Targeting Immunotherapeutic Compounds to Pararectal Lymphatic System to Improve Treatment of Rectal Cancer

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Background: Pararectal lymphatic system is essential for immune surveillance and metastasis of rectal cancer. Imiquimod (IMQ), a TLR7 agonist, activates dendritic cells and enhances antitumor immunity, but shows poor lymphatic uptake via conventional routes. Rectal administration of nano- or micro- sized drug delivery systems can achieve direct lymphatic access while minimizing systemic exposure. Peyer's patches in the rectal mucosa serve as key lymphatic entry points, where M cells facilitate nanoparticle transport to antigen-presenting cells and regional lymph nodes.

This study aims to develop IMQ-loaded lipid nano- and micro-particles (LNPs and LMPs) for enhanced lymphatic targeting via Peyer's patches, improving immune activation and antitumor efficacy in rectal cancer.

Methods: IMQ LNPs were formulated using microfluidics with two lipid systems: DPPC/cholesterol in ethanol (LNP-DP) with Pluronic F127 in buffer (pH 5.0, 7.4, or 8.5), and acetyl palmitate/DSPE-PEG2000 in ethanol (LNP-CP) with Pluronic F68 in water (pH 7.4), at IMQ concentrations of 0.5 or 1.0 mg/mL. Nanoparticles were characterized for size, PDI, zeta potential, encapsulation efficiency (EE%), and drug loading (DL%). TEM was used to assess morphology. Ex vivo evaluation of rectal tissue uptake and lymphatic distribution using confocal microscopy and HPLC is ongoing. In vivo studies to assess biodistribution and pharmacokinetics following rectal administration in rats are planned. Microparticle formulations for higher drug loading are also being developed.

Results: The LNP-DP formulation remained stable across varying aqueous phase pH conditions. However, no significant changes were observed in particle characteristics, and the encapsulation efficiency and drug loading were relatively low (EE%: 13.76 ± 6.80, DL%: 0.65 ± 0.32 at pH 7.4), indicating limited potential for IMQ incorporation. In contrast, the optimized LNP-CP formulation demonstrated significantly improved drug-loading performance. these nanoparticles have uniform and spherical morphology confirmed by TEM with mean particle diameter of 132 ± 45 nm. At an IMQ input concentration of 0.5 mg/mL, the formulation achieved the EE% of 32.02 ± 3.74% and the DL% of 0.67 ± 0.17%. When the IMQ input was increased to 1.0 mg/mL, the EE% slightly decreased to 28.64 ± 4.93%, while DL% increased to 1.01 ± 0.25%, indicating improved drug payload delivery at higher input levels despite minor efficiency loss.

Conclusions: Microfluidic formulation of IMQ-loaded LNPs yields stable nanoparticles suitable for lymphatic targeting via rectal administration. Drug loading levels did not compromise colloidal stability or particle characteristics. Further optimization of formulation and administration parameters is underway to improve lymphatic delivery and therapeutic outcomes.