Synergism between Human Annulus Cells and Human Nerve Cells Results in Significantly Elevated Neurotrophin Levels in Local Microenvironments

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Disclosures:

Introduction: Nerves grow into tissues in response to neurotrophins (signaling molecules involved in neuron survival, differentiation, migration and outgrowth). Recent findings show that disc cells can also produce neurotrophins, including nerve growth factor, brain-derived neurotrophic factor (BDNF), and neurotrophin 3 (NT3) (1,2). There are only a few previous studies looking at disc-nerve interactions (3,4). We used in vitro models to explore whether these two cell types could interact and contribute to enhanced local neurotrophin levels in the cell microenvironment. Our objective was to quantify levels of BDNF and NT3 produced by human nerve cells, by human annulus cells, and by co-cultured nerve and annulus cells.

Methods: Following Institutional Review Board approval, human annulus cells were isolated from surgical specimens and expanded in culture. Annulus cells were derived from a Thompson grade II disc, a grade III disc, and two grade IV discs. Neuroblastoma (SH-SYSY) and Schwann cells (sNF94.3) were utilized. Experimental designs used a) Cells mixed together and plated in monolayer; b) Disc or nerve cells layered upon each other, and c) Cells cultured in the same well but with one cell type on the bottom of the well, and the other cultured on a cell-well insert. Experiments used nerve and disc cells at a concentration 10^5 cells/well (24 well plates). Cultures grew for 8 days without media changes on the last 4 days of culture. Conditioned media was harvested for ELISA measurement of BDNF and NT3 (BDNF level of detection = 7.8 pg/ml; 3.9 pg/ml for NT3). Assays were run in triplicate and results averaged. Standard statistical analysis were utilized for calculation of means ± s.e.m., and ANOVA; p<0.05 was the level of significance.

Results: No significant difference was identified when results were evaluated from the three different in vitro designs. Data are presented here for cell insert experiments. BDNF data for neuroblastoma and Schwann-like cell experiments were analyzed separately. No differences were found in outcomes; both types of nerve cells showed significant ANOVA results (p = 0.042 and 0.027) with significantly greater BDNF levels in co-cultured conditioned media. Therefore, combined BDNF nerve data are presented. Figure 1A presents data for disc cells alone, nerve cells alone, and disc-nerve co-culture levels (means ± s.e.m), p = 0.02 by ANOVA. Nerve and disc cell co-culture produced significantly greater BDNF levels than that seen for disc cell culture alone, p < 0.05. NT3 experiments utilized neuroblastoma nerve cells only. Results were significant with ANOVA, p = 0.0002 (Figure 1B). NT3 levels from co-cultures were significantly greater than levels from either disc cells alone (p<0.001), or nerve cells alone (p<0.01). Figure 2 illustrates histology features for annulus cells in monolayer (Fig.2A), SH-SYSY cells in monolayer (Fig. 2B) and the admixture of disc-nerve cells in 3D in an H&E section; nerve cells are more rounded (arrow) and stained more darkly (Fig. 2C).

Discussion: Findings showed that nerve and disc co-culture significantly elevated production of BDNF and NT3. NT3 studies showed that co-culture produced levels even greater than levels produced by nerve cells alone (p<0.01), suggesting possible synergy between the two cell types. These findings lend support to our hypothesis that nerve cells and disc cells may interact within the disc and contribute to heightened neurotrophin levels in the matrix surrounding disc cells. An important, clinically relevant consequence could be increased pain levels related to increased nerve ingrowth into the disc.

Significance: These findings lend support to our hypothesis that nerve cells and disc cells may interact within the disc and contribute to heightened neurotrophin levels in the matrix surrounding disc cells. An important, clinically relevant consequence could be increased pain levels related to increased nerve ingrowth into the disc.

Acknowledgments: We thank the Brooks Back Pain Research Endowment for general lab support. We thank Darla Morton for expert technical assistance with ELISA data.

References: