Differential Effects of Nicotine and Tobacco Smoke Condensate on Human Annulus Fibrosus Cell Metabolism

INTRODUCTION. Tobacco smoking increases the risk of intervertebral disc degeneration (IDD) and back pain1,2, but the mechanisms underlying the adverse effects of smoking are largely unknown. Current hypotheses predict that smoking contributes to IDD indirectly through nicotine-mediated vasoconstriction which limits the exchange of nutrients between the discs and their surroundings3. However, it is conceivable that direct contact of disc cells, i.e., cells in the outermost annulus and those present along fissures in degenerating discs, with the vascular system containing soluble tobacco smoking constituents could perturb normal metabolic activities resulting in IDD. In this study we tested this alternative hypothesis by comparing the effects of direct exposure of human disc cells to smoke condensate and nicotine on cell viability and metabolic activity. The influence of nicotine exposure using bovine disc cell culture on matrix synthesis has previously been reported4, but the effects of tobacco smoke, which contains a mixture of many other toxic chemicals found in smokers, on human disc cellular metabolism have not been investigated.

METHODS. Human annulus fibrosus (AF) cells isolated from surgical specimens were grown in monolayer in F-12/D-MEM containing 10% FCS, 1% PS, and 25 μg/mL L-ascorbic acid at 37°C/5% CO2. Cells were cultured until 80% confluence and serum starved overnight before treated with nicotine (NS260, Sigma) or tobacco smoke condensate (TSC). TSC was prepared first by collecting particulates from tobacco smoke on a membrane filter (Pallflex or nicotine, percent cell viability was assayed by MTT colorimetric method6. Gene expression was measured by semi-quantitative real time RT-PCR using the ∆∆Ct method and the housekeeping gene GAPDH as a reference control. Proteoglycan (35S-sulfate incorporation) and collagen (3H-proline from competitive binding ELISA assay (Parameter PGE2 High sensitivity EIA kits) were measured. Levels of PGE-2 in culture media of TSC and nicotine treated cells were quantified using a competitive binding ELISA assay (Parameter PGE2 High sensitivity EIA kits). 0.5mg/ml TSC treatment increased the proinflammatory cytokine (IL-1β, IL-6, TNF-α) and decreased the anti-inflammatory cytokine (IL-4, IL-10, TGF-β) expression. 0.3mg/ml nicotine treatment modestly suppressed the matrix gene expression decreased two and three fold for aggrecan and collagen type II, respectively. 0.5mg/ml nicotine treatment modestly suppressed gene expression but had little effect on TIMP or MMP expression.

RESULTS. TSC and nicotine altered disc cell morphology and decreased cell viability in a dose-dependent manner, with the lethal dose (LD50) of ~0.8 mg/ml for TSC and ~8 mg/ml for nicotine after 72 hours of exposure (Fig. 1). TSC also reduced collagen and proteoglycan syntheses in a dose dependent manner, but nicotine at 0.3mg/ml, a concentration comparable to the reported plasma nicotine level in heavy smokers, had no significant effect on matrix synthesis. At 0.5mg/ml TSC, a dose equivalent in heavy smokers, matrix gene expression decreased two and three fold for aggrecan and collagen type 1, respectively. 0.5mg/ml TSC exposure also resulted in a twofold reduction in expression of the anticitabolic factors TIMP1 and TIMP3 but several fold increase in expression of the matrix metalloproteinase MMP1 (~60 fold) and MMP3 (~6 fold) (Fig. 2B). 0.3mg/ml nicotine treatment modestly suppressed matrix gene expression but had little effect on TIMP or MMP expression (Fig. 2C). 0.5mg/ml TSC treatment increased the proinflammatory mediator PGE-2 level in cell condition media six fold while 0.3mg/ml nicotine slightly decreased PGE-2 level (Fig. 2C).

DISCUSSION. This study demonstrates that smoke condensate, which has the advantage of containing all of the compounds inhaled by smokers8, exerts far greater detrimental effects on human disc cell viability and metabolism than nicotine exposure. Smoke condensate strongly pertursbs gene expression program controlling matrix homeostasis through downregulating matrix structural genes and upregulating the catabolic MMP genes; this imbalance might explain the prolonged recovery time after disc surgery in smokers. In vivo, cells in the outermost disc AF tissue and those along the fissures and cracks commonly found in aging and degenerating discs could come in direct contact with the many soluble tobacco chemicals in smokers’ circulatory systems9. Hence it is possible that tobacco smoking increases the risk of IDD through this direct contact mechanism in addition to the previously proposed indirect mechanism of nicotine-induced vasoconstriction leading to restricted nutrient exchange.