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

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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.\(^2\)
- In vivo studies suggest TRAIL exhibits protective effects on the endothelium, although the mechanism of TRAIL-mediated vasoprotection remains poorly understood.\(^3\)
- 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.

Methods

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

<table>
<thead>
<tr>
<th>Experimental Conditions 1</th>
<th>Experimental Conditions 2</th>
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<tbody>
<tr>
<td>Control Untreated media x24 hrs</td>
<td>Control Untreated media x24 hrs</td>
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<tr>
<td>TRAIL Media + TRAIL 100mg/ml x24 hrs</td>
<td>TRAIL Media + TRAIL 100mg/ml x24 hrs</td>
</tr>
<tr>
<td>TNF alpha Media + TNF alpha 100mg/ml x24hrs</td>
<td>H(\text{G})) Media + Dextrose 30mmol x24hrs</td>
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<tr>
<td>TNF + TRAIL Media + TNF alpha 100mg/ml + TRAIL 100mg/ml x 24hrs</td>
<td>H(\text{G}) + TRAIL Media + Dextrose 30mmol + TRAIL 100mg/ml x 24hrs</td>
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- 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 500\(\mu\)L FACS buffer and read for 10,000 events using the BD FACS Aria.
- Results were analysed using FlowJo software.

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