

A CELL PERMEANT PEPTIDE INHIBITOR OF MAPKAP KINASE II REDUCES INTIMAL HYPERPLASIA IN AN ORGAN CULTURE MODEL OF HUMAN SAPHENOUS VEIN

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Development of intimal hyperplasia involves smooth muscle cell proliferation, migration, and extracellular matrix deposition. These responses require dynamic reorganization of the actin cytoskeleton, a process known to involve expression and phosphorylation of the small heat shock protein, HSP27. We developed a cell permeant peptide inhibitor of the kinase known to phosphorylate HSP27 (MAPKAP kinase II, "MK2i"). MK2i (10 μ M) inhibited arsenite (500 μ M) or lysophosphatidic acid (LPA, 25 μ M) induced increases in the phosphorylation of HSP27 and LPA induced connective tissue growth factor (CTGF) expression ($p < 0.05$, $n = 3$ separate experiments) in A7R5 cells. MK2i also significantly inhibited transforming growth factor β (1.25 ng/ml) or LPA induced migration in a scratch assay of A7R5 cells ($p < 0.05$, $n = 3$ separate experiments). MK2i inhibited intimal thickening in human saphenous vein (HSV) rings after 14 days in organ culture with 30% FBS (42 \pm 15% reduction compared to control $p < 0.05$, $n = 6$). Western blotting of lysates from the HSV demonstrated that MK2i significantly reduced the phosphorylation of HSP27 and the expression of CTGF ($n = 6$, $p < 0.05$). To determine if HSP27 per se could lead to intimal hyperplasia, a fusion protein containing a cell penetration domain linked to recombinant HSP27 was used in the organ culture model. Treatment with recombinant HSP27 (600 μ g/mL) increased the intimal thickness by (39% \pm 10%, $p < 0.05$, $n = 3$), compared to control rings after culture. These results implicate HSP27 in the development of intimal hyperplasia and suggest that reduction of HSP27 phosphorylation by MK2i treatment is a potential strategy to inhibit intimal hyperplasia.

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