Platelet-derived growth factor-BB induces apoptosis in cultured vascular smooth muscle cells derived from human saphenous vein

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Apoptosis is increasingly recognized as an important factor in vascular remodelling and disease [1]. Platelet-derived growth factor-BB (PDGF-BB) is a potent mitogen in many cell types, including human vascular smooth muscle cells [2], and has been implicated in the vascular response to injury and atherogenesis [3]. Induction of apoptosis is known to be influenced by the presence of survival factors such as basic fibroblast growth factor (bFGF) [4] and insulin-like growth factor (IGF-1) [5]. PDGF-BB has also been reported to act as a survival factor in human cultured aortic and coronary arterial smooth muscle cells [5]. The intracellular signals responsible for cell survival are incompletely understood, however the small GTP-ase Rho binds to PDGF-receptor upon stimulation [6] and is required for G1 progression [7]. In addition inactivation of rho causes apoptosis in smooth muscle cells [8]. Lovastatin, an inhibitor of HMG CoA reductase prevents isoprenylation of small GTP-ases and disrupts their function [9]. In this study, we investigated the effect of lovastatin on apoptosis of human vascular smooth muscle cells stimulated by PDGF-BB.

Primary human saphenous vein smooth muscle cells were isolated from explants of normal saphenous vein surplus to material used for coronary artery bypass grafts as previously described [10]. The cells were routinely cultured in DMEM supplemented with sodium pyruvate (1.2mM), penicillin (120IU/ml), streptomycin sulphate (120mg/ml), gentamicin (30μg/ml) and 15% (v/v) foetal calf serum (FCS). Cells were seeded at ~1x10^5 cells/cm² in 9cm Petri dishes and growth-arrested for 7 days in NCTC-109 serum-free medium (pH 7.4), supplemented with L-glutamine (4mM), 15mM HEPES, and bovine serum albumin (2.5mg/ml). Cultures were incubated with PDGF-BB (20ng/ml) alone, lovastatin (10μM) alone, vehicle alone, or co-incubated with both PDGF-BB and lovastatin in serum-free medium for up to 48 hours. Following exposure, cells were harvested and processed using Cell Test® Plus DNA reagent kit (Becton Dickinson, CA USA). Cell cycle phases and the hypodiploid, apoptotic (A0) region were then quantified [11]. The presence of apoptotic cells was also confirmed with DAPI staining, and immunocytochemically using a terminal deoxynucleotide transferase-mediated dUTP-FITC end labelling (TUNEL) reagent (Boehringer Mannheim, Germany).

Stimulation with PDGF-BB alone increased the proportion of cells in S-phase from 4.2±1% to 8.0±1% (n=4) at 24 hours (Fig. 1.). Levels of apoptosis also increased from 11±1% to 24±1% (n=4) at 48h (Fig. 2.). Lovastatin alone had no significant effect on either S-phase or apoptosis compared with vehicle (Fig. 1. & 2.). In the presence ofLovastatin PDGF-BB had no significant effect on S-phase entry or apoptosis. (Fig. 1. & 2.).

These data indicate that stimulation of growth-arrested vascular smooth muscle cells derived from human saphenous vein with PDGF-BB increased S-phase and was associated with increased apoptosis. Co-incubation with lovastatin prevented these effects. Lovastatin alone had no effect on apoptosis. The data suggest that apoptosis following application of PDGF-BB in these cells may be related to progression through the cell cycle. Surprisingly, cell survival appears not to be affected by inhibition of HMG CoA reductase in these cells.