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Rationalizing Nanodeliverables for Effective L-Asparaginase Delivery in Chemotherapy: Update 2020

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ABSTRACT

L-asparaginase is used as an effective line of treatment for acute lymphoblastic leukemia and lymphoma, however, the high cost for this treatment restricts the wide-scale clinical application. Genetic engineering technology is used to recombinantly express L-asparaginase, with high yield, which reduced the costs of production, and overall therapy. Despite the effectiveness of this therapy, intravenous injection of L-asparaginase has some side effects, such as allergic reactions, immunosuppression, pancreatitis, neurotoxicity, hepatitis, coagulopathy, and failure to produce antibodies. To overcome this limitation, Nano-delivery systems using nanoparticles as the carrier of L-asparaginase is used as a new type of drug carrier delivery system. It can increase the permeability of biofilms, enhance drug efficacy, reduce drug toxicity, change the distribution of drugs in the body, and improve bioavailability. In this review, we summarized the application of a variety of nano-lipid and polymers as protein recombinant drug carrier systems, and these nano-carrier systems facilitate to increase the blood circulation time, reduce side effects, and improve the biocompatibility of L-asparaginase medicine. The review specifically adds value as there is no article available that has attempted to elevate the emerging Nanodeliverables and their significance in delivering L-asparaginase safely by achieving maximum therapeutic efficacy.

Keywords

L-asparaginase, Recombinant enzyme, Nanoformulations, Lipid, Polymers, Delivery.

Introduction

Acute lymphoblastic leukemia (ALL) is a blood cancer prevalent in children where the neoplastic cells have a defect in the synthesis of amino acid L-asparagine (Asn) due to low expression of asparagine synthetase (*ASNS* gene). The discovery of the antitumor activity of L-asparaginase in 1964 has marked the beginning of novel therapy and research in ALL [1,2]. Finally, in 1978, the first L-asparaginase drug was approved by the U.S. Food and Drug Administration (FDA) for the treatment of acute lymphoblastic leukemia (ALL) and it has become an effective line of treatment and [3,4], and important combination chemotherapy for treating childhood acute lymphoblastic leukemia.

L-asparaginase has a wide range of natural sources such as bacteria, actinomycetes, and fungi [5-9]. However, application of genetic engineering, microbial fermentation methods, gene expression in

E. coli can increase the production of L-asparaginase by 100-1000 times, of which *E.coli* expression system is most popular in the pharmaceutical industry due to high-density fermentation and low culture cost [10]. Besides, using fungi as an expression host can effectively reduce the side effects of allergic reactions caused by bacteria-derived enzymes.

L-asparaginase can be divided into two types: L-asparaginase I and L-asparagine II. L-asparaginase I is expressed in the cytoplasm and can hydrolyze L-asparagine (L-Asn) and L-glutamine (L-Gln), but has a low affinity for L-Asn whereas, L-asparagine II is induced to express under hypoxic conditions, secreted into the periplasmic cavity, and has a high specific hydrolytic activity for L-Asn [11]. Among them, L-asparaginase II is mainly used to treat chronic lymphocytic leukemia, lymphosarcoma, and other tumor diseases [12]. Since tumor cells cannot synthesize L-asparagine by themselves, but they need a large amount of L-asparagine to complete rapid growth, L-asparaginase kills tumor cells by degrading L-asparagine in the human body.

L-asparaginase as a heterologous protein in the human body often causes serious side effects and importantly it is susceptible to the hydrolytic action of host proteolytic enzymes leading to decreased or diminished efficacy [2]. Therefore, finding a highly efficient and biocompatible nano delivery system has become an important direction for the development of L-asparaginase drugs in recent years as summarized in Table 1. Several studies have reported severe acute allergic reactions in 24% of children and 29% of adults during the first time treatment of ASNase [13–16]. This review focused on summarizing the current advances in polymer-carriers available for recombinant protein drugs and highlights their applications in recombinant L-asparaginase drug delivery systems using relevant case studies from recent studies. Finally, we provided an outlook for the future development of the L-asparaginase-based drug nano delivery system, and some emerging alternative systems to overcome the militations of nanocarrier systems.

Polymers used for designing nanodeliverables for recombinant enzymes

Over the past few decades, researchers have been exploring and discovering recombinant protein drug carriers, and the advent of polymer nanodeliverable carriers has opened up a new prospect for carrier research for these drugs. Recombinant enzymes refer to a class of drugs that use recombinant DNA or recombinant RNA technology to express protein or polypeptide drugs *in vitro* [17]. Compared with traditional active molecules, polypeptide and protein drugs have several outstanding advantages, including small dosage, good curative effect, low toxic and side effects. However, protein drugs are easily degraded, and the short half-life greatly limits the effects of such drugs in the body [18]. Therefore, the drug delivery systems that can effectively prevent the hydrolysis of recombinant enzymes and maintain the activity of enzymes have become the main focus of recombinant enzyme drug development. Thomas *et al*. used polymer nano-carriers (PNC) composed of polyethylene glycol and poly-lactic/poly-glycolic acid (PEG–PLGA) to encapsulate catalase and peroxidase. This new type of targeted enzyme-carrier combination can effectively prevent catalase and peroxidase from being hydrolyzed and maintain the activity of the enzyme [19]. In another study, PPX(Poly(*p*-xylylene))-coated nanofibers demonstrate excellent ability to continuously release and preserve the enzymatic activity of luciferase compared to the uncoated PVA (poly(vinyl alcohol)) nanofibers [20]. In addition to these, other nano delivery systems used in the recombinant enzyme research and therapy are discussed further.

Table 1: List of nanoencapsulation strategies reported for L-Asparaginase.

Nanocarrier	Materials	Size	Purpose of the study	References
Liposome	l-asparaginase from Escherichia coli, Soybean phospholipid, and Chitosan hydrochloride	<100nm	ACLNs shows higher anti-cancer activities than free ASP	[31]
Liposome	soy lecithin, cholesterol, and charge liposomes	43.2, 35.6, and 65.8 μm respectively for neutral, positive, and negative nanoparticles	Different releasing efficiency on different charge LNP (positive, negative, and neutral)	[36]
Liposome	phosphatidylcholine dehydration-rehydration vesicles (sDRV) or extruded vesicles (VET)	85 to 250 nm	Lipid phosphatidylcholine and cholesterol and either stearyl amine, phosphatidylinositol, or monosialoganglioside resulted in a high encapsulation efficiency	[37]
Emulsification Nanoparticles	poly(lactide-co-glycolide) (PLG) nanoparticles	200 nm	Effect of carboxyl-end groups on entrapment and releasing effciency of PLG nanoparticles	[38]
Emulsification Nanoparticles	poly(3-hydroxybutyrate-co-3-hydroxyvalerate) (PHBV) nanocapsules	around 750 to about 200 nm	The blood circulation of enzymatic activities in modified PHBV nanoparticles is longer than in unmodified ones	[39]
Emulsification Nanoparticles	poly(d,l-lactide-co-glycolide) nanospheres	374 ± 26 nm	Additives to improve the biological activity of l-asparaginase during preparation and storage	[40]
Microspheres	microparticles of polyacrylamide	50 nm	The route of administration of the asparaginase-encapsulated microsphere carrier, intramuscular/subcutaneous showed a better activity retention time	[41]

Liposomes

The most important characteristics of the asparaginase carrier system are strong stability and remain in blood circulation for a long time. Liposomes are ultra-mini spherical delivery system which is formed by encapsulating drugs in a lipid bilayer and are categorized based on size and structure such as small unilamellar vesicles, large unilamellar liposome vesicles, and multilamellar vesicles. Liposomes, as a carrier for peptide and protein drugs, can effectively protect the biological activities of the protein, improve drug stability, extend half-life, and release period. Moreover, it can adsorb, fuse, endocytosis, and lipid exchange with human cells, greatly promoting drug absorption and enhancing drug cell targeting [21]. For instance, recombinant human epidermal growth factor (rhEGF) encapsulated into Dipalmitoylphosphatidylcholine (DPPC) liposome exhibits greater penetration efficiency than Phosphatidylcholine (PC) liposome. Besides that, the area under the concentration-time curve (AUC) of rhEGF increased 1.7- and 2.5-fold in PC and DPPC liposomes compared with the solution [22]. Liposomes have continued to show bright prospects in the pharmaceutical industry since the first FDA-approved nano-drug, Doxil® which is a PEGylated nano-liposome-based anti-tumor drug used in the treatment of ovarian cancer and multiple myeloma [23]. In the vaccine industry, *Inflexal® V*, a lipid-based vaccine delivery system is extensively studied for efficacy and safety which showed promising results [24].

Microemulsions and multiple emulsions

In addition to liposomes, microemulsions are another effective delivery pathway that researchers have explored in recent years to improve productivity and scale up production. Microemulsion refers to an emulsion with a droplet diameter of smaller than 140 microns, while multiple emulsions refer to a composite emulsion in which the dispersed phase contains two emulsions. In an emulsion, the dispersion of droplets is large which aids in the rapid absorption and effect of the drug with increased bioavailability [25]. Mumuni *et al.* reported a new type of oral microemulsion delivery system that uses mucin to encapsulate insulin in which the encapsulation rate reaches more than 70%, and can lower the blood sugar of diabetic mice for more than 8 hours *in vivo* experiments [26].

Nanoparticles

Unlike the above two carrier systems, the nanoparticle carrier system has unique characteristics of polymer-based protein carriers such as selective targeting and high cellular uptake. The nanoparticle delivery system offers the flexibility of chemical surface modification on the surface to overcome the limitations of other types of carrier systems. Nanoparticles are defined as a 10-1000 nm particle size colloidal drug delivery system that can easily pass through the smallest capillary in the human body to achieve sustained and targeted drug release. After entering the systemic circulation, the nanoparticles are mainly phagocytosed by white blood cells, monocytes, and macrophages in the reticuloendothelial system (RES), thereby targeting the liver, spleen, bone marrow, and other organs in the RES [27]. There are various methods for the preparation of nanoparticles for peptides and proteins. Wei Shan *et al*. reported a zwitterions-based NPs could effectively penetrate mucus and epithelium barriers and improve 4.5-fold cellular uptake than PEGylated NPs. Figure 1 represents a graphic abstract on the zwitterions-based NPs penetration of the biological barrier *in vivo.* This work provides a potential oral delivery system for protein therapeutics [28].

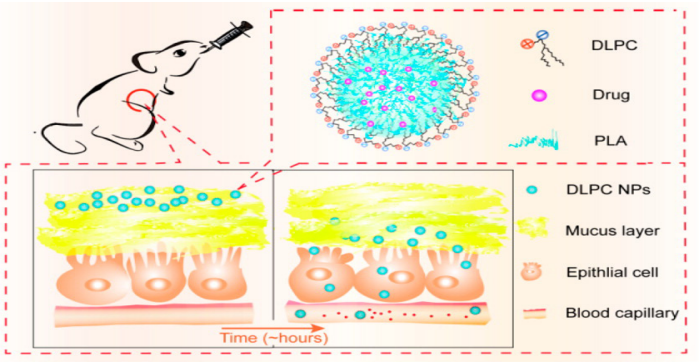

Figure 1: Graphic abstract for DLPC NPs penetration *in vivo.* Reprinted from reference [28].

Microspheres

The microsphere carrier is a tiny spherical entity with a diameter of 1-250 um formed by dissolving or dispersing the drug in a polymer material. The biodegradable polymer has been commonly used as the backbone material of the microsphere in the microsphere drug delivery system of peptides and protein drugs. The main types of microspheres are starch, gelatin, dextran, albumin, polylactic acid (PLA), polylactic acid-glycolic acid copolymer (PLGA), etc. Microspheres are also widely used in slow and controlled release injection and non-injection drug delivery systems [29].

Polymer-based nanoformulations for effective delivery of L-Asparaginase alone or with other drugs in the treatment of cancer or other therapies

With the continuous innovation and development of protein drug carriers, an increasing number of protein and peptide drugs are expected to be the key active moieties in the development of anti-tumor drugs in the future. Asparaginase is a mature anti-tumor target drug and its wide application has driven the research on its carrier system. In this section, we summarize the developments in the current research on asparaginase carriers using specific case studies. Unlike solid tumors, acute lymphoblastic leukemia is often found in the blood and bone marrow which requires the nano delivery system to facilitate the asparaginase existence in the blood circulation for a long duration [30]. Shengli Wan *et al*. reported a lipid-based nanoparticle delivery system that encapsulates L-asparaginase and surface modified with chitosan. It was the first time that chitosan modification lipid nanoparticles system utilized in L-asparaginase encapsulation and the result showed an obvious improvement in enzymatic activity and stability which exhibited better anti-tumor properties *in vitro* compared to free L-asparaginase (free ASP). Figure 2 shows the *invivo* kinetic features of L-asparaginase containing chitosan modified nanovesicles (ACLNs) where they showed increased

stability compared to free ASP in rats [31]. This study depicted that ACLNs could maintain ASP concentrations for a prolonged time due to sustained depletion of plasma asparagine to achieve better therapeutic effects.

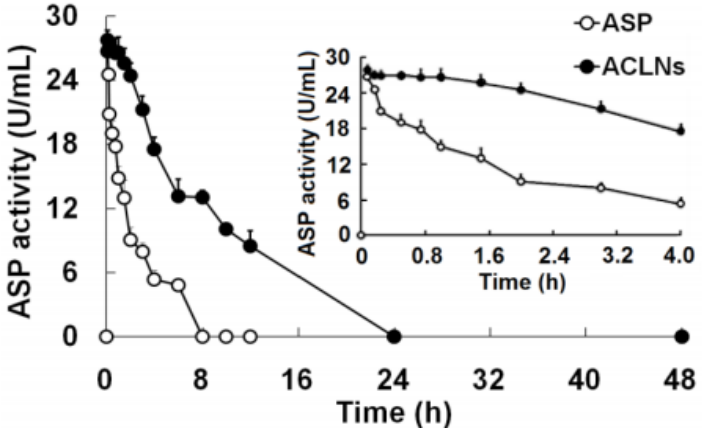

Figure 2: *In vivo* features of ACLNs in rat plasma. The plasma concentration-time curves of ACLNs and free ASP after intravenous administration of ASP dose of 2000 U/kg. Data presented as mean ± standard deviation (n=6) [31].

G.Baskar *et al.* used zinc oxide nanoparticles conjugated with L-asparaginase to treat the MCF-7 cell line and found that the cell viability decreased from 64.3% to 35.02% [32]. Some other complex combinations, such as PEG coating [33] and magnetic modification [34], also have been used in nanoencapsulation strategies for L-asparaginase drug formulation. X.Mu *et al.* showed the application of effective surface modification by using polymer-modified magnetic nanoparticles for immobilization of L-asparaginase which resulted in the retention of more than 95.7% activity after 10 repeated uses and 72.6% activity after 10 weeks of storage as represented in Figure 3.

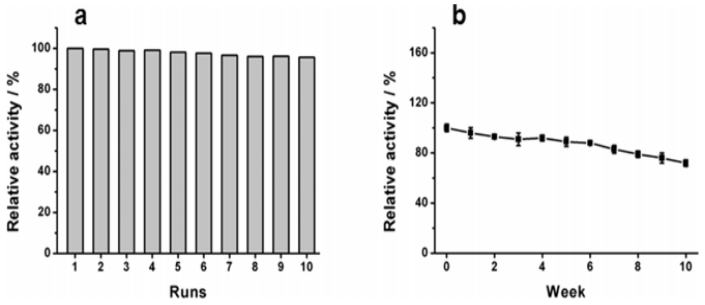

Figure 3: Application of surface modification in the nanocarrier system of L-asparaginase. (a) Reusability test of L-asparaginase immobilized magnetic nanoparticles modified with a reactive polymer; (b) stability test of L-asparaginase immobilized magnetic nanoparticles modified with reactive polymer [34].

In addition to the *in vitro* and preclinical model-based research for L-asparaginase delivery, much progress has also been made in clinical research for asparaginase delivery and modifications [32]. Vassilios I. Avramis *et al*. reported that 118 children diagnosed with acute lymphoblastic leukemia were randomly vaccinated with native asparaginase (native ASNase) and PEG-modified asparaginase (Pegaspargase). The result indicates that the elimination half-life of PEG-modified asparaginase in patients is increased from 26 hours to 5.5 days compared with the half-life of native asparaginase [35]. Figure 4 represents the enzymatic activity of asparaginase in sera over time profiles in pediatric patients with ALL after native ASNase and pegaspargase administration [35].

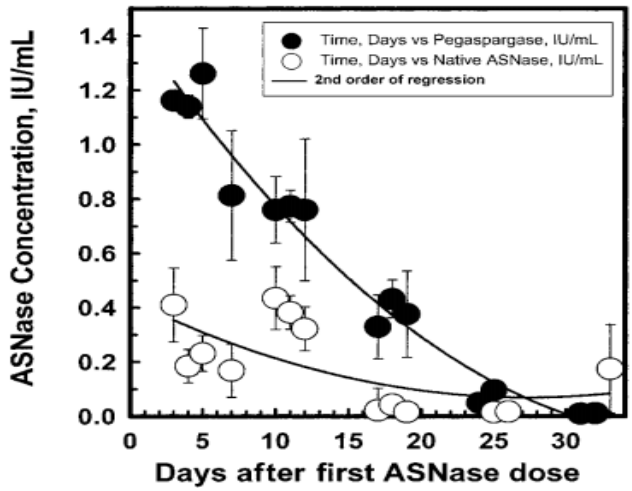

Figure 4: Asparaginase enzymatic activity in sera over time profiles in pediatric patients with ALL after native asparaginase and pegaspargase administration. (Symbols: mean ± SEM) [35].

Patents on L-asparaginase

The application and the role played by L-asparaginase therapy in treating cancers along with the application in the food industry has drawn attention to commercial/clinical application. Several patents have been filed with a focus on L-asparaginase application in the pharmaceutical and food industry. Table 2 represents the list of patents that are filed so far based on the polymer-based formulation of L-asparaginase for the increased *in vitro* and *in vivo* activity, biological carrier-based L-asparaginase, the methods used in the treatment of the cell proliferative disorders using L-asparaginase, and increased thermal activity/stability of L-asparaginase. Protein drug conjugate where polyethylene glycol is conjugated with L-asparaginase to form Pegylated L-asparaginase is patented. This conjugate has increased *in vitro* activity and half-life *in vivo* and is proposed to be used as a second line of therapy for ALL patients who have developed hypersensitivity or relapse of ALL after treatment with other L-asparaginase drugs. This patented form has also shown decreased immunogenicity than the native form [42]. Another patent invention has demonstrated the efficacy of L-asparaginase encapsulated in the red corpuscles in mouse pancreatic tumor xenograft model. The data suggested an increased pharmacokinetic of the enzyme with the use of red corpuscles compared to the native or pegylated form. The invention relates to a suspension of red corpuscles encapsulating asparaginase as a medicament intended for the treatment of pancreatic cancer, containing an effective quantity of such suspension of red corpuscles [43]. Research and invention are also focused on the

Table 2: List of patents on L-Asparaginase.

Jurisdiction	Publication Number	Publications Date	Application Date	Title	Applicants	Reference
France	US 2020/0347374 A1	Nov 5, 2020	May 18, 2020	Pegylated L-Asparaginase	Jazz Pharmaceuticals II SAS	[42]
France	2009/080837	July 2, 2009	Dec 24, 2008	Asparaginase encapsulated in red corpuscles for treatment of cancer of the pancreas	Erytech Pharma	[43]
United States	US 2011/0229984 A1	Nov 10, 2015	Jun 2, 2011	Materials and methods directed to Asparagine synthetase and asparaginase therapies	Department of Health and Human Services, USA	[44]
Denmark	US 2010/0221385 A1	Jan 18, 2011	May 18, 2010	Asparaginases	Novozymes A/S	[45]

materials and methods for use in treating cell proliferative disorders related to asparagine metabolism. This invention has described the decrease of cell proliferation by the combination therapy of asparagine synthetase (ASNS) antagonist and at least one of a L-asparginase (L-ASP) and a pegytlated L-ASP than stand-alone treatment with one these therapeutic agents. This is used in the treatment of cell proliferative disorders that include cancers like leukemia, ovarian cancers, melanomas, renal cancers, breast and brain cancers. Methods include the use of RNA interference targeted at asparagine synthetase to enhance the efficacy of L-asparaginase therapies [44]. Recombinant technology has also focused on the invention of the new asparaginases that possess improved properties of thermotolerance which includes improved activity at high temperatures and/or improved thermostability. The invention also related to the DNA sequences encoding such improved asparaginases, their production in a recombinant host cell, as well as methods of using asparaginase, in particular for reduction of acrylamide in foods which is formed during heating at high temperatures. The invention furthermore relates to methods of generating and preparing asparaginase variants having improved properties [45].

Future perspectives

Despite the importance and application of the nanocarrier systems in the area of drug delivery and targeting, the side effects have always challenged this line of therapy. To effectively overcome this limitation, biological drug carriers have recently emerged as an interesting alternative to systems. L-asparaginase encapsulated within erythrocytes has much longer blood circulation than the native form of L-asparaginase in adults and children with ALL, which is a classic example of biological drug carriers. Though it is premature to speculate the growth of biological drug carriers in parallel to the nanocarrier systems, the research in this field could open new horizons to overcome some of the limitations of nanocarrier systems.

Conclusion

L-asparaginase II is an enzyme that is widely used for the treatment of hematopoietic diseases such as acute lymphoblastic leukemia. This enzyme can mitigate the growth of the asparagine-dependent tumors by degrading circulating L-asparagine. However, native ASNase II is susceptible to proteolytic degradation by the proteases of the host organism. Besides, this enzyme therapy is also associated with a high incidence of allergic reactions. Many efforts are focused to overcome these limitations to decrease the side effects and increase the *invivo* half-life of ASNase II. Nanotechnology has shown a significant promise to overcome these limitations and in this review, we have summarized the types of nanocarrier biopolymer delivery systems for the effective delivery of the protein and peptide drugs with emphasis on the L-asparaginase therapy. The ASNase II confinement in nanocarrier systems has resulted in improved properties, especially specificity, half-life enhancement, increased proteolytic resistance, and immunogenicity. There are numerous *in vitro* and preclinical studies that have evaluated the various nanocarrier systems and their long-term impact, however, there is a need for a much thorough investigation focused on immunogenicity in clinical therapy. Also, modifications at the surface of a nanomaterial as well as physical and chemical modifications may enhance the drug loading capacity and delivery however such modifications can be challenged by the overall stability of the delivery system and dysregulated accumulation in the cancer cells. These aspects must be investigated in controlled *in vitro* and *in vivo* experiments.

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