THE INFLUENCE OF NICOTINE ON FRACTURE REPAIR IN RATS

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**Introduction**

The effects of smoking on fracture healing have largely been investigated in clinical studies of spine fusions and pseudarthroses. The nonunion rate after spinal fusion in smokers has been reported to be three to four times that of nonsmokers. With respect to diaphyseal fractures, Schmitz et al. demonstrated that in the treatment of tibia fractures, there was a 43% delay in healing and a 70% longer time to complete healing in smokers compared to nonsmokers. Of the more than 4000 constituents isolated from cigarette smoke, nicotine, the most potent vasconstricting substance in smoke, has been implicated as a cause of poor bone and neural healing. The hypothesis that this toxic alkaloid and ganglionic stimulant has detrimental effects on fracture repair was investigated using biomechanical testing, histology, osteocalcin measurements, and histomorphometry.

**Methods**

The protocol was approved by the Yale Animal Care and Use Committee. Mature, female, Sprague-Dawley rats weighing 240–10 g were divided into three groups of sixteen animals. One group served as a control while the other groups received either low-dose or high-dose nicotine. Nicotine (or normal saline in the controls) was administered continuously via a subcutaneously implanted mini-osmotic pump (Alzet, Palo Alto, CA). On day 1, a 0.24 mm K-wire was drilled down one of the femoral canals. A closed, transverse, mid-diaphyseal fracture was produced over a fulcrum. After confirmation of the position of the K-wire and location of the fracture with a radiograph, a mini-osmotic pump was implanted subcutaneously in the interscapular region. The pumps were filled with either normal saline (control), low-dose nicotine (3.0 mg/kg-d), or high-dose nicotine (9.0 mg/kg-d). The low dose corresponded to a dose in the range received by humans smoking one to two packs of cigarettes per day, and the high dose corresponded to five to six packs per day.

Half of the animals in each group were sacrificed at three weeks and the remaining at six weeks. Fourteen rats were randomly selected for histologic study of the fracture site, and the rest were used for biomechanical testing of their fractured and intact femurs. All had their tibias opposite the fractured side harvested for histomorphometry. Sera were sent for measurements of nicotine and osteocalcin levels using gas chromatography and radioimmunoassay, respectively. Osteocalcin is a low molecular weight, non-collagenous bone protein that is secreted by osteoblasts into the circulation and is therefore accepted as a marker for osteoblastic activity. Biomechanical testing involved torsional testing to failure in external rotation on a machine. The data was recorded and translated into a load-deformation curve where peak torque, torsional stiffness, and time and angle to peak torque. The data suggest that the information presented here will provide some insight into the influence of nicotine on fracture repair.

**Results**

Of the 48 rats entered into the study, there were no perioperative deaths, infections, or other complications. All animals were ambulating on the first postoperative day, and any residual limb swelling was gone by the first week after surgery. All rats progressively gained weight throughout the study. The mean semin level in the control group receiving normal saline from the pumps was 1.50 ng/ml. The low dose had a mean level of 31.83 ng/ml and the high dose group a mean level of 104.17 ng/ml. Torsional testing demonstrated that at 3 weeks, the fractured femur of the high dose group had a significantly lower torque to failure (p<.01) and torsional stiffness (p<.01) than the control group. The intact side at 3 weeks showed no effect affecting torque or stiffness. At 6 weeks, there were no significant differences between groups in both fractured and intact sides with respect to peak torque, torsional stiffness, and time and angle to peak torque. The mean serum osteocalcin level at three weeks after the fracture was 60.44 ng/ml, 67.30 ng/ml, and 62.13 ng/ml in the control, low dose, and high dose groups, respectively. At 6 weeks, the mean levels were 57.13 ng/ml, 60.38 ng/ml, and 53.75 ng/ml, respectively. There were no significant differences between the groups at both 3 and 6 weeks.

Bone volume was lowest in the high dose group (p<.05) at 3 weeks; there were no differences in bone volume at 6 weeks. There were no differences in osteocalcin and osteocalcin numbers at both 3 and 6 weeks. The bone formation rates were significantly lower in the high dose groups compared to the controls at both time points (p<.03 and .02, respectively). The bone formation rate was 118.25 %/year in the control compared to 83.83 %/year in the high dose group at 3 weeks. At 6 weeks, the rate was 79.26 %/year in the control compared to 53.06 %/year in the high dose group.

**Discussion**

Data demonstrated that the fracture callus of the high dose group at 3 weeks was weaker than its control and that there was no loss of strength of intact bone. The data suggest that by 6 weeks, the callus of the high dose group had progressed to the biomechanical stage of the control. There were no significant differences in osteocalcin levels. It is possible that the expected decrease with the lowered bone formation rate was offset by the fractured state of the animals. Since the majority of newly synthesized osteocalcin binds to the mineral in bone, it is possible that this process was affected leading to more protein in the circulation.

Lowered bone formation rates with no differences in osteocalcin numbers suggest that nicotine impaired osteoblastic activity throughout the 6 weeks post-fracture. At 3 weeks, the bone volume in the high dose group was the lowest of all groups, consistent with the lower bone formation rate. There was a significant rise in bone volume in the high dose group from 3 weeks to 6 weeks. This was accompanied by an increase in trabecular thickness and an increase in peak torque. In the situation of a lowered formation rate, a steady bone volume may be explained by a lowered resorption rate. Supporting this hypothesis, two formulas by Recker' using the histomorphometric values available showed lower resorption rates in the high dose group compared to the controls; however, these values should be viewed with some caution since they were derived and not direct measurements. Lowered bone resorption rates with no differences in osteocalcin numbers suggest that nicotine impaired osteoblastic activity in this later part of the healing period.

We can conclude from the data that nicotine weakens the fracture callus early in the healing cycle, lowers the bone formation rate during the early and later parts of the healing cycle, and does not significantly affect the strength of intact bone.

The limitations of this rat model in this particular study should be kept in mind when extrapolating the findings to humans. However, the physiologic observation of fracture healing is similar, and we therefore believe that the information presented here will provide some insight into the influence of nicotine on fracture repair.

**Acknowledgments**

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**References**

5. Recker RR. Bone histomorphometry 1983; CRC Press, Inc., FL.

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