DIBUTYL PHTHALATE INDUCES MIGRATION AND ANGIOGENESIS OF EA.HY926 HUMAN ENDOTHELIAL CELLS THROUGH GPER/ERK1/2 SIGNALING

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INTRODUCTION

Cell adhesion and **migration** represent two balanced functions of **endothelial cells** (ECs) involved in maintaining **stability and angiogenesis**, which has key role in the **pathophysiology of various human diseases**.

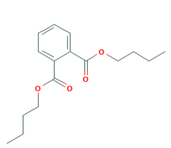
Evidence of **possible association between exposure to dibutyl phthalate** (DBP; a man-made chemical widely used in many industrial and consumer products) **and cardiovascular diseases** (CVDs) noted in studies.

The impact that DBP exerts on EC adhesion, migration and angiogenesis remains unclear.

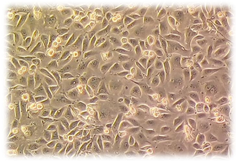
OBJECTIVES

•Examinating of the **impact that acute exposure to DBP exerts on EC** adhesion to extracellular matrix (ECM) migration and angiogenesis

•Investigating the **signaling pathways involved** in these processes.



DBP



ENDOTHELIAL CELLS

METHOD / DESIGN

EA.hy926 cells were exposed to either vehicle (0.05% DMSO – control) **or three concentrations of DBP** (10^{-6}, 10^{-5}, and 10^{-4} M DBP in 0.05% DMSO) for up to 48 h.

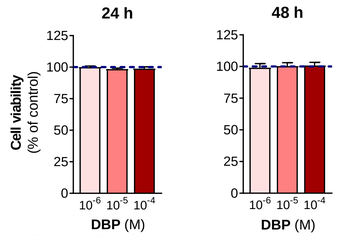
Inhibitors:

ICI 182 780 - estrogen receptor (ER) down-regulator
G15 - G-protein-coupled ER (GPER) antagonist
Wortmannin - inhibitor of the phosphoinositide-3-kinase – protein kinase B/Akt pathway
U0126 - an inhibitor of the extracellular signal-regulated kinase 1/2 (ERK1/2) pathway

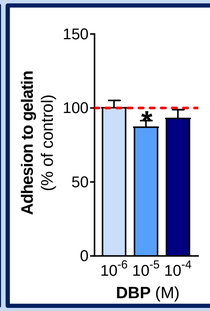
Assays:

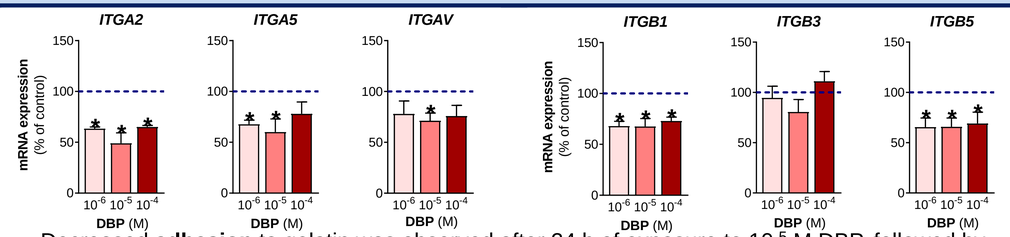
Cell viability - **alamarBlue™ assay**
Cell adhesion - **adhesion assay on gelatin-coated plates**
Cell migration - **wound-healing (“scratch”) assay**
Angiogenesis - **endothelial tube formation assay**
MMP activity - **gelatin zymography**
mRNA relative expression levels - **qRT-PCR**
Protein expression - **Western blotting (WB)**

RESULTS

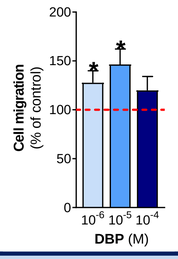


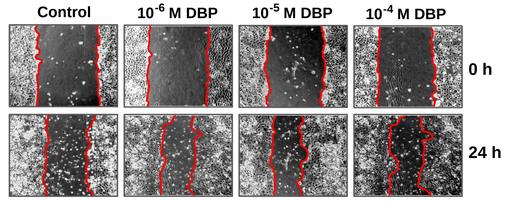
Viability of EA.hy926 cells was not affected after 24 h and 48 h of exposure to DBP.

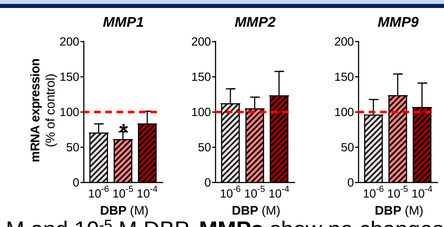


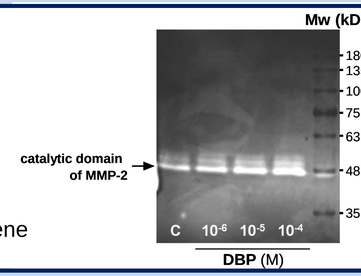


Decreased **adhesion** to gelatin was observed after 24 h of exposure to 10^{-5} M DBP, followed by diminished expression of five genes encoding the **integrin** family of proteins**: *ITGA2***, ***ITGA5***, ***ITGAV***, ***ITGB1***, and ***ITGB5***.

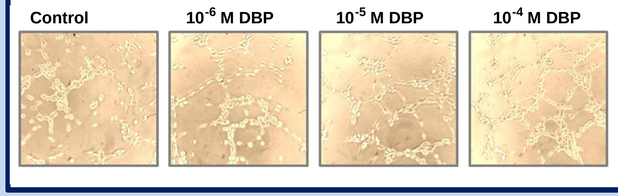




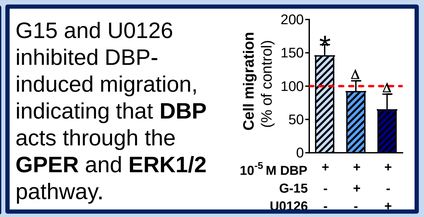




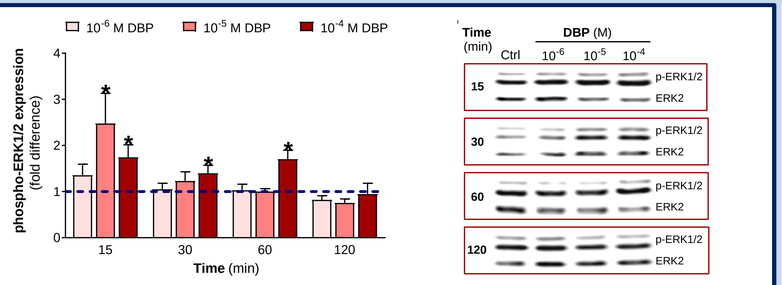
Cell migration was increased after 24 h of exposure to 10^{-6} M and 10^{-5} M DBP. **MMPs** show no changes in gene expression, but rather in their activity.



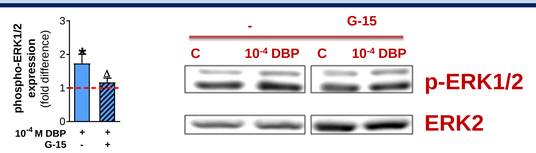
Angiogenesis was increased after 24 h of DBP pre-treatment and an additional 20 h-long exposure to 10^{-4} M DBP.



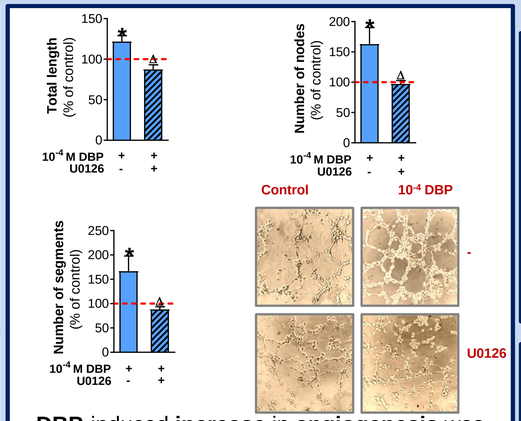
G15 and U0126 inhibited DBP-induced migration, indicating that **DBP** acts through the **GPER** and **ERK1/2** pathway.



WB analysis confirmed **activation of ERK1/2** in **DBP**-exposed EA.hy926 cells.



ERK1/2 activation **was GPER dependent**, as confirmed on WB by using G15.



DBP-induced **increase** in **angiogenesis** was **ERK1/2 dependent**, as shown by U0126-inhibited tube formation.

CONCLUSIONS

Obtained results suggest that **DBP acts through GPER** in EA.hy926 cells to **increase ERK1/2** activity, cell **migration**, and **angiogenesis**, thereby providing novel information regarding the molecular mechanism of DBP’s action in human vascular ECs. Understanding the molecular mechanisms behind different diseases with vascular etiology is critical for formulating effective prevention strategies and identifying novel therapeutic interventions. Additional studies are needed in order to provide a more detailed insight into the exact mechanism of DBP’s action in the vasculature.

ACKNOWLEDGEMENTS

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