Multipotent Poly(Tertiary Amine-Oxide) Micelles for Efficient Cancer Drug Delivery

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The cancer drug delivery process involves a series of biological barriers, which require the nanomedicine to exhibit different, even opposite properties for high therapeutic efficacy. The prevailing design philosophy, i.e., integrating these properties within one nanomedicine via on-demand property transitions such as PEGylation/dePEGylation, complicates nanomedicines' composition and thus impedes clinical translation. Here, polyzwitterionic micelles of poly(tertiary amine-oxide)-block-poly(ε-caprolactone) (PTAO-PCL) amphiphiles that enable all the required functions are presented. The zwitterionic nature and unique cell membrane affinity confer the PTAO micelles long blood circulation, efficient tumor accumulation and penetration, and fast cellular internalization. The mitochondrial targeting capability allows drug delivery into the mitochondria to induce mitochondrial dysfunction and overcome tumor multidrug resistance. As a result, the PTAO/drug micelles exhibit potent anticancer efficacy. This simple yet multipotent carrier system holds great promise as a generic platform for potential clinical translation.

1. Introduction

Chemotherapy is an effective way to treat many cancers but can be confounded by severe adverse effects and drug resistance.[1] Nanomedicine is one of the promising strategies that may address these issues.[2] Of particular interest are those formulated in polymeric micelles. Typical polymeric micelles are self-assembled from amphiphilic polymers and feature core-shell structures.[3] These structural characteristics enable good solubilization of hydrophobic agents encapsulated in the lipophilic core and long blood circulation time provided by the hydrophilic shell.[4] Polymeric micelles also hold advantages in easy preparation and high scale-up feasibility.[5] Notably, a poly(ethylene glycol)-block-poly(lactic acid) micelle formulation of paclitaxel (Genexol-PM) has been approved for clinical use.[6] While the micellar formulation can mitigate many adverse effects, it fails to enhance the therapeutic efficacy.[7]

To sufficiently elicit the therapeutic activity, an ideal micellar nanomedicine should be capable of long blood circulation, specific tumor accumulation, deep tumor penetration, readily cellular internalization, precise subcellular localization if applicable, and efficient drug release.[8] However, the properties required to achieve these capacities are generally conflicting. Taking commonly used poly(ethylene glycol)-poly(ε-caprolactone) (PEG-PCL) micelles as an instance, while the stealthy PEG coating facilitates long blood circulation, it compromises cellular internalization. Rational design to enable spontaneous property transitions in response to various endogenous and/or exogenous stimuli at specific delivery nodes is a common strategy to tackle these dilemmas.[9] For example, the
introduction of a tumor-acid-labile linker between the PEG chain and the hydrophobic segment could achieve dePEGylation in the tumor microenvironment, resolving the PEG dilemma. However, such all-into-one strategies may compromise the composition of nanomedicines and decrease the clinical translation potential. Moreover, the heterogeneous and dynamic nature of tumors may disable the designed micelles to achieve the supposed property transitions.

We have recently reported a phospholipid-affinitive poly(tertiary amine oxide) (PTAO), poly(2-(N-oxide-N,N-diethylamino)ethyl methacrylate) (OPDEA). The polyzwitterionic nature renders OPDEA non-fouling toward proteins, enabling long blood circulation. The phospholipid affinity allows OPDEA to weakly bind to cells, which can trigger transcytosis-mediated active extravasation and tumor penetration, as well as efficient cellular internalization. Compared with passive diffusion-based extravasation and penetration that relies on the leaky tumor vasculatures and is generally limited by the dense and heterogeneous tumor microenvironment, transcytosis-mediated processes leverage the intrinsic active transport pathways of endothelial cells and tumor cells and can significantly improve the tumor accumulation and infiltration of nanomedicines. OPDEA also exhibits a remarkable mitochondrial targeting ability. Mitochondria produce adenosine triphosphate (ATP) for ATP-binding cassette transporters such as P-glycoprotein to function as drug efflux pumps and may have mitochondrial DNA mutations conferring drug resistance. Targeting mitochondria to disrupt ATP synthesis and induce DNA injury can be a straightforward way to overcome multidrug resistance (MDR) of tumor cells.

Prompted by these distinguished properties of OPDEA, we propose here to develop PTAO micelles as a drug delivery platform, which may prolong the blood circulation time, increase drug accumulation in tumors, promote tumor penetration and cellular internalization, and even overcome the MDR of tumor cells, given the critical role of mitochondria involving MDR. Ultimately achieving significant therapeutic outcomes. For proof of concept, we chose PCL as the hydrophobic segment and constructed two kinds of PTAO-PCL block copolymer (OPDMA-PCL and OPDEA-PCL) as carriers for doxorubicin (DOX) delivery (Scheme 1). The DOX-loaded micelles could reverse the MDR of MCF-7/ADR tumors and achieve enhanced anticancer efficacy.

2. Results and Discussion

2.1. Preparation and Characterization of DOX-Loaded PTAO-PCL Micelles

OPDMA-PCL and OPDEA-PCL were synthesized as shown in Scheme S1 (Supporting Information). We used to prepare PTAO through post-oxidation of poly(tertiary amine) using hydrogen peroxide or meta-chloroperoxybenzoic acid (mCPBA), which requires strict control of reaction conditions. In this work, we developed a more facile method to produce PTAO-containing block copolymers with defined structures (Figure 1a). TAO monomers (ODMA and ODEA) were first produced by oxidizing N,N-dimethylaminoethyl methacrylate (DMA) and N,N-diethylaminoethyl methacrylate (DEA). mCPBA was selected as the oxidizing agent, as its activity does not affect the double bond of methacrylate. After column purification, ODMA and ODEA were obtained as colorless crystals. The chemical structures of these two monomers were confirmed by 1H-NMR spectra (Figures S1 and S2, Supporting Information). Next, heterobifunctional PCL was synthesized via ring-opening polymerization of ε-caprolactone using 5-(Boc-amino)-1-pentanol as the initiator and diphenyl phosphate as the catalyst (Figure S3, Supporting Information), which then reacted with 2-bromoisobutyl bromide to yield PCL-Br (Figure S4, Supporting Information). Subsequently, atom transfer radical polymerization of ODMA and ODEA was performed to produce OPDMA-PCL and OPDEA-PCL copolymers with PCL-Br as the initiator. Each block of the copolymers was controlled to be ~5 kDa (Figures S5 and S6, Supporting Information). Meanwhile, a PEG-PCL block copolymer was synthesized as the control (Figure S7, Supporting Information). All the polymers were labeled with 1.0 wt.% of Cyanine 5 (Cy5) to afford fluorescently labeled carriers (OPDMA-Cy5-PCL, OPDEA-Cy5-PCL, and PEG-Cy5-PCL; Scheme S2, Supporting Information).

The OPDMA-PCL and OPDEA-PCL copolymers could self-assemble into well-defined micelles in aqueous solutions. The respective diameters were 29 and 25 nm, and the zeta potentials were −2.08 and −3.46 mV (Table S1, Supporting Information). For comparison, the PEG-PCL micelles had an average size of 31 nm and zeta potential of −2.85 mV. Encapsulation of DOX into these micelles was achieved through a nanoprecipitation method. The average sizes of the OPDMA-PCL/DOX, OPDEA-PCL/DOX, and PEG-PCL/DOX micelles were 34.5, 27.7, and 37.4 nm, respectively, as determined by dynamic laser scattering (DLS) and transmission electron microscope (TEM; Figure 1b–d). Notably, the DOX-loaded PTAO micelles were slightly negatively charged (Table S1, Supporting Information), which might benefit the stealth of the nanomedicines in blood circulation. The drug loading contents of OPDMA-PCL/DOX and OPDEA-PCL/DOX were 15.7% and 14.9%, respectively, with the corresponding loading efficiency of 82.3% and 80.8%, comparable to that of PEG-PCL/DOX (16.3%; 85.4%; Table S1, Supporting Information). OPDMA-PCL and OPDEA-PCL also had a similar critical micelle concentration (CMC) of ~20 μg mL⁻¹, lower than that of PEG-PCL (Figure S8, Supporting Information). Due to the polyzwitterionic corona and the low CMC values, the OPDMA-PCL/DOX and OPDEA-PCL/DOX micelles could keep stable in phosphate-buffered saline (PBS) or full culture medium for one week, with no significant change in particle size (Figure 1e; Figure S9, Supporting Information).

The drug release kinetics of OPDMA-PCL/DOX, OPDEA-PCL/DOX, and PEG-PCL/DOX was investigated in PBS at pH 7.4 and 5.0 to mimic different physiological environments. All the micelles showed sustained release profiles. At pH 7.4, the DOX release ratios of OPDMA-PCL/DOX, OPDEA-PCL/DOX, and PEG-PCL/DOX after 24 h incubation were 46.2%, 43.6%, and 36.2%, respectively (Figure 1f). Acidic pH accelerated DOX release from all the formulations (Figure 1g) due to the protonation of DOX at acidic pH, which greatly increased its water solubility and rendered it squeezed out from the hydrophobic micelle cores. Once the DOX concentration in the medium is close to that inside the micelles, the concentration difference-driven drug release will be slowed down, resulting in no complete release. Notably, the drug release rates of PTAO-PCL/DOX were slightly fast than PEG-PCL/DOX, possibly due to the more hydrophilic
PTAO corona, which makes DOX more likely to diffuse from the cores.

2.2. In Vitro Cytotoxicity

The in vitro cytotoxicity of the micelle carriers, DOX-loaded micelles, and free DOX was first evaluated on adherent cell culture models using the MTT (3-[4,5-dimethylthiazol-2-yl]-2,5 diphenyl tetrazolium bromide) assay. The OPDMA-PCL and OPDEA-PCL carriers had minimal effects on cell proliferation in vitro, even at a concentration as high as 100 μg mL⁻¹ (Figure S10, Supporting Information). After DOX loading, OPDMA-PCL/DOX and OPDEA-PCL/DOX showed higher cytotoxicity than free DOX and PEG-PCL/DOX against a panel of cancer cell lines including 4T1, HepG2, HeLa, A549, BxPC-3, and even drug-resistant MCF-7/ADR (Figure 2a; Figure S11, Supporting Information). The IC50 values are listed in Table S2 (Supporting Information).

We also used the resazurin assay to further assess the cell-killing effects of OPDMA-PCL/DOX and OPDEA-PCL/DOX on 3D multicellular tumor spheroids (MTSs). MTS models closely resemble solid tumors in many aspects, including the heterogeneous architecture, internal gradients of signaling factors, nutrients, and oxygenation. The cells in MTSs mimic intercellular and cell-matrix interactions and exhibit greater chemotherapeutic resistance than monolayer-cultured cells. On a dense MTS model of BxPC-3 cell line (Figure 2b), the otherwise effective free DOX lost its cytotoxicity precipitously with an over 10-fold
increase in IC50, compared to that on the 2D model. Notably, OPDMA-PCL/DOX and OPDEA-PCL/DOX exhibited superior cytotoxicity, and their IC50 values were less than one-fifth of those for DOX and PEG-PCL/DOX. Moreover, free DOX showed moderate cytotoxicity against MCF-7/ADR MTs, whereas OPDMA-PCL/DOX and OPDEA-PCL/DOX still displayed potent cell-killing effects. The corresponding IC50 values were 9.93 μg mL\(^{-1}\) and 16.48 μg mL\(^{-1}\), respectively, much lower than that of free DOX (41.68 μg mL\(^{-1}\)) and PEG-PCL/DOX (117.20 μg mL\(^{-1}\)).

2.3. Cellular Uptake and Subcellular Distribution of PTAO-PCL Micelles

Zwitterionic polymers are generally ultralow fouling and can shed potential protein or cell adsorption, thus suffering from unfavorable cellular uptake.\(^{[22]}\) Interestingly, OPDEA, albeit zwitterionic, can be easily internalized by cells.\(^{[12,14]}\) We investigated whether the micellar formulations of OPDMA and OPDEA still retain this property. Fluorescently labeled micelles, OPDMA-Cy5PCL, OPDEA-Cy5PCL, and PEG-Cy5PCL, were used to avoid interference from the release and fluorescence self-quenching issues of DOX. Confocal microscopy showed bright Cy5 fluorescence inside PTAO micelle-treated cells and significantly weaker Cy5 intensity in the PEG-PCL group after 1 h incubation (Figure 2c,d). Flow cytometry analysis demonstrated that 66.13% and 60.57% of the cells treated with the OPDMA-Cy5PCL and OPDEA-Cy5PCL micelles were already Cy5 positive after 30-min incubation, whereas only 23.13% were positive in the PEG-Cy5PCL group. After 1 h incubation, the corresponding values increased to 95.40%, 93.17%, and 47.70%, respectively (Figure 2e; Figure S12, Supporting Information). These results indicated that PTAO-PCL micelles could be internalized by cancer cells much faster than similarly antifouling PEG-PCL micelles.
We then sought to clarify how the PTAO-PCL micelles were internalized. We incubated MCF-7/ADR cells at 4 °C or with a variety of endocytic inhibitors, including filipin III (an inhibitor of caveolae-mediated endocytosis), chlorpromazine (an inhibitor of clathrin-mediated endocytosis), wortmannin (an inhibitor of macrodipinocytosis), and cytochalasin D (an inhibitor of macrocinopinocytosis/phagocytosis),[23] and used flow cytometry to analyze their effects on cellular uptake. The results demonstrated that the cellular uptake of all the micelles was greatly blocked by low temperature, pointing to energy-dependent pathways. While filipin III and chlorpromazine had no significant impacts on the internalization of OPDMA-Cy5PCL and OPDEA-Cy5PCL micelles, wortmannin and cytochalasin D exhibited similarly significant inhibitory effects (Figure S13, Supporting Information). These results suggested macropinocytosis-mediated pathways for the cellular uptake of PTAO-PCL micelles. In contrast, PEG-Cy5PCL micelles were mainly internalized into cells via the clathrin- and caveolae-related pathways.

Subsequently, we investigated the subcellular distribution of the micelles using confocal microscopy. The PEG-Cy5PCL micelles were found primarily localized to lysosomes (Figure S14, Supporting Information). In contrast, the Cy5 signals of the OPDMA-Cy5PCL and OPDEA-Cy5PCL micelles had weak correlations with the LysoTracker Green signals but overlapped the MitoTracker Green signals remarkably with the overlapping degrees as high as 76% and 77%, respectively (Figure 3a–c). The DOX-loaded Cy5-free micelles also exhibited the same subcellular distribution profiles (Figure S15, Supporting Information). These results indicated that the intracellular PTAO-PCL micelles were highly localized to mitochondria. The distinct subcellular
Intracellular trafficking of Cy5-labeled micelles in MCF-7/ADR cells. a) Colocalization of PEG-Cy5PCL, OPDMA-Cy5PCL, and OPDEA-Cy5PCL with mitochondria after 2-h incubation. Scale bar: 25 μm. b) Overlapping profiles of Cy5 fluorescence with MitoTracker Green fluorescence along the selected line across the cell (indicated by a red line in the zoomed-in image). c) Manders’ correlation coefficients of Cy5 and MitoTracker Green calculated by pixel intensity using ImageJ. Cells were cultured with PEG-Cy5PCL, OPDMA-Cy5PCL, and OPDEA-Cy5PCL (Cy5-eq. dose: 0.1 μg mL⁻¹) for 2 h. The Cy5 fluorescence was shown in red, and MitoTracker Green was in green. Scale bar: 25 μm. d) The JC-1 assay of the ΔΨm in MCF-7/ADR cells. The cells were incubated with DOX formulations at a DOX-eq. dose of 0.5 μg mL⁻¹ or CCCP (1 mM) for 2 h. e) Intracellular ATP levels of MCF-7/ADR cells exposed to DOX formulations at a DOX-eq. dose of 0.5 μg mL⁻¹ or CCCP (1 × 10⁻³ M) for 12 h.

Localizations of the PTAO-PCL and PEG-PCL micelles are likely to result from the different endocytic pathways they underwent. While it is well appreciated that the clathrin-mediated pathway may end up at lysosomes, how macropinosomes bypass the endosomal/lysosomal fusion but traffic to mitochondria, supposing the PTAO-PCL micelles were still contained, is unknown, which we are working on.

As DOX may act on mitochondrial DNA to induce mitochondria disruption and cell apoptosis, mitochondria-targeted DOX transportation may promise higher potency. We used...
Figure 4. In vitro transcellular transport of micelles. 

a) The exocytosis kinetics measured by flow cytometry. ECDHCC-1 cells were incubated with Cy5-labeled micelles for 3 h and then re-cultured in a fresh medium for timed intervals before flow cytometric analysis. 

b) Transepithelial transport of Cy5-labeled micelles across the ECDHCC-1 cell monolayer on a transwell membrane. 

c) Cumulative transport of the micelles. The Cy5-labeled micelles at a Cy5-eq. dose of 0.5 μg mL⁻¹ were added onto the apical side, and the micelle concentrations in the basolateral compartment were measured at timed intervals by detecting the Cy5 fluorescence. 

d) Cellular uptake of the transported micelles in MCF-7/ADR cells on the basolateral side after 24 h incubation. Cy5-eq. dose: 0.5 μg mL⁻¹. Scale bars: 100 μm. Cell nuclei were shown in blue and Cy5 in red. 

e) Cy5 fluorescence intensity of MCF-7/ADR cells quantified by flow cytometry.

f) Intercellular transport of Cy5-labeled micelles between MCF-7/ADR cells. The cells (first batch) were cultured with micelles at a Cy5-eq. dose of 1 μg mL⁻¹ for 6 h, washed with PBS, and imaged (i); the cells were then cultured in fresh medium for 12 h, and the medium was harvested to incubate the second batch of cells for 12 h, followed by washing and imaging (ii); the same procedures were implemented for another two rounds (iii and iv). Scale bar: 50 μm. 

g) Distribution of PEG-Cy5-PCL, OPDMA-Cy5-PCL, and OPDEA-Cy5-PCL in MCF-7/ADR MTSs and the effects of
reaching up to 61% and 64% for OPDMA-Cy5PCL and OPDEA-Cy5PCL, with 24 h secretion ratios of that of DOX- (63%) and PEG-PCL/Dox (36%)-treated groups (Figure S17, Supporting Information). Moreover, the ratio of JC-1 aggregates to monomers significantly decreased at a low temperature, suggesting an energy-dependent process. Since the macropinocytosis-mediated pathway was involved in the endocytosis of the PTAO-PCL micelles, inhibiting microinocytosis by using wortmannin or cytochalasin D could drastically retard the transcellular transport. Moreover, disrupting Golgi apparatus by domesin or blocking Golgi protein transport by brefeldin A both decreased the transport rates of PTAO-PCL micelles, pointing to a Golgi apparatus-involved transcytosis process (Figure S18, Supporting Information).

In the next experiment, we aimed to investigate if the micelles undergoing the transcellular transport could still be internalized by cancer cells. We set up the experiment conditions as the same as the previous one but seeded MCF-7/ADR cells on the basolateral side of the device. Confocal microscopy images demonstrated extensive internalization of the PTAO-Cy5PCL micelles by MCF-7/ADR cells, as opposed to the barely observable fluorescence in the PEG-Cy5PCL micelle-treated cells (Figure 4d). An 11.9- and 12.3-fold higher fluorescence intensity in the OPDMA-Cy5PCL and OPDEA-Cy5PCL groups than that in the PEG-Cy5PCL group was determined by flow cytometry analysis (Figure 4e). These results suggest that PTAO-PCL micelles may undergo transcytosis-mediated serial transport.

To validate this transport mode of PTAO-PCL micelles, we performed an “infection” assay. MCF-7/ADR cells were treated with micelles for 6 h, followed by another 12-h incubation in the micelle-free medium. Afterward, the medium was collected for incubation with another batch of cells. These procedures were performed for three rounds in total. As shown in Figure 4f, Cy5 signals could be observed in all the batches of cells in the PTAO-Cy5PCL groups in a batch-dependent fashion. In contrast, only the first batch of cells in the PEG-Cy5PCL group fluorescence weakly in the Cy5 channel. The induction of transcytosis by PTAO-PCL micelles is likely to arise from the rapid cellular internalization via macropinocytosis, followed by the translocation from the leak macropinosomes to the Golgi apparatus for efficient exocytosis.

As a final test, we challenged the PTAO-PCL micelles with MCTS models, where the abundant matrix may hinder the delivery of nanomedicines. MCF-7/ADR MTSs with a diameter of 1 mm were cultured with OPDMA-Cy5PCL, OPDEA-Cy5PCL, or PEG-Cy5PCL micelles for 4 h and then transferred onto glass-bottomed Petri dishes for imaging (Figure 4g). Weak Cy5 fluorescence of the PEG-Cy5PCL micelles could be observed only around the periphery of the MTSs and hardly in the interior. In contrast, the whole MTSs receiving the OPDMAB.
**2.5. Blood Clearance, Biodistribution, and In Vivo Penetration**

RBC hitchhiking has been demonstrated as an efficient method for drug delivery that can prolong blood circulation and enhance drug accumulation in the targets.[11] Despite the polyzwitterionic nature, OPDMA and OPDEA on the micelles’ surface could adhere to RBCs. Compared with free DOX or PEG-PCL/DOX micelles, RBCs incubated with OPDMA-PCL/DOX or OPDEA-PCL/DOX micelles showed stronger DOX fluorescence, which overlapped well with the FITC-WGA-labeled RBC membrane (Figure S21, Supporting Information). Additionally, adsorption of PTAO-PCL/DOX micelles did not significantly change RBCs' morphology, integrity, and dispensability. Therefore, PTAO-PCL micelles may hitchhike on RBCs via cell binding to avoid the clearance by the mononuclear phagocyte system (MPS), which promises favorable pharmacokinetics and biodistribution profiles.

We first studied the blood clearance kinetics of PTAO-PCL/DOX micelles. DOX or DOX-loaded micelles were intravenously injected into mice, and blood samples were drawn at timed intervals to quantify the DOX concentrations using HPLC (Figure 5a). After administration, free DOX was cleared rapidly from the bloodstream, with only 0.3% of the given dose remaining after 8 h, whereas the micelle formulations circulated long in blood, with over 14% remaining after 8 h. Moreover, the OPDMA-PCL/DOX and OPDEA-PCL/DOX micelles had similar blood clearance kinetics to that of the long-circulating PEG-PCL/DOX micelles, with comparable AUC values and half-life times (Table S3, Supporting Information).

Next, we validated the in vivo tumor penetration ability of PTAO-PCL micelles. Mice bearing MCF-7/ADR tumors of ≈40 mm³ were intravenously injected with PEG-Cy5-PCL, OPDMA-Cy5-PCL, OPDEA-Cy5-PCL micelles, and the tumor regions were imaged using confocal microscopy. As shown in Figure 6f, OPDMA-Cy5-PCL and OPDEA-Cy5-PCL gradually extravasated from the blood vessels and infiltrated into the distal tumor sites. In contrast, PEG-Cy5-PCL micelles were essentially confined in the blood vessels even after 60 min post-injection. The line-scan analysis showed that OPDMA-Cy5-PCL and OPDEA-Cy5-PCL could diffuse into tumor tissues as far as 100 μm away from the vessels while the PEG-Cy5-PCL signal was already undetectable at distances 40 μm away from the vessels (Figure 6g). As a result, the overall fluorescence intensity of PTAO-PCL micelles along the selected line increased linearly with time, reaching over 27 times the intensity of PEG-Cy5-PCL micelles at 60 min (Figure S22, Supporting Information). These results demonstrated that the PTAO-PCL micelles could extravasate quickly into tumor tissues and possess superior tumor permeability over PEG-PCL micelles.

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**Figure 5.** Blood clearance, biodistribution, and tumor penetration of micelles in MCF-7/ADR tumor-bearing mice. a) The blood clearance kinetics of DOX after intravenous administration of DOX, PEG-PCL/DOX, OPDMA-PCL/DOX, or OPDEA-PCL/DOX at a DOX-equ. dose of 4 mg kg⁻¹ (n = 3). b) Distribution of DOX in tumors and major organs at 24 h post-injection of DOX, PEG-PCL/DOX, OPDMA-PCL/DOX, or OPDEA-PCL/DOX. c) In vivo real-time imaging of tumor-bearing mice after a single intravenous injection of PEG-Cy5-PCL, OPDMA-Cy5-PCL, or OPDEA-Cy5-PCL (Cy5-equ. dose of 0.5 mg kg⁻¹). The tumor regions were circled in white. d) Ex vivo imaging of tumors and organs (heart, liver, spleen, lung, and kidney) excised from the above mice. e) Quantification of average Cy5 fluorescence intensity in tumors and organs. f) Confocal imaging of time-resolved extravasation and penetration of PEG-Cy5-PCL, OPDMA-Cy5-PCL, and OPDEA-Cy5-PCL into MCF-7/ADR tumors (Cy5-equ. dose of 0.5 mg kg⁻¹). BALB/c nude mice were inoculated with MCF-7/ADR cells (5 x 10⁶ cells per mouse) near the subcutaneous venous vessel in the abdomen. When the tumor volume reached ≈40 mm³, the mice were anesthetized, and the tumor was exposed and fixed for imaging. Scale bar: 50 μm. g) Cy5 fluorescence intensity of PEG-Cy5-PCL, OPDMA-Cy5-PCL, and OPDEA-Cy5-PCL as a function of the distance from a blood vessel into an in-depth tumor region marked by the rectangular frames. NS, no significance; "**"p < 0.01.
2.6. In Vivo Antitumor Activity

The antitumor activities of the PTAO-PCL/DOX micelles were evaluated in an MCF-7/ADR orthotopic tumor model. Tumor-bearing mice were intravenously administered with PBS, DOX, PEG-PCL/DOX, OPDMA-PCL/DOX, or OPDEA-PCL/DOX at DOX equivalent dose of 4 mg kg\(^{-1}\) every 3 days for a total of five treatments (Figure 6). Compared with the PBS group, each DOX formulation led to obvious tumor growth inhibition. Nonetheless, the antitumor efficacy of DOX and PEG-PCL/DOX was moderate, with tumor inhibition rates (TIRs) of 51.6% and 40.1%, respectively. In contrast, OPDMA-PCL/DOX and OPDEA-PCL/DOX basically suppressed tumor growth during the whole experimental period with TIRs as high as 87.6% and 81.3%, respectively (Figure 6a–c; Figure S23, Supporting Information). Histological examination of the tumor tissue sections showed abundant apoptotic cells with features including extensive vacuolization, severe nucleus shrinkage, and decreased cellularity in the OPDMA-PCL/DOX and OPDEA-PCL/DOX groups. In comparison, much fewer apoptotic events were observed in the DOX and PEG-PCL/DOX groups (Figure 6e). These results demonstrated that compared with the traditional PEGylated micelles, PTAO micelles, with the efficient tumor-penetration ability and remarkable mitochondrial targeting ability, could reverse cancer cells’ MDR and thus achieve high therapeutic outcomes.

Importantly, no significant change in body weight occurred on the mice receiving OPDMA-PCL/DOX and OPDEA-PCL/DOX.
treatments, whereas the mice treated with DOX suffered from severe weight loss (up to 20%), though gradually rebounded after intraperitoneal administration with 1% (w/v) glucose solution (Figure 6d). Histological analysis showed no noticeable damages or lesions in the major organs resected from the mice in the PEG-PCL/DOX, OPDMA-PCL/DOX, and OPDEA-PCL/DOX groups, whereas free DOX caused gross damage to cardiomyocytes, evidenced by the disordered and even fractured cardiac muscle fibers (Figure 6e; Figure S24, Supporting Information). These results indicated the high biosafety of the DOX-loaded PTAO-PCL micelles.

3. Conclusion

In summary, we have presented PTAO-PCL micelles for efficient drug delivery into tumors. Due to the zwitterionic nature and unique cell membrane affinity, the PTAO micelles exhibited long blood circulation, efficient tumor accumulation and penetration, and fast cellular internalization. Moreover, the DOX-loaded PTAO micelles could target the mitochondria and lead to mitochondrial dysfunction, thus reversing the multidrug resistance of tumor cells. These abilities enabled potent therapeutic efficacy against MCF-7/ADR tumors. We envision that these PTAO polymers, as superior alternatives to PEG, may be applied to other drug delivery platforms such as liposomes and dendrimers for higher therapeutic outcomes.

Supporting Information

Supporting Information is available from the Wiley Online Library or from the author.

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Conflict of Interest

The authors declare no conflict of interest.

Data Availability Statement

The data that support the findings of this study are available in the supplementary material of this article.

Keywords

cell membrane affinity, micelle, mitochondria targeting, poly(tertiary amine-oxide), transcytosis

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