Research report

Protective effect of propofol on noise-induced hearing loss

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Abstract

Purpose: Iatrogenic noise produced by mastoid or craniotomy drills may cause hearing damage, which is induced by the generation of reactive oxygen species (ROS) and the reduction of cochlear blood flow (CoBF). This study investigated whether propofol could reduce noise-induced hearing loss (NIHL) in a guinea pig model.

Methods: Sixty-four male pigmented guinea pigs were randomly and equally divided into 4 groups: control, noise, propofol and propofol + noise. Propofol was infused intravenously for 20 min prior to noise exposure with a loading dose of 5 mg kg⁻¹ for 5 min and a maintenance infusion of 20 mg kg⁻¹ h⁻¹ for 135 min. For noise exposure, an octave band noise at a 124 dB sound pressure level (SPL) was administered to animals for 2 h. The mean arterial pressure (MAP) and CoBF were monitored continuously. Auditory function was measured by the level of distortion product otoacoustic emission (DPOAE) before and at 1 h, 72 h and 240 h after noise exposure. Cochlear levels of 8-iso-Prostaglandin F₂α (8-iso-PGF₂α) were measured immediately after the termination of noise exposure. Cochlear silver nitrate staining and outer hair cell (OHC) counting were performed after the final functional test.

Results: Noise exposure caused decreases in the CoBF and DPOAE amplitudes, over-generation of 8-iso-PGF₂α and the loss of OHCs. Pre-treatment with propofol significantly increased the CoBF and DPOAE amplitudes, decreased 8-iso-PGF₂α and the loss of OHCs.

Conclusions: Propofol exerted protective effects against NIHL in this animal model by suppressing a lipid peroxidation reaction and improving CoBF.

Keywords: Noise-induced hearing loss, Propofol, 8-iso-PGF₂α, Cochlear blood flow

1. Introduction

Iatrogenic noise generated by medical instruments has been implicated as a cause of noise-induced hearing loss (NIHL) after ear surgery or craniotomy (Tos et al., 1984). Minimizing the duration of the drilling time is reported to be the most effective way to avoid NIHL (Farzanegan et al., 2010); however, it is often impracticable.

The generation of reactive oxygen species (ROS) and decreases in the cochlear blood flow (CoBF) are thought to contribute to NIHL (Henderson et al., 2006; Miller et al., 2003). Outer hair cells (OHCs) are one of the most important populations of sensory cells in the auditory system (Lim and Melnick, 1971). As a result of their large plasma membrane structure and high energy consumption, OHCs are easily attacked by ROS (Hu et al., 2002) and suffer from compromised CoBF (Nakashima et al., 2003). Interventions that target the prevention of OHC death have been proven to have protective effects against NIHL.

The oto-protective effects of several volatile anesthetics have been reported by Chung (Chung et al., 2007). Propofol is the most widely used intravenous general anesthetic as a result of its chemical similarity to the endogenous antioxidant α-tocopherol (vitamin E) (Aarts et al., 1995); it has been reported to have promising effects on the amelioration of oxidative injury in various organs (Adaramoye et al., 2013; Bickler et al., 2012; Gokcinar et al., 2013). However, to our knowledge, no study has examined the influence of propofol on NIHL. In the present study, we investigated the effects of propofol on NIHL and its potential mechanism.

2. Results

2.1. Effects of propofol on mean artery pressure (MAP) and cochlear blood flow (CoBF) during noise exposure

In the noise group, the MAP began to increase 5 min after noise onset compared with the control group (t = 2.621, p = 0.016). The
MAP in the propofol + noise group was significantly lower than the noise group \((t = 5.463, p < 0.001)\), but higher than the propofol only group \((t = 2.785, p = 0.015)\) (Fig. 1a).

In the noise group, the CoBF began to decrease gradually 30 min after noise onset \((t = 2.634, p = 0.016)\). The CoBF reached its lowest point \((24\% \text{ below baseline})\) at the end of noise exposure. The CoBF in the propofol + noise group also showed the decreasing trend, and was lower than the control group in the last 25 min of noise exposure \((t = 2.246, p = 0.047)\). However the CoBF in the propofol + noise group was significantly higher compared with the noise group throughout the entire exposure period \((t = 4.012, p = 0.001)\) (Fig. 1b).

### 2.2. Distortion product otoacoustic emission (DPOAE)

At 1 h after noise exposure, DPOAE amplitudes in the noise group decreased significantly at frequencies of 4, 6 and 8 kHz \((t = 5.756, 6.283, 6.445; \text{all } p < 0.001)\) and recovered gradually during the following test. However, amplitudes remained significantly lower than the control group at 240 h after noise exposure \((t = 2.774, 3.021, 2.528; \text{all } p < 0.050)\). Pretreatment with propofol increased DPOAE amplitudes at frequencies of 4, 6 and 8 kHz at 1 h \((t = 3.125, 3.498, 4.021; \text{all } p < 0.010), 72 \text{ h} (t = 2.716, 2.978, 3.013; \text{all } p < 0.050) \text{ and } 240 \text{ h} (t = 2.445, 2.563, 2.528; \text{all } p < 0.050) \text{ after noise exposure compared with noise only group (Fig. 5).}

### 2.3. Cochlear 8-iso-Prostaglandin \(F_{2\alpha}\) (8-iso-PGF2\(\alpha\))

Immediately following the cessation of the noise, 8-iso-PGF2\(\alpha\) significantly increased in the noise group compared with the control group \((t = 7.554, p < 0.001)\). Pretreatment with propofol decreased the cochlear 8-iso-PGF2\(\alpha\) content compared with the noise group \((t = 6.892, p < 0.001)\) (Fig. 2).

### 2.4. OHCs nitrate staining and counting

In control and propofol groups, three rows of OHCs were arranged orderly with no obvious missing cells. In the noise group approximately 30% OHCs were missing. The lesion mainly occurred in the middle to high frequency region \((\text{at approximately } 50–100\% \text{ distance from the apex})\). OHCs loss significantly decreased in propofol + noise group compared with noise only group \((t = 6.324, p < 0.001)\), nonetheless, it remained significantly higher than the control group \((t = 2.439, p = 0.042)\) (Figs. 3 and 4).

### 3. Discussion

The results of this study show that propofol pretreatment can exert promising oto-protective effects against NIHL in guinea pigs. We propose that the mechanisms by which propofol may reduce the pathological consequences of damaged OHCs are associated with a lower generation of ROS in the cochlea and improvements in the CoBF during noise exposure, which occur by decreasing the formation of vasoactive lipid peroxidation 8-iso-PGF2\(\alpha\). The intervention dose chosen for propofol and the time interval for tests came from one of our previous related study \((Xiao et al., 2014)\). The reason why we started the noise exposure 20 min after the infusion was because MAP and CoBF became stable after this period of infusion.

Exposure to intense sound can damage the auditory organ and result in hearing loss \((Tsuprun et al., 2003)\). Noise produced by medical instrument such as mastoid drilling can reach an intensity of 125 dB sound pressure level (SPL), with a constant mean above 100 dB SPL \((Stromberg et al., 2010)\), and by surgical aspirators range from 100 to 129 dB SPL \((Yin et al., 2011)\). This high-level noise may damage not only the operated ear but also the contralat-

![Fig. 1. MAP and CoBF. (a) MAP; (b) CoBF. Data are expressed as the means ± SD \((n = 16 \text{ per group})\). "p < 0.05” noise vs. control; "p < 0.05” propofol vs. control; "p < 0.05” propofol + noise vs. noise; "p < 0.05” control vs. propofol + noise. (MAP: mean artery pressure; CoBF: cochlear blood flow).](image)

![Fig. 2. Cochlear 8-iso-PGF2\(\alpha\) content. Data are expressed as the mean ± SD \((n = 8 \text{ per group})\). p < 0.05 for noise vs. all groups. There were no significant differences between the propofol, propofol + noise, or control groups.](image)
eral ear due to negligible attenuation of sound by the intact skull (da Cruz et al., 1997; Karatas et al., 2007). Minimizing the number of burr holes and the duration of drilling time were reported to be the most effective ways to avoid NIHL (Farzanegan et al., 2010), however, they are often difficult to achieve due to the patient’s condition and the limitation of the operator’s skill. For these reasons, some prophylactic drugs or complementary approaches are needed during these surgeries to alleviate such injuries.

The generation of ROS with concomitant increased metabolic activity is thought to contribute to NIHL (Henderson et al., 2006). ROS can break down lipid and protein molecules and trigger sensory cells death. Another mechanism includes a lipid peroxidation process to form other downstream compounds. Among these compounds, the presence 8-iso-PGF2α has been demonstrated following noise exposure. 8-iso-PGF2α was generated by free-radical-catalyzed peroxidation of arachidonic acid and was also identified as a potent vasoconstrictor in the cochlea, which, in turn, caused the reduction of CoBF (Wang et al., 2007). Insufficient blood supply acts also as one mechanism which accounts for NIHL. As results of increased metabolic activity and decreased blood flow, the inner ear homeostasis is disturbed (Shi, 2011). However, reperfusion after the restoration of blood flow aggravates the disturbance, potentially resulting in the burst generation of ROS (Seidman et al., 1999). Thus, the deterioration of blood supply and the over-production of ROS form a loop to intensify this damaging process. Propofol was reported to ameliorate oxidative injury in a variety of organs partly via directly scavenging free radicals (Adaramoye et al., 2013), inhibiting lipid peroxidation (Li et al., 2014). In the present study, the cochlear 8-iso-PGF2α in the noise group was found to be 4 times higher than the control group. CoBF decreased 30 min after the onset of noise and to 24% below baseline at the end of noise exposure. The constant infusion of propofol significantly decreased 8-iso-PGF2α formation in the cochlea which might underlie the increased CoBF during noise exposure compared with noise only group. However, in the propofol + noise group, the CoBF still showed a decreasing trend, and in the last 25 min, it decreased below baseline. These findings may be due to another mechanism, for example, propofol may not completely block the activation of sympathetic nervous system (SNS) caused by this noxious stimulation, and therefore, may result in the elevation of circulatory noradrenaline (NA), which may then decrease the CoBF due to its vasoconstrictive effect. This effect may also underlie the slight increase in the MAP in the propofol + noise group compared with the propofol group during the exposure. However, in one of our previous studies, we found that dexmedetomidine exerted a hearing protection effect by fully suppressing the activation of the SNS caused by noise exposure (Wen et al., 2014).
OHCs are known to be most vulnerable to noise in the mammalian cochlea, and the loss of OHCs is considered to be the principle physiopathological mechanism underlying NIHL (Yang et al., 2004). In our present study, significant OHCs loss was observed 10 days after noise exposure. The lesion was predominantly located at the region approximately 50–100% of the distance from the apex (i.e., the middle and basal turn of the organs of corti). DPOAE amplitudes also showed a substantial decrease at 4–8 kHz, which corresponded to the cochlear lesion. Our results manifested the typical character of NIHL and were consistent with a previous study (Minami et al., 2007). Pretreatment with propofol significantly alleviated the damage to OHCs and improved the DPOAE amplitudes at frequencies of 4–8 kHz, with no clear decrease in DPOAE amplitudes observed 10 days after noise exposure. Two possible mechanisms might underlie this phenomenon. First, the maintenance of blood flow during noise exposure caused by propofol. Second, the presence of an antioxidant effect as a result of propofol’s chemical similarity to the endogenous antioxidant vitamin E may be involved, which offered OHCs both direct protection against the damage of ROS and disruption of the vicious cycle caused by ROS. The 10 day time interval we chose to perform the OHCs counting was based on the study of Yamashita (Yamashita et al., 2008), who found the lesion of OHCs and hearing function stabilized 10 days after noise exposure.

There are two main limitations to this study. First, it is well known that both apoptosis and necrosis are involved in the pathways leading to the death of OHCs (Yang et al., 2004). However, we did not determine whether the protective mechanisms of propofol occurred via an inhibition of apoptosis or by necrosis of OHCs. Second, we did not investigate the extent to which propofol suppressed the activation of SNS by noise. These two questions will be addressed in a future study.

Conclusions: Systemic administration of propofol showed a promising auro-protective effect against NIHL, which was evidenced by amelioration of lipid peroxidation damage and improvement of CoBF during noise exposure. These findings could have clinical relevance, and therefore, the therapeutic mechanism of propofol deserves further investigation.

4. Experimental procedure

4.1. Subjects

Sixty-four male pigmented guinea pigs (500–600 g) with a normal Preyer’s reflex were obtained from the Experimental Animal Center of the Medical College of Xian Jiaotong University. Otomicroscopy examination was performed to exclude the possibility of middle-ear pathology. Animals were housed in cages and maintained in environmentally controlled rooms with a 12-h light/dark cycle; food and water were available ad libitum. All experiments were approved by the Institutional Animal Care and Use Committee of Xian Jiaotong University. Efforts were made to minimize animal suffering and to reduce the number of animals that were used.
4.2. Surgery and preparation

Animals were initially anaesthetized with an intraperitoneal (i.p.) injection of sodium pentobarbitone (40 mg kg\(^{-1}\)), which was re-administered 60 min later with half of the initial dose. Animals were tracheotomized, paralyzed with vecuronium bromide, and artificially ventilated. Inspiratory and expiratory \(P_O_2\) and \(P_CO_2\) values were monitored continuously on a gas analyzer. Core temperature, which was measured by a rectal probe coupled to a thermal sensor, was maintained at 38.0 ± 0.2 °C with a heated blanket.

The right femoral artery was cannulated to measure the MAP and sample the arterial blood for gas analysis. MAP was measured by a pressure processor amplifier after the cannulation of the artery, and was recorded continuously. A second microcatheter was inserted into the left jugular vein for the administration of drugs.

4.3. Noise exposure

This study utilized the noise exposure model described by Masuda (Masuda et al., 2006). Animals were exposed to 124 dB SPL octave band noise, centered at 4 kHz, for 2 h. The intensity and frequency resembled the noise condition generated by a mastoid or craniotomy drill. Noise was generated (AA-67N; RION, Tokyo, Japan), amplified (SRP-P150; SONY, Tokyo, Japan), and delivered monaurally through an earphone (DT48, Beyer Dynamic, Germany), which was placed in both external auditory meatus. The SPL was monitored by a PE tube attached to the earphone.

4.4. Laser Doppler Assessment of CoBF

The CoBF in the right ear was recorded continuously before and during sound exposure, using Attanasio’s method (Attanasio et al., 2001). For each animal, the head was fixed in a moveable head holder. The right cochlea was exposed using a ventrolateral approach, while leaving the ossicular chain and tympanic membrane intact. The mucosa overlaying the cochlea was gently removed, with care taken to keep the bone dry. The CoBF was measured with a laser Doppler flowmeter (PeriFlux PF3 Perimed, Sweden). The needle-shaped probe was placed on the lateral wall of the basal turn of the cochlea with a micromanipulator. Blood flow signals were recorded online using a three-channel chart recorder. Changes in CoBF were expressed as percent changes from baseline.

4.5. DPOAE

Under an otomicroscope, animals were examined for external ear canal and middle ear obstruction, and the external auditory meatus was cleaned. The tympanic membrane was punctured at the anteroinferior quadrant with a capillary needle (diameter of 30 μm) to balance the pressure inside and outside the tympanic membrane (Ohihata et al., 2000). The DPOAE was recorded by an otoacoustic emission analyzer (intelligent hearing system SmarktOAE, Miami, FL, USA). The test was performed in an indoor double shielded room with ambient noise <30 dB SPL and a normal body temperature. An Etymotic 10B+ probe (Etymotic Research, Elk Grove Village, IL, USA) was inserted into the external ear canal. The bandwidth of the cubic DPOAE responses (2f1–f2) was set to a frequency range from 0.5 to 8.0 kHz, and 6 points were sampled at a rate of 128 kHz using a 16-bit D/A converter. Frequencies were acquired with an f2:f1 ratio of 1:22. The intensity of primary tones was set to L1 = 70 dB SPL, L2 = 65 dB SPL. A valid DPOAE data point measurement was considered positive with a response amplitudes of 3 dB above background. Data from the left ear were analyzed in the present paper.

4.6. ELISA assay for 8-iso-PGF\(_{2\alpha}\)

Animals were deeply anaesthetized with sodium pentobarbitone (40 mg kg\(^{-1}\), i.p.) and then decapitated. The temporal bones were quickly dissected, and the bulla was opened to expose the otic capsule. Using microdissection techniques, the lateral walls were removed and placed in ice-cold Hanks’ balanced salt solution, and the organs of Corti, including the modiolus, were isolated, frozen in liquid nitrogen, and stored at −80 °C. At the time of assay, each frozen cochlear tissue was homogenized in 0.5 ml phosphate buffer (pH 7.0) using micro-tissue grinders. The homogenate was centrifuged at 9000 g for 15 min at 48 °C. Protein concentrations of the supernatant were measured using a BCA protein quantitative assay kit (Pierce, USA). Ethanol was added to the supernatant and the samples were then homogenized. After centrifugation at 2000g for 10 min, 0.05 N NaOH was added to the supernatant and incubated at 40 °C for 1 h. The sample was applied to a Sep Pak C18 column for solid-phase purification processing and was tested by an ELISA kit (Promega, Madison, WI, USA) for 8-iso-PGF\(_{2\alpha}\).

4.7. Cochlear silver nitrate staining and OHCs counting

The same methodology described for the 8-iso-PGF\(_{2\alpha}\) assay was used to obtain the cochlear tissue. The oval window, the round window, and the apex of the cochlea were opened with a fine steel needle under a stereomicroscope. The cochlea was perfused with a 0.5% silver nitrate solution from the tip of the cochlea 5 times. After the cochlea was washed with distilled water, it was filled with 10% formaldehyde solution 10 times, and then immersed in the same formaldehyde solution for 3 h at room temperature. The basilar membrane of the well fixed cochlear tissue was dissected under the stereomicroscope and exposed to sunlight for approximately 2 h. Specimens were mounted in glycerin on glass slides and examined under an optical microscope. OHCs were counted, and cochleograms were generated. The total length of each basilar membrane for the counted OHCs was no less than 400 μm. The number of OHCs was quantified per 10% distance from the apex to the basal end of the cochlea using an ocular grid system and a 20× objective lens. OHCs without stereociliary or gaps between the normal geometric arrays were considered to be lost and were not included in the counts. The loss of OHCs was calculated as the percentage of the number of intact cells in normal fragments. Results were entered into a database and analyzed using quantitative analysis software.

4.8. Procedure

The 64 animals were randomly and equally assigned to one of the following four treatment groups (n = 16 per group): the control group, which received intravenous (i.v.) saline vehicle (20–25 μl); the noise group, which received saline (20–25 μl) and noise trauma for 2 h without propofol; the propofol group, which received propofol only; and the propofol + noise group, which was pretreated with i.v. propofol followed by noise treatment. Propofol was injected i.v. with a loading dose of 5 mg kg\(^{-1}\) over 5 min followed by a maintenance infusion of 20 mg kg\(^{-1}\) h\(^{-1}\) administered via an infusion pump. The total infusion lasted 140 min. Noise exposure started 20 min after propofol infusion and lasted for 120 min. The DPOAE was measured in all animals before sound exposure to obtain baseline values; it was measured again in eight animals at 1 h, 72 h and 240 h after the stop of noise. The CoBF and MAP were measured continuously in all animals during noise exposure. The cochlear tissues of 8 animals in each group were harvested for an enzyme immunoassay to assess 8-iso-PGF\(_{2\alpha}\) immediately after the cessation of noise. After the final functional test of DPOAE, the remaining 8 animals in each group were eutha-
nized for cochlear basilar membrane silver nitrate staining and OHCs counting.

4.9. Data collection and statistical analysis

Data are expressed as the means ± SD. One-way analysis of variance (ANOVA) for repeated-measures was used to compare the values of CoBF and MAP between and within the treatment groups. One way ANOVA was used to analyze the OHCs counting and 8-iso-PGF2α between the groups. When statistical significance was present, the post-hoc Student-Newman-Keuls test was used for multiple comparisons. Differences were considered statistically significant at the 5% level (P < 0.05).

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References


