces the bleeding time, enhances cell division and hastens wound healing^{89,90}. Topical hemocoagulase solution has been used in oral and maxillofacial surgery as a hemostatic agent and promoter of wound healing⁸⁹.

Hemocoagulase is suggested to play a good hemostatic role in the hemorrhagic capillary in abdominal incision, in cases of cleft palate and septum deviation during plastic surgery, and in the control of intraocular bleeding during vitreous surgery⁹¹. Prophylactic use of hemocoagulase in mechanically ventilated neonates is effective against pulmonary hemorrhage^{92,93}.

Jun-Min *et al.*⁹³ have conducted phase III clinical trial to evaluate the effect of hemocoagulase agkistrodon obtained from chinese moccasin snake venom. It is found to possess good hemostatic and coagulative function and is safe for arresting capillary hemorrhage during abdominal surgery.

Bromelain— Bromelain is a crude, aqueous extract obtained from the pineapple (*Ananas comosus* Merr.) containing a mixture of proteolytic enzymes, referred to as sulfhydryl proteases. They are considered to have a range of beneficial properties such as anti-inflammatory, analgesic, antithrombotic and fibrinolytic

effects^{31,88}. Bromelain is used for acute inflammation and sports injuries and also used as an alternative treatment to NSAIDs for patients with osteoarthritis⁹⁴. Bromelain and papain, the two natural proteolytic enzymes are used to neutralize snake envenomation³¹.

Pillai *et al.*⁹⁵ have demonstrated that bromelain has the potential of being used as an effective anticancer agent for malignant peritoneal mesothelioma. During the treatment of patients with burn wounds, surgical escharotomy may cause considerable blood loss. Debridase, a bromelain derived enzymatic preparation, is capable of lysing the burn eschar within 4 h, obviating the need for surgical debridement. It has an affinity to burned necrotic tissue and does not damage healthy skin⁹⁶. Deep burns are associated with the formation of an eschar, which delays healing and increases the risk of infection. A synopsis of enzyme therapy is given in Table 1.

Conclusion

Enzyme therapy is used in the treatment of cardiovascular, oncological, intestinal, viral and hereditary diseases. Some enzymes are in different phases of clinical trials. Biotechnological progress has encouraged pharmaceutical companies to produce safer and cheaper enzymes with improved potency and specificity. Enzymes in combination with drugs have potential to induce synergistic effect for treatment of various diseases and to reduce the side-effects of particular drugs.

Acknowledgements

One of the authors, TH gratefully acknowledges the Council of Scientific and Industrial Research for award of CSIR for Senior Research Associateship.

References

- Hemalatha T, UmaMaheswari T, Krithiga G, Sankaranarayanan P & Puvanakrishnan R, Enzymes in clinical medicine: An overview. *Indian J Exp Biol*, 51 (2013) 777.
- Jain I, Kumar V & Satyanarayana T, Xylooligosaccharides: an economical prebiotic from agroresidues and their health benefits. *Indian J Exp Biol*, 53 (2015) 131.
- Chandra AK, Goswami H & Sengupta P, Effects of magnesium on cytomorphology and enzyme activities in thyroid of rats. *Indian J Exp Biol*, 52 (2014) 787.
- Jayamurthy H, Sajna KV, Dastagar SG & Pandey A, Antifungal potentials of extracellular metabolites of Western Ghats isolated *Streptomyces* sp. NII 1006 against moulds and yeasts. *Indian J Exp Biol*, 52 (2014) 1138.
- Maachia BS, Rafik E, Chérif M, Nandal P, Mohapatra T & Bernard P, Biological control of the grapevine diseases 'grey mold' and 'powdery mildew' by *Bacillus* B27 and B29 strains. *Indian J Exp Biol*, 53 (2015) 109.
- Velho-Pereira S & Kamat NM, Actinobacteriological research in India. *Indian J Exp Biol*, 51 (2013) 573.
- Mario Roxas ND, The Role of Enzyme Supplementation in Digestive Disorders. *Alt Med Rev*, 13 (2008) 307.
- Kaur R & Sekhon BS, Enzymes as drugs: an overview. *J Pharm Educ Res*, 3 (2010) 29.
- Ramundo J & Gray M, Enzymatic wound debridement. *J Wound Ostomy Continence Nurs*, 35 (2008) 273.
- Ramundo J & Gray M, Collagenase for enzymatic debridement: a systematic review. *J Wound Ostomy Continence Nurs*, 36 (2009) S4.
- Coleman S, Gilpin D, Kaplan FT, Houston A, Kaufman GJ, Cohen BM, Jones N & Tursi JP, Efficacy and safety of concurrent collagenase *Clostridium histolyticum* injections for multiple Dupuytren contractures. *J Hand Surg Am*, 39 (2014) 57.
- Cuylits N, News in the treatment of Dupuytren's disease: from surgery to collagenase's injection. *Rev Med Brux*, 34 (2013) 283.
- Jain R & Jain PC, Production and partial characterization of collagenase of *Streptomyces exfoliatus* CFS 1068 using poultry feather. *Indian J Exp Biol*, 48 (2010) 174.
- Strauss BH, Osheroov AB, Radhakrishnan S, Mancini GB, Mannes A, Sparkes JD & Chisholm RJ, Collagenase Total Occlusion-1 (CTO-1) trial: a phase I, dose-escalation, safety study. *Circulation*, 125 (2012) 522.
- Wu Z, Wei LX, Li J, Wang Y, Ni DH, Yang P & Zhang Y, Percutaneous treatment of non-contained lumbar disc herniation by injection of oxygen-ozone combined with collagenase. *Eur J Radiol*, 72 (2009) 499.
- Kato M, Hattori Y, Kubo M & Maitani Y, Collagenase-I injection improved tumor distribution and gene expression of cationic lipoplex. *Int J Pharmaceut*, 423 (2012) 428.
- Levine LA, Peyronie's disease: A contemporary review of non-surgical treatment. *Arab J Urol*, 11 (2013) 278.
- Sikkens ECM, Cahen DL, Kuipers EJ & Bruno MJ, Pancreatic enzyme replacement therapy in chronic pancreatitis. *Best Pract Res Clin Gastroenterol*, 24 (2010) 337.
- Nakajima K, Oshida H, Muneyuki T & Kakei M, Pancrelipase: an evidence-based review of its use for treating pancreatic exocrine insufficiency. *Core Evid*, 7 (2012) 77.
- Löhr JM, Hummel FM, Pirilis KT, Steinkamp G, Korner A & Henniges F, Properties of different pancreatic preparations used in pancreatic exocrine insufficiency. *Eur J Gastroenterol Hepatol*, 21 (2009) 1024.
- Fieker A, Philpott J & Armand M, Enzyme replacement therapy for pancreatic insufficiency: present and future. *Clin Exp Gastroenterol*, 4 (2011) 55.
- Supkamonseni N, Thinkratok A, Meksuriyen D & Srisawat R, Hypolipidemic and hypoglycemic effects of *Centella asiatica* (L.) extract *in vitro* and *in vivo*. *Indian J Exp Biol*, 52 (2014) 965.
- Somaraju UR & Solis-Moya A, Pancreatic enzyme replacement therapy for people with cystic fibrosis. *Cochrane Database Syst Rev*, 10 (2014) CD008227.
- Hasan F, Shah AA & Hameed A, Industrial applications of microbial lipases. *Enzyme Microb Tech*, 39 (2006) 235.
- Jagtap S, Gore S, Yavankar S, Pardesi K & Chopade B, Optimization of medium for lipase production by *Acinetobacter haemolyticus* from healthy human skin. *Indian J Exp Biol*, 48 (2010) 936.

- 26 Foukis A, Stergiou PY, Filippou M, Koukouritaki M, Parapouli M, Theodorou LG, Hatziloukas E, Afendra A, Pandey A & Papamichael EM, Kinetic constraints and features imposed by the immobilization of enzymes onto solid matrices: A key to advanced biotransformation. *Indian J Exp Biol*, 52 (2014) 1045.
- 27 Domínguez-Muñoz JE, Pancreatic enzyme therapy for pancreatic exocrine insufficiency. *Gastroenterol Hepatol*, 7 (2011) 401.
- 28 Dhanasekaran R & Toskes PP, Pancrelipase for pancreatic disorders: An update. *Drugs Today (Barc)*, 46 (2010) 855.
- 29 Gaudet D, Methot J & Kastelein J, Gene therapy for lipoprotein lipase deficiency. *Curr Opin Lipidol*, 23 (2012) 310.
- 30 El-Safory NS, Fazary AE & Lee CK, Hyaluronidases, a group of glycosidases: Current and future perspectives. *Carbohydr Polym*, 81 (2010) 165.
- 31 Gomes A, Das R, Sarkhel S, Mishra R, Mukherjee S, Bhattacharya S & Gomes Aparna, Herbs and herbal constituents active against snake bite. *Indian J Exp Biol*, 48 (2010) 865.
- 32 Silverstein SM, Greenbaum S & Stern R, Hyaluronidase in ophthalmology. *J Appl Res*, 12 (2012) 1.
- 33 Gandorfer A, Enzymatic vitreous disruption. *Eye (Lond)*, 22 (2008) 1273.
- 34 Guedan S, Rojas JJ, Gros A, Mercade E, Cascallo M & Alemany R, Hyaluronidase expression by an oncolytic adenovirus enhances its intratumoral spread and suppresses tumor growth. *Mol Ther*, 18 (2010) 1275.
- 35 Rizzari C, Conter V, Starý J, Colombini A, Moericke A & Schrappe M, Optimizing asparaginase therapy for acute lymphoblastic leukemia. *Curr Opin Oncol*, 25 (2013) Suppl 1:S1.
- 36 Kumar S, Venkata Dasu V & Pakshirajan K, Studies on pH and thermal stability of novel purified L-asparaginase from *Pectobacterium carotovorum* MTCC 1428. *Microbiology*, 80 (2011) 355.
- 37 Singh Y & Srivastava SK, Statistical and evolutionary optimization for enhanced production of an anti-leukemic enzyme, L-asparaginase, in a protease-deficient *Bacillus aryabhatai* ITBHU02 isolated from the soil contaminated with hospital waste. *Indian J Exp Biol*, 51 (2013) 322.
- 38 Saxena A, Upadhyay R & Kango N, Isolation and identification of actinomycetes for production of novel extracellular glutaminase free L-asparaginase. *Indian J Exp Biol*, 53 (2015) 786.
- 39 Narta UK, Kanwar SS & Azmi W, Pharmacological and clinical evaluation of L-asparaginase in the treatment of leukemia. *Critic Rev Oncol Hematol*, 61 (2007) 208.
- 40 Kumar K, Kaur J, Walia S, Pathak T, Aggarwal D, L-asparaginase: an effective agent in the treatment of acute lymphoblastic leukemia. *Leuk Lymphoma*, 55 (2014) 256.
- 41 Emadi A, Zokaee H, Sausville EA, Asparaginase in the treatment of non-ALL hematologic malignancies. *Cancer Chemother Pharmacol*, 73 (2014) 875.
- 42 Sarada KV, Production and applications of L-Glutaminase using fermentation technology. *Asia Pac J Res*, 1 (2013) 1.
- 43 Balagurunathan R, Radhakrishnan M & Somasundaram S T, L-glutaminase producing actinomycetes from marine sediments-selective isolation, semi quantitative assay and characterization of potential strain. *Aust J Basic Appl Sci*, 4 (2010) 698.
- 44 Elzainy T A & Ali T H, Detection of antitumor glutaminase-asparaginase in the filamentous fungi. *J Appl Sci*, 6 (2006) 1389.
- 45 Sabu A, Sources, Properties and Applications of Microbial Therapeutic Enzymes. *Ind J Biotechnol*, 2 (2003) 334.
- 46 Simpson G, Roomes D & Heron M, Effects of streptokinase and deoxyribonuclease on viscosity of human surgical and empyema pus. *Chest*, 117 (2000) 1728.
- 47 Pradeepkumar PI & Höbartner C, RNA-Cleaving DNA enzymes and their potential therapeutic applications as antibacterial and antiviral agents, In: *From Nucleic Acids Sequences to Molecular Medicine RNA Technologies*, (Eds. VA Erdmann & J Barciszewski, Springer, Berlin Heidelberg), 2012, 371.
- 48 Schmidts T, Marquardt K, Schlupp P, Dobler D, Heinz F, Mäder U, Garn H, Renz H, Zeitvogel J, Werfel T & Runkel F, Development of drug delivery systems for the dermal application of therapeutic DNazymes. *Int J Pharm*, 431 (2012) 61.
- 49 Abera G, Berhanu G & Tekewe A, Ribozymes: nucleic acid enzymes with potential pharmaceutical applications—a review, *Pharmacophore*, 3 (2012) 164.
- 50 Puerta-Fernández E, Romero-López C, Barroso-delJesus A & Berzal-Herranz A, Ribozymes: recent advances in the development of RNA tools. *FEMS Microbiol Rev*, 27(2003) 75.
- 51 Grassi G, Dawson P, Guarnieri G, Kandolf R & Grassi M, Therapeutic potential of hammerhead ribozymes in the treatment of hyper-proliferative diseases. *Curr Pharm Biotechnol*, 5 (2004) 369.
- 52 Burnett JC & Rossi JJ, RNA-based therapeutics: current progress and future prospects. *Chem Biol*, 19 (2012) 60.
- 53 Lee CH, Kim JH & Lee SW, Prospects for nucleic acid-based therapeutics against hepatitis C virus. *World J Gastroenterol*, 19 (2013) 8949.
- 54 Crippa MP, Molecules in focus: Urokinase-type plasminogen activator. *Int J Biochem Cell Biol*, 39 (2007) 690.
- 55 Bouros D, Tzouveleakis A, Antoniou KM & Heffner JE, Intrapleural fibrinolytic therapy for pleural infection. *Pulm Pharmacol Ther*, 20 (2007) 616.
- 56 Kunamneni A, Ravuri BD, Ellaiah P, Prabhakar T & Saisha V, Urokinase-a strong plasminogen activator. *Biotechnol Mol Biol Rev*, 3 (2008) 58.
- 57 Bulpa P, Carbutti G, Osselaer JC, Lawson G, Dive A & Evrard P, Low-dose urokinase in massive pulmonary embolism when standard thrombolysis is contraindicated, *Chest*, 136 (2009) 1141.
- 58 Banerjee A, Chisti Y & Banerjee UC, Streptokinase—a clinically useful thrombolytic agent. *Biotechnol Adv*, 22 (2004) 287.
- 59 Bouros D, Antoniou K & Light RW, Intrapleural streptokinase for pleural infection. *BMJ*, 332 (2006) 133.
- 60 Kunamneni A, Abdelghani TT & Ellaiah P, Streptokinase--the drug of choice for thrombolytic therapy. *J Thromb*, 23 (2007) 93.
- 61 Nir S, Gadi L, Mony S, Meir M, Jaime G, Amir E, Uzi I, Gil L, Yaron I & Neville B, Successful use of streptokinase for the treatment of empyema thoracis during advanced pregnancy: A case report. *Respiratory Med CME*, 2 (2009) 21.
- 62 Chasman DI, New pathway for tissue-type plasminogen activator regulation. *Arterioscler Thromb Vasc Biol*, 34 (2014) 964.

- 63 Maroo A & Topol EJ, Clot busters!!-discovery of thrombolytic therapy for heart attack & stroke. *J Thromb Haem*, 2 (2004) 1.
- 64 Bhagat S, Agarwal M & Roy V, Serratiopeptidase: a systematic review of the existing evidence. *Int J Surg*, 11 (2013) 209.
- 65 Jadav SP, Patel N H, Shah TG, Gajera MV, Trivedi HR & Shah BK, Comparison of anti-inflammatory activity of serratiopeptidase and diclofenac in albino rats. *J Pharmacol Pharmacother*, 1 (2010) 116.
- 66 Garg R, Aslam S, Garg A & Walia R, A prospective comparative study of serratiopeptidase and aceclofenac in upper and lower limb soft tissue trauma cases. *Int J Pharmacol Pharmaceut Technol*, 1 (2012) 11.
- 67 Nakamura S, Hashimoto Y, Mikami M, Yamanaka E, Soma T, Hino M, Azuma A & Kudoh S, Effect of the proteolytic enzyme serrapeptase in patients with chronic airway disease. *Respirology*, 8 (2003) 316.
- 68 Al-Khateeb TH & Nusair Y, Effect of the proteolytic enzyme serrapeptase on swelling, pain and trismus after surgical extraction of mandibular third molars. *Int J Oral Maxillofac Surg*, 37 (2008) 264.
- 69 Mohankumar A & Hari Krishna Raj R, Production and characterization of serratiopeptidase enzyme from *Serratia marcescens*. *Int J Biol*, 3 (2011) 39.
- 70 Mecikoglu M, Saygi B, Yildirim Y, Karadag-Saygi E, Ramadan SS & Esemnli T, The effect of proteolytic enzyme serratiopeptidase in the treatment of experimental implant-related infection. *J Bone Joint Surg Am*, 88 (2006) 1208.
- 71 Parenti G, Treating lysosomal storage diseases with pharmacological chaperones: from concept to clinics. *EMBO Mol Med*, 1 (2009) 268.
- 72 Parenti G, Pignata C, Vajro P & Salerno M, New strategies for the treatment of lysosomal storage diseases. *Int J Mol Med*, 31 (2013) 11.
- 73 Lachmann RH, Enzyme replacement therapy for lysosomal storage diseases. *Curr Opin Pediatr*, 23 (2011) 588.
- 74 Germain DP, Boucly C, Carlier RY, Caudron E, Charlier P, Colas F, Jabbour F, Martinez V, Mokhtari S, Orlikowski D, Pellegrini N, Perronne C, Prigent H, Rubinsztajn R, Benistan K, Enzyme replacement therapy of lysosomal storage diseases. *Rev Med Int*, 31 (2010) S279.
- 75 Ohashi T, Enzyme replacement therapy for lysosomal storage diseases. *Pediatr Endocrinol Rev*, 10 (2012) 26.
- 76 Schreibelt G, Horssen JV, Rossum SV, Dijkstra CD, Drukarch B & de Vries HE, Therapeutic potential and biological role of endogenous antioxidant enzymes in multiple sclerosis pathology. *Brain Res Rev*, 56 (2007) 322.
- 77 Sachdeva M, Chadha R, Kumar A, Karan M, Singh T & Dhingra S, Hepatoprotective effect of trimethylgallic acid esters against carbon tetrachloride-induced liver injury in rats. *Indian J Exp Biol*, 53 (2015) 803.
- 78 Johnson F & Giulivi C, Superoxide dismutases and their impact upon human health. *Mol Aspects Med*, 26 (2005) 340.
- 79 Johansson BP, Shannon O & Björck L, IdeS: A bacterial proteolytic enzyme with therapeutic potential. *PLoS ONE*, 3 (2008) e1692.
- 80 Leissring MA, The ABCs of A β -cleaving proteases. *J Biol Chem*, 283 (2008) 29645.
- 81 Pasut G, Sergi M & Veronese FM, Anti-cancer PEG-enzymes: 30 years old, but still a current approach. *Adv drug deliv rev*, 60 (2008) 69.
- 82 Joseph B & Rajan SS, L-lysine α oxidases from fungi as an anti-tumour agent. *Adv Biotech*, 10 (2011) 27.
- 83 Richard A, Chondroitinase and central nervous system repair. *Sci Topics*, (2008, July 30) (retrieved) [http://www.scitopics.com/Chondroitinase and central nervous system repair.htm](http://www.scitopics.com/Chondroitinase%20and%20central%20nervous%20system%20repair.htm).
- 84 Bradbury EJ & Carter LM, Manipulating the glial scar: chondroitinase ABC as a therapy for spinal cord injury. *Brain Res Bull*, 10 (2011) 306.
- 85 Denholm E, Lin Y & Silver P, Anti-tumor activities of chondroitinase AC and chondroitinase B: inhibition of angiogenesis, proliferation and invasion. *Eur J Pharmacol*, 416 (2001) 213.
- 86 Wong TY, Preston LA & Schiller NL, Alginate lyase: review of major sources and enzyme characteristics, structure-function analysis, biological roles, and applications. *Annu Rev Microbiol*, 54 (2000) 289.
- 87 Alkawash MA, Soothill JS & Schiller NL, Alginate lyase enhances antibiotic killing of mucoid *Pseudomonas aeruginosa* in biofilms. *APMIS*, 114 (2006) 131.
- 88 Lamma JW & Griswold KE, Alginate lyase exhibits catalysis-independent biofilm dispersion and antibiotic synergy. *Antimicrob Agents Chemother*, 57 (2013) 137.
- 89 Aslam S, Francis PG, Rao BHS, Ummar M, Issac JK & Nair RB, A double blind study on the efficacy of local application of hemocoagulase solution in wound healing. *J Contemp Dent Pract*, 14 (2013) 394.
- 90 Shenoy AK, Ramesh KV, Chowta MN, Adhikari PM & Rathnakar UP, Effects of botropase on clotting factors in healthy human volunteers. *Perspect Clin Res*, 5 (2014) 71.
- 91 Shi Y, Zhao J, Tang S, Pan F, Liu L, Tian Z & Li H, Effect of hemocoagulase for prevention of pulmonary hemorrhage in critical newborns on mechanical ventilation: A randomized controlled trial. *Indian Pediatr*, 45 (2008) 199.
- 92 Lodha A, Kamaluddeen M, Akierman A & Amin H, Role of hemocoagulase in pulmonary hemorrhage in preterm infants: a systematic review. *Indian J Pediatr*, 78 (2011) 838.
- 93 Jun-min W, Ming-wei Z, Zhong-tao Z, Zhen-geng J, Xiao-dong H, Yuan-lian W, Shan W, Dian-rong X, Yun T, Jie L, Jing-yong X & Qing-shan Z, A multicenter, phase III trial of hemocoagulase Agkistrodon: hemostasis, coagulation, and safety in patients undergoing abdominal surgery. *Chin Med J*, 123 (2010) 589.
- 94 Brien S, Lewith G, Walker AF, Middleton R, Prescott P & Bundy R, Bromelain as an adjunctive treatment for moderate-to-severe osteoarthritis of the knee: a randomized placebo-controlled pilot study. *Q J Med*, 99 (2006) 841.
- 95 Pillai K, Akhter J, Chua TC & Morris DL, Anticancer property of bromelain with therapeutic potential in malignant peritoneal mesothelioma. *Cancer Invest*, 31 (2013) 241.
- 96 Krieger Y, Rosenberg L, Lapid O, Glesinger R, Bogdanov-Berezovsky A, Silberstein E, Sagi A & Judkins K, Escharotomy using an enzymatic debridement agent for treating experimental burn-induced compartment syndrome in an animal model. *J Trauma*, 58 (2005) 1259.