

Prevention of glucocorticoid induced-apoptosis of osteoblasts and osteocytes by protecting against endoplasmic reticulum (ER) stress.

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Increased oxidative stress, such as with excess of glucocorticoids (GC) or during aging, has been associated with endoplasmic reticulum (ER) stress, due to accumulation of misfolded or unfolded proteins, leading to cellular apoptosis. The double-stranded RNA-activated protein kinase-like ER kinase (PERK) is activated to alleviate ER stress and phosphorylates the eukaryotic translation initiation factor 2 alpha subunit (eIF2 α). Phosphorylated eIF2 α in turn inhibits global protein translation to provide time to the ER to recover from the unfolded protein load, promoting cell viability. We hypothesized that the pro-apoptotic effect of GC on osteoblasts and osteocytes are at least in part due to induction of ER stress. To test this hypothesis, we used MLO-Y4 osteocytic cells, OB-6 osteoblastic cells, and primary osteoblastic cells derived from neonatal murine calvaria. We found that the synthetic GC dexamethasone (DEX) significantly increased the percentage of apoptotic cells in cultures of MLO-Y4, OB-6, and primary osteoblastic cells. Similarly, the specific ER-stress inducing agents brefeldin A, an inhibitor of ER-golgi apparatus vesicle transport, and tunicamycin, a protein glycosylation inhibitor, significantly increased OB-6 cell apoptosis. We then tested the effect of salubrinal, an agent that protects against ER stress by inhibiting the dephosphorylation of eIF2 α , on bone cell apoptosis. Salubrinal blocked apoptosis induced by the ER stressors brefeldin A and tunicamycin in OB-6 cells. Salubrinal was also effective in blocking apoptosis induced by DEX in MLO-Y4, OB-6 and primary osteoblastic cells. Optimal responses were found at 10 μ M salubrinal, after either 6 or 24 h. Guanabenz, another inhibitor of eIF2 α dephosphorylation, also blocked DEX and tunicamycin-induced apoptosis of primary osteoblastic cells. Furthermore, addition of DEX to mineralizing OB-6 or primary osteoblastic cells markedly decreased mineral deposition and hydroxyapatite formation. In contrast, treatment with guanabenz increased mineralization of OB-6 cell cultures and prevented the inhibitory effect of DEX. We conclude that part of the pro-apoptotic actions of GC on osteoblastic cells are mediated through ER stress and that interventions that prevent dephosphorylation of eIF2 α could potentially prevent the deleterious effects of GC on bone.

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