General Abstract

CCI-001 is a novel computationally developed colchicine derivative, showing increased efficacy and selectivity compared to its parent compound. However, its clinical application is limited by low water solubility and non-specific distribution, resulting in undesirable side effects. The use of nanoparticles (NPs) to encapsulate and deliver CCI-001 may address its limitations and increase tolerability. CCI-001 encapsulation has been reported by Spada et al., who designed albumin-based NPs with improved anticancer efficacy but relatively low encapsulation efficacy and a burst release of almost the total amount of encapsulated drug in the first 24h. This work explores novel encapsulation strategies to enhance drug loading, stability, and controlled release of CCI-001 while preserving its pharmacological activity.

For this reason, two different encapsulation strategies were attempted, the first based on the nanoprecipitation/self-assembly method to obtain nNPs, and the second based on oil-in-water (O/W) emulsions to obtain eNPs.Material and methods are discussed in **Section 3**.

Section 4 focuses on the design and optimization of nNPs. To maximize drug encapsulation, two different polymers were tested as the core formers: the widely used commercial polyester Poly(D,L-lactide-co-glycolide) (75:25), mol wt. 66–107 KDa (PLGA), and a proprietary poly-ε-caprolactone (PCL)-based polyurethane (NS-HC2000). The nNPs comprise a polymeric core, a lipidic monolayer protecting the core composed of of L-α- phosphatidylglycerol (Egg, Chicken) (sodium salt) (EGG-PG) and 1, 2-Distearoyl-sn-glycero-3-phosphoethanolamine-Poly (ethylene glycol) (DSPE-PEG). The optimized nNPs showed small sizes (160–180 nm), narrow size distributions (PDI < 0.3), negative zeta potential (~ -35 mV), and stability in water at 4 °C for up to one week. NS-HC2000 outperformed PLGA, achieving a higher EE under the same preparation conditions (6% vs. 1%).

NS-HC2000-CCI-001-nNPs (CCI-001-nNPs) were further tested on three cancer cell lines, namely U87MG (human glioblastoma multiforme), Mia-PaCa-2 (human pancreatic adenocarcinoma), and OVCAR-3 (human ovarian carcinoma). Cell internalization (FACS and confocal imaging), cell viability (CellTiter 96® AQueous One Solution Cell Proliferation Assay), Caspase 3/7 activation (Apo-ONE® Caspase-3/7 assay), and proliferation inhibition (colony formation assay) were assessed using different concentrations of free drug and encapsulated drug. While U87MG cells were more resistant, CCI-001-nNPs significantly reduced Mia-PaCa-2 and OVCAR-3 viability (to ~30% and ~25%, respectively) after 72 hours at 500 nM. Apoptosis induction and reduced colony formation were confirmed, with minimal toxicity toward healthy fibroblasts (HFF-1 viability >70% at 500 2

nM). These promising results supported the advancement of CCI-001-nNPs to *in vivo* biodistribution and therapeutic studies.

Section 5 investigates the development of PEGylated oil-in-water (O/W) emulsion-based polymeric nanoparticles (eNPs) for the effective delivery of CCI-001. A polymeric-core nanoparticle system was obtained via the O/W emulsion solvent evaporation method. CCI-001 was encapsulated in a hydrophobic poly-ε-caprolactone (PCL)-based polyurethane matrix (NS-HC2000) and coated with polyethylene glycol (PEG) to enhance stealth properties. Different formulation parameters were optimized, leading to PEG-CCI-001-eNPs with an average size of 272 nm, a PDI below 0.3, a zeta potential of approximately -24 mV, and a significantly higher encapsulation efficiency of 25%.

CCI-001-loaded PEGylated nanoparticles (PEG-CCI-001-eNPs) were tested *in vitro* on U87MG, Mia-PaCa-2, and OVCAR-3 cells. While U87MG cells were confirmed to be resistant to treatment in the short term, PEG-CCI-001-eNPs elicited a decrease in viability to around 40% and 36% at 500 nM after 72 hours in Mia-PaCa-2 and OVCAR-3 cells, respectively. Moreover, colony formation assays and Caspase 3/7 activation confirmed that treatment with PEG-CCI-001-eNPs effectively prevented *in vitro* relapses even in the more resistant U87MG cells.

Section 6 provides a comparative evaluation of the physicochemical and *in vitro* results obtained with hybrid core-shell nanoparticles (CCI-001-nNPs), and polymeric nanoparticles (CCI-001-eNPs), critically discussing properties and performances.

Section 7 presents the *in vivo* evaluation of eNP and nNPs in terms of biodistribution patterns and therapeutic efficacy on SCID-CB17 mice bearing subcutaneous tumor xenografts (Mia-PaCa-2). Biodistribution studies using Cyanine7-labeled NPs (Cy7-nNPs and Cy7-eNPs) revealed that both systems exhibited extended systemic circulation and preferential tumor accumulation via enhanced permeability and retention (EPR) effect. Notably, Cy7-nNPs showed progressive tumor uptake, while Cy7-eNPs demonstrated early tumor targeting. Therapeutic efficacy was assessed in tumor-bearing mice treated with a single dose of free CCI-001, CCI-001-nNPs, or CCI-001-eNPs (3 mg/kg). All treatments significantly delayed tumor growth compared to untreated controls, extending the time to reach maximum tumor volume from 14 days (control) to 26 days (treated groups), without observable systemic toxicity or weight loss.

Overall, the study confirmed the suitability of both NPs platforms as they maintained the efficacy of CCI-001, while improving tumor targeting via EPR effect and warrants their further investigation in more relevant tumor models and/or in combinatorial treatment approaches.