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Effects Of Nicotine On Rabbit Bone Marrow Derived Osteoblastic Cells

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EFFECTS OF NICOTINE ON RABBIT BONE MARROW DERIVED OSTEOBLASTIC CELLS

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Introduction:

Spinal fusion is commonly performed to correct spinal instability in patients. The rate of nonunion after spinal fusion has been reported to be up to 3-4 times higher in patients who use tobacco products. Nicotine, the major constituent of cigarettes, is thought to cause this increased non-union. Nicotine levels in tobacco users are 10-70ng/ml in the blood (1) and as high as 1.56 mg/ml in the saliva of smokeless tobacco users (2).

In previous *in vivo* studies conducted in our laboratory (3) posterolateral spinal fusion with autologous bone graft was performed in New Zealand white rabbits that were administered with nicotine via patches. The serum concentration of nicotine was maintained at 78±49ng/ml. Fusion was assessed via blinded manual palpation by two surgeons and a radiographic score according to Boden et al., 1995 (4). The nicotine group showed significantly increased fusion rates (66.0 vs. 37.5, $p < 0.05$) over controls and a higher radiographic score (1.91 vs. 1.56). The overall goal of this research is to investigate why nicotine significantly increased the level of fusion *in vivo*. Previously, we had optimized a system in which we isolated cells from bone marrow of New Zealand white rabbits and cultured them in media that encouraged these cells to proceed down the osteoblastic phenotype (5). In this study, we exposed these cells to various levels of nicotine and evaluated the ability of these cells to express various markers of osteoblastic activity.

Methods:

Bone marrow was isolated from adult male New Zealand white rabbit femurs. They were expanded in monolayer culture adapted from standard protocols developed for rat models (5) (6) (7). Approximately 3×10^3 cells were placed in each treatment well of a 24 well plate and culture medium containing Minimum essential medium (MEM) and 40ng/mL Dexamethasone were changed every three days. All treatments were given 2.8×10^{-4} M concentration of L-ascorbic acid and 10mM concentration of β -glycerophosphate after day 7 and with every media change after that (8) (9). Cells were then exposed to one of six concentration of nicotine: 20, 40, 80ng/ml and 10, 100, 250 μ g/ml. Control cells were not exposed to nicotine. The media was changed every three days. Wells were stained with alkaline phosphatase staining kit (Sigma, St. Louis, MO) to determine the activity of the alkaline phosphatase enzyme, an early indicator of osteogenesis (10) and von Kossa staining (11), which indicates mineralization, a late stage of osteogenesis

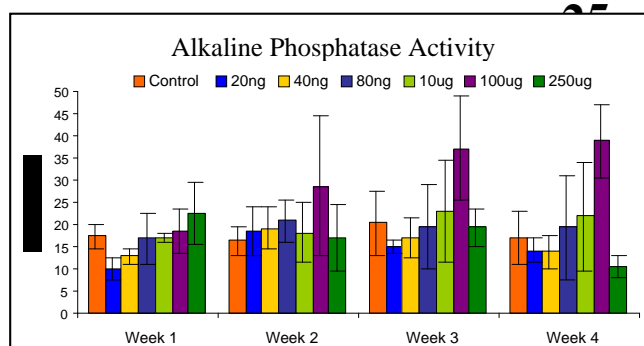


Fig 1: Rabbit bone marrow derived cells were cultured in monolayer under different conditions and tested for alkaline phosphatase activity at different time points. Alkaline Phosphatase enzyme activity was significantly enhanced by nicotine at 100 μ g/ml dose over the control at $p < 0.05$.

Essential Results:

Control cells behaved as expected, with increasing alkaline phosphatase activity up to three weeks and dropping off slightly at 4 weeks. As expected, there was no appreciable von Kossa staining up to three weeks, but after 4 weeks, the control cells stained positively for von Kossa. A two-way ANOVA using dose and time as variables showed that the 100 μ g/ml dose of nicotine significantly enhanced ($p < 0.05$) alkaline phosphatase activity over control (Figure 1). Von Kossa staining was not observed at week 1 – 3 sampling time points. Week 4 showed a positive stain for Von Kossa (Figure 2). A one-way ANOVA using dose as the variable was performed and the 100 μ g/ml and 250 μ g/ml dose had significantly greater ($p < 0.05$) mineralization than control.

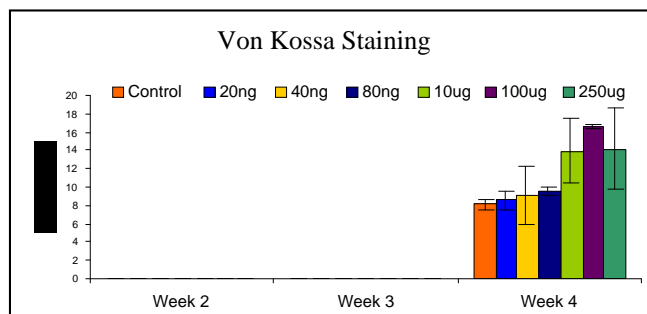


Fig 2: Rabbit bone marrow derived cells were cultured in monolayer and tested for Calcium deposition by Von Kossa staining at different time points. Von Kossa staining was not observed through day 21 but appeared at day 28. 100 μ g/ml and 250 μ g/ml treatments showed significantly higher mineralization than control on day 28 [$p < 0.05$]

Discussion:

This work demonstrated that bone marrow derived osteoblastic cells increase alkaline phosphatase activity in the presence of nicotine, although statistically significant effects are seen for high doses in the microgram range. Maximum effect was observed at 100 μ g/ml dosage. Mineralization is enhanced when nicotine is administered with higher dosages resulting in more mineralization. In a similar study nicotine has been shown to have a significant dose-dependent effect on the activity of osteoblastic cell lines with increased alkaline phosphatase activity and deposition of calcium (12). Thus, increased fusion rates we observed in nicotine rabbits *in vivo* might be explained by enhanced osteoblastic activity due to nicotine.

Interestingly, in other models of posterior spinal fusion, nicotine has been shown to decrease fusion rates. Decreased angiogenesis measured by decreased vascular ingrowths into autogenous cancellous bone grafts (13) and inhibition of expression of cytokines associated with neovascularization (14) have been observed in these models. However in many other models such as in tumors, or ischemia models, nicotine is a potent enhancer of angiogenesis (15). Therefore further investigation needs to be performed to fully understand the relationship between nicotine and angiogenesis in bone repair. The overall relationship between fusion rates and nicotine may depend on many factors, which include both angiogenesis and osteoblastic activity.

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