NeuroToxicology 34 (2013) 42–50

Contents lists available at SciVerse ScienceDirect

NeuroToxicology

Korean red ginseng ameliorates acute 3-nitropropionic acid-induced cochlear damage in mice

Chunjie Tian [a], Young Ho Kim [b,*], Young Chul Kim [c], Kyung Tae Park [b], Seung Won Kim [a], Youn Ju Kim [a], Hye Jin Lim [a], Yun-Hoon Choung [a,**]

[a] Department of Otolaryngology, Ajou University School of Medicine, Suwon, Republic of Korea
[b] Department of Otolaryngology, Seoul National University, Boramae Medical Center, Seoul, Republic of Korea
[c] Department of Physiology, Chungbuk National University, College of Medicine, Cheongju, Republic of Korea

ARTICLE INFO

Article history:
Received 1 September 2012
Accepted 12 October 2012
Available online 16 November 2012

Keywords:
Cochlea
Fibrocyte
Spiral ligament
Spiral ganglion cell
Korean red ginseng
3-Nitropropionic acid

ABSTRACT

3-Nitropropionic acid (3-NP), a mitochondrial toxin, has been reported to induce an acute cochlear damage. Korean red ginseng (KRG) is known to have protective effects from some types of hearing loss. This study aimed to observe the protective effect of KRG in an ototoxic animal model using 3-NP intratympanic injection. BALB/c mice were classified into 5 groups ($n = 15$) and dose-dependent toxic effects after intratympanic injection with 3-NP (300–5000 mM) on the left ear were investigated to determine the appropriate toxicity level of 3-NP. For observation of the protective effects of KRG, 23 mice were grouped into 3-NP (500 mM, $n = 12$) and KRG + 3-NP groups (300 mg/kg KRG for 7 days before 500 mM 3-NP administration, $n = 11$). Auditory brain response (ABR) and cochlear morphological evaluations were performed before and after drug administration.

The ABR thresholds in the 800–5000 mM groups exceeded the maximum recording limit at 16 and 32 kHz 1 day after 3-NP administration. The ABR threshold in the 500 mM 3-NP + KRG group was significantly lower than that in the 500 mM 3-NP group from post 1 week to 1 month. The mean type II fibrocyte counts significantly differed between the control and 3-NP groups and between the 3-NP and 3-NP + KRG groups. Spiral ganglion cell degeneration in the 3-NP group was more severe than that in the 3-NP + KRG group. This animal model exhibited a dose-dependent hearing loss with histological changes. KRG administration ameliorated the deterioration of hearing by 3-NP.

1. Introduction

Mitochondria play an important role in the life and death of cells. They are the intracellular organelles mainly responsible for cellular adenosine triphosphate (ATP) production by oxidative phosphorylation, and also have a series of specific functions, including apoptosis and oxidative stress control (Kokotas et al., 2007). Mitochondrial dysfunctions are critically involved in cellular processes underlying necrotic and apoptotic cell deaths, which are thought to have a major role in the pathogenesis of neurodegenerative diseases (Beal et al., 2000). Apoptosis and formation of reactive oxygen species (ROS) have been reported to be involved in pathophysiological mechanisms of cochlear damage by ischemia, ototoxins, and noise (Huang et al., 2000; Yamane et al., 1995). Mitochondrial dysfunction in the cochlea is thought to be an important cause of sensorineural hearing loss (SNHL). The frequency of mitochondrial hearing loss is unknown. It has been estimated that 1% of prelingual deafness cases result from constitutional mutations in mtDNA (Marazita et al., 1993). As it is for postlingual hearing loss, it has been estimated that 20% may be caused by mutations in the mitochondrial genome (Estivill et al., 1998).

3-Nitropropionic acid (3-NP) is an irreversible inhibitor of succinate dehydrogenase (Ludolph et al., 1991a) and complex II respiratory enzyme required for mitochondrial energy production (Nony et al., 1999). It is a compound found in crops contaminated with a naturally occurring neurotoxin produced by legumes of the genus *Astragalus* and *Arthrium* fungi (Ming, 1995) that causes neurotoxicity in animals and humans (Ludolph et al., 1991b), inducing ATP exhaustion by mitochondrial dysfunction (Pang and Geddes, 1997). Mitochondrial Ca^{2+} homeostasis is impaired along with these serial cascades and results in elevation of intracellular Ca^{2+} levels and impaired buffering capacity of intracellular Ca^{2+} in astrocytes and neurons (Calabresi et al., 2001; Nasr et al., 2003).

* Corresponding author at: Department of Otolaryngology, Seoul National University, Boramae Medical Center, 39, Boramae-Gil, Dongjak-Gu, Seoul 156-707, Republic of Korea. Tel.: +82 2 870 2442; fax: +82 2 870 3863.
** Corresponding author at: Department of Otolaryngology, Ajou University School of Medicine, San 5, Wonchon-dong, Yeongtong-gu, Suwon 443-721, Republic of Korea. Tel.: +82 31 219 5263; fax: +82 31 219 5264.
E-mail addresses: yhkiment@gmail.com (Y.H. Kim), yhc@ajou.ac.kr (Y.-H. Choung).

0161-813X/$ – see front matter © 2012 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.neuro.2012.10.008

An animal model of acute cochlear energy failure induced by administering 3-NP, the mitochondrial toxin, into the round window niche of the rat cochlea was established (Hoya et al., 2004). In one study using the permanent auditory threshold shift model induced by 3-NP, a remarkable degeneration was detected in type II fibrocytes in the spiral ligament and marginal/intermediate cells in the stria vascularis after 3-NP administration (Mizutari et al., 2008). These results indicate that a permanent auditory threshold shift caused by acute cochlear mitochondrial dysfunction is primarily mediated by cellular degeneration in the cochlear lateral wall and suggest that therapy for hearing loss due to acute energy failure may be achieved through protection or regeneration of the cochlear lateral wall.

Ginseng refers to the root of some species of the genus *Panax* (C.A. Meyer Araliaceae). Among these species, *Panax ginseng* is mainly cultivated in Korea and China and is the most widely used ginseng. It has a cultural and medical history of more than 5000 years. The active ingredients in ginseng are called ginsenosides or ginseng saponins (Liu and Xiao, 1992; Back et al., 1996). According to their structural differences, they were classified into 3 major groups: the panaxadiol (Rb1, Rb2, Rb3, Rc, Rd, Rg3, Rh2, and Rs1), panaxatriol (Re, Rf, Rg1, Rg2, and Rh1), and oleanolic acid groups (Ro) (Wen et al., 1996; Tackikawa et al., 1999). To date, more than 40 ginsenosides, the major components of ginseng extract, have been isolated from several species of ginseng (Lee et al., 2008). Red ginseng refers to the type that is dried after being steamed, which may have healthful elements not found in fresh ginseng. Korean red ginseng (KRG) has various health-promoting effects on the human body, such as reducing the incidence of cancers, protective effects on gastric ulcer, anti-inflammatory and antioxidative effects, enhancing the mind, stimulating libido, and positive effects on patients with Alzheimer disease (Calabresi et al., 2001; Nasr et al., 2003; Yun et al., 2010; Oyagi et al., 2010; Hong and Lyu, 2011; Im et al., 2010a). In addition, KRG containing 42 natural minerals offers a wide variety of beneficial nutrients to the body. Accumulative studies showed that KRG had protective effects on hearing loss induced by cisplatin, gentamicin, and noise through the protection of outer hair cells; pretreatment with ginseng extract significantly attenuated the cisplatin-induced increase in ROS and also inhibited the expression of caspase-3 and poly-adenosine diphosphate-ribose polymerase related to cisplatin-induced apoptosis in HEI-OC1 auditory cells (Lee et al., 2008; Choung et al., 2011a; Kang and Chung, 2010). Given the results of the previous studies, some forms of hearing loss may be prevented through preservation of sensory hair cells, spiral ganglion cells, and nonsensory cells like fibrocytes in the cochlear lateral wall. However, there is no current report on the protective role of red ginseng against 3-NP-induced cochlear damage. We speculated that KRG could protect from hearing loss due to 3-NP-induced acute cochlear damage. In the present study, the animal model of acute cochlear damage using intratympanic injection of 3-NP was established and the preventive effect of KRG against cochlear damage induced by 3-NP was investigated.

2. Materials and methods

2.1. Animals

Male BALB/c mice (6 weeks, 25–30 g) involved in this study were housed in the temperature-controlled (23 ± 2 °C) room with 12-h light/dark cycles and provided with free access to standard laboratory food and tap water. Institutional Animal Care and Use Committee approved the surgical procedures in accordance with the guidelines regarding the care and use of animals for experimental procedures. All efforts were made to minimize the number of animals and their suffering.

2.2. Korean red ginseng

KRG was provided from Korea Ginsengs Center (KGC®, Korea). This 100% powderized ginsenosides comprise the roots (70%) and hair roots (30%) of ginseng at 6 years of plant age. The whole ginsenoside components of KRG are summarized in Table 1. KRG was melt with tap water, and given to animals at a dose of 300 mg/kg for 7 days before and after 3-NP administration.

2.3. Study design

This study was performed in 2 phases as follows:

Phase I: investigation of 3-NP dose-dependent toxic effects on auditory function.

The study was performed with 15 BALB/c mice, which were randomly assigned to 5 groups. For each animal, the intratympanic injection of 3-NP (7 μl, Sigma, St. Louis, MO, USA) in concentration of 300, 500, 800, 1000, and 5000 mM was performed on left ear and the right ear was used as control. Auditory brainstem response (ABR) test for the assessment of hearing loss was undertaken before and on post 1 day, 1 week, and 1 month after 300, 500, 800, 1000, and 5000 mM of 3-NP administration. Cochlear organs were harvested for morphological evaluation 1 month after 500, 800, and 1000 mM of 3-NP administration.

Phase II: determining the protective effect of KRG on 3-NP-induced hearing loss with animal model established in phase I.

Twenty-three mice were classified into 3-NP group ($n = 12$) and KRG-treated 3-NP group ($n = 11$). For 7 days before 3-NP administration, mice in KRG-treated 3-NP group were pretreated with KRG (300 mg/kg; 0.8 ml/each animal; once a day; per os). All mice in both groups were administrated with 3-NP (500 mM; 7 μl; intratympanic injection) and the contralateral ears remained as controls. After 3-NP administration, additional KRG treatment was performed for 7 days in the KRG-treated 3-NP group. Auditory function was evaluated 2 days before 3-NP treatment, then 1 day, 1 week, and 1 month after 3-NP treatment. Cochleae in each group were harvested 1 month 3-NP treatment for morphological evaluation.

2.4. Intratympanic administration of 3-NP

Animals were anesthetized by intraperitoneal injection of a mixture of xylazine 10 mg/kg (Rompun®, Bayer-Korea, Korea) and Zolazepam–Tiletamine 30 mg/kg (Zoletile®, Virvac, France). Under an operating microscope, 3-NP solution (7 μl/each animal) was given slowly through the anterosuperior quadrant of the left tympanic membrane with a 0.45-gauge dental needle to fill the middle ear cavity. The mouse was then kept in the same position with the left ear facing up for more than 30 min.

Table 1
Whole ginsenoside components of the Korean red ginseng used in this study.

Ginsenoside components	Rb1	Rg1	Rc	Re	Rb2	Rf	Rh1	Rg2(s)	Rg2(r)	Rd	Rg3(s)	Rg3(r)	Total
Content (mg/g)	5.16	2.89	2.22	2.16	1.82	0.93	0.13	0.37	0.21	0.47	0.14	0.08	16.58
Component ratio (%)	(31.1)	(17.4)	(13.4)	(13.0)	(11.0)	(5.6)	(0.8)	(2.2)	(1.3)	(2.8)	(0.8)	(0.5)	(100)