Submitted: 23 September, 2022 Accepted: 30 November, 2022 Published: 15 February, 2023 DOI:10.22514/ejgo.2023.012

ORIGINAL RESEARCH

European Journal of Gynaecological Oncology

GLUT1-mediated magnetic liposomes for targeting bone metastatic breast cancer

Ze Zhao[1,*], Yi Zhao[2,*], Changqing Chen[1], Changwei Xie[1]

[1]Department of Orthopedics, the First Affiliated Hospital of Henan Polytechnic University (the Second People's Hospital of Jiaozuo City), 454001 Jiaozuo, Henan, China
[2]Department of Translational Medicine Center, the First Affiliated Hospital of Zhengzhou University, 450052 Zhengzhou, Henan, China

***Correspondence**
zezhao0910@163.com
(Ze Zhao);
zhaoyi0910@163.com
(Yi Zhao)

Abstract

Bone metastatic breast cancer is a malignant tumor in bone due to the metastasis of breast cancer, and its incidence is increasing worldwide. Treatment of cancer metastasized to bone is still a challenge because of the anticancer drugs lack target specificity. Finding an effective treatment for bone metastasis remains an urgent issue. In order to enhance the delivery of paclitaxel (PTX) to the bone metastases lesions, a novel glucose derivative was designed and synthesized in this work, which was used as liposome ligand to develop the magnetic liposome G-MLip (Glucose-modified magnetic liposome). The liposome could improve the drug formulations in the bone metastases mediated by glucose transporter 1 (GLUT1) and then target cancer cells. The PTX-loaded magnetic liposome PTX-G-MLip was prepared by the film hydration-ultrasound method. And the characterizations, such as size, zeta potential, encapsulation efficiency, release profile, stability, hemolysis, were well evaluated. What's more, the enhanced target ability was also investigated *in vitro* and in mice. The metastatic bone-targeted capacity was confirmed that the PTX concentration from PTX-G-MLip in the bone metastases lesions was markedly increased in the presence of magnetic field (MF) compared with the free PTX and other liposomes. Inspired by the enhanced targeting ability, glucose-modified magnetic liposomes could serve as an effective drug delivery system for targeting and treating bone metastases.

Keywords

Bone metastases; GLUT1; Magnetic liposomes; Warburg effect

1. Introduction

Bone metastatic is a malignant tumor in bone due to the easily metastatic characteristic, and its incidence is increasing worldwide [1–3]. The cancer cells, such as breast carcinoma and prostatic carcinoma, metastasize easily to bone tissue due to the suitable proliferation conditions of bone tissue for carcinoma cells [4–7].

The patients with bone metastasis suffer bone pain, hypercalcemia, and pathological fractures, resulting in reduced quality of life [8]. Surgery is the first-line treatment option bone metastases, but for patients with bone metastase advanced, surgery is difficult to completely remove the tumor and requires combination therapy with other antitumor treatments. In the past, numbers of chemotherapeutic agents have emerged for the treatment of bone metastase, such as paclitaxel (PTX), doxorubicin (Dox) [9, 10]. While, the application of chemotherapeutic drug is limited due to the poor targeting ability, and the low blood flows at bone further blocks the effect of chemotherapy. Therefore, it is urgently needed to develop novel strategies to deliver drugs to the metastasis bone tissue [11].

It is well known that cancer cells need more glucose than normal cells to support the normal life state of cells, which was named as Warburg effect [12]. Hence, compared with the normal cells, the bone metastatic breast cancer cell has a high expression of glucose transporters (GLUTs), especially GLUT1. The study for targeting the Warburg effect and treatments for bone metastase is a hot research topic in the medical field. What's more, among the hydroxyls of glucose, the C-6 position glycosylation is suitable for the transport by GLUT1 as our previous report [13, 14]. And our previous study also has reported a combretastatin A-4 derivative (CA4D) modified with glucose, which could target the cancer cells and then release CA4D with the hydrolase, hence improving the concentration and reducing the dose and toxic side effects [14].

Liposome, as a novel drug delivery system, has the advantages of high targeting ability, slow release behavior, reducing drug toxicity and improving drug stability [15]. The PEGylation can prevent the liposome from getting trapped by the reticuloendothelial system, which contributes to the long-circulating [16]. What's more, magnetic nanoparticles (MNPs), chemically iron oxide (Fe_3O_4), have been regarded as new delivery tools used to diagnosis, gene therapy, and targeted therapy [17–19]. And Fe_3O_4 magnetic nanoparticles are also used in the drug delivery system to increase the therapeutic efficacy based on their super-paramagnetic properties.

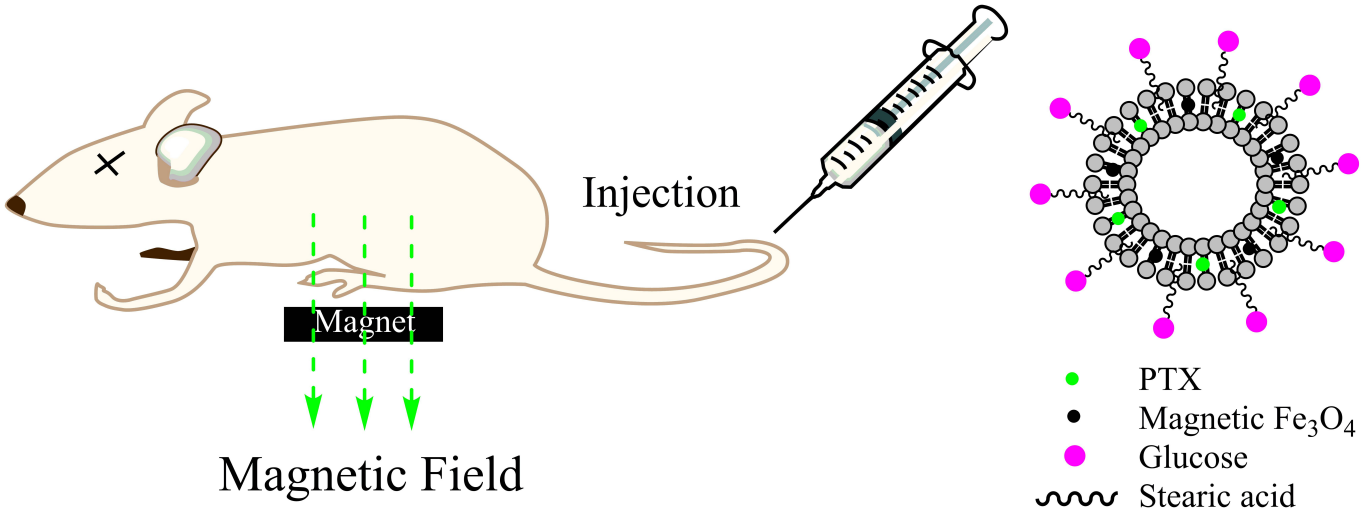

FIGURE 1. The illustration of PTX-G-MLip to target bone metastatic breast cancer. PTX: paclitaxel; Fe_3O_4: chemically iron oxide.

Magnetic liposome, which encapsulates the MNPs inside, has attracted attention as controlled release drug delivery agents because of the permeability under low frequency magnetic fields.

In the present work, a novel liposome ligand, compound 6, was designed and synthesized. And PTX-loaded magnetic liposomes were prepared by the lipid film hydration-ultrasound method, and a systematic study has been conducted to characterize the properties. Improved bone metastasis accumulation was detected through *in vivo* studies (Fig. 1).

2. Materials and methods

2.1 Chemistry

2.1.1 Synthesis of compound 2

Sodium hydride (NaH, 1.33 g, 55.51 mmoL) was dissolved in anhydrous dimethyl formamide (DMF, 100 mL), and glucose (1.00 g, 5.55 mmoL) was added into the above solution, then the mixture was stirred at room temperature. After 0.5 h, benzyl bromide (BnBr, 4.94 mL, 41.63 mmoL) was added, and the reaction was kept to be stirring for another 24 h. The reaction was quenched by adding excess methyl alcohol (CH_3OH), and the solvent was removed with rotary evaporators. The residue was re-dissolved in 100 mL dichloromethane (CH_2Cl_2), which was then filtrated and washed with saturated sodium chloride (NaCl) solution. After removing CH_2Cl_2, purification of the residue was performed through column chromatography to give the intermediate 2 (2.74 g, 78.37%) as a white solid (mp 88–90 °C). ^{1}H NMR (Nuclear magnetic resonance) (400 MHz, $CDCl_3$ (Deuterium chloroform), ppm): δ 3.47–3.57 (m, 2H), 3.65 (t, 2H, J = 7.2 Hz), 3.74 (d, 1H, J = 4.8 Hz), 3.77–3.80 (m, 1H), 4.52–5.02 (m, 11H), 7.13–7.41 (m, 25H). Elemental Analysis: calculated C, 78.07; H, 6.71, found C, 78.00; H, 6.79.

2.1.2 Synthesis of compound 3

The acetic acid-acetic anhydride (AcOH-Ac_2O, 1:5, 15 mL) was added into liquated zinc chloride ($ZnCl_2$, 1.08 g, 7.92 mmoL), and then the mixture was cooled to 0 °C. The compound 2 (1.00 g, 1.59 mmoL) in Ac_2O (5 mL) was added into the above reaction slowly at 0 °C. Subsequently, the mixture was heated up to 25 °C and stirred for another 1.5 h. Then, 50 mL ice water was added. After filtration, the filtrate was concentrated under vacuo, and the residue was purified to get intermediate 3 (0.75 g, 81.33%) as a white solid (mp 113–115 °C). ^{1}H NMR (400 MHz, $CDCl_3$, ppm): δ 2.05 (s, 3H), 3.48–3.58 (m, 3H), 3.66 (t, 1H, J = 8.8 Hz), 4.22–4.97 (m, 11H), 7.24–7.38 (m, 20 H). Elemental Analysis: calculated C, 74.21; H, 6.57, found C, 74.27; H, 6.52.

2.1.3 Synthesis of compound 4

To the solution of 10 mL CH_3OH containing sodium methoxide (CH_3ONa, 25 mg, 0.48 mmoL) was added compound 3 (0.50 g, 0.86 mmol), and then the reaction solution was stirred for 5 h at room temperature. Then, water (20 mL) was added to quench the reaction, and then filtrated. After washing with saturated sodium bicarbonate ($NaHCO_3$) aqueous solution, the filtrate was concentrated to get compound 4 (0.44 g, 95.17%) as a white solid (mp 104–106 °C). High resolution mass spectrum (HRMS): electrospray ionization (ESI+) calculated for $C_{34}H_{36}O_6Na$ $[M+Na]^+$ 563.2410, found 563.2415. Elemental Analysis: calculated C, 75.53; H, 6.71, found C, C, 75.59; H, 6.77.

2.1.4 Synthesis of compound 5

Stearic acid (1.35 g, 4.75 mmoL) was dissolved in CH_2Cl_2 (20 mL), and dicyclohexylcarbodiimide (DCC, 1.31 g, 6.33 mmoL) and dimethylaminopyridine (DMAP, 77 mg, 0.63 mmoL) were added into the above solution. Then the mixture was stirred at 0 °C. After 0.5 h, compound 4 (1.71 g, 3.17 mmoL) in tetrahydrofuran (THF, 20 mL) was added slowly, and the reaction was kept to be stirring for another 5 h at ambient temperature. Filtration was performed and the filtrate was concentrated with rotary evaporators, which was further purified through chromatography to give intermediate 5 (1.90 g, 74.35%) as a white solid. ^{1}H NMR (400 MHz, $CDCl_3$, ppm): δ 0.89 (t, 3 H, J = 7.2 Hz), 1.24–1.29 (m 28 H),

1.60–1.65 (m, 2 H), 2.31–2.35 (m, 2 H), 3.50–3.70 (m, 4 H), 4.24–4.98 (m, 11 H), 7.25–7.39 (m, 20 H). HRMS: (ESI+) calculated for $C_{52}H_{70}O_7Na$ $[M+Na]^+$ 829.5019, found 829.5016. Elemental Analysis: calculated C, 77.38; H, 8.74, found C, 77.45; H, 8.62.

2.1.5 Synthesis of compound 6

Compound 5 (68 mg, 0.084 mmoL) and 10 mg Pd/C (10%) were added into 10 mL CH_3OH, and the mixture was stirred under hydrogen pressure at ambient temperature for 7 h. Then, the Pd/C was removed by filtrating and the filtrate was concentrated with rotary evaporators, which was further purified through chromatography to give ligand 6 (34 mg, 92.13%) as a white solid. ^{1}H NMR (400 MHz, dimethyl sulfoxide-d_6 (DMSO-d_6), ppm): δ 0.85 (d, 3 H, J = 6.8 Hz), 1.23 (s, 28 H), 1.49 (s, 2 H), 2.26 (t, 2 H, J = 6.8 Hz), 2.99–3.05 (m, 1 H), 3.11–3.13 (m, 1 H), 3.73–3.77 (m, 1 H), 3.95–4.00 (m, 1 H), 4.25 (d, 1 H, J = 11.2 Hz), 4.54 (d, 1 H, J = 6.8 Hz), 4.77 (d, 1 H, J = 4.4 Hz), 4.89 (d, 1 H, J = 3.2 Hz), 5.05 (d, 1 H, J = 5.6 Hz), 6.35 (d, 1 H, J = 4.4 Hz). HRMS: (ESI+) calculated for $C_{24}H_{46}O_7Na$ $[M+Na]^+$ 469.3141, found 469.3145. Elemental Analysis: calculated C, 64.54; H, 10.38, found C, 64.65; H, 10.43.

2.2 Preparation of Fe_3O_4 MNPs

The monodisperse Fe_3O_4 MNPs were prepared according to our previous report [19]. That is to say, $Fe(acac)_3$ (353 mg, 1 mmoL), 1,2-hexadecanediol (1.29 g, 5 mmoL), oleic acid (0.85 g, 5 mmoL) and oleylamine (0.80 g, 3 mmoL) were dissolved in 10 mL phenyl ether, and the mixture was stirred at 200 ℃. After 30 min, the reaction was stirred at 265 ℃ for another 30 min. Then, the mixture was cooled to ambient conditions, and 20 mL ethyl alcohol (C_2H_5OH) was added. Subsequently, the precipitate was separated through centrifugation (5000 rpm, 10 min) and the product was re-dissolved in hexane with 25 μL oleic acid and 25 μL oleylamine. Then, the precipitation and dissolution process is repeated, resulting in Fe_3O_4 MNPs in hexane.

2.3 Preparation of PTX-loaded magnetic liposomes (PTX-G-MLip)

The PTX-loaded magnetic liposome (PTX-G-MLip) was made using the film hydration-ultrasound method as our previous report [13]. Soybean phospholipids (SPC, 0.29 g) (Shanghai Taiwei Chemical Company, Shanghai, China), cholesterol (77 mg), liposome ligand 6 (13.5 mg) and PTX (10 mg, Sangon Biotech (Shanghai) Co., Ltd., Shanghai, China), Fe_3O_4 MNPs in hexane (1.5 mL) were dissolved in chloroform ($CHCl_3$, 20 mL). And then, the mixture was dried to form a thin layer of uniform film on a rotary evaporator, which was further dried in vacuum for 4 h. Subsequently, the film was hydrated in 10 mL distilled water with gentle oscillating at 37 ℃ for 0.5 h to obtain crude liposomes, which was further sonicated intermittently by a probe sonicator at 80 W for 80 s. At last, the un-encapsulated magnetic nanoparticles was separated by magnet. The entrapment efficiency (EE%) of drug was carried out using the high performance liquid chromatography (HPLC) system (Agilent, Palo Alto, CA, USA). The mean size and zeta potential of liposomes were detected by Malvern Zeta sizer Nano ZS90 (Malvern Instruments LTD, Malvern, UK).

2.4 Drug release behavior *in vitro*

The drug release behavior *in vitro* was evaluated through dialysis [2]. Briefly, 0.4 mL liposomes loading with paclitaxel or naked paclitaxel were placed into dialysis bags (8000–14,000 Da), which was then cultured in phosphate buffer (PBS) containing 0.1% (v/v) Tween 80. Then, the released drugs were detected with HPLC at 0, 1, 2, 4, 8, 12, 24 and 48 h.

2.5 Stability of liposomes in serum *in vitro*

The serum stability of liposomes in fetal bovine serum (FBS, Shanghai Yuanye Biotechnology Co., LTD, Shanghai, China) was evaluated by measuring the turbidity variations [13]. Namely, the mixture of equal volume liposomes and FBS was incubated at 37 ℃ with shaking (45 rpm). Then, measuring the transmittance at 0, 1, 2, 4, 8, 12, 24 and 48 h was carried out at 750 nm on a microplate reader (SpectraMAX i3x, Molecular Devices, Sunnyvale, USA).

2.6 Hemolysis assays

The safety of ligands-modified liposomes was further evaluated through hemolysis assay [2]. Briefly, separation of the red blood cells from the fresh mouse blood was carried out and the cells were further washed with PBS for several times. Afterwards, the cells were diluted with PBS at 2% (v/v). The liposomes (0.4 mL) with different concentrations were cultured with 0.1 mL RBCs (red blood cells) solutions at 37 °C, and the liposomes mixed with PBS or 1% (v/v) Triton X-100 were used as negative and positive controls respectively. After 2 h, the mixture was centrifuged for 10 min (10,000 rpm), and the absorbance was measured with at 540 nm. The hemolytic rate was calculated according to the equation below:

The percent rate (%) = $\frac{A_{Sample}-A_{Negative}}{A_{Postive}-A_{Negative}} \times 100\%$, where A is the absorbance at 540 nm.

2.7 Cytotoxicity assay

The cytotoxicity assay of liposomes was evaluated with the 3-(4,5-Dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide (MTT, Beyotime Institute Biotechnology, Haimen, China). In briefly, Dulbecco's Modified Eagles Medium (DMEM) containing 10% FBS, streptomycin penicillin was used to the cancer cells MDA-MB-231 cells at 37 °C in incubator. The cells seeded in a 96-well plate with a density of 5×10^3 cells/well were incubated for 24 hours, and the liposomes with various concentrations (0.1–20 μg/mL) were added into the well. After culturing for another 24 h, MTT solution (20 μL, 5.0 mg/mL) was added and the mixture was cultured for 4 h at 37 °C. Afterwards, DMSO (150 μL) was added into the wells and the absorbance was measured with at 490 nm. The cell survival rate was calculated according to the equation below:

The cell survival rate (%) = $A_{sample}/A_{control} \times 100\%$, where A is the absorbance at 490 nm.

2.8 Targeting metastatic bone in mice

The ability of liposomes that targeting metastatic bone *in vivo* was evaluated on the Kunming mice bearing MDA-MB-231 tumors in the tibia. The mice were purchased from Chengdu Dashuo Experimental Animal Co., Ltd (Chengdu, China). And the tumor-bearing mice model was established according to our previous report [6]. The mice were randomly divided into four groups, PTX, PTX-Lip, PTX-G-MLip in absence of magnetic field and PTX-G-MLip in presence of magnetic field (0.5 T). The mice were administered with PTX (calculated as PTX, 10 mg/kg) via the tail vein. After injection, the mice were sacrificed through cervical dislocation at the predetermined time 0.5, 1, 2, 4 and 8 h. The bone metastatic lesions were excised, rinsed with 0.9% NaCl, dried over filter paper and weighed. The bone tissues were digested with aqua regia and the concentration of PTX was analyzed using the HPLC conditions mentioned above.

2.9 Statistical analysis

GraphPad 8.0 (San Diego, USA) was used to analyze the date. Statistical comparisons were performed by Student's *t*-test. Mean ± standard deviation (SD), n = 3. *p*-values less than 0.05 were considered significant. *, **, *** indicates $p < 0.05$, 0.01, 0.001 of PTX-Lip, PTX-G-MLip or PTX-Lip, PTX-G-MLip + MF compared with PTX group. #, ## indicates $p < 0.05$, 0.01 of PTX-G-MLip + MF compared with PTX-G-MLip group.

3. Results and discussion

3.1 Chemistry

The preparation of liposomes ligand 6 was in Fig. 2.

Briefly, the hydroxyl groups of glucose (compound 1) was etherified with benzyl bromide under alkaline condition (NaH) to obtain the intermediate 2, followed by the selectively acetolysis of the C-6 to get compound 3. Subsequently, the C-6 was deacetylated in the presence of CH_3OH to give the intermediate alcohol 4, which was further coupled with stearic acid in the presence of DCC and DMAP to give compound 5. At last, treatment of compound 5 with 10% Pd/C in the hydrogen atmosphere to get the desired liposome ligand 6. The title compound and important intermediates were characterized by their respective nuclear magnetic resonance (NMR) (Varian INOVA 400) and mass spectrum (MS) (Waters Micromass GCT).

3.2 Preparation and characterization of liposomes

The particle size, polymer dispersity index (PDI), EE% and zeta potential were characterized for the liposomes and the results were showed in Table 1. As the results shown, the size of the liposomes was about 120 nm, and the transmission electron microscopy (TEM) indicated that PTX-G-MLip exhibited spherical shape (Fig. 3A). What's more, the size of PTX-G-MLip was a little larger compared with uncoated liposomes PTX-Lip, which was likely because of the encapsulation of magnetic Fe_3O_4 nanoparticles. The PDI of the both liposomes was about 0.2, which revealed that these liposome had a good mono-dispersion. And the EE% was over 80%. All these results suggested that these had suitable physical and chemical properties that was crucial to *in vivo* study.

TABLE 1. The characterization of different PTX-Lip and PTX-G-MLip (n = 3).

Liposomes	PTX-Lip	PTX-G-MLip
Size (nm)	108.5 ± 4.2	120.3 ± 2.5
PDI	0.215 ± 0.019	0.227 ± 0.023
EE (%)	91.45 ± 3.26	85.58 ± 2.87
Zeta potential (mV)	−16.5 ± 1.3	−20.7 ± 1.9

PTX: paclitaxel; PDI: polymer dispersity index; EE: entrapment efficiency; PTX-G-MLip: PTX-loaded glucose-modified magnetic liposome.

As the Fig. 3B showed, the naked paclitaxel had a rapid release behavior, and this group released more 80% PTX after culturing for 12 h. While, the liposomes groups exhibited the slow release behaviors. And only about 60% paclitaxel was released from the PTX-loaded liposomes over 48 h-incubation. What's more, there was no significant difference between PTX-Lip and PTX-G-MLip in the release characteristics, and both of the liposomes showed burst initial release patterns.

Transmittance of the liposomes cultured in PBS containing 50% FBS was detected. And the results were showed in Fig. 3C. Even after 48 hours of culture, the transmittances of the two groups were still over 90%, which suggested that the liposomes had good stability to avoid interacting with serum protein.

Hemocompatibility is an important factor that influences the applications of liposomes *in vivo*. The results of hemolysis assays suggested that both of the two liposomes had limited hemolysis with phospholipids concentration up to 600 nmoles (Fig. 3D). Therefore, the magnetic liposome PTX-G-MLip could be considered as innoxious, which revealed that it had a good biosecurity.

3.3 Cytotoxicity assay

The results of cytotoxicity assay for different liposomes on MDA-MB-231 cells was showed in Fig. 4. The naked paclitaxel had a higher inhibition rate on cancer cells than that of the liposomes loading with paclitaxel, which maybe because the naked PTX could be transported into cells through passive diffusion, without the release process. It was also noticed that PTX-G-MLip could significantly inhibit the proliferation of MDA-MB-231 compared with PTX-Lip group, which maybe attribute to the modification of glucose on the surface that mediated by transporter GLUT1.

3.4 Targeting metastatic bone in mice

To evaluate the targeting ability for metastatic bone in mice, the metastasis mice model was established and the paclitaxel concentration was detected after administration. As expected, it was interesting found the concentrations of liposomes groups were markedly higher than that of naked paclitaxel group.

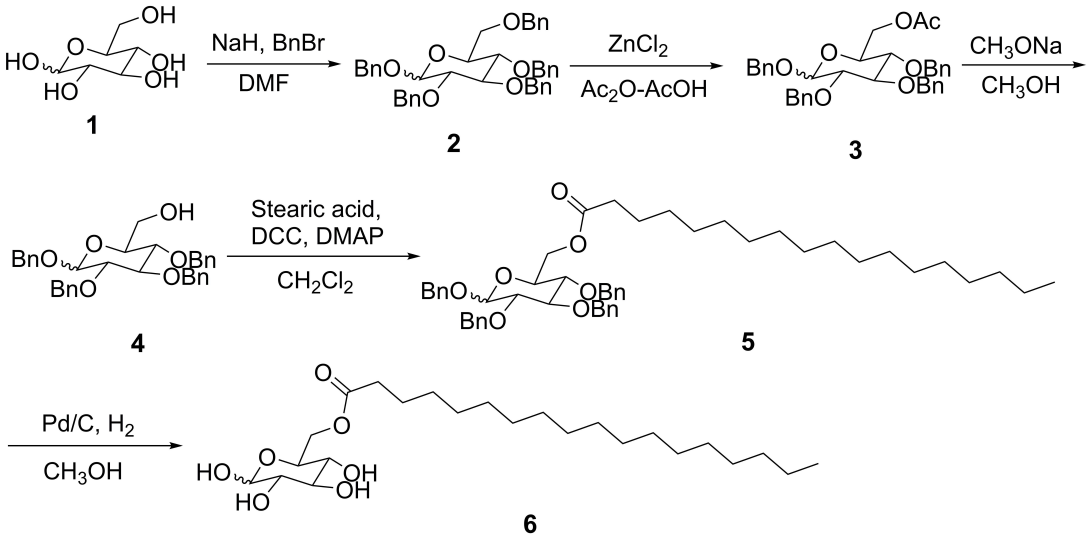

FIGURE 2. Preparation of liposomes ligand 6. NaH: Sodium hydride, BnBr: Benzyl bromide, DMF: Dimethyl Formamide, ZnCl2: zinc chloride, AcOH-Ac_2O: acetic acid-acetic anhydride, CH_3ONa: Sodium methoxide, CH_3OH: methyl alcohol, DCC: Dicyclohexylcarbodiimide, DMAP: Dimethylaminopyridine, CH_2Cl_2: Dichloromethane, Pd/C: palladium on carbon, H_2: hydrogen.

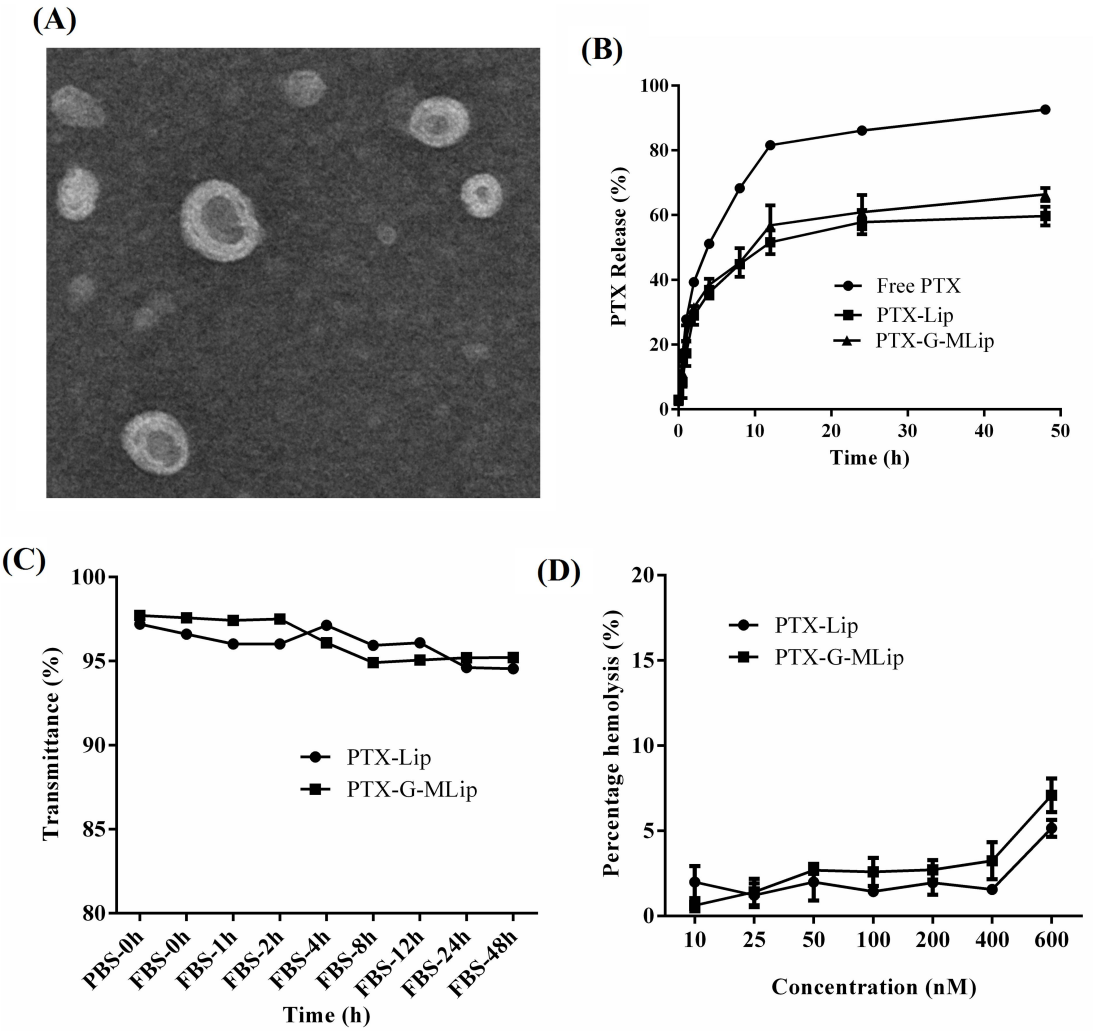

FIGURE 3. The characteristics of liposomes *in vitro*. (A) TEM of PTX-G-MLip. (B) The PTX release behavior of naked PTX, PTX-Lip and PTX-G-MLip (C) The transmittance of PTX-Lip and PTX-G-MLip in 50 % FBS. (D) Hemolysis percentage of PTX-Lip and PTX-G-MLip. (n = 3, mean ± SD). PTX: paclitaxel; FBS: fetal bovine serum; PTX-G-MLip: PTX-loaded glucose-modified magnetic liposome.

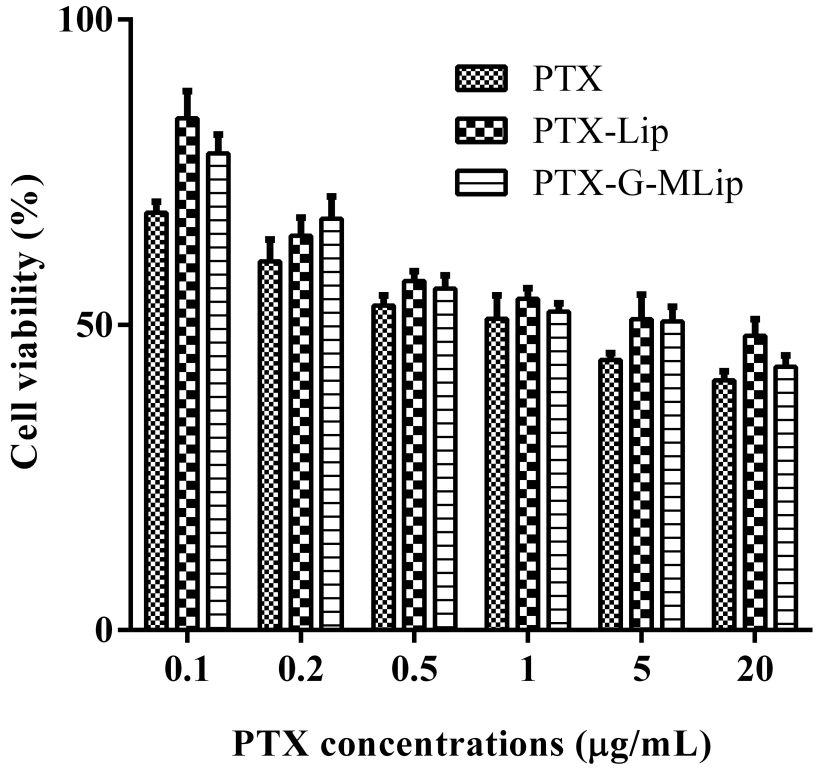

FIGURE 4. The cell cytotoxicity of naked PTX, PTX-Lip and PTX-G-MLip against MDA-MB-231 cells. The results were showed as means ± standard deviation (SD), n = 3. PTX: paclitaxel; PTX-G-MLip: PTX-loaded glucose-modified magnetic liposome.

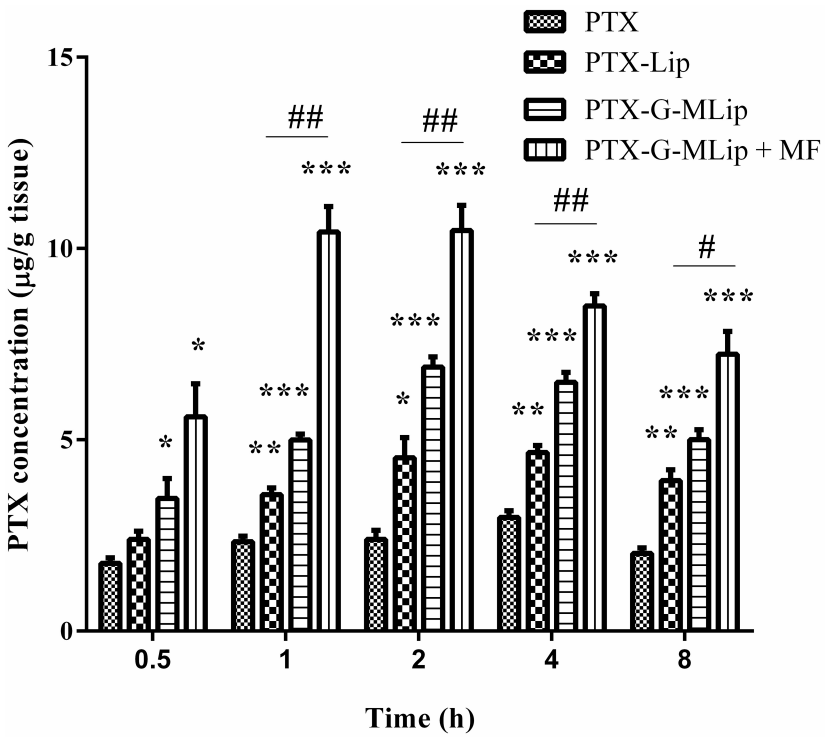

FIGURE 5. The paclitaxel concentration of free PTX, PTX-Lip, PTX-G-MLip, and PTX-G-MLip in the presence of magnetic field (PTX-G-MLip + MF) at 0.5, 1, 2, 4 and 8 h. The results were showed as means ± standard deviation (SD), n = 3. MF, magnetic field (0.5 T). *, **, *** indicates $p < 0.05$, 0.01, 0.001 of PTX-Lip, PTX-G-MLip or PTX-Lip, PTX-G-MLip + MF compared with PTX group. #, ## indicates $p < 0.05$, 0.01 of PTX-G-MLip + MF compared with PTX-G-MLip group. PTX: paclitaxel; PTX-G-MLip: PTX-loaded glucose-modified magnetic liposome.

And the PTX concentration in the metastatic bones of PTX-G-MLip groups in absence of or presence of magnetic field were dramatically enhanced compared with PTX-Lip group. This was attributed to the cancer targeting ability of glucose and high transport efficiency of GLUT1. What's more, PTX-G-MLip group in presence of magnetic field (PTX-G-MLip + MF) had an increased concentration than that of PTX-G-MLip group absence of magnetic field. The Fig. 5 also revealed that the concentration paclitaxel of PTX-G-MLip + MF group was 2–5-fold higher compared with the naked paclitaxel group, 2–3-fold higher compared with the PTX-Lip group, and 1–2-fold higher compared with the PTX-G-MLip group. All the results suggested that PTX-G-MLip in the magnetic field could enhance the targeting-delivery of drug to the metastatic bone, which may contribute to reducing the side effect on other tissues and improving the therapeutic effect.

4. Discussion

Bone metastasis is a malignant tumor in bone due to the metastasis of cancer in other organs, especially breast cancer and prostate cancer. And it is a common complication of breast cancer patients. The increased number patients with breast cancer could develop into bone metastases during the course [17]. However, bone metastasis is still a great challenge to treat because of the low blood flows in bone tissue and low targeting ability of chemotherapeutic drugs. Recently, numerous studies have reported to use PTX as chemotherapeutics to treat bone metastasis [2, 6, 20, 21]. Despite the advances in surgical techniques and chemotherapy in the treatment, the cure rate for advanced bone metastasis remains low and the mortality rate remains high. Hence, there is an urgent need to develop novel strategies to deliver PTX to the site of metastasis for the treatment of bone metastasis.

Most drugs are rarely distributed to the bone and have little effectiveness on the treatment for bone metastases. The low blood flows in bone tissue prevents the drug PTX from achieving the metastasis site. To date, many bone bone-targeting ligands, such as tetracyclines, bisphosphonates, and acidic amino acid have been developed and used to deliver anti-tumor drugs. Despite the bone-targeting ability could be achieved, the above bone-targeting moieties lack the cancer-targeting affinity, which limits the entrance of drugs into cancer cells. Therefore, the improved delivery of PTX to bone and uptake on cancer cells could battle this issue. In our previous studies, we designed and prepared the bone targeted liposomes using RGD (Arg-Gly-Asp) tripeptide or folic acid as ligands, and the results suggested that these liposomes could increase the uptake on cancer cells [2, 6].

As we all know, the cancer cells need more glucose than normal cells to maintain their normal life state, which is called Warburg effect. Therefore, the cancer cells have an overexpress of GLUT1 to uptake more glucose as energy. Hence, targeting the Warburg effect is a hot topic in the medical field in recent years. In our previous study, we also used glucose mediated by GLUT1 to increase the uptake of cancer cell [14].

Magnetic liposome is a physical targeting agent developed rapidly in recent years. Magnetic liposomes are made by mixing ferromagnetic substances (such as Fe_3O_4, γ-Fe_2O_3, *etc.*) into the liposomes. When the liposome enters the body, it is guided and located the effect of external magnetic field, which then releases the drug in the magnetic field area, so as to play the role of local concentration of the target area or the target area interception. Magnetic liposome has the advantages of lipid carrier and magnetic guidance, which makes its targeting and specificity stronger and faster, so as to achieve high efficiency, fast and low toxicity effect.

Here, in this study, a novel cancer-targeting glucose derivative 6 was synthesized, which was used as liposome ligand. The liposome had superior character, such as the suitable size, PDI and potential, the slow release behavior, the stability in serum, the low hemolysis. Also, we found that PTX-G-MLip could significantly enhance the uptake on MDA-MB-231 cells to induce the cell death. Interestingly, the results of targeting metastatic bone showed that PTX-G-MLip remarkably increased the concentrations of PTX in the metastatic bones under the external magnetic field. Compared with our previous study, the conventional cancer-targeting liposomes (RGD-Lip and FA (folic acid)-Lip) could only improve the drug concentration in bone with 1.6–3.5 times [2, 6], which is inconsistent with PTX-G-MLip without magnetic field. While, the concentration paclitaxel of PTX-G-MLip + MF group was 2–5-fold higher compared with the naked paclitaxel group. In conclusion, the study is very promising and some more work is being done, namely, the target ability and activity evaluations *in vitro* and *in vivo*. And we will report the significant results in due time.

5. Conclusions

In summary, the magnetic liposome coated with glucose, PTX-G-MLip, was designed and prepared for achieving the targeting-delivery of PTX to bone metastasis. The characterizations, such as size, zeta potential, encapsulation efficiency, release profile, stability, hemolysis, and cytotoxicity, were well evaluated. And the results suggested that the liposomes had the superior physicochemical and biological characteristics. What's more, the results of targeting ability in mice for bone metastasis showed that the magnetic liposomes PTX-G-MLip could enhance the concentration of PTX in metastatic bone tissues. In conclusion, the study is very promising and some more work is being done.

AVAILABILITY OF DATA AND MATERIALS

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

AUTHOR CONTRIBUTIONS

ZZ and YZ—designed the research study. ZZ and YZ—performed the research. CC and CX—analyzed the data. ZZ and YZ—wrote the manuscript. All authors read and approved the final manuscript.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

All the animal procedures were performed after being approved by the Experiment Administrative Committee of the Second People's Hospital of Jiaozuo City (the First Affiliated Hospital of Henan Polytechnic University) (KY2022-03-003).

ACKNOWLEDGMENT

Not applicable.

FUNDING

This work was supported by the Henan Medical Science and Technology Joint Building Program (No. LHGJ20220417 & LHGJ20210925), the Henan Key Project of Research and Development Plan (Science and Technology) (No. 222102310091), and the Fundamental Research Funds for the Universities of Henan Province (No. NSFRF210319).

CONFLICT OF INTEREST

The authors declare no conflict of interest.

REFERENCES

[1] Verbruggen ASK, McCarthy EC, Dwyer RM, McNamara LM. Temporal and spatial changes in bone mineral content and mechanical properties during breast-cancer bone metastases. Bone Reports. 2022; 17: 101597.

[2] Zhao Z, Zhao Y, Xie C, Chen C, Lin D, Wang S, *et al*. Dual-active targeting liposomes drug delivery system for bone metastatic breast cancer: Synthesis and biological evaluation. Chemistry and Physics of Lipids. 2019; 223: 104785.

[3] Luo B, Yuan Y, Zhu Y, Liang S, Dong R, Hou J, *et al*. microRNA-145-5p inhibits prostate cancer bone metastatic by modulating the epithelial-mesenchymal transition. Frontiers in Oncology. 2022; 12: 988794.

[4] Isik A, Soran A, Grasi A, Barry N, Sezgin E. Lymphedema after sentinel lymph node biopsy: who is at risk? Lymphatic Research and Biology. 2022; 20: 160–163.

[5] Işık A, Grassi A, Soran A. Positive axilla in breast cancer; clinical practice in 2018. European Journal of Breast Health. 2018; 14: 134–135.

[6] Yang Y, Zhao Z, Xie C, Zhao Y. Dual-targeting liposome modified by glutamic hexapeptide and folic acid for bone metastatic breast cancer. Chemistry and Physics of Lipids. 2020; 228: 104882.

[7] Zhang X, Liu Q, Zhang T, Gao P, Wang H, Yao L, *et al*. Bone-targeted nanoplatform enables efficient modulation of bone tumor microenvironment for prostate cancer bone metastasis treatment. Drug Delivery. 2022; 29: 889—905.

[8] Hendriks LE, Hermans BC, van den Beuken-van Everdingen MH, Hochstenbag MM, Dingemans AM. Effect of bisphosphonates, denosumab, and radioisotopes on bone pain and quality of life in patients with non-small cell lung cancer and bone metastases: a systematic review. Journal of Thoracic Oncology. 2016; 11: 155–173.

[9] Zhao Z, Chen C, Xie C, Zhao Y. Design, synthesis and evaluation of liposomes modified with dendritic aspartic acid for bone-specific targeting. Chemistry and Physics of Lipids. 2020; 226: 104832.

[10] Mu X, Zhang M, Wei A, Yin F, Wang Y, Hu K, *et al*. Doxorubicin and PD-L1 siRNA co-delivery with stem cell membrane-coated polydopamine nanoparticles for the targeted chemoimmunotherapy of PCa bone metastases. Nanoscale. 2021; 13: 8998–9008.

[11] Shao H Shao H, Varamini P. Breast cancer bone metastasis: a narrative review of emerging targeted drug delivery systems. Cells. 2022; 11: 388.

[12] Bose S, Zhang C, Le A. Glucose metabolism in cancer: the warburg effect and beyond. Advances in Experimental Medicine and Biology. 2021; 1311: 3–15.

[13] Zhao Y, Peng Y, Yang Z, Lu J, Li R, Shi Y, *et al*. PH-redox responsive cascade-targeted liposomes to intelligently deliver doxorubicin prodrugs and lonidamine for glioma. European Journal of Medicinal Chemistry. 2022; 235: 114281.

[14] Zhao Y, Zhao Z, Cui Y, Chen X, Chen C, Xie C, *et al*. Redox-responsive glycosylated combretastatin A-4 derivative as novel tubulin polymerization inhibitor for glioma and drug delivery. Drug Development Research. 2021; 82: 1063–1072.

[15] Siyal FJ, Memon Z, Siddiqui RA, Aslam Z, Nisar U, Imad R, *et al*. Eugenol and liposome-based nanocarriers loaded with eugenol protect against anxiolytic disorder via down regulation of neurokinin-1 receptors in mice. Pakistan Journal of Pharmaceutical Sciences. 2020; 33: 2275–2284.

[16] Han B, Yang Y, Chen J, Tang H, Sun Y, Zhang Z, *et al*. Preparation, characterization, and pharmacokinetic study of a novel long-acting targeted paclitaxel liposome with antitumor activity. International Journal of Nanomedicine. 2020; 15: 553–571.

[17] Chen R, Chen X, Zhou Y, Lin T, Leng Y, Huang X, *et al*. "Three-in-one" multifunctional nanohybrids with colorimetric magnetic catalytic activities to enhance immunochromatographic diagnosis. ACS Nano. 2022; 16: 3351–3361.

[18] Peng Y, Gao Y, Yang C, Guo R, Shi X, Cao X. Low-molecular-weight poly(ethylenimine) nanogels loaded with ultrasmall iron oxide nanoparticles for T_1-weighted MR imaging-guided gene therapy of sarcoma. ACS Applied Materials & Interfaces. 2021; 13: 27806–27813.

[19] Zhang L, Zhao Y, Yue Q, Fu Q, Hai L, Guo L, *et al*. Preparation and characterization of GLUT1-mediated novel brain targeting magnetic nanoparticles. Letters in Drug Design & Discovery. 2018; 15: 1308–1313.

[20] Zhu M, Liu X, Qu Y, Hu S, Zhang Y, Li W, *et al*. Bone metastasis pattern of cancer patients with bone metastasis but no visceral metastasis. Journal of Bone Oncology. 2019; 15: 100219.

[21] Kim JW, Lee S, Kim HS, Choi YJ, Yoo J, Park KU, *et al*. Prognostic effects of cytokine levels on patients treated with taxane and zoledronic acid for metastatic breast cancer in bone (BEAT-ZO) (KCSG BR 10–13). Cytokine. 2021; 142: 155487.

How to cite this article: Ze Zhao, Yi Zhao, Changqing Chen, Changwei Xie. GLUT1-mediated magnetic liposomes for targeting bone metastatic breast cancer. European Journal of Gynaecological Oncology. 2023; 44(1): 98-105. doi: 10.22514/ejgo.2023.012.