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L-asparaginase: Need for an Expedition from an Enzymatic Molecule to Antimicrobial Drug

Archana Vimal[1] · **Awanish Kumar**[2]

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Abstract
Enzymes play a vital role in the biological system as a catalyst for biochemical reactions. They have a wide range of commercial applications in many areas like the pharmaceutical industry, food industry, etc. L-asparaginase is one of the commercial enzymes with prime importance in anticancer therapy. It is mainly used in chemotherapy; however, it has the potential to cure autoimmune disorders and infectious diseases also. Previous studies reported the antimicrobial potential of L-asparaginase. Therefore, we have discussed the possibility and challenges of the antimicrobial application of L-asparaginase in the treatment of infectious diseases. This is followed by a discussion on the effective delivery of this enzyme using biopolymeric nanocarriers that ensure safe and on target action. The present article gives a perspective on the L-asparaginase molecule that could be developed/established as an approved antimicrobial drug in the future.

Keywords L-asparaginase · Experimental molecule · Antimicrobial potential · Biopolymeric nano-delivery · Futuristic antimicrobial drug

Introduction

Infectious diseases are caused by a large no. of pathogens/infectious agents (bacteria, fungi, helminths, protozoan parasites, and viruses). The emergence of various infections is a global problem and it causes a large no. of morbidity and mortality every year (Institute of Medicine (US) Forum on Microbial Threats 2009). Very few drugs are available against infectious disease because pharmaceutical companies have limited interest to develop a drug against infections. Anti-infectious agents are short-course drugs and are not as profitable in comparison to those drugs that are used to treat lifestyle diseases such as Alzheimer's, diabetes, high blood pressure, etc. Therefore, poor financial return to drug companies is associated with antimicrobials as compared to the drugs of lifestyle diseases (Purssell 2019). Another fact is the path of drug discovery is also very long and the associated cost burden is over a billion-dollar from initial investigation to clinical trials and final approval. In this scenario, drug repurposing is a fascinating idea that may save time and could short-circuit the process of new antimicrobial agent discovery.

L-asparaginase is an effective line of therapy and has been used since a long time ago for the treatment of acute lymphoblastic leukemia and lymphoma and this enzyme is isolated from natural resources (actinomycetes, bacteria, fungi, etc.) (Vimal and Kumar 2017). We have noticed an interesting fact from the literature that L-asparaginase was explored as an anti-infectious agent also (Vimal and Kumar 2021; Abd El-Baky and El-Baroty 2020; Meganathan 2016). Therefore, we have tried to provide an outlook for the further development of biopolymer-based L-asparaginase nanoformulation as an emerging and alternative therapy against microbial infections. We have discussed some challenges associated with the treatment of infectious diseases, prospects of L-asparaginase as a promising antimicrobial agent, and its effective delivery using biopolymeric nano-carriers to understand the need for an expedition of L-asparaginase molecule to be explored as an antimicrobial drug.

✉ Awanish Kumar
drawanishkr@gmail.com; awanik.bt@nitrr.ac.in

[1] Department of Bioengineering, Integral University, Lucknow, UP, India

[2] Department of Biotechnology, National Institute of Technology, Raipur, Chhattisgarh 492010, India

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Challenges in the Treatment of Infectious Diseases

Some fatal infectious diseases like SARS, AIDS, TB, etc. have emerged in the world and along with them some other threatening infections (caused by bacteria, virus, fungi, and other parasites) exist in our society that causes various infectious diseases in humans. These have led to major changes in public health activities, clinical practices, and biomedical research (Institute of Medicine (US) Forum on Microbial Threats 2009). To treat infectious diseases successfully, effective antimicrobial agents are needed. Antimicrobial agents belong to different molecular classes and suppress or kill the growth of microorganisms (bacteria, fungi, viruses, etc.). Big challenges are associated with the development of satisfactory agent/molecule for the treatment of infectious disease due to a lot of issues related to antimicrobial agents (complex structure, difficult validation processes, unspecified dose, low bioavailability, etc.). Poor membrane transport of the active molecule to the intracellular site of an infectious agent, presence of active efflux pump that expels the antimicrobials from microbes if it penetrates inside of the microbial cell, drug resistance development by microbes against particular antimicrobials, inactivating the drug by microbial enzymes (antimicrobial degrading enzymes), alternate functional pathways development by microbes to overcome the effect, toxicity caused by antimicrobials on healthy tissues of the host, rapid clearance of drugs from host tissue, etc. stands a great challenge to treat infectious diseases (Purssell 2019). Combination treatment using different anti-infectious agents belonging to different categories with different mechanisms of action is a good approach to combat infectious diseases. Further, pharmaceutical nanotechnology is emerged to resolve the challenge of delivering drugs effectively via on-site targeting. Multidisciplinary studies should be done by scientists to overcome the challenges and the development of effective antimicrobial agents.

L-asparaginase as a Promising Antimicrobial Agent

L-asparaginase converts L-asparagine to L-aspartic acid and ammonia in the cell. Its anticancerous potential has been well established, therefore, it is widely used as a chemotherapeutic agent. Attention is increasing to explore the potential of this enzyme as an antimicrobial agent in recent years. Some previous research work described the antimicrobial efficacy of L-asparaginase and advocated for its future application in infection control. Meganathan has reported the antimicrobial potential of L-asparaginase (Meganathan 2016). Antibacterial and antifungal activity of L-asparaginase was also reported by Raj and Sathiyamurthy (2016) and they have anticipated its significance for therapeutic application against pathogenic microbes. We have identified higher L-asparaginase producer strains (Vimal and Kumar 2017) and deciphered the antimicrobial potency of L-asparaginase against a wide range of gram-positive/gram-negative bacteria (*Klebsiella pneumoniae, Listeria monocytogenes, Proteus vulgaris, Pseudomonas aeruginosa, Staphylococcus aureus, Salmonella typhimurium*). The antimicrobial property of L-asparaginase was further enhanced by encapsulating this enzyme inside the biopolymeric chitosan nanoparticle for its effective delivery (Vimal and Kumar 2021). In the direction of enhancement of the antimicrobial property, half-life and serum stability of a therapeutic molecule is important parameters. The half-life and serum stability of L-asparaginase could be possible and prolonged by ascertaining the certain modification in this enzyme. N-Bromosuccinimide-modified L-asparaginase has shown greater stability and prolonged half-life (144 h) in vitro to proteolytic digestion as compared to unmodified L-asparaginase enzyme (93 h) (Mohan Kumar et al. 2014). Recently antiviral activity of this enzyme was shown against Coxsackie B3 Virus (Abd El-Baky and El-Baroty et al. 2020). They have advocated the antiviral capability of L-asparaginase via inhibiting attachment, blocking the adsorption, and penetrating viral replication. These data support the scientific rationale of its further exploration as a futuristic antimicrobial agent.

These studies showed that antimicrobial potential is associated with L-asparaginase that motivates a researcher to further do in-depth investigation to establish it as an antimicrobial drug. We know that path of drug discovery and development is very long and passes through many steps of experimental validation. Above discussed studies indicated L-asparaginase as a lead antimicrobial molecule which considers it as an antimicrobial innovative candidate and now the process of its development as an antimicrobial drug (Fig. 1) should be speed-up. Further preclinical research (In vitro, In vivo, and Ex vivo assays) is needed to understand its bioavailability, best dosage, adverse effect on different race/ethnicity groups or gender, interaction with other treatments, effectiveness compared to similar drugs, etc. The subsequent section of this article discusses its effective delivery and enhanced bioavailability. The next step is clinical development with different phases of drug trials which is followed by filling for Investigational New Drug (IND)/New Drug Application (NDA) application to regulatory authorities [Food and Drug Administration (FDA), USA; Medicines and Healthcare Products Regulatory Agency (MHRA), UK; Therapeutic Goods Administration (TGA), Australia; Central Drug Standard

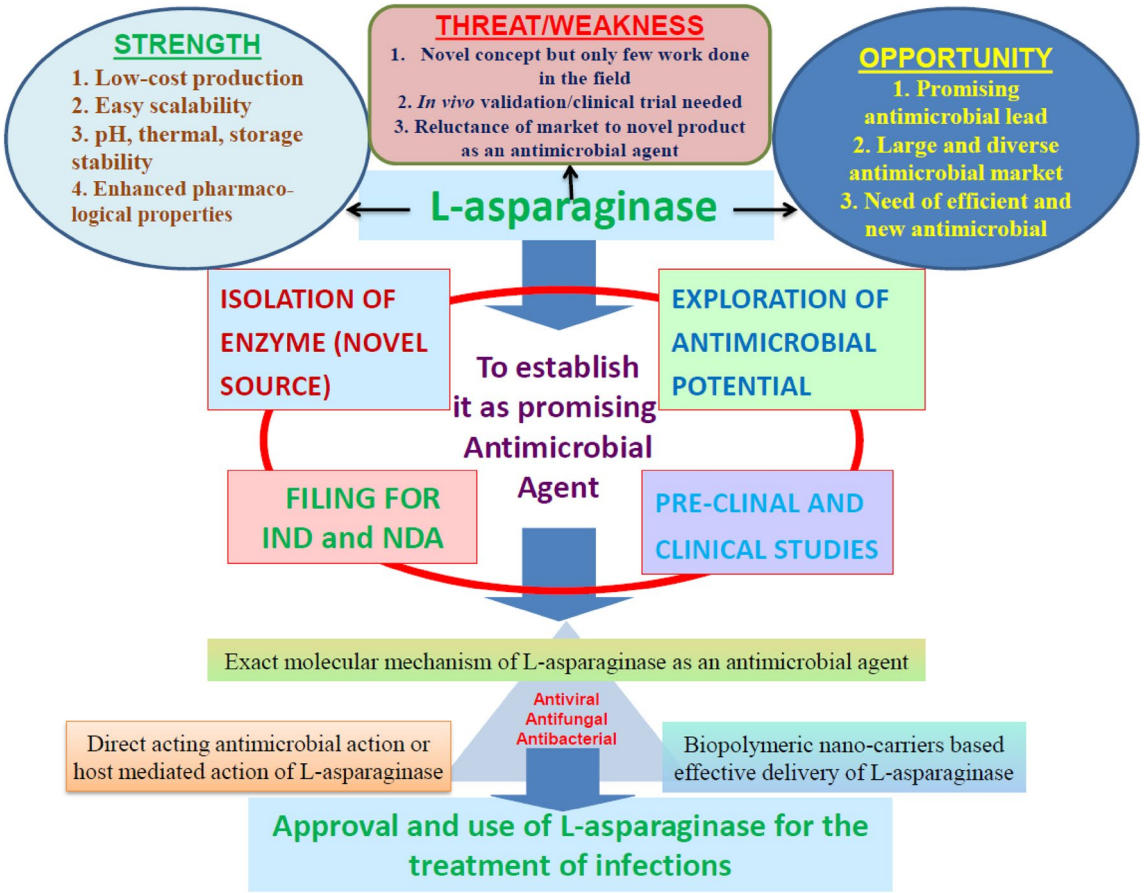

Fig. 1 Strength, opportunity, weakness, processes, and possible rationale in the development of L-asparaginase as a futuristic antimicrobial drug

Control Organization (CDSCO), India, etc.] associated with final approval for marketing of drug and post-market safety monitoring. Certain weaknesses and threats are associated with L-asparaginase to develop it as an antimicrobial drug, but many strengths and opportunities are also associated with it that further urge to develop it as successful antimicrobial medicine in the future (Fig. 1).

Although the exact mechanism of L-asparaginase as an anti-infective agent is not clear we discuss a possible mechanism of L-asparaginase. Penicillin-binding proteins (PBP) are the enzymes that mediate bacterial cell wall synthesis through peptidoglycan biosynthesis. The in silico study suggests that L-asparaginase is interacting with PBP (Vimal and Kumar 2021). It is presumed that the bactericidal action of this amidohydrolase enzyme is because it defaces the bacterial cell wall (Fig. 2). It is similar to another antimicrobial enzyme lysozyme that hydrolyzes the glycosidic bond linking N-acetylmuramic acid (NAM) and N-acetylglucosamine (NAG) present in the cell wall of bacteria. It disturbs the bacterial cell wall integrity leading to cell death (Vermassen et al. 2019).

Biopolymeric Nano-Carriers for Effective Delivery of L-Asparaginase

Many drugs have problems of low selectivity, water insolubility, poor stability, high toxicity, and some other side effects. Good drug carriers play a significant role in resolving these problems. Biopolymer (alginate, chitosan, collagen, hyaluronic acid, silk proteins, etc.) base nanoparticles are proficient drug carriers with wide development potential and have the advantage of slow or controlled release of drug at the site of the action. These biopolymeric nanocarriers are reported to exhibit excellent in vivo stability/tunability in size, charge, and drug release properties. They are preferred as a good carrier for the delivery of antimicrobial agents (Idrees et al. 2020). Modifications of biopolymers are also possible without changing their fundamental skeleton. This acquires new improved properties like enhanced microbial cell permeation, specific organ targeting, enhanced solubility in comparison to mother molecules, and offers an excellent drug delivery system.

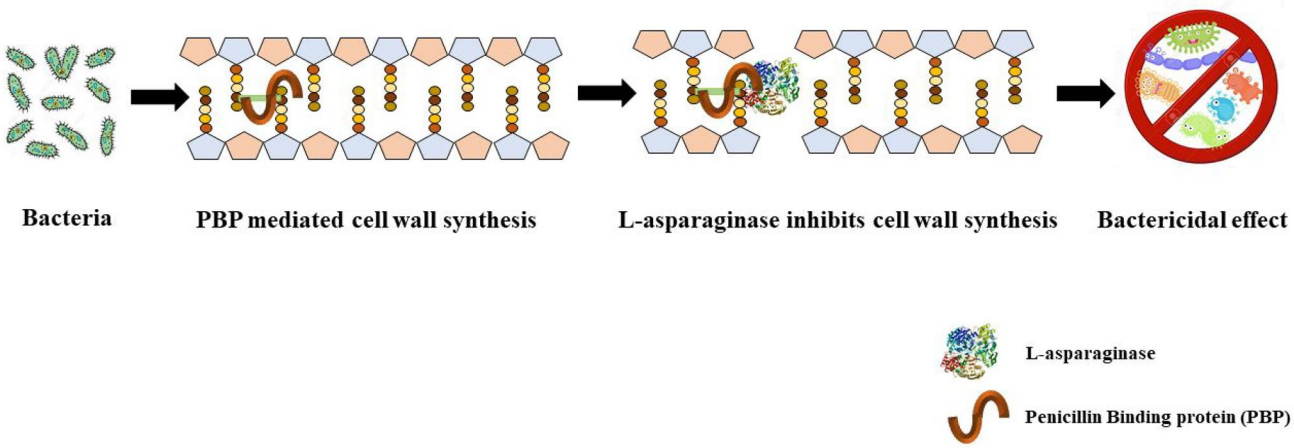

Fig. 2 Suggested action mechanism of L-asparaginase on bacterial cell wall disruption

In recent years biopolymeric nanoparticles emerge as a potential drug carrier due to their advantageous properties [like excellent mobility (due to small size) biocompatibility, non-toxicity, biodegradability, and a functional group that can be conveniently modified as per the need] that they offer over conventional drug carriers. It also enhances the absorption and bioavailability of the immobilized drug as well as provides prolonged systemic circulation. It can be used for loading a wide variety of drugs including gene drugs, protein drugs, and chemical drugs (Patra et al. 2018). Biopolymeric nanoparticles can be synthesized through various approaches successfully through coacervation method, coprecipitation method, emulsification solvent diffusion method, an emulsion-based solvent evaporation method, ionotropic gelation, microemulsion method, reverse micellar method, etc. (Sundar et al. 2010). To provide physical and thermal stability, L-asparaginase can be encapsulated inside the biopolymeric nanoparticles. The nanocomposite led to L-asparagine delivery in infection facilitating its direct release from the nanostructures into the bloodstream as well as reducing the enzyme proteolytic degradation and antibody recognition compared to free L-asparaginase. The combinatorial antimicrobial effect of enzymes and nanoparticles especially with chitosan could also increase the efficacy of the enzymatic drug L-asparaginase because chitosan itself has antimicrobial activity (Kumar et al. 2016) and chitosan was also suggested as a promising drug delivery system in the literature. Chitosan has significant applications in buccal drug delivery, ocular drug delivery, per-oral delivery, pulmonary drug delivery, mucosal drug delivery, nasal drug delivery, vaginal drug delivery, etc. (Huang et al. 2017; Casettari and Illum 2014, Bernkop-Schnürch and Dünnhaupt 2012). But one hindrance associated with chitosan is that there is no approval of chitosan-based drug delivery systems for mass application. A conceptual understanding of biological responses to L-asparaginase and chitosan nanoparticles is needed in-depth for the development of novel methods of drug delivery. Therefore, much effort is needed in this direction to establish chitosan as a drug delivery system for mass application. However, chitosan is approved for dietary use, drug, wound dressing applications, and cartilage formulations by US-FDA due to its biodegradability, biocompatibility, structural variability, low toxicity, good efficacy in dose form, and natural origin (Li et al. 2018). These are the basis and possible rationale for the future use of chitosan as a promising drug delivery vehicle for diagnostics/therapeutics and work with chitosan as delivery vehicles are under the different phases of clinical trials. For example, Rylomine™ (chitosan-based intranasal morphine) is under Phase 2 clinical trial in UK and EU, whereas, it is in Phase 3 clinical trial in the USA that indicates the future availability of chitosan bases drugs and delivery (Mohammed et al. 2017). Some other biodegradable polymers (silk fibroin, silk sericin, poly-d,l-lactide-co-glycolide, alginate-graft-PEG, chitosan-tripolyphosphate, etc.) are also explored as nano delivery systems for L-asparaginase effective delivery. We are not discussing the pros and cons of all biopolymeric nano-carrier for delivery of L-asparaginase because this is a short article and length does not permit to cover the whole issue of L-asparaginase delivery with nano-career of the above-mentioned biopolymers.

Conclusions

L-asparaginase is an approved drug for the treatment of acute lymphoblastic leukemia. ELSPAR, ERWINASE, KIDROLASE, and ONCASPAR are the brand names of marketed L-asparaginase. Based on the remarkable achievement in the field of medicine of L-asparaginase, a lot of research work

should be expedited to establish it as an antimicrobial drug. Further its validation in therapeutic development along with effective delivery for the treatment of infectious disease is necessary to establish it as a safe, cost-effective, and potent antimicrobial agent. As an L-asparaginase carrier, biopolymeric nanocomposites facilitate the ease of drug delivery through various routes that include oral, nasal, intravenous, and ocular by decreasing proteolytic degradation. If the parent molecule of the biopolymers-based drug delivery system faces some problem, then it can be modified also to achieve the goal. The modified biopolymeric nanoparticle could facilitate the controlled and sustained L-asparaginase release and targeted drug delivery of this enzyme at the site of infection. Although it offers several benefits but some shortcomings are also associated with its practical implementation. Biopolymeric nanoparticle prepared with chitosan is a little bit complicated to encapsulate hydrophilic drug-like L-asparaginase in its unmodified. However, with the help of simple modification techniques, such problems can be easily addressed. The creation of efficient formulation and use of new eco-friendly techniques using safe biomaterials can also contribute to the establishment of L-asparaginase as an official antimicrobial drug. The moto of writing this perspective is to explore the possibility of L-asparaginase to develop it as an approved antimicrobial agent and motivate researchers in this direction for the success to work with different suggested approaches (to know its antimicrobial mode of action, directly acting phenomena on microbes or host-directed, biopolymer mediated nano-delivery potential, etc.) (Fig. 1) that could be a promising way to attain L-asparaginase as successful antimicrobial therapeutics in future.

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Declarations

Conflict of interest All the authors declared that they have no conflict of interest.

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