Effect of Korean Red Ginseng on Early Vestibular Function Restoration after Unilateral Labyrinthectomy

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Objectives: Vertigo is a common condition. Definitive treatment is to induce vestibular compensation. Currently, no medications have been discovered that enhance vestibular functional restoration. The current study was conducted to evaluate the ability of ordinary Korean red ginseng (KRG) to induce vestibular compensation.

Methods: Twelve Sprague-Dawley rats were divided into two groups. Five rats (KRG group) were fed KRG extract (100 mg/kg) for 2 weeks before undergoing unilateral labyrinthectomy (ULx). The remaining seven rats (control group) were untreated before ULx. After surgery, all animals were housed in the same environment without being fed additional extract. To evaluate vestibular function, gain of the horizontal nystagmus to 0.2 Hz with a peak velocity of 100°/second sinusoidal rotation was compared and analyzed before ULx as well as 3 and 7 days after surgery.

Results: Before the operation, gain of the control and KRG group were 0.81±0.05 and 0.88±0.08, respectively, with 0.2-Hz stimulation. This value decreased to 0.43±0.08 and 0.53±0.08, respectively on 3 days after operation (p=0.047), and it was 0.40±0.06 and 0.68±0.11, respectively on 7 days after surgery. The difference of gain between the two groups was statistically significant at each 3 and 7 days (p<0.05). By confirming c-Fos protein expression in medial vestibular nuclei, the functional effect of KRG causing vestibular modulation was confirmed.

Conclusions: Rats treated with KRG showed more rapid and complete recovery after acute vestibular loss compared to untreated animals. Therefore, KRG could be one of candidate for the useful medication of vestibular diseases.

Keywords: Compensation; Ginseng; Vertigo; Vestibule

INTRODUCTION

Vertigo is experienced by 30% of all people more than once during their lifetime [1]. Morbidity associated with this condition tends to increase among the elderly [2]. Pathologic vertigo is caused by complex factors such as degenerative disorders, viral infection, or ischemic brain injury [3]. The degeneration of vestibular hair cells, one cause of vertigo, cannot
be reversed in mammals but can be in birds [4]. Therefore, for function recovery, the definitive treatment tool is to induce vestibular compensation with exercise for early adaptation [5]. Early vestibular compensation after unilateral vestibular loss is induced by decreased efficacy of postsynaptic N-methyl-D-aspartate (NMDA) receptor responses of vestibular nucleus neurons to gamma-aminobutyric acid (GABA) agonists [6]. If the total compensation courses are unsuitable, chronic vertigo occurs and the return to daily activities is also delayed [7]. Vertigo tends to arise in the elderly, a population with decreased cognitive abilities and diminished locomotion [8]. Thus, it is not easy for these individuals to effectively complete the vestibular rehabilitation exercises [9]. In cases of certain diseases such as depression or anxiety disorders, treatment with anti-depressants and vestibular suppressants delay the recovery of vestibular function because vestibular compensation is disturbed [10]. Nevertheless, studies evaluating possible pharmacological methods for reinforcing vestibular homeostasis or preventing vestibular imbalance have been rare until now [11].

Korean red ginseng (KRG) may possibly serve as a medication for treating or preventing vestibular disorders. This plant has been used as a traditional medicine in Asia for more than 2,000 years and recently been proven to enhance memory while increasing plasticity and boost cognitive functions [12]. Especially, saponin, a compound found in KRG, is a complex material that contains different types of ginsenosides. Saponin is known to have beneficial activities such as antioxidative [13,14], anti-apoptotic [15], and immune regulatory functions [16,17]. All of these properties could influence neuromodulation and vestibular compensation after acute vestibular loss. Additionally, ginsenoside-Rb1 is believed to exert antivertigo effects primarily on the peripheral vestibular system through the inhibition of calcium influx into vestibular hair cells, thereby regulating hair-cell afferent vestibular transmission [10,18,19].

The main purpose of the current study was to evaluate the useful effect of KRG on vestibular compensation. This investigation is the first to assess protection against and recovery from vestibular function following acute vestibular loss induced by unilateral labyrinthectomy (ULx) in an animal model.

MATERIALS AND METHODS

1. Animals

All animal experiments were conducted according to the guidelines of the Institutional Animal Care and Use Committee of Gachon University, Incheon, Korea (GIACUCR-009). Twelve- to 16-weeks-old Sprague-Dawley rats (Koatec, Seoul, Korea) weighing up to 250 to 350 g were used for this study. Five of the 12 rats were in the KRG group and seven were the control group (Fig. 1). All animals were housed with 100 lx of light with a 12-hour light/dark cycle at 23°C, 50% humidity, and a noise level below 50 dB.

2. Ginseng Administration

KRG extract was provided by Korea Ginseng Corporation (Daejeon, Korea). The extract contained Rb1 (0.83%), Rb2
(0.32%), Rc (0.39%), Rd (0.11%), Re (0.26%), Rf (0.16%), Rg1 (0.20%), Rg2 (0.14%), Rg3 (0.01%), and Rh1 (0.10%), and other minor ginsenosides. The KRG group was treated with 100 mg/kg of KRG extract orally once a day for 2 weeks. The control group was under the same condition and these animals were treated with the same volume of saline at the same times as the KRG group. After the treatment period, all rats underwent ULx.

3. Unilateral Labyrinthectomy

Surgery was performed with the animals under gas anesthesia. The rats were placed in a prone position and inhaled isoflurane (Aerane; Ilsung Pharm Corp., Seoul, Korea). To maintain a constant physiological state during anesthesia, the following conditions were carefully monitored: body temperature, pulse, electrocardiogram signals (THM 100; Indus Instruments, Webster, TX, USA), and O2 saturation (Ohmeda Biox 3760; Datex-Ohmeda, Madison, WI, USA). A vertical incision almost 1 cm in length was made following the right retroauricular sulcus and the eardrum was exposed. A small perforation was made in the middle ear space using a 23-gauge needle (Shingchang Medical Corp., Gumi, Korea) on the vestibular lateral wall positioned at the upside of the fallopian canal to later identify the pterygopalatine artery and stapes (Fig. 2). After observing perilymph leakage, the small perforation was extended and the vestibular lateral wall was completely opened. Next, suction was applied until there was no more leakage of lymphatic fluid. To maintain the opening, the hole was filled with collagen (Helitene; Intergra Life Sciences Corp., Plainsboro, NJ, USA). ULx completeness was verified by a tail hanging test and the appearance of spontaneous nystagmus, skew, and head tilting. Furthermore, auditory brainstem responses (ABR) were double-checked by the ABR workstation (Tucker-Davis Technologies, Alachua, FL, USA) objectively.

4. Head Fixation and Sinusoidal Rotation Stimulation

While under anesthesia, a 3-cm-long flat head bolt (30-mm tab screw; Junghwa Metal Corp., Hanam, Korea) was fixed on the skull of the rats with six tiny brass Phillips head screws (#1106; SM Medical, Seoul, Korea) and glue (Loctite 401, 20 g; Henkel, Dublin, Ireland) using a stereotaxic alignment system (Model 900 with a #1929B rat anesthesia mask; KOPF, Tujunga, CA, USA). For permanent fixation, dental cement (Vertex Self-Curing; Vertex-Dental BV, Zeist, the Netherlands) was placed overhead bolt. After bolt implantation, we waited 1 week for the animals to stabilize. The rat was then placed in a restrainer (S44-RR; Plas Labs Inc., Lansing, MI, USA) in a prone position and a bolt placed on the top of skull was inserted into the holder of the sinusoidal animal rotator (M-YS4080FN001; NSK, Ukiha, Japan) for complete fixation. A restrainer was also attached to the rotator stage with a machine screw to limit the movement of the body and head. A micro-control x-, y-, z-linear trans-
Fig. 3. The set-up used for nystagmus recording. After the permanent implantation of a head bolt on the skull (A, B), the rats were placed in a prone position in a restrainer with the neck and head fixed on an animal rotator to prevent movement of the body and head (C). (D) A marker was attached on the right pupil with glue under a microscope, and the upper and lower eyelids were sutured open to prevent blinking (D).
the skulls and postfixed them with the same solution used for initial fixation for 4 hours at room temperature (RT). We immersed the fixed brains in a 30% sucrose/phosphate-buffered solution (PBS) for 2 days at 4°C for cryoprotection. We then cut sections at a thickness of 35 μm with a freezing microtome (Leica, Wetzlar, Germany), incubated them for 20 minutes at RT with 3% hydrogen peroxide (H₂O₂), rinsed them three times for 5 minutes with 10-mM PBS, incubated them for 20 minutes with 0.5% Triton X-100 dissolved in 10-mM PBS and incubated them for 1 hour at RT with 5% skim milk. We then washed the sections in 10-mM PBS plus 0.05% Triton X-100 (PBST) and incubated them overnight at 4°C with rabbit polyclonal anti-c-Fos antibody (1:3,000; Oncogene Research Products, San Diego, CA, USA) in antibody diluent solution (Invitrogen, Carlsbad, CA, USA). The following day, we rinsed the tissue sections with PBST, incubated them with biotinylated goat anti-rabbit secondary antibody (1:1,000; DAKO, Glostrup, Denmark) for 1 hour at RT and incubated them for 1 hour at RT with avidin-biotin complex (Vector Laboratories, Burlingame, CA, USA). We visualized the neurons that were positive for FLP by incubating the sections with 0.05% diaminobenzidine (DAB; Invitrogen) plus 0.003% H₂O₂. After the DAB reaction was completed, we rinsed the sections with 0.1-M PB and mounted them on gelatin-coated slides. The slides were then air-dried, dehydrated, xylene-cleared, and mounted on coverslips with Permount (Fisher Chemical, Fairlawn, NJ, USA). We examined the brain tissue sections using bright-field microscopy (Olympus BX50; Olympus, Tokyo, Japan) to identify FLP-positive neurons. We captured and digitalized images of the dark-brown, FLP-positive neurons using a microscope-mounted digital camera (Olympus DP70, Olympus) and image-capture software (Image-Pro Plus; MediaCybernetics Inc., Rockville, MD, USA).

7. Data Analysis

A nystagmus analysis program was developed with LabVIEW (National Instruments, Austin, TX, USA). The x-axis represented time (second) and the y-axis represented the distance the eyeball moved (°). Basically, this program calculated gain, phase, and symmetry of three axes to monitor nystagmus. With horizontal nystagmus about sinusoidal rotational stimulation of KRG group and control group, the amount of gain was evaluated with descriptive statistics (mean±standard deviation). Data are presented as a box and whisker plot. Normality of the data was assessed with a Kolmogorov-Smirnov test before performing repeated-measures (RM) ANOVA. No variables were rejected, indicating that they maintained a normal distribution. We also used a one-way RM ANOVA to identify mean differences between the two groups for three replicate experiments. The Greenhouse-Geisser method was used to correct the violation of sphericity of the covariance. The p-values less than 0.05 were considered statistically significant. All statistical analyses were performed with IBM SPSS Statistics ver. 21.0 (IBM Corp., Armonk, NY, USA).

RESULTS

Before surgery, gain of the control and KRG groups were 0.81±0.05 and 0.88±0.08, respectively, with 0.2-Hz stimulation. The difference between the two groups was not significant. Gain of both groups observed 3 days after surgery was 0.43±0.08 for the control group and 0.53±0.08 for the KRG group. Seven days after surgery, gain was recovered more rapidly in the KRG group than in control animals; gain was 0.40±0.06 for control group and 0.68±0.11 for the KRG group (Fig. 4). RM ANOVA analysis of between-subjects effects showed that the gain was significantly higher for the KRG group than the control group (F[1,10]=19.91, p<0.001). Analysis of the within-subjects effects demonstrated that the linear and quadratic trends among replication measurements were statistically significant (linear: F[1,10]=217.13, p<0.001; quadratic: F[1,10]=67.71, p<0.001). The interaction effect between group and replication measurements (Fig. 5) was also significant (F[2,20]=9.61, p<0.001).

Immunohistochemical staining of FLP expression in bilateral MVN in coronal midbrain sections on 2, 6, and 12 hours after right side ULx in control group (Fig. 6B-D), and in KRG group (Fig. 6E-G) showed that KRG group did not express any FLP (Fig. 6A is negative control).
Fig. 4. Vestibule ocular reflex summation graphs of raw data of one animal in the control group (A) and five animals (B–F) in Korean red ginseng (KRG) group for sinusoidal rotation stimulation. Graphs in the left column present the data acquired immediately before unilateral labyrinthectomy (ULx) while graphs in the middle column present the data acquired 3 days after ULx and the graphs in the right column present the results obtained 7 days after surgery. No spiking nystagmus at one side was observed in the control animal 3 or 7 days after ULx. However, a nystagmus spiking on same side was seen 3 days after ULx in the KRG group. In each graph, the X-axis represents the frames. POD, postoperative day.
DISCUSSION

In the present study, consumption of KRG extract significantly attenuated nystagmus induced by ULx. Gain in the vestibular system after ULx was also restored more rapidly. This study is the first to investigate the protection and recovery of vestibular function against acute vestibular loss induced by ULx in an animal model.

In our pilot study, we found that animals treated with KRG recovered more rapidly from ULx than the untreated group. No spiking nystagmus was observed at one side oscillation in the control group 3 and 7 days after ULx while this spiking on same oscillation was observed 3 days after ULx in the KRG group (Fig. 7). This phenomenon was observed in all KRG-treated rats (Fig. 4).

KRG is a complex material that contains numerous types of ginsenosides. The effect, mechanisms of action, and characteristics of each ginsenoside were not clearly demonstrated in this study. Nevertheless, the authors speculate that KRG exerts beneficial effect on the vestibular system through several different mechanisms. The vestibular system has a distinctive compensation mechanism for repairing defective balance functions. Vestibular compensation consists of two stages: inhibition of the contralesional MVN (contra-MVN) activities during the acute stage after unilateral vestibular loss, and recovery and maintenance of spontaneous ipsilesional MVN ( ipsi-MVN) activities during the chronic stage. In this overall vestibular compensation process, activated neurons in the ipsi-MVN project their axons into the flocculus to inhibit contra-MVN neurons via NMDA.
Fig. 7. The timely serial changes of nystagmus for the sinusoidal rotation stimulation in control (A) and Korean red ginseng (KRG) treated animal (B) for 22 days after unilateral labyrinthectomy (ULx). In the dotted box, no spiking at one side was observed in the control group 3 and 7 days after ULx. However, spiking at same side was seen 3 days after ULx in the KRG group. In each graph, the x-axis represents the frames. PO, postoperative.

Receptor, nitric oxide, and/or GABA-mediated signaling, thereby restoring intervestibular nuclear activities. During the chronic stage after unilateral vestibular loss, the flocculus suppresses the inhibition of the ipsi-MVN neur ons via protein phosphatase, protein kinase C, and glutamate receptors to promote the recovery and maintenance of ipsi-MVN activities [20].

An ideal medication for enhancing vestibular compensation should decrease the efficacy of postsynaptic NMDA receptor responses of vestibular nucleus neurons in the contra-MVN. Alternatively, neuronal activity could be augmented through increased glutamate release at the ipsi-MVN, and induce the decreasing sensitivity of GABA agonists at ipsi-MVN. Non-competitive antagonists of the NMDA receptor such as MK-801 have been shown to induce vestibular compensation. However, use of these reagents is limited due to psychotomimetic side effects and acute neurotoxicity both in vitro and in vivo [21]. It has been demonstrated that KRG modulates glutamate release through GABAergic receptor antagonists in mice [22].

Additionally, the administration of KRG significantly increases the expression of proteins that influence plasticity including phospho-NMDA receptor 1, phospho-calcium-calmodulin-dependent kinase II, phospho-PKA catalytic beta subunit, phospho-cAMP response element binding protein, and brain-derived neurotrophic factor in the hippocampus [12,23,24]. This neuromodulatory effect of KRG could improve the vestibular tonic imbalance. During this process, antioxidant [13,14], antiapoptotic [15], and immune regulatory [16,17] mechanisms could influence the outcomes.

Another well-known benefit of KRG extract is the ability to
block calcium channels in the central nervous system of patients with ischemic disease [18,25]. Reagents that block calcium channels are a representative class of medications that alleviate acute vertigo symptoms.

Although the neuroregulation system is different, the cochlear and vestibular have similar mechanoelectrical transduction systems and communicate with each other. Several trials evaluating the effect of KRG on inner ear diseases such as noise-induced [26] or sudden hearing loss [27] have been performed and proposed several action mechanisms [25]. Nevertheless, it is nothing to study about medicine for strongly induced vestibular compensation preventively.

In the present study, we documented some evidence for unknown beneficial effect. Additional investigations should be performed to further investigate this topic. For determining preventive effects of KRG on vertigo, there was no sufficient information about period, number, and concentration of KRG. In this study, through the review of literatures [12,28,29], the amount of KRG is determined to be taken once each day for 2 weeks by 100 mg/kg concentration, used frequently in the study of nervous system.

In conclusion, oral administration of KRG in rats led to better and faster recovery after acute vestibular loss compared to untreated control animals. Our findings indicated that KRG can promote early vestibular compensation after acute vestibular loss. Therefore, KRG could be one of candidate for the useful medication of vestibular diseases.

CONFLICT OF INTEREST

No potential conflict of interest relevant to this article was reported.

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REFERENCES