The Role Of TCDD In Smoking-mediated Bone Healing Inhibition

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Disclosures:

Introduction: Smoking is known to inhibit bone healing and lead to increased rates of pseudoarthrosis after spine surgery and extremity fractures. While this is a well-known clinical phenomenon, the mechanism of action behind these effects is controversial. Historically, it was postulated that such inhibitory effects were a result of the lower oxygen-carrying capacity of the bloodstream in smokers. Some research has implicated nicotine in the inhibition of bone healing in pre-clinical studies, whereas other studies have found that long-term exposure to nicotine does not affect bone healing and osteointegration. A group of environmental contaminants called dioxins have also been suggested to inhibit osteogenesis. Dioxins are stable, lipophilic, and resistant to biodegradation. 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD), a potent dioxin and carcinogenic component of cigarette smoke, has been shown to negatively impact bone quality and is suggested to affect osteoblast differentiation. We hypothesized that dioxin treatment would inhibit spinal fusion capacity in a rat posterolateral arthrodesis model, and that its effects are mediated by the aryl hydrocarbon receptor (AHR). The role of AHR was elucidated in vitro by co-treatment with the receptor blocker, alpha-Naphthoflavone (ANF). We evaluated parameters important to osteogenesis after vehicle and TCDD treatment of various pre-osteoblast cells.

Methods: Female Long-Evans rats were treated with weekly IP injections of TCDD or vehicle control for 6 weeks. The animals were then anesthetized and a posterior lumbar bilateral fusion across the transverse processes of the L4 and L5 vertebrae was performed using 1μg rhBMP-2 on an absorbable collagen sponge (ACS). TCDD/vehicle treatments continued until sacrifice at 4 weeks post-op. Spines were evaluated using radiographs, micro CT, and fusion scoring. Fusion scores were determined via manual palpation by 3 blinded observers, with an established scoring system whereby 0=no bridging bone; 1=unilateral bridging; 2=bilateral bridging. Spines with an average fusion score of ≥1.0 were considered fused. RNA was isolated from rat livers for quantitation of CYP1A1, a marker of TCDD exposure. To probe AHR involvement in TCDD-mediated inhibition of osteogenic differentiation, rat bone marrow stromal cells (BMSCs) were grown under standard or osteogenic (ascorbic acid/β-glycerophosphate/dexamethasone) conditions and co-treated with TCDD and ANF. A wound assay, whereby cells are allowed to migrate across a scratched monolayer over 24 hours, was performed to evaluate the rate of cell migration after treatment with DMSO vehicle, TCDD, nicotine, ANF, or TCDD+ANF. The percent wound closure was measured at 0, 8, 15, and 24 hours. An alkaline phosphatase (ALP) assay was also performed to quantify the enzyme activity with cells in both standard and osteogenic media treated with control, TCDD, ANF, and TCDD + ANF.

Results: Qualitative radiographs showed decreased bridging bone formation in TCDD-treated rats. Fusion scores in TCDD-treated rats were significantly lower than DMSO controls (1.73 vs 0.71, respectively; p<0.001, Figure 1A). Fusion rates were similarly reduced in TCDD-treated animals (100% vs 50%, respectively; p<0.01; Figure 1B). MicroCT quantification and 3-D reconstruction showed no change in fusion mass in the TCDD treated animals as compared to controls (Figure 1C). TCDD significantly inhibited cell migration in vitro, whereas nicotine did not show the same effect (Figure 2). The effects of ANF rescued the inhibitory effect of TCDD in rodent BMSCs. TCDD was shown to decrease the amount of active ALP when compared to control, ANF, or ANF + TCDD (Figure 3).

Discussion: A multitude of factors may inhibit bone healing after spine surgery and extremity fractures. This study evaluated the impact of TCDD on spine fusion in a rat arthrodesis model. We found that dioxin significantly lowered fusion rates, but spines that fused had no difference in new bone formation between groups. Our in vitro studies suggest that TCDD inhibits pre-osteoblast cell migration through activation of the AHR, as the AHR antagonist, ANF, rescued the effect of TCDD in those studies. Additionally, TCDD was shown to have an inhibitory effect on the activity of membrane-bound ALP activity, indicating TCDD effects cell recruitment as well as cell activity in the inhibition of bone healing.

Significance: Although smoking is known to cause complications in bone healing after surgery, the mechanisms remain elusive. Our study suggest that dioxin-like compounds may be responsible for the inhibitory effect of smoking, and that agents which block the AHR may have therapeutic potential in the smoking population.

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References: Brown CW, Orme TJ, Richardson HD. The rate of pseudarthrosis (surgical nonunion) in patients who are smokers...
and patients who are nonsmokers: a comparison study. Spine 1986; 11(9): 942-3.

Figure 1: Mechanical palpation scores (A) and cartilage area (B) for control- and TCDD-treated rats. *p<0.05, **p<0.01 (1) microC=indicator of new bone formation across L4-L6.

Figure 2. Average percent wound closure for rodent BMSOs after treatment with vehicle control, nicotine, TCDD, ANF, and ANF + TCDD. Distances were calculated 15 hours post-scratch. *p<0.05, **p<0.01 TCDD vs all other groups.
Figure 3. ALP measured in cells treated with control, TCDD, ANF or TCDD + ANF. *p<0.05 TCDD vs all other groups