International Journal of Pediatric Otorhinolaryngology 78 (2014) 995–999

Contents lists available at ScienceDirect

International Journal of Pediatric Otorhinolaryngology

journal homepage: www.elsevier.com/locate/ijporl

Observation of permeability of blood–labyrinth barrier during cytomegalovirus-induced hearing loss

Xuanyi Li [a,1], Xi Shi [b,1], Yuehua Qiao [a,b,*], Kailin Xu [c,*], Lingyu Zeng [c], Caiji Wang [a], Zhou Xu [d], Haichen Niu [b]

[a] Department of Otolaryngology, The Affiliated Hospital of Xuzhou Medical College, Xuzhou 221006, China
[b] Institute of Audiology and Speech Science of Xuzhou Medical College, Xuzhou 221006, China
[c] Laboratory of Transplantation Immunity of Xuzhou Medical College, Xuzhou 221006, China
[d] School of Basic Education Science of Xuzhou Medical College, Xuzhou 221006, China

ARTICLE INFO

Article history:
Received 29 November 2013
Received in revised form 17 March 2014
Accepted 18 March 2014
Available online 27 March 2014

Keywords:
Cytomegalovirus
Blood–labyrinth-barrier
Permeability
Inflammation

ABSTRACT

Objective: Congenital cytomegalovirus (CMV) infection is the most common infectious cause of sensorineural hearing loss in children. This study aims to investigate the pathogenesis CMV-induced hearing loss from the view of integrity of blood–labyrinth-barrier (BLB).
Methods: Newborn BALB/c mice were randomly divided into three groups ($n = 22$, respectively): CMV group, control group and normal group. The CMV group and control group were intracerebrally injected with equal volume (15 μl) of murine CMV (MCMV; 10^4 IU/0.1 ml) and PBS, respectively, and normal group did not receive any treatment. After three weeks, auditory-evoked brainstem response was assessed, and permeability of BLB was evaluated by Evans blue method. Means between groups were compared using *t*-test.
Results: We observed that mice injected with MCMV had a hearing loss and it was connected with the permeability changes of BLB. Besides, using hematoxylin–eosin staining, we noticed hyperaemia in stria vascularis and spiral ligament and bleeding in scala vestibule and scala tympani in CMV group.
Conclusion: All these data indicated the possible association between CMV-induced hearing loss and BLB dysfunction with the characteristics of inflammation. Our data provide a possible path to investigate the mechanism of CMV-induced hearing damage.

1. Introduction

Cytomegalovirus (CMV), a member of the herpes virus group, is the most significant infectious cause of developmental disorders induced by intrauterine infection in humans, with an average incidence of 1% of all live births [1,2]. It is assessed that 10% of infected infants have asymptomatic congenital infection, and will subsequently suffer from brain disorders, including sensorineural hearing loss (SNHL), mental retardation, visual disorders, epilepsy, and seizures [3,4], and about 5–10% of infected infants have symptomatic congenital CMV infection at birth with the disabilities of microcephaly, periventricular calcification, and microphthalmia [5–7]. Annually, billions of dollars cost by providing specialized services for surviving infants and children with congenital CMV infection [8]. Thus the urgency and essentiality of learning the mechanisms of CMV-induced hearing loss is beyond doubt.

During CMV infection, it is reported that neutrophils and macrophages are recruited as primary innate defense mechanism against viral infection, which play important roles in controlling virus proliferation and dissemination [9]. Macrophages aid in tissue repair, cytokine production, and removal of cytopathic debri, but they can also be harmful to neighboring cells by their overactivation and the production of reactive oxygen species (ROS). Increases in macrophage ROS have been described during acoustic inner ear trauma and contributed to the lethality of spiral ganglion neurons (SGNs) and hair cells in the mammalian cochlea [10]. Still, very little is known about the mechanisms of pathogenesis of CMV-induced hearing loss.

The normal conduction of auditory signals is dependent on the normal physiological functions of auditory pathway which further based on the orderly and strict work of the microcirculation and

* Corresponding authors. Address: No. 99 Huaihai West Road, Xuzhou 221006, China. Tel.: +86 0516 85802300; fax: +86 0516 85802121.
E-mail address: oto8558@163.com (Y. Qiao).
[1] These authors are co-first authors.

http://dx.doi.org/10.1016/j.ijporl.2014.03.013
0165-5876/

blood–labyrinth barrier (BLB). The cochlear BLB, located in the stria vascularis, is essential for cochlear solute homeostasis and prevents the influx of toxic substances into the inner ear [11,12]. Destruction of the BLB is associated with the pathogenesis of a number of hearing disorders, such as Meniere's disease, autoimmune inner ear disease, meningitis-associated labyrinthitis and other genetic diseases [13–16]. In our study, we propose a hypothesis that CMV inflection might affect the integrity of BLB, and further induce the destruction of the microcirculation and internal environment homeostasis. Using auditory brainstem response (ABR) audiometry, we found the latency period of wave I of mice infected with murine CMV (MCMV) was lengthened and the wave amplitude was reduced as well as the auditory threshold was increased as compared with those in mice injected with PBS or mice in normal control group. Besides, permeability of BLB in MCMV infected group was much higher than that in normal control or PBS injected group. Our data might provide some insights into the general mechanism underlying CMV-induced hearing loss.

2. Materials and methods

2.1. Cells and virus

The MCMV (Smith strain; originated from Beijing Air Force General Hospital, PLA) passaged in mouse embryonic fibroblast (MEF) 3T3 cells were kindly provided by Microbiology Laboratory of Shandong Academy of Medical Sciences, Shandong, China. The virus was quantified by plaque assay with MEF and 50% tissue culture infective dose ($TCID_{50}$) was calculated as 10^4 IU/0.1 ml.

2.2. Experimental animals and intraperitoneal infection

Newborn, specific pathogen-free (SPF) BALB/c mice were purchased from the Laboratory Animal Center of Xuzhou Medical College, Jiangsu, China. The CMV-induced hearing loss model was established as reported previously [17]. Briefly, the neonatal mice were randomly and equally divided into three groups (22 mice per group): CMV group, control group and normal group. Mice in CMV group were subjected to intracerebral inoculation with 15 μl MCMV per mouse within 24 h after born. Mice in control group were intracerebrally injected with equal volume of phosphate buffered saline (PBS) per mouse within 24 h after born, and mice in normal group did not receive any treatment. All animals received breast feeding and were daily examined for their developmental status. All animal experiments were approved by the Biomedical Research Ethics Committee of Xuzhou medical college.

2.3. ABR audiometry

Three weeks after intracerebral inoculation of MCMV, ABR were obtained under inhalational ether anesthesia as described previously [18]. The active electrodes were inserted subcutaneously beneath the mastoid process of the measured ear, the reference electrodes were inserted beneath the skin on the top of the head and the ground electrodes were inserted into the toe. The following parameters were recorded: short sound and rarefaction wave stimulation, 13 Hz frequency, 80 dB intensity, 2000 repetition rate, 200 removing width, 1 ms/D scanning rate, and 2 μV/D sensitivity [17]. The auditory thresholds were started at 90 dB or 100 dB sound pressure level (SPL) and gradually decreased 10 dB SPL steps. The threshold was defined as the lowest intensity level at which a clear waveform was visible in the evoked trace and was determined by visual inspection of the responses [19]. The latency period of wave I, amplitude and threshold in each group were recorded.

2.3.1. Detection of permeability of BLB by Evans blue tracer assay

Twenty mice in each group were subjected to test permeability of BLB using Evans blue (EB) tracer assay. Mice were anesthetized by injecting with 10% chloral hydrate (35 ml/kg body weight). Evans blue dye (2%, 40 mg/kg; Sigma Chemical Co., St. Louis, MO, USA) was injected into the tail vein of mice. Two hours later, mice were carried out thoracotomy. Heart perfusion was performed with normal saline until a colorless liquid was observed to flow out. Then mice were sacrificed by decollation and acoustic vesicles of both sides were taken out. Acoustic vesicles were opened to expose the cochleas. Cochleas was weighted and added 1 ml formamide and further placed in 50 °C water-bath for 24 h. Evans blue was quantified in the cochlea by spectrofluorophotometer (excitation wavelength 620 nm, emission wavelength 680 nm).

2.3.2. Hemotoxylin and eosin staining

The remaining two mice were performed hematoxylin and eosin (HE) staining. After dissection of the bone tissues surrounding cochlea, cochlea was fixed in 10% formaldehyde for 3 days, decalcified in PBS containing 10% ethylendiaminetetraacetic acid (EDTA) for 7 days, and embedded in paraffin. The specimens were then sectioned, mounted, and stained for microscopic examination.

2.4. Statistical analysis

The data regarding ABR wave I and wave amplitude (mean ± standard deviation) were analyzed using SPSS13.0 software and compared between groups using *t*-test. All P values <0.05 were considered as statistically significant.

3. Results

3.1. General status of mice in each group

The health status was observed after treatment. Mice in control group and normal group were presented with smooth and shiny body hair and active reflexes, while mice in the CMV group had dull, messy hair and slower reflexes.

3.2. Detection of the hearing status through ABR audiometry

Auditory thresholds of mouse are considered mature at 3 weeks age [20]. At 3 weeks after injection, the hearing thresholds measured using ABR were determined for mice from each group. We observed that the latency period of wave I of mice infected MCMV was significantly lengthened, and the wave amplitude was significantly reduced as well as the auditory threshold was significantly increased as compared with those in control group and normal group (Fig. 1 and Table 1). The data provided us some tips that MCMV induced a loss or injury of hearing.

3.3. Evaluation of the permeability of BLB

In our study, we wondered whether CMV-induced hearing loss was associated with the permeability of BLB. By measuring the EB content of the cochleas, we observed the content of EB in MCMV

Table 1
Detection of the hearing status through ABR audiometry ($\bar{X} \pm SD$).

Groups	Number	Latency of wave I (ms)	Wave amplitude (uv)	Auditory threshold (dB)
CMV group	20	$1.92 \pm 0.13^*$	$1.80 \pm 0.38^*$	$61.87 \pm 4.47^*$
Control group	20	1.29 ± 0.13	4.82 ± 0.24	23.84 ± 3.93
Normal group	20	1.32 ± 0.13	4.79 ± 0.29	24.10 ± 2.70

SD, standard deviation.
* $P < 0.05$ compared with group normal or injected with PBS.