Introduction: Although oxidative stress causes apoptosis in many cell types, its effect on the apoptosis of notochordal cell and anti-apoptotic effects of caspase inhibitors on the oxidative stress-induced apoptosis are unknown. The purpose of this study was to demonstrate the apoptotic effect of oxidative stress and the anti-apoptotic effects of caspase inhibitors on rat notochordal cells.

Materials and Methods: Cultured rat notochordal cells were exposed to oxidative stress (500 μM of hydrogen peroxide [H2O2]). To determine the oxidative stress-induced apoptotic pathways, activations of caspases (-3, -8, and -9) as well as cleavages of Bid and poly (ADP-ribose) polymerase (PARP) were evaluated with Western blotting 6 hours after oxidative stress. To elucidate the anti-apoptotic effects of caspase inhibitors on the oxidative stress-induced apoptosis, apoptotic rates of notochordal cells with or without treatment of specific caspase inhibitors (z-IETD-fmk for caspase-8, z-LEHD-fmk for caspase-9, and z-DEVD-fmk for caspase-3) were quantified by flow cytometry.

Results: Oxidative stress significantly increased apoptosis of rat notochordal cells (2.1% versus 4.75%, P = 0.008) and led to activations of initiators of intrinsic (caspases-9) and extrinsic (caspase-8) pathways as well as their common executioner (caspase-3). It also caused cleavages of Bid and PARP. Flow cytometric analysis showed that inhibition of only one of the intrinsic and extrinsic pathways by caspase-9 inhibitor (4.75% versus 3.56%, P = 0.31) and caspase-8 inhibitor (4.75% versus 5.24%, P = 0.84) did not significantly suppress the oxidative stress-induced apoptosis. However, inhibition of both pathways by caspase-3 inhibitor significantly reduced the oxidative stress-induced apoptosis (4.75% versus 2.64%, P = 0.008) to the control level (2.1% versus 2.64%, P = 0.15).

Discussion: Oxidative stress caused apoptosis of rat notochordal cells via both intrinsic and extrinsic (type I and Type II) pathways. Because caspase inhibitors are being used in clinical trials, inhibition of both pathways using caspase inhibitors might be of future therapeutic importance in oxidative stress-induced apoptosis of notochordal cells. Our results suggest that inhibition of inappropriate or premature oxidative stress-induced apoptosis of notochordal cells may delay the starting point of disc degeneration.