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Nitric oxide synthase affects on renal fibrosis via endothelin system in vitro.

Yoon Kyung Chang[1]*, Hyun Su Choi[2], Jin Young Jeong[3] , Dae Eun Choi[3]**

Department of Internal Medicine, Catholic University of Korea[1], Clinical Research Center of Dae-Jeon St. Mary's hospital[2] , Department of Internal Medicine, Chun-nam National University of Korea[3],

Background: Renal inflammation and fibrosis are major pathomechanisms of chronic kidney disease. In the animal UUO kidney, pressure-induced tubular cell injury, activation of rennin-angiotensin system (RAS), and various inflammation- and fibrosis-inducing proteins such as MCP-1 and TGF-β induce the infiltration of macrophages and fibroblasts, leading to renal inflammation and fibrosis. Especially it is well known RAS inhibition attenuates renal inflammation and renal fibrosis, but the endotheilin-1 (ET-1) inhibition on renal fibrosis is not well known. The authors tried this study to manifest the NOS activation via ET-1 inhibitions in HK2 cells under conditioned like renal fibrosis process, with experiences based on previous study in vivo results.

Methods : The human kidney (HK) 2 cells were treated TGF-β (5ng/ml) and they were used as a model of renal fibrosis cellular condition. TGF-β treated HK2 cells were divided into 4 groups, and they were cultured within normal media as control, with valsartan(as RAS inhibitor) 10ug/ml, with bosentan (as endothelin-1 inhibitor) 1ug/ml, and with both valsartan 10ug/ml and bosentan 1ug/ml. They were culture for 3 days and 6 days. The HK2 cell groups were checked with SMA-α alpha, E-Cadherin, Collagen IV, cTGF, eNOS and iNOS in each group with western blot. Fibroblast s pecific protein-1 (FSP-1) was used for checking epithelial mesenchymal transition (EMT) of TGF-beta treated HK2 cells by immunostaining method.

Results: TGF-β treated HK2 cells showed loss of E-cadherin expression, decreased expression of eNOS and iNOS, and significantly increased expression of SMA, Col IV, and cTGF compared to normal control HK2 cells. Similar to previous UUO study in vivo, TGF-β treated HK2 cell group with valsartan and TGF-β treated HK2 cell group with bosentan, TGF-β treated HK2 cells with both inhibitors showed decreased expression of SMA, Col IV, and cTGF compared to TGF-β treated HK 2 cells with vehicle, and between 3 groups there were not significant differences. TGF-β treated HK2 cell group with bosentan, TGF-β treated HK2 cells with both inhibitors showed increased expression of eNOS and iNOS, between 2 groups there was no difference.

Conclusions: Bosentan and valsartan independently attenuated renal fibrosis process in vivo and in vitro, too. Differently from RAS inhibition, ET-1 inhibition in renal fibrosis attenuates the progression via NOS activation in TGF-β treated HK 2 cells. This study supports the possibility of ET-1 inhibitors for use to attenuate renal fibrosis in vivo and in vitro.

Keywords : endothelin-1, renin-angiotensin system, NOS, renal fibrosis