KEY WORDS: Doxorubicin, Membrane dynamics, Cholesterol, Fluorescence microscopy, fluorescence lifetimes.

ABSTRACT

Methods of fluorescence microscopy, including fluorescence spectra, images and decay kinetics are used to examine the uptake of doxorubicin and its dependency of membrane dynamics. Doxorubicin, an anthracycline antibiotic, is a drug used in cancer chemotherapy, such as breast cancer, bronchial carcinoma and lymphoma, and is known as an inducer of apoptosis. Based on its fluorescence properties we were able to monitor the uptake and intracellular localisation of doxorubicin in MCF-7 human breast cancer cells. Furthermore we treated part of our cells with methyl-β-cyclodextrin to reduce cholesterol concentration by about 50% resulting in some lowering of membrane stiffness. By comparing cells with natural and decreased cholesterol levels after 2 and 24 h incubation with doxorubicin (2 µM), we observed that higher fluorescence intensities (Fig.1) and shortened fluorescence lifetimes (Fig.2) are concomitant with lower membrane stiffness.

Figure 1: Fluorescence spectra of MCF-7 cells, incubated with doxorubicin [2 µM, 24 h]; cells with natural cholesterol content (lower curve) and cells with lower cholesterol content (upper curve). λ.ex= 470 nm.

Figure 2: Fluorescence lifetime of MCF-7 cells, incubated with doxorubicin [2 µM, 2 h and 24 h]; cells with natural cholesterol content (left bars) and cells with lower cholesterol content (right bars). λ.ex= 470 nm.

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