Enhanced antitumor activity using doxorubicin-loaded micelles based on poly(ethylene oxide)-poly[(R)-3-hydroxybutyrate]- poly(ethylene oxide) triblock copolymers.

Tae Hee Kim[1], Christopher Mount[1], Wayne Gombotz[2], and Suzie Hwang Pun[1]
[1]Department of Bioengineering, University of Washington, Box 355061, William H. Foege Building, Seattle, WA 98195
[2] Omeros Corporation, 1420 Fifth Avenue, Suite 2600, Seattle, WA 98101

Statement of Purpose: Doxorubicin (DOX) is one of the most effective chemotherapeutics for wide range of cancer treatment. However, its clinical use is limited by poor tumor tissue penetration and severe side effects such as cardiotoxicity as well as inherent and acquired drug resistance of tumors [1, 2]. Therefore, development of delivery systems for DOX is in need to improve its therapeutic efficacy with minimizing side effects. DOX encapsulation within self-assembled polymeric micelles has the potential to enhance the specificity of drug accumulation in the tumor via the enhanced permeability and retention effect (EPR) [3]. In this study, poly(ethylene oxide)-poly[(R)-3-hydroxybutyrate]-poly(ethylene oxide) (PEO-PHB-PEO) triblock copolymers were synthesized and DOX was encapsulated within polymeric micelles using this copolymers. DOX-loaded micelles were characterized and their antitumor activities were evaluated in both monolayer cells and 3-D multicellular spheroids (MCS). Biodistribution and tumor reduction *in vivo* was further studied.

Methods: PEO-PHB-PEO triblock copolymers were synthesized as previously described [4]. In brief, PHB diol (MW 2500, measured by NMR) prepared by transesterification of high molecular weight PHB was conjugated to methoxy-PEG-carboxylic acid (MW 5000, from Laysan Bio) with standard DCC coupling in the presence of DMAP. Molecular weight and purity was confirmed by gel permeation chromatography (GPC) and NMR. A solvent evaporation method was employed to encapsulate DOX into the micelles after complexing DOX with triethylamine. DOX-micelles were characterized by measuring particle sizes, drug loading efficiency, and drug release profile. DOX delivery studies were conducted in both monolayer cultured cells and in 3-D MCS. The delivery mechanism of DOX from micelles to monolayer SiHa cells, human cervical carcinoma was assessed by confocal microscopy. DOX Penetration and growth inhibition was studied with MCS composed of SiHa cells. MCS of 400 μm diameter were incubated with drugs and/or micelles for 30 minutes. For growth inhibition study, MCS was cultured for an additional 7 days. As an indication of cell proliferation, MCS diameter was measured each day by using an imaging analysis program after images were taken from optical microscope with a 10x objective. Free DOX and DOX-micelles were administered to SiHa tumor bearing mice by both intratumoral and intravenous injection, and tumor volume was measured twice a week. For biodistribution study, tissues were collected 4 and 24 hrs post-injection and analyzed for doxorubicin content by fluorescence.

Results: DOX was successfully encapsulated within PEO-2.5kPHB-PEO micelles with ~70% loading efficiency and DOX-micelle had an ideal size ranging from 30-40 nm for passive tumor targeting. Free DOX was quickly released from the dialysis tubing whereas the rate of DOX release was highly decreased by micelle encapsulation. DOX delivered by micelle showed strong nuclear fluorescence indicating efficient DOX delivery to the nucleus from micelle-mediated delivery. Intracellular uptake of DOX was enhanced when DOX was delivered to DOX-resistant cells via the micelle system. As shown in Figure 1, DOX penetration in the 3-D spheroids was found to be limited to the outer few cell layers of the MCS. In contrast, DOX delivered in micelle formulations efficiently penetrates to the core of MCS within 30 minutes post-delivery. The cytotoxicity of empty micelles, free DOX and DOX-micelles to MCS was then investigated using a growth inhibition assay. Both free DOX and DOX-micelles treatment significantly inhibit spheroid growth (Fig.2).

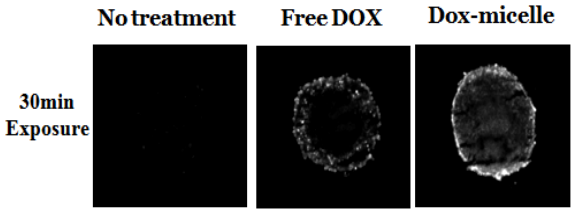

Figure 1 DOX fluorescence in representative sections from multicellular SiHa spheroids treated for 30min with free DOX and DOX-loaded micelle

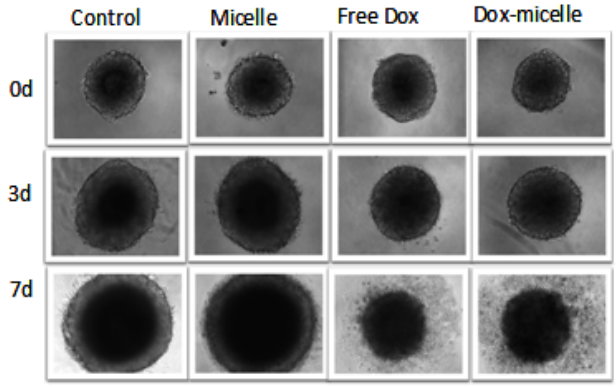

Figure 2 Growth inhibition assay of MCS. Representative images of MCS treated with unloaded micelles, free DOX and DOX-micelle for 30min.

Conclusions: We demonstrated that PEO-PHB-PEO micelle system is a promising vehicle to deliver DOX for improved cancer therapy.

References:

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