317d The Correlation of the Cell Cycle-Dependent Cytotoxicity and Drug Penetration into Three Dimension Tissue

Byoung-jin Kim and Neil S. Forbes

Specificity of chemotherapeutic drugs is based on the characteristics of larger fraction of proliferating cells in tumor tissue. Whereas 5-fluorouracil and paclitaxel specifically act on the cells in S- and G2/M phase by inhibiting DNA synthesis or microtubule de-assembly, doxorubicin induces apoptosis of proliferating cells by intercalating DNA/RNA, producing free radicals and inhibiting topoisomerase □. In addition to the specificity of drug itself, drug penetration into tissue is an critical factor affecting drug effectiveness and also the development and characterization of novel drug and drug carrier system since most of the tumor tissue in vivo has a diffusion-limited region imposed by chaotic vasculature structure around it. In this study, cell cycle specificity and penetration of anti-cancer drug were investigated using cylindroid model prepared from multicellular spheroid. The drug effectiveness depending on the cell cycle was estimated using monolayer culture. Mouse embryonic fibroblast cells were incubated in the media containing low glucose and low serum to control the growth fraction. Cell cycle were analyzed using flow cytometry with propidium iodide (PI) and acridine orange (AO) stains before/after doxorubicin treatment and the cell cytotoxicity was measured with 3,3'-dioctadecyloxacarbocyanine (live) and propidium iodide (dead) stains. With cylindroid model, the distribution of proliferating cells in cylindroid was measured with AO and DAPI based on the DNA and RNA contents. The doxorubicin penetration and cytotoxicity in cylindroid were investigated by observing fluorescence from doxorubicin and vital stain *in situ*. These studies provided a better understating of drug effects in three dimensional tissue by considering the two factors, growth fraction and drug penetration, simultaneously, which are important for the improvement in chemotherapeutic cancer treatment.