Oncology Advances **2024** vol. 2(1) | 1–9
DOI: 10.14218/OnA.2023.00009

Original Article

In *Vitro* and Circulation Kinetic Studies on π-π-stacked Poly (ε-caprolactone)-based Micelles Loaded with Olaparib

Yu Yang[1,2,3#], Yingli Wu[1,2,3#], Xujin Gong[1,2,3], Xiaolin Lv[1,2,3], Tingting Zhao[1,2,3,4], Lei Liu[1,2,3,4], Yanling Mu[1,2,3], Yanna Liu[1,2,3*] and Qingqiang Yao[1,2,3,5*]

[1]*School of Pharmacy and Pharmaceutical Sciences & Institute of Materia Medica, Shandong First Medical University & Shandong Academy of Medical Sciences, Jinan, China;* [2]*NHC Key Laboratory of Biotechnology Drugs (Shandong Academy of Medical Sciences), Jinan, China;* [3]*Key Lab for Rare & Uncommon Diseases of Shandong Province, Jinan, China;* [4]*Shandong Xiandai University, Jinan, China;* [5]*Jining Medical University, Jining, China*

Received: May 30, 2023 | **Revised:** August 01, 2023 | **Accepted:** December 15, 2023 | **Published online:** March 20, 2024

Abstract

Background and objectives: Olaparib is a selective poly (ADP-ribose) polymerase inhibitor. However, its clinical application is hindered by low solubility and undesired pharmacokinetic profiles (e.g., relatively short circulation). Therefore, the present study aims to exploit polymeric micelles as a safe solubilizer and nanocarrier of olaparib, in order to improve its solubility and pharmacokinetics.

Methods: Poly (ε-caprolactone)-*co*-poly (benzyl 5-methyl-2-oxo-1,3-dioxane-5-carboxylate), i.e., benzyl-functionalized trimethylene carbonate)-*b*-poly (ethylene glycol) (P(CL-*co*-TMC-Bz)-PEG), was synthesized by ring-opening polymerization, and used to prepare the π-π-stacked polymeric micelles for olaparib encapsulation. A series of olaparib-loaded micelles with different polymer concentrations and wt% loadings were prepared using different methods to investigate the effect of formulation variables on the size of polymeric micelles and drug loadings. In addition, the *in vitro* release of olaparib from the micelles, and the cytotoxicity of micellar olaparib formulations on the SKOV3 tumor cell line were evaluated by UV spectrophotometry and CCK-8 assay, respectively. Finally, the blood circulation kinetics and side effects of the incorporated olaparib in the micelles and free olaparib were investigated in SD rats using ultra-high performance liquid chromatography analysis and H&E staining, respectively.

Results: It was found that P(CL_{11}-*co*-TMC-Bz_5)-PEG micelles served as a safe and excellent solubilizer for olaparib, and that the solubilization capacity was easily tailored by adjusting the polymer concentration. In addition, when loaded in micelles, olaparib exhibited a sustained release behavior *in vitro*, and obvious cytotoxicity on SKOV3 cells. The *in vivo* studies revealed that olaparib incorporated in P(CL_{11}-*co*-TMC-Bz_5)-PEG polymeric micelles exhibited prolonged circulation ($t_{1/2}$ = 2.00 hours), when compared to free olaparib ($t_{1/2}$ ≤ 0.25 hours), and excellent safety. However, in terms of taking advantage of the EPR effect of the micelle delivery system to achieve the targeted olaparib delivery, the circulation time of olaparib in the micelles remained rather short.

Conclusion: Improvements, such as chemical crosslinking and drug conjugation, are required to improve the retention of olaparib-loaded polymeric micelles in blood circulation, and benefit from the use of micelles as a targeted delivery system.

Keywords: Circulation kinetics; Drug loading; *In vitro* release; Olaparib; Polymeric micelles.

Abbreviations: CCK-8, cell counting kit-8; H&E staining, hematoxylin and eosin staining; LE, loading efficiency; LC, loading capacity; PEG, poly (ethylene glycol); P(CL-*co*-TMC-Bz)-PEG, poly (ε-caprolactone)-co-poly (benzyl 5-methyl-2-oxo-1,3-dioxane-5-carboxylate; PBS, phosphate buffered saline; Zave, Z-average hydrodynamic diameter; PDI, polydispersity index; PBS, phosphate buffered saline.

***Correspondence to:** Yanna Liu, Shandong First Medical University, No. 6699 Qingdao Road, Huaiyin District, Jinan 250117, China. ORCID: http://orcid.org/0000-0002-5017-5728. Tel: +86 (0531)-59567220. E-mail liuyanna@sdfmu.edu.cn; Qingqiang Yao, Jining Medical University, No. 133 HeHua Road, Jinan 272067, China. Tel: +86-18668969013, E-mail: qqyao@sdfmu.edu.cn

[#]These authors contributed equally to this work.

How to cite this article: Yang Y, Wu Y, Gong X, Lv X, Zhao T, Liu L, *et al*. In *Vitro* and Circulation Kinetic Studies on π-π-stacked Poly (ε-caprolactone)-based Micelles Loaded with Olaparib. *Oncol Adv* 2024;2(1):1–9. doi: 10.14218/OnA.2023.00009.

Introduction

Olaparib is a poly (ADP-ribose)polymerase (PARP) inhibitor with high selectivity to inhibit DNA damage repair and induce apoptosis.[1] Clinically, this has been approved for the treatment of advanced ovarian cancer.[2] However, due to poor water solubility, the dosage forms of olaparib on the market are mainly tablets and

capsules, which have not yet achieved the expected therapeutic effect.[3,4] Furthermore, due to its intrinsic pharmacokinetics, the clinical dosage of olaparib required for achieving a therapeutic effect remains quite large, e.g., 600 mg (tablets, twice daily) per day for patients with newly diagnosed advanced ovarian cancer,[5] leading to high costs, and hindering its clinical application to some extent. On the other hand, the widespread distribution of olaparib in normal tissues resulting from non-specificity can lead to common adverse reactions, such as vomiting, nausea, diarrhea, and anemia.[3] Therefore, the development of a new olaparib dosage form is required to improve its solubility, and intrinsic pharmacokinetic profiles.

In recent years, nano-delivery systems have attracted attention in anti-cancer drug research due to their ability to improve drug solubility, pharmacokinetics, efficacy, and pathological tissue targeting.[6–8] Among them, polymeric micelles have been one of the most commonly used nanocarriers due to their advantages in drug delivery.[9] First, polymeric micelles have a "shell-core" structure, which is formed by the spontaneous assembly of amphiphilic polymers dispersed in an aqueous solution. Poorly soluble drugs are loaded in the hydrophobic core of micelles, thereby improving their solubility in water. Second, the hydrophilic shell, which is most commonly based on poly (ethylene glycol) (PEG), provides a long circulation time of micelles by reducing the uptake of micellar particles through the reticuloendothelial system (RES), thereby allowing the micelles to eventually accumulate in the tumor microenvironment.[10,11] Third, micelles can be obtained in customizable sizes, typically <200 nm, making them favorable for the passive targeted delivery of cargos to tumor sites via the enhanced permeability retention (EPR) effect.[12,13]

We previously reported that polymeric micelles constituted of poly (ε-caprolactone)-*co*-poly (benzyl 5-methyl-2-oxo-1,3-dioxane-5-carboxylate, i.e., benzyl-functionalized trimethylene carbonate-*b*-poly (ethylene glycol) (P(CL-*co*-TMC-Bz)-PEG), was not only an excellent and safe solubilizer of a hydrophobic photosensitizer but also displayed prolonged circulation times in mice, as a result of the π-π stacking interactions of the micellar core.[14] In the present study, π-π stacked P(CL-*co*-TMC-Bz)-PEG micelles were used to investigate its capability as a solubilizer of olaparib to improve the solubility, while ensuring its safety to different organs. Meanwhile, the micelles were evaluated as a drug delivery system for the prolonged circulation time of olaparib responsible for the improvement of its tumor site accumulation through the EPR effect. To this end, the P(CL-*co*-TMC-Bz)-PEG block copolymer was synthesized and characterized by gel permeation chromatography (GPC) and ^{1}H-NMR spectroscopy. A series of olaparib-loaded micelles with different polymer concentrations and wt% loadings were prepared using different methods, in order to investigate the effect of formulation variables, including the preparation methods and type of solvent of the polymer, on the size of polymeric micelles and drug loadings. In addition, the *in vitro* release of olaparib from the micelles, and the cytotoxicity of the micellar olaparib formulations on the SKOV3 tumor cell line were evaluated by UV spectrophotometry and CCK-8 assay, respectively. Finally, the blood circulation kinetics and side effects of the incorporated olaparib in micelles and free olaparib were investigated in Sprague-Dawley (SD) rats using ultra-high performance liquid chromatography analysis and H&E staining, respectively.

Materials and methods

Materials

The 2,2-Bis (hydroxymethyl)propionic acid (bis-MPA) (98%), triphosgene (99%), methanesulfonic acid (≥99.0%), benzyl bromide (98%), and pyridine (99.8%) were obtained from MACKLIN (Shanghai, China). The ε-caprolactone (CL) and methoxy-poly (ethylene glycol) (mPEG-OH, 5,000 g/mol) were obtained from Sigma-Aldrich (Shanghai, China). The human ovarian carcinoma SKOV3 cell line was purchased from the Chinese Academy of Sciences Cell Bank (Shanghai, China). The potassium hydroxide (KOH), *N,N*-dimethylformamide (DMF), ethyl acetate, n-hexane, ammonium chloride, sodium bicarbonate, 4Å molecular sieves, dimethylsulfoxide (DMSO), tetrahydrofuran (THF), and dichloromethane (DCM) were obtained from Sinopharm Chemical Preparation Co., Ltd. (China). The hydrochloric acid, toluene, and ether were purchased from Yantai Far East Fine Chemical Co., Ltd. (China). All the above reagents were used as received, except for the DCM, toluene and DMF dried on 4Å molecular sieves before use.

The ^{1}H-NMR spectra were recorded using a Bruker NMR spectrometer (400 MHz; Billerica, MA, USA). The reference line at 7.26 ppm was used for the residual solvent of the chloroform.

Synthesis of the monomer and polymer

Synthesis of the monomer

Synthesis of benzyl 2,2-bis (hydroxymethyl)propionate

The benzyl 2,2-bis (hydroxymethyl)propionate was synthesized according to a previous report.[15] Briefly, the mixture of bis-MPA (50 g, 0.37 mol) and KOH (21.2 g, 0.38 mol) in DMF (280 mL) was heated to 100°C for one hour, followed by the addition of benzyl bromide (55 mL, 0.45 mol). After 16 hours, the reaction solution was concentrated by reduced pressure distillation, and extracted by ethyl acetate (350 mL), n-hexane (250 mL), and water (250 mL). Then, the collected organic layer was concentrated to obtain the crude product (73.5 g) as white powder. The pure product was obtained as a white needle-like crystal after the recrystallization of the crude product from toluene (53.9 g, yield: 64%).

Synthesis of benzyl 5-methyl-2-oxo-1,3-dioxane-5-carboxylate

The synthesis of benzyl 5-methyl-2-oxo-1,3-dioxane-5-carboxlate (i.e. the trimethylene carbonate functionalized by the benzyl group, abbreviated as TMC-Bz) was carried out based on a previous report.[15] Briefly, pyridine (55 mL, 0.67 mol) was added to 2,2-bis (hydroxymethyl)benzyl propionate (25 g, 0.11 mol) in DCM (330 mL). Then, the mixture was cooled down to −78°C under a N_2 atmosphere, followed by the slow addition of the triphosgene (16.5 g, 0.06 mol) solution in DCM (40 mL). Next, the mixed solution was allowed to react at −78°C for 30 minutes, and subsequently at room temperature for 1.5 hours. Afterwards, the reaction solution was terminated by the addition of the saturated NH_4Cl solution (270 mL). After being washed with 1 M of hydrochloric acid and the saturated $NaHCO_3$ aqueous solution, and dried by anhydrous Na_2SO_4, the organic layer was collected and concentrated to yield the product as a white solid (25.2 g, yield: 91%). Then, the product was further purified by recrystallization from the ethyl acetate before polymerization. ^{1}H-NMR (400 MHz, $CDCl_3$): δ 7.39 (m, OCO$CH_2C_6H_5$), 5.21 (s, OCO$CH_2C_6H_5$), 4.69 (m, COOCH_2CCH_2OCO), 4.22 (m, COOCH_2CCH_2OCO), and 1.34 (s, OCH_2CCH_3).

Synthesis of the P(CL-*co*-TMC-Bz)-PEG copolymer

The poly (ε-caprolactone)-*co*-poly (benzyl-5-methyl-2-oxo-1,3-dioxane-5-carboxylate)-*b*-poly (ethylene glycol) (P(CL-*co*-TMC-Bz)-PEG) was synthesized, as previously described.[14] Briefly,

CL (502.2 mg, 4.4 mmol), TMC-Bz (600.6 mg, 2.4 mmol), and mPEG-OH (2,000.0 mg, 0.4 mmol) were dissolved in dry DCM (4.8 mL), followed by the addition of methanesulfonic acid (50.0 mg, 0.5 mmol) with stirring. The reaction proceeded under a N_2 atmosphere at 37°C for 24 hours. Then, the cooled reaction solution was added dropwise to an excess of cold ether (−20°C), and the collected precipitate was dried under vacuum to obtain the final product. ^{1}H-NMR (400 MHz, $CDCl_3$): δ 7.33 (m, $OCOCH_2C_6H_5$), 5.12 (s, $OCOCH_2C_6H_5$), 4.29 (m, $COOCH_2CCH_2OCO$), 4.10 (m, $OHCH_2CH_2CH_2$), 3.37 (s, CH_3O), 2.27 (m, $CH_2CH_2CH_2OCO$), 1.63 (m, $CH_2CH_2CH_2CH_2CH_2$), 1.37 (m, $CH_2CH_2CH_2CH_2CH_2$), and 1.26 (s, OCH_2CCH_3).

Characterization of the polymer

The average degree of polymerization (DP) of CL or TMC-Bz was determined by ^{1}H-NMR, as previously described.[14] Briefly, DP was calculated using the ratio of the integral of the CH_2 protons of CL at 1.38 ppm, or the CH_2 protons of benzyl groups in the poly (TMC-Bz) at 5.16 ppm, to the CH_3 protons of mPEG-OH at 3.36 ppm. The average molecular weight (M_n) of the polymer was calculated based on the molecular weight of the DP of each unit.

The M_n, weight average molecular weight (M_w), and polydispersity (M_w/M_n) of the synthesized polymer were detected by gel permeation chromatography (GPC). The used column was the Shodex™ KD803 column (300 × 8 mm, including a guard column, 10.0 × 4.6 mm), and the detector was a differential refractive index (RI) detector. The poly (ethylene glycol)s of narrow molecular weights (840–30,000 g/mol) were used as the calibration standard. The eluate was DMF with 10 mM of LiCl, the elution rate was 1.0 mL/min, and the column temperature was set to 65°C.

Preparation and characterization of empty micelles

The following methods were used to prepare the empty micelles: the thin-film hydration and nanoprecipitation of polymer dissolved in THF or DMSO.[14,16,17] For example, in order to prepare the micelles with 10 mg/mL of polymer by thin-film hydration, the block copolymer (10 mg) was dissolved in DCM (1 mL). Then, the solvent was removed using rotovap to form a thin film. Afterwards, the block copolymer film was hydrated in deionized water (1 mL), followed by being heated at 65°C for five minutes, in order to form a uniform micellar dispersion.

For the first nanoprecipitation method, the block copolymer (10 mg) was dissolved in DMSO (100 μL), followed by being heated up to 70°C for five minutes. Then, the cooled solution was added dropwise to deionized water (900 μL) at a 1:9 volume ratio with gentle shaking, in order to form a homogenous micellar dispersion. Afterwards, the micellar dispersion was dialyzed using dialysis tubing (MWCO = 1 kDa) against deionized water at room temperature for 12 hours to remove the DMSO.

For the second nanoprecipitation method, the copolymer dissolved in THF (10 mg/mL) was added dropwise to deionized water at a 1:1 volume ratio with gentle agitation. Then, the mixed solution was kept at room temperature for 36 hours to remove the THF.

The micellar dispersions obtained using the above methods were filtered through a 0.2 μm syringe filter. Dynamic light scattering (DLS) was used to determine the Z-average hydrodynamic diameter (Zave) and polydispersity index (PDI) of the resulting micelles. The scattering angle was fixed at 173°, and the measured temperature was at 25°C using a Malvern nano ZS (Malvern, Surrey, UK). The morphologic characteristics of the micelles were determined by transmission electron microscopy (TEM; JEOL JEM-2100Plus, Japan).

Preparation and characterization of olaparib-loaded micelles

The olaparib-loaded micelles (at different loading percentages) were prepared by thin-film hydration, as described above, with slight modifications. Briefly, a certain volume of olaparib solution in DCM (the additional volume was based on the predetermined wt% loading) was added to the polymer, followed by the addition of a certain volume of DCM, in order to obtain the predetermined polymer concentration. Then, the solvent in the mixture was evaporated to form a homogeneous film of polymer and olaparib, and this was hydrated and heated to form the olaparib-loaded micelles.

In addition, as described in above, with slight modifications, the olaparib-loaded micelles (at different loading percentages) were prepared through the nanoprecipitation of the polymer dissolved in DMSO using water as the non-solvent. Briefly, the olaparib solution in DMSO (the additional volume was based on the predetermined wt% loading) was added to the weighted polymer, followed by the addition of DMSO, to obtain the final polymer concentration (10 mg/mL). Then, the mixture of olaparib and polymer in DMSO was heated, and the cooled solution was added to deionized water (1:9 volume ratio) with gentle shaking, followed by dialysis against deionized water.

The resulting olaparib-loaded micelles were filtered through a 0.2 μm syringe filter to remove the non-encapsulated and thus precipitated Olaparib. The absorbance of micellar dispersions diluted with acetonitrile at 276 nm was measured using the UV-2006i Shimadzu Spectrophotometer (Kyoto, Japan). A series of olaparib solutions in acetonitrile was prepared as standard solutions to determine the loading of olaparib. The loading efficiency (LE) and loading capacity (LC) of olaparib were calculated according to equations (1) and (2), respectively:

$$LE\% = \frac{\text{Olaparib loaded (mg)}}{\text{Olaparib in the feed (mg)}} \times 100\% \quad (1)$$

$$LC\% = \frac{\text{Olaparib loaded (mg)}}{\text{polymer used (mg)} + \text{Olaparib loaded (mg)}} \times 100\% \quad (2)$$

In vitro release of olaparib-loaded micelles

The *in vitro* release of olaparib from micelles, with 10 wt% loading at 10 mg/mL of polymer and 3.5 wt% loading at 30 mg/mL of polymer (equivalent to approximately 1 mg/mL of olaparib), was explored using a dialysis tube (MWCO: 50 kDa) at 37°C in PBS (pH = 7.4) containing Tween-80 (0.5%, w/w), with shaking (900 rpm). Typically, 2 mL of olaparib-loaded micellar dispersion was dialyzed against 60 mL of release media. At predetermined intervals, 2 mL of release medium was withdrawn and renewed with an equal volume of fresh medium. As a control, the release of free olaparib was conducted following a similar procedure, with the replacement of olaparib-loaded micelles with the olaparib solution in DMSO. The concentration of olaparib in the medium was measured using the UV-2006i Shimadzu spectrophotometer (Kyoto, Japan) at 276 nm.

Cytotoxicity of empty and olaparib-loaded micelles

Human ovarian cancer SKOV3 cells were cultured in Dulbecco's modified Eagle's medium (DMEM) containing high glucose, which was supplemented with 10% (v/v) fetal bovine serum (FBS) and 1% (v/v) antibiotics (1,000 U/mL of penicillin and 1,000 μg/mL of streptomycin). Then, the cells were cultured at 37°C in a humidified 5% CO_2 atmosphere.

The empty and olaparib-loaded micelles were formed in water at a polymer concentration of 10 mg/mL using the thin-film hydra-

tion method, as described in above. The stock dispersions were diluted in DMEM medium to the predetermined polymer concentration before being exposed to cells.

SKOV3 cells were seeded into 96-well plates at a density of 6,000 cells/well, and incubated overnight at 37°C in a humidified 5% CO_2 atmosphere. Then, the culture medium (100 μL) in the wells was replaced by the aforementioned empty or olaparib-loaded micelles (100 μL). Cell viability was determined after 24 hours of incubation by CCK-8 assay, according to manufacturer's instructions. Briefly, 100 μL of culture medium containing 10% CCK-8 solution (SparkJade, Shandong, China) was added to each well, and the absorbance was determined at 450 nm after one hour of incubation using the Tristar^2 S LB 942 multifunction microplate reader (Berthold, Germany). Untreated cells were used as the negative control group, and the assay was repeated at least three times.

Circulation kinetics and side effects of olaparib-loaded micelles in SD rats

The olaparib-loaded P(CL-*co*-TMC-Bz)-PEG micelles, with 20 and 7 wt% drug feeds at 10 and 30 mg/mL polymer concentrations, respectively (the final olaparib concentration was approximately 2 mg/mL), were prepared, as described in above. The free olaparib solution was prepared through the dilution of the olaparib stock solution (80 mg/mL) in DMSO using DMSO/polyethylene glycol 400/PBS (2:10:88 v/v/v), and a final olaparib concentration of 2 mg/mL was obtained. This was equivalent to the injected dose of olaparib in the micellar formulation.

The animal experiments were carried out according to the Guidelines for the Feeding, Management and Use of Laboratory Animals, and approved by the Ethics Committee of the Institute of Materia Medica, Shandong Academy of Medical Sciences (Jinan, China; Approval no.: 2021011). All surgeries were performed under sodium pentobarbital anesthesia, and all efforts were made to minimize suffering. The female SD rats (weighing 195–206 g) were obtained from Jinan Pengyue Laboratory Animal Breeding Co., Ltd. (Jinan, China). There rats were housed in natural light conditions and ventilated cages at 25°C with 55% humidity. Food and water were given *ad libitum*.

Three groups of SD rats (each group = 4) were intravenously (i.v.) administrated with free olaparib or olaparib-loaded micelles via the tail vein at an injection dose of 10 mg of olaparib/kg, corresponding to approximately 2 mg of olaparib in approximately 500 μL per rat. Then, blood samples were collected at one minute (used as the control for 100% injection), and at 1, 2, 6, 8 and 12 hours after injection. After 24 hours from the i.v. administration of the formulations, these rats were sacrificed, and the heart, liver, spleen, lungs and kidneys were excised for H&E staining, to evaluate the drug toxicity. Rats treated with PBS were used as the control group.

The collected blood samples were centrifuged at 1,000 × g for 15 minutes at 4°C to obtain the plasma supernatant. To determine the amount of olaparib, 40 μL of the collected plasma was vortex-mixed with 160 μL of acetonitrile for one minute. Then, the mixture was centrifuged at 15,000 × g for 10 minutes, and the clear supernatant was collected and analyzed by ultra-high performance liquid phase chromatography (UPLC). The UPLC system consisted of a ACQUITY UPLC BEH C18 1.7 μm 2.1 × 50.0 mm column, with acetonitrile/water (60/40, v/v) as the mobile phase, using a flow rate of 0.2 mL/min. The injection volume was 7 μL, and olaparib was detected using a UV detector set at 276 nm. The measuring range was 10–100 μg/mL, and the detection limit was approximately 0.1 μg/mL. A calibration curve was obtained from a series of standard solutions of olaparib in DMSO, in which 120 μL of SD rat plasma was added, followed by olaparib extraction using acetonitrile and UPLC analysis, as described above.

Results and discussion

Synthesis and characterization of the monomer and polymer

The monomer trimethylene carbonate containing benzyl group (TMC-Bz) was synthesized using benzylation of 2,2-bis (hydroxymethyl)propionate with benzyl bromide, followed by the reaction with triphosgene, as previously described (Fig. 1a).[15] The structure of TMC-Bz was confirmed by ^{1}H-NMR spectroscopy.

The P(CL_{11}-*co*-TMC-Bz_5)-PEG block copolymer was synthesized using the ring-opening polymerization of CL and TMC-Bz, with mPEG-OH (5,000 Da) as the initiator and methanesulfonic acid as the catalyst, at a CL/TMC-Bz/initiator/catalyst molar ratio of 11.0/6.0/1.0/1.3 (Fig. 1b). The copolymer was obtained as a white solid, with a yield of approximately 69%. The characteristics of the obtained copolymer are presented in Table 1. The composition of the resulting copolymer was calculated by ^{1}H-NMR, and the results were in good agreement with that expected from the ratios of monomers in the feed. The GPC analysis revealed that the M_n of the synthesized polymer (6,844 Da) was close to that calculated in the ^{1}H-NMR analysis (7,507 Da), and the marrow molecular distribution (M_w/M_n = 1.08) suggested the absence of significant side reactions.

Preparation and characterization of empty micelles

In order to investigate the effect of the preparation method and type of organic solvent of the polymer on micellar sizes, empty polymeric micelles based on P(CL_{11}-*co*-TMC-Bz_5)-PEG were prepared at a fixed polymer concentration of 10 mg/mL using the thin-film hydration and nanoprecipitation methods, respectively. The TEM revealed that the empty P(CL_{11}-*co*-TMC-Bz_5)-PEG micelles prepared using the thin-film hydration method were close to a spherical morphology, and had an average size of approximately 40 nm (Fig. 2a). This was in line with that determined by DLS (Table 2). In addition, the size of the micelles prepared using the thin-film hydration method was similar to that prepared using the nanoprecipitation method, with DMSO as the solvent of the polymer (Table 2). In contrast, for the polymeric micelles prepared using the nanoprecipitation method, with THF as the solvent of the polymer, although the PDI was narrow, the particle size was significantly larger, suggesting that the particle size is dependent on both the method and solvent used for the polymer. Opposite to the present observation, Mahsa *et al*. reported that polymeric micelles based on the mPEG-*b*-poly (*N*-2-benzoyloxypropyl methacrylamide) (mPEG-*b*-p(HPMA-Bz)) prepared using the nanoprecipitation method, with THF as the solvent of the polymer, had smaller sizes, when compared to particles prepared with DMSO as the solvent of the polymer, suggesting that the property of the polymer plays a role in the size of the particles.[17,18]

Preparation and characterization of olaparib-loaded micelles

The P(CL_{11}-*co*-TMC-Bz_5)-PEG micelles prepared by thin-film hydration and nanoprecipitation, with DMSO as the solvent of the polymer, had comparable small sizes (Table 2). Therefore, both micelle preparation methods were used to prepare a series of olaparib-loaded polymeric micelles, to determine the effect

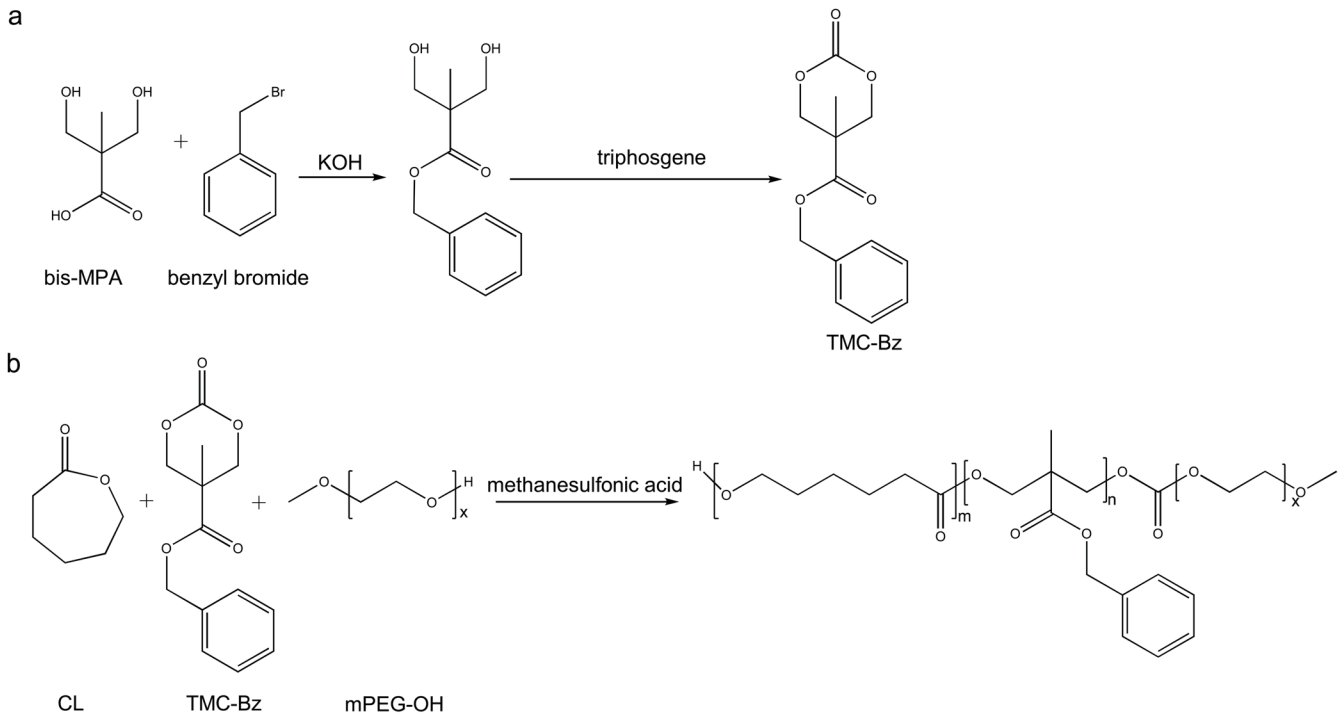

Fig. 1. Synthesis of the TMC-Bz monomer (a) and P(CL-*co*-TMC-Bz)-PEG copolymer (b). TMC-Bz, benzyl 5-methyl-2-oxo-1,3-dioxane-5-carboxlate; P(CL-*co*-TMC-Bz)-PEG), poly(ε-caprolactone)-*co*-poly (benzyl 5-methyl-2-oxo-1,3-dioxane-5-carboxylate.

of these preparation methods on the drug loading. As shown in Figures 2b and c, regardless of the preparation method and drug feeding, olaparib-loaded micelles had comparable particle sizes as the empty micelles (micelles without drug loading), indicating that drug encapsulation had a negligible effect on the particle size.

The UV-Vis analysis revealed that when olaparib was loaded in the micelles using the thin-film hydration method, the loading efficiency of olaparib at 10 mg/mL of polymer decreased from 100% to 50%, with the increase in olaparib feed ratio from 0.5% to 30%, and this reached a plateau (Fig. 3a). As a result, the loading capac-

Table 1. Characteristics of the synthesized P(CL-*co*-TMC-Bz)-PEG copolymer

Composition of the obtained copolymer[a]	Aimed molecular weight (Da)	M_n (Da, ^{1}H-NMR)	GPC		
			M_w (Da)	M_n (Da)	M_w/M_n
P(CL_{11}-*co*-TMC-Bz_5)-PEG	7,757	7,507	7,402	6,844	1.08

[a]Given numbers are degrees of polymerization of CL and TMC-Bz as determined by ^{1}H-NMR.

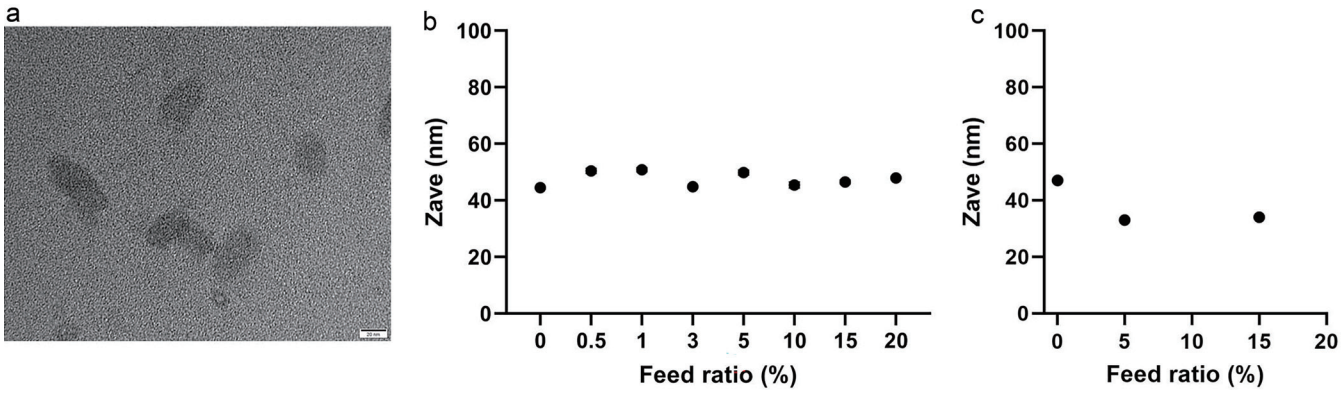

Fig. 2. (a) TEM image of P(CL_{11}-*co*-TMC-Bz_5)-PEG micelles prepared using thin-film hydration (scale bar = 20 nm), and (b and c) the Zave of olaparib-loaded micelles with various wt% loadings prepared using the thin-film hydration and nanoprecipitation methods, respectively. TEM, transmission electron microscopy; Zave, Z-average hydrodynamic diameter.

Table 2. Zave and PDI of empty polymeric micelles formed using different methods

Method	Solvent of the polymer	Zave (d.nm)	PDI
Thin-film hydration	DCM	44.00 ± 0.18	0.34 ± 0.00
Nanoprecipitation	DMSO	47.00 ± 1.04	0.37 ± 0.01
	THF	255.00 ± 2.26	0.10 ± 0.03

Zave, Z-average hydrodynamic diameter; PDI, polydispersity index.

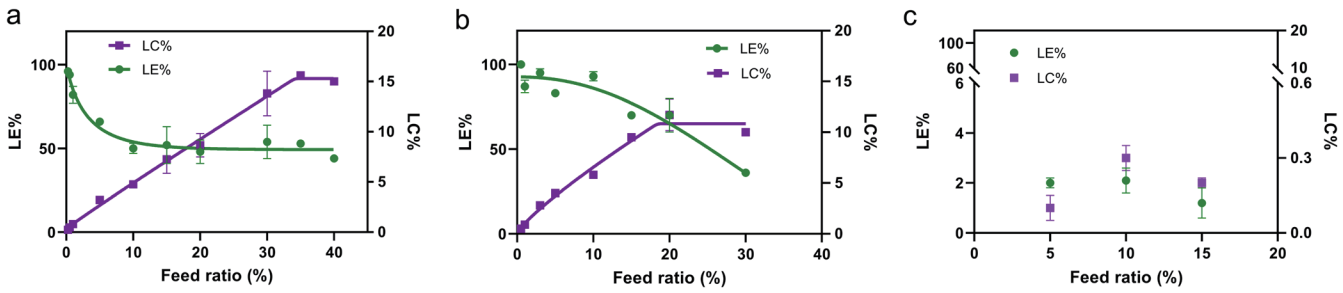

Fig. 3. LE and LC of PCL$_{11}$-*co*-TMC-Bz$_5$)-PEG for olaparib at different feed ratios (weight%) prepared using thin-film hydration at polymer concentrations of 10 mg/mL (a) and 30 mg/mL (b), and using nanoprecipitation at 10 mg/mL of polymer (C); *n* = 3. LE, loading efficiency; LC, loading capacity.

ity linearly increased until a maximum of 15% was reached at a feed ratio of 35% (Fig. 3a). Similarly, when the polymer concentration was increased to 30 mg/mL, the loading capacity continued to increase with the increase in olaparib feed ratio, reaching a plateau at 12% at a feed ratio of 20% (Fig. 3b). It is noteworthy that at the present tested condition, the solubility of olaparib in the micelles formed in the aqueous solution reached 4 mg/mL. Compared to that of olaparib in the micelles prepared using the thin-film hydration method, the loading efficiencies of P(CL$_{11}$-*co*-TMC-Bz$_5$)-PEG micellar dispersions at olaparib feed ratios of 5%, 10%, and 15% (formed using nanoprecipitation, with DMSO as the solvent of the polymer) were remarkably lower (≤2.1%), and the corresponding loading capacities were less than 0.35% (Fig. 3c). Therefore, the thin-film hydration method was superior to the nanoprecipitation method in improving the solubility of olaparib in the P(CL$_{11}$-*co*-TMC-Bz$_5$)-PEG micelles. Overall, these results suggest that the solubility of olaparib in an aqueous solution can be improved by using P(CL$_{11}$-*co*-TMC-Bz$_5$)-PEG polymeric micelles as the solubilizing excipient and that the solubilization capacity can be tailored by adjusting the polymer concentration, as needed.

In vitro release of olaparib-loaded micelles

The *in vitro* release of olaparib from micelles, with 10 wt% loading at 10 mg/mL of polymer and 3.5 wt% loading at 30 mg/mL of polymer (equivalent to approximately 1 mg/mL of olaparib), which was formed using thin-film hydration, was investigated in PBS (pH 7.4) supplied with 0.5% Tween-80 at 37°C. Then, this was compared with free olaparib under the same condition. As shown in Figure 4, the free olaparib demonstrated a rapid release in the first hour, which was indicated by approximately 70% olaparib in the medium, and a total of >80% of olaparib released into the medium within six hours. However, when olaparib was loaded in P(CL$_{11}$-*co*-TMC-Bz$_5$)-PEG micelles, the release of olaparib slowed down, and this release became significantly slower as the polymer concentration increased. For instance, the amount of released olaparib from the micelles was merely approximately 40% at 10 mg/mL of polymer, and 25% at 30 mg/mL of polymer in the

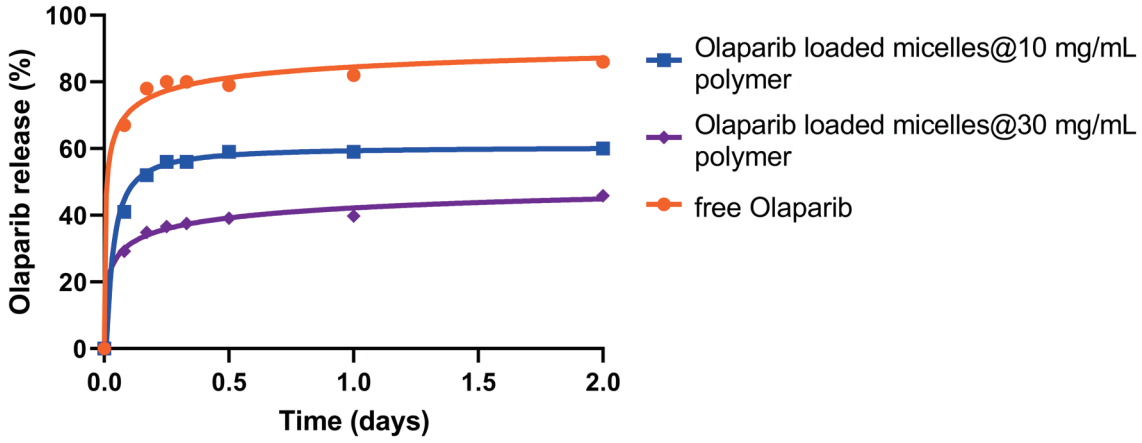

Fig. 4. Release profiles of olaparib-loaded polymer micelles and free olaparib obtained in PBS (pH 7.4) supplied with 0.5% Tween-80 at 37°C.

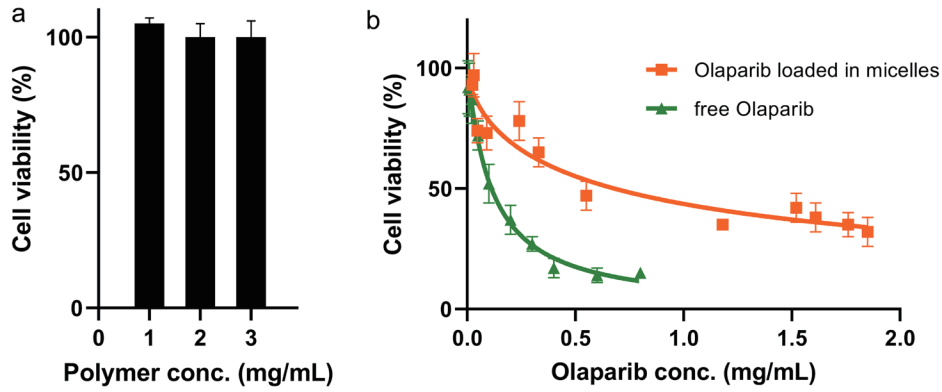

Fig. 5. Cytotoxicity assessed by CCK-8 assay of empty micelles composed of 1, 2 and 3 mg/mL of P(CL11-co-TMC-Bz5)-PEG polymer (a), and olaparib-loaded in micelles at a fixed polymer concentration of 1 mg/mL (b) on SKOV3 cells after 24 hours. For olaparib-loaded micelles, micelles with different loading amounts at a polymer concentration of of 10 mg/mL were prepared, and each micelle was diluted with DMEM for 10 times to obtain the corresponding olaparib concentration. CCK-8, cell counting kit-8; P(CL-*co*-TMC-Bz)-PEG), poly(ε-caprolactone)-*co*-poly (benzyl 5-methyl-2-oxo-1,3-dioxane-5-carboxylate.

first hour. Then, this gradually increased to approximately 60% and 40%, respectively, within 12 hours, and remained stable. The higher release of olaparib in micelles at low polymer concentration is probably due to the overloading of the core of the micelles with olaparib, and this can also be observed in photosensitizer-loaded micelles.[16] Overall, these results suggest that the retention of olaparib was improved by the encapsulation in micelles, while the extent of improvement relied on the loading percentage of olaparib-loaded micelles, i.e., polymer concentration.

Cytotoxicity of empty and olaparib-loaded micelles

Cell viability assays were conducted on the SKOV3 tumor cell line to determine the cytotoxicity of empty and olaparib-loaded micelles. As shown in Figure 5a, no toxic effect was observed when SKOV3 cells were exposed to empty micelles at a polymer concentration of up to 3 mg/mL. This demonstrated the excellent cytocompatibility that was also observed on HeLa and A431 cells.[14] After 24 hours of incubation, regardless of the formulation, the viability of SKOV3 cells treated with olaparib exhibited a dose-dependent decrease (Fig. 5b). It is noteworthy that the IC_{50} values for the micellar preparations of olaparib on SKOV3 cells were slightly higher than those for free olaparib (Table 3). Furthermore, a higher IC_{50} value was previously observed in photosensitizer-loaded P(CL-*co*-TMC-Bz)-PEG micelles, when compared to its free formulation.[14] This might be attributed to the less efficient cellular uptake of PEGylated micelles, and/or the relatively time-consuming degradation of polymers to release olaparib.[16,19,20]

Circulation kinetics and side effects of olaparib-loaded micelles in SD rats

The concentrations of olaparib in plasma were analyzed after the injection of free olaparib or olaparib-loaded P(CL_{11}-*co*-TMC-Bz_5)-PEG micelles containing ~2 mg/mL Olaparib at polymer concentrations of 10 mg/mL (20 wt% drug feed) and 30 mg/mL (7 wt% drug feed), respectively (Fig. 6). It was clearly observed that the free olaparib was quickly eliminated from the blood circulation, and no olaparib was detected in blood after 0.5 hours (Fig. 6, green line). In contrast, when loaded in micelles, olaparib exhibited a significantly slower elimination in circulation. The elimination more significantly slowed down with the increase in polymer concentration from 10 to 30 mg/mL (Fig. 6, blue vs. red line). As a result, the half-life times ($t_{1/2}$ values) of olaparib improved from approximately 0.6 to 2.0 hours, with the injected polymer concentration increasing from 10 to 30 mg/mL. This is in line with the *in vitro* release of olaparib from micelles (Fig. 4, blue vs. purple line). Furthermore, the more rapid elimination of olaparib at low polymer concentration is probably related to the overloading of the core of the micelles with olaparib (20 wt% drug loading). It is noteworthy that although the circulation time of olaparib was prolonged by encapsulation in the P(CL_{11}-*co*-TMC-Bz_5)-PEG polymeric micelles, this remained rather short for achieving the target drug delivery through the "EPR" effect. Thus, this needs to be further improved by e.g., chemical crosslinking, drug-polymer conjugation, or increasing the amount of aromatic rings.[21–23]

The *in vivo* toxic effect of the different olaparib formulations in the organs of rats was also determined. To this end, rats treated with PBS, free olaparib, or olaparib-loaded micelles (30 mg/mL of initial polymer) for 24 hours were sacrificed. Then, the heart, liver, spleen, lungs, kidneys, and brain were dissected and prepared for H&E staining. As shown in Figure 7, no obvious pathologic changes were observed in the organs of rats, regardless of the treatment groups, suggesting the safety of the micellar olaparib formulation.

Table 3. IC_{50} for free olaparib and olaparib-loaded micelles on SKOV3 cells (obtained from the curves in Figure 5b)

Compound	Free olaparib	olaparib-loaded micelles
IC_{50} (mg/mL)	0.11	0.68

Conclusions

The present study revealed that polymeric micelles based on P(CL_{11}-*co*-TMC-Bz_5)-PEG can improve the solubility of olaparib to up to 4 mg/mL in water, and that the solubilization capacity can be further improved by adjusting the polymer concentration, as needed. Therefore, P(CL_{11}-*co*-TMC-Bz_5)-PEG polymeric micelles can be considered as a safe solubilizer for olaparib. In addition, olaparib-loaded micelles had a good sustained release ability of olaparib *in vitro*, and had obvious cytotoxicity on SKOV3 cells. Furthermore, the *in vivo* pharmacokinetic studies revealed the prolonged blood circulation of olaparib incorporated in the micelles, when compared to free olaparib. However, in terms of taking advantage of the EPR effect of the micelle delivery sys-

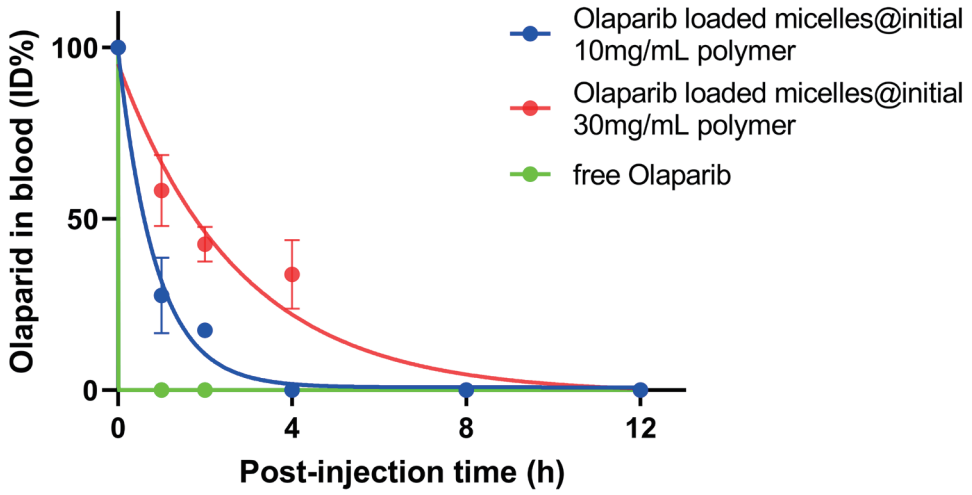

Fig. 6. *In vivo* pharmacokinetics of free olaparib and olaparib-loaded micelles (the injected polymer concentration was 10 and 30 mg/mL, respectively) upon tail vein administration in SD rats (10 mg of olaparib per kg bodyweight of rat, i.e., ~2 mg of olaparib per rat, with an injection volume of approximately 500 μL). Blood samples taken at the predetermined time points were used to quantify the percentage of olaparib of the injected dose (%ID) present in systemic circulation. The data are presented as mean ± standard deviation (SD), *n* = 3–4.

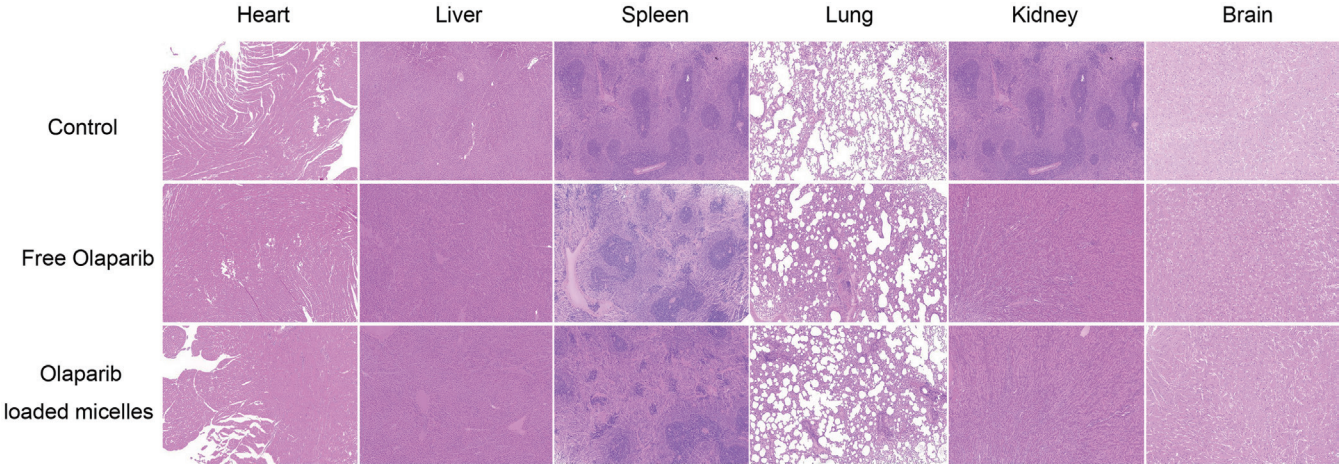

Fig. 7. Evaluation of side effects: H&E staining of the heart, liver, spleen, lung, kidneys and brain obtained from rats treated with PBS (control), free olaparib, and olaparib-loaded micelles (initial polymer concentration of 30 mg/mL, magnification: ×200). H&E staining, hematoxylin and eosin staining; PBS, phosphate buffered saline.

tem to achieve targeted olaparib delivery, the circulation time of olaparib in the micelles remained rather short. Therefore, further optimization by e.g. chemical crosslinking and drug conjugation, is needed to improve the retention of olaparib-loaded polymeric micelles in the blood circulation, and benefit from these as carriers, in order to achieve the targeted delivery of the payload in pathological tissues.

Acknowledgments

None.

Funding

This work was supported by the Natural Science Foundation of Shandong Province (ZR202103020117), and Academic Promotion Program of Shandong First Medical University (No. 2019 LJ003).

Conflict of interest

The authors have no conflicts of interest related to this publication.

Author contributions

Study concept and design (YL, WL); acquisition of data (YY, WL, GJ); analysis and interpretation of data (WL, YY, GJ); drafting of the manuscript (YY, WL); critical revision of the manuscript for important intellectual content (YL); administrative, technical, and material support (XL, TZ, LL, YM); study supervision (YL, QY). All authors made a significant contribution to the study, and approved the final manuscript.

Data sharing statement

The data (figures) used in support of the findings of the study are included within the article.

Ethics statement

The animal experiments were carried out according to the Guidelines for the Feeding, Management and Use of Laboratory Animals, and approved by the Ethics Committee of the Institute of Materia Medica, Shandong Academy of Medical Sciences (Jinan, China; Approval no.: 2021011). All surgeries were performed under sodium pentobarbital anesthesia, and all efforts were made to minimize suffering.

Reference

[1] Menear KA, Adcock C, Boulter R, Cockcroft XL, Copsey L, Cranston A, *et al*. 4-[3-(4-cyclopropanecarbonylpiperazine-1-carbonyl)-4-fluorobenzyl]-2H-phthalazin-1-one: a novel bioavailable inhibitor of poly (ADP-ribose) polymerase-1. J Med Chem 2008;51(20):6581–6591. doi:10.1021/jm8001263, PMID:18800822.

[2] Maiorano BA, Maiorano MFP, Maiello E. Olaparib and advanced ovarian cancer: Summary of the past and looking into the future. Front Pharmacol 2023;14:1162665. doi:10.3389/fphar.2023.1162665, PMID:37153769.

[3] Liu JF, Barry WT, Birrer M, Lee JM, Buckanovich RJ, Fleming GF, *et al*. Combination cediranib and olaparib versus olaparib alone for women with recurrent platinum-sensitive ovarian cancer: a randomised phase 2 study. Lancet Oncol 2014;15(11):1207–1214. doi:10.1016/S1470-2045(14)70391-2, PMID:25218906.

[4] Bochum S, Berger S, Martens UM. Olaparib. Recent Results Cancer Res 2018;211:217–233. doi:10.1007/978-3-319-91442-8_15, PMID:30069770.

[5] DiSilvestro P, Banerjee S, Colombo N, Scambia G, Kim BG, Oaknin A, *et al*. Overall Survival With Maintenance Olaparib at a 7-Year Follow-Up in Patients With Newly Diagnosed Advanced Ovarian Cancer and a BRCA Mutation: The SOLO1/GOG 3004 Trial. J Clin Oncol 2023;41(3):609–617. doi:10.1200/JCO.22.01549, PMID:36082969.

[6] Khizar S, Alrushaid N, Alam Khan F, Zine N, Jaffrezic-Renault N, Errachid A, *et al*. Nanocarriers based novel and effective drug delivery system. Int J Pharm 2023;632:122570. doi:10.1016/j.ijpharm.2022.122570, PMID:36587775.

[7] Malik JA, Ansari JA, Ahmed S, Khan A, Ahemad N, Anwar S. Nanodrug delivery system: a promising approach against breast cancer. Ther Deliv 2023;14(5):357–381.

[8] Sung YK, Kim SW. Recent advances in polymeric drug delivery systems. Biomater Res 2020;24:12. doi:10.1186/s40824-020-00190-7, PMID:32537239.

[9] Perumal S, Atchudan R, Lee W. A Review of Polymeric Micelles and Their Applications. Polymers (Basel) 2022;14(12):2510. doi:10.3390/polym14122510, PMID:35746086.

[10] Welbank T, Kurien M. To PEG or not to PEG that is the question. Proc Nutr Soc 2021;80(1):1–8. doi:10.1017/S002966512000703X, PMID:32441238.

[11] Ibrahim M, Ramadan E, Elsadek NE, Emam SE, Shimizu T, Ando H, *et al*. Polyethylene glycol (PEG): The nature, immunogenicity, and role in the hypersensitivity of PEGylated products. J Control Release 2022;351:215–230. doi:10.1016/j.jconrel.2022.09.031, PMID:36165835.

[12] Maeda H. Toward a full understanding of the EPR effect in primary and metastatic tumors as well as issues related to its heterogeneity. Adv Drug Deliv Rev 2015;91:3–6. doi:10.1016/j.addr.2015.01.002, PMID:25579058.

[13] Maeda H, Wu J, Sawa T, Matsumura Y, Hori K. Tumor vascular permeability and the EPR effect in macromolecular therapeutics: a review. J Control Release 2000;65(1-2):271–284. doi:10.1016/s0168-3659(99)00248-5, PMID:10699287.

[14] Liu Y, Fens MHAM, Lou B, van Kronenburg NCH, Maas-Bakker RFM, Kok RJ, *et al*. π-π-Stacked Poly(ε-caprolactone)-b-poly(ethylene glycol) Micelles Loaded with a Photosensitizer for Photodynamic Therapy. Pharmaceutics 2020;12(4):338. doi:10.3390/pharmaceutics12040338, PMID:32283871.

[15] Pratt RC, Nederberg F, Waymouth RM, Hedrick JL. Tagging alcohols with cyclic carbonate: a versatile equivalent of (meth)acrylate for ring-opening polymerization. Chem Commun (Camb) 2008;(1):114–116. doi:10.1039/b713925j, PMID:18399418.

[16] Wennink JWH, Liu Y, Mäkinen PI, Setaro F, de la Escosura A, Bourajjaj M, *et al*. Macrophage selective photodynamic therapy by meta-tetra(hydroxyphenyl)chlorin loaded polymeric micelles: A possible treatment for cardiovascular diseases. Eur J Pharm Sci 2017;107:112–125. doi:10.1016/j.ejps.2017.06.038, PMID:28679107.

[17] Bagheri M, Bresseleers J, Varela-Moreira A, Sandre O, Meeuwissen SA, Schiffelers RM, *et al*. Effect of Formulation and Processing Parameters on the Size of mPEG- b-p(HPMA-Bz) Polymeric Micelles. Langmuir 2018;34(50):15495–15506. doi:10.1021/acs.langmuir.8b03576, PMID:30415546.

[18] Du JZ, Tang LY, Song WJ, Shi Y, Wang J. Evaluation of polymeric micelles from brush polymer with poly(epsilon-caprolactone)-b-poly(ethylene glycol) side chains as drug carrier. Biomacromolecules 2009;10(8):2169–2174. doi:10.1021/bm900345m, PMID:19722555.

[19] Xie J, Xu C, Kohler N, Ho Y, Sun S. Controlled PEGylation of monodisperse Fe_3O_4 nanoparticles for reduced non-specific uptake by macrophage cells. Adv Mater 2007;19(20):3163–3166. doi:10.1002/adma.200701975.

[20] Terashima T, Sugita T, Fukae K, Sawamoto M. Synthesis and single-chain folding of amphiphilic random copolymers in Water. Macromolecules 2014;47(2):589–600. doi:10.1021/ma402355v.

[21] van Nostrum CF. Covalently cross-linked amphiphilic block copolymer micelles. Soft Matter 2011;7:3246–3259. doi:10.1039/C0SM00999G.

[22] Ekladious I, Colson YL, Grinstaff MW. Polymer-drug conjugate therapeutics: Advances, insights and prospects. Nat Rev Drug Discov 2019;18(4):273–294. doi:10.1038/s41573-018-0005-0, PMID:30542076.

[23] Shi Y, van der Meel R, Theek B, Oude Blenke E, Pieters EH, Fens MH, *et al*. Complete Regression of Xenograft Tumors upon Targeted Delivery of Paclitaxel via Π-Π Stacking Stabilized Polymeric Micelles. ACS Nano 2015;9(4):3740–3752. doi:10.1021/acsnano.5b00929, PMID:25831471.