THE SUSCEPTIBILITY OF STRUCTURAL ALVEOLAR CELLS TO CERAMIDES-INDUCED AUTOPHAGY

Dorothy K. Ndishabandi¹³, Daniela Petrusca², Kenneth Ndebele¹ and Irina Petrache²

¹Department of Biology, Jackson State University, Jackson, MS 39217, USA
²Indiana University School of Medicine, Indianapolis, IN 46206, USA
³Summer Research Fellowship Program

Abstract: Pulmonary emphysema is a chronic obstructive pulmonary disease (COPD), a highly prevalent disease. Emphysema, which is typically caused by cigarette smoking in susceptible individuals, has no effective treatments. Utilizing murine models of emphysema, we identified ceramide, a bioactive sphingolipid which causes cell apoptosis as a potential target for therapy in emphysema. Ceramides are upregulated in the lungs of COPD patients. In the research experiment, Cells such as the Human Microvascular Endothelial Cell (HMVEC-L) and Small Airways Epithelial Cells (SAEC) comprising the alveolar membrane are used in the ceramide treatment as well as the dose response as administered. The purpose of the experiment is to find whether alveolar compartment cells die in response to ceramide via autophagy. Autophagy is a process where a cell breaks down its own components. In order to evaluate autophagy, HMVEC-L and SAEC from smokers and non-smokers donor were grown on gelatin-coated cover slips and treated with ceramide (Cer 6:0, Cer 16:0) for 6 hours. As a control, cells growing in complete media (BSA EBM 2) or treated with dihydroceramide C8:0,a ceramide precursor which does not cause cell death. Cells are then stained with acridine orange (AO) whereby cells containing autophagic punctae are observed by fluorescent microscopy. A dose response of chloroquine, a specific inhibitor of lysosomal enzymes, is administered to the cells and western blotting performed to optimize the visualization of certain proteins using specific autophagosome markers (LC3I/LC3II). From the results of the data conducted, cells treated with ceramide show autophagy by the presence of punctae. In the two different cell lines of this study, ceramide stimulate autophagy causing cells to die. From the results, Ceramide induced acridine orange punctae which were inhibited by 3MA in both Human Microvascular Endothelial cell and Small Airways Epithelial Cells. The Cells from nonsmokers exhibited more punctae in response to Ceramide. As far as western Blotting is concerned, Chloroquine was not particularly helpful in enhancing the presence of LC3II on the blot. In conclusion, the changes in shape exhibited by cells with autophagic activity suggest the onset of autophagic cell death in response to ceramide. From the data, cigarette smoking did not appear to increase the susceptibility of alveolar epithelial cells to ceramide-induced autophagy although both alveolar cell types studied are susceptible to ceramide induced autophagy.

Keywords: Alveolar cells, ceramides, autophagy

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