ENZYMES AS DRUGS: A NOVEL THERAPEUTIC APPROACH

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Summary

Enzymes are biocatalysts that catalyze many reactions in the body. These enzymes are divided in to six groups. Recently enzymes are used to treat a number of diseases. Enzyme replacement can be done in enzyme deficiency disorders like gaucher's disease, fabry disease, pompe disease, mucopolysaccharidosis, Severe combined immunodeficiency, α_1 Antitrypsin deficiency etc. Enzymes like streptokinase, urokinase, alteplase, reteplase etc are used in the treatment of thrombosis. L asparginase can be used as an anticancer agent. Enzymes like hyaluronidase can be used to facilitate the action of other drugs like local anesthetics. Streptodornase can be used to dissolve purulent or fibrinous secretions from infections. In modern drug therapy enzymes have a vital role to play.

Key Words: Enzyme replacement therapy, Thrombolytics, Streptodornase, Hyaluronidase.

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Introduction

Enzymes are biologic polymers that catalyze the chemical reactions that make life.[1] They are also called biocatalysts. A catalyst is defined as a substance that increases the velocity or rate of a chemical reaction without itself undergoing any change in the overall process.[2]

Enzymes are grouped in to six classes -
1. Oxidoreductases - Catalyze oxidation and reductions.
2. Transferases - Catalyze transfer of moieties such as glycosyl, methyl, or phosphoryl groups.
3. Hydrolases – Catalyze hydrolytic cleavage of C-C, C-O, C-N and other bonds.
4. Lyases – Catalyze cleavage of C-C, C-O, C-N and other bonds by atom elimination, leaving double bonds.
5. Isomerases – Catalyze geometric or structural changes within a molecule.
6. Ligases – Catalyze the joining of two molecules coupled to the hydrolysis of ATP.

Enzymes have many therapeutic applications.

Enzymes in replacement therapy

1. Gaucher's disease

Gaucher's disease is the most common lysosomal storage disorder known and results from a genetic deficiency of the enzyme glucocerebrosidase (glucosylceramidase). It is caused by recessive mutation in a gene located on chromosome 1. Enzyme deficiency results in accumulation of glucocerebroside within the reticuloendothelial system. It may present with hepatosplenomegaly, bone marrow suppression, and bone lesions.[3]

The ability to safely and effectively use enzyme therapy to inhibit or reverse visceral-disease progression and involvement has provided impetus for design of new enzyme therapies, and creation of substrate depletion and pharmacological chaperone strategies. Intravenous glucocerebrosidase (Imiglucerase) can administered for type I and most type 3 patients. Therapy can reduce liver and spleen size, reduce skeletal muscle abnormalities, and reverse other manifestations.[4]

2. Fabry disease

It is an inherited disorder which occurs due to deficiency of the lysosomal hydrolase alpha-galactosidase A (αGalA) as a result of mutations in the *Gal* gene at Xq22. The result is intralysosomal accumulation of glycosphingolipids. Patients may have acute attacks of pain lasting for few minutes to days. Stroke, seizures, heart disorders (conduction disturbances, valve disease, and left heart failure) and kidney disorders (proteinuria and chronic renal failure) develop in the third or fourth decade of life.[5]

The recent introduction of enzyme replacement therapy with recombinant agalsidase α or β has been a major breakthrough in the treatment of Fabry disease.[5] Two enzyme replacement therapies are available, Agalsidase alpha (Replagal) and Agalsidase beta (Fabrazyme or Genzyme). Fortnightly infusion of enzyme will prevent disease progression and reverse symptoms.[6]

3. Mucopolysaccharidosis type I (MPS I)

Mucopolysaccharidosis type I (MPS I) is a chronic and progressive, autosomal recessive lysosomal storage disease in which degradation of the glycosaminoglycans (GAGs) dermatan and heparan sulphate is deficient. First described by Hurler in 1919, occurs due to deficiency of α-L-Iduronidase which is responsible for removing terminal iduronic acid residues during the sequential degradation of dermatan and heparan sulphates. The multi-system sequelae result in clinical manifestations that vary between individuals but may include mental retardation, skeletal abnormalities, enlarged liver and spleen, respiratory problems, heart disease and reduced life expectancy.[7] Clinical trials of enzyme-replacement therapy in MPS I have shown clinical benefit in patients with considerable preexisting disease. Therapy can be started as early as 5 months with laronidase(α-L-Iduronidase), this may significantly delay or prevent the onset of the major clinical signs, substantially modifying the natural history of the disease.[8]

4. Mucopolysaccharidosis type II(MPSII)

Mucopolysaccharidosis type II (MPS II; Hunter syndrome) is a rare X-linked recessive disease caused by deficiency of the lysosomal enzyme iduronate-2-sulphatase. It is charectarized by progressive accumulation of glycosaminoglycans in nearly all cell types, tissues and organs. Clinical manifestations include severe airway obstruction, skeletal deformities, cardiomyopathy and, in most patients, neurological decline. Death usually occurs in the second decade of life, although some patients with less severe disease have survived into their fifth or sixth decade.[9]

Iduronate-2-sufatase enzyme replacement therapy is of significant benefit in reducing symptoms of MPSII. Alternatively electro gene transfer for the production and release of recombinant iduronate-2-sulfatase (IDS) can be done.[10]

5. Mucopolysaccharidosis type VI(MPSVI)

It is an autosomal recessive disease, caused by a deficiency in the activity of the lysosomal hydrolase N-acetylgalactosamine 4-sulfatase, or arylsulfatase B (ARSB). Patients with MPS VI usually appear normal at birth, but occasionally may present characteristics such as dolicocephaly, wide forehead and spinal abnormalities. As the disease progresses they may develop facial infiltration, hepatosplenomegaly, growth deficit, joint contractures, cardiovascular involvement, ocular abnormalities (corneal clouding, glaucoma and papilledema with optical atrophy), neurological involvement (hydrocephalus, medullar compression; mental retardation is not a common occurrence), obstructive sleep apnea syndrome and umbilical and inguinal hernias.[11]

Enzyme replacement therapy (ERT) has been shown to clinically benefit affected individuals greater than 6 years of age. Recently it has been showed that early initiation of ERT, as early as 8 weeks can slow or prevent the development of significant pathological changes of MPS VI. Weekly 1 mg/kg recombinant human N-acetylgalactosamine-4-sulphatase (rhASB) can be administered.[12]

6. Glycogen storage disease type II(Pompe disease)

Pompe disease (acid maltase deficiency, glycogen storage disease type II) is a rare progressive autosomal recessive disorder occurs due to the deficit of lysosomal glycogen degradation enzyme acid α-glucosidase (GAA).

In infants, Pompe disease is characterized by prominent hypotonia, muscle weakness, motor delay, feeding problems, and respiratory and cardiac insufficiency.[13] Enzyme replacement therapy has recently become available and has been shown to be effective in prolonging survival and improving respiratory performance. Weekly enzyme infusions over 6 months result in degradation of lysosomal glycogen in the heart and muscle.[14]

7. Cystic fibrosis:

It is an autosomal recessive disorder caused due to mutation in the gene called cystic fibrosis transmembrane conductance regulator(CFTR) resulting in abnormal sodium and Chloride transport in several tissues. Its main clinical manifestations include bronchopulmonary infections along with gastrointestinal disorders like exocrine pancreatic insufficiency and nutritional disorders. Intense and recurrent inflammation ultimately leads to an overabundance of activated neutrophils and macrophages that contribute to free radical generation.[15] EUR-1008 (Zenpep [pancrelipase]) is a new, enteric-coated, porcine-derived pancreatic enzyme product (PEP) developed for the treatment of cystic fibrosis (CF) patients with malabsorption associated with exocrine pancreatic insufficiency (EPI). It significantly improve coefficient of fat absorption and coefficient of nitrogen absorption, and control of malabsorption and clinical symptoms of Cystic fibrosis.[16]

8. Severe combined immunodeficiency (SCID):

Caused due to Adenosine deaminase (ADA) deficiency. It is an inherited disorder of purine metabolism characterized by immunodeficiency, failure to thrive and metabolic abnormalities. Defect is seen in both cellular and humoral immunity. ADA is a predominantly cytoplasmic enzyme found in all tissues of the body where it plays an important role in the recycling of adenosine after DNA breakdown and specifically catalyzes the deamination of deoxyadenosine (dAdo) and adenosine (Ado) to deoxyinosine and inosine respectively. Lack of ADA results in a number of metabolic derangements which ultimately lead to the accumulation of toxic substrates in both intra- and extra-cellular compartments ultimately leading to defect in lymphocyte development and function.[17] Enzyme replacement therapy (ERT) with pegademase bovine (PEG-ADA) was the first pegylated protein approved by the U.S. Food and Drug administration in March 1990. It is used to treat X – linked severe combined immunodeficiency syndrome, as an alternative to bone marrow transplantation and enzyme replacement by gene therapy.[18]

9. α_1 Antitrypsin deficiency

α_1 Antitrypsin is a proteolytic enzyme that hydrolyze and destroy proteins. It has a very vital function in lungs. In normal lung the alveoli are chronically exposed to low level of neutrophil elastase released from activated and degenerating neutrophils. This proteolytic activity can destroy the elastin in the alveolar walls if unopposed by the inhibitory action of α_1 Antitrypsin. Deficiency of α_1 Antitrypsin results in emphysema. The deficiency can be reversed by weekly intravenous administration of α_1 Antitrypsin.[19]

Enzymes in therapy of thrombosis

The fibrinolytic system dissolves intravascular clots as a result of the action of plasmin, an enzyme that digests fibrin, thus inhibits pathological progression of clot during hemostasis.

Plasminogen, an inactive precursor, is converted to plasmin by cleavage of a single peptide bond. Plasmin is a relatively nonspecific protease; it digests fibrin clots and other plasma proteins, including several coagulation factors. Therapy with thrombolytic agents enhance conversion of plasminogen to plasmin which dissolve both pathological thrombi and fibrin deposits at sites of vascular injury. Therefore, the drugs are toxic, producing hemorrhage as a major side effect.[20]

1. Streptoknase

Was discovered in 1933 when it was found that filtrates of broth cultures of certain strains of Streptococcus bacteria (beta-hemolytic streptococci) could dissolve a fibrin clot. It combines with the proactivator plasminogen. This enzymatic complex catalyses the conversion of inactive plasminogen to active plasmin. Streptokinase was initially used in combating fibrinous pleural exudates, hemothorax, and tuberculous meningitis. In 1958, streptokinase was first used in patients with acute myocardial infarction.[21]

2. Urokinase

Fibrinolytic potential of human urine was first described in 1947. The active molecule was named urokinase. It is a human enzyme synthesized by the kidney. Unlike streptokinase, urokinase is not antigenic and directly activates plasminogen to form plasmin.[22]

3. Antistreplase

Consists of a complex of purified human plasminogen and bacterial streptokinase that has been acylated to protect the enzyme's active site. When administered acyl group hydrolyses, freeing the activated streptokinase-proctivator complex. It is having more clot selectivity than streptokinase alone.[23]

4. Alteplase

Alteplase (tPA, Activase) was the first recombinant tissue-type plasminogen activator and is identical to native tissue plasminogen activator. In vivo, tissue-type plasminogen activator is synthesized and made available by cells of the vascular endothelium. It is the physiologic thrombolytic agent responsible for most of the body's natural efforts to prevent excessive thrombus propagation.[24] Alteplase is FDA approved for treatment of ST-elevation myocardial infarction (STEMI), acute ischemic stroke (AIS), acute massive pulmonary embolism, and central venous access devices (CVAD).[24]

5. Reteplase

Reteplase (r-PA, Retavase) is a second-generation recombinant tissue-type plasminogen activator that work more rapidly and to have a lower bleeding risk than the first-generation agent alteplase. Reteplase does not bind fibrin as tightly as native tissue plasminogen activator, allowing the drug to diffuse more freely through the clot rather than binding only to the surface the way tissue plasminogen activator does. It is less expensive because it lacks fibrin binding domain. It is less fibrin specific than tPA.[25]

6. Tenecteplase

Tenecteplase(TNKase) was approved by the FDA as a fibrinolytic agent in 2000. This drug has a similar mechanism of action as alteplase (tPA). It is the latest thrombolytic agent approved for use in clinical practice. TNKase is currently indicated for the management of acute myocardial infarction (AMI).It is having longer half life and can be given as intravenous bolus. It is slightly more fibrin specific than tPA.[25]

Enzymes in Cancer Therapy.

L asparginase

L-aspargine is required for protein synthesis. While most normal tissues are able to synthesize L-aspargine in amounts sufficient for protein synthesis, some types of lymphoid malignancies derive the required amino acid from plasma. Asparginase is an enzyme that catalyzes the hydrolysis of aspargine to aspartic acid, deprives malignant cells of the aspargine necessary for protein synthesis, leading to cell death.[26] Pegasparginase(oncaspar) is a preparation in which enzyme is conjugated with monomethoxy polyethylene glycol. It is having longer half-life and reduced immunogenicity.[20] Pegasparginase is currently used in the treatment of acute lymphoblastic leukemia, it is also found to be useful in chronic lymphocytic leukemia, non-Hodgkin's lymphoma, Hodgkin's lymphoma, multiple myeloma and plasma cell leukemia.[18]

Miscellaneous Enzymes

1. Streptodornase

Is an enzyme (DNAase) produced by hemolytic streptococci that is used medically, often in combination with streptokinase, to dissolve purulent or fibrinous secretions from infections.[27]

2. Hyaluronidase

Hyaluronidase is an enzyme that depolymerizes hyaluronate which forms a protective barrier in the tissues. Hyaluronidase prepared from mammalian testis can be used therapeutically to enhance dispersion of drugs like local anaesthetics in various parts of the body.[28]

Conclusion

Therapeutic utility of enzymes as drugs is increasing day by day. Enzymes are substrate specific and have a relatively lower side effect profile. Further research is needed in this field so that more number of enzymes can be used in modern therapeutics for better patient care.

References

1. Robert K, Danyl K, Victor W. Harper's illustrated biochemistry. 27th edition. Boston: McGraw Hill Publishers; 2006.p.49-59

2. SatyanarayanaU, Chakrapani U. Biochemistry. 3rd edition. Vijayawada: Uppala publishers; 2007. p.84-85

3. Morales LE. Gaucher's disease: a review. *The Annals of Pharmacotherapy*. 1996; 30(4): 381-388.

4. Gregory A Grabowski. Phenotype, diagnosis, and treatment of Gaucher's disease. The Lancet. 2008; 372(9645): 1263-1271

5. Charles Masson, Idrissa Cissé, Virginie Simon. Fabry disease: A review. Joint Bone Spine. 2004; 71(5): 381-383

6. Karen JL, Hale EK, Ma L. Angiokeratoma corporis diffusum (Fabry disease). Dematology online journal. 2005; 11(4): 8.

7. David Moore, Martin J Connock, Wraith Ed. The prevalence of and survival in Mucopolysaccharidosis I: Hurler, Hurler-Scheie and Scheie syndromes in the UK. Orphanet J Rare Dis. 2008; 3: 24.

8. Gabrielli O, Clarke LA, Bruni S, Coppa GV. Enzyme-replacement therapy in a 5-month-old boy with attenuated presymptomatic MPS I: 5-year follow-up. Pediatrics. 2010 ;125(1): 183-187.

9. Edmond Wraith, Maurizio Scarpa, Michael Beck. Mucopolysaccharidosis type II (Hunter syndrome):a clinical review and recommendations for treatment in the era of enzyme replacement therapy. Eur J Pediatr. 2008; 167: 267–277

10. Friso A, Tomanin R, Zanetti A. Gene therapy of Hunter syndrome: evaluation of the efficiency of muscle electro gene transfer for the production and release of recombinant iduronate-2-sulfatase (IDS). Biochimica et biophysica acta. 2008; 1782(10): 574-80.

11. Antonio Cardoso Santos, Ana Azevedo, Simone Fagondes. Mucopolysaccharidosis type VI (Maroteaux-Lamy syndrome): assessment of joint mobility and grip and pinch strength **.** Journal de Pediatria. 2008; 84(2): 131

12. McGill JJ, Inwood AC, Coman DJ. Enzyme replacement therapy for mucopolysaccharidosis VI from 8 weeks of age - a sibling control study. Clinical Genetics. 2009: 1-7

13. Shin YS. Glycogen storage disease: clinical, biochemical, and molecular heterogeneity. Seminars in pediatric neurology. 2006; 13(2): 115-20

14. But WM, Lee SH, Chan AO. Enzyme replacement therapy for infantile Pompe disease during the critical period and identification of a novel mutation. Hong Kong Medical Journal. 2009; 15(6): 474-7.

15. O'Sullivan BP, Freedman SD. Cystic fibrosis. The Lancet. 2009; 373(9678): 1891-904

16. Wooldridge Jl, Heubi JE, Amaro-Galvez R. EUR-1008 pancreatic enzyme replacement is safe and effective in patients with cystic fibrosis and pancreatic insufficiency. Journal of cystic fibrosis. 2009; 8(6): 405-17.

17. Booth C, Gasper HB. Pegademase bovine (PEG-ADA) for the treatment of infants and children with severe combined immunodeficiency (SCID). Biologics: targets & therapy. 2009; 3: 349-58.

18. Ravishankar AC, Hiremath SV. Pegylated Protein Drugs: A Promising New Approach For Drug Delivery. Pharmacologyonline. 2009; 3: 821-826

19. Richard A Harvey, Pamela C Champe. Lippincotts illustrated reviews: Biochemistry. 3rd edition. Philadelphia: Lippincott Williams & Wilkins; 2005.p.49-50

20. Laurence L Bruton, John S Lazo, Keith L Parker. Goodman & Gilman's The Pharmacological Basis of Therapeutics. 11th edition. New York: Mc Graw Hill Publishers; 2006.p.1470

21. Sikri N, Bardia A. A history of streptokinase use in acute myocardial infarction. Tex Heart Inst J. 2007; 34(3): 318-27.

22. Ouriel K. A history of thrombolytic therapy. J Endovasc Ther. 2004; 11(2): 128-133.

23. Richard A. Harvey, Pamela C. Champe, Richard Finkel. Lippincotts illustrated reviews: Pharmacology. 4th edition. Philadelphia: Lippincott Williams & Wilkins; 2008.p.244

24. Fauci, Braunwald, Kasper, Hauser, Longo, Jameson. Harrison's Principles of Internal Medicine. 17th edition. New York: Mc Graw Hill Publishers; 2008.p.746

25. Bertram G. Katzung, Susan B. Masters, Antony J. Trevor. Basic & Clinical Pharmacology. 11th edition. New Delhi: Tata Mc Graw Hill Publishers; 2009.p.597-598

26. Appel IM, van Kessel-Bakvis C, Stigter R, Pieters R. Influence of two different regimens of concomitant treatment with asparaginase and dexamethasone on hemostasis in childhood acute lymphoblastic leukemi. Leukemia. 2007; 21: 2377.

27. Vasudevan DM, Sreekumari S. Textbook of Biochemistry. 4th edition. New Delhi: Jaypee Publishers; 2004.p.59-60

28. Bhagavan NV. Medical Biochemistry. 4th edition. New Delhi: Academic press; 2002.p.183