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RAZVOJ NAPREDNIH *IN SITU* GELIRAJUĆIH SUSTAVA ZA NAZALNU PRIMJENU DONEPEZILA

DOKTORSKI RAD

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DEVELOPMENT OF ADVANCED *IN SITU* GELLING SYSTEMS FOR NASAL DONEPEZIL DELIVERY

DOCTORAL THESIS

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SAŽETAK

Alzheimerova bolest (AB) je kronični i progresivni neurodegenerativni poremećaj. Liječenje AB-a trenutno podrazumijeva simptomatsko liječenje demencije pomoću reverzibilnih inhibitora kolinesteraze. U tu skupinu ubraja se donepezil, lijek prvog izbora za AB. Trenutno je donepezil uglavnom dostupan u obliku oralnih tableta, a takav način primjene ima određene nedostatke, poput gastrointestinalnih nuspojava i loše bioraspoloživosti donepezila u mozgu uslijed slabog prolaska kroz krvno-moždanu barijeru. Jedan od mogućih načina zaobilaska krvno-moždane barijere je nazalna primjena lijekova. Nazalna primjena omogućava izravnu dostavu lijeka u mozak prvenstveno putem olfaktornog područja i u manjoj mjeri putem respiratornog područja nosne šupljine. Učinkovitost nazalno primijenjenog lijeka uvelike ovisi o profilu nazalne depozicije u ciljano područje, vremenu zadržavanja na mjestu primjene te utjecaju farmaceutskog oblika na oslobađanje lijeka i barijerna svojstva sluznice. U ovom radu razvijeni su napredni *in situ* gelirajući sustavi za nazalnu primjenu donepezila: praškasti *in situ* gelirajući sustav – kitozansko-manitolske mikrosfere pripravljene sušenjem raspršivanjem korištenjem ultrazvučne sapnice; i tekući *in situ* gelirajući sustav - termoosjetljiva otopina donepezila, kitozana i β-glicerofosfata. Primjenom statističkog dizajna eksperimenta omogućeno je fino ugađanje formulacijskih, procesnih i/ili parametara primjene s ciljem optimiranja ključnih svojstava *in situ* gelirajućih sustava s donepezilom. Oba razvijena nazalna sustava pokazuju odgovarajući sadržaj donepezila te optimalna reološka svojstva i svojstva raspršivanja, uključujući veličinu čestica/kapljica prikladnu za nazalnu primjenu. Formulacije su optimirane i s obzirom na profil nazalne depozicije, osiguravajući dostavu visokog postotka doze lijeka u olfaktorno područje. Razvijeni farmaceutski oblici pokazuju i poželjna biofarmaceutska svojstva, uključujući biokompatibilnost, mukoadhezivnost, neiritabilnost, produljeno oslobađanje i povećani permeacijski potencijal donepezila, te su zadovoljavajućeg profila stabilnosti. Zaključno, vodeći se načelima kakvoće utemeljene kroz dizajn, razvijeni su napredni *in situ* gelirajući sustavi za nazalnu primjenu donepezila s ugrađenim potencijalom izravne dostave lijeka iz nosne šupljine u mozak. Komplementarni razvoj praškastog i tekućeg sustava omogućio je njihovu usporedbu te predstavlja čvrst temelj za buduća farmakokinetička *in vivo* ispitivanja na animalnim modelima.

Ključne riječi: donepezil, nazalna depozicija, olfaktorno područje, sušenje raspršivanjem, ultrazvučna sapnica, *in situ* gelirajući sustav, kitozan

SUMMARY

Introduction: Alzheimer's disease is a chronic and progressive neurodegenerative disease characterised by memory loss, decline in thinking skills and eventually the ability to carry out daily routine tasks. The main symptom of Alzheimer's disease is dementia. Alzheimer's disease is the most common form of dementia and may contribute to 60 – 70 % of all dementia cases. Currently, the available therapy for Alzheimer's disease is symptomatic treatment. The first drug of choice for the symptomatic treatment of dementia is acetylcholinesterase inhibitor donepezil, which is currently available predominantly in oral solid dosage forms. Donepezil oral delivery is related to several drawbacks such as first pass metabolism, gastrointestinal adverse effects, and low brain delivery efficiency due to low blood-brain barrier penetration. A viable alternative of bypassing the blood-brain barrier is nasal drug administration. Nasal route of administration enables local and systemic drug delivery. Moreover, it is an attractive route of administration for neurological drugs that can be delivered directly from the nasal cavity to the brain, thus circumventing the blood-brain barrier. For efficient delivery of neurological drugs, it is necessary to ensure drug deposition in the olfactory region. The olfactory region of the nasal cavity uniquely provides direct contact of neurons with the external environment, which allows drugs to be directly transported to the brain *via* the olfactory nerve. Also, to a lesser extent, direct nose-to-brain delivery is enabled *via* the trigeminal nerve located in the olfactory and respiratory region of the nasal cavity. Neural nasal drug delivery is characterised by a rapid onset of action, low enzymatic activity and bypass of the gastrointestinal system. The main challenges of nasal drug delivery are related to the complex geometry of the nasal cavity which makes it difficult to deliver the drug to the target area. In addition, the olfactory region is located along the dorsal roof of the nasal cavity and is hindered by the nasal valve, which is the region of the highest airflow resistance. Also, another factor that interferes with the efficient therapeutic outcome of the nasally applied drug is mucociliary clearance. Mucociliary clearance limits the contact time between the drug and the mucosal surface, leading to limited availability of the drug at the site of action. In order to overcome the mentioned obstacles, advanced liquid and powder *in situ* gelling systems are being developed. Liquid *in situ* gelling systems are easy-to-produce platforms that are easily sprayed as a solution and after the deposition at the nasal mucosa they undergo a sol-gel transition in response to certain physiological stimuli (temperature, pH, ionic concentration). Gel formed at the nasal mucosa prolongs retention time and consequently increases drug absorption and bioavailability. Nasal powder *in situ* gelling systems swell in contact with the

nasal mucosa and form a gel, thus prolonging residence time at the site of deposition. Nasal powders offer improved stability profile and increased drug concentration at the nasal mucosa. Also, powders recently demonstrated a higher potential to ensure effective drug olfactory deposition. The most commonly used method for the preparation of nasal powders is spray drying. The aim of this study was to develop powder and liquid *in situ* gelling systems with biopharmaceutical properties and nasal deposition profile suitable for direct nose-to-brain delivery of donepezil. A statistical design of experiments was used to optimise the formulation, process and/or administration parameters with respect to the key properties of the *in situ* gelling systems, including nasal deposition profile. The potential of the developed formulations for efficient and safe delivery of donepezil *via* the olfactory route was compared.

Methods: A statistical design of the experiments (DoE) was implemented for the optimisation of the formulation, process and/or administration parameters, with regard to formulation drug content, physicochemical properties and targeted nasal deposition within the 3D-printed nasal cavity model. For the proper selection of appropriate excipients and in order to set suitable design space, thorough preliminary studies were performed. An advanced powder platform for donepezil nose-to-brain delivery was prepared by spray drying of donepezil, chitosan and mannitol aqueous solution. During the spray drying process, ultrasonic nozzle was used to atomise the feed solution in order to fulfill size-related requests for nasal aerosol particles. Donepezil powder formulations were characterised in terms of: drug content, particle size distribution, residual moisture content, swelling properties, flow properties, spray cone angle and *in vitro* nasal deposition profile. An advanced *in situ* gelling liquid system was prepared as a chitosan-based donepezil loaded thermogelling solution using β-glycerophosphate as a gelling agent. Donepezil thermoresponsive liquid formulations were characterised in terms of: pH, zero-shear viscosity, gelation time and temperature, spray cone angle, droplet size distribution and *in vitro* nasal deposition profile. DoE matrix revealed the optimised leading formulation (both for powder and liquid *in situ* gelling formulations) that was further characterised in terms of stability, *in vitro* release (using automated Franz diffusion cells system), *in vitro* biocompatibility and permeability (using Calu-3 cells), *ex vivo* mucoadhesion (using porcine nasal mucosa), and *in vivo* irritability (using slug mucosal irritation assay).

Results: Donepezil loaded chitosan/mannitol microspheres as dry powder delivery platform were successfully developed. DoE elucidated how formulation, process and/or administration parameters affected crucial properties of donepezil dry powder platform, in particular its nasal deposition profile. Spray drying process resulted in high process yield. Donepezil loaded

microspheres obtained by DoE were characterised by appropriate drug content, residual moisture content below 10 % and favourable swelling. Fine tuned process and formulation parameters resulted in the production of powders with adequate flow properties (Hausner ratio ranged between 1.15 ± 0.06 and 1.30 ± 0.09), appropriate spray cone angle (22.5 ± 0.2 to 28.3 ± 0.9 °) and a great majority of particles larger than 10 μm (D_v10 ranged between 6.7 ± 0.4 – 11.6 ± 0.4 μm). According to the regulatory guidelines for nasal products, the majority of nasal aerosolised particles must be above 10 μm. In this work, the specified criteria was met by using the ultrasonic nozzle in the spray drying process. Several microsphere samples prepared within the DoE matrix resulted in D_v10 above 10 μm, confirming the suitability of the ultrasonic nozzle approach to comply with size-related criteria for nasal aerosol particles. Obtained physicochemical properties of the donepezil dry powder platform, coupled with appropriate administration parameters, ensured targeted nasal deposition upon aerosolization. Optimised drug deposition profile resulted in high percentage of the applied dose (65.5 ± 0.2 %) deposited in the olfactory region. Quality by Design (QbD) approach enabled the selection of the leading donepezil loaded chitosan/mannitol microspheres. The leading formulation showed adequate morphology: microspheres were uniform in size, characterised by spherical shape and smooth surface. Also, the leading donepezil powder formulation showed prominent mucoadhesive properties, prolonged drug release (total drug release in 3 h) and permeation-enhancing properties by reversible opening of tight junctions due to the presence of chitosan and mannitol in the formulation. Biocompatibility was assessed using Calu-3 cell monolayer. Using slug mucosal irritation assay, suitable irritability profile was confirmed: the leading formulation showed no statistical difference in mucus production compared to the negative control. Comprehensive characterisation of the leading donepezil loaded chitosan/mannitol microspheres proved that the formulation fulfilled the needs for efficient brain-targeted delivery.

Liquid *in situ* gelling system for donepezil nose-to-brain delivery was succesfully developed as a thermosensitive solution of donepezil, chitosan and β-glycerophosphate. Namely, in the presence of β-glycerophosphate, chitosan-based formulations exhibit thermogelling properties at the temperature of the nasal mucosa. By varying the aforementioned formulation parameters, thermogelling donepezil formulation was optimised regarding formulation viscosity, gelling and spray properties, as well as its nasal deposition profile within the 3D-printed nasal cavity model. DoE matrix generated moderate viscous solutions (35.03 ± 0.82 - 232.21 ± 2.30 mPa s) that exhibited gelation at the temperature of the nasal mucosa (gelation time ranged between

0.0 ± 0.0 and 14.9 ± 0.2 min; and temperature of instant gelation ranged between 32.2 ± 0.9 and 39.9 ± 0.1 °C). Also, donepezil-loaded thermogelling formulations were characterised by appropriate spray properties (spray cone angle: 15.1 ± 0.3 and 26.6 ± 1.2°; the majority of aerosolised droplets above 10 µm: D_v10 ranged between 23.6 ± 1.4 and 72.3 ± 3.2 µm). All observed properties were largely influenced by the concentration of donepezil and chitosan in the system. By coupling administration and formulation parameteres, the applied DoE resulted in the olfactory deposition reaching a remarkably high 71.8 ± 0.8 % of the applied dose. Nasal deposition studies revealed the potential of the strategy employed to deliver the effective donepezil dose to the targeted region of the nasal cavity. Guided by the QbD principles, leading donepezil liquid *in situ* gelling formulation was identified. For the optimised formulation no cytotoxic effect was observed at the Calu-3 cell line. In addition, the formulation exhibited prolonged drug release ($t_{1/2}$ about 90 min), suitable mucoadhesive properties and prominent permeation enhancing effect in relation to the corresponding donepezil solution. Donepezil-loaded chitosan-based thermogelling formulation showed a potential for safe nasal delivery, demonstrated by an acceptable irritability profile obtained in the slug mucosal irritation assay.

Both powder and liquid donepezil *in situ* gelling systems were proven to be easy-to-produce platforms, with suitable stability profiles. Furthermore, both formulations showed optimal physicochemical and biopharmaceutical properties which are of great importance for an efficient donepezil nose-to-brain delivery. Indeed, nasal deposition studies revealed a high olfactory deposition for both systems. The comprehensive complementary approach is beneficial from a patient point of view, where patients have a choice of choosing the preferred type of formulation and thus maximise adherence to the therapy.

Conclusion: DoE approach enabled the development of donepezil nasal formulations with built-in quality attributes in a cost and time-saving manner. By fine tuning of the formulation, process and/or administration parameters, the manufacturing process resulted in final powder and liquid advanced donepezil *in situ* gelling formulation of desirable properties. High olfactory deposition for both systems indicates the formulation potential to achieve its desired therapeutic outcome in the central nervous system. The systematic and overall approach to the development of the advanced donepezil nasal systems proposed within this work showed encouraging results that can be a starting point for extending the study to an appropriate *in vivo* model for final proof-of-concept.

Keywords: donepezil, nasal deposition, olfactory region, spray drying, ultrasonic nozzle, *in situ* gelling system, chitosan