DEVELOPMENT OF AN IN VITRO MODEL OF CHRONIC HYPEROSMOTIC STRESS REVEALS THAT GLUCOSE AND MANNITOL HAVE DIFFERENTIAL EFFECTS ON MESOTHELIAL CELL ADHESION, MIGRATION AND PROLIFERATION

Vassilios Liakopoulos[1], Vasiliki Peppa[2], Konstantinos Leivaditis[1], Chrissi Hatzoglou[2], Michail Daniilidis[1], Sotirios Zarogiannis[2]

[1]Peritoneal Dialysis Unit, 1st Department of Internal Medicine, School of Medicine, Aristotle University of Thessaloniki, Greece
and
[2]Department of Physiology, School of Medicine, University of Thessaly, Larissa, Greece

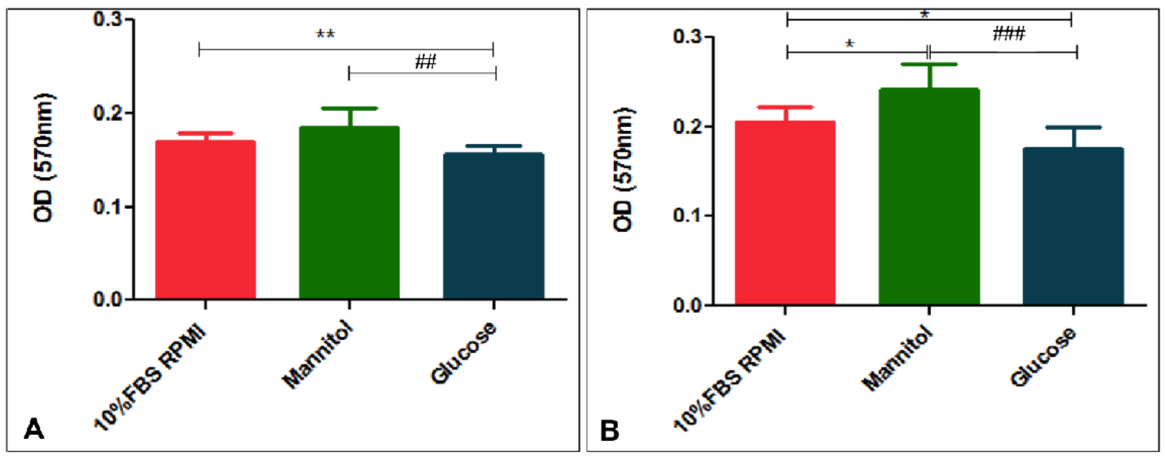
Introduction

Exposure of peritoneal mesothelial cells to dialysis solutions exerts chronic inflammation that leads to peritoneal fibrosis and modality dropout. Hyperosmotic shock has been previously shown in *in vitro* studies to promote fibrotic responses. However, the models used were studying acute effects of hyperosmotic shock to mesothelial cells. The aim of our study was to investigate using a new in vitro model, the effect of chronic osmotic stress, on cell adhesion, migration and proliferation of mesothelial cells.

Materials and Methods

Mesothelial cells (MeT-5A) were constantly exposed to glucose (100mM) and mannitol (100mM) supplemented 10% FBS RPMI 1640 media and cell adhesion, migration and proliferation assays were performed on the first and second passage after the first application of osmotic stress. In control experiments cells were cultured in 10% FBS RPMI 1640 media. In cell adhesion experiments $2.5x10^4$ synchronized cells/well were left to adhere for 90 minutes in fibronectin coated 48 well plates. After washing, adhering cells were fixed with PFA and followed by staining with 0.5% crystal violet solution in PBS for 10 minutes. Subsequently 10% acetic acid was used for destaining and the extracted dye underwent Optical Density (O.D.) measurement at 590 nm. Cell migration experiments were performed with the wound scratch assay. Cells ($1.5x10^5$) were grown to confluency at 48 well fibronectin coated plates and a scratch was done in the middle of the well with a 20μLt sterile pipette tip. An image was taken at t=0 and cells were then incubated for 8 hours and another image was taken and the assay was stopped. The areas of the images were calculated using Image J software and the migration index (M.I.) was calculated using the formula: $MI = A_0 - A_8/A0$. For the cell proliferation experiments $2x10^4$ cells were placed on 4 different 96 well plates and O.D. measurements were taken at different time points (3, 24, 48, and 72 hours) after plating. Cell proliferation data on 3 hours served as the control and was placed as 100%. Statistical analysis was done by GraphPad Prism 6.0 software.

Results



1st Passage Adhesion Experiment 2nd Passage Adhesion Experiment

Figure 1. Mannitol increases cell adhesion of MeT-5A cells, while glucose stress leads to decreased ability of MeT-5A cells to adhere and this is seen in both generations of cells (*p<0.05 vs. Control group; **p<0.01 vs. Control group, ##p<0.01 vs. Glucose group, ###p<0.001 vs. Glucose group).

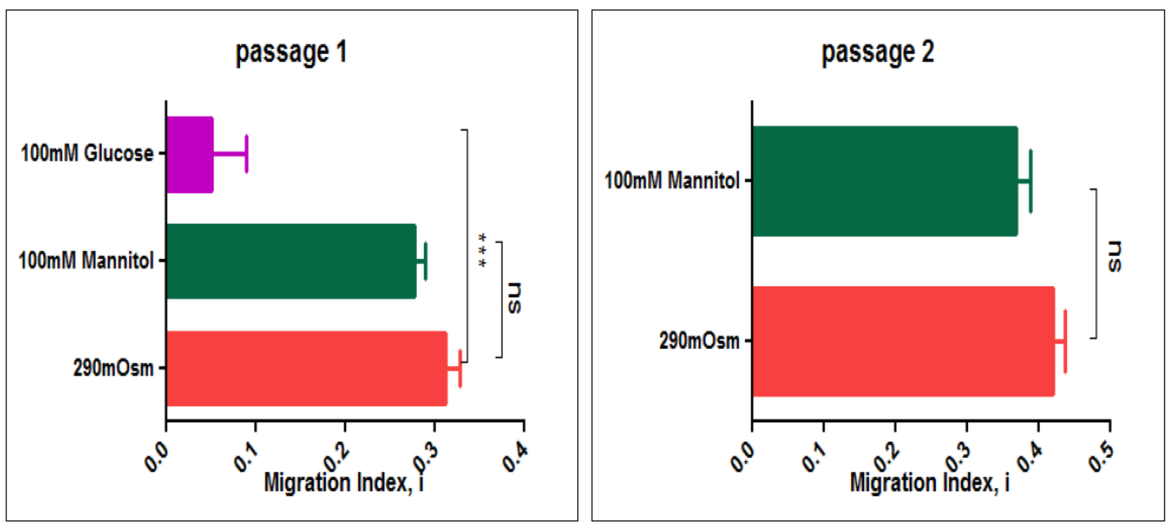
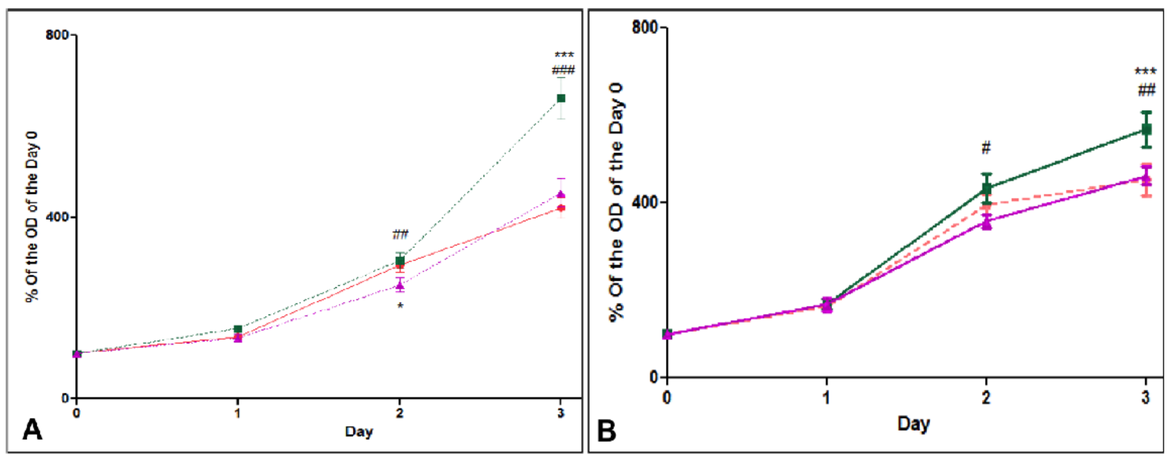


Figure 2. Mannitol does not influence MeT-5A cell migration in neither generation of cells. Glucose significantly decreases cell migration on passage 1 while on passage 2 the cells were lifting from the plate and the experiment could not be performed (***p<0.001 vs. Control group).



1st Passage Proliferation Experiment 2nd Passage Proliferation Experiment

Figure 3. Mannitol (green plot) increases the rate of cell proliferation in both generations of cells under chronic stress. Glucose (purple plot) does not show significant differences than Controls (orange plot) in both passages (*p<0.05 vs. Control group; #p<0.05 vs. Glucose group).

Conclusions

Our results indicate that chronic exposure to osmotic stress affects mesothelial cell functions but high concentration of mannitol caused a significantly milder effect than high concentration of glucose. This new in vitro model is mimicking the exposure of cells to chronic hyperosmotic shock to a better extent with possible clinical implications for peritoneal dialysis.

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Vassilios Liakopoulos
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