Indoxyl sulfate induces aortic calcification in human aortic smooth muscle cells

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Background: Vascular calcification is common in chronic kidney disease (CKD), and recognized as surrogate marker for cardiovascular disease (CVD). Indoxyl sulfate (IS) is a protein bound uremic toxin to exacerbate vascular calcification in CKD patients, and its therapeutic target has been searched for. We hypothesized that Notch signal pathway alterations caused by IS is involved in vascular calcification because Notch signal activation increase cell survival of vascular cells in developmental and pathological status.

Methods: IS (200 mg/kg/day in drinking water) or vehicle was administered to Dahl salt-resistant normotensive rats (DN) and Dahl salt-sensitive hypertensive rats (DS) to evaluate vascular calcification and the expression of Notch-1 and 3 with immunohistochemistry. Human arterial smooth muscle cells (HASMCs) was culture under various concentration of IS, and the expression of Notch 1 and 3, and apoptosis were assessed with RT-PCR, caspase activity assay, and TUNEL staining. Inorganic phosphate-induced calcification in HASMCs was also evaluated with or without IS and pharmacological inhibition of Notch and apoptosis signaling (gamma-secretase inhibitor, DAPT; 20 μM; caspase inhibitor, ZV AD 100 μM).

Results: Aortic calcification was observed solely in IS-administered DS rats. The expression of Notch-1 and 3 was slightly increased in aortic SMCs from vehicle-treated DS rats compared to vehicle treated DN mice. IS induced the expression of Notch-1 and 3 in aorta from both DN and DS rats, and strong signal was especially observed in IS-administered DS rats. Notably the expression of Notch-1 and 3 was fainter in vascular calcification in IS-treated DS rats. In cultured HASMCs, the expression of Notch1 and 3 was peaked at 24 h after administration of IS (1000 μM), and fainted within 72 h. The expression of Notch-1 and 3 was also peaked at the concentration of 500 μM of IS and fainted less than 1000 μM. Exposure to IS increased TUNEL-positive cells and caspase 3/7 activity in a dose- and time-dependent manner. IS accelerated inorganic phosphate-induced calcification in HASMCs, and the effect was canceled by pharmacological inhibition of Notch and apoptotic signal.

Conclusion: IS transiently activates Notch signal in vascular smooth muscle cells, but the effect was fainter in accordance to higher concentration and longer duration of exposure to IS. The decreased Notch activity induced formation of apoptotic body and calcified lesions. Thus, Notch signal would be a novel therapeutic target for vascular calcification in CKD patients.

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