SILICA-INDUCED RESISTANCE OF CULTURED FIBROBLASTS TO FAS-INDUCED APOPTOSIS: IMPLICATIONS FOR SCLERODERMA

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Background: Systemic sclerosis (SSc, scleroderma) is a rare autoimmune disease that has been associated with exposure to specific chemicals, particularly silica and solvents. The mechanisms by which certain chemicals may cause SSc are unknown. Dermal fibroblasts cultured from SSc patients show an altered phenotype that includes the over-expression of collagen and a reduced susceptibility to Fas-induced apoptosis, both of which may exacerbate fibrosis. Fas resistant populations of fibroblasts can be selected using repeated cytokine treatment. Treatment of cells with silica has previously been reported to induce expression of transforming growth factor (TGF)β1 and other cytokines which may provide a simple pathway from exposure to resistance. Hence the hypothesis of this study was that repeated treatments with silica may be able to induce/select for the resistant phenotype.

Methods: MRC-5 human lung fibroblasts were treated repeatedly with medium alone, or medium plus sonicated or unsonicated quartz silica. Sonication is a method of disrupting particles using ultrasound. Cells were allowed to recover between treatments. An MTT assay was carried out following each treatment to determine the level of cell death in response to CH11, an antibody that stimulates Fas-induced apoptosis. These MTT assays, together with caspase assays, determined the total level of cell death and whether this death was apoptotic.

Results: Sonicated silica (250µg/ml) induced 50% cell death after 24 hrs. Caspase activity increased 3.5 fold, demonstrating that apoptotic death was taking place. After a single treatment, there was a similar level of cell death induced by CH11 in cells treated with unsonicated or sonicated silica when compared to medium controls: medium (7.9±6.7%), unsonicated (5.2±2.8%), sonicated (6.7±3.1%). Following repeat treatments, cell death induced by CH11 varied between cells treated with unsonicated (40.9%±3.1%) or sonicated silica (19.6%±4.5%). The cell death induced in medium treated cells was 40.4%±4.7%; the result for sonicated silica is statistically significant compared to this, with p=0.01. Overall, sensitivity to CH11 increased as the cells aged.

Conclusions:
1. An increased level of resistance to apoptosis was induced or selected for following repeated treatments with sonicated silica compared to unsonicated silica or medium only.
2. This suggests a possible mechanism by which cells in genetically susceptible individuals may become more resistant following a prolonged low-level exposure to silica.
3. Sonication exposes fresh surfaces of the silica, resulting in a greater production of reactive oxygen species, which is a possible cause of this effect.

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