The Effect of Transdermal Nicotine on Fracture Healing in a Rabbit Model

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Objective: Cigarette smoking inhibits fracture healing and places the patient at a higher risk of delayed union and nonunion. Nicotine has been implicated as the primary ingredient responsible for these effects. However, an analysis of current published investigations reveals conflicting data, with some evidence that nicotine alone does not significantly affect healing. We undertook an animal study of the effects of transdermal nicotine on fracture healing.

Methods: Twenty-two adult male New Zealand white rabbits were randomly assigned to the nicotine group or the control group. A midshaft tibial osteotomy was performed on the left tibiae of all 22 rabbits. The nicotine rabbits were exposed using a 10.5-mg transdermal patch applied daily to the ear. Radiographs were obtained, and the area of fracture callus was assessed. Rabbits were euthanized at 21 days. Fractures were stressed to failure, and load/deformation curves were recorded.

Results: The average area of callus formation was greater in the control group (Control: 0.158 cm^2, Nicotine: 0.124 cm^2), but the difference was not statistically significant ($P = 0.30$). There was a significant difference between the 2 groups for mean normalized torque to failure (Nicotine: 36% of nonfractured side, Control: 69% of nonfractured side, $P = 0.028$). The control group mean normalized stiffness was significantly greater than that for the nicotine rabbits (Control: 87%, Nicotine: 43%, $P = 0.036$). There were 3 nonunions in the nicotine group (27%) compared with none in the control group ($P = 0.062$).

Conclusions: In a rabbit model of fracture healing, transdermal nicotine exposure resulted in decreased mechanical strength of healing fractures at 21 days and a higher rate of nonunion at 21 days compared with that of controls.

Key Words: nicotine, fracture, fracture healing, smoking

(*J Orthop Trauma* 2012;26:724–727)

INTRODUCTION

Fracture healing is a complex process affected by local and systemic factors. Clinical studies have shown that cigarette smoking is an inhibitor of fracture healing that places the patient at a higher risk of delayed union and nonunion.[1–8] Nicotine has been implicated as the primary ingredient in cigarette smoke responsible for these effects.[9–17] Many of the previous studies of the effect of nicotine on fracture healing used levels of nicotine higher than those observed in average smokers and levels much higher than those found in individuals using a nicotine patch for nicotine replacement therapy.[11,18,19] Other animal studies and in vitro studies using serum nicotine levels that better approximate those in human smokers showed no significant effect on the mechanical strength of healing fractures.[9,19–22]

We undertook an animal study of the effects of transdermal nicotine on fracture healing as an initial step in determining whether nicotine replacement therapy would be helpful in lowering the risk of delayed union or nonunion in smokers with fractures. Our null hypothesis was that transdermal nicotine exposure would not significantly inhibit bone healing compared with that in controls without nicotine exposure.

MATERIALS AND METHODS

Groups

A power analysis was performed based on the mechanical results in the study of Skott et al,[20] and based on these results, 11 rabbits in each group were designated for mechanical testing. All the rabbits were New Zealand White males, at least 10 months old, weighed 3.5–4.2 kg, and were supplied by Myrtle Rabbitry, Thompson, TN. Thus, a total of 22 New Zealand White Rabbits were randomly assigned to 1 of 2 groups: group 1: Nicotine patch; group 2: control group with no exposure. The animals were killed at 21 days.

Surgery

A midshaft tibial osteotomy, stabilized with an Orthofix M-120 metacarpal minifixator with 40/15 conical screws, was performed on each rabbit. This model has been established and reported on by Fredericks et al.[23,24] The surgical approach to the tibia was identical in all rabbits. All operative procedures were performed in a surgical suite using inhalation anesthesia and aseptic techniques. Isoflurane (2%–4%) was delivered in O_2. The antibiotic, Cephalothin (13 mg/kg), was

Accepted for publication August 21, 2012.
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Presented in part at the Annual Meeting of the Orthopedic Trauma Association, 2011, San Antonio, TX.
The authors report no conflicts of interest.
The study was approved by the University of Iowa Animal Care and Use Committee, #0802021, 2/20/2008.
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used before surgery and twice a day for 2 days postoperatively. Analgesics were administered as individually needed based on observation to ensure that the animals were comfortable. Throughout the experiment, the animals were individually caged. The animals were monitored closely for signs of discomfort or pain (ie, excessive swelling or heat at the surgical site, lack of appetite, etc.).

Nicotine Administration and Serum Cotinine Level Assessment

Nicotine exposure was performed using a 10.5-mg transdermal patch applied daily to the ear as described by France et al.[25,26] The patch was chosen due to its ability to produce sustainable levels comparable with those found in human smokers and the similarity between the patch and the true clinical scenario.

Group 1 rabbits had a 10.5-mg nicotine patch attached to the ear starting postoperation day 1, and patches were changed daily until euthanasia on day 21. Clear Nicotine Transdermal Patch (Walgreens) 21 mg of Nicotine Polacrilex was cut into half to create the 10.5-mg patch. Blood draws were performed on all the rabbits from group 1 at 14 days after fracture and at sacrifice to ensure that cotinine levels were similar to those found in humans using the nicotine patch for nicotine replacement therapy. Cotinine levels, rather than nicotine levels, were monitored due to the longer half-life of cotinine (16–20 hours compared with 2 hours) and the decrease in fluctuation of cotinine levels.[19] Cotinine levels were assessed using liquid chromatography tandem mass spectrometry by an outside laboratory with expertise in performing such an analysis (IIT Research Institute, Chicago, IL).

Radiographs

Anteroposterior and lateral radiographs were taken at weekly intervals to evaluate callus formation and assess adequacy of fixation. The area of periosteal callus and diaphyseal bone at the osteotomy site was measured using digital x-rays. Periosteal callus was outlined along the bone between the 2 innermost screws in both the lateral and anteroposterior films, and the area was calculated.

Euthanization

Rabbits were euthanized at 21 days with 120 mg/kg of Euthasol.

Mechanical Testing

After euthanasia was administered, the left and right tibiae (right tibiae for an internal control) of 11 rabbits per group underwent destructive biomechanical testing. The tibiae were harvested, cleaned of soft tissues, and immediately tested on an MTS machine. Special care was taken during dissection to preserve the fracture callus on each specimen and throughout biomechanical testing. The bone was kept moist with saline throughout. The proximal and distal ends of the harvested tibiae were placed in polyvinyl chloride sleeves and encased with polymethylmethacrylate cement (PMMA). Approximately 1.5–2.0 cm of bone was left exposed superiorly and inferiorly between the defect site and the PMMA. All fixation screw holes were filled and covered with PMMA to eliminate those weakened areas as potential fracture initiation sites. Mechanical tests were then performed using an MTS Mini-Bionix torsional testing machine (MTS, Minneapolis, MN). The fracture sites were stressed to failure and load/deformation curves recorded for statistical evaluations. Torsion tests were conducted at a rate of 1.76°/s until failure occurred and the torque versus angular deformation data recorded. Torsional strength was defined as the maximum torque before the first abrupt drop in the torque versus angular deformation curve (newton meter). Stiffness, which is the slope of the rising portion of the curve, was calculated as the ratio of the torsional strength to the angle of failure (newton meter per degree). A percentage of the normal side was calculated for each fractured tibia for torque and stiffness. Specimens with 0 torsional strength were classified as nonunions.

Results and Statistical Analysis

Data from radiographic callus measurement and mechanical testing were compared between the nicotine patch and control groups. The *T*-test was used for comparing radiographic callus formation and mechanical testing results, and χ^2 analysis for incidence of clinical nonunion (Microsoft Excel 2007). Alpha was set at 0.05.

RESULTS

Serum cotinine values at 14 days averaged 93 ppb (parts per billion = nanograms per milliliter) and ranged from 29 to 145 ppb. Values before killing (day 21) averaged 224 ppb and ranged from 57 to 462 ppb. The average of all serum cotinine levels assessed was 177 ppb. Cotinine blood concentrations average about 250–300 ppb in groups of cigarette smokers, with a range up to 900 ppb.[19] Cotinine levels in subjects using transdermal nicotine replacement are usually 50%–70% of the levels detected in smokers.[19]

The average area of periosteal callus in the nicotine group was 0.124 cm^2 compared with 0.158 cm^2 for the control rabbits (Fig. 1). Although the control mean was higher, the difference was not statistically significant ($P = 0.30$).

The average torque to failure of the nicotine exposed fractured tibiae at 21 days was 36% of the nonfractured side (normalized torque to failure), with a range from 0% to 83%. This was significantly less than the average normalized torque to failure in the control group, which was 69%, with a range from 25% to 146% ($P = 0.028$). The average normalized stiffness was also significantly lower in the nicotine group compared with that in the control group (Nicotine: 43%, range 0%–127%; Control: 87%, range 35%–184%; $P = 0.036$) (Fig. 2).

There were 3 gross nonunions in the nicotine group (27%) and none in the control group. The χ^2 analysis was performed and failed to reach statistical significance with a P value of 0.062.

DISCUSSION

Cigarette smoking or other tobacco use is an accepted risk factor for delayed fracture healing, although the mechanism of the effect is not well understood.[27] Smokers have

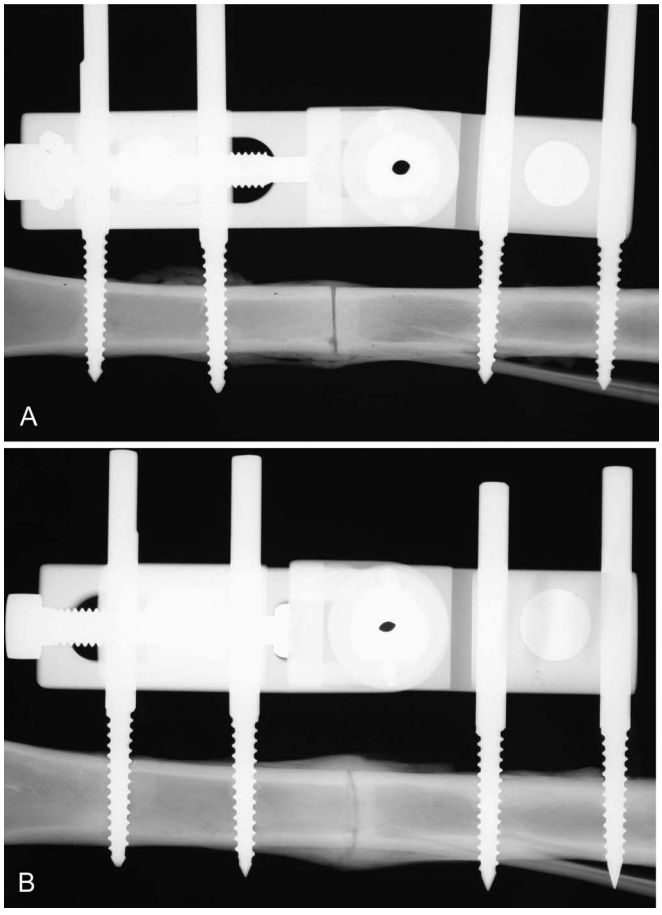

FIGURE 1. Comparison of callus in nicotine specimen (A) and control specimen (B) at 21 days after osteotomy. There is modest callus at the nicotine specimen osteotomy site (A) compared with the abundant callus seen in the control specimen (B) at the osteotomy site.

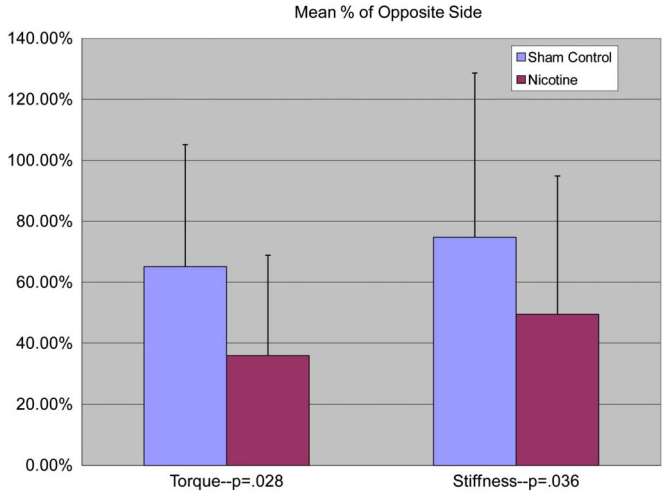

FIGURE 2. Mechanical testing results demonstrated a significantly higher torque to failure and stiffness values in the control group.

been found to have a longer time to clinical union and higher incidence of nonunion of tibial shaft fractures.[4] Nicotine has been implicated as a major contributor to the clinical effects of smoking on fracture healing. The scientific literature is inconsistent and incomplete in evaluating the effects of nicotine on fracture healing, however. There are no published clinical studies that assess the impact of nicotine alone on fracture healing. Animal studies are few in number and have had variable results.

In their study published in 1998, Raikin et al[15] describe plating tibial osteotomies in rabbits exposed to nicotine via miniosmotic pumps. They found that, compared with control animals, nicotine exposure resulted in radiographic, biomechanical, and clinical inhibition of fracture healing. Of note, the average serum nicotine level in their study was 61 ng/mL, which is more than that observed in smokers (10–50 ng/mL, cotinine 250–300 ng/mL) and 3–6 times more than that observed in subjects using a nicotine patch for smoking cessation (10–20 ng/mL, cotinine 125–210 ng/mL).[19]

Since the study by Raikin et al, however, there have been in vitro studies that have questioned the inhibitory effect of nicotine on fracture healing. Gullihorn et al[21] found that smoke condensate but not nicotine alone reduced all indices of osteoblastlike cell metabolic activity. Rothem et al[22] found toxic and antiproliferative effects at high nicotine levels and stimulatory effects at lower nicotine levels.

In vivo animal studies have also indicated that nicotine alone may not inhibit fracture healing.[28–30] Of particular note is the study by Skott et al,[20] in which they compared the effects of nicotine and tobacco extract without nicotine on the mechanical strength of healing femoral fractures in a rat model. They found that tobacco extract without nicotine significantly decreased the mechanical strength of the healing fractures at 21 days of healing. Nicotine alone did not significantly affect stiffness or torque to failure. Serum nicotine levels were 40–50 ng/mL, consistent with levels seen in persons smoking 1–2 packs per day.

These findings would indicate a potential role for nicotine replacement therapy for smokers with fractures at risk for delayed union. However, with serum cotinine levels lower than those in the studies cited above, we still witnessed a statistically significant inhibitory effect of transdermal nicotine on fracture healing in this rabbit model. Thus, our findings support those reported by Raikin et al by demonstrating biomechanical effects with nicotine exposure levels that are more realistic for smokers and especially patch users.

Our findings are contrary to the implications of the in vitro studies outlined above that demonstrate a stimulatory effect of nicotine on bone cells. It may be that, in vivo, nicotine's vasoconstrictive effects outweigh the cellular stimulation witnessed in vitro. Zheng et al[31] studied the effect of nicotine on bone healing in a rabbit model of distraction osteogenesis. They found that nicotine exposure inhibited bone formation and decreased blood flow. The mechanisms they identified for these effects were decreased expression of BMP-2 in osteoblasts and vasoconstriction.

Weaknesses of the study include small numbers, although the differences seen for biomechanical testing were

still statistically significant. However, the difference in clinical nonunions did not reach statistical significance with $P = 0.062$. In addition, although an animal model has many advantages, the effects of nicotine on fracture healing seen in rabbits may not translate to humans.

Further research to identify reasonable alternatives for smokers with fractures would be worthwhile. Possible areas of research would include the use of partial nicotine agonists currently being used for smoking cessation [Varenicline (Chantix)], or the use of fracture healing augmentation substances to overcome the inhibitory effect of smoking.

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