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VIBRATION DISRUPTS THE ENDOTHELIAL BARRIER OF RAT-TAIL ARTERIES
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Introduction

We have utilized the rat-tail vibration model to study the acute effects of vibration and explore mechanisms for vascular pathology in HAVS. We reported previously that 4-hr continuous vibration produces vasoconstriction in rat-tail arteries, tight folding and breaks in the internal elastic membrane, and endothelial cell vacuolization injury.¹,² The present study investigated whether the vasoconstriction and cell damage caused by a single 4-hr vibration exposure disrupts the integrity of the endothelial barrier.

Methods

Morphometric evaluation: Male Sprague-Dawley rats, weighing 275-300 gm had their tails vibrated at 60 Hz, 49 m/s² acceleration for 4 hr and were allowed to recover for 0, 1 or 7 days (Immediate, 1d and 7d survival groups), using our rat-tail model.³ Control sham rats were treated similar to the vibration immediate group except not exposed to vibration. Tail arteries were aldehyde fixed, embedded in epoxy resin and semithin cross sectioned (0.5 µm) and stained for morphometric analysis. Vasoconstriction was determined by lumen size measurement using version 1.28v Image J software (NIH). The total numbers of endothelial vacuoles were counted to assess cell injury.

Evan’s Blue (EB) functional assay: To test if vibration disrupts the barrier function of the endothelial layer in the tail arteries, 32 rats were randomly assigned to EB-sham, EB-immediate, EB-1d survival and EB-7d survival groups and treated as described above. Following vibration, rats were anaesthetized, and EB (20 mg/kg body wt) was injected into the systemic circulation via the liver blood supply. After 15 min, a systemic arterial blood sample was collected via cardiac puncture for measurement of the circulating EB concentration. The serum was separated and stored for spectrophotometric assay of EB content using an EB albumin standard curve. Rats were euthanized and perfused with phosphate buffer to clear all EB from the vascular network, and the tail arteries were removed and quick frozen for microscopic and biochemical analysis. A weighed segment of artery was incubated in 100 µl of formamide for 24 hr at 4º C to extract EB. The dye content in the formamide was measured spectrophotometrically at the wavelength of 595 nm and normalized to the EB serum content for the same animal.
Results

Figure 1: Compared to sham exposure, vibration caused a significant decrease in lumen size immediately following exposure (** p<0.01). Vasoconstriction persisted 1 day post-exposure but was not different from sham at 7 days. Vibration increased endothelial cell (EC) vacuoles, a injury index, immediately following exposure compared to sham (**p<0.001). Vacuoles persisted in significant numbers in the 1 day survival group (* p<0.05 compared to sham and 0 day). At day 7, vacuole numbers were not different from sham.

Figure 2: Extravasation of EB into the artery (arrows) was detected on day 0 by immunofluorescence imaging of artery cross sections and confirmed quantitatively by spectrophotometric measurement of EB content in artery extracts (**p<0.01). EB levels were not different from sham by day 1 recovery, even though EC vacuoles persisted.

Conclusions

1. A 4 hr exposure to vibration causes persistent vasoconstriction and endothelial cell injury which are reversed by 7 days.
2. Immediately post vibration, the endothelial barrier was breached because circulating EB dye entered the artery wall. The barrier was restored within 24 hr.
3. A single bout of vibration induces arterial pathologies of reduced lumen size and break down of the endothelial barrier. If daily repeated vibration exposure were to sustain these pathologies, the long term consequence would be reduced blood flow and vascular fibrosis predicted for vibration white finger.

References