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Research paper

Effect of low-frequency but high-intensity noise exposure on swine brain blood barrier permeability and its mechanism of injury

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ABSTRACT

Objectives: Vibroacousitic disease (VAD) is caused by excessive exposure to low-frequency but high-intensity noise. The integrity of the brain blood barrier (BBB) is essential for the brain. The study aimed to investigate the effect of noise exposure on the BBB.
Methods: Healthy male Bama swine were exposed to 50, 70, 100, and 120 Hz, 140 dB noise for 30 min. After exposure, CT brain imaging and ex vivo fluorescent imaging of parenchymal EB leakage were performed (each group consisted of N = 3 swine). The human cerebral microvascular endothelial cells were exposed to 70 Hz, 140 dB noise for 5 min.
Results: The BBB permeability assay showed that 50, 70, and 100 Hz with 140 dB noise exposure accelerated BBB permeability, and the BBB opening at 70 Hz was most serious and reversible. Additionally, CT images demonstrated that the noise-induced opening of the BBB caused no intracerebral hemorrhage. This noise-induced BBB opening was related to the downregulation of zo-1 and occludin. Finally, cysteinyl leukotriene receptor 1 (CysLT1 receptor) was found to regulate noise-induced tight junction defects in vitro.
Conclusions: In conclusion, noise exposure accelerates the formation of a high-permeability BBB with leaky tight junctions through a CysLT1-mediated mechanism, which warrants additional research.

1. Introduction

Vibroacoustic disease (VAD) is a noise-induced, cumulative pathology, caused by excessive and undue exposure to low-frequency but high-intensity sound. If exposure to noise exceeds certain levels, then negative health outcomes can be observed. These negative outcomes have been identified in environments with high-intensity noise over 110 dB (dB) coupled with frequencies below 100 Hz (Hz), such as aeronautical technicians, military pilots, cabin crew members, disc-jockeys, etc [1]. Evidence of the non-auditory effects of environmental noise exposure on public health is growing [2]. Observational and experimental studies have shown that VAD mainly affects brain functions and causes a series of central nervous system (CNS) symptoms such as dizziness, severe vertigo, non-convulsive neurological deficits and late-onset epilepsy [1,3], although the specific neuropathological changes of human VAD remain to be clarified [4].

The blood–brain barrier (BBB) provides both anatomical and physiological protection for the CNS, strictly regulating or impeding the entry of many substances and blood-borne cells into nervous tissue [5]. An increase in BBB permeability causes vasogenic brain edema and aggravating neuronal injury [6]. In the CNS, blood vessels are separated from the brain by the BBB and the lumen is vulnerable to sonic wave-induced damage [7]. Low-frequency but high-intensity noise may increase the permeability of the BBB, and noise-induced inflammatory proteins in the peripheral blood become toxic to the CNS. Several studies have shown that low-frequency but high-intensity noise exposures induce CNS impairment, such as axonal degeneration [8], excessive microglial activation, pro-inflammatory cytokines, neuron death [9]. and apoptotic cell death [10]. Noise-impaired learning and memory abilities were also improved in several rodent models [10]. However, less research has focused on sound-induced BBB damage. Focused ultrasound has been proven to have great utility for drug delivery by opening the BBB [11], with the activation of astrocytes and microglia [12] or an increase in hippocampal neurogenesis [13]. Focused

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ultrasound-mediated BBB opening has demonstrated that the BBB is susceptible to sound waves. Notably, studying the characteristic parameters of noise that cause damage to the BBB is still an intractable research subject. Additionally, the degree of noise-induced BBB impairment and the mechanism of injury still remain unknown. Because of the emerging association between BBB function and noise exposure, we performed this study to determine whether the exposure to low-frequency but high-intensity noise resulted in normal or abnormal structure and function of the BBB in Bama miniature swine whose anatomical structures are similar to those of humans.

2. Subjects and methods

2.1. Animals

Healthy male Bama miniature swine are provided and fed by the Department of Zoology, Third Military Medical University. Swine were randomly divided into a noise-exposure group (50 Hz, 90 Hz, 100 Hz, or 120 Hz in 140 dB) and control group (noise-free group). Each group consisted of 3 swine. Before sound exposure, both groups were under anesthesia induced by 1 mg/kg nembutal (2% solution, Sigma-Aldrich, St. Louis, MO). The maintenance and care of experimental animals and all procedures described comply with the Animal Ethics and Wellbeing Committee of Zhengzhou University and Third Military Medical University, China.

2.2. The noise device

The low-frequency sound generation system (College of Electronic Science and Engineering, National University of Defense Technology, Changsha, China) in the present study included a high-pressure siren (acoustic power 10 kw, frequency between 50 Hz and 300 Hz) driven by three air compressors (atlas XAVS900) and an exponential horn (cut-off frequency 35 Hz). The high-intensity acoustic chamber consisted of a large standing wave tube with cross-sectional area of 0.7225 m^2. A microphone (B & K 4941) was placed in the chamber and is connected with a data collection system (B & K 3050). Noise with a frequency of 50–120 Hz and a pressure level of 140 dB was used in this study. The frequency and pressure levels were maintained for 30 min in animal exposure experiments and 5 min in cell exposure experiments and were monitored by the data collection system.

2.3. BBB permeability assay

We performed a BBB assay using Evans blue (EB). The methods employed for EB injection and collection of samples were performed as reported in the previous study [14]. Two hours before sacrifice, swine were anesthetized and injected via the carotid artery with 80 mg/kg EB (Sigma-Aldrich, St. Louis, MO) using a 2% solution of EB in saline solution (0.9% sodium chloride; Kelun Pharmaceutical, China). Immediately after sacrifice, the pigs were perfused with cold saline solution containing sodium heparin (10 IU/mL, Chengdu Hepatunn Pharmaceutical, China). Firstly, the EB staining images of brains were obtained using the Pre-clinical In Vivo Image System (IVIS Spectrum) (Caliper Life Sciences, USA). Fluorescence images were retrieved by measuring the spectrum with different excitation wavelengths at 620 and 740 nm and using a filter of 535 nm. Data were analyzed using Living Image 3.2 software (Caliper Life Sciences, USA). Secondly, the concentration of EB in the brain was determined spectrophotometrically at 620 nm. Lastly, brains were processed for histological evaluation and then were examined by fluorescence microscope. The sections responsible for EB uptake were investigated because of the advantage of light EB presence. Unstained sections are observed by using bright field microscopy, and red auto fluorescence of EB was observed on the same slides using the excitation and emission filters for rhodamine fluorescence (Zeiss D-7082 inverted fluorescence microscope, Zeiss, Oberkochen, Germany).

2.4. Computed tomography (CT)

A head CT scan was done for swine at 0, 36 and 84 h post-noise (70 Hz,140 dB) using a CT scanner (Philips, Amsterdam, Holland).

2.5. Cell culture

The human cerebral microvascular endothelial (hCMEC/D3) cells [15] were plated into a 6-well plate coated with 0.1 mg/ml rat tail collagen type I (BD Biosciences) for 1 h, at 37 °C. For culture, the cells were seeded at a concentration of 27,000 cells/cm^2 and grown in EBM-2 medium (Lonza, Basel, Switzerland) supplemented with the EGM-2-MV bullet kit (Lonza, Basel, Switzerland) containing vascular endothelial growth factor, R3-insulin-like growth factor-1, human epidermal growth factor, human fibroblast growth factor-basic, hydrocortisone, 2.5% fetal bovine serum and 100 μg/ml penicillin/streptomycin. Cell culture medium was changed every 2-3 days. Cells were maintained at 37 °C under an atmospheric pressure of 5% CO_2. When indicated, confluent cells were treated with 20 nM leukotriene D4 (LTD4) in deuterium-depleted (dd) water for 24 h, with 0.6 μM montelukast sodium in (dd) water for 24 h, or with noise exposure for 10 min.

2.6. Western blot analysis

Swine hippocampus and cultured cells were collected after noise exposure experiments. Total protein was extracted using RIPA lysis buffer (PBS, 1% NP40, 0.5% sodium deoxycholate, 0.1% SDS, 0.25 mM PMSF, 5 mg/ml aprotinin, and 1 mM sodium orthovanadate). Proteins were extracted using a compartment protein extraction kit (Millipore, Massachusetts, USA) according to the manufacturer's instructions. The concentrations of protein extracts were determined using the BCA protein assay kit (Beyotime, Beijing, China). After electrophoresis in 10% sodium dodecyl sulfate, sodium salt (SDS) polyacrylamide gels, proteins in the gels were transferred onto nitrocellulose membranes and were incubated with the following antibodies: anti-zo-1 (1:1000, Abcam), anti-occludin (1:1000, Santa Cruz), anti-CysLT1 Receptor (1:500, Santa Cruz), anti-CysLT2 Receptor (1:500, Santa Cruz), or anti-β-actin (Santa Cruz Biotechnology). The membranes were washed and then incubated with appropriate secondary antibody conjugated to horseradish peroxidase. Immunoreaction signals were visualized with ECLTM or ECL plus TM. Western blotting detection reagent and exposed to the LAS-3000 Mini Bio-imaging Analyzer System (Clubio, Gel Catcher2850). Signal intensity was determined using MultiGauge software (FUJIFILM). The data were normalized to β-actin expression and further normalized to the control.

2.7. Statistical analyses

All values are displayed as means ± standard error of the mean (SEM) (n = 3). For statistical analysis, GraphPad Prism 5.0 (GraphPad Software Inc., San Diego, CA, USA) was used. The statistical significance of differences among groups was determined by analysis of variance (ANOVA) followed by Dunnett's Multiple Comparison Test. Differences between two groups were analyzed with Student's *t*-test, and two-way ANOVA with repeated measures was used to analyze the difference between the co-treatment and the single treatment in cell experiments. For all tests, differences with $P < 0.05$ were considered significant.