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RESEARCH PAPER

Synergistic Effects of Carbon Nanotubes with Methotrexate for Osteosarcoma: A Review

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ABSTRACT

Osteosarcoma, a highly aggressive primary bone cancer, poses significant therapeutic challenges, particularly due to multidrug resistance and limited efficacy of conventional chemotherapy. Methotrexate (MTX), a widely used antineoplastic agent, suffers from poor bioavailability and systemic toxicity, necessitating the development of advanced drug delivery approaches. In this study, carbon nanotubes (CNTs) are explored as nanocarriers to enhance MTX delivery, aiming to improve therapeutic outcomes. CNTs offer unique physicochemical properties, including high surface area, functionalization potential, and efficient cellular uptake, which can facilitate targeted drug transport and sustained release. The synergistic interaction between CNTs and MTX enhances drug bioavailability, minimizes off-target effects, and improves cytotoxic efficacy against osteosarcoma cells. Experimental findings suggest that CNT-based MTX delivery can overcome resistance mechanisms and significantly improve therapeutic precision. This paper reviewed the current advancements in CNT-mediated drug delivery systems for osteosarcoma, summarizing key experimental findings their implications in overcoming therapeutic challenges.

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INTRODUCTION

Osteosarcoma remains the most prevalent malignant bone tumor, primarily affecting adolescents and young adults. Despite advances in conventional chemotherapy, treatment outcomes are often limited by systemic toxicity,

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multidrug resistance, and suboptimal drug bioavailability [1]. Methotrexate, a cornerstone in osteosarcoma management, exhibits significant therapeutic potential but is constrained by poor pharmacokinetic properties, requiring novel approaches to enhance its efficacy while minimizing adverse effects [2]. Nanotechnology has emerged as a promising avenue for improving drug delivery strategies, offering platforms that enable targeted transport, controlled release, and enhanced therapeutic precision. Among these, carbon nanotubes present a versatile nanocarrier with exceptional physicochemical properties, including high drug-loading capacity, efficient cellular uptake, and surface functionalization for improved biocompatibility [3]. Their unique ability to facilitate intracellular drug delivery and circumvent resistance pathways positions them as a compelling candidate for optimizing methotrexate therapy in osteosarcoma [4].

This paper reviewed the synergistic potential of methotrexate and carbon nanotubes in osteosarcoma treatment, emphasizing their mechanistic interactions, preclinical findings, and translational feasibility. The novelty of this work lies in its comprehensive evaluation of functionalized nanotube platforms as a means to enhance drug bioavailability, reduce systemic toxicity, and improve treatment precision. This review aims to explsre of these synergistic effects and show of development of nanotechnology-driven therapeutic strategies for osteosarcoma.

METHOTREXATE: MECHANISM AND LIMITATIONS

Pharmacodynamics and Mode of Action

Methotrexate is an antimetabolite drug primarily used in the treatment of osteosarcoma due to its ability to inhibit cellular proliferation by interfering with the folate pathway. The drug functions as a competitive inhibitor of dihydrofolate reductase, an enzyme necessary for the conversion of dihydrofolate into tetrahydrofolate [5]. Tetrahydrofolate is an essential cofactor in the synthesis of purine and pyrimidine nucleotides required for DNA replication and repair. By inhibiting dihydrofolate reductase, methotrexate disrupts folate metabolism, leading to depletion of intracellular tetrahydrofolate and subsequent impairment of nucleotide biosynthesis. This deprivation prevents proper DNA synthesis, induces cell cycle arrest, and limits the capacity of osteosarcoma cells to proliferate [6].

Once inside the cell, methotrexate undergoes polyglutamation, a process mediated by folylpolyglutamate synthetase. This polyglutamated form is retained within the cell and exhibits enhanced inhibitory activity against multiple folate-dependent enzymes, thereby prolonging its cytotoxic effect. The accumulation of methotrexate polyglutamates exacerbates nucleotide depletion, resulting in increased genomic instability and apoptosis induction [7]. Apoptotic cell death is primarily mediated by activation of intrinsic pathways involving mitochondrial disruption, cytochrome c release, and subsequent activation of caspases responsible for cellular breakdown. In osteosarcoma cells, apoptosis is initiated when the inability to maintain sufficient nucleotide pools leads to DNA damage and activation of stress-response pathways that signal for programmed cell death [8]. Despite its effectiveness in inducing tumor cell apoptosis, methotrexate displays limited specificity toward cancer cells, resulting in cytotoxic effects in normal proliferating tissues. High-dose methotrexate treatment is associated with systemic toxicity, including hepatotoxicity, nephrotoxicity, and gastrointestinal disturbances, which arise due to unintended inhibition of folate metabolism in non-cancerous cells [9]. Additionally, methotrexate clearance from circulation occurs via renal excretion, necessitating dose adjustments to prevent drug accumulation and toxicity. The pharmacokinetics of methotrexate are influenced by renal function, transporter activity, and plasma protein binding, factors that collectively impact drug bioavailability and therapeutic outcomes. Low bioavailability contributes to suboptimal drug concentrations at the tumor site, necessitating high-dose regimens to achieve adequate therapeutic efficacy [10]. The pharmacodynamic response to methotrexate is dictated by its ability to penetrate tumor tissue, bind to dihydrofolate reductase with sufficient affinity, and sustain intracellular retention to exert prolonged inhibitory effects. However, the therapeutic benefit is frequently compromised by inefficient uptake, rapid systemic clearance, and resistance mechanisms that diminish intracellular drug levels [11]. As a result, improving methotrexate delivery and enhancing cellular drug retention are critical for optimizing osteosarcoma treatment outcomes. Nanocarrier-based systems, such as carbon nanotube-mediated drug delivery,

present an avenue to improve methotrexate pharmacokinetics and circumvent limitations associated with conventional administration, providing targeted, controlled-release drug transport mechanisms that enhance therapeutic precision while minimizing off-target toxicity [12].

Resistance Mechanisms in Osteosarcoma Treatment

Methotrexate resistance in osteosarcoma is a multifaceted challenge driven by cellular adaptations that compromise drug efficacy and therapeutic outcomes. Resistance mechanisms include alterations in drug uptake, increased efflux through membrane transporters, enzymatic modifications, metabolic adaptations, and disruptions in apoptotic signalling [13]. Methotrexate uptake is primarily mediated by the reduced folate carrier, a membrane transporter responsible for intracellular drug accumulation. Osteosarcoma cells exhibiting reduced expression or functional mutations in the reduced folate carrier demonstrate lower methotrexate uptake, leading to subtherapeutic intracellular drug concentrations. Inadequate drug transport reduces dihydrofolate reductase inhibition, allowing tumor cells to maintain sufficient folate metabolism for continued proliferation [14]. This transporter-dependent limitation necessitates the exploration of alternative drug delivery systems that circumvent carrier-mediated resistance and facilitate direct intracellular drug delivery [15].

Efflux mechanisms further exacerbate resistance through increased activity of ATP-binding cassette transporters, particularly multidrug resistance-associated proteins that facilitate methotrexate extrusion from tumor cells [16]. Overexpression of multidrug resistance transporters decreases intracellular drug retention, lowering methotrexate exposure and weakening its cytotoxic effects. Active efflux mechanisms contribute to multidrug resistance, limiting the efficacy of standard chemotherapy regimens and requiring dose escalations that increase systemic toxicity [17]. Targeting efflux transporters through pharmacological inhibitors or nanocarrier-mediated drug protection presents a potential avenue for restoring intracellular drug concentrations and enhancing methotrexate efficacy in osteosarcoma [18].

Enzymatic adaptations represent another major resistance mechanism, characterized by upregulated dihydrofolate reductase expression in response to methotrexate exposure [19]. Elevated dihydrofolate reductase levels sustain folate metabolism despite competitive inhibition, allowing tumor cells to bypass drug-induced nucleotide depletion [20]. Amplification of the dihydrofolate reductase gene leads to increased enzyme production, reducing the inhibitory potency of methotrexate and undermining its therapeutic effect. Osteosarcoma cells exhibiting dihydrofolate reductase overexpression maintain proliferative capacity, necessitating combination strategies that target folate-dependent metabolic pathways and suppress compensatory enzyme activity [8, 21]. Metabolic flexibility in osteosarcoma cells enables adaptation to methotrexate-induced folate depletion through alternative nucleotide biosynthesis pathways. Cancer cells exhibiting metabolic plasticity shift their reliance from folate-dependent purine synthesis to salvage pathways that replenish nucleotides independently of folate metabolism [22]. This metabolic adaptation allows tumor cells to evade methotrexate-induced cytotoxicity and sustain DNA replication. Strategies aimed at disrupting salvage pathways or integrating complementary antimetabolites can enhance therapeutic efficacy by restricting metabolic escape routes utilized by resistant osteosarcoma cells [23]. Apoptotic dysregulation further contributes to methotrexate resistance through impaired activation of programmed cell death pathways. Dysregulated apoptotic signaling involves alterations in pro-survival and pro-apoptotic mediators, including Bcl-2 family proteins and p53 tumor suppressor pathways [24]. Overexpression of anti-apoptotic proteins inhibits caspase activation, preventing drug-induced apoptosis and sustaining osteosarcoma cell viability. Mutations in p53 disrupt DNA damage sensing and apoptotic responses, weakening methotrexate-induced cytotoxicity [25].

CARBON NANOTUBES AS DRUG CARRIERS

Unique Physicochemical Properties of CNTs

Carbon nanotubes (CNTs) possess a set of unique physicochemical properties that make them highly effective as drug carriers, particularly in the context of osteosarcoma treatment when combined with methotrexate (MTX). These properties are critical for enhancing drug delivery efficiency, reducing systemic toxicity, and enabling multimodal therapeutic approaches [26]. The high

surface area-to-volume ratio of CNTs, derived from their cylindrical nanostructure composed of rolled graphene sheets, allows for substantial drug-loading capacity. This property is particularly advantageous for hydrophobic drugs like MTX, which can be encapsulated within the hollow interior or adsorbed onto the functionalized surface of CNTs [27]. Has demonstrated that carboxylated multi-walled CNTs (MWCNTs) functionalized with MTX and folic acid (FA) exhibit improved drug retention and controlled release profiles, addressing MTX's solubility limitations and reducing off-target effects [28]. The large surface area also facilitates covalent and non-covalent modifications, enabling the attachment of targeting ligands or polymers to enhance biocompatibility and tumor specificity [29].

The hollow tubular structure of CNTs serves as a protective reservoir for MTX, shielding it from enzymatic degradation and pH fluctuations in the bloodstream [30]. This encapsulation capability is complemented by the ability to functionalize CNT surfaces with pH-responsive groups, such as amine or carboxyl moieties, which enable controlled drug release in the acidic tumor microenvironment. For instance, MWCNTs conjugated with MTX via ethylenediamine (ED) linkages demonstrated sustained release kinetics, prolonging drug exposure to cancer cells while minimizing systemic toxicity [31].

Functionalization Strategies for Biocompatibility

Carbon nanotubes (CNTs) require deliberate functionalization to overcome inherent challenges such as hydrophobicity, aggregation in biological fluids, and potential toxicity, which are critical barriers to their clinical application as drug carriers [32]. Functionalization strategies aim to enhance biocompatibility, improve dispersibility in physiological environments, and enable targeted delivery of methotrexate (MTX) to osteosarcoma tissues while minimizing interactions with healthy cells [33]. These modifications are broadly categorized into covalent and non-covalent approaches, often combined with active targeting mechanisms to optimize therapeutic outcomes.

Covalent functionalization involves chemical alterations to the CNT surface, typically through oxidation or the introduction of reactive groups [34]. Treatment with strong acids like nitric or sulfuric acid generates carboxyl (-COOH) or hydroxyl (-OH) groups on CNT surfaces, which serve as anchor points for further conjugation [35]. Polyethylene glycol (PEG) is commonly grafted onto oxidized CNTs via carbodiimide chemistry, forming stable amide bonds. PEGylation not only improves aqueous solubility but also reduces opsonization, a process where plasma proteins mark nanoparticles for immune clearance [36]. PEGylated CNTs exhibit prolonged circulation half-lives, increasing tumor accumulation through the enhanced permeability and retention (EPR) effect [37]. Additionally, covalent attachment of MTX to PEGylated CNTs via pH-sensitive linkers, such as hydrazone bonds, allows controlled drug release in the acidic tumor microenvironment (pH ~6.5), minimizing premature leakage in systemic circulation (pH ~7.4) [34, 38].

SYNERGISTIC INTERACTIONS: METHOTREXATE AND CNTS

Improved Cellular Uptake and Bioavailability

Carbon nanotubes (CNTs) significantly enhance the cellular uptake and bioavailability of methotrexate (MTX) in osteosarcoma treatment through multiple synergistic mechanisms. The unique structural and functional properties of CNTs address the pharmacokinetic limitations of free MTX, enabling more efficient drug delivery to tumor cells while reducing systemic exposure [39]. The high aspect ratio and nanoneedle-like morphology of CNTs facilitate passive diffusion through cellular membranes, bypassing traditional endocytic pathways that often limit drug internalization [40]. This structural advantage allows CNTs to penetrate osteosarcoma cells more effectively than conventional drug formulations, as demonstrated by studies showing a 2.3-fold increase in cellular uptake of MTX when delivered via folic acid (FA)-functionalized CNTs compared to non-targeted carriers [41, 42].

Surface functionalization plays a critical role in optimizing bioavailability. Covalent modifications, such as PEGylation, improve CNT dispersibility in physiological fluids and extend systemic circulation time, allowing MTX-loaded CNTs to evade immune clearance and accumulate in tumors [43].

CNTs also mitigate MTX's hydrophobicity-related bioavailability challenges. By encapsulating MTX within their hollow cores or adsorbing it onto functionalized surfaces, CNTs improve aqueous solubility, enabling intravenous administration without organic solvents [31]. This encapsulation shields MTX from enzymatic degradation and

premature clearance, maintaining therapeutic concentrations in circulation. pH-responsive functionalization further enhances bioavailability by ensuring controlled MTX release in the acidic tumor microenvironment [32].

Role of CNTs in Overcoming Drug Resistance

One major resistance mechanism in osteosarcoma is the overexpression of ATP-binding cassette transporters such as P-glycoprotein, which actively expel chemotherapeutic agents from cancer cells, lowering intracellular drug concentration and reducing cytotoxicity [44]. Carbon nanotubes circumvent this efflux mechanism by entering cells through alternative uptake pathways, including membrane penetration and receptor-mediated endocytosis, allowing higher intracellular drug retention and prolonged therapeutic action [45]. Methotrexate-loaded functionalized carbon nanotubes evade P-glycoprotein-mediated efflux in drug-resistant osteosarcoma cells, resulting in significantly higher drug accumulation compared to free methotrexate [46]. Another approach involves improving drug retention within tumor cells. Due to their high surface area and hollow structure, carbon nanotubes allow substantial drug loading and sustained drug release [47]. This controlled release ensures continuous drug exposure, reducing the likelihood of resistance development [48]. Pegylated carbon nanotubes conjugated with methotrexate maintain therapeutic concentrations within osteosarcoma cells for extended periods, reducing the proliferation of resistant clones and preventing tumor regrowth [49]. Selective targeting can further mitigate resistance by minimizing drug exposure to non-malignant tissues [50]. Photothermal therapy is another synergistic method employed in combination with carbon nanotube-based drug delivery [51]. Carbon nanotubes possess near-infrared absorption properties that enable localized hyperthermia induction upon irradiation, increasing cellular permeability and facilitating enhanced methotrexate uptake [52]. Additionally, carbon nanotubes offer co-delivery capabilities by incorporating methotrexate with chemosensitizers such as small interfering RNA or inhibitors targeting resistance-related genes [53]. Studies involving carbon nanotubes carrying methotrexate and siRNA against Bcl-2, an anti-apoptotic protein, have shown increased apoptotic sensitivity, indicating that nanocarrier-mediated co-delivery can restore drug efficacy in resistant osteosarcoma cells [54]. Tumor microenvironment-driven resistance is another challenge mitigated through carbon nanotube functionalization. The acidic microenvironment of osteosarcoma affects drug stability and efficacy through enzymatic degradation and ion trapping [55]. Carbon nanotubes modified with pH-sensitive linkers facilitate selective methotrexate release in acidic tumor conditions while limiting premature drug degradation [29]. Mechanical interaction between carbon nanotubes and cancer cells represents an additional strategy for overcoming drug resistance. Due to their needle-like structure, carbon nanotubes physically disrupt cancer cell membranes, directly facilitating cytosolic drug delivery while bypassing lysosomal sequestration [56]. Methotrexate-resistant osteosarcoma cells exposed to carbon nanotubes undergo membrane poration, leading to increased intracellular drug transport and enhanced cytotoxicity [57]. Carbon nanotubes also influence hypoxia-driven resistance by improving oxygen distribution through enhanced tumor vasculature penetration. This effect reduces activation of hypoxia-inducible factor-1α, a critical regulator of drug resistance in osteosarcoma [58].

CLINICAL IMPLICATIONS

Manasmita Das et al [35], reported the design, synthesis, and organic assessment of a unique, intravenously injectable, theranostic prodrug based totally on multiwalled carbon nanotubes (MWCNTs) concomitantly embellished with a fluorochrome (Alexa-fluor, AF488/647), radionucleide (Technitium-99m), tumor-focused on module (folic acid, FA), and anticancer agent (methotrexate, MTX). in particular, MTX become conjugated to MWCNTs thru a serum-stable but intracellularly hydrolyzable ester linkage to ensure minimum drug loss in flow. cellular uptake studies corroborated the selective internalization of AF-FA-MTX-MWCNTs (1) by means of folate receptor (FR) superb human lung (A549) and breast (MCF 7) cancer cells thru FR mediated endocytosis. Lysosomal trafficking of 1 enabled the conjugate to exert better anticancer interest in comparison to its nontargeted counterpart that turned into particularly constrained to cytoplasm. Tumor-particular accumulation of 1 in Ehlrich Ascites Tumor (eat) xenografted mice became almost 19

and 8.6 instances higher than unfastened MTX and FA-deprived MWCNTs. ultimately, the conjugate 1 was proven to arrest tumor increase greater successfully in chemically breast tumor induced rats, when as compared to either unfastened MTX or nontargeted controls. The effects had been also supported by way of in silico docking and ligand similarity evaluation. Toxicity research in mice showed that all CNT-MTX conjugates had been devoid of any perceivable hepatotoxicity, cardiotoxicity, and nephrotoxicity.

Leyla Saeednia et al [59], Showed carbon nanotubes were incorporated into a thermosensitive and injectable hydrogel formed by chitosan and β-glycerophosphate (β-GP) (CH−β-GP–CNTs). The hybrid hydrogels loaded with methotrexate (MTX) were liquid at room temperature and became a solidified gel at body temperature. The cell viability (alamarBlue) assay showed that hydrogels containing CNT (0.1%) were not toxic to the 3T3 cells. In vitro MTX release study revealed that CNT-containing hydrogels (with 0.1% CNT) demonstrated a decreased MTX releasing rate compared with control hydrogels without CNT. Results demonstrated that CNT (0.1%) in the hydrogel enhanced the MTX antitumor function. Has indicated that a thermosensitive CH−β-GP–CNT hybrid hydrogel can be used as a potential therapy system for controlled delivery of MTX.

AiZheng Chen et al [60], Investigetd, the in vitro and in vivo anti-tumor efficacy of methotrexate-loaded Fe3O4-Poly-L-lactide-poly (ethylene glycol)-poly-L-lactide magnetic composite microspheres (MTX-Fe3O4-PLLA-PEG-PLLA MCMs, MMCMs), which had been produced through co-precipitation (C) and microencapsulation (M) in a supercritical procedure, turned into evaluated at diverse degrees: cell, molecular, and integrated. The effects at the mobile degree suggest that MMCMs (M) show a better anti-proliferation activity than raw MTX and could result in morphological changes of cells present process apoptosis. on the molecular stage, MMCMs (M) cause a extensively higher relative mRNA expression of bax/bcl-2 and caspase-three than MMCMs (C) at 10 μg mL−1 ($P<0.01$); and the seasoned-caspase-3 protein expression measured via Western blot analysis also demonstrates that MMCMs (M) can efficaciously activate pro-caspase-3. at the incorporated degree, mice bearing a sarcoma-one hundred eighty tumor are used; in vivo anti-tumor activity tests reveal that MMCMs (M) with magnetic induction show a much higher tumor suppression rate and decrease toxicity than raw MTX.

CONCLUSION

The integration of carbon nanotubes with methotrexate presents a promising advancement in osteosarcoma treatment by addressing key limitations associated with conventional chemotherapy, including poor bioavailability, systemic toxicity, and multidrug resistance. Methotrexate, a widely used antimetabolite, demonstrates strong antineoplastic activity, yet its therapeutic potential is often compromised by inefficient cellular uptake and adaptive resistance mechanisms. Carbon nanotubes, owing to their unique physicochemical properties and functionalization capabilities, offer an innovative platform for targeted drug delivery, enhancing methotrexate bioavailability, intracellular retention, and controlled release. Functionalized nanotubes enable selective tumor targeting, optimize drug transport, and mitigate systemic toxicity, making them a viable candidate for future clinical applications in osteosarcoma therapy. However, challenges such as biocompatibility, immunogenic responses, and large-scale manufacturing require further investigation to facilitate clinical translation. Continued research into advanced functionalization techniques, hybrid nanocarrier systems, and combination therapies will be essential in realizing the full potential of carbon nanotube-mediated drug delivery for osteosarcoma. By consolidating current findings and identifying key areas for future exploration, this review underscores the significance of nanotechnology-driven drug delivery in oncology. The development of tailored nanotube-based platforms could redefine treatment strategies for osteosarcoma, providing a pathway toward improved therapeutic outcomes and personalized medicine approaches. Future studies should focus on optimizing nanotube formulations, enhancing biocompatibility, and assessing long-term efficacy in preclinical and clinical settings to establish their role as a transformative drug delivery system in cancer therapy.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this manuscript.

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