EPITHEORESE KLINIKES FARMAKOLOGIAS
KAI FARMAKOKINETIKES, INTERNATIONAL
EDITION 13: 81 (1999)

Hyaluronic acid Secreted by Human Vascular Smooth Muscle Cells as a Possible Target for the Prevention of Atherosclerosis

E. Papakonstantinou and G. Karakiulakis

Department of Pharmacology, School of Medicine, Aristotle University, Thessaloniki, Greece

The proliferation of vascular smooth muscle cells (VSMC) and their migration from the tunica media to the tunica intima are essential steps in the cascade of events leading to the formation of atherosclerotic lesions. Among several growth factors and cytokines, platelet-derived growth factor (PDGF) has been shown to be essentially involved in atherosclerosis. It is generally not expressed in the normal artery, whereas it is upregulated in lesions of atherosclerosis. PDGF stimulates the proliferation of VSMC, it is a potent vasoconstrictor and can induce chemotaxis of VSMC through a variety of extracellular matrices. We have previously shown that PDGF-BB specifically stimulates proliferating VSMC to secrete a 340 kDa hyaluronic acid (HA-340). This molecule is also present in the different layers of human atheromatic aortas with a negative concentration gradient from the tunica media to the tunica intima and the atheromatic plaque. The aim of this study was to investigate the biological function of this molecule in respect with VSMC proliferation and migration, two processes that are associated with atherogenesis. The isolation of HA-340 was performed after homogenization of the individual aortic layers (atheromatic plaque, tunica intima, tunica media and tunica adventitia) by lipid extraction and extensive digestion with pronase and Dnase. The total glycans were purified from the digestion products by gel filtration on Sephadex G-25 and fractionated on a Superose 6 column. HA-340 was identified after enzymatic treatment of the ensuing glycan fractions with glycosaminoglycan-degrading enzymes, followed by electrophoresis on polyacrylamide gradient gels and cellulose acetate membranes. We observed that HA-340 inhibited the PDGF-induced proliferation of human VSMC in a dose-dependent manner (Fig. 1) and enhanced the PDGF-dependent invasion of VSMC through a basement membrane barrier (Fig. 2). These effects were abolished following treatment of HA-340 with *Streptomyces hyaluronidase* (0.25 units per µg of HA-340), indicating that the effect of HA-340 on VSMC proliferation and migration are apparently size-dependent. Furthermore, the effect of HA-340 on the PDGF-dependent invasion of VSMC coincided with increased secretion of the 72-kDA type IV collagenase by VSMC and was completely blocked by GM6001, a hydroxamic acid inhibitor of matrix metalloproteinases. HA-340 did not exert any chemotactic potency, nor did it affect chemotaxis of VSMC along a PDGF gradient. Our findings suggest that homeostasis of HA plays a pivotal role in the regulation of processes associated with atherogenesis and may offer an laternative target for pharmacological intervention in the prevention of atherosclerosis.

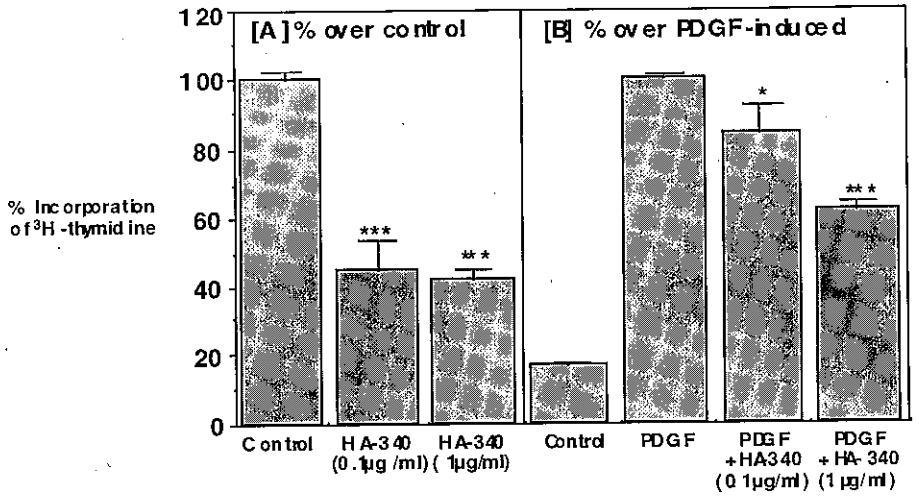

*Figure 1. Effect of HA-340 on the spontaneous and the PDGF-induced VSMC proliferation. Quiescent subconfluent VSMC (80% cell density, starved for 48 h at 0.1% FCS) were pre-incubated with various concentrations of HA-340 for 2 h prior to the addition of [^{3}H]-thymidine (0.5 µCi/ml) [A] or [^{3}H]-thymidine (0.5 µCi/ml) plus PDGF-BB (10 ng/ml) [B]. The effect of HA-340 on cell proliferation was assessed after 36 h of further incubation by measuring the incorporation of [^{3}H]-thymidine into newly synthesized DNA. In either case, the spontaneous incorporation of [^{3}H]-thymidine [A] and the mitogen-activated incorporation of [^{3}H]-thymidine [B] was set to 100%. Each bar represents the mean±SE of triplicate determinations from three independent experiments using at least four different primary VSMC lines. Student's t-test (unpaired, two sided) was used for statistical analysis; (*) indicates levels of statistical significance; *p<0.02, ***p<0.001. Control: 0.1% FCS.*

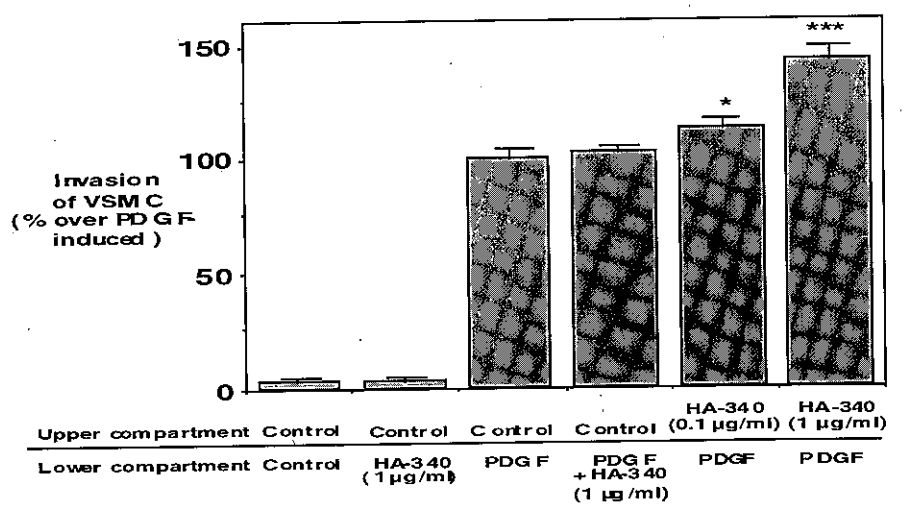

*Figure 2. Effect of HA-340 on the invasion of VSMC through an artificial BM. The effect of HA-340 on the spontaneous or the PDGF-induced VSMC invasion through a BM barrier was assessed using a Boyden-chamber-system containing a cell permeable filter membrane coated with reconstituted BM. Cells (10^4/ml) were seeded into the upper compartment of the Boyden-chamber and brought to quiescence (0.1% FCS, 48 h). Stimuli were then added to the upper or lower compartment, as indicated. Invasion of VSMC in the presence of PDGF-BB (10 ng/ml) alone in the lower compartment was regarded as 100%. Each bar represents the mean±SE of triplicate determinations from three independent experiments using at least four different primary VSMC lines. Student's t-test (unpaired, two sided) was used for statistical analysis; (*) indicates levels of statistical significance: *p<0.02; ***p<0.001. Control: 0.1% FCS.*