INTRODUCTION: Currently, an estimated 52.4 million Americans are regular smokers. Clinical studies indicate that cigarette smoking has a negative effect on bone and bone healing, as it appears to impede bone metabolism, inhibit fracture repair, increase the rate of postoperative infection, and the incidence of nonunion.\(^1,2\) Therefore, patients who smoke are at greater risk for delayed fracture healing or non-union, and would likely benefit from a therapy that accelerates and/or improves bone healing. Recombinant human bone morphogenetic protein-2 (rhBMP-2) is a potent osteoinduction factor shown to induce bone formation in many animal models. Although several clinical and preclinical studies have been initiated to evaluate orthopedic indications for rhBMP-2, few have investigated the possible effects of nicotine on bone induction by rhBMP-2. The objectives of this study were to determine, using a rabbit ulnar osteotomy model, whether fracture healing is inhibited by systemic nicotine administration, and whether treatment with rhBMP-2, delivered in an absorbable collagen sponge (ACS), overcomes this inhibition.

METHODS: The study consisted of three groups of adult male NZW rabbits (n=12-13 rabbits/group). One group (NIC) received a continuous subcutaneous infusion of nicotine (6.48 mg/kg/day) for a four-week pretreatment period, and for the duration (6 weeks) of the study. The second group (PRE-NIC) received nicotine during the pretreatment period only. The third group (SALINE) received a saline infusion for both the pre-treatment and healing periods. The nicotine and saline were delivered via mini-osmotic pumps surgically implanted in the dorsal thoracic region. After the pretreatment period, bilateral mid-ulnar osteotomies (0.5-1 mm) were surgically created using an oscillating saw, and were replaced in NIC and SALINE groups only. Pumps were replaced a third time in these two groups at week 7. In each rabbit, one osteotomy was treated with an rhBMP-2/ACS onlay (0.2mg/mL rhBMP-2, total dose 40 μg rhBMP-2), and the contralateral osteotomy remained untreated. Biomechanical testing of the healing osteotomies in all groups was performed immediately after surgery, and weekly thereafter to monitor fracture healing. Serum samples were collected at baseline, week 4, week 7, and at sacrifice to determine the levels of cotinine, the major metabolite of nicotine catabolism, in the sera of rabbits in the NIC and PRE-NIC groups. Rabbits were euthanized six weeks after creation of the osteotomies (week 10). The limbs were faxitron radiographed and scanned using peripheral quantitative computed tomography (pQCT) to assess callus area (mm\(^2\)), mineral content (BMC, g), and density (mg/cm\(^3\)). The ulna (11 rabbits/group) were then tested to failure in torsion at a quasi-static loading rate to determine failure torque (TQ, N-m), torsional stiffness (STF, N-m/mm), and energy to failure (ETF, N-m-mm). After biomechanical testing, the specimens were faxitron radiographed to determine the location of failure. The effect of nicotine treatment on osteotomy healing was evaluated in the untreated limbs using ANOVA with post-hoc testing using Fisher’s LSD. The effect of nicotine treatment on healing was evaluated using a mixed model ANOVA for repeated measures. All tests were two-tailed and differences were considered significant at p<0.05. This protocol was approved by the IACUC of Genetics Institute and all procedures were carried out according to AAALAC guidelines.

RESULTS: Cotinine levels ranged from 50-350 ng/ml, corresponding to a serum nicotine level of approximately 10-70 ng/ml (equivalent to blood levels of nicotine in humans after smoking 20-30 cigarettes/day or 1-1.5 packs/day)\(^3,4\). In the NIC group, serum cotinine levels (mean ± SD) were 277.5 ± 109.8, 208.4 ± 91.2, and 226.4 ± 88.7 ng/ml at weeks 4, 7, and 10, respectively. Serum cotinine was 282.2 ± 99.2 ng/ml in the PRE-NIC group at 4 weeks, and was undetectable thereafter. Systemic nicotine did not inhibit callus formation or healing of the osteotomy. Callus properties and biomechanical strength were similar in the untreated ulnae in the NIC, PRE-NIC, and SALINE groups (Figure 1). rhBMP-2/ACS treatment enhanced the callus formation and biomechanical properties of the healing osteotomy in all groups. The area of the mineralized callus and BMC of the callus were on average 26% and 29% greater, respectively, in the rhBMP-2/ACS treated ulnae than in the untreated, contralateral ulnae. TQ was 50-60% greater in the ulnae treated with rhBMP-2/ACS compared with the contralateral untreated ulnae (p<0.05 for all groups, Figure 1). Similarly, STF was 56-71% greater (p<0.05 for all groups), and ETF was 60-90% (p<0.05 for all groups) greater in ulnae treated with rhBMP-2/ACS. Faxitron radiographs taken after mechanical testing showed that 70-80% of untreated osteotomies fractured through the original osteotomy in all groups. In contrast, 18% (NIC), 30% (PRE-NIC), 0% (SALINE) of the rhBMP-2/ACS treated limbs fractured through the original osteotomy.

DISCUSSION: We evaluated whether osteotomy healing in rabbits was inhibited by nicotine, and whether this healing would be enhanced by rhBMP-2/ACS treatment. Despite achieving serum nicotine levels equivalent to those shown to inhibit bone healing in other animal models\(^3,4\), we found that healing of a 0.5–1 mm osteotomy was not inhibited by either pre-treatment or continuous nicotine administration. Possible explanations for the discrepancy between our results and those of others may be the characteristics of our animal model and the choice of a single sacrifice timepoint. In contrast to previous animal models used, such as spinal fusion and bone graft incorporation, an osteotomy presents a less challenging healing environment. For instance, significantly less bone is required to bridge a 0.5-1mm gap than is required to accomplish bony fusion between vertebrae. Therefore, it may be that the negative effects of nicotine on healing are relatively greater, and therefore easier to detect, in bone defects that require a more intensive healing process. An additional limitation that may have affected our conclusions was that rabbits were sacrificed at a single timepoint. Therefore, we were unable to evaluate whether nicotine may have inhibited the repair process at earlier or later stages. If rabbits were sacrificed at a later timepoint, differences may have been observed between the untreated ulnae in saline and nicotine groups, where the saline group may have eventually healed, and nicotine groups may have remained unhealed. Nevertheless, we found that nicotine administration did not limit the ability of rhBMP-2/ACS to enhance healing. The biomechanical properties of ulnae treated with rhBMP-2/ACS were 50-100% higher than those ulnae left untreated. Although we did not observe delayed healing due to nicotine administration, these data indicate that the ability of rhBMP-2/ACS to enhance fracture healing is not inhibited by systemic nicotine. Thus, rhBMP-2/ACS therapy may be useful to augment fracture healing or spinal fusion in-patients who smoke.


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