TRAIL downregulates Endothelin 1 and upregulates Superoxide Dismutase 1 in human aortic endothelial cells under proatherogenic conditions

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OBJECTIVES

- Tumour necrosis factor related apoptosis inducing ligand (TRAIL) may be involved in the pathogenesis of cardiovascular disease
- *In vivo* findings suggest that TRAIL may exhibit vasoprotective effects on the endothelium, although the mechanism of TRAIL-mediated vasoprotection remains poorly understood[1,2]
- Atherosclerosis is a chronic inflammatory condition and *in vitro* studies have demonstrated that endothelial cells exposed to disturbed/oscillatory flow adopt a proinflammatory phenotype[3]

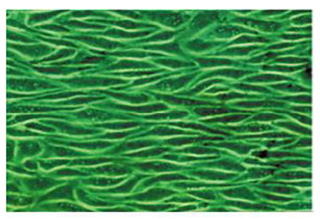


Figure 1. ECs exposed to laminar flow

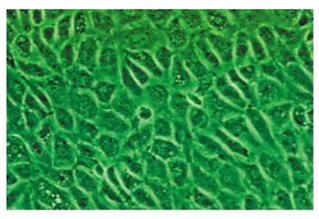


Figure 2. ECs exposed to oscillatory flow

- The aim of this study was to characterise the protective effects of TRAIL on inflammatory gene expression from vascular endothelial cells under proatherogenic conditions

METHODS

- Human aortic endothelial cells (HAECs) were grown to confluency in standardised Promocell media
- HAECs were seeded onto 0.6mm µ-slide plate at a concentration of 200,000 cells per slide
- The slide was attached to the IBIDI® µ-slide pump system (Image 1) which was connected to an air pressure control pump
- Cells were exposed to the conditions outlined below

Table 1. Experimental conditions

Control	HAECs Untreated media +/-10 dynes/cm^2 24 hours
TRAIL	HAECS Media + TRAIL 100ng/ml +/-10 dynes/cm^2 24 hours

- RNA was harvested with mirVana miRNA Isolation kit™ after 24 hours
- cDNA was synthesised using RT^2 PreAMP cDNA synthesis kit (Qiagen)
- cDNA was added to a RT^2 Profiler™ PCR array of genes involved in endothelial cell biology and analysed on ABI 7900HT Real Time PCR system

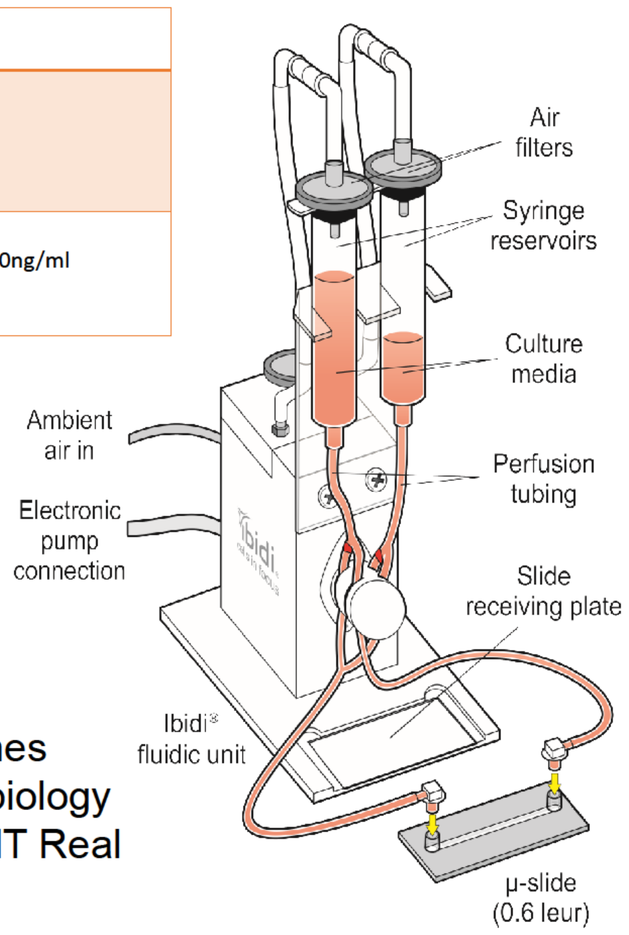


Image 1. Fluidic unit from the IBIDI µ-slide pump system. A proatherogenic environment is simulated by applying oscillatory flow to the µ-slide by separating the switching times of the valves on the fluidic unit

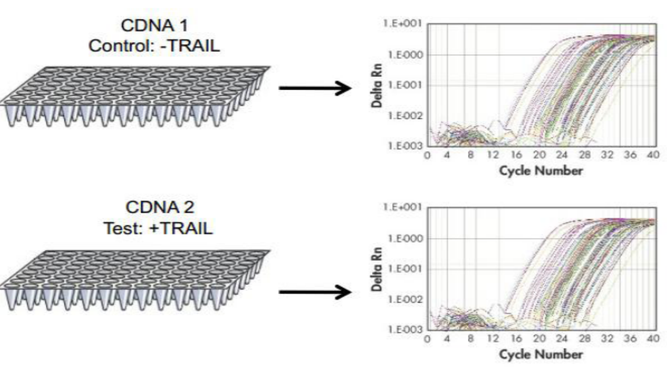
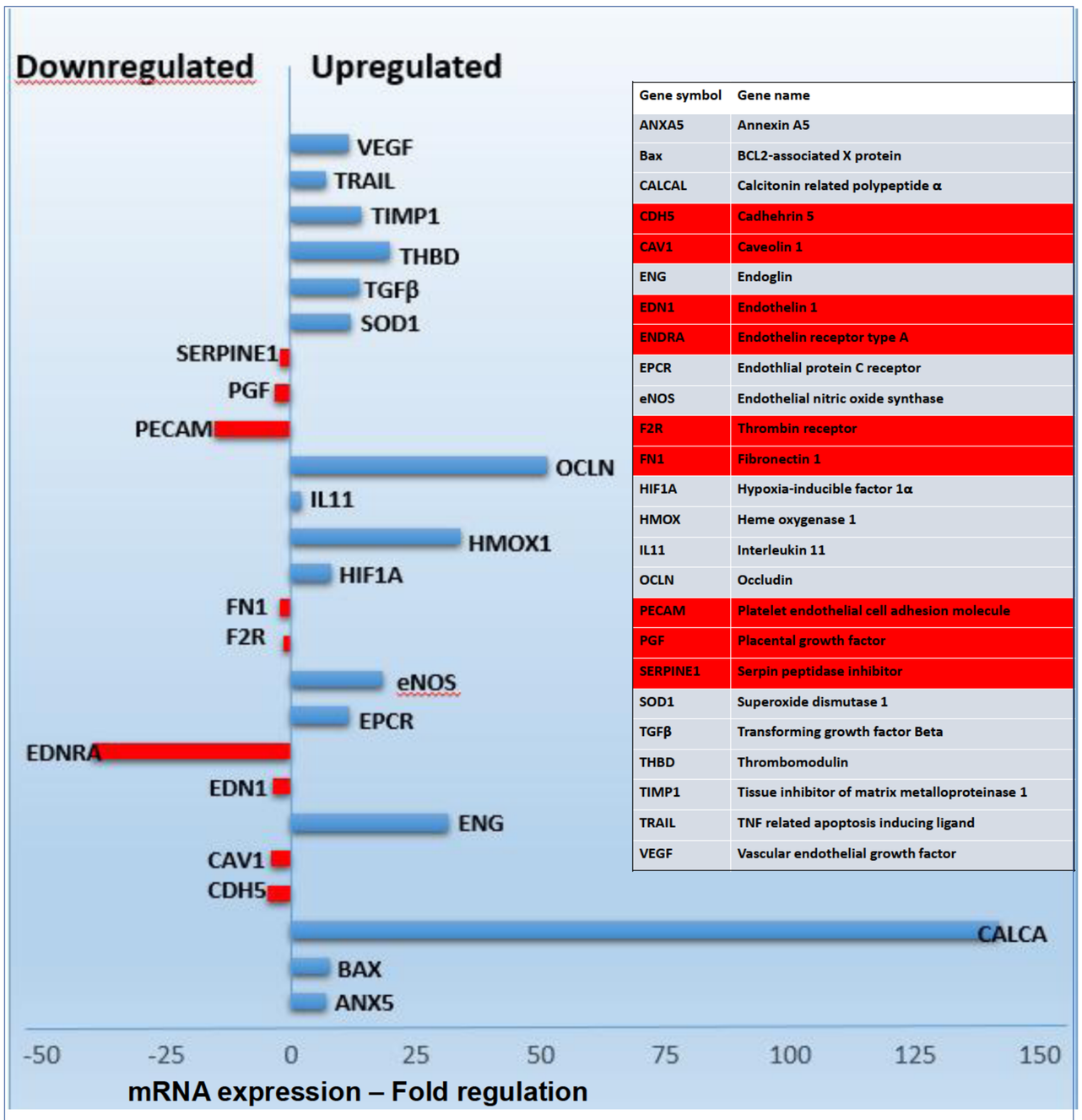


Image 2. PCR array analysis on Real Time PCR system

RESULTS



Gene symbol	Gene name
ANXA5	Annexin A5
Bax	BCL2-associated X protein
CALCAL	Calcitonin related polypeptide α
CDH5	Cadhehrin 5
CAV1	Caveolin 1
ENG	Endoglin
EDN1	Endothelin 1
ENDRA	Endothelin receptor type A
EPCR	Endothlial protein C receptor
eNOS	Endothelial nitric oxide synthase
F2R	Thrombin receptor
FN1	Fibronectin 1
HIF1A	Hypoxia-inducible factor 1α
HMOX	Heme oxygenase 1
IL11	Interleukin 11
OCLN	Occludin
PECAM	Platelet endothelial cell adhesion molecule
PGF	Placental growth factor
SERPINE1	Serpin peptidase inhibitor
SOD1	Superoxide dismutase 1
TGFβ	Transforming growth factor Beta
THBD	Thrombomodulin
TIMP1	Tissue inhibitor of matrix metalloproteinase 1
TRAIL	TNF related apoptosis inducing ligand
VEGF	Vascular endothelial growth factor

Figure 3. Effect of TRAIL on HAEC gene expression under oscillatory flow. Histogram/table shows upregulatory (blue) and downregulatory (red) effects of TRAIL on HAEC genes under oscillatory flow. Housekeeping genes for normalization include B2 macroglobulin and arachidonate 5-lipoxygenase

DISCUSSION

- TRAIL upregulated a number of genes including SOD1, which encodes the enzyme Superoxide dismutase, involved in the breakdown of reactive oxygen species (ROS)
- TRAIL downregulated EDN1 and EDNRA, which encodes Endothelin 1 and the Endothelin receptor. These potent proinflammatory proteins can mediate ROS generation through increased NADPH oxidase activity[4]
- TRAIL also downregulated the adhesion molecule genes CDH5 and PECAM, which are involved in the promotion of atherogenesis
- In conclusion, TRAIL appears to exert protective anti-inflammatory effects, in a proatherogenic environment, partly through reduction of oxidative stress

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Diabetes (to include obesity, pathophysiology & epidemiology)

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