Available online at www.sciencedirect.com
SCIENCE DIRECT
Neuroscience Letters 395 (2006) 18–22
Neuroscience Letters
www.elsevier.com/locate/neulet

Cochlear damage due to germanium-induced mitochondrial dysfunction in guinea pigs

Tatsuya Yamasoba [a,*], Yu-ichi Goto [b], Hirofumi Komaki [b], Masakazu Mimaki [b], Akira Sudo [b], Mitsuya Suzuki [a]

[a] *Department of Otolaryngology, Head and Neck Surgery, University of Tokyo, Hongo 7-3-1, Bunkyo-ku, Tokyo 113-8655, Japan*
[b] *Department of Mental Retardation and Birth Defect Research, National Institute of Neuroscience, National Center of Neurology and Psychiatry, Tokyo, Japan*

Received 24 August 2005; received in revised form 17 October 2005; accepted 17 October 2005

Abstract

This investigation addressed the effect of germanium dioxide (GeO_2)-induced mitochondrial dysfunction on hearing acuity. Guinea pigs were fed chow that contained 0%, 0.15%, or 0.5% GeO_2. The animals that were fed 0.5% GeO_2 for 2 months developed hearing impairment chiefly due to degeneration of stria vascularis and cochlear supporting cells, which exhibited electron-dense mitochondrial inclusions. Cytochrome *c* oxidase activity was decreased in the skeletal muscles and kidney, which also exhibited electron-dense mitochondrial inclusions. No apparent pathological changes were observed in the utricle, semicircular canal, or among the vestibular nerve fibers, or in the liver or heart. The untreated animals and those treated with 0.15% GeO_2 did not exhibit hearing impairment or pathological changes in any organs. These findings suggest that administration of 0.5% GeO_2 induces mitochondrial dysfunction in the stria vascularis and supporting cells in the cochlea, as in the skeletal muscles and kidney, thereby causing hearing impairment in the guinea pigs.

Keywords: Hearing loss; Mitochondrial myopathy; Mitochondrial DNA; Cochlea; Muscle; Kidney

Mutations in mitochondrial DNA (mtDNA) are reported to be closely associated with both syndromic and nonsyndromic forms of sensorineural hearing loss (SNHL) [14]. SNHL occurs in approximately 70% of the three most common mitochondrial disorders: mitochondrial myopathy, encephalopathy, lactic acidosis and stroke-like episodes (MELAS), myoclonus epilepsy associated with ragged-red fiber, and chronic progressive external ophthalmoplegia (CPEO) [14]. SNHL is also frequently manifested in subjects with other mtDNA mutations, particularly those in 12S rRNA and $tRNA^{Ser(UCN)}$ [2]. In general, SNHL due to mtDNA lesions primarily involves the cochlea, while the vestibular system and retrocochlear auditory pathway are well preserved [1,11,17]. The fact that mtDNA lesions frequently cause SNHL of cochlear origin suggests that certain sets of cochlear cells have a high metabolic demand and are strongly dependent upon mitochondrial function. Human temporal bone histopathology has been reported only in four patients with SNHL associated with mtDNA lesions [7,13,20], in which the stria vascularis (SV) exhibited degeneration but other cochlear tissues such as hair cells were not always affected. It is therefore unclear which cochlear cells are preferentially affected by chronic mitochondrial dysfunction associated with mtDNA lesions.

There are several animal models, including genetically modified mice, that exhibit certain forms of mitochondrial dysfunction [15]. Auditory function has been studied in mice with mutant mtDNA carrying a 4696-base pair deletion, which develop hearing loss when the heteroplasmy is greater than 80% [8]. To date, cochlear histopathology has not been studied in these mice. Other animal models can be created by introducing harmful agents into the mitochondria. Hoya et al. [5] applied a mitochondrial toxin, 3-nitropropionic acid (3-NP), topically to rat cochlea and found that animals treated with 500 mM of 3-NP exhibited permanent threshold shifts. Histologically, there was degeneration of fibrocytes in the spiral ligament and spiral limbus, which indicated that these tissues may be vulnerable to acute mitochondrial dysfunction. Another animal model was created by chronic application of germanium

* Corresponding author. Tel.: +81 3 5800 8924; fax: +81 3 3814 9486.
E-mail address: tyamasoba-tky@umin.ac.jp (T. Yamasoba).

0304-3940/$ – see front matter © 2005 Elsevier Ireland Ltd. All rights reserved.
doi:10.1016/j.neulet.2005.10.045

dioxide (GeO_2). This model is of special interest since in humans, long-term administration of high doses of GeO_2 causes renal failure, emaciation, and muscle weakness [3,10]. Germanium compounds are readily absorbed following oral exposure, distributed throughout the body tissues, particularly the kidney and thyroid, and excreted largely in the urine. Rats that are fed chow containing GeO_2 exhibit body weight loss, myopathy, and nephropathy, and their skeletal muscles show numerous ragged-red fibers, cytochrome *c* oxidase (COX)-deficient fibers, and accumulation of electron-dense material in the mitochondria [3,4,9,16]. These pathological findings resemble those observed in patients with mitochondrial encephalomyopathy. Although the precise mechanism of toxicity of GeO_2 is unknown, recent study demonstrated that rats treated with GeO_2 for 24 weeks showed increased free radical generation and decreased mitochondrial DNA copies in the skeletal muscles compared to controls given normal diet [6]. Thus, chronic administration of GeO_2 may provide a controlled model for assessment of mitochondrial dysfunction-induced SNHL and allow analysis of the specific histopathology underlying this clinically significant disorder.

Thirty-six albino male guinea pigs (250–300 g, approximately 4 weeks after birth) with auditory brainstem response (ABR) thresholds within the normal laboratory range were used. Twelve animals were assigned to one of the three groups and fed standard guinea pig chow containing 0% (control), 0.15%, or 0.5% GeO_2. The controls and animals that were administered 0.15% GeO_2 were allowed to survive for 6 months, but animals administered 0.5% GeO_2 were sacrificed after 2 months because many became inactive and appeared less healthy at that time. Animals were caged singularly in an ambient room under a 12-h light:12-h dark cycle beginning at 06:00 h. Food and water were available ad libitum. Clinical manifestations and the body weight of each animal were observed every 2 weeks. Pure-tone ABRs were measured every 4 weeks. The experimental protocol was approved by the Committee for the Use and Care of Animals at the University of Tokyo and conformed to the NIH Guidelines for the Care and Use of Laboratory Animals.

The method for ABR measurement has been previously described [19]. In brief, the animals were anesthetized with xylazine hydrochloride (10 mg/kg, i.m.) and ketamine hydrochloride (40 mg/kg, i.m.). Following anesthetization, needle electrodes were placed subcutaneously at the vertex (active electrode), beneath the pinna of the left ear (reference electrode), and beneath the right ear (ground). The sound stimulus consisted of a 15-ms tone burst, with a rise-fall time of 1 ms at frequencies of 2, 4, 8, and 16 kHz. The responses of 1024 sweeps were averaged at each intensity level (5 dB steps) to assess the threshold. Hearing threshold was defined as the lowest stimulus intensity that produced a reliable peak 3 or 4 in ABR waveforms. The effect of GeO_2 on the ABR thresholds was analyzed by comparing the final thresholds, measured 2 month later for animals that were administered 0.5% GeO_2 and 6 month later for those given 0.15% GeO_2 and controls, among the three groups using one-way ANOVA followed by a multiple comparison procedure (Student–Newman–Keuls method).

The controls and animals that were administered 0.15% GeO_2 were sacrificed 6 months later. The animals that were administered 0.5% GeO_2 were sacrificed after 2 months because most of them became very inactive and seemingly unhealthy at that time. Under anesthesia with xylazine hydrochloride (10 mg/kg, i.m.) and ketamine hydrochloride (40 mg/kg, i.m.), the bulla in the left ear was exposed, a small opening was made in the scala tympani, and the perilymphatic spaces were perfused with a fixative containing 2% paraformaldehyde and 2.5% glutaraldehyde in phosphate buffer. This was followed by immediate excision of the soleus and extensor digitorum longus (EDL) muscles, liver, kidney, and heart. A part of these organs was quickly frozen in isopentane chilled in liquid nitrogen, and the remaining specimens were immersed in the fixative. The left temporal bone was then excised, immersed in the same fixative, decalcified in 10% ethylenediaminetetraacetic acid, and divided into cochlear and vestibular parts. The cochlea was incised parallel to the modiolus and the utricular macula and ampulla of the lateral semicircular canal were obtained. For electron microscopy, all the fixed specimens (muscle, liver, kidney, heart, cochlea, utricle, and lateral semicircular canal) were post-fixed in 1% osmium tetroxide, dehydrated in a graded ethanol series, and embedded in epoxy resin. Ultrathin sections were double stained with uranyl acetate and lead citrate and examined with a transmission electron microscope (TEM), as previously described [18]. Serial frozen sections of the skeletal muscle, heart, kidney, and liver were also stained with hematoxylin and eosin (H&E) and an antibody to COX, as previously described [16].

In the controls and animals that were administered 0.15% GeO_2, the body weight increased gradually from 280 to 780 g during a period of 6 months. In contrast to this, the weight of animals that were administered 0.5% GeO_2 increased slightly to 310 g in the first month and reduced to 295 g in the second month (Fig. 1). The weight loss was accompanied by muscle weakness and atrophy, but none of the animals exhibited apparent neurological abnormalities such as ataxia or convulsions.

The controls and animals that were administered 0.15% GeO_2 did not demonstrate shifts in the ABR threshold compared with their baseline values at any frequency during the experiment, whereas the animals that were administered 0.5% GeO_2 exhibited significant increase ($p < 0.01$) in the ABR thresholds at all frequencies 2 months later (Fig. 1).

The most prominent findings in the animals that were administered 0.5% GeO_2 were marked degeneration of the SV and supporting cells in the organ of Corti (Fig. 2). Vacuolar degeneration, accompanied by abnormal mitochondria containing electron-dense inclusions, was predominantly observed in the SV of the basal turns of the cochlea. Electron-dense mitochondrial inclusions were also observed in the Hensen cells, Boettcher cells, and Claudius cells, all of which showed marked degenerative changes. The inner and outer hair cells (IHCs and OHCs, respectively), pillar cells, and Deiters cells, as well as the basilar and tectorial membranes, showed no obvious signs of pathology. A small amount of electron-dense material was observed along the intact cochlear nerve fibers. No electron-dense material or pathological changes were observed in the utricle, lateral semicircular canal, or among the vestibular nerve