Fluid flow shear stress induces the assembly of Cx43-forming hemichannels responsible for the release of PGE_2 in osteocytes

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Introduction: Mechanosensing bone osteocytes express large amounts of connexin (Cx) 43, yet gap junctions are only active at the small tips of their dendritic processes, suggesting another function for Cx43. Previous studies have shown that both primary osteocytes and the osteocyte-like MLO-Y4 cells respond to fluid flow shear stress by releasing intracellular prostaglandin E_2 (PGE_2).

Methods: Cells were treated with fluid flow shear stress at 16 dynes/cm^2 in the absence and presence of 18 β-glycyrrhetinic acid (β-GA), carbenoxolone or Cx43-hemichannel blocking antibody. Hemichannel activity was analyzed by dye-uptake analysis. Immunofluorescence, sucrose gradient, biotinylation and detergent-extraction were used to analyze the assembly and surface expression of Cx43-hemichannels.

Results: Cells plated at lower densities release more PGE_2 than cells plated at higher densities. This response was significantly reduced by Cx43 antisense and by the gap junction and hemichannel inhibitors, β-GA and carbenoxolone, even in cells without physical contact, suggesting the involvement of Cx43-hemichannels. Inhibitors of other channels, such as the purinergic receptor $P2X_7$ and the prostaglandin transporter PGT, had no effect on PGE_2 release. Shear stress induced the opening of hemichannels in primary osteocytes and MLO-Y4 cells, and this opening was inhibited by an antibody specific for Cx43-hemichannels. In the presence of fluid flow shear stress, Cx43 protein redistributes and migrates towards the plasma membrane. There was an increase in the connexon (hexameric) forms of Cx43 after fluid flow shear stress. Surface expression of Cx43 was also increased by shear stress. Shear stress rendered the Cx43 located at the cell surface more resistant to Triton-X-100 extraction, suggesting the formation of detergent-insoluble protein plaques, similar to previously reported gap junctional plaques.

Discussion: These results suggest fluid flow shear stress induces the assembly and translocation of Cx43 to the membrane surface and that un-apposed hemichannels formed by Cx43 serve as a novel portal for the release of PGE_2 in response to mechanical strain.