PLATFORM STANDARDIZATION FOR THE DETECTION OF HISTOPATHOLOGICAL FEATURES IN MURINE MODELS OF HEPATIC AND PULMONARY DISORDERS

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21st- 23rd September 2021

BACKGROUND

The clinical progression in both chronic liver and interstitial lung disorders leads to: 1) chronic parenchymal injury; 2) persistent activation of inflammatory response (macrophages); 3) sustained fibrogenesis (extracellular matrix - ECM accumulation) and 4) aberrant wound healing response. Therapeutic approaches still lack and in humans the treatment efficacy cannot be monitored by histopathological analysis for ethical issues, therefore animal models recapitulating human disorders are required.
Moreover nanoparticles (NPs), nanometer scale complex systems (10–1000 nm), represent an increasingly explored method for enhanced delivery of therapeutics.

AIM

1. Characterize histopathological features of inflammation and fibrosis from mouse lung and liver sections

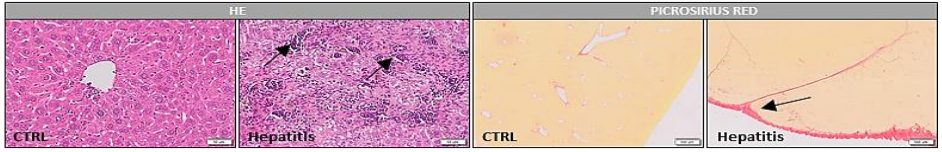
1 HISTOLOGICAL CHARACTERIZATION

METHODS

Tissue preparation

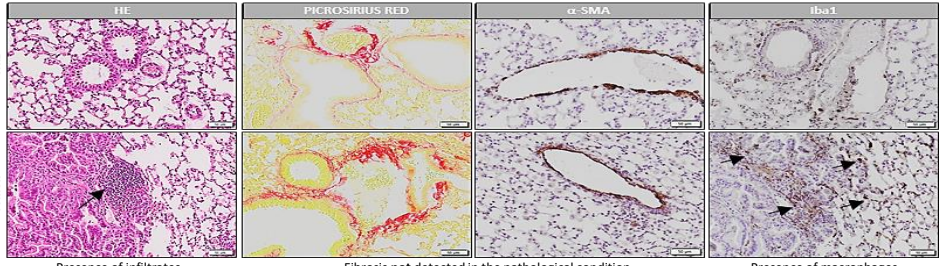
- **Integrity of tissues:** Hematoxylin-Eosin (HE) staining
- **Fibrosis marker:** Picrosirius-red (SR - collagen in red) and anti-α-SMA (smooth muscle actin)
- **Inflammation marker:** microglia/macrophage-specific markers (Iba1)

LIVER: Optimization of the staining protocol for detection of inflammatory cells and fibrosis (positive control).



Histological examination of liver sections revealed the presence of **cellular infiltrates** and subcapsular and interlobular **fibrosis** in the pathological condition (autoimmune hepatitis - arrows) when compared to the control (scale bar 50 µm HE -100 µm SR).

LUNGS: Detection of fibrotic and inflammation marker in tumor bearing mice (negative control).

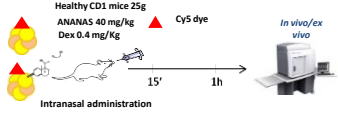


Presence of infiltrates | Fibrosis **not** detected in the pathological condition | Presence of macrophages

2 PRELIMINARY EXPERIMENTS: NANOPARTICLES BIODISTRIBTION IN LUNGS

METHODS

***In vivo* preliminary studies** were carried out on healthy mice. Mice were treated intranasally with a single instillation of the corticosteroid dexamethasone (Dex) linked to nanoparticle and sacrificed at different time points. Optical imaging and confocal microscopy were carried out to localize fluorescently labeled nanosteroids in the whole body and in lung sections.

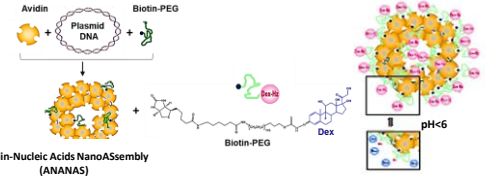


AIM

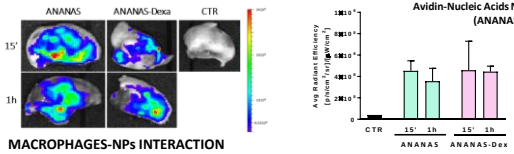
2. Determine the biodistribution of nanoparticles (NPs) in lungs and liver and their interaction with resident macrophages

NANOSTEROIDS

In order to improve the **bioavailability** of intranasally administered therapeutics, one highly efficient modification is to link the drug of interest to the NPs (in this study the steroid dexamethasone).

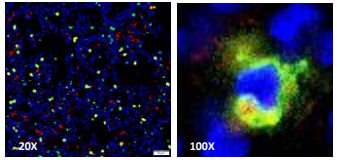


LUNG BIODISTRIBUTION



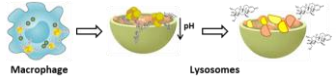
After a single intranasal instillation, an intense and protracted fluorescence signal was detected in **lungs** in both experimental groups with similar distribution and intensity up until 1 h.

MACROPHAGES-NPs INTERACTION



Hoechst (nuclei) CD68 (macrophages) ANANAS (NPs)

The formulations are efficiently internalized in **lung macrophages**. These cells, which are closely involved in inflammatory disease onset and progression of IPF, have a strong ability to recognize, ingest, and degrade foreign materials, and can thus capture circulating NPs of a different nature, making these systems the ideal drug carriers.



RESEARCH PLAN

Optimization of histological procedures on both liver and lung sections ✓

Nanoparticles biodistribution study in lung and liver

Establishment of Idiophatic Pulmonary Fibrosis (IPF) animal model

Work in progress

PROJECT DESIGN and FUTURE PLANS

- Establishment of the IPF animal model: (authorization n° 558/2021-PR)
- Evaluate the biodistribution of ANANAS and pharmacokinetics profile of Dex in IPF.
- Assess the efficacy of ANANAS-Dex in IPF mice and the interaction of the macrophages with the NPs and their role in the pathology.