Spring 2020

Analyzing the Effects of Opioids on Cortical Pore Networks in Rabbit Tibiae and Femora

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Department of [Biology]

Honors Research Project

Submitted to

The Williams Honors College
The University of Akron

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Analyzing the Effects of Opioids on Cortical Pore Networks in Rabbit Tibiae and Femora

Honors Thesis

Presented to

The Honors College at The University of Akron

In Partial Fulfillment

of the Requirements for the Degree

Bachelor of Science (Honors)

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May 2020

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I. Abstract

New Zealand White rabbits were administered morphine, transdermal fentanyl patches, saline placebo injection, and placebo tegaderm patches respectively for eight weeks to test whether opioids effect cortical bone pore network structures within femora and tibiae.

II. Introduction

Cortical bone contains pore networks which attribute to their overall strength (Ammann and Rizzoli 2003). Varying structures of pore networks such as, number of branches, length, connectivity, and thickness is dependent on several variables, which can be biological or environmental (Khosla et al. 2006). The density and connectivity of an individual's cortical pore network can vary widely depending on sex, age, diet, hormones, and substance use (Khosla et al. 2006). Bone remodeling, an ongoing tissue turnover process occurring throughout life, lends to the variation observed among pore networks between individuals. Bone remodeling is accomplished via bone effector cells – osteoblasts and osteoclasts – two cell types that reside within bone. Osteoclasts are responsible for bone-resorbing while osteoblasts are responsible for bone-forming and the two processes are in equilibrium. Although the two processes are in equilibrium bone formation lags in comparison to bone resorption, indicating a required balance (Stout and Crowder 2012). In atypical circumstances, such as in cases of metabolic bone disease or substance use (e.g., opioids), the equilibrium between the two processes can be disrupted therefore causing abnormalities within the structures of an individual's bone pore network (Chrastil et al. 2013).

There has been a sharp increase since the 1990's for written prescriptions for opioid analgesics (Paulozzi et al. 2006). Subsequently an increase in misuse of opioids followed. Recent studies estimate over one million people in the United States misuse opioids (National Institutes of Health 2018). In the United States deaths due to overdose involving opioids has increased 200% since 2002 (Rudd et al. 2016). The growing epidemic has led to a link between opioids and bone fragility. Opioid usage has been clinically demonstrated to influence the overall pore network within bones by disrupting the equilibrium the between resorption and formation of bone (Chrastil et al. 2013). Osteoblasts, bone-forming cells, possess opioid receptors which opioids interact with and inhibit bone formation. Additionally, opioids can inhibit the function of vasoactive intestinal peptide (VIP), a neuropeptide that stimulates osteogenesis and reduces osteoclast function. Neuropeptide Y (NPY) is another neuropeptide that stimulates osteogenesis and is inhibited by opioids (Coluzzi et al. 2020 Apr 6). Inhibition of bone formation can lead to deviations within pore networks. Opioids can also produce an endocrine effect that reduces estradiol levels in women and testosterone in men (Coluzzi et al. 2015). Transdermal fentanyl, which is known to have higher opioid activity than morphine, has been linked to androgen deficiency (Coluzzi et al. 2020 Apr 6). Lower estradiol levels in women naturally occur with age, which leads to an increase risk of osteoporosis (Coluzzi et al. 2015).

Previous research has failed to fully explain the effects of prolonged misuse of opioids on bone pore network microstructures. Opioids considerably reduce bone mineral density (BMD) in individuals (Kinjo et al. 2005). Since the presence of opioids disrupts bone formation it is predicted that the three-dimensional (3D) structure of pore

networks will also be disrupted. In order to explore this hypothesis using an *in vivo* live animal model, New Zealand white rabbits were utilized.

Cortical bone pore networks can be visualized in 3D using desktop micro-Computed Tomography (μCT). Through employing this method, the number of branches, the length, connectivity between branches, and the thickness of pore networks will be quantified. The objective of the present study is to identify if prolonged use of opioids will cause an abnormal structure to pore networks within bone. It is hypothesized that due to osteoblast disruption, opioid rabbit bones will display less cortical pore connectivity, shorter and fewer connecting branches, and thinner pore networks.

III. Materials and Methods

Due to this project being a part of a larger ongoing project in Dr. Janna Andronowski's lab some material and methods were excluded.

SkyScan 1172 desktop micro-CT imaging

Clean femora and tibiae (void of adhering soft tissues) were stored in a -20°C freezer and thawed in the refrigerator 24 hours prior to imaging. Due to height restrictions associated with the μCT gantry, tibiae were trimmed distally for imaging using a Buehler Isomet 1000 precision saw (Buehler, Lake Bluff, IL) with a diamond blade. The final height was not to exceed 87mm (Appendix B). To ensure no movement occurred during scanning, all bones were wrapped and secured with parafilm. Clay markers were employed to indicate the anterior and medial sides of the tibiae. Femora and tibiae were imaged at the mid-shaft using a SkyScan 1172 (Bruker, Billerica, MA) desktop μCT system at 5.49 μm resolution. Subsequent scan data was placed into a

custom workflow using various image processing software programs (e.g., Nrecon (Bruker, Billerica, MA), ImageJ (NIH, Bethesda, MD), and CTAnalyser (Omicron, Klaus, Austria)) to convert the X-ray projections into 3D models of vascular pore networks (Appendix C; Appendix D). This workflow provided an image stack of two-dimensional (2D) cross-sections (1,000-1,200 slices) of the rabbit femora and tibiae representing the pore networks within the bone. Cross-sections that were obtained represent approximately 6.5mm length of bone at the mid-shaft of the femora and tibiae.

Data Extraction

Cross-sections generated from the workflow were uploaded to Amira 6.0 (Thermo Fisher Scientific, Waltham, MA) which produced 3D renders of the pore networks (Appendix E). The auto-skeleton feature created a spatial graph of the bone's pore network throughout the cross-sections. The spatial graph was then analyzed to generate data for length of branches, number of branches, connectivity of network, and thickness of the branches. Data for each index of each individual bone was averaged to generate the mean and the median.

Statistical Analyses

Pore systems among treatment groups (control, fentanyl, morphine) were analyzed by calculating the total, mean, median, maximum, and minimum for each index. A Shapiro-Wilk test and a Levene test were conducted in JMP Pro 14 (SAS Institute, Cary, NC) to ensure normal distribution of residuals and homogeneity of variance, respectively. Variables that violated the Shapiro-Wilk test or the Levene test were log transformed. Data met the assumptions of normality once log transformed,

however the assumption of homogeneity of variance remained violated. Although the assumption of homogeneity of variance was violated parametric statistics were applied. Mixed model statistical tests are robust to normality violations thus rationalizing proceeding with the log transformed data. Indices were compared among individuals by using a Repeated Measures ANOVA (RM-ANOVA) conducted in JMP Pro 14 (SAS Institute, Cary, NC). Treatment groups were analyzed using an RM-ANOVA to account for the repeated measurement of femur and tibia. A Tukey's Honest Significance Differences (HSD) post-hoc pairwise comparison test revealed any significant differences between the treatment groups.

IV. Results

Due to this project being a part of a larger ongoing project in Dr. Janna Andronowski's lab results cannot be reported at this time.

V. Discussion

Due to this project being a part of a larger ongoing project in Dr. Janna Andronowski's lab the discussion was excluded at this time.

VI. Conclusion

Due to this project being a part of a larger ongoing project in Dr. Janna Andronowski's lab the conclusion was excluded at this time.

VII. Acknowledgements

I would like to thank Dr. Henry Astley and Dr. Brian Bagatto for taking the time to review my writing and provide feedback. In addition, I would like to thank Dr. Mary Beth Cole for her assistance in producing the image processing workflows. Lastly, I would like to thank Dr. Janna Andronowski for allowing me the opportunity to conduct this research in her lab as well as taking the time to teach me the process of scientific writing.

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