Histological and Radiographic Effects of E-cigarette Vapor on Fracture Healing in a Mouse Fibula Model

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INTRODUCTION: Although electronic cigarette vaping (ECV) is generally considered safer than cigarette smoking, the increasing usage in pediatric and adult populations is a cause for concern. Previous studies have demonstrated cytotoxic, inflammatory, and differentiation-inhibiting effects on multiple organ systems[1,2,3]. However, the effects of ECV on bone health have yet to be fully explored[4]. The present study aims to evaluate the effects of tobacco- and menthol-flavored ECV extracts on fracture healing in a mouse fibula fracture model.

METHODS: Following protocol approval by our Institutional Animal Care and Use Committee, 15 mice were assigned to 1 of 3 treatment groups: PBS, menthol-ECV (M-ECV), or tobacco-ECV (T-ECV). Only female mice were utilized for this pilot study for uniformity, but the intent would be to include animals of both sexes in a future, larger study. ECV concentrate was extracted from commercially available pods via bubbling into PBS solution. A dose escalation trial evaluating toxicity determined treatment dose to be 0.1x of the extract concentration. Bilateral fibula fractures were surgically induced, in mice receiving 200 ul intraperitoneal ECV injections 5 times per week, beginning 1 week preoperatively and continuing postoperatively. Weekly x-rays were taken following surgery. At 3 weeks after initial surgery, fibulas were harvested for microCT. Imaging analysis was completed using the ImageJ plug-in, BoneJ, to measure callus size, mineralized bone volume, and trabecular thickness. Fibulas were sectioned and stained using Masson's trichrome protocol.

RESULTS SECTION: MicroCT analysis showed no statistically significant differences in bone volume (BV), total volume (TV), BV/TV ratio, or trabecular thickness between treatment groups (Figure 1). However, histological evaluation revealed T-ECV-treated fibulas had large, collagen-filled bone defects with widespread reactive osteocytes. PBS- and M-ECV- treated groups demonstrated more complete healing with minimal reactive changes (Figure 2).

DISCUSSION: This study provides histological evidence of differences in fracture healing in animals exposed to ECV. While radiographic metrics were found to be similar across groups, microCT is unable to reliably differentiate woven from lamellar bone thus limiting interpretations regarding degree of bone remodeling present[5]. Other limitations in this study include potential physiological differences resulting from intraperitoneal administration as opposed to ECV inhalation and lack of stress testing which may have provided functional assessment of healing. Nevertheless, histological analysis revealed impaired or delayed fracture healing and increased cytotoxicity and inflammatory responses in the T-ECV group, suggesting a potential deleterious effect of ECV on bone health and regeneration. Further studies, including validation in human models, are warranted to corroborate these findings.

CLINICAL RELEVANCE: These findings demonstrate the potential harmful effects of ECV on bone healing and regeneration, with histological evidence of reduced bone regeneration and persistence of inflammatory changes. In the context of the rising usage of vaping, careful consideration must be taken when counseling patients after injury and developing treatment strategies.

REFERENCES:

1. Thanavala, Yasmin, and Maciej L. Goniewicz. "Vaping-induced severe respiratory disease outbreak: what went wrong?." *The Lancet Respiratory Medicine* 7.12 (2019): 1014-1015.

2. Seiler-Ramadas R, Sandner I, Haider S, Grabovac I, Dorner TE. Health effects of electronic cigarette (e-cigarette) use on organ systems and its implications for public health. Wien Klin Wochenschr. 2021 Oct;133(19-20):1020-1027.

3. Yu C, Liu J, Sakurai R, Wang Y, Afrose L, Gour A, Sharma A, Chandan G, Rehan VK. Perinatal nicotine vaping exposure induces pro-myofibroblastic phenotype in rat bone marrow-derived mesenchymal stem cells. Reprod Toxicol. 2024 Oct;129:108673.

4. Nicholson T, Scott A, Newton Ede M, Jones SW. (Lond). 2021 May 5;18(1):16. doi: 10.1186/s12The impact of E-cigarette vaping and vapour constituents on bone health. J Inflamm 950-021-00283-7.

5. Ip V, Toth Z, Chibnall J, McBride-Gagyi S. Remnant Woven Bone and Calcified Cartilage in Mouse Bone: Differences between Ages/Sex and Effects on Bone Strength. PLoS One. 2016;11(11):e0166476.

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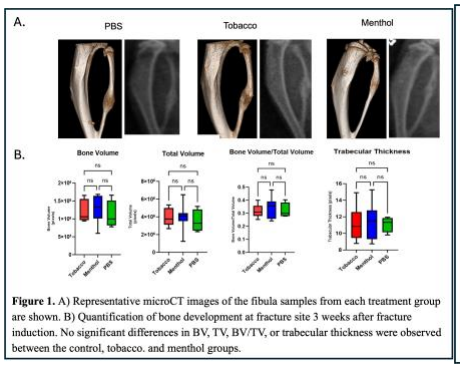

Figure 1. A) Representative microCT images of the fibula samples from each treatment group are shown. B) Quantification of bone development at fracture site 3 weeks after fracture induction. No significant differences in BV, TV, BV/TV, or trabecular thickness were observed between the control, tobacco. and menthol groups.

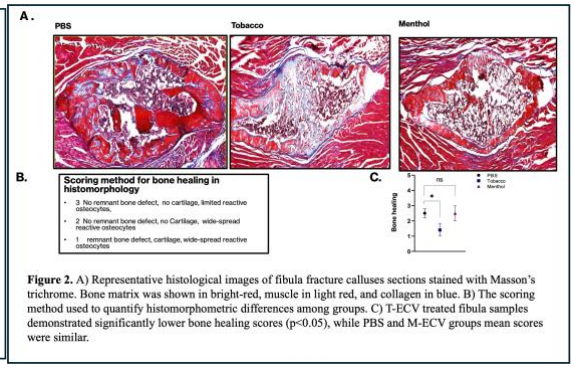

Figure 2. A) Representative histological images of fibula fracture calluses sections stained with Masson's trichrome. Bone matrix was shown in bright-red, muscle in light red, and collagen in blue. B) The scoring method used to quantify histomorphometric differences among groups. C) T-ECV treated fibula samples demonstrated significantly lower bone healing scores ($p<0.05$), while PBS and M-ECV groups mean scores were similar.