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Thermal modulation of intracellular drug distribution using thermoresponsive polymeric micelles

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Dedicated to Professor Teiji Tsuruta on the occasion of his 88th birthday (Beiju).

Abstract

Intracellular distribution of free doxorubicin (DOX) or DOX-loaded in polymeric micelles with thermoresponsive outer shells of poly(*N*-isopropylacrylamide) or its copolymers in cultured human breast cancer cells (MCF-7) were investigated by fluorescence and confocal laser scanning microscopy. Free DOX accumulated rapidly and selectively in cell nuclei, independent of temperature changes. In contrast to free drugs, the intracellular distribution of DOX-loaded in the thermoresponsive polymeric micelles was significantly affected by temperature changes across lower critical solution temperature (LCST) of the micelles. Above the micelle LCST, DOX delivered by the micelles was localized uniformly inside of MCF-7 cells. By contrast, the amount of DOX delivered to MCF-7 cells drastically decreased below the micelle LCST due to minimal interaction of the micelles with cell membrane surfaces. These results clearly showed that the mechanism of the intracellular drug localization was different between free drugs and DOX-loaded in the micelles. The thermoresponsive micelles aggressively interacted with the cells and carried DOX into the cells *via* triggered phase transition of the outer shells. In addition, much lower accumulation of free DOX was observed in the resistant cells compared to its parent sensitive MCF-7 due to the resistant mechanism. Of interest, DOX accumulation in the resistant cells was almost in the same level as with MCF-7 (sensitive) cells for the micelle system above the LCST.

Keywords: Poly(*N*-isopropylacrylamide); Thermoresponse; Polymeric micelles; Doxorubicin; Intracellular drug distribution

1. Introduction

Selective anti-cancer drug delivery to solid tumor tissues using drug carriers has been an extremely

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attractive application for cancer chemotherapy without severe toxic side effects. For this purpose, several types of drug carriers, such as water-soluble polymers [1,2], liposomes [3,4], and polymeric micelles [5], have been actively investigated.

Amphiphilic block copolymers form core-shell multi-molecular assemblies called polymeric micelles in aqueous media [6,7]. Highly hydrated outer shells of polymeric micelles provide their reliable structural stability in aqueous environments. Hydrophobic inner cores can incorporate a large amount of hydrophobic drug with maintaining their water-solubility due to the presence of the hydrophilic outer shells. Furthermore, nano-ordered diameter range of polymeric micelles (10–200 nm) can allow long circulation in the blood stream avoiding the body's defense systems (reticuloendotherial system, RES) and thus, utilize the enhanced permeability and retention (EPR) effect [8,9] at solid tumor sites for tumor targeting. We have previously reported that polymeric micelles composed of poly(ethylene oxide)-*b*-poly(L-asparate) block copolymers containing an anticancer drug, doxorubicin (DOX), selectively accumulated at solid tumor sites by the passive targeting mechanism, the EPR effect [10,11].

Recently, polymeric micelles with stimuli-responsive drug release mechanisms as a novel concept for anticancer drug delivery have been designed for applications in effective cancer chemotherapy [12–14]. The different drug release kinetics stimulated by physico-chemical signals (e.g., heat, pH, and ultrasound) may lead to maximal cytotoxic action at tumor sites, resulting in locoregional drug accumulation while reducing drug accumulation in normal tissues to inhibit undesirable side effects. These drug carrier systems combining two or more targeting methodologies is defined as multi-targeting systems. In order to accomplish these intelligent drug targeting systems, we have developed polymeric micelles possessing thermoresponsive outer shells [15–17]. Our strategy of cancer chemotherapy using the thermoresponsive polymeric micelles is as follows. Polymeric micelles with drugs circulate in the blood avoiding the RES uptake, and accumulate selectively at solid tumor tissues *via* the EPR-mediated targeting below the micelle LCST. And then the thermoresponsive outer shells of the micelles shrink and change into hydrophobic by local heating at the target sites upon the LCST. This alternation of micelle properties may induce selective drug actions at the heated target site. Simultaneously, this strategy can achieve temporal drug delivery control by local temperature increases.

Poly(*N*-isopropylacrylamide) (PIPAM) is well-known to undergo sharp coil-to-globule transitions at 32 °C in water [18]. This phase transition temperature is called a lower critical solution temperature (LCST). This polymer changes from water-soluble and hydrophilic state (coil) below its LCST to water-insoluble and hydrophobic state (globule: aggregation) above the LCST. Previously, we have already reported successful preparations of thermoresponsive polymeric micelles constructed with two block copolymers, PIPAM-*b*-poly(butyl methacrylate) [15] and PIPAM-*b*-poly(D,L-lactide) [16]. In our previous works, the DOX-loaded thermoresponsive micelles demonstrated successful controlled ON–OFF drug release and subsequent expression of *in vitro* cytotoxicity with applied temperature changes [15,17].

Here, we mainly focus on investigation of intracellular drug delivery and interactions of the thermoresponsive polymeric micelles into/with cultured human breast cancer (MCF-7) cells by fluorescence and laser scanning confocal microscopy in order to understand cytotoxic mechanisms modulated by temperature changes as well as to optimize drug carrier design for the multi-targeting systems.

2. Materials and methods

2.1. Materials

N-Isopropylacrylamide (IPAM) was kindly provided by Kohjin (Japan) and recrystallized from *n*-hexane. D,L-Lactide (LA, TCI, Japan) was purified by recrystallization from ethyl acetate. Butyl methacrylate (BMA, Tokyo Kasei Co. Ltd., Japan), *N*,*N*-dimethylacrylamide (DMAM, Wako Pure Chemicals, Japan) and 3-mercaptopropionic acid (Aldrich) were distilled under reduced pressure. Triethylamine and xylene were purchased from Wako Pure Chemicals and purified by standard methods. Benzoylperoxide (BPO, Kanto Chemical Co., Japan), *N*-ethylacetamide (TCI), *N*,*N*-dimethylacetamide (Wako Pure Chemicals), thionyl chloride (Wako Pure Chemicals), diethyl ether (Wako Pure Chemicals), 2-mercaptoethanol (Wako Pure Chemicals) and tin(II)2-ethylhexanoate (Wako Pure Chemicals) were used as received. Doxorubicin hydrochloride (DOX–HCl) was obtained from Mercian Co., Japan.