Human smokers’ serum inhibits migration and elevates levels of cardiovascular disease associated proteins in an endothelial cell in vitro model

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INTRODUCTION

Cigarette smoking is strongly associated with atherosclerosis, ischaemic heart disease and acute coronary thrombosis (1). A number of in vitro models are used to study cardiovascular disease mechanisms and to examine the role of smoking in disease initiation and progression. Particulate or aqueous cigarette smoke extracts are commonly used exposure agents for these in vitro studies. Here we explore the use of smokers’ sera as a more biologically-relevant exposure agent in studies monitoring a number of cardiovascular disease endpoints following exposure of cultured endothelial cells to sera.

METHODS

Human umbilical vein endothelial cells were grown to confluency and a scratch wound created with a pipette tip. Cells were exposed in quadruplicate for 24 hours to 50% serum (in cell culture media) obtained from 10 healthy smokers or non-smokers. Migration was monitored hourly using the IncuCyte® imaging apparatus. After 20 hours, media and cells were harvested for gene and protein expression analyses using the TaqMan® and MesoScale Discovery (MSD) platforms, respectively.

Serum Samples Sera were obtained from 10 healthy smokers and 10 healthy non-smokers as detailed below:

<table>
<thead>
<tr>
<th>Protein secretion</th>
<th>Gene expression</th>
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<tr>
<td>MCP-1</td>
<td>IL-6</td>
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Taqman® analysis RNA extracted from endothelial cell cultures were analysed for gene expression levels using the following custom cardiovascular disease-specific Taqman® array:

RESULTS

CONCLUSIONS

Scratch wound assay

Confluent endothelial cells

WoundMaker apparatus

Smoker

Non-Smoker

Figure 1. Smokers’ serum inhibited endothelial wound repair. Images were taken 4 and 20 hours after wounding, in the presence of 50% smokers’ or 50% non-smokers’ serum, as indicated.

EXPOSURE TO SMOKERS’ SERA IMPAIRED ENDOTHELIAL MIGRATION WHEN COMPARED TO NON-SMOKERS’ SERA

Exposure to smokers’ sera, when compared with non-smokers’ sera, increased the expression of a number of genes and proteins associated with cardiovascular disease including:

- secretion of both MCP-1 and IL-6 proteins
- MCP-1 and IL-6 gene expression
- expression of genes for IL1-R1, TGFβ1 and VEGFA

Smear is potentially a relevant in vitro exposure agent to examine the effects of cigarette smoke on cardiovascular disease processes

REFERENCE