Nucleoside Analogue Containing Nanoparticle for Therapeutic Management of Leukemia

Synopsis

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Leukemia represents a heterogeneous group of hematological malignancies characterized by the uncontrolled proliferation of abnormal white blood cells, leading to bone marrow failure and systemic complications. Broadly categorized into acute and chronic forms, leukemia profoundly disrupts normal hematopoiesis and compromises immune defense. Among these, Acute Myeloid Leukemia (AML) stands out as a particularly aggressive subtype, marked by the clonal expansion of immature myeloid blasts in the bone marrow and peripheral blood. Despite therapeutic advancements, AML continues to bear a dismal prognosis, with high relapse rates, multidrug resistance, and severe systemic toxicities associated with conventional chemotherapeutics.

The CD117 (c-Kit) receptor is a transmembrane tyrosine kinase protein which is notably overexpressed in a significant proportion of AML cells. This aberrant expression renders CD117 an attractive molecular target for the selective treatment of AML, as it enables the discrimination of malignant cells from their normal counterparts. In light of this therapeutic potential, the present study focuses on the design and development of an innovative nanotechnology-based approach for the targeted management of AML. Specifically, this work involves the fabrication of poly(lactic-co-glycolic acid) (PLGA) nanoparticles encapsulating the chemotherapeutic agent clofarabine and the nanoparticles are further functionalized on their surface with CD117-specific single-stranded DNA aptamers. These aptamer-conjugated, clofarabine-loaded nanoparticles (Apt-CNP) are designed to enhance the selective delivery of the drug to CD117-overexpressing leukemia cells, thereby maximizing therapeutic efficacy while minimizing off-target cytotoxicity.

This study builds on the combined advantages of nanomedicine and aptamer-based targeting to address the challenges associated with conventional chemotherapy. Clofarabine, which is a second-generation purine nucleoside analogue, has shown strong anti-leukemic activity but its use is often limited by dose-related toxicities and the development of drug resistance in patients. By encapsulating clofarabine within biodegradable PLGA nanoparticles, it becomes possible to achieve sustained and controlled drug release while protecting healthy tissues from widespread exposure. In addition, modifying the surface of these nanoparticles with single-stranded DNA aptamers that specifically recognize CD117 and bind selectively for their uptake by AML cells overexpressing the receptor. This targeted approach helps improve treatment effectiveness while reduce unwanted side effects on normal cells.

The study involved the synthesis of clofarabine-loaded PLGA nanoparticles using the multiple emulsion solvent evaporation technique, and optimization of critical parameters such as particle size, encapsulation efficiency, and drug loading. Conjugation of the CD117-specific aptamer onto the nanoparticle surface was confirmed through agarose gel electrophoresis, which revealed characteristic shifts indicative of successful surface modification. Morphological assessment using Scanning Electron Microscopy (SEM), High-Resolution Transmission Electron Microscopy (HR-TEM), and Atomic Force Microscopy (AFM) confirmed the spherical structure, uniformity, and nanoscale surface topography of the fabricated nanoparticles. Additionally, Fourier Transform Infrared (FTIR) spectroscopy confirmed the potential chemical interactions involved between the drug and the excipients.

In vitro evaluations using HL60 and U937 leukemia cells demonstrated that Apt-CNP exhibited enhanced cellular uptake and cytotoxicity towards HL60 cells compared to U937 cells as U937 cells lack the overexpression of CD117 receptor. Flow cytometry analyses revealed compelling evidence of receptor-mediated endocytosis facilitated by the aptamer's high affinity to CD117. Furthermore, the targeted nanoparticles induced significant mitochondrial depolarization, as evidenced by JC-1 dye assay, corroborating their pro-apoptotic activity at the cellular level.

In addition to in vitro validation, the in vivo pharmacokinetic profile of the Apt-CNP was also investigated. The pharmacokinetic studies revealed prolonged circulation time, enhanced plasma stability, and improved bioavailability of clofarabine when delivered through the aptamer-conjugated nanoparticle system, compared to the free drug. These findings underscore the potential of Apt-CNP in achieving sustained therapeutic levels while mitigating systemic toxicity.

Collectively, this research underscores the promise of aptamer-conjugated PLGA nanoparticles as a novel drug delivery system for targeted AML therapy. The Apt-CNP not only potentiate the efficacy of clofarabine by ensuring targeted drug delivery but also mitigate systemic toxicities and help overcome the drug resistance, thereby address critical gaps in the current AML management.

In conclusion, this study demonstrates the successful development and characterization of aptamer-conjugated, clofarabine-loaded PLGA nanoparticles as a targeted drug delivery system for AML therapy. The in vitro and in vivo findings support their potential to improve drug selectivity and reduce off-target side effects.

Future investigations will focus on scaling up the synthesis process, assessing biodistribution profiles in leukemia models, and evaluating therapeutic outcomes in xenograft models of AML. This work, therefore, contributes meaningfully to the evolving landscape of targeted cancer nanotherapeutics and offers a beacon of hope for improving AML patient outcomes.

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