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ENDOTHELIAL CELL INJURY UNDER HIGH FREQUENCY VIBRATION IN THE RAT-TAIL MODEL

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ABSTRACT

Hand-Arm Vibration Syndrome (HAVS) consists of vascular, sensorineural and musculoskeletal disorders and affects around 1.7-5.8% of industrial workers. In this study, a rat-tail vibration injury model is used to assess early vascular damage due to HAVS, manifested in the form of endothelial cell vacuolation and oxidative injury. Tails were vibrated at two frequencies 125Hz and 250Hz for 4hr/day for 1 and 5 days (49m/s^2). Hematoxylin and Eosin (H&E) staining was done to assess gross changes in artery sections and toluidine blue stain was done for vacuole counting. Immunohistochemical (IHC) methods were used to detect Nitrotyrosine, a potent biomarker of cell inflammation and oxidative stress. The vacuole count in Endothelial Cells (ECs) was not statistically significant after 1 and 5 days for any frequency. However IHC images showed significant oxidative damage in Endothelial Cells (ECs) with considerable oxidative damage being induced as early as 1 day for both 125Hz and 250Hz frequencies, with more EC damage induced by 250Hz frequency after 5 days. These findings indicate that higher frequency vibrations can cause severe oxidative damage to EC.

INTRODUCTION

Hand-Arm Vibration Syndrome (HAVS) is a complex interaction of vascular, sensorineural and musculoskeletal disorders, currently affecting 8-10 million industrial workers in USA. Rat-tail vibration model has been validated to mimic the biodynamic response of finger tissue. The current ISO 5349 (2001) standard sets vibration exposure guidelines based on frequency weighting, which underestimate the injury related to high frequency vibrations >100Hz.Several researchers have reported vibration-induced vascular damage [1-3, 5] but only recently has the focus shifted to high frequencies (125Hz, 250Hz) as they lie in the resonance frequency of fingers when maximum power is absorbed from the vibrated tool. Increased NT levels have been detected in several pathologies (cardiovascular, renal, and pulmonary) in which endothelial dysfunction plays a key role. The pattern of NT staining can provide significant insight about the onset of vibration-induced vascular damage. This study aims to assess the histological and pathological implications (oxidative stress) of mechanical vibrations by analyzing the distribution and degree of vacuolation and NT immunostaining in the ECs of rat-tail artery with time at high frequencies.

METHOD

Experimental Setup. Male Sprague-Dawley rats (250±15g) were used in this study and were randomly assigned to 5 groups: nonvibrated control group (n=2), 1-day vibrated at 125 Hz (n=1), 1-day vibrated at 250Hz (n=1), 5-day vibrated at 125Hz (n=1) and 5-day vibrated at 250Hz (n=1). The rats were placed in acrylic Broom style restrainers and the tails were strapped to the platform (Fig.1A) and subjected to an unweighted acceleration of 49m/s^2 rms.

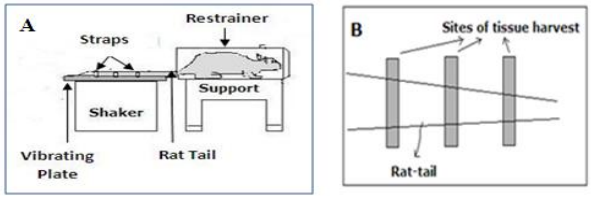

Figure 1. Schematic of the rat placed in restrainer for vibration (A) and sites of tissue harvest (B)

Tissue Processing. The ventral artery was isolated from sections shown (Fig. 1B) after euthanasing the rat and immersion-fixed in 4% paraformaldehyde. All procedures used were in compliance with the IUCAC (Institutional Animal Care and Use Committee). Three sets of tissue were processed for H&E staining, Toluidine Blue Staining and Immunostaining (NT).

Vacuole Counting. Semithin artery sections (1µm) was used for toluidine blue staining and vacuoles (2-12µm in size) were counted [1] in endothelium sections using ImageJ software (NIH, Bethesda,MD). For every rat, vacuoles were counted in five randomly sampled sections.

Immunohistochemistry. Vessels were fixed, paraffin-embedded and sectioned and then immunostained for mouse anti-NT antibody (Santa Cruz Biotechnology, 1:100 dilution) and biotin secondary antibody and then viewed under light microscope. Quantification of NT immunostaining intensity was done in Adobe Photoshop CS2, using ten random samplings of 10x10 pixels each, based on method described elsewhere [4].

Statistical Analysis. Minitab 15.0 was used to perform one-way ANOVA and Tukey's tests to compare means of different groups. All values are reported as mean ± SD. A p-value of 0.05 or less was considered significant.

RESULTS AND DISCUSSION

H&E staining. Vasoconstriction in the lumen [1] is clearly visible after 250Hz vibration but the EC damage is not clearly discernible in histological images of arterial sections (Fig.2).

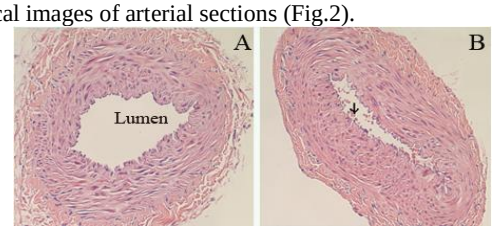

Figure 2. Photomicrographs of artery sections of rats vibrated at 250 Hz for 1 day (A) and 5 day (B). (*H&E, X200. Arrow shows platelets inside lumen)*

Vacuole Count. Light microscopic images of Toluidine Blue stained sections of arteries are shown (Fig.3A,3B).The EC vacuoles are not statistically different between Control, 125Hz and 250Hz group after 5 days (Fig.3C).

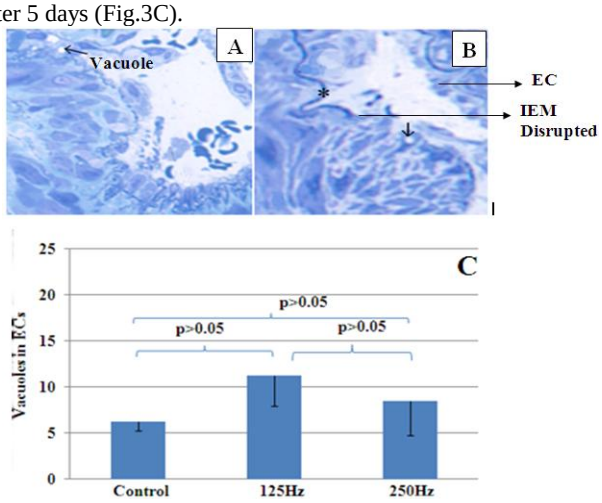

Figure 3. Photomicrographs showing ECs vacuoles (arrows) at 250Hz after 1 day (A) and 5 day (B) of vibration. (Original Magnification 400X).

Disruption of Internal Elastic Membrane(IEM, shown by *) was observed in 250Hz vibrated rats after 5 day(Figure 3B), but vacuolation alone does not give a complete assessment of intensity of vibration induced EC damage.

Immunohistochemistry. Quantification of immunostained section of arteries (Barplot in Fig. 4) shows that the damage is more for higher frequency of 250Hz after 5 days in ECs**.** We also used *anti-rabbit NT antibody* to confirm our findings of localization of signal in endothelial layer (data not shown).

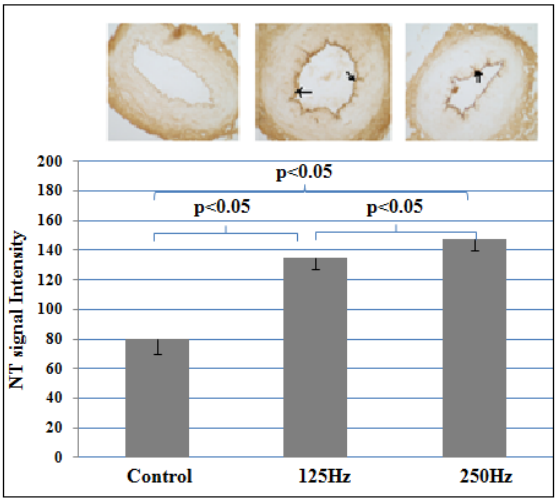

Figure 4. The photomicrographs show Nitrotyrosine staining localized in endothelium (shown by arrows)in the arteries of rats(Original Magnification 200X) exposed to control, 125Hz and 250Hz vibration for 5 days, with their corresponding NT signal quantification in the plot.

CONCLUSIONS

The present study demonstrates that EC injury in the form of vacuolation was not significantly different for between Control, 125Hz and 250Hz group. However, NT immunoreactivity was highest for 250Hz group after 5 days in ECs in the rat-tail artery. In conclusion, histological evaluation and vacuole counting alone do not give accurate assessment of EC damage under vibration at higher frequency; hence immunohistochemical expression of NT is warranted to accurately quantify oxidative injury. Vibration-induced oxidative injury was frequency dependent hence HAVS guidelines underestimate the risk of vibration frequency exposures. More number of animals are being used to further leverage the findings and to compare the temporal variation of ECs oxidative damage after 10 days or more.

ACKNOWLEDGMENT

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