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CATALYST AND ENZYMES IN ORGANIC CHEMISTRY AND ITS USES IN FORMULATION OF DRUGS

Upasana Bhatiya, Udit Narayan Rawat, Utkarsh Tiwari, Varun Kumar Jain,

Prateek Jain, Imran Khan*

Adina Institute of Pharmaceutical Sciences, Sagar (M.P.)

*Corresponding Author's E mail: imrankhanaips@gmail.com

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ABSTRACT
Enzymes are biological systems' chemical catalysts. They enable organisms to self-replicate and catalyse key metabolic events in a selective and efficient manner. Enzymes are extremely effective and selective biocatalysts found in living creatures, with the exception of ribozymes, which are a tiny group of RNA molecules exhibiting catalytic activity. They come in a wide range of types of processes that they can catalyse, including oxidation–reduction, group transfers inside and between molecules, hydrolysis, isomerization, ligation, bond cleavage, and bond creation. Enzyme-based catalyses also have a higher degree of fidelity, are performed under mild reaction conditions, and are very efficient in terms of number of steps, providing them an advantage over their chemical counterparts. Treatments based on enzyme catalytic activity can convert a wide range of target compounds, allowing for the restoration of normal physiological metabolism. Because of their affinity and specificity qualities, these medicines have significant advantages over traditional therapeutic techniques.

Keywords: Enzymes, Treatments, Formulation, Application.

INTRODUCTION

The use of enzyme technology in pharmaceutical research, development, and manufacturing is a rapidly increasing topic that has spawned several publications, reviews, and books. The scope of this review will be limited to current studies on the use of enzymes as medicines.

The therapeutic enzyme concept has been around for at least 40 years. De Duve, for example, described a therapeutic enzyme as part of replacement therapy for genetic deficiencies in the 1960s [1].

The Food and drug Administration (FDA) approved the first recombinant enzyme drug, Activase1 (alteplase; recombinant human tissue plasminogen activator), in 1987. This 'clot-buster' enzyme is used to treat heart attacks that are caused by a clot blocking a coronary artery. This was the second recombinant protein medicine to hit the market (the first was insulin, which was released in 1982). The FDA has since approved a number of additional enzymes that are used as anticoagulant or coagulant medications.

Adagen1, a polyethylene glycol (PEG)-treated version of bovine adenosine deaminase (ADA), was licenced in 1990 to treat individuals with a kind of severe combined immunodeficiency illness (SCID) caused by chronic ADA deficiency. Adagen1 (pegadamase bovine) is notable for being the first therapeutic enzyme authorised by the FDA under the Orphan Drug Act. In the United States, the Orphan Drug Act was created in 1983 to encourage pharmaceutical companies to find therapies for diseases that affect only a small number of individuals (less than 200000). Drugs granted Orphan Drug classification receive seven years of market exclusivity, among many other provisions and incentives. Similar legislation exists in Europe and Australia, providing similar protection and incentives.

The approval of Activase1 (alteplase) and Adagen1 (pegadamase bovine) signalled the start of a new era for enzymes, elevating them from a "holistic" supplement to a "therapeutic" medicine. Because therapeutic enzymes' biological impact is dependent on catalysis, a feature that increases potency, they are currently used to treat a wide range of diseases and ailments.

Enzyme therapy

The treatment of SCID with Adagen® (pegadamase bovine) is the first effective application of an enzyme therapy for an inherited disease [2]. The enzyme ADA cleaves the excess adenosine in these individuals' blood, reducing the toxicity of the increased adenosine levels on the immune system. The efficacy of the treatment [3] is contingent on the PEG modification of ADA. PEG increases the enzyme's half-life (which was previously less than 30 minutes) and minimises the risk of immunological responses caused by the drug's bovine origin see[4] and [5] for reviews on PEGylation).

In a related study, Bax et al. [6] discovered that effective trapping of native ADA in carrier erythrocytes boosts the enzyme's half-life significantly. The first enzyme replacement therapy in which an exogenous enzyme was directed to the correct bodily compartment. Brady and colleagues[7] pioneered the use of modified placental glucocerebrosidase to replace lost glucocerebrosidase in Gaucher patients (Ceredase1). After that, recombinant DNA technology enabled for the more efficient manufacturing of Ceredase1 (imiglucerase), a glucocerobrosidase that was approved in 1994.

This medical and commercial success has paved the door for the development of new enzyme treatments, particularly for other LSDs. Fabry's disease, a fat (glycolipid) storage condition caused by a deficiency ina-galactosidase, is another LSD that has piqued pharmaceutical companies' interest. Renal failure, discomfort, and corneal clouding are all symptoms of the condition, which predominantly affects the vasculature. After completing Phase III clinical trials, two businesses are seeking FDA approval (and exclusivity under the orphan medication classification) (reviewed in [8] see also Update). One business has a recombinant a-galactosidase expressed in Chinese hamster ovary cells [9], whereas the other has it expressed in human cells.

The FDA recognised a third a-galactosidase product, expressed in plants, as an orphan medicine in early 2003.

At least three mucopolysaccharide (MPS) storage disorders (a subtype of LSD) are now being studied using enzyme replacement therapy [10-11]. Aldurazyme1 (laronidase), an enzyme replacement treatment for MPS I, recently completed a Phase III clinical trial [12] and is seeking approval in the United States and Europe (see Update). A-Liduronidase deficiency is a feature of this LSD. Aryplase TM (recombinant human N-acetylgalactosamine-4sulfatase), an enzyme replacement therapy for MaroteauxLamy syndrome (MPSVI), recently completed a Phase II clinical trial. The therapy was evaluated for safety, efficacy, and possible study endpoints. Finally, in 2002, a Phase I/II clinical trial for an enzyme replacement therapy for Hunter's disease (MPS II), an LSD caused by an iduronate 2-sulfatase deficiency, was completed. The results reveal that urine glycosaminoglycan (GAG) levels are reduced in a dose-dependent manner.

A defect in a-glucosidase causes Pompe's disease, also known as glycogen storage disorder type II (GSDII), which is the topic of multiple therapeutic trials. Pompe's illness is largely a muscular disease. Preliminary results employing recombinant enzymes[13-14] have been published and are promising. In order to treat this terrible condition successfully, high doses are required, and immunological reactions in certain patients may interfere with therapy. The first muscle illness to be treated with enzyme replacement therapy could be Pompe's disease [15].

Oral and inhalable enzyme therapies

In contrast to the treatments discussed thus far, some conditions do not necessitate the intravenous injection of a human enzyme. Several disorders have responded to oral enzyme compositions, updating the age-old use of enzymes as digestive aids. Sacrosidase (Table 1), a b-fructo furanoside fructohydrolase from Saccharomyces cerevisiae that can be administered orally, can be used to treat

congenital sucrase-isomaltase deficiency (CSID). Patients with CSID are unable to consume sucrose as a disaccharide. The medicine hydrolyzes sucrose, allowing patients to eat a more normal diet. It is especially beneficial for young patients who have difficulty adhering to a sucrose-free, low-starch diet[16]. Another hereditary condition that necessitates rigorous adherence to a particular diet is phenylketonuria (PKU). Low or absent phenylalanine hydroxylase activity, which catalyses the conversion of phenylalanine to tyrosine, is the cause of PKU. PhenylaseTM, an oral therapy based on recombinant yeast phenylalanine ammonia lyase, is being developed (PAL). In the gastrointestinal system, PAL has been found to breakdown phenylalanine [17].

A combination of pancreatic enzymes, comprising lipases, proteases, and amylases, has been found to be effective in the treatment of fat malabsorption in HIV patients [18]. Pancreatic insufficiency, which affects the majority of cystic fibrosis (CF) patients, is similarly treated with these enzymes [19]. Surprisingly, the lipases created for this purpose are derived from transgenic corn. Another pancreatic enzyme mixture, TheraCLEC TotalTM (lipase, amylase, and protease mix), was classified as an orphan medicine in 2002 and uses enzyme crystallisation and crosslinking processes for its preparation (Table 2). Lower doses and higher efficacy are possible with this new formulation.

Enzyme therapies could be used to treat a variety of other ailments that are predominantly digestive in nature. Oral peptidase supplement therapy, for example, could be used to treat Celiac Sprue, often known as Celiac disease, a common small intestinal ailment characterised by an immunological reaction to the gliadin protein found in wheat products [20].

The use of inhalable enzyme compositions in the treatment of CF has been discovered. Pulmozyme1 (Dornasea), a DNase, was one of the first orphan drugs to be approved by the FDA. Dornase a liquefies mucus that has collected in the lungs [21]. Dornase a can reduce pulmonary tissue deterioration in CF patients by reducing the quantity of matrix metalloproteinases in the bronchoalveolar lavage fluid [22].

Proteolytic and glycolytic enzymes for treating damaged tissue

Previously, a variety of proteolytic enzymes from plants and microorganisms were investigated as a potential substitute for mechanical debridement (removal of dead skin) of burns. Regrettably, the results have been inconsistent, possibly due to the poor quality of the enzymes utilised [23-24].

Clinical trials are currently underway for a number of products of higher quality and purity, some of which are recombinant in origin. Debrase gel dressing, which is made up of enzymes isolated from pineapple, was approved by the US Food and Drug Administration in 2002 for a Phase II clinical trial

to treat partial-thickness and full-thickness burns. In Europe, this medication was also designated as an orphan medicine. The proteolytic enzyme Vibrilase TM (recombinant vibriolysin) from the marine bacterium Vibrio proteolyticus has been demonstrated to be effective against denatured proteins such as those seen in burned skin. A Phase I clinical research to assess the safety and tolerability of this topically applied enzyme for burn debridement was just launched (http://www.bmrn.com). A recent trial with the collagenase clostridiopeptidase in children with partial-thickness burns yielded some promising findings [25].

Chondroitinases may be utilised to treat spinal injuries, as they have been shown to aid in the regeneration of the injured spinal cord. The enzyme works by eliminating accumulated chondroitin sulphate in the glial scar, which slows axon development [26]. On chondroitin sulphate, hyaluronidase displays a comparable hydrolytic activity and may aid in the regeneration of injured nerve tissue [27].

Enzymes for the treatment of infectious diseases

Because of its capacity to break carbohydrate chains in bacteria's cell walls, lysozyme has been exploited as a naturally occurring antibacterial agent in many foods and consumer products. RNase A and urinary RNase U, which selectively digest viral RNA [28], have also been demonstrated to have activity against HIV, offering up some interesting possibilities for the treatment of HIV infection. Chitinases are another naturally occurring antibacterial agent. Chitin is a good target for antimicrobials since it is found in the cell walls of a variety of pathogenic species, including fungi, protozoa, and helminths [29]. bacteriophage-derived lytic enzymes have been used to attack the cell walls of Streptococcus pneumoniae, Bacillus anthracis, and Clostridium perfringens [30-32]. The use of lytic bacteriophages as a therapeutic for infections is also being researched, and it may be effective in the fight against new drug-resistant bacterial strains.

Enzymes for the treatment of cancer

Enzyme therapies have been used successfully in cancer research in the past. PEGylated arginine deaminase, an arginine-degrading enzyme, has been found in recent research to inhibit human melanoma and hepatocellular carcinomas, which are auxotrophic for arginine due to a lack of argino succinate synthetase activity [33].

Another PEGylated enzyme, Oncaspar1 (pegaspargase), has recently proved to be more effective than native, bacterial asparaginase in the treatment of children with newly diagnosed standard-risk acute lymphoblastic leukaemia [34]. Normal cells can synthesis asparagine, but cancer cells cannot and will

perish if this asparaginede grading enzyme is present. Despite the fact that PEG-asparaginase has a higher pharmacy cost, the overall cost of the treatment is relatively close to that of the native enzyme [35]. Asparaginase and PEG-asparaginase are efficient chemotherapy supplements.

Proliferation is another feature of the oncogenesis process. Chondroitinase AC and, to a lesser extent, chondroitinase B have been found to limit tumour growth, neovascularization, and metastasis by removing chondroitin sulphate proteoglycans [36-38].

Antibody-directed enzyme prodrug treatment (ADEPT) is an example of another way enzymes can be used as cancer therapeutics. A monoclonal antibody transports an enzyme to cancer cells, where it activates a prodrug, killing cancer cells but not normal cells [39-40]. A class of cancer therapies based on tumor-targeted enzymes that activate prodrugs is being discovered and developed using this method. This initiative will also make use of the targeted enzyme prodrug treatment (TEPT) platform, which involves enzymes with antibody-like targeting domains [41].

Hyperuricemia, a build-up of uric acid that causes gouty arthritis and chronic renal failure, is one of the side effects of cancer chemotherapy. Urate oxidase is capable of degrading uric acid, which is poorly soluble. Intriguingly, humans have the gene for this enzyme, but it has a nonsense codon. The FDA has recently given orphan drug classification to five medications that use this enzyme (Tables 1 and 2). Rasburicase recombinant is a safe and effective uricolytic drug [42-43], and the PEGylated version of the enzyme reduces immunogenicity while increasing half-life [44].

Prospective therapeutic enzymes

The most essential detoxifying enzyme in cells, superoxide dismutase, converts the highly toxic superoxide anion to the moderately harmful hydrogen peroxide. Even in its PEGylated form, this enzyme, which has been of interest to the pharmaceutical sector for some time, has never lived up to its promise [45]. Catalase, another anti-oxidant that transforms hydrogen peroxide to water and oxygen, works in the same way. Surprisingly, these enzymes have been demonstrated to extend the life of Caenorhabditis elegans, a nematode, and this effect could be transferred to mammals [46]. In hemorrhagic shock, future versions of these enzymes may aid to minimise organ harm [47].

Acetylcholine is broken down by human butyrilcholinesterase, a naturally occurring serum detoxifying enzyme. Recent findings [48] suggest that it could be effective in the treatment of cocaine overdose. The enzyme's activity toward cocaine has increased as a result of structure-based re-engineering [49]. Directed

evolution has also resulted in even more efficient butyrilcholinesterase optimization [50], and directed evolution promises to be the most potent method yet in the development of enzyme medicines [51].

CONCLUSION

To summarise, enzyme therapy is a new treatment option for a variety of diseases, including metabolic disorders. Liposomes, membrane vesicles, nanoparticles, and erythrocytes are examples of enzyme encapsulation techniques that improve in vivo half-life, tissue selectivity, and minimise immunogenicity. The use of targeted enzyme modification techniques, such as PEG conjugation, improves functional bioavailability while lowering immunogenicity. Finally, monitoring patients' immune responses may help to improve patient care and preserve therapy efficacy and safety.

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