# Enhanced Antitumor Efficacy by D-Glucosamine-Functionalized and Paclitaxel-Loaded Poly(Ethylene Glycol)-Co-Poly(Trimethylene Carbonate) Polymer Nanoparticles

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**ABSTRACT:** The poor selectivity of chemotherapeutics for cancer treatment may lead to dose-limiting side effects that compromise clinical outcomes. To solve the problem, surface-functionalized polymer nanoparticles are regarded as promising tumor-targeting delivery system. On the basis of glucose transporter (GLUT) overexpression on cancer cells, D-glucosamine-conjugated and paclitaxel-loaded poly(ethylene glycol)-co-poly(trimethylene carbonate) copolymer nanoparticles (DGlu-NP/PTX) were developed as potential tumor-targeting drug delivery system in this study. Because of the high affinity between D-glucosamine and GLUT, DGlu-NP/PTX could target to tumor tissue through GLUT-mediated endocytosis to improve the selectivity of PTX. DGlu-NP/PTX was prepared by emulsion/solvent evaporation technique and characterized in terms of morphology, size, and zeta potential. *In vitro* evaluation of two-dimensional cells and three-dimensional tumor spheroids revealed that DGlu-NP/PTX was more potent than those of plain nanoparticles (NP/PTX) and Taxol. *In vivo* multispectral fluorescent imaging indicated that DGlu-NP had higher specificity and efficiency on subcutaneous xenografts tumor of mouse. Furthermore, DGlu-NP/PTX showed the greatest tumor growth inhibitory effect on *in vivo* subcutaneous xenografts model with no evident toxicity. Therefore, these results demonstrated that DGlu-NP/PTX could be used as potential vehicle for cancer treatment. © 2014 Wiley Periodicals, Inc. and the American Pharmacists Association J Pharm Sci 103:1487–1496, 2014

**Keywords:** nanoparticles; paclitaxel; D-Glucosamine; GLUT; tumor-targeting delivery system; biomaterials; drug delivery system; biodegradable; polymers; cancer chemotherapy

## INTRODUCTION

Chemotherapy is the most common method for the treatment of cancer. However, the concentration of chemotherapeutics in tumor tissue is usually limited because of the poor selectivity of antitumor drugs. To overcome this shortcoming, copolymer nanoparticles with core–shell structure have been proposed as targeting drug delivery system with great attentions. The hydrophobic core serves as the reservoir for pharmaceutical compounds with poor solubility and/or low stability in physiological environments, whereas the hydrophilic shell provides the nanocarriers with desirable solubility in aqueous solutions.[1,2]

Although polymer nanoparticles offer many benefits in drug delivery, conventional nanoparticles suffer from limited *in vivo*-active targeting ability because of the nonspecific systemic distribution. Surface-functionalized copolymer nanoparticles by tumor-specific targeting moieties (e.g., receptor-binding ligands or antibodies) were considered as active targeting drug delivery system for tumor treatment.[3,4] Unlike normal cells, cancerous cells have acquired metabolic ability to survive in harsh microenvironment conditions (e.g., hypoxia and acidity), thus developing a more aggressive phenotype.[5] One of the characteristic alterations associated with the increased glycolytic rate of cancer cells is dramatically increased cellular glucose uptake, which is mediated by glucose transporter (GLUT), a kind of transmembrane proteins. Therefore, GLUT is highly expressed in cancers, including breast, nonsmall cell lung cancer, thyroid, head and neck, colon, and esophagus,[6] which suggests that the GLUT might be an efficient target for drug delivery to tumor tissue. Actually, because of GLUT being highly expressive on glioma cells and blood brain barrier (BBB), GLUT showed that it could mediate D-glucosamine-modified nanoparticles across BBB and glioma tissue in intracranial glioma-bearing mice model.[7]

Poly(trimethylene carbonate) (PTMC) has been widely used in biomedical field because of the tunable biodegradability without formation of acidic compounds and excellent mechanical properties.[8,9] In our previous study, we have investigated the synthesis and self-assembly behavior of poly(ethylene glycol) (PEG)–PTMC diblock copolymer. And the PEG–PTMC copolymer nanoparticles have been demonstrated to be an effective carrier for anticancer drug.[10] However, the plain PEG–PTMC nanoparticles presented only passive targeting ability to tumor tissue because of the enhanced permeability and retention effect.[11] Therefore, it is aspired to conjugate certain tumor-targeting ligands to develop active targeting nanocarrier. The surface-functionalized nanoparticles can specifically target cancerous tissues/cells because of their passive and active targeting abilities, thereby greatly enhancing the therapeutic outcomes while reducing any nonspecific systemic toxicity.[12–16]

Paclitaxel (PTX) has been demonstrated significant antitumor activity against various solid tumors such as ovarian,

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breast, lung, head, and neck cancer.[17] However, its high hydrophobicity and nonselective distribution *in vivo* seriously restrict the practical use. Thus, there is an urgent demand for targeting drug delivery system for PTX application.

In this study, we have constructed a novel PTX-loaded targeting copolymer nanoparticle for tumor-special delivery by employing D-glucosamine as targeting moiety and PEG–PTMC nanoparticle as drug carrier (DGlu-NP/PTX). DGlu-NP/PTX was prepared and characterized *in vitro*. The targeting efficiency was systematically evaluated by *in vitro* cell, *in vitro* three-dimensional (3D) tumor spheroids model and *in vivo* subcutaneous xenograft nude mice model.

## MATERIALS AND METHODS

### Materials

Paclitaxel was obtained from Xi'an San jiang Bio-Engineering Company Ltd. (Xi'an, China). $MPEG_{3K}$–$PTMC_{6K}$ and NHS–$PEG_{3.5K}$–$PTMC_{6K}$ were synthesized as described previously.[9] D-Glucosamine (2-amino-2-deoxy-D-glucose) hydrochloride, Hoechst 33342, propidium iodide (PI), and 3-(4,5-dimethyl-thiazol-2-yl)-2,5-diphenyl-tetrazolium bromide (MTT) were purchased from Sigma (St. Louis, Missouri). Oregon Green 514 palloidin, antibovine α-tubulin mouse mAb, and Alexa Fluor 633-conjugated goat antimouse IgG antibody were obtained from Molecular Probes Company (California, USA).. 1,1′-dioctadecyl-3,3,3′,3′-tetramethylindotricarbocyanine iodide (DiR) was purchased from Biotium (California, USA). Annexin V-FITC Apoptosis Detection kit and Micro BCA Protein assay kit were purchased from Beyotime® Biotechnology Company Ltd. (Nantong, China). Penicillin–streptomycin, Dulbecco's Modified Eagle's Medium (DMEM), fetal bovine serum and 0.25% (w/v) trypsin solution were purchased from Gibco BRL (Gaithersberg, Maryland). RG-2 cell line was obtained from ATCC. BALB/c mice ($20 \pm 2$ g) were supplied by Department of Experimental Animals, Fudan University (Shanghai, China). All animal experiments were carried out in accordance with guidelines evaluated and approved by the Ethics Committee of the College of Pharmacy, Fudan University.

### Synthesis and Characterization of D-Glucosamine-Conjugated PEG–PTMC Copolymer

NHS–PEG–PTMC (0.0053 mmol) and 2-amino-2-deoxy-D-glucose (0.0106 mmol) were dissolved in 10 mL dimethylsulphoxide with 0.1 mL $Et_3N$ for 48 h reaction. Then, the mixture was dialyzed (MWCO 3500 Da) against deionized water for 48 h. The final solution was lyophilized and stored at −20°C until use. The copolymer was characterized by $^1$H-NMR spectra in dimethyl sulfoxide (DMSO)-d6.

### Preparation of D-Glucosamine-Conjugated Targeting Nanoparticles

D-Glucosamine-conjugated and paclitaxel-loaded poly(ethylene glycol)-co-poly(trimethylene carbonate) copolymer nanoparticle was prepared through the emulsion/solvent evaporation technique as previously.[18–21] Briefly, 36 mg of MPEG–PTMC, 4 mg of DGlu–PEG–PTMC, and 2.8 mg of PTX in 1 mL dichloromethane (DCM) was added into 5 mL of 0.6% sodium cholate aqueous solution and then sonicated at 200 W on ice. The formed emulsion was added dropwise into 25 mL of 0.3% sodium cholate under rapid magnetic stirring. After that, DCM was evaporated by rotary vacuum at 37°C. The formed nanoparticle suspension was concentrated by ultrafiltration. After washed twice by deionized water, DGlu-NP/PTX was resuspended in 1 mL saline and kept at 4°C for further use.

1,1′-Dioctadecyl-3,3,3′,3′-tetramethylindotricarbocyanine iodide-labeled DGlu-NP was prepared with the same procedure except that the PTX was prepared by DiR.

### Characterization of Targeting Nanoparticles

The mean particle size, size distribution, and zeta potential of DGlu-NP/PTX were determined by dynamic light scattering (DLS). The morphology of DGlu-NP/PTX was observed using a Carl Zeiss Ultra 55 field emission scanning electron microscope (Oberkochen, Germany). The PTX encapsulated in nanoparticles was quantified via HPLC as described previously.[22]

### Inhibitory Effect Against Tumor Cells

As GLUT was overexpressed on brain gliomas,[23] we used rat glioma RG-2 cells as cell model to evaluate the inhibitory effect of DGlu-NP/PTX. RG-2 cells were seeded in 96-well plates at the density of $5 \times 10^4$ cells/well and cultured at 37°C for 24 h. Then, the cells were exposed to various PTX formulations, including Taxol, NP/PTX, and DGlu-NP/PTX with various concentrations. After 72 h incubation, MTT was added into the medium at 0.5 mg/mL for 4 h incubation. Afterwards, 200 μL DMSO was added into each well to dissolve any purple formazan crystals formed. The relative color intensity was measured by Tecan Safire 2 microplate reader (Männedorf, Switzerland).

### Immunofluorescence Analysis

RG-2 cells were seeded at a density of $5 \times 10^5$ cells/well in six-well plates. After 24 h incubation, cells were treated with various PTX formulations at equivalent PTX concentration (200 ng/mL) for 24 h. Then, the cells were fixed with 4% formaldehyde for 10 min and permeabilized in 0.1% Triton X-100 phosphate-buffered saline (PBS) solution that contained 1% bovine serum albumin (PBS–BSA) and RNase 100 μg/mL. After washing three times with PBS–BSA, the cells were treated with Oregon Green 514 palloidin (1:100 v/v) in PBS–BSA for 20 min and then incubated for 60 min with antibovine α-tubulin mouse mAb in PBS–BSA. After adding 2 μg/mL Alexa Fluor 633-conjugated goat antimouse IgG antibody, the cells were incubated for another 60 min. The samples were rinsed three times by PBS–BSA and treated with 100 nM PI for 5 in and then viewed by Confocal Laser Scanning Microscopy (CLSM) examination with TCS SP5 of Leica (Solms, Germany).

### Cell Apoptosis Assay

For the quantitative analysis of apoptosis, RG-2 cells were seeded into six-well plate at a density of $5 \times 10^5$ cells/well for 24 h incubation. Then, cells were treated with various PTX formulations at equivalent PTX concentration (200 ng/mL). After 24 h incubation, cells were stained using the Annexin V-FITC Apoptosis Detection kit followed by the manufacturer's instructions. The stained cells were analyzed using a FACSCalibur flow cytometer of BD Biosciences (New York, USA) taking untreated cells as blank control. Data analysis was performed using CellQuest software of Becton Dickinson (New York, USA).