The assessment of atherosclerotic potential of tobacco and nicotine products by foam cell formation assay

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Introduction

Atherosclerosis is a progressive arterial disease in which lipid-rich plaques form and may eventually contribute to thrombosis, heart attack, or stroke. Major risk factors include hyperlipidaemia, hypertension, diabetes, obesity, aging, and smoking, with elevated LDL, reduced HDL, and oxidative stress playing central roles. Elevated LDL and reduced HDL are central to plaque development. Oxidative stress also drives atherosclerosis by promoting endothelial dysfunction, monocyte infiltration, and LDL oxidation (1).

Foam cell formation is a key early step, arising when monocyte-derived macrophages take up oxidized LDL (oxLDL) via scavenger receptors such as CD36 and SR-A. This forms fatty streaks, the earliest visible atherosclerotic lesions (Figure 1).

Limitations in traditional animal models highlight the need for New Approach Methodologies (NAMs). This study aimed to establish an *in vitro* model of foam cell formation, using ImmuCYTE cultured human monocytes, integrating key cellular and molecular events to assess atherogenic potential.

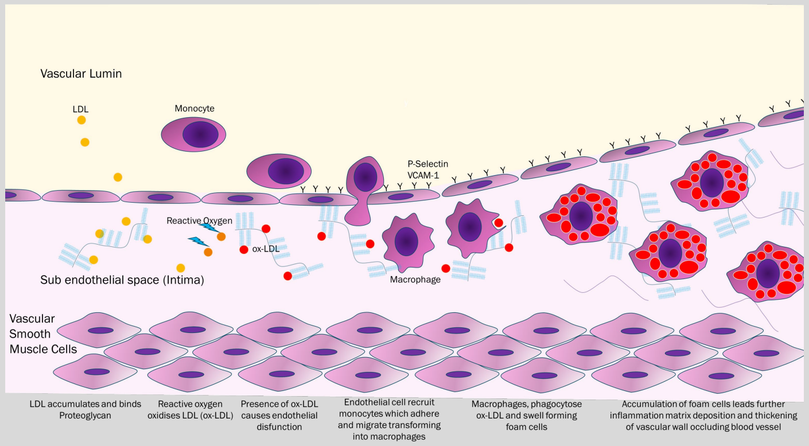

Figure 1. Process of foam cell formation in oxidative stress induced atherosclerosis.

References

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2. Bozhilova S, Baxter A, Bishop E, et al. Optimization of aqueous aerosol extract (AqE) generation from e-cigarettes and tobacco heating products for in vitro cytotoxicity testing. Tox Letters. 2020;355:51-63
3. Jin M, Earla R, Shah A, Earla RL et al. A LC-MS/MS method for concurrent determination of nicotine metabolites and role of CYP2A6 in nicotine metabolism in U937 macrophages: implications in oxidative stress in HIV + smokers. J Neuroimmune Pharmacol. 2012;7(1):289-99

Methodology

Cigarette smoke AqE Production

Cigarette smoke Aqueous Extracts (AqE) were produced by standard method (2). In brief 8 puffs (1 stick) of 1R6F reference cigarette smoke was bubbled through cell culture media via smoking engine according to Heath Canada Intense regimen, producing 100% extract.

Nicotine Quantitation

Nicotine was determined via standard methods using ABS4000 Q-trap LC-MS/MS, using protocol adapted from Onoue et al. and Jin et al. (3).

Cell-Culture

ImmuCYTE human monocytes (ImmuPHAGE™) were exposed to three test items and vehicle controls across concentrations (0.1–10% v/v), plus and minus LDL and oxLDL.

High Content/Flow Cytometry Screening

Cells were imaged using Cytek Image Stream MKII system with analysis performed using IDEAS analysis software. Deep Red LipidTox (Invitrogen) was used to visualise Neutral lipid.

Results (1)

In the absence of AqE no increase in cellular neutral lipid (oxLDL) was detectible by fluorescence. Combining oxLDL with 10% (v/v) AqE however led to a significant increase in cellular neutral lipids content following 48 h and 72 h (Figure 2).

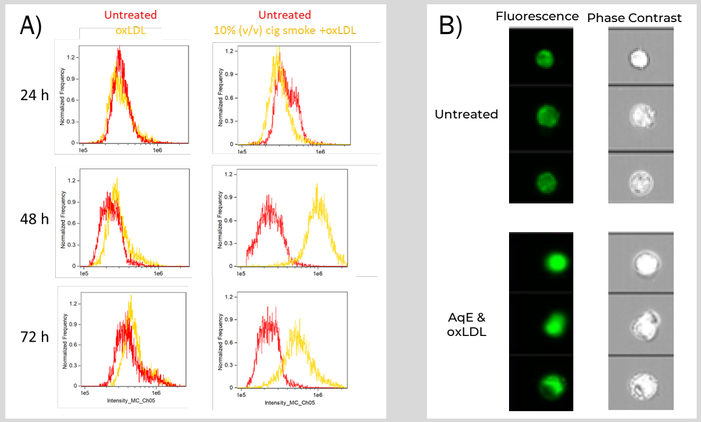

Figure 2. Change in fluorescence intensity of monocytes untreated (vehicle control) or exposed to oxLDL, with or without the addition of cigarette smoke AqE, A) Flow cytometry histogram of florescence intensity (neutral lipid) vs cumulative cell number. B) Fluorescence imaging of neutral lipid for treated and untreated cells.

Results (2)

Thresholding the cellular population by fluorescence intensity as per Figure 2, the impact on percentage of cells showing increase neutral lipid content could be determined across a time course of exposure (Figure 3). Results indicate both that 1R6F AqE induced lipid accumulation, and that 24 hours was the peak timepoint in neutral lipid accumulation.

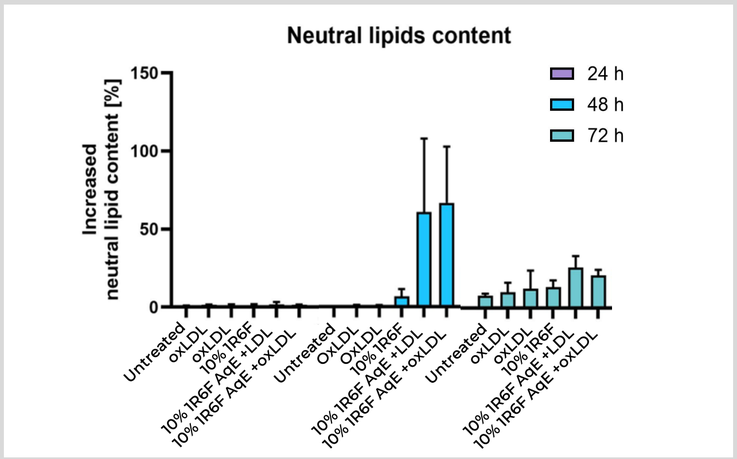

Figure 3. Percentage of cells showing increased neutral lipid content relative to untreated cells, by time and treatment conditions.

Conclusion

High content imaging demonstrates that cigarette smoke AqE acts directly on the study monocytes, promoting a phenotypic change or cellular differentiation and allowing foam cell formation. In the absence of cigarette smoke AqE, the monocyte cells in this study did not interact with either LDL or oxLDL, remaining non-phagocytotic, a characteristic reversed by the inclusion of AqE.
This model provides a sensitive platform for investigation of the atherogenic potential of tobacco and nicotine products and could be included in a panel of *in vitro* tests, along with clinical and other studies, to assess the potential atherogenic cardiovascular disease risks posed by such products.

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