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Review article

Formulation of Biologics for Alternative Routes of Administration: Current Problems and Perspectives

Sanja Stanković[1], Marija Tasić Kostov[2]

[1]*Pharmacy „Cvejić“,Niš, Serbia*
[2]*Pharmacy Department, University of Niš, Faculty of Medicine, Niš, Serbia*

SUMMARY

Introduction: Biologics (biopharmaceuticals) present new promising therapies for many diseases such as cancers, chronical inflammatory diseases and today's biggest challenge - COVID-19.
Research: Today, most biologics have been synthetized using modern methods of biotechnology, in particular DNA recombinant technology. Current pharmaceutical forms of protein/peptide biopharmaceuticals are intended for parenteral route of administration due to their instability and large size of molecules. In order to improve patient compliance, many companies are working on developing adequate forms of biopharmaceuticals for alternative, non-invasive routes of administration. The aim of this work is to review current aspirations and problems in formulation of biopharmaceuticals for alternative (non-parenteral) routes of administration and to review the attempts to overcome them. These alternative routes of administration could be promising in prevention and treatment of COVID-19, among other serious diseases.
Conclusion: The emphasis is on stabilizing monoclonal antibodies into special formulations and delivery systems; their application should be safer, more comfortable and reliable. When it comes to hormones, vaccines and smaller peptides, some companies have already registered drugs intended for nasal and oral delivery.

Keywords: **biologics, alternative routes of administration**

Corresponding author:
Sanja Stanković
e-mail: sanjastankovic278@gmail.com

INTRODUCTION

The term biotechnology refers to every technique which uses living organisms for the production or modification of a product. Today, therapeutic molecules that are biological in nature and manufactured using modern methods of biotechnology are referred to as biotechnological drugs/biopharmaceuticals (or biotech drugs) (1). More precisely, these drugs have been made through one of the biotechnological processes, such as the recombinant DNA (rDNA) technology, the control of gene expression of biologically active proteins in prokaryotic and eukaryotic cells, or the hybridoma technology (for monoclonal antibodies-mAbs) (2). This category of drugs includes proteins (enzymes, mAb, cytokines, hormones, hematopoetic growth factors), peptides and nucleic acids (genes, oligonucleotides, small interfering RNA) (3). Nowadays, two generations of protein-based biotechnological drugs can be found on the market: the first and the second.

1. First generation biopharmaceuticals are mainly the copies of endogen proteins, simple replacement proteins displaying an identical amino acid sequence to a native human protein manufactured using the DNA recombinant technology (for example, human insulin, human growth factor, etc.), or antibodies manufactured with hybridoma technology.

2. Second generation biopharmaceuticals are proteins or antibodies "improved" through engineering (at the level of their physical, chemical and pharmacokinetic characteristics) applied in order to alter a protein's immunological or pharmacokinetic profile, or in order to generate novel fusion products (for example, covalent attachment of chemical moieties such as polyethylene glycol-PEG) (4).

Biotechnological drugs have been put in place for treating and improving many life-treating diseases such as cancer, chronic inflammatory diseases (rheumatoid arthritis, juvenile idiopathic arthritis, psoriasis, psoriatic arthritis, Crohn's disease, ulcerative colitis and ankylosing spondylitis) multiple sclerosis and, most recently, novel COVID-19 (5, 6).

However, their administration is extremely challenging because of biopharmaceutical and physicochemical limitations, requiring circumventing enzymatic degradation and reducing immune reactions, while ensuring molecular stability and permeability. Parenteral administration overcomes most of the referred issues, so it appears as an obvious option; to date, a vast majority of marketed biopharmaceuticals is administered by direct injection. However, the associated cost and patient discomfort have turned the research efforts toward alternative routes of administration that will increase patient compliance.

In this paper, we will discuss biotechnological drugs for alternative, non-parenteral routes of administration, problems during their formulation, and efforts to resolve them.

Characteristics of biopharmaceuticals

The formulation and manufacturing processes of biopharmaceuticals differ from the ones for low-molecular weight drugs (so-called chemical drugs) primarily because of their complex structure. Biotech drugs are mostly proteins. For comparison, Aspirin®, a representative of conventional chemical drugs, has a relative molecular weight of 180 daltons (Da), insulin about 5800 Da, and mAbs of 150000 Da (150 kDa) (6). Because of their structure, environmental stress can easily lead to physical and chemical instabilities of biopharmaceuticals. Physical instabilities like aggregation, precipitation and adsorption can happen with drugs of all types, but denaturation is unique to proteins. Chemical instabilities, by definition, involve processes that make-or-break covalent bonds. The main types of chemical instabilities are hydrolysis, oxidation and deamidation (7). Protein instability can be caused by exposure to environmental stressors such as pH extremes, high temperatures, freezing, light, and oxygen presence (8). The effects of degradation can vary from the changes in pharmacokinetic and pharmacodynamic parameters, to the loss of biological activity, toxicity and immunogenicity (1). Characteristic of biopharmaceuticals is that they have a higher potential for causing an immunological reaction compared to chemical drugs (6). Neutralizing antibodies are detected as response of immune system to the protein application, which leads to a reduction in the efficacy of therapy and can affect the pharmacokinetic parameters of the drug (9).

Specific physical and chemical characteristics of proteins such as high molecular weight, hydrophilic nature and electric charge result in their low bioavailability. The oral route of administration of protein-based drugs is limited by the enzymatic degradation in GIT, which causes drug inactivation. The problem with other common non-parenteral

routes of drug administration could be the low absorption, caused by the size and the shape of particles. For that reason, biotech drugs are formulated for parenteral use, while an effort is made for establishing alternative needle-free administration such as oral, nasal, buccal, transdermal, pulmonal and rectal. These alternative routes of administration should achieve better patient compliance.

One of the greatest advances in the field of biotechnology has been the discovery of mAbs, which have revolutionized the field of research and medicine. At the time of writing this paper, all approved and marketed mAbs are administered systemically via the parenteral route (10). The most common ways of parenteral application are intravenous (i.v.) and subcutaneous (s.c.). Subcutaneous injections are a better choice compared to i.v. application because of the possibility of self-administration and the use of depo formulations requiring less frequent application which could contribute to better patient compliance. Extracellular matrix of hypodermis is a significant barrier for subcutaneous application. As a result, the volume of s.c. injection is limited to 1 - 2 ml. With insulin application this is not an issue, but bigger volume is usually needed for mAbs. Technology with human recombinant hyaluronidase is designed as potential solution for this problem. This enzyme is a temporarily delimited hyaluronic acid, a natural component of extracellular matrix (9). The use of human recombinant hyaluronidase in formulations intended for subcutaneous use enables better absorption of the drug and possible application of a greater volume. While bovine-derived hyaluronidase was used initially, nowadays human recombinant hyaluronidase is more prevalent since it has a lower potential of causing an immunogenic reaction (Hylenex®, approved by FDA in 2005.) (11). This human recombinant version of the enzyme is approved as a separate product and is used in combination with monoclonal antibodies and immunoglobulins (12).

ALTERNATIVE ROUTES OF ADMINISTRATION OF BIOPHARMACEUTICALS

Oral route of administration

The oral route of administration of therapeutic protein or peptide-based drugs (TPPs) is limited by the mucus and epithelial cell barriers and proteolytic enzymes in the gastrointestinal tract (GIT). These barriers block access of larger molecules like TPPs which results in their reduced bioavailability (11). There are a few different strategies for overcoming these problems. One of them is to administer the drug with protease inhibitors, which would prevent degradation by proteolityc enzymes. To prolong the interaction with the mucus layer, TPPs could be modified using PEG (11, 3).

High molecular weight of TPPs also present an issue. As mentioned before, the biggest challenge with mAbs is their size. Current strategies combine capsule for oral use as proper pharmaceutical form, with enzyme inhibitors and absorption enchancers, which could present a way to prevent degradation and resorption problems in GIT (11). Chelating agents, bile salts, surfactants, fatty acids and their salts are recognized as good permeation enhancers. These substances open up tight junctions, lower mucus viscosity and increase resorption in GIT (11, 13). Viswanathan. et al. (14) used poli(aminoamid) dendimers for the immobilization and the oral delivery of antibodies. The presumed mechanism of action is the opening of tight junctions and paracellular transport in intestine (14). One more approach for overcoming epithelial cell membrane barrier in GIT is the use of a carrier molecule such as a liposome, a microsphere or a nanosphere. Liposomes are lipid-based carrier systems. The disadvantages of these carriers include the inefficient encapsulation of hydrophilic proteins, their instability in GIT and poor permeation through mucosa. However, these problems could be overcome with modification of liposome surface with a mucoadhesive polymer chitosan and protease inhibitors like aprotinin. Additionally, polymeric microspheres are used to facilitate transport of proteins after oral administration. Nanoparticles are carriers with several advantages. They protect proteins from acid and proteolytic enzymes in GIT, enable them to cross the mucus layer and provide improved bioavailability (13).

Many trials were conducted in order to achieve a stable oral formulation of insulin. Morçöl et al. (15) proposed oral insulin delivery system phosphate-PEG-insulin-casein (CAPIC). They tested this system in mice and showed a prolonged hypoglycemic effect in diabetic mice. Furthermore, CAPIC formulation protects insulin from degradation after an oral administration until it is released in intestines where it can be absorbed (15). Oramed, Ltd. (Israel) finished phase II clinical study for orally adminis-

trated insulin (ORMD-0801-NCT02496000) and initiated the second phase 3 oral insulin study (16, 17). This oral insulin formulation relies on protease inhibitors and absorption enhancers (18).

In addition to studies on absorption enhancers, nanoparticles as carriers, and peptide modification, researches on intestinal patches and microneedles which should provide a better biopharmaceutics transfer from intestine to blood are being conducted (11).

Biopharmaceutical company Rani Therapeutics (USA) has been developing intestinal microneedles placed inside enteric polymer-coated oral capsules. The capsules are supposed to be dissolved and to release microneedles at the pH > 6 in intestine, which would then penetrate the intestinal epithelium. This could be a way to overcome possible degradation of biopharmaceuticals in GIT (11). Traverso et al. (19) showed that insulin administered via gastrointestinal injection in pigs had higher bioavailability than the subcutaneous injection, and satisfying safety profile. Even in the case when microneedles remain in the gastrointestinal tract for two months, there is no tissue damage observed (19). It is also proposed that the future microneedle devices could be coupled with an external stimulation to provide a controlled release of the drug (11).

One more approach used mucoadhesive patches shielded within capsules for protection from enzymatic degradation. After the capsule dissolution, these patches bind to intestinal mucus, causing a prolonged residence time and an enhanced absorption of the drug. Patch systems could be based on mucoadhesive polymers and hydrogels (11).

Biotechnological company Tiziana Life Science (UK) has been conducting clinical trials with enteric coated capsules containing lyophilized powder of foralumab for oral use (20). Phase 1 clinical trial for this fully human anti-CD3 mAb has been completed and phase 2 trial was planned to start in the second quartal of 2021. The drug was well-tolerated in all tested doses, even with the highest dose of 5 mg; no drug-safety issues were observed (20, 21). Other researches showed that orally administrated anti-CD3 mAb was well-tolerated, safe and biologically active in patients with non-alcoholic steatohepatitis (NASH) in patients with chronic hepatitis C virus and type 2 diabetes (22). Tiziana Life Science is also working on nasal foralumab formulation for patients with progressive multiple sclerosis and COVID-19 (20,21). It was shown that nasal administration of foralumab could reduce lung inflammation and blood inflammatory biomarkers in mild to moderate COVID-19 patients (21), so this rout of drug (mAb) administration could be of particular interest.

Biopharmaceuticals are also being used in the treatment of chronic intestinal diseases like Chron`s diseases, ulcerative colitis and colorectal cancer. Parenteral administration of these drugs also causes many serious adverse effects. Systemic exposure to biopharmaceuticals is associated with toxicity such as risk of infection due to suppression of the immune system and hypersensitivity reactions like antibodies against the biopharmaceutical resulting in loss of response. One alternative that can potentially overcome systemic adverse effects is an oral administration of biopharmaceuticals with local colon-targeted delivery (23). There are various strategies for creating colon-targeting drugs for oral administration, such as linkage of the drug with a carrier, coating with pH-sensitive polymers, and using timed-release systems (24).

Maurer et al. (25) investigated local colon-targeted delivery of infliximab tablets for oral administration as a safe and an effective dosage form of infliximab for the treatment of patients with Crohn's disease. These infliximab tablets use ColoPulseTM coating in order to achieve colon-specific release. To stabilize the antibodies during the production and storage, they were incorporated in a sugar glass containing the oligosaccharide inulin (25). Many of the antibodies in use today are formulated like lyophilizate in order to achieve better physical and chemical stability (10). The feasibility study showed that this formulation was stable and that 83% of biological activity has been maintained after 16 months of storage (25). The oral infliximab product (Celltrion Healthcare Co., Ltd., South Korea and Intract Pharma, UK) was cleared by the UK regulatory body to proceed to Phase 1b/2a clinical trials in inflammatory bowel disease patients (IBD) during the second half of 2021 (26). Intract Pharma (UK) is a company specialized in oral drug delivery with their Phloral® drug delivery technologies for precise colonic delivery and Soteria® for delivery of biotherapeutics to gastrointestinal tissue. They announced the initiation of a collaborative research study with Ferring Pharmaceuticals (Switzerland) to investigate oral delivery of a mAb for the treatment of IBD (27). Phloral® is a technology which guarantees site-specific release even if the pH in the GIT varies. Polysaccharide component used in this tech-

nology is independently digested by enzymes secreted by naturally residing bacteria in the colon. In the research study, 3/8 of the conventional pH-sensitive polymer coated tablets failed to release and were voided intact against the Phloral® coated tablets which were all released in the colon. Soteria® acts by protecting the drug from the environment of the stomach and the small intestine and by releasing the compound in the colon using the Phloral® coating technology. This technology enhances the uptake of the drug into the colon tissue from where it is able to engage the local targets or to enter the systemic circulation (28). Rapid progress in research and development renders these routes of administering biotechnologies promising, and future advancements in the field are certainly expected.

Nasal and buccal route of administration

Another form of a potential non-invasive route of drug administration is via nasal and buccal mucosa. Some of the problems that need to be overcome are the size of the molecules, the specific lipid composition of the buccal epithelia, and the small nasal surface (11). Nasal administration of novel drugs for COVID-19 is especially significant, given that the virus enters the body through the same route.

The composition of buccal mucosa could enable the diffusion of peptide-based drugs via the paracellular pathway. Buccal delivery of drugs avoids their degradation by the gastric acid and proteolytic enzymes in the GIT. The limitation of the buccal delivery is the potential degradation of protein-based drugs by the saliva. Enzymatic inhibitors such as bile salts, aprotinin or polyacrylic acid have been incorporated into formulations of biopharmaceutics for the buccal route (13). Bile salts and surfactants are added to formulations to aid solubilization and extraction of lipids from the buccal tissue and enable paracellular transport of biopharmaceuticals. Currently investigated buccal formulations for insulin are mucoadhesive films, nanoparticles and sprays (11).

Oral-LynTM (Generex, Canada) is a liquid oral spray formulation of the human insulin. This formulation of insulin for buccal absorption showed the same effects on blood glucose control in comparison with regular insulin injections (29). This clinical study is in phase III (NCT00668850) and has not been completed yet (30).

The nasal route of administration shows certain advantages thanks to the thin epithelial barrier being highly vascularized. Additionally, this way of delivery can bypass the hepatic first-pass metabolism, the blood-brain barrier and is characterized by relatively low activity of proteolytic enzymes. Bypassing the blood-brain barrier enables the delivery of drugs directly to the brain tissue or cerebrospinal fluid. Current investigations in the field of nasal route of drug administration include enzyme inhibitors, absorption enhancers and mucoadhesive formulation to improve nasal residential time. Mucoadhesive micro-/nanoparticles can be effective carriers for this way of delivery because of the longer residence time (31).

Tiziana Life Science (UK) is conducting clinical study with the aforementioned drug foralumab for the nasal route of administration. The use of this mAb incorporated in nanoparticles is explored for COVID-19 patients due to its immunomodulating abilities. Results show a positive impact on pneumonia and a decreased level of IL-6 and CRP in a group of patients who used foralumab compared to the control group. Nasally administrated foralumab effect is not directly targeting COVID-19. Anti-inflammatory effect of foralumab is through modulation of the immune system. The direct delivery of foralumab rapidly suppresses lung inflammation, as evident from the CT scans. The treatment also improved the senses of smell and taste in treated patients (32).

Phase I of clinical trials for the oral administration of foralumab and for the nasal administration have been completed (21, 32). The nasal administration of foralumab stimulated the anti-inflammatory cytokine IL-10 and suppressed the pro-inflammatory cytokine IFN-ɣ. Foralumab was well-tolerated with no drug-related toxicities. Nasal administrated foralumab passes the blood-brain barrier and activates an immune response in the cervical lymph nodes and suppress inflammation commonly associated with neurodegenerative diseases, including multiple sclerosis (21).

Intranasal administration of genetically modified IgG for SARS-CoV-2 is currently in pre-clinical trials on mice. Zhang H. et al. (33) investigated the possibility that this nasal spray could be used prophylactically. Data shows efficiency of this formulation in nose and lungs 7 days after the exposure to the virus (33). Diomics (USA) and Active Motif Inc. (USA) are also working on a formulation for a local

prophylactic effect against SARS-CoV-2. This bioabsorbable polymer should keep the mAbs in the nasal cavity, where the virus is trapped and neutralized for up to 24 h. Dioguard TM was undergoing *in vitro* testing and Diomics was planning to propose fast track authorization from FDA to accelerate human clinical trials (34).

There are some issues in formulating full-length antibodies due to their size and instability. Nanobodies® (Ablynx, Belgium) are therapeutic proteins based on single-domain antibody fragments. The discovery that llamas and camels (Camelidae) possess fully functional antibodies only consisting of heavy chains with a single variable domain sparked the development of a new generation of therapeutic molecules. These cloned and isolated single variable domains have full antigen binding capacity and they weigh only 12 - 15 kDa (35, 36), thus showing the advantages of small-molecule drugs such as size, a good stability and an ease of production. Nanobody® caplacizumab (Cablivi®, Ablynx, Belgium) for parenteral use was the first approved antibody of this type by the European Medicines Agency (37). The advantages of Nanobodies® could be used for non-parenteral routes of administration. Intranasal delivery of the nanobody PIN-21 showed good results in preventing and treating SARS-CoV-2 infection in Syrian hamsters, and further researches should be conducted (38).

Pulmonary route of drug administration

The advantages of pulmonary drug delivery are the large absorptive surface areas and the extensive vascularization in the lungs which enable a rapid absorption, as well as the minimal first-pass metabolism (11). This way of delivery also enables a high concentration of drug in the lungs which is desirable for lung diseases including asthma, lung cancer and respiratory infections. Pulmonary delivery is an attractive non-invasive alternative route of administration for the delivery of biologics. Still, there are many anatomical, physiological and immunological barriers (37). Lung surfactants must not be disrupted, and alveolar macrophages must also be avoided during this way of delivery. Administrated drug should pass a mucus layer before the enzymatic degradation and mucociliary clearance. After passing the mucus layer, the drug should be transferred through the pulmonary epithelial barrier. Small, hydrophobic molecules pass the membrane via a concentration gradient. The problem with large and polar molecules, like protein-based drugs, are tight junctions in the lung tissue and the difficulty in the paracellular absorption (11). Drug must overcome the mucus layer, the epithelial layer, the basement membrane and the capillary endothelium in order to be absorbed into the blood and to show a systemic effect. There are many different approaches to overcome these barriers: preventing degradation, enhancing barrier permeability via opening of tight junctions, disrupting the lipid bilayer, and using carriers and enabling longer resident time of the drug. Permeation enhancers are being added to formulations to aid the transport of biopharmaceuticals in the lungs. Polymers, surfactants, enzyme inhibitors, and tight junction modulators are being used like permeation enhancers (39). For example, dipalmitoylphosphatidylcholine, the main component of the lung surfactant, is used as a lung absorption enhancer for parathyroid hormone (40). Zheng J et al. (41) studied the use of natural pulmonary surfactants and their artificial substitute (phospholipid hexadecanol tyloxapol) as absorption enhancers for insulin in diabetic rats. Dry powders containing recombinant human insulin with and without surfactant were administered intratracheally. The result of this study showed that both natural and artificial surfactants might be promising absorption enhancers for pulmonary delivery of large molecules like insulin (41). Cationic polymers interact with mucosal barrier and enhance the absorption of biopharmaceuticals via tight junction modification. Chitosan and its derivatives have been used to develop mucoadhesive polymers. These permeation enhancers are biodegradable and biocompatible, prolong resident time of the drug, and thus enhance pulmonary absorption. Same as for the other non-invasive routes of administration, nanoparticles have been used as drug carriers. These carriers are enhancing absorption by overcoming mucociliary clearance and avoiding phagocytosis by alveolar macrophages (39).

Non-invasive insulin delivery has long been one of the targets in the drug delivery. Exubera® (Pfizer, USA) was approved by the FDA in 2006. This dry powder inhaler which contains insulin was available on the market for one year. After that, Exubera® was discontinued because of the high cost and concerns with side effects such as lung cancer (11). Technosphere® insulin (Afrezza®, MannKind, USA) is another dry powder inhaler with insulin

which was approved by the FDA in 2014 (37). They used the carrier fumaryl diketopiperazine for insulin to form microparticles and maintained delivery to the alveoli (42). Full-length mAbs and antibody fragments such as antigen-binding fragments (Fab), domain antibodies (dAb) and single-domain antibodies (Nanobody®) have also been explored for pulmonary delivery (37). Inhaled Nanobody® are currently under clinical development.

A number of nanobodies against SARS-CoV-2 infection has been explored (37). The SARS-CoV-2 virus is enters the human cells via angiotensin converting enzyme 2 (ACE2) receptor interacting with the receptor binding domain (RBD) of the spike protein on the viral surface (36). Inhalable RBD-specific nanobody candidates are being developed and *in vitro* activity was confirmed (37). These nebulized deliverable treatments of nanobodies against SARS-CoV-2 could be further developed into therapeutics as well as diagnostic and prevention reagents for COVID-19 (36, 43, 44).

Transdermal route of application

Transdermal route of administration is painless and advantageous for absorption due to the large available surface area and the avoidance of the first pass metabolism. However, low permeability of stratum corneum (SC) could limit the absorption of biopharmaceuticals. Absorption enhancers or disruption of the skin barrier have been used as strategies; ultrasound, iontophoresis, jet injector and microneedles effectively bypass the SC barrier and enable transfer of drugs into the systemic circulation (11). Iontophoresis using a weak electric current is one way of enhancing delivery of ionic and hydrophilic compounds across the intact skin (45). This method has already been used in physical therapy in order to achieve transdermal absorption of protein-based drugs. Transdermal administration of insulin in diabetic rats with help of iontophoresis was studied (46).

The use of microneedles is another way to effectively bypass the SC barrier. This delivery system should store, transport and deliver biopharmaceuticals by temporarily creating microscopic aqueous channels within the epidermis through which the drug molecules can diffuse into microcirculation of dermis (47). Most of the studies using this method investigated the delivery of vaccines and hormones. Yu et al. (48) reported research on a microneedle array containing glucose-responsive vesicles as a "smart" insulin patch (48). Courtenau et al. (49) tested transdermal administration of mAb bevacizumab with microneedles *in vivo*. The solution of bevacizumab was added to a mixture of gelatin, mannitol, NaCl and sucrose, and was then lyophilized. This was the first example of a high dose transdermal delivery of an antibody therapeutic in vivo using hydrogel microneedles. Bevacizumab has been detected in plasma throughout the 7 days following a single application. The drug was also detected in the skin tissue, the spleen and lymph nodes. This could be a viable option for the treatment of lymphomas and secondary metastatic tumors (49).

Carriers are another technology which can provide drug delivery through the skin. They are made from biocompatible and biodegradable polymers. Several types of potential polymeric nanocarriers like nanofibers, nanospheres, nano/microcapsules, polymersomes, polymeric micelles and nanogels should be mentioned (50).

Rectal route of administration

Rectal administration of drugs has been used for many years. Except possibility of avoiding hepatic first pass-effect, this way of application is useful when patients have nausea, vomiting or are unconscious. Also, extensive rectal vascularization and low protease activity can enhance absorption (51). N-trimethyl chitosan chloride and sodium salicylate as absorption enhancers have positive effect in insulin thermosensitive liquid suppository. However, the rectal way of insulin application does not have clinically relevant solutions (52). MAbs adalimumab and infliximab have advanced the therapy for inflammatory bowel disease. Their disadvantages are the systemic toxicity and the discomfort due to parenteral application. Aprodu et al. (53) presented hydrogel formulation as a base for the biopharmaceutical application. This hydrogel system based on the methylcellulose and hyaluronic acid had desirable characteristics for local rectal application and a potential for the target delivery of biologics in inflammatory bowel disease. The system showed a good retention and an easy application of the hydrogel loaded with the bovine serum albumin (53).

CONCLUSION

Biologics represent the future in the treatment of many diseases. Moreover, biotechnological therapeutic approaches have a special position and seem to be one of the most effective solutions for combating COVID-19; the suggested intranasal route, in addition to the intravenous, is being investigated as the more appropriate route of drug administration (particularly in the case of some mAbs) in the management of COVID-19.

However, there are many challenges and more efforts to overcome problems with formulation of biologics for non-parenteral routes of administration. Many different approaches are used to prevent degradation and enhance absorption of biotechnological drugs: nanoparticles, absorption enhancers, enzyme inhibitors, etc. For some smaller molecules in this class of drugs such as hormones and enzymes, researches already achieved stabile formulations. MAbs, as class of protein-based drugs with highest molecular weight present a particular problem. They were initially developed for i.v. administration, but efforts are made to develop stable and effective pharmaceutical forms of mAbs for non-parenteral routes of administration. Efficacy, patient's adherence to treatment and access to therapy with biopharmaceuticals could be improved with the development of stable formulations of biotechnological drugs for non-parenteral use, and it seems that the future of development of biopharmaceuticals should be redirected from injectable preparations to more user-friendly routes of drug administration.

References

1. Savić S, Milić J. Biološki lekovi: farmaceutsko-tehnološke specifičnosti. Arh Farm 2012; 62:374-91.

2. Crommelin D, Bermejo T, Bisssig M et al.Pharmaceutical evaluation of biosimilars: important differenceas from generic low molekul-weight pharmaceuticals. Eur J Hosp; Pharm 2005; 11: 11-7.

3. Savić S, Pantelić I, Savić M. Farmaceutski oblici bioloških i drugih lekova u terapiji multiple skleroze. Arh Farm 2015; 65: 237 - 55.

4. Savić S, Vuleta G, Savić M. Hormoni kao biotehnološki lekovi: humani insulin i analozi. Arhiv Farm 2010; 60(2): 207-25.

5. Alten R, Cronstein BN. Clinical trial development for biosimilars. Semin Arthritis Rheum 2015; 44 (6): S2-S8. https://doi.org/10.1016/j.semarthrit.2015.04.002

6. International alliance of patients organizations. Biologics Introduction. 2017. www.iapo.org.uk/sites/default/files/files/factsheet1.pdf

7. Nagel KM. Therapeutic Proteins. In Introduction to Biologic and Biosimilar Product Development and Analysis 2018 (pp. 31-54). Springer, Cham. https://doi.org/10.1007/978-3-319-98428-5_2

8. Emami F, Vatanara A, Park EJ, Na DH. Drying technologies for the stability and bioavailability of Biopharmaceuticals. Pharmaceutics 2018; 10(3):131. https://doi.org/10.3390/pharmaceutics10030131

9. Awwad S, Angkawinitwong U. Overview of antibody drug delivery. Pharmaceutics 2018; 10 (3), 83. https://doi.org/10.3390/pharmaceutics10030083

10. Madani F, Hsein H, Busignies V, Tchoreloff P. An overview on dosage forms and formulation

strategies for vaccines and antibodies oral delivery. Pharm Dev Technol S 2020; 25(2),133-48. https://doi.org/10.1080/10837450.2019.1689402

11. Anselmo AC, Gokarn Y, Mitragotri S. Non-invasive delivery strategies for biologics. Nat.Rev. Drug Discov 2019; 18: 19-40. https://doi.org/10.1038/nrd.2018.183

12. Knowles SP, Printz MA, Kang DW, LaBarre MJ, Tannenbaum RP. Safety of recombinant human hyaluronidase PH20 for subcutaneous drug delivery. Expert Opinion on Drug Delivery 2021 Oct 1:1-3. https://doi.org/10.1080/17425247.2021.1981286

13. Nirnoy D, Kamalika S, Hassan A. An Update on Pharmaceutical Strategies for Oral Delivery of Therapeutic Peptides and Proteins in Adults and Pediatrics. Children 2020; 7(12): 307. https://doi.org/10.3390/children7120307

14. Viswanathan P, Muralidaran Y, Ragavan G. Challenges in oral drug delivery: a nano-based strategy to overcome. In Nanostructures for oral medicine 2017; 173-201: Elsevier. https://doi.org/10.1016/B978-0-323-47720-8.00008-0

15. Morcol T, Nagappan P, Nerenbaum L, Mitchell A, Bell S J. Calcium phosphate-PEG-insulin-casein (CAPIC) particles as oral delivery systems for insulin. Int J Pharm 2004; 277(1-2): 91-7. https://doi.org/10.1016/j.ijpharm.2003.07.015

16. Eldor R, Neutel J, Homer K, Kidron M. Efficacy and safety of 28-day treatment with oral insulin (ORMD-0801) in patients with type 2 diabetes: A randomized, placebo-controlled trial. Diabetes, Obesity and Metabolism 2021;23(11):2529-38. https://doi.org/10.1111/dom.14499

17. Oramed Pharmaceuticals. Oramed Initiates Second Phase 3 Oral Insulin Study Under the FDA's Approved Dual Concurrent Protocol. PRNewswire: Oramed Pharmaceuticals-press releases 2021 March 23. Available from: https://www.oramed.com/oramed-initiates-second-phase-3-oral-insulin-study-under-the-fdas-approved-dual-concurrent-protocol/Last accessed 22.05.2021.

18. Nagaraju R, Madan S, Arora K, Arora NM, Kundu PK, Greenberg-Shushlav Y, Kidron M, Hershko A. Recombinant Protease Inhibitor Enhances Oral Insulin Pharmacodynamics in Pigs. Diabetes 2018; 67(1). https://doi.org/10.2337/db18-121-LB

19. Traverso G, Schoellhammer CM, Schroeder A, Maa R, Lauwers GY, Polat BE, Anderson DG, Blankschtein D, Langer R. Microneedles for drug delivery via the gastrointestinal tract. J Pharmaceutical Sci 2015;104(2):362-7. https://doi.org/10.1002/jps.24182

20. Tiziana Life Science. Tiziana Reports Phase 1 Clinical Data Demonstrating Oral Treatment with Foralumab, a Fully Human Anti-CD3 Monoclonal Antibody, is Well-tolerated in Healthy Volunteers. Businesswire: Tiziana Life Science;cited 2020 Jan 09. Available from:
21. https://www.businesswire.com/news/home/20200109005415/en/
.
22. Tiziana Life Science. Corporate Presentation.Tiziana Life Sience:published 2021 Jan. Available from: https://www.tizianalifesciences.com/cms/resources/tlsa-non-confidential-jan-2021.pdf

23. Ilan Y, Shailubhai K, Sanyal A. Immunotherapy with oral administration of humanized anti-CD3 monoclonal antibody: a novel gut-immune system-based therapy for metaflammation and NASH. Clin & Exp Immunol 2018 ;193(3):275-83. https://doi.org/10.1111/cei.13159

24. Vass P, Démuth B, Hirsch E, Nagy B, Andersen SK, Vigh T. et al. Drying technology for colon-targeted oral delivery of biopharmaceuticals. J Controled Release 2019; 296:162-78. https://doi.org/10.1016/j.jconrel.2019.01.023

25. Chourasia MK, Jain SK. Pharmaceutical approaches to colon targeted drug delivery systems. J Pharm Pharm Sci 2003;6(1):33-66.

26. Maurer JM, Hofman S, Schellekens RC, Tonnis WF, Duboi AO, Woerdenbag HJ et al. Development and potential application of an oral ColoPulse infliximab tablet with colon specific

release: A feasibility study. Int JPharm 2016; 505(1-2): 175-18
https://doi.org/10.1016/j.ijpharm.2016.03.027

27. Intract Pharma. Intract Pharma Limited and Celltrion Group announce collaboration for development of oral Infliximab. Biosimilar development: Intract Pharma: cited 2020 Aug 20. Available from: https://www.biosimilardevelopment.com/doc/intract-pharma-limited-and-celltrion-development-of-oral-infliximab-0001. Last accessed 16.04.2021
.
28. Intract Pharma. Intract Pharma Enters into Research Collaboration with Ferring Pharmaceuticals to Evaluate Oral delivery of Monoclonal Antibodies for Treatment of Chronic Gastrointestinal Diseases-Intract Pharma: cited 2021 Apr 01. Available from : https://www.intractpharma.com/2021/04/01/intract-pharma-enters-into-research-collaboration-with-ferring-pharmaceuticals-to-evaluate-oral-delivery-of-monoclonal-antibodies-for-treatment-of-chronic-gastrointestinal-diseases/.
Last accessed 16.04.2021.

29. Intract Pharma. Enabling Technologies for Precision Drug Delivery in the GI Tract. Available from:
https://www.intractpharma.com/
Last accessed 16.04.2021
.
30. Annabestani Z, Sharghi S, Shahbazi S, Karimi F, Taheri E, Heshmat R, Larijani B. Insulin buccal spray (Oral-Lyn) efficacy in type 1 diabetes. J Diab Metabolic Dis 2010;9:18.

31. Generex Biotechnology Corp. Active Comparator Study of Generex Oral-lyn™ Spray and Injected Human Insulin, ClinicalTrials.gov Identifier: NCT00668850, 2008. Available from:
clinicaltrials.gov/ct2/show/NCT00668850?term=NCT00668850&draw=2&rank=1

32. Bajracharya R, Song JG, Back SY, Han HK. Recent advancements in non-invasive formulations for protein drug delivery. Comput Struct Biotechnol J 2019;17:1290-308.
https://doi.org/10.1016/j.csbj.2019.09.004

33. Tiziana Life Science. Tiziana Reports Positive Data from the Clinical Study of Nasal Administration with Foralumab, its proprietary fully human anti-CD3 monoclonal antibody, in COVID-19 Patients in Brazil. GlobeNewswire: Tiziana Life Science:cited 2021 Feb 02. Available from: https://www.globenewswire.com/news-release/2021/02/02/2167825/0/en/Tiziana-Reports-Positive-Data-from-the-Clinical-Study-of-Nasal-Administration-with-Foralumab-its-proprietary-fully-human-anti-CD3-monoclonal-antibody-in-COVID-19-Patients-in-Brazil.html,
Last accessed 18.03.2021.

34. Zhang H, Yang Z, Xiang J, Cui Z, Liu J, Liu C. Intranasal administration of SARS-CoV-2 neutralizing human antibody prevents infection in mice. bioRxiv. 2020 Jan 1.
https://doi.org/10.1101/2020.12.08.416677

35. Diomics Corporation, US, 2020. Diomics aims to nose out coronavirus ahead of vaccines with nasal spray that delivers monoclonal antibody protection. Available at:
https://www.businesswire.com/news/home/20200908005196/en/Diomics-Aims-Nose-Coronavirus-Vaccines-Nasal-Spray
(Last Accessed March 24, 2021).

36. Ablynx. Understanding Nanobody Technology.Available from:
https://www.ablynx.com/technology-innovation/understanding-the-nanobody-technology/. Last accessed 09.04.2021

37. Esparza TJ, Martin NP, Anderson GP, Goldman ER, Brody DL. High affinity nanobodies block SARS-CoV-2 spike receptor binding domain interaction with human angiotensin converting enzyme. Sci Rep 2020;10(1):1-3.
https://doi.org/10.1038/s41598-020-79036-0

38. Liang W, Pan HW, Vllasaliu D, Lam JK. Pulmonary delivery of biological drugs. Pharmaceutics 2020; (11):1025.
https://doi.org/10.3390/pharmaceutics12111025

39. Nambulli S, Xiang Y, Tilston-Lunel NL, Rennick LJ, Sang Z, Klimstra WB, Reed DS, Crossland NA, Shi Y, Duprex WP. Inhalable Nanobody (PiN-21) prevents and treats SARS-CoV-2 infections in

Syrian hamsters at ultra-low doses. Sci Adv 2021;7(22):eabh0319. https://doi.org/10.1126/sciadv.abh0319

40. Ghadiri M, Young PM, Traini D. Strategies to Enhance Drug Absorption via Nasal and Pulmonary Routs. Pharmaceutics 2019; 11(3):E113. https://doi.org/10.3390/pharmaceutics11030113

41. Hoyer H, Perera G, Bernkop-Schnürch A. Hoyer H, Perera G, Bernkop-Schnürch A. Noninvasive delivery systems for peptides and proteins in osteoporosis therapy: a retroperspective. Drug Dev Ind Pharm 2010;36(1):31-44. https://doi.org/10.3109/03639040903059342

42. Zheng J, Zhang G, Lu Y, Fang F, He J, Li N et al. Effect of Pulmonary Surfactant and Phospholipid Hexadecanol Tyloxapol on Recombinant Human-Insulin Absorption from Intratracheally Administered Dry Powders in Diabetic Rats. Chem Pharm Bull 2010; 58(12):1612-6. https://doi.org/10.1248/cpb.58.1612

43. Rendell M. Technosphere inhaled insulin (Afrezza). Drugs of today (Barcelona, Spain: 1998). 2014;50(12):813-27. https://doi.org/10.1358/dot.2014.50.12.2233894

44. Ma H, Zeng W, Meng X, Huang X, Yang Y, Zhao D, Zhou P, Wang X, Zhao C, Sun Y, Wang P. Potent Neutralization of SARS-CoV-2 by Hetero-bivalent Alpaca Nanobodies Targeting the Spike Receptor-Binding Domain. J Virol 2021; 95(10):e02438-20. https://doi.org/10.1128/JVI.02438-20

45. Martinez-Delgado G. Inhaled nanobodies against COVID-19. Nature Rev Immunol 2020;(10):593. https://doi.org/10.1038/s41577-020-00443-5

46. Hasan M, Khatun A, Fukuta T, Kogure K. Noninvasive transdermal delivery of liposomes by weak electric current. Adv Drug Delivery Rev 2020; 154-155: 227-35. https://doi.org/10.1016/j.addr.2020.06.016

47. An YH, Park MJ, Lee J, Ko J, Kim SH, Kang DH, Hwang NS. Recent advances in the transdermal delivery of protein therapeutics with a combinatorial system of chemical adjuvants and physical penetration enhancements. Adv Therapeutics 2020 Feb;3(2):1900116. https://doi.org/10.1002/adtp.201900116

48. Kirkby M, Hutton AR, Donnelly RF. Microneedle mediated transdermal delivery of protein, peptide and antibody based therapeutics: current status and future considerations. Pharmaceutical Res 2020;37:1-8. https://doi.org/10.1007/s11095-020-02844-6

49. Yu J, Zhang Y, Ye Y, DiSanto R, Sun W, Ranson D, Ligler FS, Buse JB, Gu Z. Microneedle-array patches loaded with hypoxia-sensitive vesicles provide fast glucose-responsive insulin delivery. Proc Natl Acad Sci 2015;112(27):8260-5. https://doi.org/10.1073/pnas.1505405112

50. Courtenay AJ, McCrudden MTC, McAvoy KJ, Helen McCarthy HO, Donnelly RF. Microneedle-mediated transdermal delivery of Bevacizumab. Mol. Pharmaceutics 2018; 15(8):3545-56. https://doi.org/10.1021/acs.molpharmaceut.8b00544

51. Peña-Juárez MC, Guadarrama-Escobar OR, Escobar-Chávez JJ. Transdermal Delivery Systems for Biomolecules. J Pharm Innov 2021:1-4. https://doi.org/10.1007/s12247-020-09525-2

52. Bajracharya R, Song JG, Back SY, Han HK. Recent advancements in non-invasive formulations for protein drug delivery. Comput Struct Biotechnol J 2019;17:1290-308. https://doi.org/10.1016/j.csbj.2019.09.004

53. Bialik M, Kuras M, Sobczak M, Oledzka E. Achievements in Thermosensitive Gelling Systems for Rectal Administration. Int J Mol Sci 2021; 22(11):5500. https://doi.org/10.3390/ijms22115500

54. Aprodu A, Mantaj J, Raimi-Abraham B, Vllasaliu D. Evaluation of a Methylcellulose and Hyaluronic Acid Hydrogel as a Vehicle for Rectal Delivery of Biologics. Pharmaceutics 2019; 11(3): 127. https://doi.org/10.3390/pharmaceutics11030127

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Formulacije bioloških lekova za alternativne puteve administracije – trenutni problemi i perspektive

Sanja Stanković[1], Marija Tasić Kostov[2]

[1]*Apotekarska ustanova „Cvejić“, Niš, Srbija*
[2]*Univerzitet u Nišu, Medicinski fakultet, Niš, Srbija*

S A Ž E T A K

Uvod. Biološki lekovi (biofarmaceutici) predstavljaju novu, obećavajuću terapiju za mnoge bolesti, kao što su karcinomi, hronične inflamatorne bolesti i današnji najveći izazov – COVID-19. Danas se većina bioloških lekova dobija savremenim metodama biotehnologije, a pre svega tehnikom rekombinantne DNK.
Diskusija. Biofarmaceutici tipa proteina/peptida uglavnom se primenjuju parenteralno, zbog nestabilnosti i veličine molekula. Međutim, mnoge kompanije rade na razvoju adekvatnih farmaceutskih oblika biofarmaceutika za alternativne neinvazivne puteve primene, između ostalog i radi poboljšanja terapijske komplijanse. Cilj ovog rada bio je sumiranje trenutnih motivacija, ali i problema u formulacijama biofarmaceutika za alternativne, neparenteralne puteve administracije i pregled pokušaja da se isti prevaziđu. Ovi alternativni putevi primene mogu pružiti mogućnost novog načina preventive i lečenja i oboljenja izazvanog virusom COVID-a-19.
Zakljucak. Akcenat je na težnjama da se monoklonska antitela stabilizuju u specijalne formulacije i sisteme za isporuku leka, čija bi primena bila komfornija, pouzdana i bezbedna. Što se tiče hormona, vakcina i manjih peptida, neke kompanije već su registrovale nazalne sprejeve ili oralne oblike za njihovu primenu.

Ključne reči: **biološki lekovi, alternativni putevi primene**