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Effect of biomimetic shear stress on intracellular uptake and cell-killing efficiency of doxorubicin in a free and liposomal formulation

Taehee Kang[a,1], Younhee Cho[a,1], Chulhun Park[a], Soo-Dong Kim[a], Euichaul Oh[c], Jing-Hao Cui[d], Qing-Ri Cao[d], Beom-Jin Lee[a,b,*]

[a] College of Pharmacy, Ajou University, Suwon 443-749, Republic of Korea
[b] Institute of Pharmaceutical Science and Technology, Ajou University, Suwon 16499, Republic of Korea
[c] College of Pharmacy, The Catholic University, Bucheon 420-743, Republic of Korea
[d] College of Pharmaceutical Science, Soochow University, Suzhou, 215123, China

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ABSTRACT

Shear stress could be considered in the context of cellular uptake and cell-killing efficiency. Thus, mimicking the dynamic characteristics of *in vivo* environment is important in targeted drug delivery. We investigated the intracellular uptake and cell-killing efficiency of doxorubicin (DOX) in a free and liposomal form (Doxil®) under biomimetic shear stress to mimic *in vivo* environment. In this dynamic environment, cells demonstrated significantly higher fluorescence intensity than that of the static environment, and fluorescence microscopy images indicated increased intracellular uptake of DOX in the presence of fluidic shear stress. In cells treated with free DOX and liposomal Doxil®, cell-killing efficiency was affected by shear stress. Taken together, shear stress, affecting drug uptake and cell-killing efficiency, is important in intracellular drug targeting.

1. Introduction

Knowledge of the *in vivo* cell microenvironment is important for understanding and controlling cell behavior. Fluid flow is ubiquitous in the human body and is generally known to affect several cellular behaviors. The flow and forces induced by shear stress are especially important in intravenous drug delivery. Shear stress has been shown to affect many cellular processes such as cell proliferation, cytoskeleton remodeling, adhesion, and migration in various types of cells (Davies, 1995). Shear stress not only influence the cell-drug contact time, but also affects the properties of cells i.e. activation of different receptors (Chachisvilis et al., 2006; Nguyen et al., 2001). To date, limited studies have explored the dynamics of cells that are subjected to the frictional force shear stress generated by blood flow. Blood flow causes cells to align and rearrange their cytoskeleton (including actin filaments); hence, the multifarious characteristics of the in vivo environment are different from static cell cultures (Coan et al., 1993; Kim et al., 1989; Malek and Izumo, 1996). Most often, cell culture characterization is performed under static conditions using a monolayer of cells, which does not mimic the dynamic characteristics of the in vivo environment, and possibly accounts for the differences observed between cell culture and animal experiments. The consideration of shear stress in drug delivery has recently increased. However, because most of these studies focus on the endothelium, the effect of fluid shear stress on the behavior of tumor cells still remain largely unexplored.

Mechanical forces contribute to cancer metastasis. During metastasis cancer cells experience two main types of fluid shear stress: stresses exerted on circulating tumor cells by blood flow in the vascular microenvironment and interstitial flows in the tumor microenvironment (Michor et al., 2011; Swartz and Lund, 2012), with the average shear stress range from 0.5 to 30.0 dyn/cm^2 and very low shear stress (0.1 dyn/cm^2) (Mitchell and King, 2013; Tarbell and Shi, 2013). Stress generated by interstitial and blood flows have been suggested to play key role in the metastatic process, by enhancing tumor cell invasion and promoting adhesion of circulating tumor cell to blood vessels, respectively (Roberts et al., 2007). Studies conducted with cultured cancer cells (e.g.,

* Corresponding author at: College of Pharmacy, Ajou University, Suwon 443-749, Republic of Korea.
E-mail address: bjl@ajou.ac.kr (B.-J. Lee).
[1] Equally contributed.

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colon, ovarian, bladder, esophageal, and melanoma) reported that mechanical stimuli such as pressure or fluid flow, affects cancer cells' adhesion (Dong et al., 2005; Haier et al., 1999; Niedbala et al., 1985; Thamilselvan et al., 2004), migration (Slattery et al., 2005), expression of cadherin and integrin (Lawler et al., 2004), invasion capacity (Lawler et al., 2009), morphology (Chotard-Ghodsnia et al., 2007; Lawler et al., 2006), and viability (Haier and Nicolson, 2001). While these studies highlighted the importance of shear stress in the context of drug delivery, systematic assessment of drug cellular uptake and efficiency of drug delivery in epithelial cells have not been determined.

The present study used the antitumor drug, doxorubicin (DOX), which is widely used for the treatment of a broad spectrum of cancers (including bladder, breast, pancreas, colon, stomach, lung, and ovarian cancer) as the model drug (Buzdar et al., 1985; Singal and Iliskovic, 1998). The purpose of this study was to investigate the cellular uptake and cell-killing efficiency of DOX when it is a free drug or encapsulated drug as liposomal formulation in epithelial cells under biomimetic shear stress. Because epithelial cells are influenced by interstitial flow, the experiments under biomimetic dynamic conditions in this study were performed with low shear stress, 0.5 dyn/cm^2.

2. Materials and methods

2.1. Materials

Doxorubicin hydrochloride was received from Dong-A Pharmaceutical Co., Ltd., Korea. Cell media and the supplements for cell culture were purchased from Gibco (Grand Island, NY). Tube (Silicone Tubing), connector (Elbow Luer Connector), and fluidic cell chamber (μ-Slide VI 0.4) were purchased from Ibidi GmbH (Munich, Germany). Hoechst 33342 fluorescent stain and trypan blue solution were purchased from Invitrogen (USA), and paraformaldehyde (4%) was purchased from Electron Microscopy Science, EMS (USA). The other reagents were from Sigma-Aldrich (USA).

2.2. Cell culture

Four different cell lines were used. The cell lines were obtained from the Korean Cell Line Bank (KCLB, Seoul, Korea). Human Embryonic Kidney (HEK) 293T cells (KCLB No. 21573), a specific cell line originally derived from human embryonic kidney cells grown in tissue culture. Pancreatic adenocarcinoma cells, PANC-1 (KCLB No. 21469), derived from the ductal region of a pancreatic epithelioid carcinoma. HEK 293 T and PANC-1 cells were maintained in Dulbecco's Modified Eagle's Medium (DMEM), supplemented with 10% fetal bovine serum (FBS) and 1% penicillin/streptomycin. The human colorectal adenocarcinoma cell line, HT29 (KCLB No. 30038), and the human lung adenocarcinoma cell line, A549 (KCLB No. 10185), were grown in RPMI 1640 medium, supplemented with 10% FBS and 1% penicillin/streptomycin. For the biomimetic microfluidic experiment, 30 μl of cell suspension (4×10^5 cells/mL) was seeded into each channel of the fluidic cell chamber and 100 μl of the corresponding cell medium was added. Then, cells were allowed to attach and stabilize in a humidified incubator containing 5% CO_2 and 95% air, at 37 °C.

2.3. Biomimetic microfluidic experiment

After the cells were stabilized and grown for 24 h under static conditions, the fluidic cell chamber was connected to the biomimetic microfluidic system (BMS), which was calibrated in a previous study (Kang et al., 2016). The BMS, which consisted of a peristaltic pump, a bubble trap, a fluidic cell chamber, tubes, connectors and medium, was placed in an incubator (37 °C and 5% CO_2) with the exception of the pump during the fluidic experiment as shown in Fig. 1. We used commercial product for fluidic cell chamber (μ-Slide VI 0.4) which has the six channels, with the volume of 30 μl in each channel. DOX (1 μg/ml) was prepared in cell medium. Media containing DOX was run throughout the cell chamber, and the cell monolayer was subjected to shear stress (0.5 dyn/cm^2) of laminar flow by perfusion using a peristaltic pump for experiments of intracellular uptake (for 1 h) and cell-killing efficiency (for 12 h), respectively. To perform the experiments

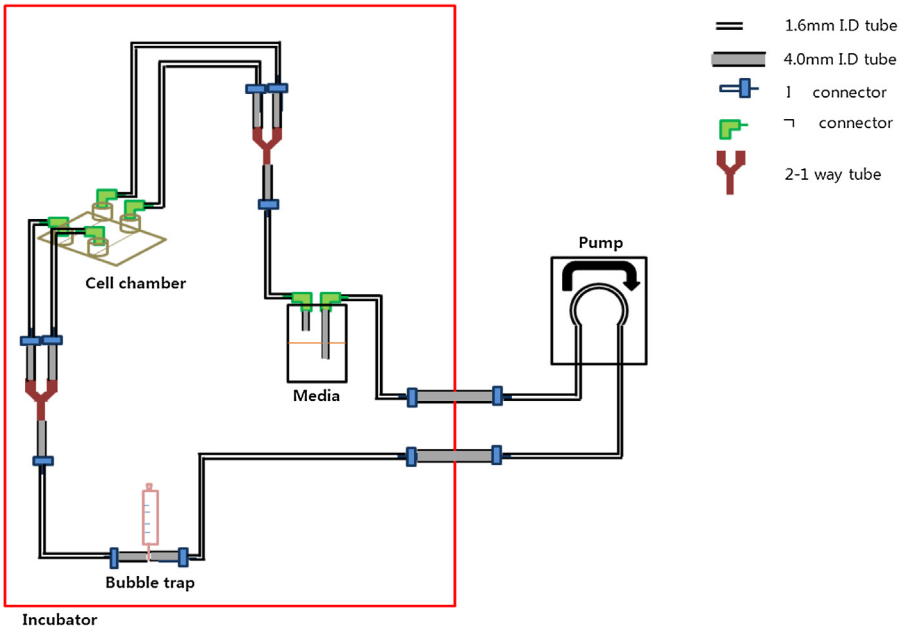

Fig. 1. Schematic of the experimental biomimetic flow system.