Atorvastatin-induced changes in micro-vascular smooth muscle function

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Lowering of blood cholesterol by atorvastatin is known to occur through up-regulation of hepatic LDL receptors and subsequent binding of circulating LDL cholesterol (Goldstein & Brown, 2009). Atorvastatin inhibits HMG-CoA reductase, an enzyme in the mevalonate pathway, the end effect of which reduces cholesterol synthesis. Mid-pathway effects reduce the synthesis of isoprenoids, non-sterol lipids (*e.g.* geranylgeraniol) which are required for the membrane localization of signalling proteins like the small GTPase RhoA. The effect of changes to the level of RhoA isoprenylation may underlie the pleiotrophic actions of HMG-CoA reductase inhibition and has been shown to alter membrane signalling for proliferation in vascular smooth muscle cells (VSMC) (Clunn *et al.*, 1997). The small GTPase, RhoA, plays well documented roles in many intracellular signalling pathways including proliferation, cytoskeletal rearrangement, cell division and contraction in VSMC, independent to changes in intracellular calcium concentration (Uehata *et al.*, 1997). The plasma membrane cholesterol content may also alter signal transduction (Potocnik *et al.*, 2007). This study aimed to discriminate between two effects of atorvastatin, reduction in cellular cholesterol content and reduced isoprenylation of RhoA, on the control of contraction.

The procedures on animals were approved by the RMIT Ethics in Animal Experimentation Committee. The contractile response of arterioles from 4 week atorvastatin (10 mg/kg/d, in drinking water) or vehicle treated male wistar rats were compared along with the effect of agonist stimulation on RhoA cellular localisation using immuno-fluorescence and dot blots of fractionated, cultured human VSMC. The cholesterol levels of micro-dissected arteries were measured and showed a consistent decrease associated with atorvastatin treatment, similar to that in VSMCs, cultured in serum free medium. VSMCs showed a redistribution of RhoA from membrane to cytosol and cytoskeleton with agonist activation. This was altered when cholesterol levels were reduced using beta-cyclodextrin and was inhibited by atorvastatin. In isolated, cannulated and pressurised arterioles about 40% of the contractile myogenic (pressure induced) tone was sensitive to the Rho-kinase inhibitor Y27632 and this proportion was not altered by atorvastatin treatment.

These findings suggest that receptor mediated signalling of contraction in VSMCs is associated with intracellular RhoA translocation which is inhibited by atorvastatin treatment but not by removal of membrane cholesterol. In intact arterioles RhoA-associated kinase contributes significantly to contraction however atorvastatin treatment does not appear to alter this contribution. The presence of circulating cholesterol and isoprenoids may prevent atorvastatin-mediated changes in cellular lipid metabolism from affecting VSMCs *in vivo*.

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