

The impact of carbon monoxide inhalation on developing noise-induced hearing loss in guinea pigs

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Abstract

Carbon monoxide (CO) poisoning is one of the most common types of fatal poisonings worldwide. Acute exposure to high levels of CO as well as chronic exposure to low levels of CO and excessive noise can lead to high frequency hearing loss. In this study, twelve guinea pigs were randomly divided into two groups: (1) exposed to noise and (2) exposed to noise plus CO. Auditory brainstem responses (ABRs) were measured prior to the experiment and immediately, 5, 10 and 15 days post exposures. There was a significant difference between the ABR thresholds before and immediately after exposure to noise at frequencies of 4, 8, and 16 kHz and the most threshold shift was observed at 8 kHz. There was also a significant difference between the ABR thresholds before and immediately after exposure to noise and CO at frequencies of 2, 4, 8, and 16 kHz which demonstrated a temporary hearing loss after exposure to noise and CO and the major impact of CO on developing noise induced hearing loss occurred at 8 kHz. No significant difference was observed between the ABR thresholds recorded before conducting the experiments and the ones obtained 5, 10 and 15 days after simultaneous exposure to noise and CO at none of frequencies. Simultaneous exposure to noise and CO contributes to transient hearing loss in guinea pigs with the most evident temporary shift at 8 kHz. The methods were accepted in the Ethics Committee of Iran University of Medical Science (registration No. CTRI/2016/01/017170) on January 18, 2016.

Key words: auditory brainstem responses; carbon monoxide; frequencies; guinea pigs; noise; noise induced hearing loss; thresholds; transient hearing loss

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INTRODUCTION

The overwhelming population growth coupled with development of industry and metropolitan technology has created too many problems for urban dwellers. Noise pollution as one of the principal environmental pollutants is considered as a key contributor in causing such problems in large cities.¹ Noise-induced hearing loss (NIHL) is not only a physical injury but also a cochlear injury caused by metabolic complications.² Metabolic disorders can increase the formation of free radicals in mitochondria.³ Such free radicals created in cochlea have been reported to be the major cause of developing NIHL.⁴ Decrease in cochlear blood flow due to the formation of free radicals as a result of exposure to noise have shown to cause oxygen deficiency in the cochlea and all these consequences ultimately lead to apoptosis and cell death.^{5,6} Meanwhile, pre-treatment of the rats with normobaric hyperoxia have contributed to a significant reduction in ABR threshold shifts, and improving the distortion-product otoacoustic emissions amplitudes.⁷

Carbon monoxide (CO) poisoning is one of the most common types of fatal poisoning in many countries around the world. Acute exposure to high levels of CO or prolonged exposure to low levels of this gas and excessive noise has

shown to increase hearing loss at high frequencies.⁸

Several studies conducted on animals have demonstrated that exposure to CO alone cannot cause hearing loss; however, if animals are simultaneously exposed to noise and CO (at concentrations > 500 ppm), CO would have a reinforcing effect for developing NIHL.^{1,3,9,10}

Various studies have investigated this reinforcing effect. Their results have indicated that the levels of oxygen free radicals in the cochlea of animals exposed to simultaneous noise and CO were significantly higher than those in the cochlea of animals exposed to noise alone. Therefore, the main aim of this study was to investigate the effect of CO inhalation on NIHL in guinea pigs using auditory brainstem response (ABR) test.^{1,9,11}

MATERIALS AND METHODS

Ethical statement

To comply with the ethical guidelines “the Laboratory Checklist for Animals” based on the Animal Health Guidelines was implemented. Thus, in terms of the number of animals, we attempted to use the least possible. The methods were accepted in the Ethics Committee of Iran University of Medical Science (Registration No. CTRI/2016/01/017170) on January 18, 2016.



Animals

Twelve adult male, white, 8-week-old Dunkin-Hartley guinea pigs weighing 250–350 g were purchased from the Pasteur Institute of Tehran, Iran. Animals were kept in the animal lab at the School of Rehabilitation Sciences, Iran University of Medical Sciences, Tehran, Iran and in the chamber according to the recommended conditions (in terms of temperature, light, food, ventilation and other physical and chemical conditions) with free access to water and food. The temperature and relative humidity were maintained at 20–25°C and approximately 45–55% and the laboratory lamp was illuminated from 7 a.m. to 7 p.m. (12:12 hours light: dark cycle).

Intervention

The animals were randomly divided into two groups ($n = 6$) according to the intervention: noise and noise + CO groups.

Exposure to noise

Animals were exposed to 4 kHz octave-band noise at 105 dB sound pressure level for 6 hours in 5 consecutive days. The desired noise was provided by a generator noise (Benaphon Electronic Company, Tehran, Iran).¹² The $100 \times 60 \times 100$ cm³ noise exposure chamber was made of glass (6 mm thick) and metal frame (galvanized iron), and speakers supported by a powerful amplifier produced the desired noise level inside the chamber. The chamber had the conditions of an acoustic reverberant field in a way that the sound pressure level was approximately the same at all points. Moreover, to ensure that the intensity was equal for both ears, the distance between the noise source and each ear was kept at around 10 cm.

Exposure to CO

Animals were exposed to CO at a certain concentration of 500 ± 5 ppm for 6 hours during 5 consecutive days in addition to noise exposure. Four small cages, each containing two guinea pigs (due to the possible animal mortality), were placed inside the chamber. The cages were positioned separately from each other and from the audio speakers. The desired concentration of CO in the chamber was dynamically generated in order to provide the animals with the minimum required ventilation within the chamber. According to the instructions from Organization for Economic Cooperation and Development standards,¹³ the rate of chamber airchange for this experiment should be maintained at 13–15 times per hour. Depending on the size of the chamber, the required flow rate for the proper ventilation was 60–80 L/min which was constantly provided by an air pump (ACO-006; Sunsun Yuting, China).

CO was introduced into the chamber through a regulator (CK14506; Hercules, Dortmund, Germany) using interface hoses and rotameter. Flow control of the inlet gas was adjustable by two parameters including regulator valve and rotameter screw. CO concentration was measured by a direct reading sensors and electrochemical sensor (MRU DELTA 65; Stack Sampler, Neckarsulm-Obereisesheim, Germany). The sensor was fixed in the chamber during animals' exposure and the CO concentration was continuously monitored throughout the

exposure period.

ABR test

Prior to performing the ABR test (Navigator pro natus, San Carlos, CA, USA), all guinea pigs were weighed using digital scales; an injection dose was calculated according to their weights. They were then anesthetized by intramuscular injection of xylazine (4 mg/kg; Pasteur Institute of Tehran) and ketamine (40 mg/kg; Pasteur Institute of Tehran).

The baseline ABR test was performed for all groups before any exposure to noise/CO. Then, the ABR test was recorded for the first group immediately after 5 days of exposure to noise and for the second group immediately after 5 days exposure to noise + CO (post 1).

The ABR test was also conducted for the first and second groups on the 5th (post 2), 10th (post 3) and 15th (post 4) days post-exposure. We made use of the ABR test in this study because of the fact that this technique is a favorable method for estimating hearing thresholds in animals and determined the statuses of the inner ear.^{14,15}

Statistical analysis

In the current study, the tables, graphs, statistical indices including central and scattering indices were employed to describe the obtained data. The data were also analyzed using analysis of variance with repeated measures. For comparing the threshold (mean \pm SD) between the two groups, we used independent samples *t*-test. *P* values of < 0.05 were considered statistically significant for the analysis. The IBM SPSS 22 statistical software package (IBM, Armonk, NY, USA) was used for the statistical analysis.

RESULTS

There was no statistically significant difference in ABR thresholds before exposure ($P > 0.05$; **Table 1**). Data analysis showed that there was a statistically significant difference in the thresholds of the auditory-evoked brainstem responses (ABR thresholds) between the noise and noise + CO groups immediately after exposure at 8 kHz ($P < 0.05$; **Table 1**). No significant difference was found between ABR thresholds of two groups at 5, 10 and 15 days after exposure at different frequencies ($P > 0.05$; **Table 1**).

DISCUSSION

Results indicated that the thresholds of the ABRs were significantly different between the ones obtained as baselines and those recorded immediately after exposure to noise at frequencies of 4, 8 and 16 kHz. In other words, exposure to noise at 105 dB sound pressure level for 5 consecutive days and 6 hours a day resulted in temporary hearing loss in animals and the highest threshold shift was occurred at 8 kHz.

Given that the highest energy of 4 kHz octave-band noise is 0.5–1 octave more than the central frequency; it is expected to find the highest threshold shift of ABR at about 8 kHz in exposure to octave-band noise centered at 4 kHz. This finding was reported in another study conducted by Yamasoba et al.¹⁶



Table 1: Change of auditory brainstem response thresholds (dB) in guinea pigs

Frequency (kHz)	Noise group	Noise + carbon monoxide group	P-value
Before exposure (baseline)			
2	28.50±4.18	25.16±12.81	1
4	20.16±3.76	22.66±9.30	1
8	16.00±6.32	11.83±4.91	1
16	13.16±5.84	12.33±7.35	0.75
Immediately after exposure			
2	41.00±4.47	45.16±8.61	1
4	41.50±10.83	46.50±10.36	1
8	40.33±13.66	50.66±13.29	0.01
16	29.33±14.71	34.00±15.81	1
5 d after exposure			
2	39.50±8.21	43.50±10.36	1
4	32.16±5.84	31.83±9.70	1
8	18.66±6.05	22.66±7.52	0.24
16	14.83±4.91	27.33±12.51	0.56
10 d after exposure			
2	39.12±3.76	39.33±6.05	1
4	31.00±3.16	30.16±8.61	1
8	13.83±5.84	16.00±8.36	1
16	13.16±5.84	16.50±8.21	1
15 d after exposure			
2	39.33±4.08	36.83±8.01	1
4	31.00±3.16	26.00±8.94	1
8	16.83±5.84	13.83±8.61	1
16	13.16±5.84	14.83±4.08	1

Note: Data are expressed as mean ± SD ($n = 6$), and were analyzed by repeated measures analysis of variance followed by independent samples *t*-test.

who concluded that the superior threshold shift occurred at 8 kHz in the guinea pigs exposed to 4 kHz octave-band noise. The ABR test was also recorded until 15 days after exposure to evaluate the recovery process. There was no significant difference in the thresholds of ABR between baseline and 5 days post exposure to noise and the thresholds almost returned to baseline value, confirming temporary hearing loss and recovery creation. This finding is consistent with that of Tan et al.¹⁷ who exposed the guinea pigs to broadband dB sound pressure level 105 noise for 12 hours and declared that the ABR thresholds were reduced immediately after exposure to noise. Moreover, a complete recovery in ABR thresholds was observed at all frequencies by performing the ABR test 4 days after exposure.

No significant difference was observed between the thresholds of ABR on the 5th, 10th and 15th days after exposure to noise, indicating that the threshold was fixed from the 5th day after exposure to noise. The following of thresholds on the 5th, 10th and 15th days and designed of CO chamber were the innovation of the study. There was a significant difference in the thresholds of ABR between baseline (before exposure to noise + CO and immediately after simultaneous exposure

to all frequencies of 2, 4, 8, and 16 kHz. In other words, the simultaneous exposure to noise + CO caused a temporary hearing loss in animals, and the highest threshold shift was at 8 kHz. Rao and Fechter¹⁸ in 2000 represented that CO at 500 ppm had a reinforcing effect on the NIHL. Furthermore, the threshold shift was seen up to the 15th day after exposure to noise + CO, but there was no significant difference in the thresholds of ABR between baseline and 5th, 10th and 15th days after exposure to noise + CO at none of the experimental frequencies. Besides, it was illustrated that recovery from the loss of exposure to noise + CO occurred at all frequencies. These results are inconsistent with those of Mortazavi et al.¹⁹ who have demonstrated that the reinforcing effect of CO at frequencies less than 2 kHz disappears over time, but this effect persists at higher frequencies.

This dissimilarity may be due to the differences in intensity level and duration of exposure to noise + CO and in auditory curve of rabbit and guinea pigs. The thresholds of ABR had greater increase in guinea pigs exposed to noise + CO compared to those exposed to noise alone at all experimental frequencies.¹⁹ Data analysis represented that there was a statistically significant difference between the thresholds of ABR of the second group immediately after the noise + CO exposure and those of the first group immediately after the noise exposure at 8 kHz.

Various studies have suggested that the increased reactive oxygen species play an important role in cochlear damage caused by exposure to noise + CO. Increased levels of superoxide, hydroxyl radical and reactive nitrogen species in cochlea as well as decreased levels of internal antioxidant enzymes including succinate dehydrogenase have been found after exposure to noise + CO.^{5,11,19,20} Therefore, the reinforcing effect of CO on NIHL in this study can be attributed to the role of this gas in increasing oxidative stress in the cochlea of animals. In summary, the findings of the present study indicated the reinforcing effect of CO on temporary NIHL at 8 kHz.

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Author contributions

FB carried out the experiment and wrote the manuscript with support from MSh. MF provided the technical support including the exposure instrumentations the systems. MK performed the analytic calculations. AR helped supervise the project. All authors read and approved the final version of manuscript for publication.

Conflicts of interest

Authors declare no conflicts of interest.

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Institutional review board statement

The study was approved and registered from Ethics Committee of Iran University of Medical Sciences (registration No. CTRI/2016/01/017170) on January 18, 2016.

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