APJ inhibition reduces flow-induced endothelial cell migration with atorvastatin but not pravastatin treatment

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Introduction:

Statins are used for cardiovascular disease prevention and influence endothelial cell properties. The APJ receptor is believed to mediate some pleiotropic effects of statins. We have previously reported that inhibition of the APJ receptor influenced pravastatin (pra) but not atorvastatin (ator) induced human umbilical vein endothelial cell (HUVEC) proliferation. We hypothesized that the observed heterogeneous effects of APJ inhibition on statin induced proliferation will also be seen for flow-induced migration. Therefore, we investigated the influence of the inhibition of the APJ receptor on the statin mediated flow-induced HUVEC migration.

Methods:

HUVEC migration was assessed by live cell microscopy for a period of 15 h under two different flow conditions (1.5 dyne/cm^2 [low] and 10 dyne/cm^2 [high]). Two concentrations (0.1 μM; 1 μM) of ator or pra were used. APJ was inhibited using the antagonist ML221 (10 μM). Images were taken at a frequency of 1 frame/15 min and analyzed using ImageJ. Per assay 20 cells were tracked and a total sample size of n=5 for each group was assessed. Statistical analysis regarding directionality, velocity, euclidian and accumulated distance was performed with mixed models.

Results:

For velocity, accumulated and euclidian distance significant main effects for flow, concentration and statin were observed (all $p < .01$). Further, for these three parameters significant statin and ML221 ($p = .01$) interaction as well as flow and ML221 ($p < .001$) interaction were seen. The triple interaction between statin, flow and ML221 was also significant ($p < .001$). Inhibition of APJ did not influence directionality in cells treated with high or low concentrations of ator and pra at high flow conditions. Under low flow conditions directionality increased with ML221 treatment in control and 1 μM ator, but not 1 μM pra. No effect for directionality was found for statins at 0.1μM. At 10 dyne/cm^2 APJ inhibition decreased migration velocity under control (by 0.085 μm/min, $p < .0001$), 1 μM ator (by 0.086 μm/min, $p < .0001$) and 0.1 μM ator (by 0.058 μm/min, $p = .0011$), while pra had no influence. A higher velocity was found under low flow when APJ was inhibited (control 0.283 μm/min vs. ML221 0.334 μm/min, $p = .0012$). Ator and pra treatment under low shear stress resulted in a similar cell behavior. Under high shear stress inhibition of APJ reduced accumulated distance in control by 75μm ($p < .0001$), with high ator by 75μm ($p < .0001$) and low ator concentration by 51μm ($p = .0009$). In pra treated cells ML221 did not change accumulated distance at 10dyne/cm^2. At low flow however, accumulated distance increased under ML221 at control and statin treatment. Same results were seen for euclidian distance.

Conclusion:

We report that the inhibition of the APJ-receptor decreases flow-induced HUVEC migration under high, but not low shear stress. Further, ator but not pra rescued ML221-induced reduction in HUVEC migration. Therefore, statins influence HUVEC migration and pleiotropic effects of some but not all statins might be mediated via the APJ-pathway.