Aldosterone Increases PAI-1 Production by Cultured Renal Fibroblasts Both Alone and Synergistically with TGF-β

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Recent data have suggested that aldosterone may play a direct role in cardiac fibrosis through its actions on collagen production. Here we ask whether addition of aldosterone to cultured renal fibroblasts increases PAI-1 production. Second, since levels of both aldosterone and the fibrogenic cytokine TGF-β are elevated in renal fibrotic disease and it has been reported that TGF-β is antagonistic to aldosterone by reducing sodium reabsorption in kidney collecting duct cells, we looked at the effect of cotreatment with aldosterone and TGF-β.

Rat renal fibroblast cells were obtained from ATCC. Cells were maintained in RPMI 1640 medium supplemented with 5% fetal bovine serum at 37℃ in a 5% CO2 incubator. Cells were plated into six-well plates. Subconfluent cells were made quiescent by 16hr incubation in serum free RPMI 1640. Treatment lasted 48h after which culture media were collected and analyzed for PAI-1 mRNA by Northern blotting and PAI-1 production by Western blotting.

Doses of aldsterone 0, 10-11M, 10-9M, 10-7M, 10-6M, 10-5M increased PAI-1 in culture media by 1.0, 1.3, 1.6, 3.0, 8.3, and 12.2 fold, respectively. Treatment for 3-24h resulted in time-dependent increase in PAI-1 mRNA. Cotreatment of fibroblasts with 10-8M, 10-7M, or 10-6M aldosterone and 20 pg/mL TGF-β1 together, resulted in culture medium levels of PAI-1 that were 1.3-, 1.8- and 2.3-fold higher than those seen with TGF-β alone. Addition of 10-6M spironolactone markedly attentuated the synergistic effect bringing PAI-1 levels with 10-8M, 10-7M, or 10-6 M aldosterone and 20 pg/mL TGF-β1 together to 1.1-, 1.2- and 1.3-fold higher than with TGF-β alone.

From these results, we conclude that aldosterone itself may have a role in renal fibrosis by increasing PAI-1 production in renal fibroblasts. This effect of aldosterone is mediated via the classic mineralocorticcoid receptor. Also, addition of TGF-β appears to synergistically increase the aldosterone effect on PAI-1 production rather than antogonizing it as has been reported for sodium reabsorption in collecting duct cells.