Comparison of Vascular Cell Adhesion Molecule-1 (VCAM-1) expression following stimulation with TNF alpha on human umbilical vein endothelial cells and EA.hy926 cells for atherosclerosis research

Kathleen Thayse[a], Nadège Kindt[a], Sophie Brogniet[a], Alexandra Tassin[b], Stéphane Carlier[a]

[a]Department of Cardiology, UMONS, Mons
[b]Department of Respiratory Physiology, UMONS, Mons

Background

Vascular cell adhesion molecule-1(VCAM-1) is a molecule that plays an important role in the initiation of atherosclerosis. It is transiently induced on activated endothelial cells and is responsible for the migration of leucocytes into the subendothelial space. VCAM-1 is a promising target for the detection of the lesions of atherosclerosis but also for targeting the activated endothelium that may develop atheromatous plaques.

Numerous endothelial cell lines are used for atherosclerosis research. Human umbilical vein endothelial cells (HUVEC) are primary cell lines sharing the most characteristics with in-vivo endothelial cells but are expensive and difficult to isolate. Immortalized cells have been developed to avoid these drawbacks but some characteristics may have changed. EA.hy926 are the most used immortal cell lines, resulting from fusion between HUVEC and human epithelial cell line A549 derived from a lung carcinoma.

The purpose of this study is to determine which endothelial cell line between HUVEC and EA.hy926 express VCAM-1 following in-vitro stimulation with tumor necrosis factor (TNF) alpha.

Methods

We have activated HUVEC and EA.hy926 with TNF alpha (10 ng/ml) during 6 and 24 hours. We have incubated them with an anti-VCAM Rabbit primary antibody overnight at 4°C and with a goat anti-rabbit secondary antibody coupled with Alexa Fluor 594 during 1h at ambient temperature. EA.hy926 have also been incubated with DAPI to see cell nuclei. Cells that haven't been exposed to TNF alpha were used as negative controls. We compare the presence of VCAM-1 in the two activated cell lines by fluorescence microscopy.

Results

There was no difference of expression of VCAM-1 between activated and non-activated EA.hy926 (mean fluorescence intensity respectively 2.7 ± 1.5 and 2.5 ± 2.5, Fig A and B) (p=0,92). On the other hand, only activated HUVEC expressed VCAM-1 (mean fluorescence intensity 6.58 ± 5.1 vs 0 ± 0 for non-activated, Fig C and D).

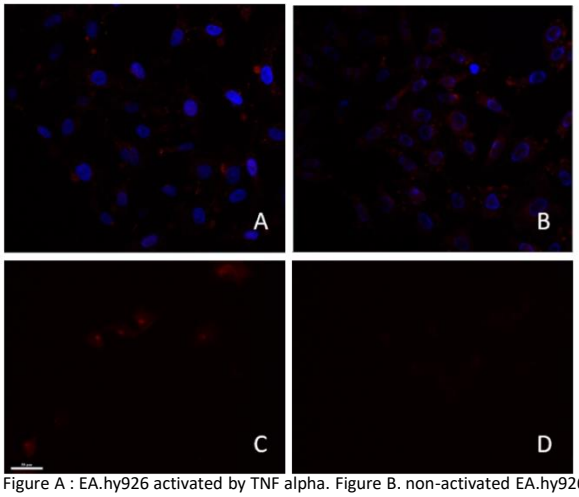

Figure A : EA.hy926 activated by TNF alpha. Figure B. non-activated EA.hy926
Figure C : HUVEC activated by TNF alpha. Figure D : non-activated HUVEC

Conclusions

Our methodology aimed to further validate non-invasively using targeted nanoparticules and MRI VCAM expression confirms that HUVEC, although expensive cell lines difficult to cultivate, express well VCAM-1 after exposition to TNF alpha, but on the contrary to some previous reports (Bouïs D et al., 2001) EA.hy926 cell lines cannot be used for bench research in atherosclerosis.