THE INFLUENCE OF SATURATED FATTY ACIDS ON HUMAN LUNG EPITHELIAL CELLS

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Introduction

Fatty acids (FAs) can be utilized in manufacturing of novel inhalable drug delivery systems for lung cancer treatment, i.e. solid lipid nano- or microparticles [1]. FAs are naturally occurring in saturated or unsaturated forms with different carbon chain lengths. It is known that unsaturated FAs (i.e. arachidonic acid) decrease cell membrane stiffness of lung epithelial cells, leading to increased drug uptake and bioavailability [2]. Unsaturated FAs are in liquid state at room temperature so they cannot be used as nano- or microparticle matrix. Saturated FAs are promising materials for fabrication of drug delivery carriers, however there is limited information on their effect on lung epithelial cells.

The aim of this study was to evaluate the influence of various saturated FAs on viability and mechanical properties of malignant and non-malignant human lung epithelial cells.

Materials and Methods

Human lung epithelial cells (malignant – A549. ATCC® CCL-185™ and non-malignant – BEAS-2B, ATCC® CRL 9609™) were cultured in DMEM supplemented with 10% FBS, 1% penicillin/streptomycin and 1% glutamine (only for BEAS-2B). Cells were seeded in 96-well plates or Petri dishes and cultured overnight prior to addition of FAs. FAs (C10:0 – C18:0) were dissolved in 99.8% ethanol and added to cell culture medium to obtain final concentrations of FAs equal to 25, 50, 75 and 100 μM (final concentration of ethanol <1%). The ratio of cell number to the amount of FAs added was constant in all the experiments. FAs uptake was determined using optical tomography (Nanolive 3D Cell Explorer). Viability of cells after 24 h incubation with FAs was evaluated using resazurin reduction assay (AlamarBlue, Sigma-Aldrich) and live/dead fluorescent staining. Cell proliferation was assessed using IncuCyte® ZOOM System (Essen BioScience) that records phase contrast images of cells every 2 h. Cell membrane stiffness was determined by atomic force microscopy in contact mode with 10 nN indentation force (MFP-3D-Bio, Assylum Research).

Results and Discussion

FAs were easily uptaken by both malignant and non-malignant cells, however the amount of fatty acids stored in lipid droplets within the cells was higher in malignant cells (FIG. 1). Metabolic activity assays showed that several saturated FAs (i.e. myristic and palmitic acids) at the lowest concentration of 25 μM decrease viability of malignant epithelial cells (<60% compared to control), but they are non-toxic for non-malignant epithelial cells. FAs such as capric and lauric acids did not affect both malignant and non-malignant cells growth even at the highest concentrations (up to 100 μM). These findings were confirmed by live/dead staining and determination of cell proliferation over 4 days of incubation with FAs.

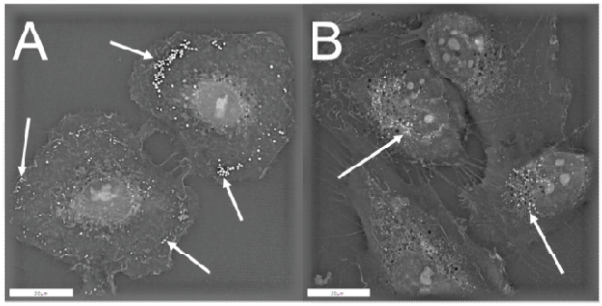

FIG. 1. Optical tomography images of malignant (A) and non-malignant (B) cells incubated with myristic acid for 24 h. Arrows indicate lipid droplets inside cells. Scale bar: 20 μm.

Cell membrane stiffness (Young's modulus) of cells incubated with FAs at 25 μM were determined based on the analyses of force-distance curves recorded using AFM. In the case of non-malignant cells, mechanical properties of cells incubated with various FAs were not significantly different from cells cultured in control conditions. However, when malignant cells were incubated with lauric, myristic and palmitic acids, the median Young's modulus of their cell membrane was almost twice lower than in control samples (FIG. 2). It will be further evaluated if changes in cell membrane properties result in increased membrane permeability.

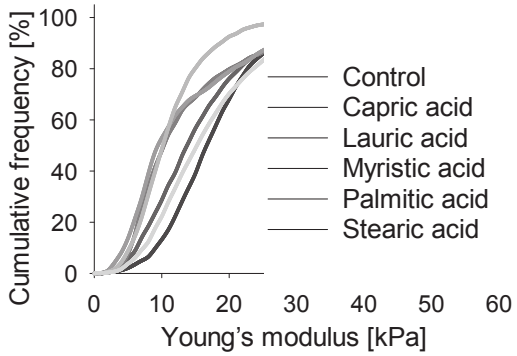

FIG. 2. Young's modulus of malignant cells incubated with saturated FAs.

Conclusions

The influence of saturated FAs on human epithelial cells was evaluated in this study. As natural substances, FAs are easily incorporated inside cells and stored in lipid droplets. The FAs uptake is more efficient in malignant cells than in non-malignant cells. Myristic and palmitic acids are toxic for malignant cells, even at low concentrations (25 μM), while being well tolerated by non-malignant cells. What is more, such FAs significantly decreased mechanical stiffness of cell membranes in malignant cells. This phenomenon may be beneficial in terms of novel lung cancer treatment, as the use of selected FAs for manufacturing of inhalable drug delivery systems can increase permeability of malignant cells, enhance drug uptake and result in more efficient treatment.

Acknowledgments

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References

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