World Journal of Acupuncture – Moxibustion 28 (2018) 55–58

Contents lists available at ScienceDirect

World Journal of Acupuncture – Moxibustion

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

Experimental Research

The effect of acupuncture at Jǐngjiājǐ (颈夹脊) on the repair and regeneration of cochlear hair cells of rats with sensorineural deafness☆

Zhongtai LI (李种泰)*, Lina CUI (崔丽娜)

Department of Acupuncture–Moxibustion, Yanbian University Hospital, Yanji 133000, Jilin Province, China (延边大学附属医院针灸科, 吉林延吉 133000,中国)

ARTICLE INFO

Article history:
Available online 17 March 2018

Keywords:
Sensorineural deafness
Jǐngjiājǐ
ABR
DPOAE
Hair cell

ABSTRACT

Objective: To observe the effect of acupuncture at Jǐngjiājǐ (颈夹脊) on the repair and regeneration of cochlear hair cells of guinea pigs with sensorineural deafness.
Methods: Sixty healthy guinea pigs were selected, 20 guinea pigs were randomly assigned to the normal control group (group A), and other guinea pigs were randomly divided into model control group (group B) and acupuncture treatment group (group C) after injection with gentamicin sulfate in order to induce deafness. No intervention was given to the guinea pigs in group A and group B, and acupuncture at Jǐngjiājǐ was given to the guinea pigs in group C for 30 days. ABR threshold, DPOAE amplitudes and hair cells counting of guinea pigs in each group were recorded after intervention for 30 days.
Results: After intervention for 30 days, ABR threshold in group C was significantly lower than that in group B (38.46 ± 7.36 vs 82.94 ± 6.47, $P<0.01$), and the DPOAE amplitudes in group C were obviously higher than that in group B (28.06 ± 5.64 vs 25.23 ± 5.38, $P<0.01$). The number of cochlear hair cells in group C increased significantly, over 50% of the hair cells survived, accounting for 66.67% of the observation cases. The number of cochlear hair cells in the 3rd and 4th gyri was close to the normal level, and plenty of proliferous sustentacular cells can be seen. Compared with group B, the number of cochlear outer hair cells in each gyrus in group C significantly increased (36.76 ± 1.97 vs 28.59 ± 2.24, $P<0.01$), indicating that acupuncture at Jǐngjiājǐ can promote the repair and regeneration of cochlear hair cells.
Conclusion: Acupuncture at Jǐngjiājǐ can promote the repair and regeneration of cochlear hair cells, thus improving the hearing of guinea pigs with deafness.

Introduction

Sudden deafness refers to the sudden sensorineural hearing loss with unclear etiology. The hearing may reduce by more than 30 dB in several minutes, several hours or 3 days [1]. It is one of the otolaryngology department emergencies with the least ideal curative effect, and its incidence is on the rise globally. Accounting for 2% of the first-visit patients in ENT department [2], the incidence will increase along with age, and the disease seriously affects people's normal life, which has become a public health problem in the world. The etiology of sudden deafness has not been clear at present. Many theories have been proposed, such as virus infection, inner ear microcirculation disturbance, endothelial dysfunction, autoimmunity, membranous labyrinth rupture and so on. The pathological cause of sudden deafness lies in the damage or degeneration of hair cells and spiral ganglion. Therefore, the study of cochlear hair cells regeneration is of great significance in treatment of sensorineural deafness. People used to think that hair cells could not repair once they were damaged. However, studies have shown in recent years that not only the avian cochlear hair cells can regenerate after injury [3], but also the cochlear hair cells of mammals are provided with certain regeneration capacity [4]. In this study, whether acupuncture can promote the repair and regeneration of damaged hair cells or not was explored through observing the cochlear hearing and hair cells changes in guinea pigs at different times after gentamicin poisoning.

Materials and methods

Experimental animals grouping and ototoxicity models building

Sixty clean and healthy guinea pigs (provided by the Experimental Animal Department of Yanbian University Medical College, SCXK (Jilin) 2011–0007) were selected, including 30 male guinea pigs and 30 female guinea pigs, and the weight was 300–400 g. These guinea pigs were provided with sensitive auricle reflex and normal tympanic membrane according to otoscopy, and the thresh-

☆ Supported by The Education Department of Jilin Province, "12th Five-year Plan" science and technology research planning project: J.J.K.H.Z. [2015] No. 33.
* Corresponding author.
E-mail address: lzt6796@163.com (Z. LI).

https://doi.org/10.1016/j.wjam.2018.03.010
1003-5257/

old of auditory brainstem response (ABR) was less than 30 dBnHL. Under the same conditions, the animals were fed in the screening animal experiment center with quiet environment and suitable temperature and humidity. After adaptive feeding for one week, 20 guinea pigs were randomly assigned to the normal control group (group A). Other guinea pigs were injected with gentamicin sulfate (Humanwell Healthcare, Yichang) in the dosage of 80 mg/kg for once a day. After injection for 7 days, the guinea pigs with absent auditory brainstem response (ABR) or the threshold shift before and after deafness more than 60 dBnHL were included in model group (n = 40). Among which, 20 guinea pigs were randomly included in model control group (group B), and other guinea pigs were included in acupuncture treatment group (group C). Intervention treatment was started. All the animal experiments followed the relevant provisions of laboratory animal management in Yanbian University Hospital.

Intervention methods

The guinea pigs in group A and group B were fed with normal diet, and fixed in the fixation shelf for 25 min in prone position for once a day to build a similar environmental stimulus to group C. In group C, the guinea pigs were fixed in the fixation shelf in prone position. By reference to *Experimental Acupuncture Science*, Jǐngjiājǐ (颈夹脊) (C2-C7) was selected. After conventional disinfection, Jǐngjiājǐ was needled by adopting a disposable filiform needle (specification: 0.27 mm × 15 mm). The needle tip was inserted toward the spine in an angle of 25–30° and with a depth of 0.5 cm. The needle was retained for 25 min, and twirling stimulus was given for once every 10 min. The treatment was conducted for once a day, and treatment for 30 days was needed.

Indices detection

(1) Detection of auditory brainstem response (ABR) threshold

SmartEP (produced by Intelligent Hearing Systems, US) was adopted to detecting the ABR threshold of anesthetized guinea pigs in the screened room. After anesthesia, the electrode of the needle was inserted into the calvarium as the recording electrode, the electrode in same side was reference electrode, and the contralateral electrode was grounded electrode. The stimulation rate was 19.1 m/s, with high pass of 300 and low pass of 3000, and average superposition of 256–512 times. The test sound intensity was 60dBnHL at the beginning, and reduced by 10 dB gradually. After closing to the threshold, it was reduced by 5 dB gradually. By taking stable HI wave with good repeatability as the benchmark, ABR threshold was determined via the average method reported by Donald Henderson [5].

(2) Detection of distortion product otoacoustic emission (DPOAE)

The intensity of two initial sounds was pure tone signal L1 = L2 = 70 dB SPL, f1/f2 = 1.22, and the range of geometric mean of f1 and f2 was 0.5–8.0 kHz. The DPOAE amplitudes at different stimulation intensities (1, 2, 4 and 6 kHz) were measured, and when the response amplitude was 3 dB larger than the background noise, the response was detected. The above tests were overlaid for 100 times.

Cochlear stretched preparation and counting

Under the state of anesthesia, the guinea pigs were decapitated rapidly, and the right auditory vesicle was taken quickly for extracting the cochlea. Under the dissecting microscope, a fiber needle was used to drill a hole in the cochlear spire, the vestibular membrane, round window membrane and oval window membrane were pricked, and the return bone bump was drilled to obtain a small hole. 0.5% silver nitrate solution was injected repeatedly from the hole in the spire to the hole in the return bone, then double distilled water was applied to wash for several times, finally, 10% formalin fixed solution was injected. After the specimens were exposed to sunlight for 2–4 hours, the basilar membrane was dissected and stretched preparation was made under dissecting microscope. Under the light microscope, the normal survival hair cells were counted from basal gyrus to parietal gyrus of the cochlea (normal outer hair cells show a clear and complete V-shape).

Table 1
Comparison of ABR threshold of the guinea pigs with sensorineural deafness after modeling and intervention for 30 days [dB nHL, mean ± SD(SE)].

Groups	Pigs	Ears	After modeling	After intervention for 30 days
A	20	40	20.24 ± 4.29	20.24 ± 4.29
B	20	40	86.45 ± 12.17	82.94 ± 6.47
C	20	40	89.72 ± 10.79	38.46 ± 7.36[a]

[a] Compared with group B, $P < 0.05$.

Statistical analysis

After all the experimental animals were coded with marks, they were randomly grouped by adopting SPSS22.0 software. Microsoft Office Excel 2011 was used for data entry, all the data were analyzed via SPSS22.0 software, and *t* test was applied. ABR and DPOAE thresholds as well as hair cells counting were expressed as mean ± SD (SE).

Results

ABR detection result

After modeling, ABR thresholds in group B and group C were significantly higher than that in group A (both $P<0.01$), indicating that the modeling was successful. The difference in ABR threshold between group C and group B was not statistically significant ($P>0.05$). Detection was performed again after intervention for 30 days, and the ABR threshold in group C was significantly lower than that in group B ($P<0.05$). The details are shown in Table 1.

DPOAE detection result

After modeling, DPOAE amplitudes at the frequency of 1, 2, 4 and 6 kHz in group B and group C reduced obviously (all $P<0.01$). The differences in DPOAE amplitudes at the frequency of 1, 2, 4 and 6 kHz between group C and group B were not statistically significant (all $P>0.05$). Detection was performed again after intervention for 30 days, and the DPOAE amplitudes at the frequency of 1, 2, 4 and 6 kHz in group C were significantly higher than that in control group (all $P<0.01$). The details are shown in Table 2.

Cochlear morphology and hair cells counting under the light microscope

Stretched preparation: the arrangement of gyri and outer hair cells in group A is regular and normal (Fig. 1a). In group B, cochlear hair cells in the 1st and 2nd gyri disappeared basically, only a few of hair cells were seen, the outer hair cells in the 1st row of the 3rd gyrus disappeared basically, and the hair cells in the 2nd and 3rd rows degenerated. Most hair cells were absent (Fig. 1b). In group C, the reproduction counting of cochlear hair cells increased obviously, and most cells in intermediate state can be seen with complete stereociliary bundle under the fine-tuning of microscope.