S-B4

Indoxyl uslfate의 골세포에 대한 영향

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Indoxyl Sulfate Induced Apoptosis and Inhibited Differentiation in Cultured Osteoblast Cells

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Background: Indoxyl sulfate (IS), an organic anion uremic toxin, promotes the progression of renal dysfunction. Several studies have suggested that IS inhibits osteoclast differentiation and suppresses parathyroid hormone (PTH)-stimulated intracellular cAMP production, decreases PTH receptor expression, and induces oxidative stress in primary mouse calvariae osteoblast cell culture. However, the direct effects of IS on osteoblast apoptosis have not been fully evaluated. Hence, we investigated whether IS acts as a bone toxin by studying whether IS induces apoptosis and inhibits differentiation in the cultured osteoblast cell line MC3T3-E1.

Methods: We assessed the direct effect of IS on osteoblast differentiation and apoptosis in the MC3T3-E1 cell line. We examined expression of organic anion transport, caspase 3/7 activity, proteins related to apoptosis, free radical production, alkaline phosphatase activity, and mRNA expression of type 1 collagen and osteonectin.

Results: OAT3 gene expressed on MC3T3-E1 cell. IS, at the concentration range of 0.1-1.5 mM, inhibited cell proliferation at 72 h using an MTT assay. Production of free radicals in osteoblasts increased depending on the concentration of IS. To determine whether IS induces apoptosis, cells were incubated with different concentrations of IS for 12 h, stained, and subjected to FACS analysis to measure apoptosis and necrosis. IS increased the proportion of apoptotic cells. To elucidate the role of caspases in osteoblasts, we first examined the activity of the executioner caspase-3/7 in response to IS in osteoblasts. The activity of caspase 3/7 peaked at 6 h of incubation with IS (1.0 mM). To determine which apoptosis-related factors may be acting upstream of caspase activation, the expression of p53, Bcl2, and Bax were measured after the addition of IS (1.0 mM). IS increased Bax and p53, acting in an apoptotic role. However, Bcl-2 was not influenced by IS (1.0 mM) at 1, 3, and 6 h. Furthermore, IS inhibited expression of mRNA type 1 collagen and osteonection and alkaline phosphatase activity. These results suggest that IS inhibits osteoblast differentiation.

Conclusion: IS acts as a bone toxin by inhibiting osteoblast differentiation and inducing apoptosis via the caspase pathway.

Key Words: 요독, 신성골이영양증, 세포자멸사
Uremic toxins, Renal osteodystrophy, Apoptosis