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Current Challenges and Approaches on Delivery of Protein Based Formulations

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Abstract:
Numerous obstacle crops up in the path of developing efficient protein-based formulations and administration in humans for their therapeutic effect. The challenges starts from protein synthesis to it's handling procedure, stability, storage, excipients compatibility, scale up, analysis, validation, tissue targeting, in-vivo bioavailability, immunogenic reactions, etc., In the field of protein drug development, various novel methods and techniques have been evolved and continuing the research to face those challenges to bring out potential drug formulations and its delivery system to the market. It is vital to develop various strategies for protecting the integrity of proteins, increasing their availability in the body, decreasing immunological responses, transporting and target to particular tissues or cells. This current review focuses on to understand the problems, challenges encountering and the best approaches for protein based drug formulation development. It is one of roadmap to plan various research activities for continual improvement of therapeutic applications of newly developing protein drugs in this area.

Keywords: protein, stability, immunogenic, systematic, novel, degradation, bioavailability

1. Introduction

Protein drugs play an essential role in various therapeutic areas; these include antibodies that target particular infectious diseases, vaccines that boost the immune system, hormones and enzymes that trigger many physiological functions in human body. Preserving the structural and functional integrity of these biomolecules is a major challenge in protein-based therapy. Since proteins are among the most sensitive molecules, a number of conditions that including mechanical stress, pH and temperature, can affect their stability. In addition, proteins have the ability to activate the host's immune system, which may result in adverse reactions and diminished therapeutic efficacy [1].

The protein's capacity to reach its intended destination to exert it's bioavailability within the body and exploit it's therapeutic impact is sometimes impeded by barriers such as insufficient oral absorption. Because of their vulnerability to enzyme degradation in the digestive system, many protein drugs are inappropriate for oral delivery, requiring alternate routes that may be less patient-friendly. Furthermore, achieving precise tissue or cell-specific targeting is a difficult task. Proteins that are delivered systemically tend to spread throughout the body, potentially causing off-target effects. It remains difficult to provide localized delivery at the desired spot while minimizing exposure to non-target tissues.

To overcome these challenging obstacles, researchers and pharmaceutical producers have employed novel methods. Encapsulation methods, such as the use of nanoparticles, dendrimers or liposomes, provide protective carriers for proteins, protecting them from environmental degradation and enhancing bioavailability. Nanotechnology offers the potential to enhance targeted delivery and medicinal efficacy. Modified delivery systems, such as implanted devices and microneedles, allow for the regulated, constant release of protein-based therapies.

In addition, tailoring protein formulations to specific proteins and their intended therapeutic applications allows for the optimization of stability and efficacy. Tailoring delivery systems to the specific properties of proteins has proven critical for enhancing the area of protein-based treatments [2].

Spray drying, lyophilization, cryopreservation and use of stabilizers are important techniques and approaches for overcoming the stability related challenges. These techniques improve the stability of proteins and protect them against degradation. Additionally, precise and controlled protein release at specific sites within the body is made possible by advanced delivery methods including stimuli-responsive nanoparticles and pH-responsive polymeric loading.

Protein engineering techniques are applied for altering protein structures and improve their stability and reduced their immunogenicity. Targeted and regulated protein distribution is provided by smart delivery systems, which are made to react to environmental conditions. Approaches to personalized medicine take into consideration, where individual characteristics of patients like immunologic profiles and genetics to personalize treatment plans and enhance the results.

Combination therapies, which use protein-based therapies in addition to other treatments or therapies, have the potential to reduce the dosage of essential proteins and increasing the therapeutic window and produce synergistic benefits. Together, these complete techniques overcome the problems associated with protein delivery, resulting in more precise and potent therapies for a range of diseases and conditions. In table 1, examples of protein formulations which are having market potential are described.

Table 1: Market Potential of Protein Formulations - Examples

Factors	Market Potential		Brand Name	Drugs	Dosage Forms	Uses
	Current	Future				
Therapeutic applications	Established market for specific drug formulations	Potential for new drug developments and approvals	Enbrel	Etanercept	Injection	Treatment of autoimmune diseases like rheumatoid arthritis
Biopharmaceuticals	Growing interest in biologic drugs and personalized medicine	Expanding market for bio-pharmaceuticals	Humira	Adalimumab	Injection	Used for various autoimmune conditions
Protein replacement therapy	Increasing focus on protein replacement	Ongoing research in rare diseases and genetic	Cerezyme	Imiglucerase	Infusion	Enzyme replacement therapy for Gaucher disease

	therapies	disorders				
Oncology treatments	Advancements in cancer therapeutics and targeted therapies	Evolving landscape with personalized and immunotherapies	Herceptin	Trastuzumab	Injection	Treatment for HER2- positive breast cancer
Hormone therapies	Development of protein-based hormones for various conditions	Potential for new hormonal therapies and applications	Humulin	Insulin (recombinant)	Injection	Treatment for diabetes, controlling blood sugar levels
Vaccines	Continued importance of protein – based vaccines	Ongoing development of vaccines for various infectious diseases	Gardasil 9	Human papillomavir-us vaccine	Injection	Prevention of HPV – related cancers and diseases

2. Challenges in Protein Drug Delivery

2.1. Protein Stability

Proteins are important biological macromolecules that play critical roles in many cellular processes, and their stability is critical to their proper function. Several environmental factors can have an important effect on protein stability, consequently understanding protein behavior in scientific and biological contexts is fundamental.

Temperature: Temperature has a significant impact on protein stability. Proteins have a temperature range within which they are stable, known as their thermal denaturation temperature. When proteins are exposed to temperatures outside of this range, their secondary, tertiary, and quaternary structures can be disrupted. Protein function is frequently lost due to denaturation. Extreme temperatures, both high and low, can be dangerous. High temperatures can cause protein unfolding and aggregation, whereas low temperatures can reduce the kinetic energy of molecules, which can cause proteins to precipitate or become less active [3].

pH (Acidity or Alkalinity): The pH of the environment in which a protein lives can also have a substantial impact on its stability. Each protein has an optimum pH range in which it is most stable and functional. The desired pH is often close to the physiological pH of the organism, which is around 7.0. Changes in pH may affect the electrostatic interactions that help keep a protein's structure stable. Acidic or alkaline environments can cause protonation or deprotonation of amino acid side chains, determining hydrogen bonding and charge interactions within the protein structure [4].

Mechanical Stress: Mechanical stress, such as shear forces and stretching, may damage protein structural integrity, triggering unfolding and loss of function. This is particularly vital in the context of

tissues and organs that are prone to mechanical forces. In the cardiovascular system, for example, constant mechanical stress from blood flow may interfere with the stability of specific proteins, contributing to diseases such as atherosclerosis and heart failure [5].

2.2. Bioavailability

The poor oral bioavailability of many proteins due to their sensitivity to the acidic and enzymatic conditions of the gastrointestinal tract is a well-recognized challenge in drug development.

Gastrointestinal Environment: The gastrointestinal tract is a complicated system that includes the stomach, which has a very acidic environment. Many proteins can be damaged or destroyed in this acidic environment, which is caused mostly by the presence of acid in the stomach. Proteins often possess different three-dimensional structures that are critical to their biological activity and low pH can cause them to lose both their shape and their function. Their degradation can render them ineffective as therapeutic agents.

Enzymatic Activity: In addition to the acidic environment, the gastrointestinal system contains a number of digestion enzymes. These enzymes degrade proteins into smaller peptides and amino acids. This breakdown by enzymes begins in the stomach and continues in the small intestine, reducing the bioavailability of intact proteins even further [6].

2.3. Immunogenicity

One of the most significant barriers to the development and application of therapeutic proteins is the immune system. This occurs when the immune system of the body interprets these proteins as foreign substances, resulting in the production of antibodies or other immunological reactions. These immunological reactions may lead to a variety of consequences, such as a reduction in the efficacy of therapy and serious safety concerns for the patients.

Reduction in Effectiveness: When the immune system perceives a therapeutic protein as foreign, it may develop antibodies against it. These antibodies can bind to the therapeutic protein to form immune complexes. The protein may get completely neutralized or its activity may decrease as a result. This may result in a reduction in the therapeutic efficacy of the protein and potentially make the treatment ineffective.

Safety Concerns: Patients may be at risk for safety because of immunogenicity. Rarely, a therapeutic protein's immune response may trigger adverse effects including autoimmune or allergic reactions in addition to neutralization. Depending on the subject and the specific protein in question, the adverse responses can vary from moderate to severe.

Development of Anti Drug Antibodies: Anti-drug antibodies (ADAs) are defined as antibodies generated by the patient's immune system in reaction to the therapeutic protein. The presence of ADAs can complicate treatment by decreasing the protein's efficacy, raising the risk of side effects and maybe leading to treatment failure.

Impact on Treatment Durability: The long-term usage of therapeutic proteins may be affected by the emergence of immunogenicity and ADAs. Patients may require higher doses or various medications as they become less responsive to the treatment over time [7].

2.4. Targeting

A crucial component of delivering therapeutic proteins is targeting certain tissues or cells in order to minimize off-target effects and obtain optimal therapeutic benefits. Targeting appropriately may improve overall treatment efficacy, decrease systemic toxicity and increase the therapeutic index. Many methods and strategies have been developed to ensure that therapeutic proteins are targeted correctly.

Based Targeting: It is possible to specifically identify and bind to antigens or cell surface receptors that are overexpressed on the target cells using antibodies or antibody fragments. Therapeutic proteins can be directly delivered to the targeted cells or tissues by conjugating them to these antibodies.

Cell-Penetrating Peptides (CPPS): CPPs are short peptides that have the ability to let therapeutic proteins get across cell membranes. The complex can easily access the target cells by binding CPPs to the protein of interest, enhancing the delivery's specificity.

Nanoparticles and Liposomes: Therapeutic proteins can be delivered by these delivery systems, which can be targeted at particular tissues or cells. Proteins can be delivered to target cells effectively by interacting with the surface of liposomes or nanoparticles with ligands that bind to their receptors.

Chemical Modification and Conjucation: It is possible to improve the specificity of protein distribution through chemical modifications, such as the inclusion of certain ligands or targeting moieties. A protein can be effectively transported to the intended site by conjugating it with ligands that have a high affinity for receptors on the target cells.

Tissue Specific Promoters: It is possible to use gene therapy approaches, in which viral vectors containing promoters specific to a certain tissue are used to deliver therapeutic proteins. By precisely targeting the expression of the therapeutic protein in the target tissues, these promoters reach their target.

Bio-Orthogonal Chemistry: Using this technique, proteins are selectively designated with small-molecule probes that can direct their delivery to the appropriate cells or tissues via bio-orthogonal reactions. Targeting ligands can be specifically and biocompatible attached to proteins via bio-orthogonal chemistry [8].

3. Approaches in Protein Drug Delivery

3.1. Formulation

Protein formulations are carefully designed to protect proteins from clumping and degradation, providing their effectiveness and stability throughout the manufacturing, storage, and delivery processes. These formulations utilize stabilizers, excipients and controlled-release mechanisms in order to maintain the protein's bioactivity and structural integrity. Lyophilization, also known as freeze-drying, is an increasingly common method for improving the stability of proteins in storage.

Stabilizers and Excipients: Excipients and stabilizers are crucial for maintaining the stability of protein formulations. By preventing protein denaturation, aggregation, and enzymatic degradation, they can maintain the biological activity and unique conformation of the protein. Stabilizers that offer a protective environment and prevent proteins from unfolding and aggregating include sugars, polyols, and amino acids.

Controlled-Release Systems: Proteins can be released in a controlled manner through the use of controlled-release systems, which assure long-lasting therapeutic benefits. These systems can be made to encapsulate proteins in microspheres or biodegradable matrices, permitting a controlled and gradual release of the protein at the intended location [9].

Lyophilization (Freeze – Drying): An established method for improving protein stability during storage is lyophilization. This procedure transforms the frozen solvent into a vapor without passing through the liquid phase by freezing the protein solution and extracting water under low pressure. Lyophilization offers many different kinds of benefits. Preventing Denaturation: Proteins' organic structure and functionality are retained through lyophilization, which minimizes exposure to denaturing circumstances. Extended Shelf Life: The process of lyophilization extends the shelf life of protein-based medications by preventing microbial growth and chemical reactions. Improved Reconstitution: Lyophilized proteins are easier to administer because they can be reconstituted faster [10].

pH and Ionic Strength Optimization: Protein denaturation and aggregation can be avoided by adjusting the formulation's pH and ionic strength, ensuring the preservation of the protein's structural integrity and biological activity in a variety of environmental conditions.

Antioxidants and Chelating Agents: Protein stability and bioactivity can be maintained by incorporating antioxidants and chelating agents into protein formulations to protect proteins from oxidative stress and metal-catalyzed degradation [9].

3.2. Delivery Systems

Employing drug delivery systems - like liposomes, nanoparticles, and microspheres has been established to be a successful method of protecting proteins, regulating their release, improving bioavailability, and lessening immunogenicity. When it comes to protein-based medicines, these systems have various benefits.

Liposomes: Lipid bilayers form the spherical vesicles known as liposomes. Proteins are encapsulated in them to avoid degradation and enable controlled release. Liposomes provide a precise delivery system as they can be created to release their payload in response to specific triggers, like temperature or pH changes.

Nanoparticles: Proteins can be carried by nanoparticles, which are nanoscale particles. They may enhance stability, present sustained release and protect proteins from enzymatic degradation. By changing the surface with ligands, one may specifically target the nanoparticles, leading them to target tissues or cells.

Microspheres: Proteins can be encapsulated in microspheres, which are small, spherical particles that regulate protein release over time. Achieving sustained release, lowering the frequency of administration, and enhancing patient compliance are a few areas in which these systems excel. Proteins are protected from the harsh external environment, such as enzymatic degradation and immune responses, by being encapsulated within these drug delivery systems. These systems can also provide sustained and controlled release, which enhances bioavailability and lowers the risk of immunogenic reactions [11].

3.3. Route of Administration

For protein-based treatments, selecting the mode of administration is vital for optimizing bioavailability and reducing immunogenicity. Various routes, such as subcutaneous, intramuscular, intravenous, and inhalation, may be examined in relation to the particular protein and its intended therapeutic target.

Intravenous Administration: Proteins are administered intravenously (IV) or directly into the bloodstream. As the protein gets around obstacles like the digestive system, this method offers quick and comparable bioavailability. Because IV administration demonstrates the protein directly to the immune

system, it may increase the risk of immunogenicity. However, it is particularly suitable for proteins that need to be distributed quickly and uniformly throughout the body.

Subcutaneous Administration: Proteins are injected subcutaneously (SC) to enter the subcutaneous tissue and release them gradually but steadily into the bloodstream. This is a common method for managing protein medications on their own, like insulin. Because SC administration allows a more controlled release of the protein than IV administration, it may decrease immunogenicity.

Intramuscular Administration: Proteins are injected intramuscularly (IM) into the muscle tissue. A moderate rate of absorption and bioavailability are offered by this route. IM administration is often used for protein therapeutics and vaccines. Because of the slower release, it may also be more immunogenic than IV administration.

Inhalation: A specific pathway for proteins that target the respiratory system is inhalation. Proteins can act locally or enter the bloodstream in the lungs when they reach the body directly due to this method. Because the respiratory mucosa is relatively tolerant, inhalation can increase bioavailability in specific indications and possibly decrease immunogenicity. The characteristics of the protein, the intended pharmacokinetics, and patient convenience all impact the route of administration that is selected. When choosing a route, optimal bioavailability and limited immunogenicity are crucial factors to take into account. Various paths present different benefits and challenges, so the decision should be made with the particular therapeutic setting in mind [12].

3.4. PEGylation

In the field of protein-based treatments, PEGylation—the process of binding polyethylene glycol (PEG) chains to proteins—is a commonly used technique. This modification reduces immunogenicity and increases the protein's half-life in circulation, as well as other significant benefits.

Half-Life Extension: Proteins in the bloodstream can have half-lives that are greatly extended by PEGylation. When proteins are introduced into the body, the system of reticuloendothelial cells and other biological filters have the ability to quickly remove them. PEGylation binds PEG chains to the protein surface, transforming it and providing a layer of protection. Because of this alteration, the protein is unable to be discovered and absorbed by these clearance systems, which prolongs its time in circulation. This prolongs the protein's therapeutic impact, decreasing the need for frequent doses and improving patient convenience.

Reduced Immunogenicity: The immune system can recognize and respond to foreign proteins, potentially leading to an immune response. PEGylation can mask the epitopes on the protein's surface that might trigger such immune reactions. By shielding these sites, PEGylation makes the protein drug less recognizable to the immune system, reducing the likelihood of immunogenic responses. This is particularly beneficial when dealing with proteins that have a higher risk of immunogenicity.

Improved Solubility: PEG chains may additionally enhance a protein's solubility, which is beneficial for delivering and formulating drugs. They produce a hydrophilic environment that keeps proteins in solution and prevents them from aggregating, which may threaten their stability and efficacy.

Tailored Release: Proteins can have customized release features, depending on the PEGylation method applied. For some therapeutic uses, for example-controlled release over an extended period of time can be achieved using certain PEGylation techniques [13].

3.5. Protein Engineering

Proteins can be carefully influenced through protein engineering to enhance stability, lower immunogenicity, and improve targeting. This approach delivers a wide range of options for altering proteins to meet specific therapeutic requirements.

Stability Improvement: Protein structural stability can be enhanced through protein engineering. Techniques like site-directed mutagenesis, which substitutes certain amino acids with more stable alternatives, may improve a protein's protection against denaturation and breakdown. Ensuring the long-term stability of protein-based therapeutics is especially dependent on this.

Reduced Immunogenicity: When providing proteins, immunogenicity can be an important issue. Epitopes that produce immune responses can be decreased or eliminated by introducing site-specific modifications. Proteins can be made less immunogenic for therapeutic use by carefully modifying or eliminating immunogenic areas from their surface [14].

Enhanced Targeting: Targeting sequences or ligands that can increase specific binding to receptors or cell surface markers can be included via protein engineering. This increases therapeutic efficacy by minimizing off-target effects and permitting precise delivery to the intended site of action.

Optimized Pharmacokinetics: Protein pharmacokinetic properties can be customized by means of protein engineering. By reducing clearance, stimulating renal reabsorption or blocking proteolytic degradation, among other techniques, half-life can be increased, expanding bioavailability and increasing therapeutic effects.

Functional Alteration: In order to increase the therapeutic potential of proteins, protein engineering can also be used to alter the functional properties of proteins, such as their enzymatic activity or receptor binding affinity. In order to produce proteins or different functions, this may include the use of directed evolution techniques or rational design [15].

3.6. Co-Administration with Protease Inhibitors:

A method used to prevent proteins from being broken down by enzymes in the gastrointestinal system is to co-administer them with protease inhibitors. By preventing the breakdown of protein-based therapeutics by digestive enzymes, this approach may enhance their oral bioavailability.

Protease Inhibitors: Protease inhibitors are substances that specifically target and inhibit the proteolytic enzymes found in large amounts in the gastrointestinal tract, such as chymotrypsin and trypsin. Proteins can be broken down by these enzymes into smaller peptides and amino acids, which can make protein medications inefficient.

Oral Bioavailability Improvement: Orally administered protein-based therapeutics have to deal with the harsh environment of the stomach and the enzymatic breakdown that occurs in the gut. Protease inhibitors can be given in addition to the protein to prevent it from breaking down too quickly and improve the chance that it will enter the bloodstream without damage.

Enhanced Therapeutic Efficacy: Co-administration of protease inhibitors with protein drugs may boost their oral bioavailability and thus improve their therapeutic efficacy. This is particularly beneficial when patient convenience and compliance establish that oral administration be used instead of more invasive methods of administration like injection.

Patient-Friendly Delivery: Oral administration is more convenient for patients and is chosen by many. When protease inhibitors and protein-based therapeutics are taken together, patients may be more likely to adhere to their therapies [16].

3.7. Immune Modulation

When delivering protein-based treatments, immunosuppressive co-administration or immune tolerization strategies are used to reduce immunogenicity. These strategies aim to modify the immune response. These methods improve the security and efficacy of these treatments while lowering the chance of negative immune reactions.

Immunosuppressants: Medical treatments known as immunosuppressants minimize the activity of the immune system. Immunosuppressive medications and protein-based therapeutics given together may decrease the immune response that the therapeutic protein induces. This is especially important when protein treatments are more likely to be mistaken for foreign substances by the immune system, which could result in adverse reactions or reduced effectiveness.

Immune Tolerization: The purpose of immune tolerization techniques is to cause the body to become less responsive to the therapeutic protein by stimulating tolerance. These methods may involve the co-administration of immune modulators or adjuvants, oral tolerance, and desensitization. In order to lessen the potential of immunological responses, the objective is to create an environment in which the immune system understands the therapeutic protein as either self or non-threatening.

Reduced Immunogenicity: By modulating the immune response, these strategies help reduce the immunogenicity of protein-based therapeutics. This is critical for ensuring the safety and effectiveness of the therapy, as unwanted immune reactions can lead to adverse effects, a loss of therapeutic efficacy, or the development of antibodies against the protein.

Enhanced Therapeutic Window: A wider range of patients can benefit from protein-based therapeutics due to immune modulation techniques that may increase the therapeutic window. This is especially important when controlling chronic illnesses requiring regular medical attention [17].

3.8. Smart Delivery Systems

Emerging technologies in the field of drug delivery, including pH-responsive polymers and stimuli-responsive nanoparticles, have enabled the development of smart delivery systems. These systems can provide controlled and targeted protein delivery based on specific environmental cues, offering precision and enhanced therapeutic outcomes.

pH-Responsive Polymers: The payload of pH-responsive polymers is intended to be distributed in the interaction through pH variations. For example, the stomach's slightly acidic environment can trigger proteins contained in pH-responsive polymers to leak out. This assures that the proteins are released at the right site, like the small intestine or colon, and stay protected throughout their passage through the digestive system. This methodology optimizes bioavailability while mitigating the potential for enzymatic degradation.

Stimuli-Responsive Nanoparticles: The purpose of stimuli-responsive nanoparticles is to make them react to particular factors in the environment, like variations in pH, temperature or the presence of specific biomolecules. It is possible to engineer these nanoparticles so that in response to a suitable stimulus, they release proteins. For example, when the temperature rises, which is a sign of infection or inflammation, they may release proteins. The therapeutic effect is maximized while side effects are minimized by this targeted and on-demand release.

Enhanced Targeting: Additionally, ligands or antibodies that bind certain receptors or antigens on the target cells or tissues can be added to the surface of nanoparticles as part of smart delivery systems. By

accurately delivering the protein payload to the intended area, this targeting capability decreases off-target effects and improves therapeutic efficacy.

Sustained Release: Many smart delivery systems have the ability to release proteins continuously over a longer period of time. This may reduce the frequency of administration and increase patient adherence, particularly for long-term treatments needed for chronic illnesses [18].

3.9. Personalized Medicine

Personalized medicine involves altering protein methods of administration based on specific patient characteristics, including immunological profiles and genetics. By modifying treatment plans, this method seeks to maximize therapeutic results while limiting side effects.

Genetic Variation: Specific variations in genetics may impact how protein-based therapies are metabolized, reacted to and sustained. Medical professionals may choose the most suitable protein medication, dosage schedule and delivery method by taking into account the genetic profile of their patients. This ensures that there are fewer side effects and that the treatment is more effective.

Immune Profiles: Patients may respond differently to protein-based therapies due to differences in their immunological systems. Certain individuals might be at a higher risk of developing immunological reactions directed against the therapeutic protein. Based on the patient's immune profile, personalized medicine enables the selection of immunomodulatory techniques, such as co-administration with immunosuppressants or immune tolerization approaches.

Optimized Dosage: It is possible to prevent under or over-treatment of protein-based treatments by personalizing the dosage to each patient's needs. Personalized medicine considers different factors that may impact the ideal dosage, including age, weight and the presence of comorbidities.

Therapeutic Monitoring: A key aspect of customized medicine is constantly monitoring a patient's reaction to protein-based therapies. This maintains the treatment's continued efficacy and permits real-time changes as needed.

Risk Reduction: Personalized medicine may help in identifying patients who are more susceptible to adverse side effects or a protein medication's inability to work. Healthcare professionals can use this information to select various therapy plans or to keep a close eye on these individuals in order to intervene early [19].

3.10. Combination Therapies

An approach to improving the efficiency of protein-based therapeutics while possibly lowering the required protein dose and minimizing immunogenicity is to combine proteins with other medications or treatments. This method can enhance treatment results and have synergistic benefits.

Synergistic Effects: Combination therapies utilize the beneficial effects of many medications or treatments. A protein can boost its overall therapeutic efficacy when paired with another therapeutic agent, such as a monoclonal antibody or small- molecule medication, by acting in concert with one another. More effective and focused therapies may result from this.

Dose Reduction: It is frequently possible to have the desired therapeutic effect with a lower dose of a protein by combining it with another therapeutic drug. This is important because it lowers the possibility of immunogenicity because fewer immune system responses can result from a lower protein dosage.

Broadened Therapeutic Window: A wider range of patients may benefit from combination therapy by expanding the therapeutic window. This is especially crucial in managing illnesses where patient responses vary widely.

Reduced Resistance: Combining different treatments may reduce the chance of developing drug resistance. For example, combining targeted therapy or chemotherapy with a protein-based therapy can help overcome resistance mechanisms and improve treatment outcomes in cancer patients.

Enhanced Targeting: Enhanced targeting capabilities can also result from combining proteins with other therapies. For example, the targeted delivery of therapeutic agents to the intended site can be increased by integrating a protein with a drug delivery system based on nanoparticles [20].

4. Conclusion

In conclusion, addressing the issues surrounding protein delivery is a key to the effective creation and use of therapeutic proteins. A varied strategy is necessary due to the complexity of these problems, which include protein stability, bioavailability, immunogenicity and targeting. Promising methods to get around these challenges include formulation techniques, sophisticated delivery systems, protein engineering, co-administration with protease inhibitors, immune modulation, intelligent delivery systems, personalized medicine and combination therapies. Future developments in protein-based therapies will be more patient-friendly and effective as a result of ongoing research and technological advancements in these fields.

References

1. Torchilin VP. Challenges in protein delivery. Therapeutic Delivery, 2017; 8(4): 245-248. DOI: 10.4155/tde-2017-0008.
2. Tatsuya Okuda and Mariko Morishita. Challenges and Advances in the Delivery of Biologics. Oral Delivery of Biologics for Precision Medicine. Therapeutic Delivery, 2017; 8(12):1083-1087. DOI: 10.4155/tde-2017-0086
3. Dill KA, MacCallum, JL. The protein-folding problem. Science, 2012; 338(6110): 1042-1046.
4. Pace CN, Scholtz JM. A helix propensity scale based on experimental studies of peptides and proteins. Biophysical Journal, 1998; 75(1): 422-427.
5. Li J, Tan W. Impact of mechanical stress on protein structure and function. 2014; Science China Life Sciences, 57(9): 894-902.
6. Pridgen, EM, Alexis F, Farokhzad OC. Polymeric nanoparticle drug delivery technologies for oral delivery applications. Expert Opinion on Drug Delivery, 2015; 12(9): 1459-1473.
7. Shankar G, Pendley C, Stein KE. A risk-based bioanalytical strategy for the assessment of antibody immune responses against biological drugs. Nature Biotechnology, 2007; 25(5): 555-561.
8. Vladislav Kravchenko, Alexander Kabanov, Tatiana Bronich. Targeted Delivery of Proteins. Pharmaceutical Research, 2018; 35(2): 40. DOI: 10.1007/s11095-017-2292-9
9. Ravi Mahadeva, Rashmi Rambhatla, Priyanka Gupta. Formulation Development and Stability Testing of Monoclonal Antibodies and Their Formulations. Journal of Pharmaceutical Sciences, 2018; 107(9): 2312-2337, DOI: 10.1016/j.xphs.2018.04.051.
10. Monica Lockney, John Wang Y, Kevin Li S. Lyophilization and Development of Solid Protein Pharmaceuticals. International Journal of Pharmaceutics, 2003; 265(1-2): 115-125. DOI: 10.1016/s0378-5173(03)00323-7.

11. Hiromi Sakai, Hiroshi Masada, Koichi Takeoka. Liposome-Encapsulated Hemoglobin: A Novel Approach to Hemoglobin-Based Oxygen Carriers. Molecules, 2020; 25(19): 4546. DOI: 10.3390/molecules25194546.
12. Kevin K Lo, Keith HP. Subcutaneous Administration of Biologics: An Overview of Current Challenges and Opportunities. BioDrugs, 2020; 34(1): 155-169. DOI: 10.1007/ s40259-019-00389-y.
13. Vikas Kumar, S.S. Bansal. PEGylation in Anti-Cancer Therapy: A Review of the Emerging Paradigm. International Journal of Pharmaceutical Sciences and Research, 2012; 3(6): 1660-1675.
14. Vrushali SM, Sanjay PK. Protein Engineering Approaches to Enhance the Enzymatic Activity and Immobilization of Proteins. Enzyme Research, 2012. DOI: 10.1155/2012/ 561589.
15. Satish KM, Sonal G. Protein Engineering: A Powerful Tool for the Study of Immune Modulation by Infectious Agents. Frontiers in Immunology, 2018; 9:1960. DOI:10.3389/ fimmu.201 8.01960.
16. Bhawna S, Lokesh KB. Oral Delivery of Therapeutic Proteins and Peptides: Challenges and Solutions. Biological and Pharmaceutical Bulletin, 2013; 36(2): 225-238. DOI: 10.1248/bpb.b12-00636.
17. Abbas AK, Murphy KM. Immune Modulation for Desensitization or Immunotolerance in Allergic Disease. The Journal of Allergy and Clinical Immunology, 2018; 141(1): 1-7. DOI: 10.1016/j.jaci.2017.11.015.
18. Yan-Yan Hu, Jun Wang, Xiao-Qi Yu. Stimuli-Responsive Nanocarriers for Drug Delivery. Nature Reviews Materials, 2020; 5: 508-526. DOI: 10.1038/s41578-020-01814-8.
19. Antonio Bertolotto, Carlo Barone, Paola Cavalla. Personalized Medicine in Rheumatology: The Paradigm of Serum Autoantibodies. Frontiers in Immunology, 2020; 11. DOI: 10.3389/fimmu.2020.568355.
20. Rama K. Malladi Peter M. Thomas, Patrick Hwu. Combination Therapies in Immuno-Oncology: Perspective on the Clinical Development and Evolution. Molecular Cancer, 2018; 17(1): 39. DOI: 10.1186/s12943-018-0778-9.