THE INFLUENCE OF NICOTINE ON FRACTURE REPAIR IN RATS

*+ABULENCIA, A.E., FRIEDLAENDER, G.E., TROIANO, N.W., AND PATEL T.C., *+Department of Orthopaedics and Rehabilitation, Yale School of Medicine, 800 Howard Avenue, New Haven, CT 06511, (203) 785-2579, FAX (203) 785-7132, gary.friedlaender@yale.edu

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[1]. With respect to diaphyseal fractures, Schmitz et al.[2] 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 vasoconstricting substance in smoke, has been implicated as a cause of poor bone and wound 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 +/- 10g 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 (3.0mg/kg-d), or high-dose nicotine (9.0mg/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[3].

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 at peak torque were recorded. Specimens for fracture histology were examined in a blinded fashion under light microscopy and assigned a numerical grade for the maturity of the callus based on a previously used grading system[4]. All rats were pulse-labeled with Calcein (2,7 bis[bis(carboxymethyl) – aminomethyl]-fluorescein) (Merck) intraperitoneally (30 mg/kg) 11 and 1 days prior to sacrifice. For histomorphometry, sections were examined in a blinded fashion measuring light and fluorescent parameters at a constant distance from the growth plate of the tibia.

Statistical analysis was performed using analysis of variance with $p < .05$ as being statistically significant.

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 limp was gone within the first week after surgery. All rats progressively gained weight throughout the study.

The mean serum 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 differences in peak 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.

Examination of fracture histology showed no significant numerical differences between the groups, though there was some suggestion that the fracture calluses of the nicotine groups at 3 weeks were more immature.

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 ($p=.10$) at 3 weeks; there were no differences in bone volume at 6 weeks. There were no differences in osteoblast and osteoclast 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

The data demonstrate 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 sigificant 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[3].

Lowered bone formation rates with no differences in osteoblast 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[5] 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 osteoclast numbers suggest that nicotine impaired osteoclastic 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 physiological process of bone remodeling is similar[6], and we therefore believe that the information presented here will provide some insight into the influence of nicotine on fracture repair.

Acknowledgment

Supported in part by the New Haven Foundation.

References

1. **Brown CW, et al.** *Spine.* 1986; 11:942-943.
2. **Schmitz M, et al.** Presented at the Annual Meeting of the OTA, 1995.
3. **Calvo MS, et al.** *Endocrine Reviews* 1996; 17(4):333-368.
4. **Huo MH, et al.** *J Orthop Res* 1991; 9:383-390.
5. **Recker RR.** Bone histomorphometry 1983; CRC Press, Inc., FL.
6. **Vignery A, Baron R.** *Anat Rec* 1980; 196:191-200.

☐ One or more of the authors have received something of value from a commercial or other party related directly or indirectly to the subject of my presentation.

☒ The authors have not received anything of value from a commercial or other party related directly or indirectly to the subject of my presentation.