The long-term effects of chronic passive cigarette smoking on gene expressions of mouse tail disc tissues

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Introduction: Low back pain (LBP) is a major medical problem and socio-economic concern in the United States. It afflicts 52 million individuals and results in over 100 million lost work days per year[1]. The increased risk of LBP among cigarette-smokers has been known for 20 years and the odds ratio compared with non-smokers has been 1.3 to 2.5[2]. Degeneration of intervertebral disc (IVD) has been implicated as a primary etiologic factor for LBP. Substantial emerging evidence implicates that decrease of matrix components in disc, primarily proteoglycan and type II collagen, is the major initial trigger for disc degeneration[3]. The molecular mechanism of IVD degeneration has been investigated mainly in vitro but few studies in vivo have been reported[4] [5]. The objective of this study was to test the hypothesis that chronic passive cigarette smoke promotes IVD degeneration using quantitative IVD gene expression in smoke exposed and control mice.

Methods: The TE-10 machine (Teague Enterprises), a semiautomated mainstream and sidestream cigarette smoke producing system, was used for small animal smoke exposure. Mice were exposed 5 days a week, 6 hours a day. The smoking device provided uniform smoking of cigarettes under identical conditions. Carbon monoxide was monitored. Five mice each, housed for 3 months and 6 months with and without passive cigarette smoking, were allocated to a smoking group and a control group, total 20 mice. Total RNA was extracted from annulus fibrosus (AF) and nuleus pulposus (NP) tissues of tail discs of New Zealand White (NZW/LacI) mice. After synthesis of cDNA, the quantitative analysis of type I, type II collagen and aggrecan gene expression was performed by the real time PCR.

Results: After 3 months of passive smoking, the gene expressions of type II collagen and aggrecan were downregulated and type I collagen was upregulated in NP and AF tissues as compared to the gene expressions of those in the nonsmoking group. Type II collagen (4.83±0.33 vs 10.04±3.8, P< 0.01), two way ANOVA analysis, n=5), aggrecan (0.41±0.02 vs 0.48±0.05 ,P< .05, two way ANOVA analysis, n=5), and type I collagen (0.21±0.02 vs 0.14±0.05, P< .05, two way ANOVA analysis, n=5) (Fig.1). After 6 month passive smoking, the gene expressions of type II collagen and aggrecan were further downregulated and type I collagen was further upregulated in NP and AF tissues as compared to that of 3 month data or controls (P<.05, two way ANOVA analysis) (Fig.1). We compared the gene expressions of type II collagen, aggrecan, and type I collagen between the 3 month and 6 month nonsmoking groups. There were no significant difference between two groups (P >.05) so we combined this two groups together as a single control in Fig.1.

Discussion: Normal NP consists of type II collagen with minimal quantities of type I collagen [6]. In degenerating IVDs, type II collagen is replaced with the more fibrosis type I collagen [7], resulting in a stiffer NP with disruption of load dissipation properties [8]. This study showed that passive smoking could result in decreasing type II collagen and aggrecan gene expressions and increasing type I collagen gene expression in disc tissues. These gene changes increased with prolonged smoking exposure. Uei et al [4] reported that collagen genes ( type I, II and IX) were down-regulated 4 weeks of smoking and down-regulated remarkably after 7 weeks of smoking. Aggrecan also started to be up-regulated at 4 week. Our data are similar to Uei’s on type II collagen. In contrast, we found type I collagen increased in AF over time and NP and AF aggrecan gene expression decreased in smoke exposed mice vs. controls over time. Our results are more correlated to previously described changes in gene expression associated with degenerative disc disease [7]. This study also demonstrated that it was possible to quantitatively analyze gene expression at mRNA levels using a small amount of mouse tail disc tissue. To our knowledge, this study is the first to show gene expression changes of discs for as long as 6 month passive cigarette smoking. We will further investigate protein changes and histological change of disc after chronic passive cigarette smoking.

Keywords: passive cigarette smoking, gene expression.

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Reference: