Tumour necrosis factor related apoptosis inducing ligand (TRAIL) reduces reactive oxygen species production by human aortic endothelial cells exposed to inflammatory stimuli

Forde H[1,2], Davenport C[1,2], Rochfort KD[2], Cummins PM[2], Smith D[1]
1. Beaumont & RCSI Medical school, Beaumont, Dublin 9
2. School of Endothelial biology, DCU, Glasnevin, Dublin 9

Introduction

- Increased oxidative stress is a significant contributor to endothelial dysfunction in vascular disease
- During atherosclerotic plaque formation, reactive oxygen species (ROS) production is increased within endothelial cells exposed to pathological disturbances in laminar blood flow such as that which occurs at arterial bifurcations and curvatures[1]
- Tumour necrosis factor related apoptosis inducing ligand (TRAIL), is a member of the TNF alpha superfamily which may be involved in the pathogenesis of CVD
- *In vivo* studies suggest TRAIL exhibits protective effects on the endothelium, although the mechanism of TRAIL-mediated vasoprotection remains poorly understood[2,3]
- Preliminary studies from this group have demonstrated that TRAIL treatment of aortic endothelial cells under oscillatory shear stress, shifts the net gene expression towards an **antioxidant** phenotype, allowing us to hypothesize that TRAIL may be atheroprotective through reducing oxidative stress (Figure 1)
- The aim of this study therefore, was to characterise the effects of TRAIL on vascular endothelial cells *in vitro* under conditions of increased oxidative stress

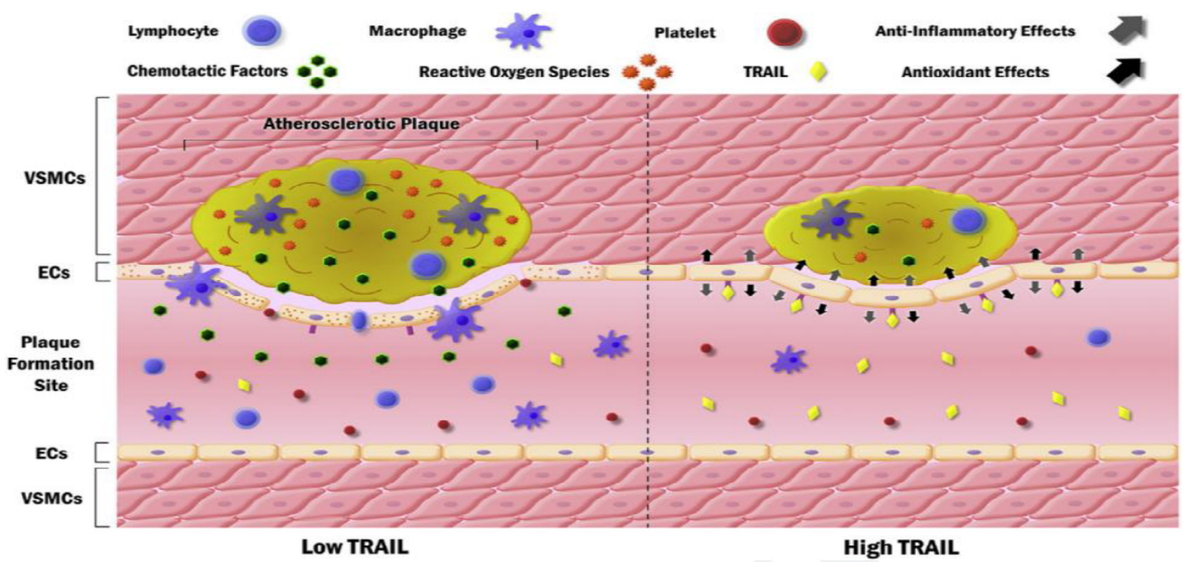

Figure 1. Hypothetical mechanism of TRAIL mediated vasoprotection. TRAIL may enhance the antioxidant properties of endothelial cells which may impact on smooth muscle cells and leukocytes in the vicinity, to ultimately reduce plaque formation

Methods

- Human aortic endothelial cells (HAECs) were grown to confluency in standardised Promocell media
- HAECs were seeded onto 6 well plates and exposed to the experimental conditions listed below to induce oxidative stress

Experimental Conditions 1	
Control	Untreated media x24 hrs
TRAIL	Media + TRAIL 100ng/ml x24 hrs
TNF alpha	Media + TNF alpha 100ng/ml x24hrs
TNF + TRAIL	Media + TNF alpha 100ng/ml + TRAIL 100ng/ml x 24hrs

Experimental Conditions 2	
Control	Untreated media x24 hrs
TRAIL	Media + TRAIL 100ng/ml x24 hrs
HG	Media + Dextrose 30mmol x24hrs
HG + TRAIL	Media + Dextrose 30mmol + TRAIL 100ng/ml x 24hrs

- Cells were labelled with dihydroethidium (DHE) for 30 mins prior to completion of treatments
- DHE permeates the cell membrane and upon reaction with superoxide anions, forms a red fluorescent product called 2-hydroxyethidium, which intercalates with DNA[4]
- Cells were trypsinised, pelleted and washed with FACS buffer
- Cells were then suspended in 500µL FACS buffer and read for 10,000 events using the BD FACS Aria
- Results were analysed using FlowJo software

Results

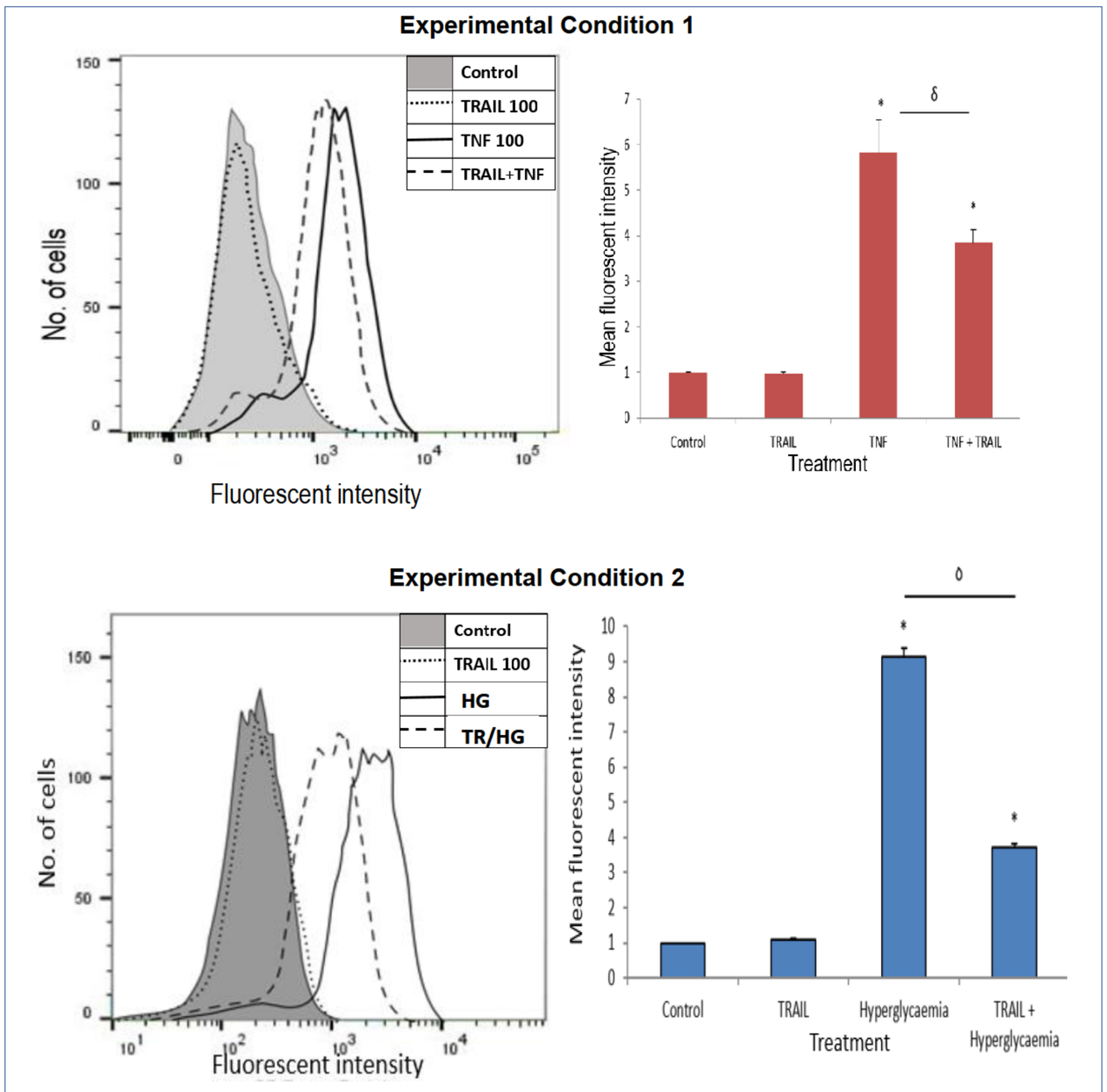

Figure 2. Effect of TRAIL on ROS generation by HAECs. Histogram/table shows effect of TRAIL on ROS generation by HAECs under basal and stimulated (TNF alpha and Hyperglycaemia) conditions. * = $p<0.05$, compared to control condition; δ = $p<0.05$ compared to TNF/HG condition; TNF – Tumour necrosis factor; HG – Hyperglycaemia (30mmol); TR – TRAIL.

Discussion

- TRAIL has no effect on ROS generation by HAECs under basal conditions
- TNF alpha and hyperglycaemia are both potent inducers of ROS
- TRAIL significantly reduces TNF alpha and hyperglycaemia induced ROS formation
- This suggests that TRAIL has an antioxidant effect in conditions of increased oxidative stress, and this effect is independent of TNF alpha blockade
- This study also provides evidence that TRAILs ability to upregulate antioxidant genes in stressed endothelial cells, translates into functional changes within the cell
- Further studies are required to determine the specific pathway through which TRAIL mediates oxidative stress

References

1. Chen X, Andresen BT, Hill M, Zhang J, Booth F, Zhang C. Role of reactive oxygen species in tumor necrosis factor-alpha induced endothelial dysfunction. *Current hypertension reviews*. 2008 Nov;4(4):245.
2. Di Bartolo BA., Chan J., Bennett MR., Cartland S., Bao S., Tuch BE., Kavurma MM. (2011). TNF-related apoptosis-inducing ligand (TRAIL) protects against diabetes and atherosclerosis in Apoe−/− mice. *Diabetologia*, *54*(12), 3157-3167
3. Watt V., Chamberlain J., Steiner T., Francis S., Crossman D. (2011). TRAIL attenuates the development of atherosclerosis in apolipoprotein E deficient mice. *Atherosclerosis*, *215*(2), 348-354.
4. Zhao, H. et al. Detection and characterization of the product of hydroethidine and intracellular superoxide by HPLCand limitations of fluorescence. *Proc Natl Acad Sci U S A* 102, 5727-32 (2005).