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Research paper

Long-term administration of magnesium after acoustic trauma caused by gunshot noise in guinea pigs

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ABSTRACT

In a previous study we observed that a 7-day post-trauma magnesium treatment significantly reduced auditory threshold shifts measured 7 days after gunshot noise exposure. However this improvement was only temporary, suggesting that it could be potentially beneficial to prolong this treatment. The aim of the present study was to evaluate the efficacy of a long-term (1 month) magnesium treatment after an impulse noise trauma, in comparison with either a 7-day magnesium treatment, an administration of methylprednisolone (conventional treatment), or a placebo (NaCl). Guinea pigs were exposed to impulse noise (three blank gunshots, 170 dB SPL peak). They received one of the four treatments, 1 h after the noise exposure. Auditory function was explored by recording the auditory brainstem response (ABR) and measuring the distortion product otoacoustic emissions (DPOAE) over a 3-month recovery period after the gunshot exposure. The functional hearing study was supplemented by a histological analysis. The results showed that a 1-month treatment with magnesium was the most effective treatment in terms of hair cell preservation. The DPOAE confirmed this effectiveness. Methylprednisolone accelerated recovery but its final efficacy remained moderate. It is probable that magnesium acts on the later metabolic processes that occur after noise exposure. Multiple mechanisms could be involved: calcium antagonism, anti-ischaemic effect or NMDA channel blockage. Regardless of the specific mechanism, a 1-month treatment with magnesium clearly attenuates NIHL, and presents the advantage of being safe for use in humans.

1. Introduction

Loss of hearing is a major health condition affecting more than 278 million people worldwide. It is estimated that 16% of disabling loss of hearing in adults worldwide is due to occupational noise (Nelson et al., 2005). Exposure to high-intensity noise can cause irreversible hearing damage, as a result of direct mechanical or secondary metabolic effects. The noise causing the trauma may be continuous (concerts etc.), repetitive impulsive (industrial noise, pneumatic drills etc.) or pure impulsive (explosions, gunshot noise). The latter case particularly affects hunters, victims of terrorist attacks and military personnel. The military is one of the world's most noise-hazardous occupations, and noise-induced hearing loss (NIHL) in the military continues to be a severe and costly problem despite hearing conservation programs. Auditory damage from acoustic trauma accounted for up to 47% of all wounded in action evacuations from Operation Iraqi Freedom and Operation Enduring Freedom, and is the fourth leading reason for medical referral for military personnel returning from deployment (Kopke, 2005).

There are many unresolved issues concerning noise-induced hearing loss. For instance, uncertainties exist over treatment. The current post-exposure treatment for acute acoustic trauma by the US Military includes removing the patient from the noise hazard and maintaining him in an effectively quiet environment for 21 days. In other countries including France, the conventional therapy is based on corticosteroid administration (1.5 mg/kg methylprednisolone, IV during 6 days). Since the discovery that noise-induced metabolic processes (including oxidative stress, ischaemia, ionic disturbance and excito-toxicity) play a significant role in cochlear injury, research has defined a variety of potential therapies which are effective in reducing permanent hearing loss associated with acoustic overexposure in animal

Abbreviations: ABR, auditory brainstem response; dB SPL, decibel sound pressure level; DPOAE, distortion product otoacoustic emission; GR, glucocorticoid receptor; IHC, inner hair cell; NIHL, noise-induced hearing loss; NMDA, *N*-methyl-D-aspartate; OHC, outer hair cell; PTS, permanent threshold shifts; SEM, standard error of the mean; TTS, temporary threshold shifts.

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models (see Le Prell et al. (2007b) for review). Among pharmacological agents, magnesium could present relative therapeutic efficiency. Whilst magnesium is well known as a preventative treatment for sound trauma (see Cevette et al. (2003) and Sendowski (2006a) for reviews), a study by Scheibe et al. (2001) showed that it may also be useful therapeutically: in animals submitted to a series of impulses (167 dB SPL, $1s^{-1}$, 38 min), the administration of magnesium by the systemic route significantly reduced hearing loss after 7 days. This treatment was more effective if it was quickly instigated. Threshold shift reduction 7 days after acoustic trauma in guinea pigs was confirmed using DPOAE (Haupt et al., 2003). In another study (Sendowski et al., 2006c) it has been shown that a 7-day post-trauma magnesium treatment reduced auditory threshold shift measured 7 days after gunshot noise exposure. However, this improvement was temporary, suggesting that it could be potentially beneficial to prolong magnesium administration. The aim of the present study was therefore to evaluate the efficacy of a long-term (1 month) magnesium treatment after gunshot noise trauma, in comparison with either a 7-day magnesium treatment, an administration of methylprednisolone or a placebo (NaCl).

2. Materials and methods

Sixty-five female albino guinea pigs (outbred Dunkin Hartley, 400–500 g, Charles River France) with normal Preyer reflex were used in this study. The protocol of this work was approved by the local animal care and use committee (no. 2005/14.0).

2.1. General protocol

The animals were randomly divided into four groups: two groups treated with magnesium (7-day and 1-month treatments), one group treated with methylprednisolone (as the reference treatment) and a placebo group (NaCl). The guinea pigs were exposed to gunshot noise and received the treatment 1 h after exposure. Auditory function was evaluated by auditory brainstem responses (ABR) and distortion products otoacoustic emission (DPOAE). These functional tests were performed before trauma (control), and after 20 min, 7 days, 14 days, 1 month and 3 months of recovery. A histological analysis, conducted at the end of the experiment, completed these functional data.

2.2. Auditory thresholds

The auditory thresholds were determined by ABR under light anesthetic (Ketamine chlorhydrate 40 mg/kg with xylazine 10 mg/kg IP). The body temperature of the animal was maintained at 38 ± 1 °C using an isothermic heating pad. The response signals to 12 frequencies from 2 to 32 kHz were recorded by subcutaneous stainless-steel needle electrodes. The difference in potential was measured between an electrode on the vertex and an electrode behind the ipsilateral ear, with a neck electrode serving as ground. The equipment used to generate the sound stimuli and record the ABR included two microprocessors and a programmable attenuator, driven by Siggen and Biosig software (system 3; Tucker–Davis Technologies (TDT); Gainsville, FL, USA). The sound stimuli consisted of bursts of pure tone (duration 13 ms, rise time 1.5 ms), presented 15 times/sec, the levels of which ranged from 0 to 90 dB SPL in 5 dB steps. The sound was delivered by an earphone (Sennheiser type P ×100) placed near the ear channel. Cochlear responses were amplified (×100,000) by a differential amplifier (GRASS RPS107; Astromed; Trappes; France), filtered (0.1 Hz–3 kHz), averaged (1024 samples) and stored in a Pentium PC. Threshold, tested separately for each ear, was defined as the lowest intensity of stimulation to yield a repeatable waveform based on an identifiable ABR wave III or IV. ABR wave III is the most robust component of the guinea pig waveform (Le Prell et al., 2007a). At threshold intensity, at least two sequences of recording were carried out to confirm response reproducibility. Each time, threshold shifts relative to the control before trauma were calculated.

2.3. DPOAE measurements

The measurement of DPOAEs was performed just before the ABR determination (under light anesthesia on a heating blanket). The equipment used to generate the two primary tones (f1 and f2) and to detect the 2f1–f2 distortion product included a microprocessor, an electrostatic speaker driver and a microphone amplifier (system 3, TDT; Gainsville, FL, USA). Two short plastic tubes connected the transducer outputs (EC1, TDT) to the OAE probe (ER10B+; Etymotic Research), which contained the miniature low-noise microphone for emission detection. The end of the probe was deeply and tightly inserted into the external ear canal. The entire stimulation and detection process was automatically controlled using a PC computer and driven by a MATLAB™ script. The software, based on Fourier transform calculation, generated the stimulations through two independent channels and computed the complex response at 2f1–f2. Our standard procedure was to generate a "DP gram" that is scan of DPOAE amplitude from 2 to 22 kHz (f2 frequency) with 8 frequencies per octave. The levels of the primary tones were 65 dB SPL for f2 and 60 dB SPL for f1, and the ratio f2/f1 was set at 1.20. The stimulus parameters were chosen according to published data about DPOAEs in guinea pigs (Michaelis et al., 2003). Data are described with respect to f2 frequency since the generator site of the 2f1–f2 distortion product has been most closely correlated with the f2 frequency place in the cochlea (Brown and Kemp, 1984).

Before each test, the entire system was calibrated using a Brüel & Kjaer conditioner (B&K; nexus; bandwidth 140 kHz) connected to a 0.5-in. microphone (B&K type 4191). An artificial ear (Brüel and Kjaer type 4153), adapted to guinea pigs, was used to calibrate the speakers. During calibration, each stimulus was recorded on a digital recorder (Odyssey OD100; Nicolet technologies) and both the frequency and the intensity were analysed using special software (Proview; Nicolet technologies).

Reliability of this method over time has been prior assessed on non-traumatised animals ($n = 4$), measuring the DPOAE amplitude three times with 2 weeks intervals.

2.4. Sound trauma and treatment

The acoustic trauma was obtained in the morning (at around 10 a.m.) by exposing the animal to three blank gunshots from a FAMAS F1 rifle, and was performed in an anechoic room. The guinea pig was placed in a hammock 60 cm from the barrel of the gun, corresponding to a 170 dB SPL (peak value). A microphone (1/8-in.; B&K type 4231), the membrane of which was placed parallel to the eardrum of the animal, enabled the noise perceived in the ear to be estimated. The signal was conditioned (Nexus, B&K) and recorded on the Odyssey system (OD200 – 10 MS/s).

Treatment began 1 h after the trauma. The magnesium protocol we chose was the one successfully used previously which resulted in an increase of blood and perilymph magnesium concentrations (Scheibe et al., 2002; Sendowski et al., 2006c). The animals belonging to the magnesium treated groups received subcutaneous injections of magnesium for 3 days (35 mg $MgSO_4$/100 g) and were given drinking water supplemented with magnesium (3.7 g $MgCl_2$/l) for 1 week ("7-day Mg" group) or 1 month ("1-month Mg" group). The control animals received injections of physiological saline under the same conditions ("NaCl" group), with no