**Arginase-1 Responsive Lipid Nanoparticles For TNF-α-siRNA Delivery To Alleviate Colitis**

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**Background and aims.** Oral delivery of small interfering RNA (siRNA) remains a major challenge in therapeutic applications due to rapid degradation in the harsh gastrointestinal (GI) environment. Developing a robust delivery system to protect siRNA from enzymatic degradation and ensure its effective intracellular release is critical for clinical translation. Ulcerative colitis (UC), characterized by elevated arginase 1 (Arg1) expression at inflamed colonic sites, presents a unique opportunity for targeted therapy. This study aims to design Arg1-responsive lipid nanoparticles (siRNA-LANPs) using L-arginine-conjugated chitosan (ACS) to achieve tumor necrosis factor-alpha (TNF-α) silencing for UC treatment.

**Methods.** siRNA-LANPs were developed by conjugating ACS with lipid components to enable Arg1-responsive siRNA release. The release kinetics of siRNA-LANPs were evaluated in Arg1-enriched buffer (1 U/mL). Structural integrity and siRNA bioactivity were assessed after 12-hour exposure to simulated GI fluids. Lysosomal escape efficiency and TNF-α silencing capability were analysed *in vitro* using macrophages, with Lipo3000 transfection reagent as a positive control group. For *in vivo* validation, dextran sulfate sodium salt (DSS)-induced colitis mice were treated with siRNA-LANPs, and therapeutic outcomes were compared with sulfasalazine, a first-line UC drug.

**Results.** The Arg1-responsive design enabled rapid siRNA release, with 61.30% cumulative release within 2 h. siRNA-LANPs retained 76.3% structural integrity and 72.3% siRNA bioactivity post-GI fluid exposure. The nanoparticles achieved 29.7% lysosomal escape efficiency, leading to 55.4% TNF-α silencing *in vitro*, comparable to Lipo3000 (58.0%). In DSS-induced colitis mice, siRNA-LANPs outperformed sulfasalazine, significantly reducing disease severity through TNF-α suppression.

**Conclusion/Discussion.** This work presents an innovative, orally administrable siRNA delivery platform leveraging disease-specific enzyme responsiveness. siRNA-LANPs demonstrated exceptional GI stability, targeted release, and potent therapeutic efficacy in UC models, surpassing conventional treatments. The Arg1-triggered design offers a promising strategy for enhancing siRNA bioavailability in inflammatory bowel diseases, highlighting its potential for clinical adaptation.

**Acknowledgement.** This study was supported by the Research Committee of the University of Macau (No.MYRG-GRG2024-00240-ICMS-UMDF), the Science and Technology Development Fund, Macau SAR (No.005/2023/SKL), and the National Natural Science Foundation of China (No.82304411).