EXPOSURE OF HUMAN MESOTHELIAL CELLS TO MILD HYPEROSMOTIC STRESS INDUCED BY GLUCOSE, MANNITOL OR NACL INFLUENCES CELL ADHESION AND MIGRATION BUT NOT PROLIFERATION

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Introduction

Exposure of mesothelial cells of the peritoneal membrane 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. In contrast with severe hyperosmotic stress (> 400 mOsm) the effects of mild hyperosmotic stress (350 mOsm) on physiological cellular functions has not been extensively studied. The aim of our study was to assess the effect of acute exposure to mild hyperosmotic stress on cell adhesion, migration and proliferation of the human mesothelial cell line MeT-5A.

Materials and Methods

Mesothelial cells (MeT-5A) were grown on supplemented 10% FBS RPMI 1640 cell culture medium and exposed to hyperosmotic stress (350mOsm) by addition of glucose (50 mM), mannitol (50mM) or NaCl (25 mM) to the media. 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 considered as 100%. Statistical analysis was done by GraphPad Prism 6.0 software.

Results

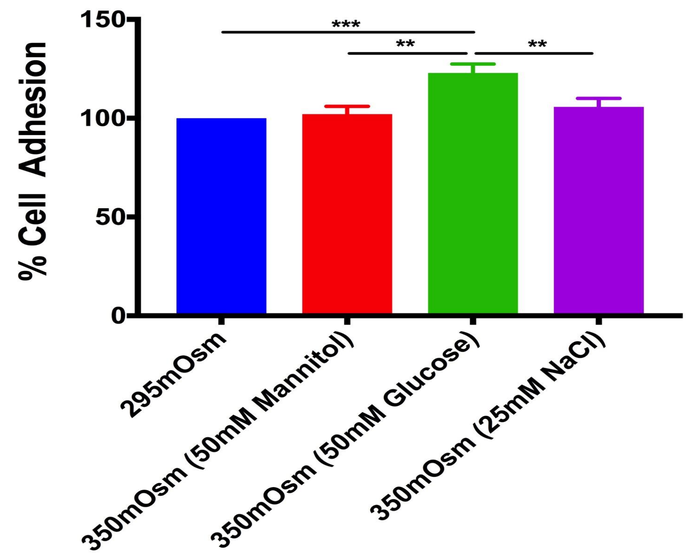

Figure 1. Glucose increases cell adhesion of MeT-5A cells, while mannitol and NaCl do not alter the ability of MeT-5A cells to adhere (***p<0.001 vs. Control group; **p<0.01 vs. Glucose group.

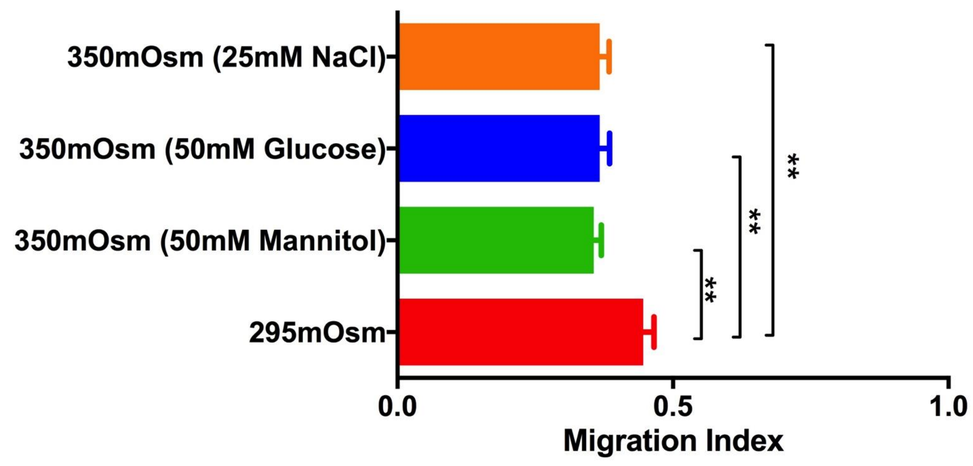

Figure 2. Glucose, mannitol and NaCl significantly reduce the migration of MeT-5A cells (**p<0.01 vs. Control group).

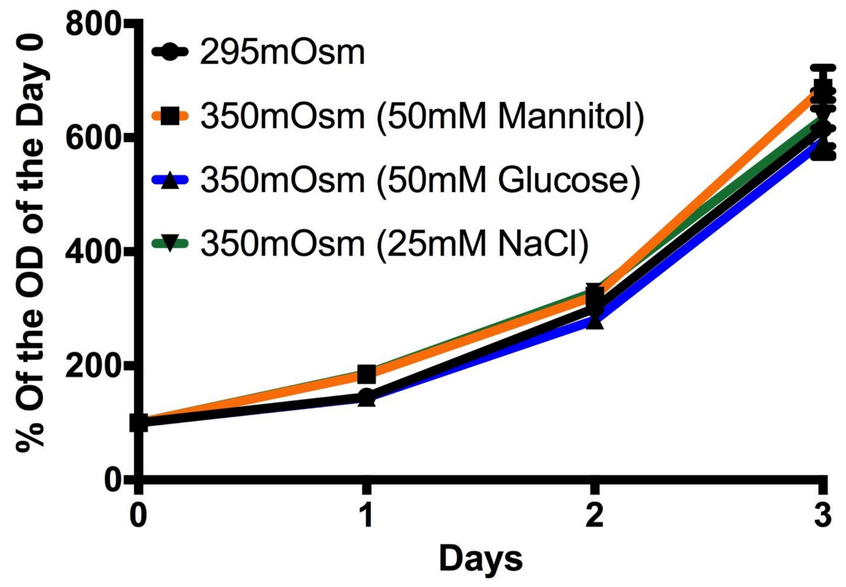

Figure 3. Glucose, mannitol and NaCl do not have differences in the cell proliferation rate as compared to control group.

Conclusions

In conclusion, our results indicate that exposure of human mesothelial cells to glucose induced mild hyperosmotic stress significantly increased cell adhesion, while mannitol or NaCl had no effect. Cell migration was reduced irrespective of the source of hyperosmolarity. Cell proliferation was unaffected. The underlying mechanisms of our results require further investigation for unraveling the reasons why mesothelial cells exhibit a less migratory phenotype when under mild hyperosmotic stress.

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