Materials Research Express

PAPER

A feasible strategy of fabricating camptothecin (SN38)-loaded holmium ferrite nanocarrier delivery for glioma treatment

Yu Zeng, Huihui Chen, Fan Yang, Huiyong Li and Pengxiang Yang

Department of Neurosurgery, the First People’s Hospital of Wenling, Wenling 317500, People’s Republic of China

Mailing address: No. 333, Chuanan South Road, Chengzi Street, Wenling-317500, China.

E-mail: pengxiang.y@yahoo.com

Keywords: biocompatibility, holmium ferrite, cytotoxicity, glioma cancer, SN38

Abstract

Malignant gliomas are the most prevalent and deadly primary brain tumors. The life expectancy of people with gliomas only slightly increases through surgical procedures, radiation, and chemotherapy. Magnetic nanocarriers must be developed to enable drug delivery using a magnetic field. A utilized to fabricate holmium ferrite nanoparticles is described herein. β-Cyclodextrin-polyethylene glycol (PEG) conjugate is used as a coat for the holmium ferrite nanoparticles. X-ray diffraction, energy dispersive x-ray spectroscopy, and x-ray photoelectron spectroscopy are all used to study the nanoparticles. This size range of nanoparticles is optimal for efficient drug delivery. The in vitro cytotoxicity of the fabricated nanoparticles was examined using U87MG and LN229 glioma cancer cells. The acridine orange/ethidium bromide and nuclear staining methods examined the morphological changes in the U87MG and LN229 glioma cells. The mode of cell death mechanism was investigated by Annexin V-FITC/PI flow cytometry methods. The possibility for successful SN38 delivery for the treatment of glioma cancer exists with the SN38@HF-β-CD-PEG.

1. Introduction

Cancer is still the leading cause of death from the disease worldwide. Despite progress, the death rate from cancer remains disturbingly high. There were 33,000 new instances of central nervous system (CNS) malignancies in 2016, resulting in 2,27,000 fatalities [1–3]. Glioma is the most frequent primary central nervous system tumor, at a rate of 3–8 new cases per 1,00,000 persons per year (40%–50%). Gliomas are divided into four stages by the World Health Organization (WHO): Malignant brain tumors of grades I and II include astrocytomas, oligodendroglomas, dysmembryoplastic neuroepithelial tumors, gangliogliomas, and mixed gliomas [4]. High-grade gliomas (malignant gliomas, MG) such as anaplastic astrocytoma, oligodendroglioma, ependymoma, glosarcoma, and glioma are classified as WHO grades III and IV. More aggressive and with a worse prognosis is MG [5]. Less than 5% of patients diagnosed with grade IV survived for at least five years. Clinical treatment often consists of one or more standard methods: surgery, radiation therapy, or chemotherapy [6–8]. However, precise excision is challenging due to the tumor’s infiltrative growth pattern [9]. Further, radiation and chemotherapy have significant and unfavorable effects on quality of life [10].

The FDA-approved anticancer drug irinotecan (camptothecin-11) is metabolized into the active metabolite SN38 (7-ethyl-10-hydroxyl camptothecin). Carboxylesterase-mediated cleavage in the liver converts inactive irinotecan to the bioavailable form SN38 after irinotecan intake [11]. So, in humans, only 1%–9% of an intravenous dosage of irinotecan gets changed into SN38. A hundred to a thousand times as effective as irinotecan is SN38. Its cytotoxic properties arise from its inability to perform topoisomerase I inhibition, an enzyme essential for properly regulating DNA replication and transcription [12]. Due to its high efficacy, SN38 is considered a treatment for numerous types of cancer, including lung, ovarian, breast, and colorectal. Since SN38 is not well soluble in water or other common pharmaceutical solvents, its therapeutic utility is limited [13]. Preventing the inactivation of anticancer medications of the camptothecin family by metabolism to the
carboxylate form, which occurs under normal body conditions, is a significant challenge [14–16]. A tight lactone ring in SN38’s structure makes it active at pH 5.0, but at physiological pH, the ring opens, transforming SN38 into an inert carboxylate system [17]. Therefore, therapeutic advantages and clinical efficacy must be achieved to design a formulation capable of retaining the active form of SN38, in addition to tackling the formulation difficulties of SN38 linked to its solubility in an aqueous solution [18].

Holmium orthoferrite (HoFeO₃), a member of the perovskite family with a distorted ABO₃ orthorhombic structure of YFeO₃ type, is the focus of much recent research. HoFeO₃’s magnetic and dielectric behavior is due to the symmetry of its structure, which has led to its potential use in a wide range of industries and applications [19]. One such application is magnetotherapy products, which have effectively alleviated acute and chronic pain associated with several different injuries and fractures. In addition, HoFeO₃ as a dopant in different composite ceramics, has been the subject of much research [20]. It is well-known that the phase composition, structural characteristics, size, shape, and morphology of the resulting nanocrystals all play a significant role in determining the functional qualities of these materials [21]. Though there are many established techniques for making nanostructured ferrites and orthoferrites (including mechanochemical, sonochemical, hydrothermal, sol-gel, and other syntheses), achieving the desired functional properties of the resulting nanocrystals requires the use of sophisticated equipment and a wide range of synthesis parameters [22]. The powders of hexagonal orthoferrites of different compositions have been produced in various ways, as evidenced by numerous published publications on the subject [23]. In contrast to the strategies, the glycine-nitrate combustion process yields a powder in only a few minutes, has excellent phase homogeneity in the end product, and necessitates no elaborate or costly apparatus [24]. In contrast to other methods of producing rare-earth orthoferrites, solution combustion synthesis (SCS) has the added benefit of yielding both the orthorhombic modification and the metastable hexagonal form [25]. The authors achieved many hexagonal orthoferrite nanopowders, previously produced only by thin-film synthesis, using SCS [26–28]. Despite widespread interest in using glycine-nitrate combustion to make functional materials, no comprehensive studies have been conducted to determine how the combustion conditions affect the phase and chemical composition, morphology, structural, and magnetic features of hexagonal and orthorhombic HoFeO₃ [29–31].

Here, we describe the fabrication and characterization of holmium ferrite nanoparticles (NPs), their coating with a β-cyclodextrin (CD)-PEG conjugate, and the loading and release of the drug 7-ethyl-10-hydroxycamptothecin (SN38), and an anticancer investigation of the SN38-loaded NPs against glioma cancer cell lines. The cytotoxicity and cellular internalization of the SN38-loaded NPs in glioma cancer cells in vitro. The purpose of taking both U87MG and LN229 cells was to assess the efficiency of the proposed nanoformulation in glioma cells to gain a solid sense of the effectiveness of the nanoformulation for glioma cancer treatment. Various apoptosis staining methods examined the cancer cells’ morphological properties.

2. Experimental methods

2.1. Reagents and materials

β-Cyclodextrin, p-toluene sulfonyl chloride, polyethylene glycol; (MW 200), ethylene diamine, ammonium chloride, aqueous ammonia solution, and 7-Ethyl-10-hydroxycamptothecin (SN38) were obtained from Sigma-Aldrich (USA). Dimethyl sulfoxide (DMSO) was acquired from Shanghai Aladdin Bio-Chem Technology Co., Ltd (China). Dichloromethane (DCM) and other solvents were obtained from Shanghai Macklin Biochemical Co., Ltd (China). Formulated Dulbecco’s modified eagle’s medium (DMEM), fluorescein isothiocyanate (FITC), 4’,6-diamidino-2-phenylindole (DAPI), acridine orange/ethidium bromide (AO/EB) were ordered from Thermo Fisher Scientific, Inc. (USA). 3-(4,5-dimethylthiazol-2-yl)-2,5-Diphenyltetrazolium bromide (MTT) was acquired from Shanghai Aladdin Bio-Chem Technology Co., Ltd (China).

The final product’s transmission electron microscopic (TEM) image was taken using a JEOL-2100F transmission electron microscope (JEOL, Japan). Fourier transform infrared (FT-IR) KBr/sample pellet spectra were measured from a Nicolet 6700 FT-IR spectrometer (Thermo Fisher Scientific, USA). The crystallization form was analyzed using an XRD-7000 x-ray diffractometer (Shimadzu, Japan) with CuKα radiation (λ = 1.5406 Å). Surface chemistry was determined by x-ray photoelectron spectroscopy (XPS) using an ESCALAB 250Xi x-ray photoelectron spectrometer (Thermo Fisher Scientific, USA). The nanoparticles’ hydrodynamic size and zeta potential were measured through dynamic light scattering (DLS) via Zetasizer Nano ZS90 (Malvern Instruments, UK).

The human glioma cancer cells (U87MG and LN229) were acquired from the Cell Bank of the Chinese Academy of Sciences (Shanghai, China). U87MG and LN229 glioma cancer cells were cultured in Dulbecco’s Modified Eagle’s Medium (DMEM) medium supplemented with 10% (v/v) fetal bovine serum (FBS) and 1% antibiotics (penicillin/streptomycin, 10,000 U ml⁻¹) at 37 °C in a humidified atmosphere containing 5% CO₂.
2.2. Fabrication of holmium ferrite NPs (HFNPs) and β-CD-PEG conjugate

A solution containing 1 M holmium nitrate and 2 M ferric nitrate was synthesized. The addition of ammonium hydroxide was followed by stirring with a magnetic stir bar. For the next 18-h, the solution was heated to 180 °C within a hermetically sealed autoclave. The acetone was used to clean the solution and disperse the NPs that had been suspended in it.

PEG-bis-(tosylate) was synthesized according to a previously published method. Polyethylene glycol (1.0 ml), triethylamine (3.2 ml), and DCM (250 ml) were typically mixed at 45 °C for 35 min. We next added tosyl chloride (3.9 g) to 150 ml of DCM and stirred the reaction mixture for 4-h. Products were cleaned and dried. DCM was added to a mix of bis-sulfonate-PEG (1 g) dissolved in ammonia (30%) and agitated for 1-h. H2N-PEG-NH2 was precipitated after the solvent was removed by evaporation and diethyl ether addition. In DMSO (5 ml), PEG-bisamidine (3.0 g) and 6-p-toluenesulfonyl β-cycloextrin (1.0 g) were heated to 65 °C for 12-h before being cooled and put into acetone. Filtration, acetone washing, and drying were all used to finalize the product. The HFNPs (10 mg) were added to 20 mg of the β-CD-PEG polymer in a slightly basic aqueous medium (pH 8.0, adjusted using NaOH).

2.3. Drug loading and release

On a microbalance, SN38-load HFNPs were weighed and dispersed in Phosphate Buffer Saline (PBS), maintained at pH 7.4. The concentration of SN38 was calculated by measuring its absorbance at 368 nm. The percentage of loaded drugs is given by

\[
\text{% Adsorption efficiency} = \left( \frac{\text{Weight of SN} - 38 \text{ in HFNPs}}{\text{Weight of HFNPs}} \right) \times 100
\]

The SN38-loaded NPs were suspended in dialysis bags, dipped in PBS solution (pH 7.4 and 6.0 separately), and a mechanical shaker was shaken at room temperature. The solutions released from the bags were withdrawn at pre-planned time intervals. The concentrations of the solutions were determined using UV-vis spectroscopy. The average values obtained from three different intervals were utilized for plotting the drug release rate [32].

The in vitro SN38 release profile was studied, employing the previous method’s reported method. SN38-loaded HFNPs were put in dialysis bags with a molecular weight cut-off range of 100 Da. The buffer solution PBS was utilized, and two different pHs, 7.4 and 6.0, were set up to measure the release of drugs of the pHs. UV-vis absorbance values were calculated for the solutions withdrawn.

2.4. Evaluation of in vitro cytotoxicity

The MTT analysis was performed to determine whether the optimized formulation (SN38@HF-β-CD-PEG) was cytotoxic to U87MG and LN229 cell lines and normal HEK293 l929 cell lines in vitro. Results were compared to those obtained with unconfined SN38. Cells were grown on a 96-well culture plate and DMEM culture medium (10% cells per well). After a 24-h incubation in an incubator, cell seeds were treated with free HFNPs, SN38, and SN38@HF-β-CD-PEG (at different concentrations) before being re-incubated for 24-h. Each cultured cell was removed after incubation. Following a 4-h incubation in a microplate solution, cells were treated with dimethyl sulfoxide solution. The percentage of live cells was determined using a microplate reader at 540 nm. The viability percentage was examined by previous literature [33–36].

2.5. Morphological examination

The efficacy of SN38 and SN38@HFNPs to trigger apoptosis in U87MG and LN229 cells were assessed by the AO/EB dual staining approach. U87MG and LN229 cells were plated in 6-Well plates (10⁶ cells) and incubated with HFNPs, free SN38, and SN38@HF-β-CD-PEG at 1C₅₀ concentration. The plates were incubated for 24-h at 37 °C with 5% CO₂. After incubation with AO/EB dual staining, the cells were analyzed using a fluorescence microscope to determine apoptotic cell death [37–39].

Nuclear morphological alterations, such as fragmentation or condensation, linked with apoptosis can be evaluated using DAPI labeling. In this assay, U87MG and LN229 cells were seeded at a density of 10⁶ cells well⁻¹ in six-well plates before being treated with the indicated concentrations of HFNPs, free SN38, and SN38@HF-β-CD-PEG and incubated for 24-h at 37 °C in 5% CO₂. Following incubation, the cells were rinsed twice with PBS for 15 min. After incubation with a DAPI staining solution, the cells were analyzed using a fluorescence microscope to determine apoptotic cell death [40–42].

2.6. Apoptosis investigation by flow cytometry

U87MG and LN229 cells were seeded at a density of 10⁶ cells well⁻¹ in six-well plates before being treated with the indicated concentrations of HFNPs, free SN38, and SN38@HF-β-CD-PEG and incubated for 24-h at 37 °C in 5% CO₂. After treatment with cells, 100 μl of staining buffer (5 μl of annexin-V and 5 μl propidium iodide (PI)
were added to each flow cytometry tube and incubated at 4 °C in the dark for 15 min. Samples were examined using the SYSMEX flow cytometer [43–45].

3. Results and discussion

3.1. Nanoparticles characterization

Nanoparticles’ pharmacokinetic profile and absorption in the blood are greatly improved when they are adequately sized and have their surfaces coated with organic or inorganic material. Particle size in the 10 to 200 nm range allowed for optimal penetration of cancer cells and increased cancer cell inhibition [46–48]. The potential therapeutic use of drug-loaded HFNPs (SN38@HF-β-CD-PEG) depends on their excellent drug-loading efficiency, extended drug release, cytotoxic profile, cellular internalization, and morphological changes (figure 1).

β-CD-PEG was used to coat the NPs. X-ray powder diffraction was used to characterize the polymeric-coated NPs (HF-β-CD-PEG) (figure 2(A)). It may be inferred from the sharpness of the peaks that the ferrite is created in a suitable crystallinity manner. The coated polymer produces a diffraction pattern with a curvature of less than 20°. No distinct holmium oxide phase is generated, as the peaks do not coincide with those previously described for Ho2O3. They are consistent with the XRD pattern for holmium-doped Fe2O3 that has been described. In the presence of Ho3+, the lattice of Fe3O4 (JCPDS file, No. 19-0629) undergoes deformation, producing peaks at 2θ values of 20, 33, 36, 53, 58, 61, 63, and 65° and doping with Ho3+ causes distortion in the fcc crystal. The adsorbent of Ho3+ ions alone does not arise because it does not induce distortion in the lattice. The transmission electron micrograph (TEM) image of HF-β-CD-PEG (SN38@HFNPs) is shown in figure 2(C). The NPs’ surface coating of thin polymer seems clear. The NPs appear somewhat spherical and measure 75 ± 6 nm in size. The polydispersity index (PDI) and zeta potential of β-CD-PEG-HFNPs were displayed at 0.105 ± 0.05 and -11 ± 2.4 mV, respectively. As-prepared HFNPs EDX is shown in figure 2(B), and Ho, Fe, and O can all be displayed. No further lanthanide impurities were found in the HF. The ferrite is identified as HoFe2O4 using ICP-AES. β-cyclodextrin-polyethylene glycol (β-CD-PEG) was used to coat the NPs. TGA results for the coated NPs are presented in figure 2(D). The HF-β-CD-PEG and SN38@HF-β-CD-PEG nanoparticles were stable under an aqueous solution and cell culture media on different days. The stability of the fabricated nanoparticles was examined by the dynamic light scattering (DLS) measurement (figure 3). This good stability is essential for using GNP probes in biological sample studies. Up to 180 °C, the polymer releases water, resulting in a weight loss of around 10%. Up to 370 °C, further weight loss of 18% occurs when the surface polymer melts. The subsequent...
Figure 2. Nanoparticles characterization. (A) X-ray diffraction profile. (B) EDX spectral analysis of fabricated HFNPs. (C) TEM image of β-CD-PEG-HFNPs. (D) Thermogravimetric (TGA) analysis.

Figure 3. The stability of the nanoparticles (β-CD-PEG-HFNPs and SN38@β-CD-PEG-HFNPs) was examined by the dynamic light scattering (DLS) method. The nanoparticles were incubated with an aqueous solution and cell culture DMEM media on different days.
mass loss (530 °C–800 °C) results from β-CD-PEG polymeric degradation. The XPS of the polymer-coated HFNPs is interpreted as follows: The 2P$_{3/2}$ and 2P$_{1/2}$ electrons show peaks at 711.1 and 724.4 eV, respectively. These values correspond to the typical values of Fe$^{3+}$ in ferrite. The indicative regions of Ho 4d binding energies are observed in the spectrum, viz., 163.0 and 160.8 eV, typical of Ho 4d$_{5/2}$ and Ho 4d$_{3/2}$, respectively. The XPS spectrum of O 1 s shows that the peaks correspond to the Fe–O oxygen and the oxygens in the coated polymer. The Fe–O peak is observed at 530.1 eV, the C–O peak at 532.8 eV, and the C=O peak at 531.2 eV. Similarly, the C 1 s peaks correspond to C–C (284.9 eV), C–O–C (286.9 eV), and O–C=O (288.3 eV) bonds (figures 4(A)–(D)).

The FT-IR spectral analysis of the HFNPs is displayed in figure 5(A). The HFNPs appear to have a modest absorption band at 1350–1750 cm$^{-1}$. The β-CDPEG-coated HFNPs display absorption peaks from C–C stretching (1421 cm$^{-1}$) and –C–N stretching (1631 cm$^{-1}$). The Fe–O absorption peaks are found at 581 cm$^{-1}$. These show that the HFNPs are covered with a layer of β-CD-PEG. Additionally, the vibration and stretching bands at roughly 1650 and 3500 cm$^{-1}$ are attributed to the stretching and bending vibration of the water peaks.

3.2. Drug loading and the release of drug
The anticancer drug SN38 was loaded in the carrier HF-β-CD-PEG (SN38@HFNPs) as per the procedure discussed in the experimental section. The drug loading percentage, determined by equation (1), is 92% UV-vis spectroscopy was employed to calculate the concentration of SN38, measuring the absorbance at 385 nm.

The in vitro release profiles of SN38 are shown in figure 5(C). The cumulative release of SN38 as a function of time reveals that the release is slow and sustained. The initial rapid release rate of SN38 becomes near constant after 20-h when the pH is 7.4. The cumulative release percentage is about 39%. A decrease in pH modulates the drug release. The cumulative release percentage increases, reaching a plateau of about 66% after 18-h. This result reveals that the release of SN38 is slow and sustained and occurs well above 48-h. Further, the release of the drug is tunable by varying the pH value.

3.3. Magnetic properties
The as-prepared HFNPs exhibit the sigmoidal magnetization curves (figure 5(B)) characteristic of superparamagnetic NPs, with no hysteresis loop. We find a Ms value of 43 emu g$^{-1}$ for the saturation magnetization. In other words, superparamagnetic NPs quickly relax when an external magnetic field is
removed. It can be used for medication aided by magnetic fields and magnetic hyperthermia therapy for cancer. Adding a $\beta$-CD-PEG coating on as-prepared HFNPs reduces the Ms and the magnetization shape.

3.4. In vitro cell viability assay
Assessing the NP’s cytotoxicity toward cancer cells after production is essential for drug delivery. Figure 6 shows that the cytotoxicity of SN38@HF-$\beta$-CD-PEG, free HFNPs, and free SN38 was tested using an MTT assay against U87MG and LN229 glioma cancer cell lines. Cytotoxicity toward U87MG cells was lower for HFNPs without SN38 compared to SN38@HFNPs. More than 70% cell viability was displayed with HFNPs, but SN38@HF-$\beta$-CD-PEG demonstrated much higher toxicity than free SN38 or HFNPs at the same dose. For U87MG cells, the IC$_{50}$ value for SN38@HF-$\beta$-CD-PEG was determined to be 15.25 ± 2.54 $\mu$g ml$^{-1}$. Comparatively, the toxicity of HFNPs to LN229 cells was much lower than that of U87MG cells. However, the cytotoxicity of SN38@HF-$\beta$-CD-PEG and SN38 on LN229 cells was much more significant. Similarly, U87MG and LN229 cells were more sensitive to SN38@HF-$\beta$-CD-PEG than free SN38 and HFNPs. For SN38@HF-$\beta$-CD-PEG, we measured an IC$_{50}$ of 16.29 ± 2.87 $\mu$g ml$^{-1}$. The data from both cell lines imply that SN38@HFNPs may have clinical use in treating glioma cells. The cytotoxic effects of SN38@HF-$\beta$-CD-PEG, free HFNPs, and free SN38 on normal cell line HEK293 and L929 cells were also determined by the MTT assay. Cells were grown for 24 h and treated dose-dependent (figure 7). The treatment of the highest SN38@HF-$\beta$-CD-PEG, free HFNPs, and free SN38 caused cytotoxicity on HEK293 cells, whereas treatment with high SN38 showed cell cytotoxicity observed. Overall, the fabricated nanoparticles don’t affect the normal cells from this investigation.

3.5. Morphological examination of glioma cells
The AO-EB double-staining approach was used to examine the morphological alterations associated with apoptosis after NPs exposure. At 24 h at the IC$_{50}$ concentration, apoptotic characteristics such as condensed nuclei, membrane blebbing, and apoptotic bodies were seen in U87MG and LN229 cells treated with SN38,
HFNPs, and SN38@HF-β-CD-PEG (figure 8). Viable, early apoptotic, late apoptotic, and necrotic phenotypes were assigned to the labeled cells. Because of nuclear fragmentation and chromatin condensation, early apoptotic cells fluoresced a fluorescent solid green, while late apoptotic cells fluoresced an orange because of nuclear condensation, nuclear shrinkage, and blebbing [52]. The red necrotic cells were easily spotted. However, the nuclei of the control cells remained unchanged and resembled green (viable). The AO/EB staining outcomes reveal that the SN38@HF-β-CD-PEG efficiently kills the glioma cancer cells by observing fluorescence microscopy.

Cells were investigated independently using DAPI staining methods to establish that the apoptotic cell death produced by HFNPs, free SN38, and SN38@HF-β-CD-PEG was linked with nuclear condensation and fragmentation. U87MG and LN229 cells were stained with DAPI and evaluated under a fluorescence microscope following exposure to HFNPs, free SN38, and SN38@HF-β-CD-PEG for 24-h at the IC50 concentration. Intercalating agent DAPI attaches to DNA in the spaces between individual bases. In this investigation, SN38@HF-β-CD-PEG produced nuclear alterations in cells undergoing apoptosis, as evidenced by the increased number of PI-positive cells (figure 9). The results of DAPI staining demonstrate that the SN38@HFNPs shows efficiently kill the glioma cancer cells.
3.6. Apoptosis investigation by flow cytometry

Cells treated with HFNPs, free SN38, and SN38@HF-β-CD-PEG were labeled with Annexin V-FITC and PI for flow cytometric analysis to determine the mechanism of cell death (figure 10). The transfer of phosphatidyl serine (PS) from the inner to the outside leaflet of the plasma membrane is a prominent feature of early apoptosis. Using fluorescently tagged Annexin V-FITC is a simple way to identify apoptotic cells because of Annexin V-FITC’s strong affinity for PS. In contrast, PI is selectively permeable through damaged cell membranes, allowing it to detect necrotic cells. Figure 10 reveals that the percentage of early apoptotic cells in treated U87MG and LN229 cells with SN38@HF-β-CD-PEG reveals more significant apoptosis than in control cells. Late apoptotic and necrotic populations are not significantly different between treated and control cells in U87MG and LN229.

4. Conclusion

We present a holmium ferrite-mediated nanocarrier capable of delivery of 7-ethyl-10-hydroxyl camptothecin (SN38). The β-CD-PEG-coated HFNPs demonstrate a remarkable drug-loading capacity. The SN38 release from the nanocarrier is pH-dependent and prolonged. SN38 showed broad intracellular distribution on the U87MG and the LN229 cell lines, suggesting a biocompatible holmium ferrite nanocarrier composition was designed for prolonged and specific cell-targeted delivery of SN38 to glioma cancer cell lines. The formulation’s efficacy in inhibiting the growth of human glioma cancer cells suggests it may be used in therapeutic settings. The acridine orange/ethidium bromide and nuclear staining methods examined the morphological changes in the glioma cells. These data support further investigation into the feasibility of SN38@HF-β-CD-PEG as a
delivery vehicle for SN38 in treating glioma cancer. However, more in vivo research on appropriate animal models is required before it can be successfully translated into clinical research.

**Data availability statement**

No new data were created or analysed in this study.

**Author contributions**

The manuscript was written through the contributions of all authors. All authors have approved the final version of the manuscript.

**Disclosure statement**

The authors reported no potential conflict of interest.

**ORCID iDs**

Pengxiang Yang https://orcid.org/0000-0002-7054-245X

**References**

[1] Han S, Liu Y, Cai S J, Qian M, Ding J, Larion M, Gilbert M R and Yang C 2020 IDH mutation in glioma: molecular mechanisms and potential therapeutic targets Br. J. Cancer 122 1580–5

[2] Wang J, Hjelmeland A B, Nabors L B and King P H 2019 Anti-cancer effects of the HuR inhibitor, MS-444, in malignant glioma cells Cancer Biol. Ther. 20 979–88

[3] Guo X, Pan Y and Gutmann D H 2019 Genetic and genomic alterations differentially dictate low-grade glioma growth through cancer stem cell–specific chemokine recruitment of T cells and microglia Neuro. Oncol. 21 1250–62

[4] Poff A, Koutnik A P, Egan K M, Sahebjam S, D’Agostino D and Kumar N B 2019 Targeting the Warburg effect for cancer treatment: Ketogenic diets for management of glioma Semin. Cancer Biol. (Elsevier) 135–48

[5] Muskens I S, de Smith A J, Zhang C, Hansen H M, Morimoto L, Metayer C, Ma X, Walsh K M and Wiemels J L 2020 Germline cancer predisposition variants and pediatric glioma: a population-based study in California Neuro. Oncol. 22 864–74

[6] Du B and Waxman D J 2020 Medium dose intermittent cyclophosphamide induces immunogenic cell death and cancer cell autonomous type I interferon production in glioma models Cancer Lett. 470 170–80

[7] Wang Q, Hu B, Hu X, Kim H, Squatrito M, Decarvalho A C, Lyu S, Li P and Li Y 2017 Erratum: tumor evolution of glioma-intrinsic gene expression subtypes associates with immunological changes in the microenvironment Cancer Cell 32 42–56

Wang Q, Hu B, Hu X, Kim H, Squatrito M, Decarvalho A C, Lyu S, Li P and Li Y 2018 Cancer Cell. 33 152
[8] Silver D J and Latia H D 2018 Revealing the glioma cancer stem cell interaction, one niche at a time J. Pathol. 244 260–4

[9] Miller A D, Miller C R and Rossmeissl H J 2019 Canine primary intracranial cancer: a clinicopathologic and comparative review of glioma, meningioma, and choroid plexus tumors Front. Oncol. 9 1151

[10] Ma Q, Long W, Xing C, Chu J, Luo M, Wang H Y, Liu Q and Wang R F 2018 Cancer stem cells and immunosuppressive microenvironment in glioma Front. Immunol. 9 2924

[11] Chi Y, Wang Z, Wang J, Dong W, Xin P, Bi J, Jiang T and Chen C-P 2020 Dimeric camptothecin-loaded mPEG-PCL nanoparticles with high drug loading and reduction-responsive drug release Colloid. Polym. Sci. 298 51–8

[12] Zle T, Yang L, Yu F-Q, Dong W, Ma R-J, Li Y, Zhou J, Ding Z-T, Jiang Z-H and Liu J-M 2019 Synthesis and antitumor activity of biotinylated camptothecin derivatives as potent cytotoxic agents Biorg. Med. Chem. Lett. 29 234–7

[13] Zhang S, Kobuya Y, Oritia H, Ishimine M, Kobayashi T, Chua S M B, Nakaoka H, Inoue I, Hino O and Yokomizo T 2019 Sensitization of gastric cancer cells to irinotecan by p53 activation PVB Reports. 2 130–3

[14] Lerchen H G et al 2022 Molecule–drug conjugate (SMDC) consisting of a modified camptothecin payload linked to an oV83 binder for the treatment of multiple cancer types Cancers (Basel) 14 391

[15] Zhang Q, Zhang F, Li S, Liu R, Jin T, Dou Y, Zhou Z and Zhang J 2019 A multifunctional nanotherapeutic for targeted treatment of colon cancer by simultaneously regulating tumor microenvironment Theranostics. 9 3732

[16] Wu G, Mai X, Liu F, Lin M, Dong X, Xu Q, Hao C, Zhang L, Yu R and Jiang T 2019 Synthesis of novel 10, 11-methylenedioxy-camptothecin glycidoxy derivatives and investigation of their anti-tumor effects in vivo RSC Adv. 9 11142–50

[17] Tsuchihashi Y, Abe S, Miyamoto I, Tsunematsu H, Izu A, Hatanaka H, Okuno K, Yamane M, Yasuoka T and Ikeda Y 2020 Novel hydrophilic camptothecin derivatives conjugated to branched glycerol trimer suppress tumor growth without causing diarrhea in murine xenograft models of human lung cancer Mol. Pharm. 17 1049–58

[18] Huang Q, Liu X, Wang H, Liu X, Zhang Q, Li K, Chen Y, Zhu Q, Shen Y and Sui M 2022 A nanotherapeutic strategy to overcome chemoresistance to irinotecan-7/ethyl-10-hydroxy-camptothecin in colorectal cancer Acta Biomater. 137 262–75

[19] Kumar G et al 2021 Recent advances in processing, characterizations and biomedical applications of spinel ferrite nanoparticles Mater. Res. Rev. 112 62–120

[20] Laurent S, Broidot J-L, Vander Elst L and Muller R N 2010 Magnetic iron oxide nanoparticles for biomedical applications Future Med. Chem. 2 427–49

[21] Yu S, Zhang H, Zhang S, Zhong M and Fan H 2021 Ferrite nanoparticles-based reactive oxygen species-mediated cancer therapy Front. Chem. 9 651053

[22] Katoch G, Kana G, Singh M, Garcia-Páezs A, Bhardwaj S, Sharma I, Sharma P and Kumar G 2021 Recent advances in processing, characterizations and biomedical applications of spinel ferrite nanoparticles Ferrite Nanostructures with Tuneable Props. Divers. Appl. 112 62–120

[23] Kershi R M, Ali F M and Sayed M A 2018 Influence of rare earth ion substitutions on the structural, optical, transport, dielectric, and magnetic properties of superparamagnetic iron oxide nanoparticles J. Adv. Ceram. 7 218–28

[24] Fopase R, Saxena V, Seal P, Borah J P and Pandey L M 2020 Ytrrium iron garnet for hyperthermia applications: synthesis, characterization and in vitro analysis Mater. Sci. Eng. C 116 111163

[25] Martinsson K D, Kondrashkova I S, Chebanenko M I, Kiselev A S, Kiseleva T Y and Popkov V I 2022 Morphology, structure and magnetic behavior of orthorhombic and hexagonal HoFeO3 synthesized via solution combustion approach J. Rare. Earths. 40 296–301

[26] Mokhosi S R, Mlalosele W, Nhlapo A and Singh M 2022 Advances in the synthesis and application of magnetic ferrite nanoparticles for cancer therapy Pharmacinetics. 14 937

[27] Ahmed M, Akhtar M J, Alhaqdaq H A, Khan M A M and Alroykan S A 2015 Comparative cytotoxic response of nickel ferrite nanoparticles in human liver HepG2 and breast MCF-7 cancer cells Chemosphere. 135 275–88

[28] Akhavan O, Meidanchi A, Ghaderi E and Khoei S 2014 Zinc ferrite spinel-graphene in photothermal-photodynamic therapy of cancer J. Mater. Chem. B 2 3360–14

[29] Pradhan P, Girj I, Banerjee R, Bellare J and Bahadur D 2007 Preparation and characterization of manganese ferrite-based magnetic liposomes for hyperthermia treatment of cancer J. Magn. Magn. Mater. 311 208–15

[30] Al-Qubaisi M S, Rasedee A, Flaifel M H, Ahmad S H J, Hussein-Al-Ali S, Hussein M Z, Eid E E M, Zainal Z, Saeed M and Ilowefah M 2019 Impact of aliphatic acyl and aromatic thioamide substituents on the anticancer activity of Ru(II) acylthiourea ligands Coord. Chem. Rev. 391 1366–75

[31] Swaminathan S, Haribabu J, Mohamed Subarkhan M K, Gayathri D, Balakrishnan N, Bhuvanesh N, Echeverria C and Karvembo R 2021 Effect of aliphatic acyl and aromatic thioamide substituents on the anticancer activity of Ru(ii)-p-cymene complexes with acylhioaurous ligands—in vitro and in vivo studies Dalton. Trans. 50 16311–25

[32] Sathiyas K, Mohamed Subarkhan M, Fathima Safwana C K, Sruhtl S, Sathiya Kamatchi T, Keerthana B and Kumar S L A 2022 New organoruthenium(II) complexes containing N, N-donor (X = O, S) heterocyclic chelators: synthesis, spectral characterization, in vitro cytotoxicity and apoptosis investigation Inorg. Chem. Acta. 535 120863

[33] PillaiRadugula R, Haribabu J, Mohamed Subarkhan M K, Echeverria C, Karvembo R and Gopalakrishnan N 2021 Effect of morphology and (Sn, Cr) doping on in vitro antiproliferation properties of hydrothermally synthesized 1D GaO3O2 nanostructures J. Sci. Adv. Mater. Devices. 6 631–63

[34] Kershi R M, Ali F M and Sayed M A 2018 Influence of rare earth ion substitutions on the structural, optical, transport, dielectric, and magnetic properties of superparamagnetic iron oxide nanoparticles J. Adv. Ceram. 7 218–28

[35] Pradhan P, Girj I, Banerjee R, Bellare J and Bahadur D 2007 Preparation and characterization of manganese ferrite-based magnetic liposomes for hyperthermia treatment of cancer J. Magn. Magn. Mater. 311 208–15

[36] Al-Qubaisi M S, Rasedee A, Flaifel M H, Ahmad S H J, Hussein-Al-Ali S, Hussein M Z, Eid E E M, Zainal Z, Saeed M and Ilowefah M 2019 Impact of aliphatic acyl and aromatic thioamide substituents on the anticancer activity of Ru(ii)-p-cymene complexes with acylhioaurous ligands—in vitro and in vivo studies Dalton. Trans. 50 16311–25

[37] Sathiyas K, Mohamed Subarkhan M, Fathima Safwana C K, Sruhtl S, Sathiya Kamatchi T, Keerthana B and Kumar S L A 2022 New organoruthenium(II) complexes containing N, N-donor (X = O, S) heterocyclic chelators: synthesis, spectral characterization, in vitro cytotoxicity and apoptosis investigation Inorg. Chem. Acta. 535 120863
[42] Wang Y, Jin J, Shu L, Li T, Lu S, Subarkhan M K M, Chen C and Wang H 2020 New organometallic ruthenium(II) compounds synergistically show cytotoxic, antimitastatic and antiangiogenic activities for the treatment of metastatic cancer Chem. – A Eur. J. 26 15170–82

[43] Li H, Tian J, Wu A, Wang J, Ge C and Sun Z 2016 Self-assembled silk fibroin nanoparticles loaded with binary drugs in the treatment of breast carcinoma Int. J. Nanomedicine. 11 4573–80

[44] Li J, Wang Y, Xue S, Sun J, Zhang W, Hu P, Ji L and Mao Z 2016 Effective combination treatment of lung cancer cells by single vehicular delivery of siRNA and different anticancer drugs Int. J. Nanomedicine. 11 4609–24

[45] Vodnik V V et al 2021 Development of genistein-loaded gold nanoparticles and their antitumor potential against prostate cancer cell lines Mater. Sci. Eng. C 124 112078

[46] Mohammad A S, Griffith J I, Adkins C E, Shah N, Sechrest E, Dolan E L, Terrell-Hall T B, Hendriks B S, Lee H and Lockman P R 2018 Liposomal irinotecan accumulates in metastatic lesions, crosses the blood-tumor barrier (BTB), and prolongs survival in an experimental model of brain metastases of triple negative breast cancer Pharm. Res. 35 31

[47] Kydd J, Jadia R, Velpurisiva P, Gad A, Paliwal S and Rai P 2017 Targeting strategies for the combination treatment of cancer using drug delivery systems Pharm. 9 46–57

[48] Varela-Moreira A, Shi Y, Fens M H A M, Lammers T, Hennink W E and Schielflers R M 2017 Clinical application of polymeric micelles for the treatment of cancer Mater. Chem. Front. 1 1485–501

[49] Kang C, Sun Y, Zhou J, Li W, Zhang A, Kuang T, J X and Yang Z 2016 Delivery of nanoparticles for treatment of brain tumor Curr. Drug Metab. 17 745–54

[50] Padmanabhan P, Kumar A, Kumar S, Chaudhary R K and Gulyas B 2016 Nanoparticles in practice for molecular-imaging applications: An overview Acta Biomater. 41 1–16

[51] Revia R A and Zhang M 2016 Magnetite nanoparticles for cancer diagnosis, treatment, and treatment monitoring: recent advances Mater. Today 19 157–68

[52] Hu Y, Yu D and Zhang X 2021 9-amino acid cyclic peptide-decorated sorafenib polymeric nanoparticles for the efficient in vitro nursing care analysis of hepatocellular carcinoma Process Biochem. 100 140–8