Original Article

Observation of vestibular compensation after unilateral labyrinthectomy in a rat model of type I insulin-dependent diabetes mellitus

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Received February 8, 2016; Accepted March 9, 2017; Epub May 15, 2017; Published May 30, 2017

Abstract: This study was aimed to explore the influence of diabetes duration on cervical vestibular-evoked myogenic potential (cVEMP) and vestibular compensation after unilateral labyrinth injury in streptozocin (STZ)-induced diabetic rats. SD male rats (with the weight of 300-350 g) were injected (55 mg/kg) with STZ intraperitoneally. At 4, 8 and 12 weeks after the induction of diabetes in model rats; cVEMP was detected by auditory evoked potential equipment (Audera V2.7, GSI, USA). Rats were anesthetized with 2% pentobarbital sodium (0.2 mL/100g i.p.). When recorded, the head of rat was lifted and moved forward and upward to achieve hyperextension of the neck muscles. cVEMP was detected before unilateral labyrinthectomy and four selected imbalance symptoms for scoring (head deflection, limb outreach, forced annular motion or roll and nystagmus) were recorded at different time points after surgery. The cVEMP results indicated that there were no significant difference in N1 latency periods (ms) in the Week 4 and Week 8 groups compared with that in the control group (P > 0.05), while the N1 latency period was significantly increased in the Week 12 group (P < 0.01). There were no significant difference in the amplitude between P1-N1 of cVEMP in the control group and diabetes groups (P > 0.05). There was no significant difference in the stimulation threshold and P1 latency period (ms) of the Week 4 and control groups (P > 0.05), while those of the Week 8 (P < 0.05) and Week 12 (P < 0.01) groups were significantly increased. There were no significant difference in the imbalance symptom scores in the Week 4 and Week 8 groups compared with those in the control group (P > 0.05), while the scores were significantly higher in the Week 12 group at 12 h after surgery or later (P < 0.05). These results suggested that the rate of abnormal cVEMP (increased stimulation threshold and extended wave latency) increased with the duration of diabetes course or uncontrolled blood glucose, although there was no significant difference in wave amplitude. Furthermore, longer diabetes course had a negative influence on vestibular compensation after unilateral labyrinthectomy, leading to extension of compensation time.

Keywords: Diabetes, vestibular compensation, vestibular, rat, cVEMP, labyrinthectomy

Introduction

Maintenance of physiological balance, especially maintenance and coordination of dynamic balance, requires the input of peripheral sensory information supported by vestibular and visual senses and proprioception, which are collectively known as the equilibrium triad. Specifically, these three reflex pathways are initiated at the vestibular end organs, which collect actuating signals from the head and body. These signals are transmitted to dendrites of first level bipolar vestibular ganglion cells and then to multiple subsets of vestibular nucleus neurons, which are distributed in the brainstem. The second level vestibular nucleus neurons transmit signals to the third level neurons, cortical functional area and spinal motor neurons to control gaze, position and equilibrium. The vestibulo-ocular reflex controls visual focus during movement by adjusting movement of six eye muscles. The vestibulo-spinal reflex controls body and limb movements to maintain position and equilibrium. Furthermore, some of the vestibular nucleus neurons project onto the oculomotor nucleus complex to connect with spinal nucleus veins through the medial longitudinal fasciculus, which may be associa-
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Characteristics of the vestibular signaling pathway. Movements of the head and eyes are influenced by multiple factors, including trauma, drugs, tumors, underlying diseases, such as diabetes, with approximately 10% of adult Chinese diabetes patients affected according to the latest statistics from the World Health Organization. Diabetes is associated with microangiopathy-related pathological changes, such as ocular fundus pathology and neuropathy. It has been reported that diabetes patients are at increased risk of auditory and vertigo-based diseases, with the annual incidence of falls in these patients (aged > 65 years) reaching 39% [1]. Based on detection of nystagmus, Liu et al. [2] reported vestibular dysfunction in 80.8% of 120 diabetes patients. Among them, vestibular dysfunction accounted for 66%, while central nervous system dysfunction accounted for 16.5%. The main influence of diabetes on equilibrium function is on perception, transmission and integration of vestibular and visual signals and proprioception. Lack of proprioception in diabetes patients is very common and microangiopathy and peripheral neuropathy are considered to be the main causes. Reports indicated an incidence of decreased proprioception in 15% of insulin-dependent diabetes patients and 13% of non-insulin-dependent diabetes patients [3]. Lack of proprioception can influence static and dynamic postural stability, and decreased proprioception is often accompanied with peripheral neuropathy. This leads to decreased peripheral nerve conduction velocity and perceptive function, as well as decreased perceptive function of the foot pressure sensor, ankle joint and plantaris motion sensor, further increasing the risk of falls [4, 5]. Diabetes can result in retinopathy, and the vestibulo-ocular reflex plays an important role in equilibrium regulation and maintenance as well as the development of vestibular compensation. Direct damage to vestibular organs is due to the characteristics of blood supply in the inner ear and microangiopathy caused by diabetes. Microangiopathy not only causes peripheral vestibular hypofunction, but also destroys the central vestibular system, which is often not detected until the damage is in an advanced stage. However, most clinical studies reported to date do not provide any clarification of the mechanism underlying the damaging effects of diabetes on the vestibular signaling pathway. Furthermore, variations among clinical studies in terms of patient age, gender, diet, education, and living environment, as well as the duration and type of therapeutic drug interventions, make it very difficult to identify appropriately matched control patients for comparison with diabetes patients. Although it is widely accepted that diabetes has a negative effect on the vestibular system, its influence on vestibular compensation after unilateral peripheral vestibular injury caused by disease or trauma remains to be determined. An improved understanding of the influence of diabetes on vestibular recovery is of critical importance in identifying effective therapies.

In the present study, the influence of diabetes on vestibular function and vestibular compensation was investigated by measuring cVEMP and imbalance symptom scores after unilateral labyrinthectomy in a rat model of diabetes induced by streptozotocin (STZ). This information will provide theoretical references for evaluation of the prognosis and recovery after vestibular injury in patients with diabetes.

Materials and methods

Animals and grouping

Male Sprague-Dawley (SD) rats (300-350 g; n = 60) with normal tympanic membrane and auricle reflex, were provided by the Animal Experiment Center of Southern Medical University (China). The rats were randomly divided into the control and diabetes groups, and the diabetes group was further divided into Week 4, Week 8 and Week 12 groups.

Rat model of diabetes

Preparation for diabetes model: SD male rats (n = 50) were maintained at 18-22°C with free access to food and water. After acclimatization for 1 week, STZ (Sigma-Aldrich, USA) was injected (55 mg/kg) intraperitoneally. After 1 week, blood glucose was detected in samples obtained from the caudal vein; successful diabetes model induction was confirmed by blood glucose > 16.7 mmol/L. Blood glucose measurements were repeated every 2 weeks. The failed animal models were excluded, and rats were added as required to maintain the number in each group.
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Preparation for unilateral labyrinthectomy: The rats were anesthetized by intraperitoneal (i.p.) injection of chloral hydrate (Reagent Center of Southern Medical University, China) (5 mL/kg). The skin behind the ear was prepared by disinfection and exposure of the area behind the anterior-auricle incision. The artery under the stapes was then identified and blocked. The stapes base plate was removed and the vestibular window was opened and disrupted with a hook-type device before injection of chloroform (Reagent Center of Southern Medical University, China) into the vestibular window through an angled hypodermic needle. Gelatin fragments were used to fill the vestibular window, and the incision was sutured to complete the model. The rats were placed under a light-source during recovery. After the recovery of consciousness, rats began to develop head deflection, limb stretching, rolling towards the side of the surgery and spontaneous nystagmus.

Recording of vestibular dysfunction

After the unilateral vestibular resection, dynamic changes in symptoms of imbalance were recorded at 0, 4, 12, 24, 48, and 72 h after surgery.

Detection of CVEMP threshold, latency period and amplitude of each wave

The cVEMP was detected by auditory evoked potential equipment (Audera V2.7, GSI, USA). Rats were anesthetized with 2% pentobarbital sodium (0.2 mL/100g i.p.). When recorded, the

<table>
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<tr>
<th>Table 1</th>
<th>Analysis of imbalance behavior scores in the different groups</th>
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<tbody>
<tr>
<td>Group</td>
<td>0 h</td>
</tr>
<tr>
<td>Control</td>
<td>8.00</td>
</tr>
<tr>
<td>Week 4</td>
<td>8.00</td>
</tr>
<tr>
<td>Week 8</td>
<td>8.00</td>
</tr>
<tr>
<td>Week 12</td>
<td>8.00</td>
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<tr>
<td>F</td>
<td>1.78</td>
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<tr>
<td>P</td>
<td>P &gt; 0.05</td>
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</table>

Note: Compared with the control group, *P < 0.05.

<table>
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<tr>
<th>Table 2</th>
<th>Comparison of cVEMP in the control and diabetes groups</th>
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<tbody>
<tr>
<td>Group</td>
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<tr>
<td>Control</td>
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<td>Week 8</td>
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<td>Week 12</td>
<td>20</td>
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<tr>
<td>F</td>
<td>25.24</td>
</tr>
<tr>
<td>P</td>
<td>P &lt; 0.01</td>
</tr>
</tbody>
</table>

Note: Compared with the control group, *P < 0.05.

Preparation for unilateral labyrinthectomy: The rats were anesthetized by intraperitoneal (i.p.) injection of chloral hydrate (Reagent Center of Southern Medical University, China) (5 mL/kg). The skin behind the ear was prepared by disinfection and exposure of the area behind the anterior-auricle incision. The artery under the stapes was then identified and blocked. The stapes base plate was removed and the vestibular window was opened and disrupted with a hook-type device before injection of chloroform (Reagent Center of Southern Medical University, China) into the vestibular window through an angled hypodermic needle. Gelatin fragments were used to fill the vestibular window, and the incision was sutured to complete the model. The rats were placed under a light-source during recovery. After the recovery of consciousness, rats began to develop head deflection, limb stretching, rolling towards the side of the surgery and spontaneous nystagmus.

Imbalance behavior score: According to the methods reported by Petrosini [6], four imbalance symptoms were selected for scoring (head deflection, limb outreach, forced annular motion or roll and nystagmus). In a two-point system the presence of one of the following symptoms was scored: 1) spontaneously persistent roll towards the injured side; 2) angle of head deflection > 45°; 3) posterior limb outreach; and 4) persistently spontaneous nystagmus towards uninjured side. In a one-point system the presence of one of the following symptoms was scored: 1) spontaneous downward eye movement on the injured side. No limb outreach. Disappearance of annular and spiraling movement, rats could escape after stimulation, though the rats moved slowly, but could move in a straight line. Disappearance of nystagmus). The total score ranged from 0 to 8.

Detection of CVEMP threshold, latency period and amplitude of each wave

The cVEMP was detected by auditory evoked potential equipment (Audera V2.7, GSI, USA). Rats were anesthetized with 2% pentobarbital sodium (0.2 mL/100g i.p.). When recorded, the
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head of rat was lifted and moved forward and upward to achieve hyperextension of the neck muscles. A silver recording electrode was inserted into the bilateral back cervical extensor at the C3 cervical spine level, and a reference electrode was inserted into the middle of occipital bone, while an earth electrode was inserted into the foot. An earphone was inserted into the external auditory canal and fixed at 0.5 cm depth. The stimulation sound was generated with an alternating wave at a stimulation strength of 100 dB nHL, which was decreased progressively by 10 dB nHL. The repeat rate was 5.00 Hz, and scanning performed 204 times at 40 s intervals, with band-pass filtering of 10-1,000 Hz. Response to cVEMP was recorded at different time points after the induction of diabetes. The cVEMP was recorded as the average of two measurements in each ear. The first diphasic wave was recorded as P1 (positive wave) and N1 (negative wave) and the appearance was considered to represent the elicitation of cVEMP. The threshold was defined as the minimum stimulation strength eliciting cVEMP, and the absolute amplitude between peaks was regarded as the amplitude between P1 and N1. The relationships between cVEMP threshold, P1 and N1 latency period, and amplitude in the different groups were analyzed.

Statistical analysis

SPSS19.0 was used to analyze the data. Measurement data were expressed as mean ± standard deviation. One-way ANOVA was used to compare the parameters of cVEMP in the diabetes groups and the control group. P < 0.05 was considered to indicate statistical significance.

Results

Blood glucose detection after modeling

STZ was intraperitoneally injected with the dose of 55 mg/kg. After 1 week, blood glucose was detected from caudal vein, and

Figure 1. Diagram and threshold of cVEMP in the control group.
blood glucose > 16.7 mmol/L suggested a successful modeling. Blood glucose from caudal vein was detected every 2 weeks, and the values were all higher than 16.7 mmol/L.

**Behavior observation**

Although the rats showed autonomic limb activity, pain reactivity, efforts to stand, reduced movement and cowering in the first few minutes of recovered consciousness after unilateral labyrinthectomy, the rats did not exhibit spontaneous nystagmus, rolling or head deflection. Subsequently, nystagmus towards the uninjured side was observed, followed by autonomous rolling, head deflection and even dorsiflex and tonic limb extension. Animals did not eat or drink on the day of surgery. With the extension of compensation time, spontaneous rolling disappeared first. After being stimulated by sound or touch, the rats moved in an annular manner, followed by forward movement in a spiral manner either subsequently or concurrently. Nystagmus and head deflection disappeared after the rolling motion and before the disappearance of spiral forward movement. Although in the late stage of compensation, the angle of head deflection was significantly decreased or even disappeared, neck torsion was still apparent. These phenomena persisted for at least 3 months. Neck torsion did not affect the ability of rats to identify the mouthpiece of the water bottle provided, or to walk in straight lines. At 24-36 h after unilateral labyrinthectomy, spontaneous nystagmus of the rats in the control group disappeared, and the head deflection was significantly alleviated. After acclimatization, the rats in control group exhibited avoidance behavior when stimulated by sound or touch. This behavior was presented as the movement in a linear direction, although the action was slow. There were no significant differences in the compensation scores among all the groups within 4 h after surgery. There were no significant differences in the scores of the Week 4 and Week 8 groups compared with...
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that in the control group ($P > 0.05$); however, significantly higher scores were observed in the score of the Week 12 group at 12 h after surgery or later ($P < 0.05$) (Table 1).

**CvEMP in neck muscle**

There cvEMP thresholds (dB nHL) in the control and diabetes groups were significantly different ($P < 0.01$). ANOVA showed that there was no significant difference in the thresholds of the Week 4 group and control groups ($P > 0.05$), while those of the Week 8 ($P < 0.05$) and Week 12 ($P < 0.01$) were significantly increased. The P1 latency periods (ms) of cvEMP in the control and diabetes groups were significant different ($P < 0.01$). ANOVA showed that there was no significant difference in the P1 latency periods (ms) of the Week 4 and control groups ($P > 0.05$), while those of the Week 8 ($P < 0.05$) and Week 12 ($P < 0.01$) groups were significantly increased. A similar trend was observed in the N1 latency periods (ms) of cvEMP in the control and diabetes groups. There were no significant differences in the amplitude between waves of cvEMP in the control and diabetes groups ($P > 0.05$) (Table 2; Figures 1-4).

**Discussion**

According to the latest statistics from the World Health Organization, diabetes is becoming one of the most common chronic diseases. The complications of diabetes involve many systems, including the eye, kidney, heart and cerebral vessels and central nervous system, as well as the vestibular system. Myers et al. [7, 8] observed a reduction in the diameter of vestibular nerve fibrosis and thinning of the peripheral myelin in diabetic rats. Furthermore, the severity of these changes increased with the duration of disease. Nicholoson et al. [9] reported abnormalities in the slow phase velocity of the vestibulo-ocular reflex and gaze test compared with the control group. Currently, direct evaluation of vestibular pathway signaling...
involves the detection of vestibular nystagmus, cVEMP and ocular vestibular-evoked myogenic potential (oVEMP). cVEMP is widely used in China. There have been some reports on the detection for vestibular function in diabetes. Bektas et al. [10] evaluated cVEMP in 12 diabetes patients with peripheral neuropathy and 13 diabetes patients without peripheral neuropathy as well as healthy individuals. No significant difference in either latency period or amplitude was observed between the groups. Konukseven et al. [11] assessed oVEMP and cVEMP both in 30 diabetes patients at the early stage and 31 healthy controls. In the diabetes group, P1 and N1 latency periods of oVEMP were 30.4% and 37.5%, and those of cVEMP were 53.7% and 59.3%, respectively. The inconsistency between the findings of these two studies may be due to differences in patient age, gender, diet, education, and living environment, as well as the duration and type of therapeutic interventions.

The aims of this study were to explore the influence of both diabetes and the disease duration on vestibular dysfunction and vestibular compensation after unilateral labyrinth injury in a rat model of diabetes induced by STZ. Our results showed that diabetes negatively influences the process of vestibular compensation, and this effect is exacerbated with disease duration. In the present study, there were no significant difference in the imbalance scores after unilateral labyrinthectomy of the Week 4 and Week 8 groups compared with that in the control group ($P > 0.05$); however, the rats in the Week 12 group showed significant increases at 12 h after surgery or later ($P < 0.05$), with animals showing the disappearance of spontaneous nystagmus or spiral moving forward. Above all, the development of vestibular compensation in the control group was significantly faster than that in the diabetes groups and furthermore, this difference was increased with the disease course. Most symptoms were static vestibular abnormalities, such as spontaneous nystagmus and head deflection. Annular motion around a single point and spiral forward movement were also observed as dynamic symp-
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toms of vestibular dysfunction. Previous studies indicated that disappearance of static symptoms was earlier than that of dynamic symptoms, and that the dynamic symptoms also took longer to disappear [12].

In combination with the preoperative cVEMP results, the P1 latency periods (ms) of the Week 8 ($P < 0.05$) and Week 12 ($P < 0.01$) groups were shown to be significantly increased compared with those of the control group, and a significant increase in the N1 latency period (ms) was observed in the Week 12 ($P < 0.01$) group. Figures 1-4 indicated that higher threshold of sound stimulation increased with disease duration, suggesting that injury to the sacculus increases simultaneously. Previous studies have suggested that sensorineural hearing loss has no significant influence on cVEMP, suggesting that sound stimulation acts directly on the sacculus. The sacculus is the only recognized region with the function of phonosensitive function as the peripheral vestibular terminal organ; therefore, the increase in the sacculus threshold indicates the possibility of injury. Zuniga et al. [13] found that there were no significant difference in cVEMP latency periods in patients with migraine vertigo and Menière’s disease compared with the control group. Amplitude in the MD group was significantly changed. In comparisons of VEMP in Phex-Hyp-Duk/y (vestibule abnormality in mice with endolymphatic hydrops) and control mice, Sheykholeslami et al. [14] found that there was no significant difference in P1 and N1 latency periods. All conclusions indicated that the diseased sacculus had no significant influence on the potential latency of cVEMP. Combined with our results showing that the stimulation threshold was increased with the disease course, it can be concluded that the cVEMP signaling pathway influences all or most of the sacculus- or post-sacculus-related pathways. However, the absence of significant differences in amplitude and wave elicitation ratio indicates that the degree of injury degree had not reached the level of that associated with Menière’s disease or vestibular neuritis. Indeed, our observations were continued only until Week 12 after the induction of diabetes, which suggests that the negative effect on the cVEMP reflex pathway occurs at an early stage. Although we did not further explore the involvement of the post-sacculus pathway, it might be possible to make some deductions based on our existing evidence. The vestibular nucleus behind the sacculus plays a very important role in maintaining normal vestibular function. Under normal physiological conditions, the vestibular nucleus collects the signals uploaded by the bilateral vestibular end organs, integrates the information from visual, proprioception or other systems in appropriate time and space, finally transmitting the signals to the third neuron, cortical functional areas and spinal motor neurons. Although there have been no report on the influence of diabetes on the vestibular nucleus and post-nucleus pathway, previous studies have indicated that vestibular compensation is closely related to the vestibulo-ocular reflex, cognitive ability, long-term potentiation and long-term derepression (LTD) in the cerebellum, and the change and re-equilibrium of excitability in the bilateral vestibular nucleus. However, the close correlation of retinopathy, aging of the human brain and Alzheimer’s disease caused by diabetes may form the basis of the pathological mechanism underlying the influence of diabetes on vestibular compensation [15-20]. Combined with the imbalance symptom score, the slowest recovery of compensation was observed in the Week 12 group, which is in accordance with characteristics of the influence diabetes-related microangiopathy and metabolic disorders, including diabetes, on the nervous system.

In summary, the findings of the present study allow us to conclude the rate of CV abnormality increased with the course of diabetes or uncontrolled blood glucose status, and is associated with increased stimulation threshold and wave latency extension, but no significant change in amplitude. Furthermore, although compensation was observed in most of the static symptoms, full assessment of the dynamic compensation was not completed in our study due to the experimental schedule. In terms of the duration of static symptom recovery, that of the Week 12 diabetic group was significantly longer than that of the normal control group. However, observation of the dynamic symptoms showed in the Week 12 diabetic group was significantly slower than that in the control group. At least in terms of the duration of static symptom recovery, that of 12-week diabetic group was significantly longer than that of normal control group significantly. However, in the dynamic observation, rats changed from the annular motion to adopt a pattern of movement in a straight line.

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Although rats did not recover a fast and flexible state of motion, the recovery was significantly extended in Week 12 group compared with that in the control group. The findings of the present study have practical reference value for treating patients with diabetes combined with vertigo, as well as for vestibular rehabilitation. The influence of diabetes on the vestibular reflex pathway is complicated and multi-level, and the detailed site and mechanism of the negative influence on the pathway require further elucidation.

Disclosure of conflict of interest

None.

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