Ritonavir and Lopinavir Boosted with Ritonavir Induce Endothelial dysfunction and Premature Senescence in Cultured Human Coronary Artery Endothelial Cells

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Background
Clinical studies have shown that treatment with some protease inhibitors is associated with atherogenic lipid profiles and endothelial dysfunction and could result in early cardiovascular diseases. In vitro studies demonstrated that short-term treatment with some PIs could directly alter endothelial function. However, the long-term effect of PIs on endothelial function and senescence has not been evaluated.

Methods
We studied the sequential impact on endothelial function and senescence of a chronic incubation of human coronary artery endothelial cells with ritonavir (RTV, 7.5 microm) or lopinavir (10 microM) plus ritonavir (2 microM) (LPV/r). Endothelial cell dysfunction was assessed at 10, 20 and 30 days, by nitric oxide (NO) production (DAF fluorescence), oxidative stress (CM-H2DCFDA oxidation, NBT reduction), inflammation (IL-6, IL-8, MCP-1 secretion) and production of adhesion molecules (VCAM, ICAM) and PAI-1. Proliferation, replication and senescence were measured by the population doubling level, BrDU incorporation into DNA, senescence-associated beta-galactosidase activity and altered cell morphology. The protein expression of the senescence marker prelamin A was also determined.

Results

1) Long-term treatment with RTV and LPV/r resulted in progressive endothelial cell dysfunction

a) Decreased NO production and NO synthase expression
b) Increased oxidative stress
c) Increased inflammatory mediator secretion: IL6, IL-8 and MCP1
d) Increased adhesion molecule ICAM, VCAM and PAI-1 secretion

2) Long-term treatment with RTV and LPV/r progressively induced cellular senescence

a) Decreased cell proliferation and replication
b) Increased senescence-associated beta-galactosidase activity
c) Increased number of dysmorphic nuclei
d) Increased prelamin A accumulation

Conclusion
- Long-term exposure of human coronary artery endothelial cells to RTV and LPV/r progressively induced endothelial dysfunction at different levels: altered production of NO and adhesion molecules, increased oxidative stress and inflammation.
- Endothelial cell dysfunction was associated with increased expression of the senescence protein prelamin A and signs of early cellular senescence.
- We suggest that PI-induced endothelial dysfunction might result in stress-induced premature cell senescence.
- This could participate to early cardiovascular diseases and aging occurring in some PI-treated patients.