**THE EFFECT OF NICOTINE ON GENE EXPRESSION DURING SPINE FUSION**

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**Introduction:** Nicotine has been shown in both clinical trials and animal models to decrease the rate of successful spine fusion. Yet, the mechanism of this inhibition is unknown. Previously, we have described the temporal and spatial pattern of gene expression of various bone related proteins during the spine fusion process in a rabbit model. We hypothesize that the addition of systemic nicotine in the rabbit posterolateral fusion model will decrease the expression of growth factors associated with neovascularization. In addition, we studied the expression of osteoblast related genes during nicotine inhibited and "normal" spine fusions in order to assess the effect of systemic nicotine on osteoblast function.

**Methods:** Twenty-eight New Zealand white rabbits underwent single level posterolateral spine fusion using autogenous bone. Fourteen rabbits received systemic nicotine delivered through a subcutaneous mini-osmotic pump, resulting in serum nicotine levels equivalent to smoking two to three packs per day. Fusion masses were harvested at 0, 2, 5, and 7 days, as well as 2, 3, and 4 weeks post arthrodesis. The fusion masses were divided into the outer zones, or the bone directly adjacent to the transverse processes, and the central zone, or the bone between the transverse processes. Following harvest, the mRNA was extracted from the homogenized specimen using a 6M guanidine solution and separated on a CsCl gradient. After several washings, the mRNA of various bone related proteins was determined using RT/PCR. Specifically, we amplified the genes for collagen I and II, VEGF and bFGF, proteins associated with angiogenesis, and BMP 2, 4, and 6. The RT/PCR product was then separated on a 12 % acrylamide gel, dried and quantitated on a phosphorimager screen. All intensities were normalized to that of GAPDH. Quantification was performed on a minimum of two fusion masses at each time point, with the RT/PCR results duplicated for each specimen. Values were expressed as fold increase +/- standard error of the mean/time zero. Analysis of differences in mRNA levels was performed using a one-way analysis of variance with post hoc multiple comparison tests when appropriate. Statistical significance was tested for p<0.05.

**Results:** First, expression of all proteins appeared first in the outer zones, with a lag effect noted in expression in the central zone. This is consistent with previously reported results of gene expression patterns in “normal” spine fusions and was present in both the nicotine and control animals. Second, nicotine significantly inhibited the expression of several genes, mainly in the central zone of the fusion mass. Expression of type I and type II collagen were both reduced in the central zone of the fusion mass of the nicotine animals at 2 weeks. This was particularly true of type II collagen, which was expressed at levels 70 +/- 12 fold less in the nicotine animals (p<0.005). BMP-6 was the first bone morphogen to be expressed in both groups, with peak expression at day 2. Peak levels were significantly greater in the control animals (30 +/- 2 fold increase) compared with the nicotine animals (10 fold increase, p<0.01). BMP-4 expression was also noted to be decreased by 20 +/- one fold in the nicotine animals at one week, when mRNA levels were at their peak (p<0.0005) (Figure 1). Finally, BMP-2 mRNA was expressed in greatest amounts at week 4 in the central zone, with an 80 +/- six fold increase in the control animals, compared to an 8 +/- five fold in the nicotine animals (p<0.005) (Figure 2). Expression of bFGF and VEGF were also decreased in the nicotine animals. VEGF expression peaked at day 5, but was expressed at an 8 +/- two fold increase in the nicotine animals, compared with a 175 +/- 25 fold increase in the controls.

**Discussion:** These results suggest a molecular basis for the inhibition of spine fusion by nicotine involving inhibition of cytokines known to be important for both angiogenesis and osteoblast function. Previous experiments have postulated that decreased angiogenesis is responsible for this effect. This is best supported by the decreased expression of VEGF seen at day 5. In addition to decreased angiogenesis, it appears that osteoblasts are also directly suppressed by nicotine. This is supported by significantly decreased expression of BMP-2, 4, and 6 in the nicotine group. This data also confirms the spatial and temporal pattern of BMP expression seen during spine fusion which has previously been reported. Understanding the basic mechanism of nicotine suppression of spine fusion should help devise strategies to counteract these negative effects.

**References:**

**Acknowledgements:**
This work was supported by a research fellowship and grant from The North American Spine Society.