

Magnetically manipulated and transferrin-modified liposomes for efficient brain delivery of harmine and glioblastoma therapy

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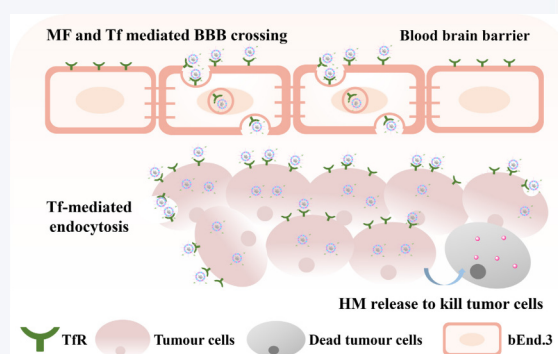
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ABSTRACT: Glioblastoma, originating from glial cells in the brain's neuroepithelial tissue, are the most common primary malignant tumors of the central nervous system (CNS). Due to their invasive growth characteristics, most glioblastoma are difficult to completely resect, leaving residual tumor cells that become the source of recurrence. Harmine (HM), an active compound extracted from *Peganum harmala* L. seeds, exhibits a particularly notable in its anti-glioblastoma activity. However, HM suffers from poor selectivity, low bioavailability, and a tendency to enter the normal brain tissue, where it reversibly and competitively inhibits monoamine oxidase A (MAO-A), leading to neurotoxic side effects such as tremors and convulsions. To improve the therapeutic efficacy and reduce toxicity, this study fabricated a delivery system-based magnetic nanoliposomes (MNLs) formulation modified with transferrin (Tf) on the surface (HM@MNLs-Tf). The high expression of TfR on glioma cells and blood-brain barrier endothelial cells (BMECs) facilitates the efficient crossing of the blood-brain barrier (BBB) and targeting of glioma cells. Additionally, the presence of iron oxide nanoparticles within HM@MNLs-Tf enhances BBB penetration when subjected to an external magnetic field (MF). This mechanism improves the selectivity of HM and reduces the neurotoxicity associated with MAO-A inhibition, thereby increasing its clinical applicability. *In vitro* and *in vivo* results demonstrated that the approach employed by this study significantly improved the therapeutic effect of HM to glioblastoma while minimizing its neurotoxicity and side effects.

KEYWORDS: harmine, magnetic nanoliposomes, blood-brain barrier, glioblastoma, central nervous system toxicity



1 Introduction

Glioblastoma, originating from glial cells in the brain, are the most common primary intracranial tumors. With an annual incidence of 3 to 6.4 per 100,000, they account for approximately 23.3% of all central nervous system (CNS) tumors and 78.3% of malignant CNS

tumors [1]. Glioblastoma are highly invasive, grow rapidly, and exhibit enhanced angiogenesis. Conventional surgical methods cannot achieve complete resection, resulting in poor prognosis and short survival times [2, 3]. Recent studies have shown that the immunosuppressive microenvironment of gliomas can be modulated through intracellular molecular aggregation strategies, such as the use of tumor microenvironment-specific peptide-porphyrin conjugates (e.g., Gi-F-CAA) to induce ferroptosis and enhance glutathione peroxidase 4 (GPX4) inhibition and oxidative stress [4, 5]. For the immunosuppressive microenvironment, chemotherapy drug remains essential to control tumor progression and extend survival in glioma patients in the treatment of glioma either as a monotherapy or in combination with other therapeutic

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strategies. Moreover, the presence of biological barriers, including the blood-brain barrier (BBB) and the blood-tumor barrier (BTB), severely limits the penetration of nearly 98% of small-molecule drugs into glioma tissues [6]. Novel delivery systems, such as membrane fusion lipid nanovesicles (MF-LNVs), can directly deliver large molecules into the cytoplasm via endocytosis-mediated membrane fusion, bypassing the leakage issues of traditional endocytic pathways [7]. Additionally, the self-renewal capacity, high migratory ability, and invasiveness of the heterogeneous tumor cell population significantly contribute to the high recurrence rate of glioblastoma [8–10].

Peganum harmala L., commonly known as wild rue, belongs to the family Zygophyllaceae and the genus *Peganum*. Harmine (HM), an active compound extracted from the seeds of this perennial herb, exhibits a wide range of pharmacological effects [11]. These include promoting pancreatic β -cell proliferation [12], exhibiting antibacterial activity [13, 14], demonstrating anti-glioblastoma efficacy [15, 16], and significantly inhibiting the activities of monoamine oxidase (MAO) and acetylcholinesterase [17, 18]. However, like other chemotherapeutic agents, such as Carmustine, Temozolomide, and Nitrosoureas [19–21], HM has poor water solubility, resulting in low bioavailability. At higher doses, HM readily crosses the BBB, leading to significant adverse neurotoxic effects, including tremors, convulsions, paroxysmal seizures, involuntary movements, and muscle rigidity [22, 23]. To reduce neurotoxicity, design principles from non-cationic lipid nanoparticles (NC-TNPs) can be adopted, wherein hydrogen bonding between thiourea groups and mRNA phosphate groups replaces traditional electrostatic compression, significantly reducing inflammation [24]. These challenges underscore the urgent need for an innovative drug delivery platform to improve the therapeutic efficacy of HM against malignant glioblastoma.

In chemotherapy for glioblastoma, directly injecting therapeutic agents into the brain is an invasive procedure that carries a risk of injury [25]. Intravenous administration (IV), on the other hand, offers a safer alternative by effectively transporting therapeutic agents to the brain without causing significant side effects. However, one of the primary challenges in brain-targeted drug delivery via IV is the BBB, which significantly reduces delivery efficiency [25–26]. To overcome this obstacle, it is essential to develop carriers capable of bypassing or penetrating the BBB, enabling the successful delivery of therapeutic agents to the brain via IV administration. Currently, the design of novel drug delivery systems and carriers, such as liposomes (Lps) or polymer nanoparticles (NPs), has become a global research focus. Surface engineering strategies, such as transferrin modification, can enhance the targeting ability of nanoparticles. The mechanism is similar to the mannose receptor (CD206) $\times$ C-X-C chemokine receptor type 4 (CXCR4) synergistic targeting strategy in bispecific fluorescence probes (bsProbe) [27–28]. These approaches aim to facilitate targeted drug delivery while minimizing cytotoxicity and side effects [29–31].

Magnetic nanoliposomes (MNLs) have emerged as rapidly developing and highly valuable nanoparticles in recent years, with increasing applications in various biomedical fields, such as magnetic targeting and magnetic resonance imaging [32–35]. MNLs have also demonstrated breakthrough potential in brain tumor therapy, with a core innovation in overcoming the delivery limitations of conventional liposomes through a physical magnetic targeting mechanism [36]. In comparison to traditional liposomes

relying on passive permeability (enhanced permeability and retention (EPR) effect) or receptor-mediated active targeting, MNLs, guided by an external magnetic field, achieve a 3.1-fold improvement in BBB/BTB penetration efficiency [37]. In 2.8 times the drug accumulation in glioblastoma models and a 74.3% tumor inhibition rate (control < 20%) [38]. MNLs can also realize the encapsulation of chemotherapeutic drugs with small molecule, and exhibit enhanced ability to cross biological barriers such as blood vessel walls and cell membranes with the application of an external magnetic field (MF) near the focus [39–41]. Intracellular aggregation strategies, such as enzyme-triggered self-assembly, can improve drug retention at tumor sites and enhance immunogenic cell death effects [5, 42]. Additionally, surface modification of MNLs with targeting ligand further improves the delivery efficiency of chemotherapy drug to tumor tissues [43–44]. Transferrin receptors (TfR) are promising targets for glioblastoma, as they are expressed on both tumor cells and brain microvascular endothelial cells (HBMECs) [45–46]. Therefore, transferrin (Tf) has the ability to serve as a useful ligand for targeted delivery in glioblastoma therapy. Through TfR-mediated endocytosis, Tf demonstrates high permeability across the BBB and exhibits strong targeting capability to glioblastoma cells, resulting in significantly enhanced accumulation at the tumor site [47–49].

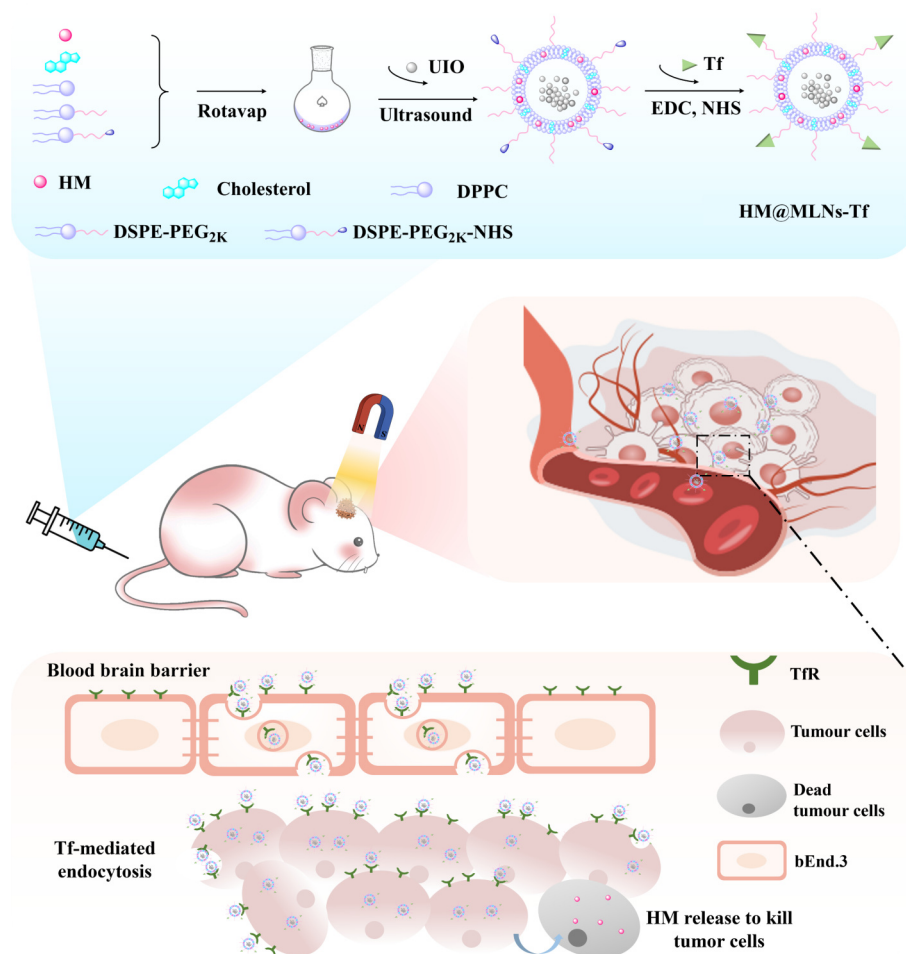
To preserve the antitumor activity of HM while reducing the toxicity, this study fabricated a delivery system-based MNLs formulation with the modification of Tf on its surface (HM@MNLs-Tf, Scheme 1). For the high expression of TfR on the surface of glioma cells and BMECs, HM@MNLs-Tf can efficiently across BBB and target to glioma cells. Additionally, with the application of an external MF, the iron oxide nanoparticles within the HM@MNLs-Tf can greatly enhance the BBB penetration ability. This mechanism effectively enhances the selectivity of HM and reduces the reversible and competitive inhibition of MAO-A by HM, thereby improves its toxicity to the CNS and increases its possibility to apply in clinic.

2 Experimental

2.1 Materials and reagents

Materials used in this work include: HM (Nanjing Zeran Pharmaceutical Technology Co., Ltd., China), Tf (Sigma, USA), fluorescein isothiocyanate (FITC) (Sigma-Aldrich, USA), IR820 (Shanghai McLean Co., Ltd.), $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$ and $\text{FeCl}_2 \cdot 4\text{H}_2\text{O}$ (McLean Biochemical Technology Co., Ltd., China), phosphatidylcholine (PC), L- α -dipalmitoyl phosphatidylcholine (DPPC), 1,2-distearoyl-sn-glycero-3-phosphoethanolamine-N-[methoxy(polyethylene glycol)-2000] (DSPE-PEG_{2K}) (Shanghai Avet Pharmaceutical Technology Co., Ltd., China), DSPE-PEG_{2K}-NHS (Xi'an Ruixi Biotechnology Co., Ltd., China), cholesterol (Avantipid, USA), Dulbecco's modified Eagle medium (DMEM) (Nanjing Vicente Biotechnology Co., Ltd., China), fetal bovine serum (FBS) (Beijing Baioboyuan Biological Co., Ltd., China), Penicillin-Streptomycin solution (Hyclone, USA), Mouse MAO, serotonin (5-HT), 5-hydroxyindoleacetic acid (5-HIAA), tryptophan hydroxylase (TPH), and norepinephrine (NE) enzyme-linked immunosorbent assay kit (Jiangsu Jingmei Biotechnology Co., Ltd.).

Mouse brain microvascular endothelial cells (bEnd.3) and human glioblastoma cells (U87-MG) were provided by the



Scheme 1 Synthesis of HM@MNLS-Tf and schematic diagram of its anti-glioma effect.

American Typical Cultures Conservation Center (ATCC). 5-week-old female BALB/c-nude mice as well as 5-week-old female BALB/c mice were purchased from the Beijing Viton Lever Laboratory Animal Co. Ltd. and kept in the National Center for Nanoscience Laboratory Animals. The Animal Ethics Committee of the National Center for Nanoscience reviewed the experiments (Ethics No. NCNST21-2020-10).

2.2 Preparation and characterization of iron oxide magnetic nanoparticles ($\text{Fe}_3\text{O}_4\text{@CA}$)

3.5 mmol $\text{FeCl}_3\cdot 6\text{H}_2\text{O}$ and 2 mmol $\text{FeCl}_2\cdot 4\text{H}_2\text{O}$ were dissolved in HCl solution (2 mL, 1 mM) which was placed in 250 mL of three-necked round-bottomed flask containing 160 mL of deionized water with 40 °C oil bath. After continuous stirring for 30 min, 15 mL of concentrated ammonia (28%) was quickly added. Rapid stirring was kept for 30 min, and then, 0.5 mM citric acid was added and the reaction was stirred for another 30 min. Next, the reaction solution was warmed to 85 °C and kept slow stirring for 2 h. N_2 was kept on during the whole experiment. At the end of the reaction, the obtained product was dialyzed in a Spectra/Por (MWCO: 12,000–14,000) dialysis bag against deionized water for 3 days (8 changes) to obtain the iron oxide magnetic nanoparticles ($\text{Fe}_3\text{O}_4\text{@CA}$). The hydrodynamic size and zeta potential of $\text{Fe}_3\text{O}_4\text{@CA}$ were determined by a Zetasizer Nano ZS laser particle sizer (Malvern Instruments Ltd., UK). A double copper mesh was

used to observe the morphology of $\text{Fe}_3\text{O}_4\text{@CA}$ by a Tecnai G2 20 S-TWIN TEM (FEI, USA).

2.3 Preparation of HM@MNLS-Tf

Liposomes (Lps) were prepared by reverse-phase evaporation method. Briefly, we weighed PC, DPPC, cholesterol, DSPE-PEG_{2K}, DSPE-PEG_{2K}-NHS, and HM (12.81:13.62:12.86:0.50:0.50:6.12–24.50 μmol ratio), dissolved them in a chloroform:methanol mixture (2:1, v/v), and rotary-evaporated the solution to form a thin film. We then added 4 mL of deionized water and 1.2 mM $\text{Fe}_3\text{O}_4\text{@CA}$, and dissolved the thin film by sonication. Next, we added specific amounts of Tf (0.065, 0.130, 0.260, and 0.520 μmol) and 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide/N-hydroxysuccinimide (EDCI/NHS) for activation, and stirred the mixture magnetically for 24 h. At the terminal point of reaction, HM@MNLS-Tf were collected by centrifuging the reaction system at 12,000 rpm/min for 10 min and washed 2 to 3 times by the deionized (DI) water. The synthesis of FITC@MNLS-Tf or IR820@MNLS-Tf for imaging experiments was performed in the same way as the description above, except that FITC or IR820 was used to replace HM. HM@MNLS-Tf aqueous solution was diluted to the appropriate concentration, and then the particle size and potential were determined by a Zetasizer Nano ZS type laser particle sizer. The morphology of HM@MNLS-Tf was observed by the same way described above.

2.4 Encapsulation efficiency and loading efficiency

A series of purified HM solution was taken to record the absorption at 320 nm with a Lambda-950 UV-visible spectrophotometer (Perkin Elmer, USA) and make the work curve. Then, certain amount of methanol was added to destroy the Lps membrane of HM@MNLs-Tf, and the absorbance was measured to calculate the drug concentration via the work curve with the MNLs-Tf NPs as the blank control. The encapsulation efficiency was calculated according to the equation (Eq. (1)), the same volume of HM@MNLs-Tf nanoparticles and MNLs@ Tf nanoparticles were weighed, freeze-dried, and the loading efficiency was calculated according to the equation (Eq. (2)):

$$\text{Encapsulation efficiency (\%)} = \frac{\text{Amount of drug loaded (mg)}}{\text{Amount of drug input (mg)}} \times 100\% \quad (1)$$

$$\text{Loading content (\%)} = \frac{\text{Amount of drug loaded (mg)}}{\text{Total mass of the drug delivery system (mg)}} \times 100\% \quad (2)$$

2.5 Stability

Three batches of HM@MNLs-Tf were prepared and placed at 4 °C for 0, 1, 2, 3, 4, 5, 6, 7, and 14 days to measure their hydrodynamic size and observe the stability.

2.6 Drug release *in vitro*

5 mL of HM@MNLs-Tf was taken, the drug content was determined and then packed in a dialysis bag with a MWCO of 1.4 KDa, clamped at both ends and placed in phosphate buffer saline (PBS) containing 40 mL of different pH values (5.0/7.2), the temperature was maintained at 37 ± 0.5 °C, and the rate was 500 rpm/min. At certain time points, 1 mL of samples was taken out, and an equal amount of fresh PBS at the same temperature was replenished. The concentration of the drug was determined by the ultraviolet (UV) method and the cumulative release rate was calculated. This experiment was repeated 3 times.

2.7 Magnetic resonance imaging (MRI) imaging capability

A series of concentration gradients of Fe₃O₄@CA, HM@MNLs-Tf, and cells after co-incubation with HM@MNLs-Tf were prepared, and the relaxation efficiencies were calculated by MRI imaging assay with a BioSpec 70/20USR (Bruker, Germany).

2.8 Intracellular localization

1 mL logarithmically grown U87-MG were spread on confocal dishes at a concentration of 2 × 10⁴ cells/mL and placed in an incubator at 37 °C, 5% CO₂, and saturated humidity for 24 h. The cells were cultured in DMEM supplemented with 10% FBS and 1% antibiotics (100 U/mL penicillin and 100 µg/mL streptomycin). After discarding the culture medium, FITC-labeled Lps were added to the cells of each group, and a blank control group was set up simultaneously. After incubation for 4 h, the culture medium of the experimental group was discarded. The cells were washed with PBS 3 times, 1 mL of Lyso-TrackerRed staining solution (Lyso-TrackerRed:PBS = 1:1000) was added into each well for lysosome staining, 4% paraformaldehyde fixation, and the nuclei of the cells

were stained with 4',6-diamidino-2-phenylindole (DAPI) staining solution (DAPI:PBS = 1:1000). Cells were visualized using a Zeiss 710 laser scanning confocal microscopy (LSCM, Zeiss Optical Group, Germany), and the experiment was repeated thrice.

2.9 Cell uptake

Flow cytometry (FCM) observation of cellular uptake: 1 mL logarithmically grown U87-MG were spread in 6-well plates at a concentration of 2 × 10⁶ cells/mL and placed in an incubator at 37 °C, 5% CO₂, and saturated humidity for 24 h. After discarding the culture medium, FITC-labeled Lps were added to the cells of each group, and a blank control group was also set up. The cells were incubated for 30 min, 1 h and 4 h. At the end of the incubation, the culture medium was discarded, the cells were washed with PBS by 3 times, then collected, and the fluorescence intensity was measured by Accuri C6 FCM (BD, USA) within 1 h. In addition, cellular uptake of FITC-labeled Lps with different Tf input volumes (0.065, 0.130, 0.260, and 0.520 µmol) was assessed by LSRFortessa FCM (BD, USA) to determine the effect of Tf modification on targeting efficiency.

2.10 Antitumor activity of HM@MNLs-Tf *in vitro*

Cytotoxicity was determined by cell counting kit-8 (CCK-8) assay. 200 µL logarithmically grown U87-MG were inoculated in 96-well culture plates at a concentration of 5 × 10³ cells/mL and placed in an incubator at 37 °C, 5% CO₂, and saturated humidity for 24 h. After discarding the culture medium, experimental groups were added with HM of 40, 20, 10, 5, 2.5, 1.25, and 0.625 µg/mL, and a blank control group was set simultaneously. After incubation for 24, 48, and 72 h, the culture medium was discarded, and 100 µL of freshly prepared CCK-8 solution (CCK-8:DMEM = 1:9) was added to each well. The absorbance was measured at 450 nm using the Enspire multi-mode microplate detection system (Perkin Elmer, USA), and the cell viability was calculated according to the equation (Eq. (3)).

$$\text{Cell viability (\%)} = \frac{\text{Experimental group} - \text{Blank control}}{\text{Negative control} - \text{Blank control}} \times 100\% \quad (3)$$

Propidium iodide (PI)/Annexin V staining method was used for detection of apoptosis. 1 mL logarithmically grown U87-MG were spread in 6-well plates at a concentration of 2 × 10⁵ cells/mL and placed in an incubator at 37 °C, 5% CO₂, and saturated humidity for 24 h. After discarding the culture medium, an experimental group with HM of 10 µg/mL and a blank control group were set to incubate for 24 h, and the cell morphology was observed under an inverted microscope. At the end of the incubation, the cells were collected and adjusted to 1 × 10⁶ cells/mL, 5 µL of Annexin V and 5 µL of PI were added for labeling, and the fluorescence intensity was measured by FCM within 1 h. The experiment was repeated 3 times and analyzed by FlowJo software.

2.11 Across the BBB ability of HM@MNLs-Tf *in vitro* and *in vivo*

Establishment of BBB model: 1 mL logarithmically grown bEnd.3 were inoculated in Transwell 6-well plates at a concentration of 6.7 × 10⁴ cells/mL. The cells were cultured in DMEM supplemented with 10% FBS and 1% antibiotics (100 U/mL penicillin and 100 µg/mL streptomycin). The plates were incubated at 37 °C in a

humidified atmosphere containing 5% CO₂. The culture medium was changed every 2 days until the cells formed a completely dense monolayer. The trans-epithelial electrical resistance (TEER) value of the cells was monitored by Millicell-ERS Epithelial Resistance Meter (Millipore, USA) at different days of growth. When the TEER value was more significant than 200 Ω·cm², the cells could be used for trans-membrane transport experiments.

In vitro trans-BBB study: After the cells formed a completely dense monolayer, the experimental groups were added to the upper chamber of the Transwell (FITC: 2 μg/mL), a magnet was added or not under the plate of the FITC@MNLs-Tf group. Then, 100 μL of samples were taken from the lower chamber at 0, 30, 60, 90, 120, 150, 180, and 240 min. The FITC content was analyzed by replenishing 100 μL of N-2-hydroxyethylpiperazine-N-2-ethane sulfonic acid (HEPES) buffer, and the permeability was calculated. The buffer of the upper chamber was discarded at 240 min, and the upper and bottom Transwell plate were placed in an *in vivo* imaging system (IVIS) spectrum small animal *in vivo* imager (Perkin Elmer, USA) for fluorescence imaging (excitation wavelength: 485 nm, emission wavelength: 515 nm).

2.12 In vivo trans-BBB study

In order to evaluate the *in vivo* BBB permeability of HM@MNLs-Tf, the experimental group was labeled with IR820 by the previous report method [50]. Then, 100 μL HM@MNLs-Tf (IR820 0.2 mM) was injected into BALB/c-nude mice by tail vein (*n* = 5), and the control group was injected with an equal amount of IR820 dye. A magnetic field with a strength of 1 T was applied to the IR820@MNLs-Tf group for 10 min after the administration of HM@MNLs-Tf. Brain fluorescence images were acquired at 1, 12, and 24 h after drug administration using an IVIS spectral imaging system (Ex: 675 nm, Em: 700 nm).

2.13 MRI imaging *in vitro* and *in vivo*

A series of concentration gradients of Fe₃O₄@CA, HM@MNLs-Tf, and co-incubation solutions of cells and HM@MNLs-Tf were prepared by using a multiplicative dilution method, and the relaxation efficiencies were calculated by MRI imaging assay with a BioSpec 70/20USR (Bruker, Germany).

U87-MG in the logarithmic growth phase were collected and 2 × 10⁶ cells/mL inoculated with in the right lower limb of BALB/c-nude mice to establish a subcutaneous tumor model. When the tumor volume grew to ~ 500 mm³, MNLs@Tf (200 μL, 2 mg Fe/mL) was injected into the tail vein. After the administration of MNLs@Tf, an external MF with a strength of 1 T was applied to the tumor tissue for 10 min. Magnetic resonance imaging of HM@MNLs-Tf was performed using a small animal MRI machine (imaging parameters: TR = 3000 ms, TE = 40 ms, slice thickness = 1 mm, FoV = 35 × 35) at 0, 1, 2, 4, 8, and 24 h.

2.14 In vivo anti-tumor activity study

BALB/c-nude mice were inoculated with 0.2 mL of logarithmic growth phase U87-MG at 2 × 10⁶ cells/mL subcutaneously in the right lower limb. After inoculation, the growth and size of the tumors were observed and recorded. When tumor volume arrived at 40–50 mm³, mice were randomly divided into 8 groups: control, Lps, HM, HM@Lps, HM@MNLs, HM@MNLs+MF, HM@Lps-Tf, and HM@MNLs-Tf+MF group. 4 mice in each group were injected into the tail vein with 200 μL of the samples, and HM content is 2.0 mg/kg in the corresponding group. HM@MNLs-Tf

group was administered with an external MF with a strength of 1 T for 10 min immediately after administering the drug. The drugs were administered 10 times once every 2 days. The lengths of the longest diameter of the mouse tumor (*a*) and the short diameter perpendicular to each other from the long diameter (*b*) were measured by digital vernier calipers and the tumor volume was calculated according to the equation (Eq. (4)):

$$V = \pi \cdot a \cdot b^2 / 6 \quad (4)$$

At the end of treatment, fresh blood of mice was taken to determine blood routine by BC-5300 Myriad Veterinary Automatic Blood Cell Analysis (Shenzhen Myriad Biological Company), and blood biochemical indexes were determined by BS-240 Automatic Biochemical Analyzer (Shenzhen Lei She Life Science and Technology).

Hematoxylin and eosin (H&E) staining of organs was taken to observe the pathological changes of each tissue, and tumor tissues were taken to detect terminal deoxynucleotidyl transferase-mediated DUTP Nick end labeling (TUNEL) and Ki67 by immunohistochemistry.

2.15 Safety evaluation of HM@MNLs-Tf+MF

Tremor experiment: HM and HM@MNLs-Tf (HM: 20 mg/kg) were intraperitoneally injected to BALB/c healthy mice, and each group with 10 mice. The HM@MNLs-Tf group was given an external MF with a strength of 1 T for 10 min immediately after drug administration, and the observation was started 5 min after the injection and continued for 30 s at each time point. According to the tremor scoring scale (Table 1), a tremor score was given to each mouse.

Temperature change curve: After the administration of BALB/c mice, each group consisting of 8 mice, the anal temperature of mice with a thermistor probe inserted into 1.0 cm of rectum lubricated with liquid paraffin oil was detected every 15 min using an ordinary electronic thermometer, and the body temperature measured at different time points was subtracted from the baseline temperature (anal temperature measured 15 min before the injection of the drug) to obtain the curve of the change in body temperature.

Neurotransmitter assay: BALB/c healthy mice were injected intraperitoneally with 0.9% NaCl, HM, and HM@MNLs-Tf (HM content 20 mg/kg), and the HM@MNLs-Tf group was subjected to an external MF immediately after administration of the drug for 10 min. Mice were killed by cervical dislocation method at 0.5, 1, 24 and 168 h, respectively, and the whole brains were removed and weighed. The whole brain of mice was washed with PBS and put into a grinding tube with steel beads, 1 mL of radioimmuno-precipitation assay (RIPA) lysate (containing inhibitor) was added and then put into a low-temperature grinder, centrifuged at 12,000× *g* for 10 min. The supernatant was taken and tested for the content of 5-HT, 5-HIAA, TPH, NE, and the activity of MAO by

Table 1 Tremor scale

Score	Animal behavior
0	No tremor
1	Mild rare tremor
2	Moderate intermittent tremor
3	Severe and frequent tremors
4	Severe persistent tremor

using an enzyme-linked immunosorbent assay (ELISA) kit. Each time point included 8 mice per group for each indicator.

2.16 Statistical methods

Student's t-test was used for the statistical differences between the two groups. For comparisons involving multiple groups, one-way analysis of variance (ANOVA) was applied, followed by post hoc tests with appropriate multiple comparison corrections (e.g., Bonferroni correction) to control the Type I error rate. * indicates $P < 0.05$, ** indicates $P < 0.01$, *** indicates $P < 0.001$.

3 Results and discussion

3.1 Characterization of HM@MNLS-Tf

This study synthesized iron oxide nanoparticles (Fe_3O_4) via hydrothermal method in the hot aqueous solution of citric acid, resulting in citric acid-coated iron oxide NPs ($\text{Fe}_3\text{O}_4\text{@CA}$). The particle size of $\text{Fe}_3\text{O}_4\text{@CA}$ was measured using a particle size analyzer, which showed an average size of approximately 50 nm with a polymer dispersity index (PDI) less than 0.3. Transmission electron microscope (TEM) images revealed good dispersion of $\text{Fe}_3\text{O}_4\text{@CA}$, with particle sizes around 10 nm (Fig. S1 in the Electronic Supplementary Material (ESM)). HM@MNLS-Tf were prepared using the thin-film ultrasonic method (Fig. 1(a)), and the effects of varying amounts of HM and Tf on the properties of the

HM@MNLS-Tf were evaluated to optimize the formulation. Firstly, we changed the carrier and drug ratio to evaluate the drug content efficiency. The lipid content in MNLS was fixed at 40.29 μmol , and the HM input amount was varied. Encapsulation efficiency and drug content were used as evaluation parameters. The intersection of encapsulation efficiency and drug content at different HM input amounts is shown in Fig. S2 in the ESM. When the amount of HM exceeded 20.20 μmol , the drug content of liposomes (Lps) continued to increase with the drug input, but the encapsulation efficiency began to decrease, indicating that more drugs are not loaded. Therefore, the optimal HM input amount in this study was determined to be 20.20 μmol . Under conditions where both lipid and HM contents remained constant, the grafting rate of Tf was used as an evaluation metric to screen the input ratio of Tf, ensuring that Tf was conjugated to the Lps for targeted delivery *in vivo*. As shown in Fig. S3 in the ESM, the highest utilization rate of Tf (17.2%) was achieved with 0.13 μmol . Notably, when the Tf input amount exceeded 0.13 μmol , the utilization ratio of Tf was no longer increased and even showed a slight decline. Characterization of HM@MNLS-Tf prepared under the optimal conditions revealed that their particle size was approximately 200 nm, exhibiting good dispersion and a regular spherical shape. The addition of HM and $\text{Fe}_3\text{O}_4\text{@CA}$ increased the size of the nanoparticles when compared with the Lps and HM@Lps-Tf (Fig. 1(b)). For the HM@Lps-Tf, the encapsulation efficiency was 76.00%, the drug loading content was 7.05%, and the zeta potential was approximately -27.60 mV. In contrast, the HM@MNLS-Tf exhibited an encapsulation efficiency

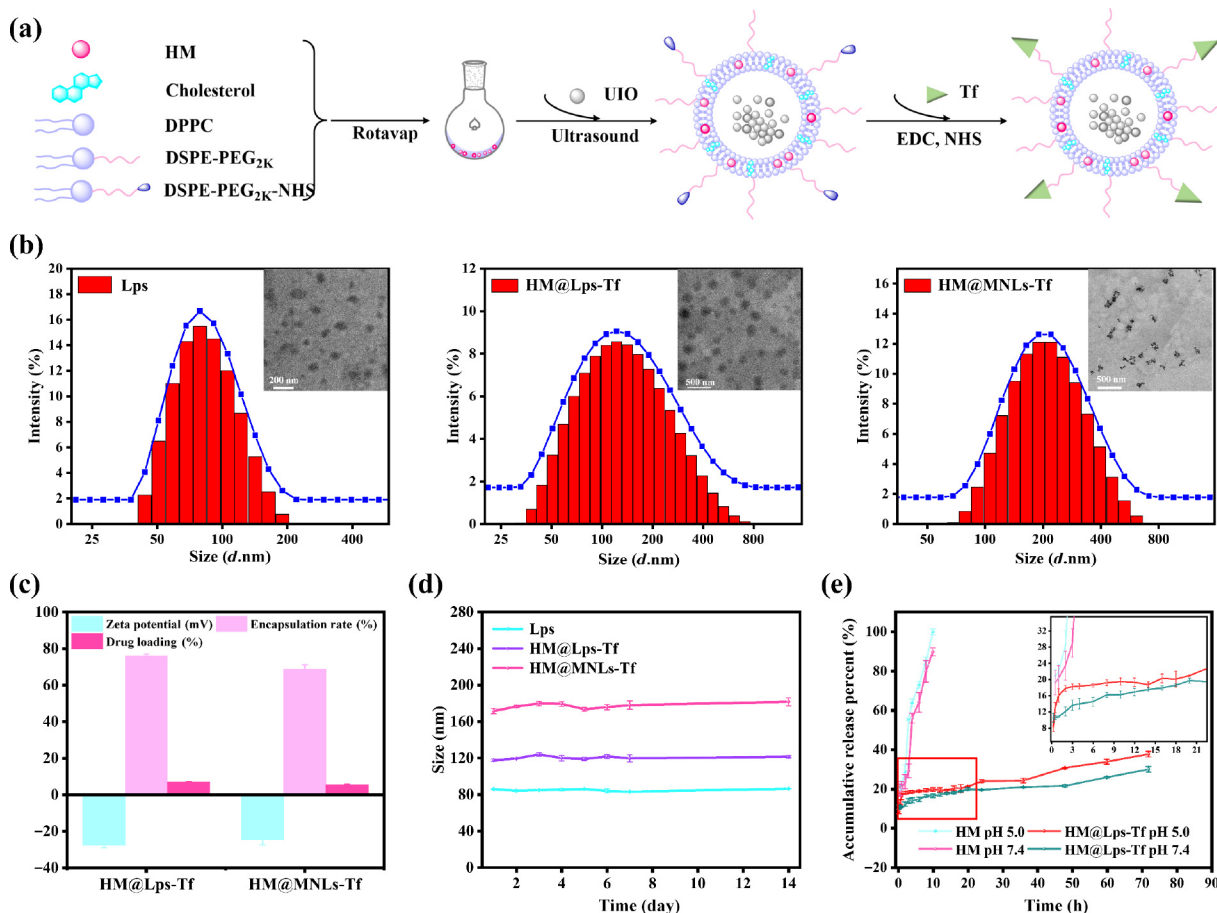


Figure 1 Synthesis and characterization of HM@MNLS-Tf. (a) Schematic diagram of the synthesis process of HM@MNLS-Tf. (b) Hydrated size and morphology of various Lps, scale bars: 200 nm (left), 500 nm (right two). (c) Zeta potential, encapsulation efficiency and loading content of HM@Lps-Tf and HM@MNLS-Tf ($n = 3$). (d) Stability of various Lps ($n = 3$). (e) Cumulative release of HM and HM@Lps-Tf under different pH buffer ($n = 3$).

of 68.65%, a drug loading content of 5.57%, and a zeta potential of approximately -24.60 mV (Fig. 1(c)). Additionally, these Lps demonstrated excellent stability, without significant changes in particle size observed after 14 days of storage (Fig. 1(d)). The release behavior of free HM and HM@Lps-Tf under different pH conditions was further studied. As shown in Fig. 1(e), free HM released over 95% release within 10 h at different pH values. In contrast, HM in HM@Lps-Tf displayed the sustainable release behavior, and arrived at $\sim 40\%$ after 72 h. Under the lysosomal conditions (pH 5.0), the release rate of HM was faster compared to normal physiological conditions (pH 7.4).

3.2 Targeting evaluation of HM@MNLs-Tf *in vitro*

To further investigate the targeting ability of the Tf modification on the Lps, we firstly observed the expression of Tf on the surface of U87 via the western blot (WB) analysis. The results confirmed the high expression of TfR in U87-MG cells (Fig. S4 in the ESM). Then, FITC-labeled NPs were used, and we further explored the effects of different Tf grafting ratio on U87 cells uptake. The results indicated that cellular uptake of FITC@Lps-Tf did not significantly improved with Tf input amount greater than 0.13 μmol (Fig. S5 in the ESM). Therefore, 0.13 μmol of Tf was selected as the optimal input amount for subsequent experiments, balancing maximal grafting efficiency with effective targeting. After that, the uptake of them by U87-MG at different time points was measured using FCM. The results indicated that U87-MG showed a time-dependent uptake of the Lps, and the uptake rate of Tf-modified Lps was significantly higher than that of unmodified Lps (Fig. 2(a)). Meanwhile, the LSCM observations were consistent with the FCM data and confirmed the targeting ability of Tf. After 4 h, U87-MG treated with FITC@Lps-Tf exhibited significantly enhanced green fluorescence intensity, and the Lps showed good co-localization with the red-fluorescent-labeled lysosomes, indicating that endocytosis is the main cell uptake manner (Fig. 2(b)).

3.3 Antitumor evaluation of HM@MNLs-Tf *in vitro*

To investigate the resistance of HM@MNLs-Tf against glioblastoma, human glioblastoma cells (U87-MG) were selected as the glioma cell model. CCK-8 assay results showed that blank Lps did not exhibit noticeable toxicity against U87-MG (Fig. S6 in the ESM). Both free HM and HM@MNLs-Tf demonstrated significant inhibitory effects on U87-MG (Fig. 3(a)). After 24 h, the IC₅₀ value of free HM was 9.7 $\mu\text{g/mL}$, while that of HM@MNLs-Tf was 104.5 $\mu\text{g/mL}$, showing a significant difference. Interestingly, the IC₅₀ value of the free HM group is 2.3 $\mu\text{g/mL}$ after 72 h treatment, and that value of the HM@MNLs-Tf is decreased to 9.1 $\mu\text{g/mL}$. This gradual decrease in IC₅₀ for HM@MNLs-Tf corresponds well with the cumulative release profile shown in Fig. 1(e); specifically, the 72-h IC₅₀ decrease aligns with a $\sim 40\%$ cumulative release at pH 5.0, suggesting that the acidic lysosomal environment enhances the sustained release of HM and contributes to its increased cytotoxicity. These results indicate that the efficiency of HM is increased more than 4.2- and 11-fold in free HM and HM@MNLs-Tf group, respectively. We think the reduced difference is due to the sustained release behavior of HM from HM@MNLs-Tf. The apoptosis results from FCM were consistent with the results of CCK-8 assay (Fig. 3(b)). After 72 h, early apoptosis in U87-MG treated with free HM was 8.7%, while late-stage apoptosis reached 70.5%. In contrast, the cells treated with HM@MNLs-Tf showed 17.4% early apoptosis and 49.7% late-stage apoptosis. Besides, the morphology of cells in different treatment groups has been observed to further demonstrate the results of FCM. As shown in Fig. 3(c), untreated U87-MG appeared as epithelial-like clusters, whereas free HM-treated cells exhibited shrinkage and deformation, losing their epithelial-like morphology, which aligns with the apoptosis results. The HM@MNLs-Tf treated U87-MG appeared to be in early apoptosis, with nuclear shrinkage and decreased cell aggregation, showing a more dispersed appearance while maintaining their epithelial morphology.

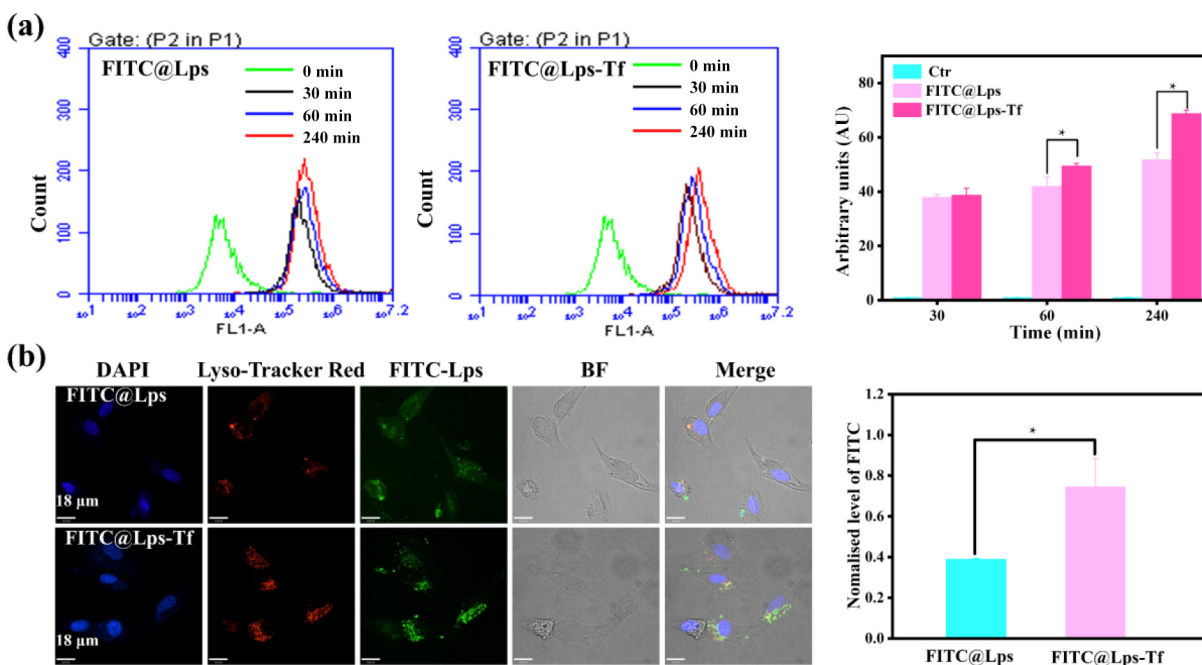


Figure 2 Cell uptake pathways and cellular localization. (a) FCM measurement of U87 cell uptake of Tf-modified and unmodified Lps at different time points, shown in flow cytometry plots (left) and statistical chart (right) ($n = 3$). (b) LSCM observation of U87 cell localization of Tf-modified and unmodified Lps, shown in fluorescence images (left) and statistical chart (right). Blue: nucleus, red: lysosome, green: Lps, scale bars: 18 μm , $n = 3$.

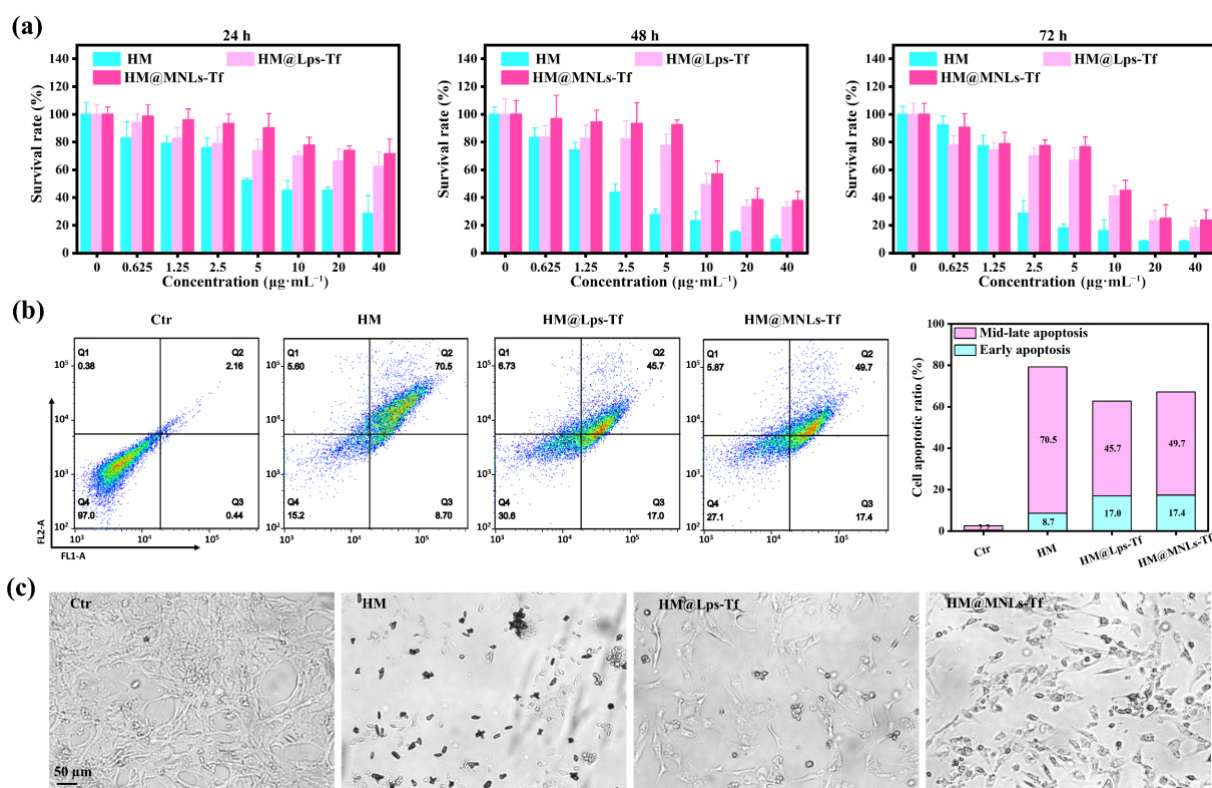


Figure 3 *In vitro* evaluation of anti-tumor effects. (a) Survival rate of U87 cells at different time points ($n = 6$). (b) Apoptosis of U87 cells after treatment with different samples, shown in flow cytometry plots (left) and statistical chart (right) ($n = 3$). (c) Morphology of U87 cells with different samples treatment under the light microscope.

3.4 Evaluation of cross-BBB properties of HM@MNLs-Tf

To further assess the passive targeting effect of the external magnetic field (MF), the study employed a monolayer of mouse brain microvascular endothelial cells (bEnd.3) as an *in vitro* BBB model. Utilizing the high expression feature of Tf receptors on bEnd.3 cells and the glioma cells, combination with the strong guiding ability of the external MF, MNLs-Tf may own the strong ability to cross BBB (Fig. 4(a)). The resistance values change before and after incubation showed no significant variation, indicating that the BBB structure remained intact, thus confirming the successful establishment of the BBB penetration model (Fig. S7 in the ESM). Then, FITC was used to substitute HM and evaluate the BBB penetration ability of HM@MNLs-Tf *in vitro*. Fluorescence intensity in the upper and lower chambers of the bEnd.3 monolayer was measured to evaluate the targeting ability of the MNLs-Tf (Fig. 4(b)). After 4 h of incubation, the green fluorescence intensity in the lower chamber of Tf-modified nanoparticles was significantly higher than that of unmodified nanoparticles. The permeability of FITC@Lps-Tf was twice that of FITC@Lps. Under the influence of the external MF, the permeability of FITC@MNLs-Tf was 3.28-fold of FITC@Lps and 1.60-fold of that of FITC@Lps-Tf, demonstrating that the external MF significantly enhanced the targeting ability of MNLs (Fig. 4(e)).

Next, we employed the near-infrared dye new indocyanine green (IR820) as a substitute for FITC to further explore the ability of MNLs-Tf to cross BBB *in vivo*. The accumulation of IR820 in the brain under the influence of an external MF was measured to assess the nanoparticles' BBB penetration. As shown in Figs. 4(c) and 4(f), Tf-modified Lps exhibited a greater accumulation and stronger fluorescence in the mouse brain compared to non-Tf-modified Lps after 12 h. Under the external MF, IR820@MNLs-Tf showed

significantly increased accumulation in the brain after 48 h later, illustrating a prolonged retention time and a notable difference in fluorescence intensity compared to IR820@Lps-Tf. These results further confirmed that the external MF can guide MNLs to the tumor site. To further assess IR820 accumulation in the brain, mouse brain tissue was collected 12 h post-injection. The *ex vivo* results were consistent with the *in vivo* findings, with significant differences in brain accumulation between Tf-modified and non-modified Lps. Under the external MF, the accumulation of MNLs in the brain increased significantly, and the fluorescence intensity showed a notable difference when compared to IR820@Lps (Figs. 4(d) and 4(g)).

Additionally, iron-based materials are widely used as contrast agents in T_2 -weighted MRI [51–52]. Therefore, this study further investigated the imaging capabilities of the MNLs. According to Fig. S8 in the ESM, both Fe_3O_4 @CA and HM@MNLs-Tf exhibited strong T_2 signals. The r value for Fe_3O_4 @CA was 391.80 s^{-1} , while for HM@MNLs-Tf, it was $234.40 \text{ s}^{-1} \cdot \text{mM}^{-1}$, indicating that HM@MNLs-Tf has a stronger T_2 effect compared to Fe_3O_4 @CA, likely due to a denser distribution of Fe_3O_4 within the Lps. The T_2 -weighted images of U87-MG co-incubated with HM@MNLs-Tf becomes darker along with the increased nanoparticle concentration, indicating the good concentration-dependent property (Fig. S9 in the ESM). Further, magnetic resonance imaging confirmed the targeting ability of the MNLs in glioma (Fig. S10 in the ESM). Under the influence of an external MF, mice in the MNLs-Tf group showed a significant darkening effect at the tumor site compared to the MNLs group. The darkening effect peaked at 8 h, with the images showing the most distinct results. Afterward, the MNLs were gradually metabolized, confirming that MNLs-Tf plus MF exhibited excellent imaging capabilities and targeting ability in T_2 imaging.

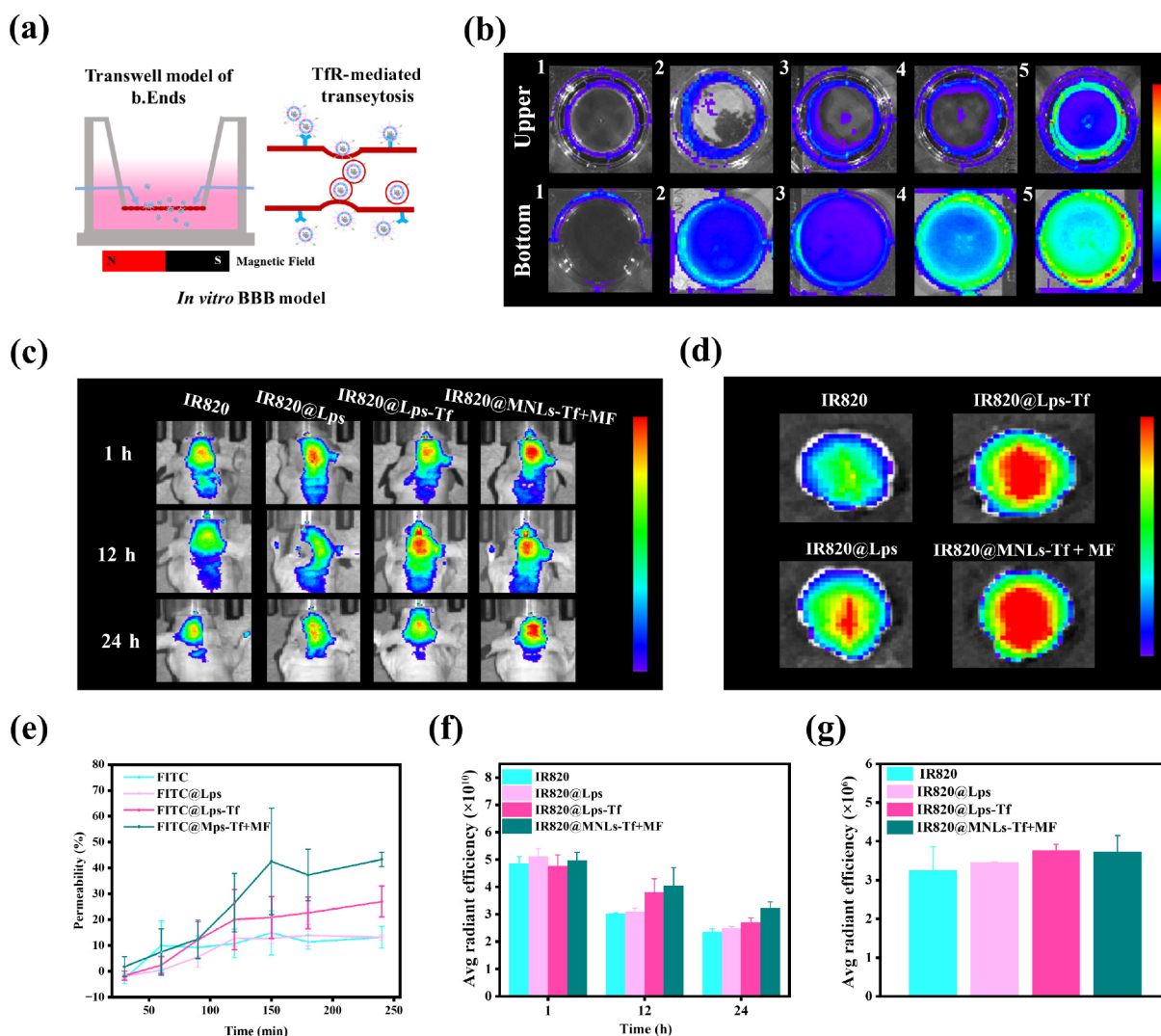


Figure 4 Evaluation of BBB crossing efficiency. (a) Schematic diagram of BBB crossing *in vitro*. (b) Fluorescence images of the bEnd.3 monolayer and the basolateral chamber after 4 h incubation with different materials, 1: Ctr, 2: FITC, 3: FITC@Lps, 4: FITC@Lps-Tf, 5: FITC@MNLs-Tf. (c) Fluorescence imaging of different materials in healthy mice at different time points. (d) *In vitro* fluorescence imaging of the mouse brain at 12 h post-administration. (e) *In vitro* fluorescence penetration statistics ($n = 4$). (f) *In vivo* fluorescence statistics ($n = 4$). (g) Fluorescence statistics of the brain *ex vivo* ($n = 4$).

3.5 Antitumor activity study of HM@MNLs-Tf *in vivo*

The *in vivo* therapeutic effect of HM@MNLs-Tf was assessed using a U87-MG-bearing mouse model. Mice were treated with different NPs at an equivalent HM dose of 2 mg/kg, administered every two days for 21 days. Figure 5(a) shows no significant change in the body weight of tumor-bearing mice before and after treatment. However, tumor weight exhibited significant differences (Fig. 5(b)). The tumor weight of the HM@MNLs-Tf group was significantly lower compared to the HM@Lps and control groups. The free HM group showed less difference in tumor weight compared to the control group, likely due to the poor selectivity of free HM, which failed to effectively reach the tumor. In contrast, the HM@Lps-Tf group exhibited a significant reduction in tumor weight compared to the HM@Lps group. Under the external MF, the tumor weight of the HM@MNLs-Tf group was significantly lower compared to both the HM@MNLs and HM@Lps-Tf groups, indicating the combined effect of MF and Tf-modification (Bonferroni correction $P < 0.05$). The HM@MNLs group under MF also showed lower tumor weight compared to the HM@MNLs group without MF,

and the HM@MNLs-Tf group exhibited the best therapeutic effect. These data demonstrate that both MF and Tf-modification were effective strategies to improve the therapeutic effect of HM. The photograph of tumors in different groups and tumor growth curves shown in Figs. 5(c) and 5(d) further verified this conclusion.

Caspase-3, a key apoptosis executor, is activated by the release of cytochrome c (Cyt-c), and Bcl-2 plays a crucial role in apoptosis regulation. Immunohistochemical detection was performed to assess the expression of Caspase-3, Bcl-2, and the proliferation marker Ki67 in tumor tissues (Figs. 5(e) and 5(g), and Fig. S11 in the ESM). These results revealed that in the HM@MNLs-Tf group under the external MF, Caspase-3 expression significantly increased, while Bcl-2 and Ki67 expression significantly decreased. Caspase-3 expression in the HM@MNLs-Tf group was significantly higher compared to the HM@Lps group, while Bcl-2 expression was significantly lower, indicating effective tumor cell apoptosis and inhibition of proliferation (Bonferroni correction $P < 0.05$). TUNEL apoptosis assay results were consistent, showing a significant increase in TUNEL-positive tumor cells in the HM@MNLs-Tf group under MF. H&E staining showed fewer and less dense nuclei

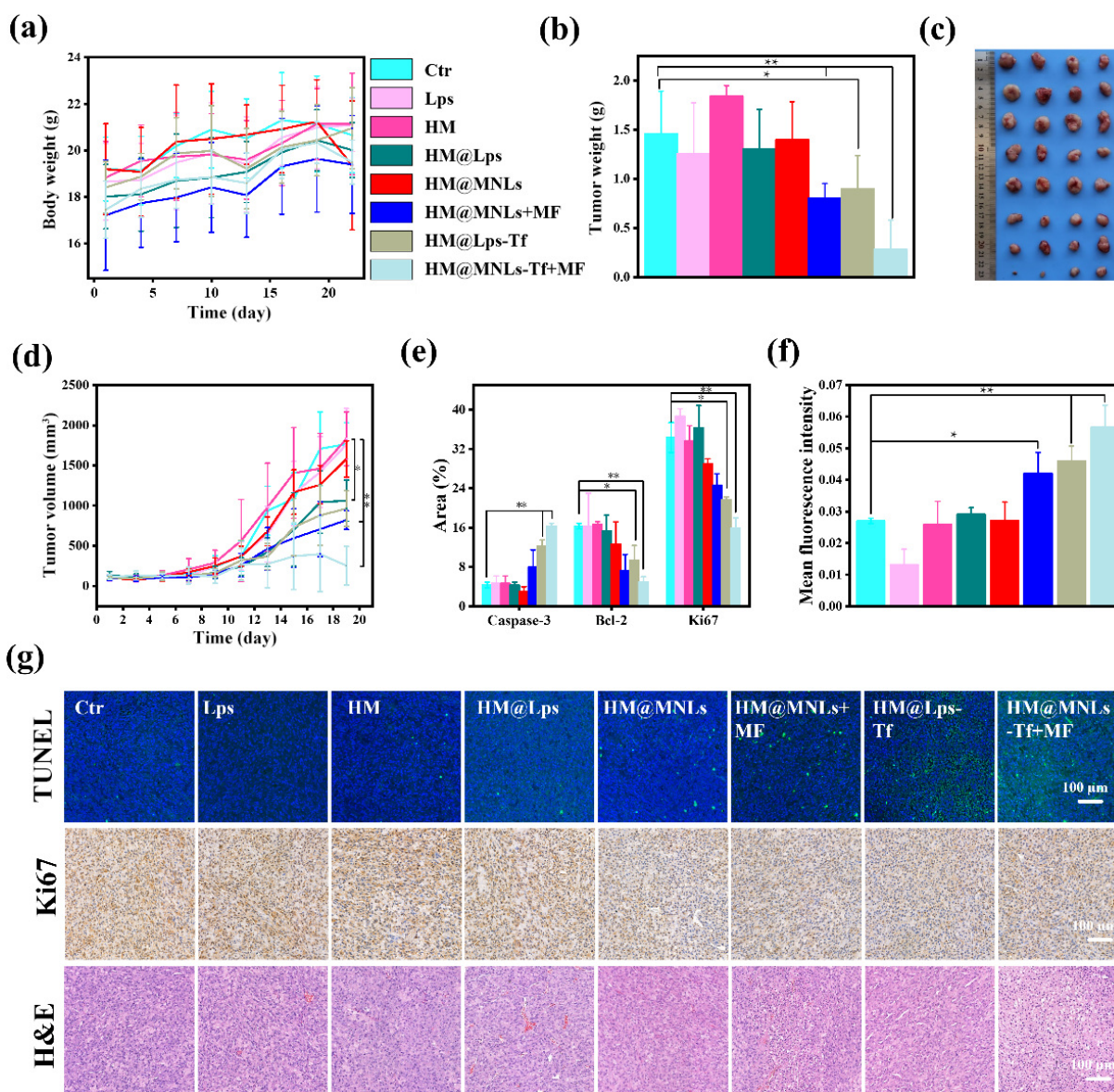


Figure 5 Evaluation of antitumor effects *in vitro*. (a) Weight change curve of tumor-bearing mice treated with different materials at various time points ($n = 4$). (b) Statistical analysis of tumor weight after treatment with different materials ($n = 4$). (c) *In vitro* tumor images ($n = 4$). (d) Tumor volume change curve at different treatment times with different materials ($n = 4$). (e) Immunohistochemical analysis of Caspase-3, Bcl-2, and Ki67 expression ($n = 3$). (f) Immunohistochemical analysis of TUNEL ($n = 3$). (g) Immunohistochemical images of TUNEL and Ki67, and H&E staining of the tumor, scale bars: 100 μ m.

in treated groups, especially in HM@MNLs-Tf, indicating reduced tumor cell number and activity (Figs. 5(f) and 5(g)).

Blood routine tests (Fig. S12 in the ESM) showed no significant differences in red blood cell and platelet counts between the experimental and control groups. White blood cell count was relatively lower, especially neutrophils, possibly due to mild inflammatory response in the tumor group. This suggests that the lipid-based HM formulation has some anti-inflammatory effects, enhanced by MF, particularly in the HM@MNLs-Tf group. Biochemical analysis showed a significant decrease in aspartate aminotransferase (AST) levels, indicating reduced liver burden with MF treatment. No significant renal toxicity was observed. MF treatment also led to decreased myocardial enzymes in the HM@MNLs-Tf group, suggesting cardiac protection. H&E staining showed no noticeable fibrosis, necrosis, or inflammatory infiltration in major organs (Fig. S13 in the ESM).

3.6 CNS toxicity study

HM is a β -carboline alkaloid that selectively and reversibly inhibits MAO-A, preventing the degradation of 5-HT and NE in the brain, leading to toxic side effects, including tremors and hypothermia (Fig. 6(a)). In this study, we investigated whether improving the targeting property of HM could alleviate its toxicity to the CNS. Initially, we observed the tremor behavior of mice injected with HM and HM@MNLs-Tf under the influence of an external MF. As shown in Fig. 6(b), after injection of free HM, the tremor score remained above 2, indicating moderate intermittent tremors in the mice. In contrast, the tremor scores for the HM@MNLs-Tf group remained below 2, suggesting mild and rare tremors, thus indicating that the formulation of HM used in this study indeed mitigated its toxicity. Temperature measurements revealed that, within 15 min of injection, the free HM group showed a rapid decrease in temperature, whereas the HM@MNLs-Tf group

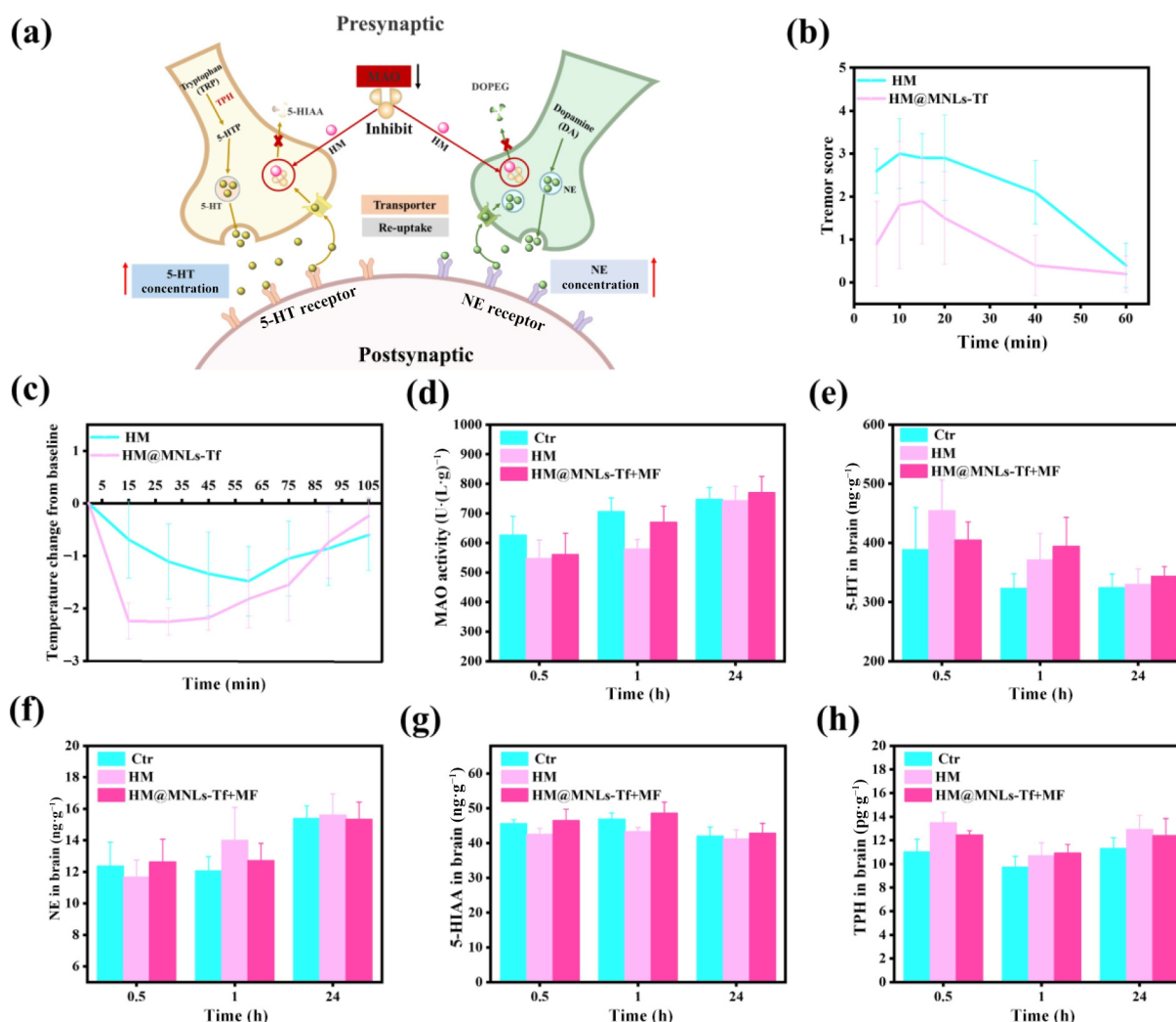


Figure 6 Evaluation of CNS toxicity. (a) Schematic diagram of CNS toxicity produced by HM. (b) Tremor scores of mice administered HM and HM@MNLs-Tf+MF for 60 min at different time points ($n = 8$). (c) Statistical results of the absolute temperature decrease in mice administered HM and HM@MNLs-Tf+MF for 105 min at different time points ($n = 8$). (d) Detection of MAO activity in the brain of mice at different time points in each group ($n = 8$). (e) Detection of 5-HT content in the brain of mice at different time points in each group ($n = 8$). (f) Detection of NE content in the brain of mice at different time points in each group ($n = 8$). (g) Detection of 5-HIAA (a 5-HT metabolite) levels in the brain of mice at different time points in each group ($n = 8$). (h) Detection of TPH (the rate-limiting enzyme in 5-HT synthesis) expression levels in the brain of mice at different time points in each group ($n = 8$).

exhibited a more gradual decline and a quicker recovery (Fig. 6(c)). We also assessed the MAO levels in the brains of mice at different time points. As shown in Fig. 6(d), both the free HM and HM@MNLs-Tf groups exhibited lower MAO levels than the control group at 0.5 h post-injection, indicating that both formulations inhibited MAO to some extent. However, the MAO levels in both groups began to rise over time, and the HM@MNLs-Tf group shows significantly higher MAO levels than the free HM group at 1 h. The MAO levels in both groups then leveled off, approaching those of the control group. The selective inhibition of MAO-A by HM prevents the effective degradation of 5-HT, leading to an increase in 5-HT levels in the brain. As shown in Fig. 6(e), at 0.5 h post-injection, the 5-HT levels in the brains of the HM group were elevated compared to the control group, likely due to the ability of free HM to easily cross the BBB. However, the 5-HT levels in the HM group returned to control levels after 1 h, while the HM@MNLs-Tf group showed a significant increase in 5-HT at 1 h compared to the control group. These results suggest that the MNLs-encapsulated HM, in combination with Tf targeting, leads to

a prolonged circulation in the brain. After 24 h, the HM in the experimental groups was largely metabolized, and the 5-HT levels were comparable to the control group. Regarding NE (Fig. 6(f)), the free HM group showed a decrease in NE levels at 0.5 h, whereas the HM@MNLs-Tf group maintained NE levels similar to the control group. At 1 h, the NE levels in the free HM group were significantly higher than that in the control group, indicating that free HM inhibited NE degradation. However, no difference was observed between the HM@MNLs-Tf group and the control group. Additionally, we measured the expression levels of the metabolite of 5-HT, 5-HIAA, and the rate-limiting enzyme TPH in the mice (Figs. 6(g) and 6(h)). At 0.5 and 1 h, the HM group showed a marked decrease in 5-HIAA levels compared to the control group, while the HM@MNLs-Tf group showed 5-HIAA levels similar to the control group. This is likely due to the inhibition of MAO-A by HM, which prevents the effective degradation of 5-HT, thus lowering 5-HIAA levels. The TPH expression levels were consistent with the 5-HT results, showing an increase at 0.5, 1, and 24 h post-injection compared to the control group (Fig. 6(h)). These results

demonstrate that the MNLs drug delivery system plus MF effectively reduces the acute toxicity of HM to the CNS.

4 Conclusions

Glioma is the most common primary malignant brain tumor derived from glial cells, characterized by its high invasiveness and complexity. Due to the intricate integration of glioma cells with normal brain tissues, treatment remains highly challenging. Current therapies have limited efficacy and may induce severe side effects. To improve treatment outcomes and reduce side effects, recent research has focused on drug delivery systems (such as Lps) and targeted therapies. HM, a bioactive alkaloid with significant anti-tumor activity, is widely found in the seeds of *Peganum harmala* L. Despite its strong anti-cancer properties, HM's poor water solubility results in low bioavailability and notable side effects, particularly toxicity to the CNS. To enhance the therapeutic effects of HM and reduce its neurotoxicity, we have explored magnetic-targeted TfR-mediated Lps drug delivery systems (HM@MNLs-Tf), allowing for precise targeting of glioma cells and minimizing impacts on normal brain tissue.

In this study, an innovative drug delivery method based on liposomal drug carriers was employed, wherein HM was encapsulated in Lps and surface-modified with Tf. This modification facilitated specific binding to the TfR that are highly expressed on glioma cells, promoting Lps uptake. Additionally, by applying an external MF, the aggregation of the Lps at the targeted region was enhanced, thereby improving the precision of drug delivery. In our research, Fe_3O_4 @CA magnetic nanoparticles were prepared using a hydrothermal method to serve as the magnetic components within the Lps, enhancing drug targeting under magnetic influence. The study successfully synthesized magnetic nanoparticles with good dispersion and small particle size (~ 200 nm), and negative surface charge (~ -28 mV). Lps, prepared via the thin-film hydration method, achieved a drug encapsulation efficiency of over 70%, with excellent stability. Drug release studies *in vitro* showed that HM@Lps-Tf exhibited a significantly higher release rate in the acidic pH 6.5 tumor microenvironment compared to the neutral pH 7.4 physiological environment, indicating that the Lps could respond to the tumor microenvironment for sustained drug release. A 1 T magnetic field was used in this study, which can effectively guide liposomal accumulation at tumor sites by enhancing the magnetic gradient force and improving tissue penetration. This strength of magnetic field has been shown to significantly enhance nanoparticle deposition and antitumor efficacy, overcoming blood flow resistance and improving particle migration to deep tissues [53].

Through LSCM and FCM, it was observed that Tf-modified Lps (FITC@Lps-Tf) were taken up by glioma cells more than the unmodified Lps (FITC@Lps). Moreover, Lps displayed good colocalization with lysosomes, suggesting efficient cellular entry and drug release within the cells. The CCK-8 cell proliferation assay revealed a dose-dependent inhibition of glioma cells by HM@MNLs-Tf. Compared to free HM, HM@MNLs-Tf had a higher IC₅₀ value, but as the Lps released the drug slowly, their effect gradually approached that of free HM, indicating that this delivery system can effectively keep the sustained drug release and reduce side effects. Flow cytometry further confirmed that HM@MNLs-Tf significantly induced apoptosis in glioma cells, similar with that of free HM.

To verify whether the liposomal system could overcome the BBB, a bEnd.3 mouse brain microvascular endothelial cell model was constructed. The results demonstrated that Tf-modified, under the influence of an external MF, significantly enhanced the permeability across the BBB. The fluorescence signal intensity of MNLs in the brain tissue was also notably higher than that of unmodified Lps, further confirming the improved brain targeting capability of the fabricated drug loading system in this study. In the glioma mouse model, HM@MNLs-Tf exhibited good anti-tumor effects, significantly inhibiting tumor growth, with minimal changes in body weight and no noticeable side effects. Immunohistochemistry and TUNEL assays showed significant apoptosis in tumor tissues and reduced cell proliferation, confirming effective tumor growth inhibition.

To assess whether HM@MNLs-Tf could reduce the neurotoxicity of HM, tremor, body temperature, and 5-HT metabolism were examined. The results revealed that loaded with HM, under MF application, significantly reduced tremor and temperature drops caused by HM, while regulating 5-HT levels, thus decreasing CNS toxicity. This finding highlights the substantial advantages of liposomal drug delivery systems in minimizing side effects.

This study presents an innovative drug delivery system HM@MNLs-Tf that effectively delivers HM to glioma cells and enhances drug accumulation at the targeted site through MF. This system not only improves therapeutic efficacy but also significantly reduces HM's neurotoxic side effects, demonstrating the promising clinical application possibility. Furthermore, the research provides a new approach for glioma treatment, especially in the fields of precise drug delivery and targeted therapy. In conclusion, the magnetically controlled TfR targeted liposomal system offers a solution for HM to be used as the brain glioma treatment drug. Future research could further use this drug delivery system to improve the therapeutic effect of other drugs extracted from plants.

Electronic Supplementary Material: Supplementary material (including detailed characterization data such as size distribution of Fe_3O_4 @CA nanoparticles, optimization of HM and Tf loading parameters, cellular affinity assays, cytotoxicity evaluations, MRI performance, blood compatibility tests, and histological analyses) is available in the online version of this article at <https://doi.org/10.26599/NR.2025.94908011>.

Data availability

All data needed to support the conclusions in the paper are presented in the manuscript and the Electronic Supplementary Material. Additional data related to this paper may be requested from the corresponding author upon reasonable request.

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Declaration of competing interest

All the contributing authors report no conflict of interests in this work.

Author contribution statement

X. H. T.: Conceptualization, data curation, formal analysis, investigation, methodology, writing – original draft; S. H. L.: Conceptualization, data curation, formal analysis, investigation, methodology; R. B.: Supervision, project administration, validation; R. R. Q.: Data curation, validation, investigation; T. L.: Data curation, validation, investigation; J. K. Z.: Data curation, validation, investigation; C. Y. C.: Data curation, validation, investigation; M. W.: Supervision, funding acquisition, project administration; H. G. Z.: Supervision, funding acquisition, project administration, methodology, writing – review & editing.

Informed consent

Not applicable.

Ethics statement

The animal experiments were carried out following the guidelines of the National Centre for Nanoscience and Technology of China Committee for the Care and Use of Laboratory Animals and were approved by the Animal Ethics Committee of the National Centre for Nanoscience and Technology of China (Ethics No. NCNST21-2020-10).

Use of AI statement

None.

References

- [1] Sung, H.; Ferlay, J.; Siegel, R. L.; Laversanne, M.; Soerjomataram, I.; Jemal, A.; Bray, F. Global cancer statistics 2020: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA Cancer J. Clin.* **2021**, *71*, 209–249.
- [2] Barthel, L.; Hadamitzky, M.; Dammann, P.; Schedlowski, M.; Sure, U.; Thakur, B. K.; Hetze, S. Glioma: Molecular signature and crossroads with tumor microenvironment. *Cancer Metast. Rev.* **2022**, *41*, 53–75.
- [3] Matsumae, M.; Nishiyama, J.; Kuroda, K. Intraoperative MR imaging during glioma resection. *Magn. Reson. Med. Sci.* **2022**, *21*, 148–167.
- [4] Hou, D. Y.; Cheng, D. B.; Zhang, N. Y.; Wang, Z. J.; Hu, X. J.; Li, X.; Lv, M. Y.; Li, X. P.; Jian, L. R.; Ma, J. P. et al. *In vivo* assembly enhanced binding effect augments tumor specific ferroptosis therapy. *Nat. Commun.* **2024**, *15*, 454.
- [5] Hou, D. Y.; Wang, H. R.; Wang, Y. Z.; Cheng, D. B.; Tang, B. Z.; Xu, W. H. Intracellular aggregation of exogenous molecules for biomedical applications. *Chem. Soc. Rev.* **2025**, *54*, 6412–6447.
- [6] Mitusova, K.; Peltek, O. O.; Karpov, T. E.; Muslimov, A. R.; Zyuzin, M. V.; Timin, A. S. Overcoming the blood-brain barrier for the therapy of malignant brain tumor: Current status and prospects of drug delivery approaches. *J. Nanobiotechnol.* **2022**, *20*, 412.
- [7] Zhao, C. Y.; Wang, C. R.; Shan, W. B.; Wang, W. P.; Deng, H. Z. Fusogenic lipid nanovesicle for biomacromolecular delivery. *Nano Lett.* **2024**, *24*, 8609–8618.
- [8] Fawal, M. A.; Jungas, T.; Davy, A. Inhibition of DHFR targets the self-renewing potential of brain tumor initiating cells. *Cancer Lett.* **2021**, *503*, 129–137.
- [9] Ravasio, R.; Ceccacci, E.; Minucci, S. Self-renewal of tumor cells: Epigenetic determinants of the cancer stem cell phenotype. *Curr. Opin. Genet. Dev.* **2016**, *36*, 92–99.
- [10] Safa, A. R.; Saadatzadeh, M. R.; Cohen-Gadol, A. A.; Pollok, K. E.; Bijangi-Vishehsaraei, K. Glioblastoma stem cells (GSCs) epigenetic plasticity and interconversion between differentiated non-GSCs and GSCs. *Genes Dis.* **2015**, *2*, 152–163.
- [11] Zhang, L.; Li, D. C.; Yu, S. L. Pharmacological effects of harmine and its derivatives: A review. *Arch. Pharm. Res.* **2020**, *43*, 1259–1275.
- [12] Kumar, K.; Wang, P.; Sanchez, R.; Swartz, E. A.; Stewart, A. F.; DeVita, R. J. Development of kinase-selective, harmine-based DYRK1A inhibitors that induce pancreatic human β -cell proliferation. *J. Med. Chem.* **2018**, *61*, 7687–7699.
- [13] Chen, D. Y.; Tian, X. Y.; Zou, X.; Xu, S. J.; Wang, H. R.; Zheng, N.; Wu, Z. W. Harmine, a small molecule derived from natural sources, inhibits enterovirus 71 replication by targeting NF- κ B pathway. *Int. Immunopharmacol.* **2018**, *60*, 111–120.
- [14] Shi, Y. J.; Chen, X. D.; Shu, J. Y.; Liu, Y.; Zhang, Y.; Lv, Q. H.; Wang, J. F.; Deng, X. M.; Liu, H. T.; Qiu, J. Z. Harmine, an inhibitor of the type III secretion system of *Salmonella Enterica* serovar typhimurium. *Front. Cell. Infect. Microbiol.* **2022**, *12*, 967149.
- [15] Hu, Y. H.; Yu, X. L.; Yang, L.; Xue, G. M.; Wei, Q. L.; Han, Z. J.; Chen, H. Research progress on the antitumor effects of harmine. *Front. Oncol.* **2024**, *14*, 1382142.
- [16] Zhu, Y. G.; Lv, Y. X.; Guo, C. Y.; Xiao, Z. M.; Jiang, Q. G.; Kuang, H.; Zhang, W. H.; Hu, P. Harmine inhibits the proliferation and migration of glioblastoma cells via the FAK/AKT pathway. *Life Sci.* **2021**, *270*, 119112.
- [17] Jiang, B.; Meng, L. Y.; Zou, N.; Wang, H. X.; Li, S. P.; Huang, L. F.; Cheng, X. M.; Wang, Z. T.; Chen, W. S.; Wang, C. H. Mechanism-based pharmacokinetics-pharmacodynamics studies of harmine and harmaline on neurotransmitters regulatory effects in healthy rats: Challenge on monoamine oxidase and acetylcholinesterase inhibition. *Phytomedicine* **2019**, *62*, 152967.
- [18] Li, S. P.; Wang, Y. W.; Qi, S. L.; Zhang, Y. P.; Deng, G.; Ding, W. Z.; Ma, C.; Lin, Q. Y.; Guan, H. D.; Liu, W. et al. Analogous β -carboline alkaloids harmaline and harmine ameliorate scopolamine-induced cognition dysfunction by attenuating acetylcholinesterase activity, oxidative stress, and inflammation in mice. *Front. Pharmacol.* **2018**, *9*, 346.
- [19] Nozhat, Z.; Heydarzadeh, S.; Shahriari-Khalaji, M.; Wang, S. B.; Iqbal, M. Z.; Kong, X. D. Advanced biomaterials for human glioblastoma multiforme (GBM) drug delivery. *Biomater. Sci.* **2023**, *11*, 4094–4131.
- [20] Ashby, L. S.; Ryken, T. C. Management of malignant glioma: Steady progress with multimodal approaches. *Neurosurg. Focus* **2006**, *20*, E3.
- [21] Prados, M. Temozolomide in combination with other cytotoxic agents. *Semin. Oncol.* **2001**, *28*, 24–33.
- [22] Sun, Q. Q.; Liu, C.; Jiang, K. X.; Fang, Y. S.; Kong, C. C.; Fu, J.; Liu, Y. G. A preliminary study on the neurotoxic mechanism of harmine in *Caenorhabditis elegans*. *Comp. Biochem. Physiol. C: Toxicol. Pharmacol.* **2021**, *245*, 109038.
- [23] Lv, Y.; Liang, H. Y.; Li, J.; Li, X. X.; Tang, X. H.; Gao, S. Y.; Zou, H.; Zhang, J.; Wang, M.; Xiao, L. Central inhibition prevents the *in vivo* acute toxicity of harmine in mice. *J. Toxicol. Sci.* **2021**, *46*, 289–301.
- [24] Wang, C. R.; Zhao, C. Y.; Wang, W. P.; Liu, X. Q.; Deng, H. Z. Biomimetic noncationic lipid nanoparticles for mRNA delivery. *Proc. Natl. Acad. Sci. USA* **2023**, *120*, e2311276120.
- [25] Warner, J. S.; Kinsey, C. M.; Bates, J. H. T.; Mori, V. Intratumoral chemotherapy: The effects of drug concentration and dose apportioning on tumor cell injury. *Bioengineering (Basel)* **2024**, *11*, 809.
- [26] Marchan, E. M.; Harrell, A. C.; Waddle, M. R.; Miller, R. C.; Miller, D.; Stross, W.; Kaleem, T.; Ruiz-Garcia, H.; Peterson, J. L.; Quinones-

- Hinojosa, A. et al. Comparative utilization analysis of radiation modalities among adults with primary brain tumors in the united states from 2004 to 2015. *Int. J. Radiat. Oncol.* **2018**, *102*, E351–E352.
- [27] Hou, D. Y.; Zhang, N. Y.; Zhang, P.; Li, X. P.; Wu, J. C.; Lv, M. Y.; Wang, Z. J.; Hu, X. J.; Liang, J. X.; Wang, H. L. et al. *In vivo* self-assembled bispecific fluorescence probe for early detection of bladder cancer and metastasis. *Sci. Bull.* **2025**, *70*, 407–418.
- [28] Huang, P.; Wang, C. R.; Deng, H. Z.; Zhou, Y. F.; Chen, X. Y. Surface engineering of nanoparticles toward cancer theranostics. *Acc. Chem. Res.* **2023**, *56*, 1766–1779.
- [29] Topcu, B. T.; Bozdağ Pehlivan, S.; Akdağ, Y.; Mut, M.; Öner, L. Antibody conjugated nano-enabled drug delivery systems against brain tumors. *J. Pharm. Sci.* **2024**, *113*, 1455–1469.
- [30] Miao, Y. B.; Zhao, W.; Renchi, G.; Gong, Y.; Shi, Y. Customizing delivery nano-vehicles for precise brain tumor therapy. *J. Nanobiotechnol.* **2023**, *21*, 32.
- [31] Ganjeifar, B.; Morshed, S. F. Targeted drug delivery in brain tumors-nanochemistry applications and advances. *Curr. Top. Med. Chem.* **2021**, *21*, 1202–1223.
- [32] Li, X. X.; Li, W. Y.; Wang, M. N.; Liao, Z. H. Magnetic nanoparticles for cancer theranostics: Advances and prospects. *J. Control. Release* **2021**, *335*, 437–448.
- [33] Dadfar, S. M.; Roemhild, K.; Drude, N. I.; Von Stillfried, S.; Knüchel, R.; Kiessling, F.; Lammers, T. Iron oxide nanoparticles: Diagnostic, therapeutic and theranostic applications. *Adv. Drug Deliv. Rev.* **2019**, *138*, 302–325.
- [34] Umut, E.; Coşkun, M.; Pineider, F.; Berti, D.; Güngüneş, H. Nickel ferrite nanoparticles for simultaneous use in magnetic resonance imaging and magnetic fluid hyperthermia. *J. Colloid Interface Sci.* **2019**, *550*, 199–209.
- [35] Shasha, C.; Krishnan, K. M. Nonequilibrium dynamics of magnetic nanoparticles with applications in biomedicine. *Adv. Mater.* **2021**, *33*, 1904131.
- [36] Xu, S. B.; Zhang, G. X.; Zhang, J. M.; Liu, W.; Wang, Y. C.; Fu, X. Y. Advances in brain tumor therapy based on the magnetic nanoparticles. *Int. J. Nanomed.* **2023**, *18*, 7803–7823.
- [37] Lu, Y. J.; Hsu, H. L.; Lan, Y. H.; Chen, J. P. Thermosensitive cationic magnetic liposomes for thermoresponsive delivery of CPT-11 and SLP2 shRNA in glioblastoma treatment. *Pharmaceutics* **2023**, *15*, 1169.
- [38] Wu, H. A.; Liu, L.; Ma, M.; Zhang, Y. Modulation of blood-brain tumor barrier for delivery of magnetic hyperthermia to brain cancer. *J. Control. Release* **2023**, *355*, 248–258.
- [39] T. S, A.; Shalumon, K. T.; Chen, J. P. Applications of magnetic liposomes in cancer therapies. *Curr. Pharm. Des.* **2019**, *25*, 1490–1504.
- [40] Alawak, M.; Mahmoud, G.; Dayyih, A. A.; Duse, L.; Pinnapireddy, S. R.; Engelhardt, K.; Awak, I.; Wölk, C.; König, A. M.; Brübler, J. et al. Magnetic resonance activatable thermosensitive liposomes for controlled doxorubicin delivery. *Mater. Sci. Eng. C* **2020**, *115*, 111116.
- [41] Thébault, C. J.; Ramniceanu, G.; Boumati, S.; Michel, A.; Seguin, J.; Larrat, B.; Mignet, N.; Ménager, C.; Doan, B. T. Theranostic MRI liposomes for magnetic targeting and ultrasound triggered release of the antivascular CA4P. *J. Control. Release* **2020**, *322*, 137–148.
- [42] Zhao, C. Y.; Wang, C. R.; Shan, W. B.; Wang, Z. L.; Chen, X. Y.; Deng, H. Z. Nanomedicines for an enhanced immunogenic cell death-based *in situ* cancer vaccination response. *Acc. Chem. Res.* **2024**, *57*, 905–918.
- [43] Zhang, P.; Ye, G. H.; Xie, G. F.; Lv, J.; Zeng, X. H.; Jiang, W. Research progress of nanomaterial drug delivery in tumor targeted therapy. *Front. Bioeng. Biotechnol.* **2023**, *11*, 1240529.
- [44] Baker, A.; Khan, M. S.; Iqbal, M. Z.; Khan, M. S. Tumor-targeted Drug Delivery by Nanocomposites. *Curr. Drug Metab.* **2020**, *21*, 599–613.
- [45] Li, J. R.; Zhang, Z. X.; Zhang, B. L.; Yan, X. Y.; Fan, K. L. Transferrin receptor 1 targeted nanomedicine for brain tumor therapy. *Biomater. Sci.* **2023**, *11*, 3394–3413.
- [46] Guo, Z. L.; Zhang, Y. R.; Fu, M. P.; Zhao, L.; Wang, Z.; Xu, Z. S.; Zhu, H. F.; Lan, X. L.; Shen, G. X.; He, Y. et al. The transferrin receptor-directed CAR for the therapy of hematologic malignancies. *Front. Immunol.* **2021**, *12*, 652924.
- [47] Kang, Z. Y.; Zeng, C. L.; Tian, L.; Wang, T. R.; Yang, S.; Cheng, Q.; Zhang, J.; Meng, Q. B.; Zhang, C. H.; Meng, Z. Transferrin receptor targeting segment T7 containing peptide gene delivery vectors for efficient transfection of brain tumor cells. *Drug Deliv.* **2022**, *29*, 2375–2385.
- [48] Xiao, W.; Wang, Y. Z.; Zhang, H. L.; Liu, Y. W.; Xie, R.; He, X. Q.; Zhou, Y.; Liang, L. Q.; Gao, H. L. The protein corona hampers the transcytosis of transferrin-modified nanoparticles through blood-brain barrier and attenuates their targeting ability to brain tumor. *Biomaterials* **2021**, *274*, 120888.
- [49] Ramalho, M. J.; Loureiro, J. A.; Coelho, M. A. N.; Pereira, M. C. Transferrin receptor-targeted nanocarriers: Overcoming barriers to treat glioblastoma. *Pharmaceutics* **2022**, *14*, 279.
- [50] Du, B. L.; Qu, C. R.; Qian, K.; Ren, Y.; Li, Y. S.; Cui, X. H.; He, S. Q.; Wu, Y. F.; Ko, T.; Liu, R. Q. et al. An IR820 dye-protein complex for second near-infrared window and photoacoustic imaging. *Adv. Opt. Mater.* **2020**, *8*, 1901471.
- [51] Rahmati, S.; David, A. E. A review of design criteria for cancer-targeted, nanoparticle-based MRI contrast agents. *Appl. Mater. Today* **2024**, *37*, 102087.
- [52] Geraldes, C. F. G. C. Rational design of magnetic nanoparticles as T₁–T₂ dual-mode MRI contrast agents. *Molecules* **2024**, *29*, 1352.
- [53] Saadat, M.; Manshadi, M. K. D.; Mohammadi, M.; Zare, M. J.; Zarei, M.; Kamali, R.; Sanati-Nezhad, A. Magnetic particle targeting for diagnosis and therapy of lung cancers. *J. Control. Release* **2020**, *328*, 776–791.



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