MENTION « SCIENCES DU MÉDICAMENT »
Parcours M2 : PHARMACOTECHNIE ET BIOPHARMACIE

Informations indispensables

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Titre du Sujet : *Controlled Drug Release from Liquid Crystalline Lipid Nanoparticles for Neuroprotection*

-Scientific context

Among other neurodegenerative diseases, Alzheimer's and Parkinson's diseases are causing a medical and socio-economic burden on an estimated one billion people worldwide and 6.8 million deaths annually. The progressive loss of neurons that results in dysfunction of the cognitive, sensory, behavioral, and motor nervous systems characterize these disorders. Oxidative stress, which results in the production of reactive oxygen species (ROS) and free radical formation, is a common feature of these diseases. This can lead to neurodegeneration and possibly the formation of plaque in the central nervous system.

Lipid-based nanoparticles (LNPs) with inner liquid crystalline organization comprise a new strategy for drug delivery and modulation of the ROS levels in cells and tissues towards neuroprotection and neuro-regeneration. Lyotropic lipid-based nanoparticles (cubosomes, hexosomes, and liposomes) are of interest for antioxidant compounds delivery because their structures favor enhanced encapsulation efficacy and entrapment of active pharmaceutical ingredients. Nanocarriers of cubosome, liposome and hexosome types may improve the drug bioavailability and protect unstable drug molecules, which can be either hydrophilic or hydrophobic substances. Among other phytochemicals with neuroprotective properties, quercetin is a multifunctional compound of low solubility requiring delivery carriers to reach the target site of action.

Controlled release from liquid crystalline lipid nanoparticles (LCNPs) is a recent field of nanomedicine research. Experiments are currently expanded to provide data, which can serve for kinetic modeling of drug release from such controlled drug delivery systems (*e.g.*, using the zero-order model, the first-order model, Higuchi, Korsmeyer-Peppas, Hixson-Crowell, Baker-Lonsdale, Weibull, or Hopfenberg models).

In this analysis, the effects of the lipid nanoparticle shape and structural organization for the drug release profiles are considered crucial. For instance, the contact area of cellular membranes with nanoparticles, studied as drug delivery carriers, is expected to increase for particles of elongated shapes *versus* spherical ones.

-Objectives

The objective of the M2 research is to develop and characterize liquid crystalline lipid nanoparticles (LCNPs) for controlled release of drugs aimed at neuroprotection (*e.g.*, flavonoids such as quercetin). Quercetin (Que) is known for its antioxidant and neuroprotective properties and LCNPs may enhance Que bioavailability by providing controlled drug release. The performed literature review should identify the unresolved questions in the context of controlled release mechanisms from LCNPs for efficient neuroprotective Que delivery. The performed physico-chemical experiments should establish the parameters and the relationships between structure and shape of the LCNPs, their stability, and the cumulative percent drug release over time. Flow cytometry analysis of selected quercetin-loaded LCNPs nanoformulations will be performed in view of potential functional efficacy of the nanoformulations.

-Working program and tasks

1) Lipid nanoparticle preparation

Liquid crystalline lipid nanoparticles (LCNPs) will be prepared by the method of hydration of a lyophilized thin lipid film. The thin film will include different lyotropic lipids and the neuroprotective drug compounds, including quercetin. The components will be mixed at desired proportions. The solvent will be evaporated under a stream of nitrogen gaz to create a thin film, which will be lyophilized overnight using a lyophilizer. After hydration of the thin-film samples, the amphiphilic mixtures will be dispersed using an ultrasonic ice bath and vigorous vortexing in repetitive cycles of agitation.

2) Physico-chemical characterization of LCNPs and investigation of controlled drug release

Physico-chemical experiments will be performed to establish the optimized formulation parameters (*e.g.*, lipid-to-drug ratio, surfactant concentration) determining the stability and drug loading efficiency of the LCNPs. The presence and type of the liquid crystalline lipid phases will be evidenced by small-angle X-ray scattering data. For flavonoid (Que) quantification, UV-Vis and HPLC measurements will be performed.

The effect of the lipid materials (*e.g.,* glyceryl monooleate, fatty acids, and phospholipids mixed in different ratios) on the mesophase structure of the self-assembled LCNPs and controlled drug release profiles will be studied. The role of the stabilizing surfactants (*e.g.*, Poloxamer 407) for the kinetics of the drug release will be evaluated as well. The nature of the release medium will be varied in terms of composition and viscosity in order to establish tendencies or correlations of interest for Que administration to the central nervous system.

Flow cytometry analysis for selected quercetin-loaded LCNPs nanoformulations will be performed. Quercetin has an inhibitory effect on inflammatory responses. Quercetin treatment may inhibit inflammation-induced mitochondrial fission and promote mitochondrial fusion, which are important processes involved in neuronal cell survival. Therefore, some initial experiments will be performed with bioassays to evaluate the functional properties of the quercetin-loaded LCNPs.

3) Analytical evaluation and data analysis

The obtained results will be interpreted in the context of controlled release and neuroprotection. For statistical analysis, appropriate statistical methods to analyze data will be used (e.g., ANOVA, t-tests). The release-kinetics-profile data will be analyzed using different models (*e.g.,* Higuchi model for the rate of drug release from matrix nanodevices, or the applicability of Korsmeyer-Peppas or Weibull mathematical models) in order to understand the flavonoid (Que) drug release mechanisms from LCNPs with different compositions and structural organizations.

-References

[1] Akanchise, T.; Angelov, B.; Angelova, A. Nanomedicine-mediated recovery of antioxidant glutathione peroxidase activity after oxidative-stress cellular damage: insights for neurological long COVID. *J. Med. Virol*. 2024; e29680. https://doi.org/10.1002/jmv.29680

[2] Akanchise, T.; Angelova, A. Ginkgo Biloba and Long COVID: In Vivo and In Vitro Models for the Evaluation of Nanotherapeutic Efficacy. *Pharmaceutics*. 2023, 15, 1562. https://doi.org/10.3390/pharmaceutics15051562

[3] Wu, Y.; Angelova, A. Recent Uses of Lipid Nanoparticles, Cell-Penetrating and Bioactive Peptides for the Development of Brain-Targeted Nanomedicines against Neurodegenerative Disorders. *Nanomaterials***.** 2023, 13, 3004. https://doi.org/10.3390/nano13233004.

[4] Akanchise, T.; Angelova, A. Potential of Nano-Antioxidants and Nanomedicine for Recovery from Neurological Disorders Linked to Long COVID Syndrome. *Antioxidants* 2023, 12, 393. https://doi.org/10.3390/antiox12020393

[5] Rakotoarisoa, M.; Angelov, B.; Espinoza, S.; Khakurel, K.; Bizien, T.; Angelova, A. Cubic liquid crystalline nanostructures involving catalase and curcumin: BioSAXS study and catalase peroxidatic function after cubosomal nanoparticle treatment of differentiated SH-SY5Y cells. *Molecules*, 2019, 24 (17), 3058.

[6] Rakotoarisoa, M.; Angelov B., Garamus, V. M.; Angelova, A. Curcumin- and fish oil-loaded spongosome and cubosome nanoparticles with neuroprotective potential against H_2O_2-induced oxidative stress in differentiated human SH-SY5Y cells. *ACS Omega*, 2019, 4, 3061–3073.

[7] Angelova, A.; Drechsler, M.; Garamus, V.M.; Angelov, B., Liquid crystalline nanostructures as PEGylated reservoirs of omega-3 polyunsaturated fatty acids: Structural insights toward delivery formulations against neurodegenerative disorders. *ACS Omega*, 2018, 3, 3235–3247.

[8] Rakotoarisoa, M.; Angelova, A. Amphiphilic nanocarrier systems for curcumin delivery in neurodegenerative disorders. *Medicines* 2018, 5, 126.

[9] Angelova, A.; Garamus, V. M.; Angelov B.; Tian Z.; Li Y. Zou A. Advances in structural design of lipid-based nanoparticle carriers for delivery of macromolecular drugs, phytochemicals and anti-tumor agents. *Adv. Colloid Interface Sci*., 2017, 249, 331-345.

[10] Angelova, A.; Angelov, B. Dual and multi-drug delivery nanoparticles towards neuronal survival and synaptic repair. *Neural Regeneration Research*, 2017, 12, 886-889.

[11] Guerzoni, L.P.B.; Nicolas, V.; Angelova, A. In vitro modulation of TrkB receptor signaling upon sequential delivery of curcumin-DHA loaded carriers towards promoting neuronal survival, *Pharmaceutical Research*, 2017, 34, 492–505.

[12] Zou, A.; Li, Y.; Chen, Y.; Angelova, A.; Garamus, V. M.; Li, N.; Drechsler, M.; Angelov, B.; Gong, Y. Self-assembled stable sponge-type nanocarries for Brucea javanica oil delivery. *Colloids and Surfaces B: Biointerfaces*, 2017, 153, 310–319.

[13] Géral, C., Angelova, A., Lesieur, S. From molecular to nanotechnology strategies for delivery of neurotrophins: emphasis on brain-derived neurotrophic factor (BDNF). *Pharmaceutics* 2013, 5, 127-167.

[14] Angelova, A.; Angelov, B.; Drechsler, M.; Lesieur, S. Neurotrophin delivery using nanotechnology. *Drug Discovery Today* 2013, 18, 1263-1271.

[15] Angelova, A., Angelov, B.; Mutafchieva, R.; Lesieur, S.; Couvreur, P. Self-assembled multicompartment liquid crystalline lipid carriers for protein, peptide, and nucleic acid drug delivery. *Acc. Chem. Res*. 2011, 44, 147-156.