**INTRODUCTION:** During disc degeneration, disc cell viability and gene expression fail to adequately maintain the extracellular matrix (ECM) resulting in proteoglycan loss, disc dehydration and annular tears, which may lead to disc herniation. The human intervertebrate disc contains a small cell population that with the processes of aging and degeneration becomes even further reduced. Work by this laboratory and others has shown that apoptosis reduces disc annulus cell numbers during aging/degeneration in vivo[1, 2]. This puts the remaining cell population at high risk for any further decrease in cell function or cell numbers[3]. Anti-apoptotic reagents have potential application in the biologic treatment of disc degeneration[4]. Our hypothesis is that thymosin β4 (TB4) (a known inhibitor of apoptosis[5]) inhibits annulus cell apoptosis. Our objectives were: 1) To determine if TB4 could inhibit apoptosis induced by hydrogen peroxide or serum starvation in human intervertebral annulus cells; 2) To determine the effect of TB4 on proteoglycan production or proliferation of human annulus in three-dimensional (3D) or monolayer culture, respectively.

**METHODS:** Human annulus cells were established and grown in monolayer with or without TB4 for 24 h. Apoptosis was induced using hydrogen peroxide exposure or serum starvation. Cells were incubated for 3-5 days before terminating the experiment. At study end, annulus cells were fixed and processed for apoptosis identification. The assay for apoptosis detection was performed using the T & D systems (Minneapolis, MN) TACS TdT in situ Apoptosis Detection Kit (TUNEL). Positive and negative controls were included with each TUNEL assay[6]. The percentage of apoptotic cells (% apoptosis) was determined by cell counts. Annulus cells were also grown in monolayer or 3D culture and treated with or without TB4 for proliferation determination and proteoglycan production in vitro using the 1,9-dimethylmethylene blue (DMB) assay and histologic staining with toluidine blue. Proliferation was measured at 490nm using the Cell Titer 96 Aqueous One Solution Kit (Promega, Madison, WI). Statistical Analysis: Data were analyzed using SAS version 8.2. A p-value of less than 0.05 was considered statistically significant. Standard statistical methods were used. Data are presented as means ± SD (n).

**RESULTS:** A significant reduction in disc cell apoptosis was seen after 800 nM TB4 treatment following either hydrogen peroxide (Figure 1a) or serum starvation induced apoptosis. The percentage of cells undergoing apoptosis significantly decreased in TB4-treated cells in both apoptosis-induction designs: hydrogen peroxide induced apoptosis, 46.9% ± 10.9 (5) (mean ± S.D. (n)) in controls vs 21.4% ± 6.1 (5) in TB4-treated cells (Figure 1A *p = 0.0437); serum starvation design, 38.3% ± 8.1 (5) for controls vs 12.3% ± 8.4 (5) in TB4 treated cells (P = 0.0347). TB4-exposure, however, did not alter cell proteoglycan production as assessed with DMB measurement (0.81 mg/ml ± 0.44 (3) for controls vs 0.94 ± 0.42 (3) in TB4 treated cells data not significant) and with histology (Figure 1b). No significant change in proliferation was present following exposure to concentrations from 1-800 nM. Absorbance at 490 nm ranged from between 1.55 and 1.90 (3) ± 0.12-0.19. No dose-dependent response was present. The control value was 1.66 (3) ± 0.13. The percentage of cells undergoing apoptosis significantly decreased in TB4-treated cells in both apoptosis-induction designs: hydrogen peroxide induced apoptosis, 46.9% ± 10.9 (5) (mean ± S.D. (n)) in controls vs 21.4% ± 6.1 (5) in TB4-treated cells (Figure 1A *p = 0.0437); serum starvation design, 38.3% ± 8.1 (5) for controls vs 12.3% ± 8.4 (5) in TB4 treated cells (P = 0.0347). TB4-exposure, however, did not alter cell proteoglycan production as assessed with DMB measurement (0.81 mg/ml ± 0.44 (3) for controls vs 0.94 ± 0.42 (3) in TB4 treated cells data not significant) and with histology (Figure 1b). No significant change in proliferation was present following exposure to concentrations from 1-800 nM. Absorbance at 490 nm ranged from between 1.55 and 1.90 (3) ± 0.12-0.19. No dose-dependent response was present. The control value was 1.66 (3) ± 0.13. No significant change in proliferation was present following exposure to concentrations from 1-800 nM. Absorbance at 490 nm ranged from between 1.55 and 1.90 (3) ± 0.12-0.19. No dose-dependent response was present. The control value was 1.66 (3) ± 0.13.

**CONCLUSIONS:** We report the novel finding of the anti-apoptotic effect of TB4 on human annulus cells in vitro. Results suggest that TB4 may have potential future therapeutic application in biologic therapies for disc degeneration.

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**Figure 1**

**References**


