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Effect of serum nicotine level on posterior spinal fusion in an in vivo rabbit model

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Abstract

BACKGROUND CONTEXT: Cigarette smoking has a deleterious effect on spinal fusion. Although some studies have implied that nicotine is primarily responsible for poor fusion outcomes, other studies suggest that nicotine may actually stimulate bone growth. Hence, there may be a dose-dependent effect of nicotine on posterior spinal fusion outcomes.

PURPOSE: The purpose of this study was to determine if such a relationship could be shown in an in vivo rabbit model.

STUDY DESIGN/SETTING: This is a prospective in vivo animal study.

METHODS: Twenty-four adult male New Zealand white rabbits were randomly divided into four groups. All groups received a single-level posterolateral, intertransverse process fusion at L5–L6 with autologous iliac crest bone. One group served as controls and only underwent the spine fusion surgery. Three groups received 5.25-, 10.5-, and 21-mg nicotine patches, respectively, for 5 weeks. Serum nicotine levels were recorded for each group. All animals were euthanized 5 weeks postoperatively, and spinal fusions were evaluated radiographically, by manual palpation, and biomechanically. Statistical analysis evaluated the dose response effect of outcomes variables and nicotine dosage. This study was supported by a portion of a $100,000 grant from the Orthopaedic Research and Education Foundation. Author financial disclosures were completed in accordance with the journal's guidelines; there were no conflicts of interests disclosed that would have led to bias in this work.

RESULTS: The average serum levels of nicotine from the different patches were 7.8±1.9 ng/mL for the 5.25-mg patch group; 99.7±17.7 ng/mL for the 10.5-mg patch group; and 149.1±24.6 ng/mL for the 21-mg patch group. The doses positively correlated with serum concentrations of nicotine (correlation coefficient=0.8410, $p<.001$). The 5.25-mg group provided the best fusion rate, trabeculation, and stiffness. On the basis of the palpation tests, the fusion rates were control (50%), 5.25 mg (80%), 10.5 mg (50%), and 21 mg (42.8%). Radiographic assessment of trabeculation and bone incorporation and biomechanical analysis of bending stiffness ratio were also greatest in the 5.25-mg group. Radiographic evaluation showed a significant ($p=.0446$) quadratic effect of nicotine dose on spinal fusion.

CONCLUSIONS: The effects of nicotine on spinal fusion are complex, may be dose dependent, and may not always be detrimental. The uniformly negative effects of smoking reported in patients

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undergoing spinal fusion may possibly be attributed to the other components of cigarette smoke.

Keywords: Nicotine; Bone healing; Spinal fusion; Smoking; Pseudarthrosis; Lumbar fusion

Introduction

Numerous human and animal studies have shown that cigarette smoking is detrimental to bone health and impairs bone healing [1–5]. Nicotine has been implicated as the agent in cigarette smoke that is responsible for the ill effects of smoking on bone health. Animal studies have reported decreased bone healing and lower rates of spinal fusion in rats and rabbits exposed to nicotine [6–8]. Notably, Wing et al. [9] found that, while chronic exposure to nicotine decreased spinal fusion rates in a rabbit model, quitting improved fusion rates.

The mechanism by which nicotine affects bone health and healing has yet to be fully elucidated. Some authors suggest that it may be related to vascular changes (vasoconstriction and/or decreased vascularization) induced by nicotine exposure [10–12]. Heeschen et al. [13], however, found nicotine stimulated angiogenesis in three different animal models not involving bone, and Clouse et al. reported that coronary artery bypass grafts in a canine model were unaffected by nicotine [14].

Our group has previously reported that, in a study evaluating the effects of direct current (DC) electrical stimulation on spinal fusion in rabbits exposed to nicotine, both the nicotine control group (fusion+nicotine administration) and the DC stimulation group (fusion+nicotine+DC stimulator) had increased fusion rates compared to the negative control group (fusion alone) [15]. That study, however, did not examine a dose-response relationship. In a follow-up study, we demonstrated a dose-dependent increase in osteoblastic activity with nicotine exposure in an in vitro cell culture model [16]. We therefore hypothesized that there would likewise be a dose-dependent impact of nicotine exposure on spinal fusion in vivo. The purpose of this study was to further elucidate in an in vivo model the dose-dependent effect of nicotine on spinal fusion.

Materials and methods

All procedures used were in accordance with approval from the Institutional Animal Care and Use Committee. Twenty-four, adult (1 year), 4.0-kg, male, New Zealand white rabbits were obtained and randomly divided into four test groups (n=6). All groups received a single-level posterolateral, intertransverse process fusion at L5–L6 with autologous iliac crest bone. One group served as controls and only underwent the spine fusion surgery. Three groups received the autologous bone graft fusion and variable doses of nicotine administered via transdermal patch (5.25, 10.5, and 21 mg, respectively).

The surgical procedure was performed as previously described in the literature [7,17]. All surgeries were performed by a single operative team including the senior author and two experienced laboratory technicians. Postoperative radiographs were taken. The animals were observed during recovery, then placed in individual housing after recovery. Nicotine was administered to rabbits by way of transdermal nicotine patches (Habitrol; Novartis Consumer Health, Inc., Parsippany, NJ, USA) applied to the ear. Patches were changed daily for a period of 5 weeks after surgery. Blood was drawn from the central ear vein at 3 and 5 weeks after surgery, and serum nicotine levels were assessed by an independent laboratory (National Medical Services, Willow Grove, PA, USA).

All animals were euthanized 5 weeks (35 days) postoperatively. Five weeks has been demonstrated to be adequate time for fusion to occur and peak biomechanical tensile strength to be achieved [17]. The fusion masses and adjacent unfused segment (L4–L5) were carefully harvested from each animal. The L4 and L5 vertebrae provided a relative scale for control. Spinal fusions were evaluated radiographically, by manual palpation, and then biomechanically tested in a manner consistent with protocols from previous studies [7,12,17,18].

Anterior/posterior radiographs were obtained of the harvested vertebral segments. The radiographs were viewed and graded by two independent orthopedic surgeons blinded to the experiment. The radiographs were graded using a scale of 1 (not fused) to 3 (fused). A fused segment was defined as complete fusion with no lucent clefts or radiolucencies. An unfused segment was defined as 100% clefts. A grade of two defined partial fusion, indicated by the presence of partial clefts. This standard technique is used in the clinical setting. Trabeculation, its continuity and uniformity, and bone incorporation were indicators of fusion used in the evaluation.

The lumbar spine segments were manually palpated and evaluated for fusion on the basis of motion, similar to the evaluation standard in humans for determining nonunion and solid fusion. Each sample was tested for gross motion by two different surgeons and graded as fused or not fused (samples were not tested to failure). The presence or absence of motion determined the grade when the segments were stressed in flexion and extension. A grade of fused corresponded to a fused segment with no motion. A grade of unfused corresponded to a lack of fusion with unrestricted motion.