主論文の要旨

Decreased hearing levels at frequencies for understanding speech in tannery workers exposed to a high level of trivalent chromium in Bangladesh

（バングラデシュにおける高濃度の三価クロムに曝露された皮なめし労働者の会話音域の聴力低下）

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【Background】

Decrease of hearing level (DHL) is one of the most important public health issues that directly affect social life worldwide. Hearing levels at lower frequencies of 0.5 k-4 k Hz centered on 1 k Hz are especially important for understanding speech. Hexavalent chromium [Cr(VI)], which has a strong corrosive effect, has been reported to cause perforation of the eardrum. Trivalent chromium [Cr(III)] also has a weak corrosive effect. However, there has been no study on the effects of exposure to Cr, either Cr(VI) or Cr(III), on hearing levels in animals or humans. Our previous study revealed that >99.99% of t-Cr in the wastewater to which workers were directly exposed inside the tanneries was Cr(III), indicating that tannery workers in Bangladesh were exposed to a high level of Cr (III). In this study, we aimed to investigate the effect of exposure to Cr(III) on hearing levels in tannery workers and then further examine the etiology by animal experiments.

【Methods】

A total of 149 male subjects including 100 tannery workers and 49 non-tannery workers (office workers) in Bangladesh participated in this cross-sectional study. Basic information including the investigation area, subjects, collection of biological samples and environmental conditions inside tanneries was the same as our previous studies. Noise levels in the office of non-tannery workers and in different tannery processes were measured. Hearing levels at frequencies of 1 k, 4 k, 8 k and 12 k Hz of subjects were determined by pure-tone audiometry (PTA) with air conduction. Levels of t-Cr in humans were determined by using hair and toenail samples and measured by inductively coupled plasma mass spectrometry (ICP-MS).

In the animal study, basic chromium sulfate (BCS), which is actually used in tanneries in Bangladesh, were prepared for external treatment of eardrums with Cr(III). A unilateral eardrum of male ICR mice (3-4 weeks of age) was treated once with 10 μL of BCS-dissolved distilled water that contained the mean concentration (1.9 μg/μL) of Cr(III) in wastewater samples previously measured in Bangladeshi tanneries. A unilateral eardrum of each of the control mice was also treated once with 10 μL of distilled water. Hearing levels were measured by auditory brainstem response (ABR) before and 3 days after the treatment with ear drops. Eardrums treated and those not treated with Cr(III) were also morphologically analyzed by a scanning electron microscope (SEM) and SEM-energy dispersive X-ray (SEM-EDX). All statistical analyses were performed using SPSS 24.0.

【Results】

To examine a dose-dependent effect of Cr exposure, tannery workers were divided into a group of workers who were exposed to a low level of t-Cr (tannery workers with low Cr, n=50) and a group of workers who were exposed to a high level of t-Cr (tannery workers with high Cr, n=50) by the median levels of t-Cr in their hair and toenails. The hearing levels at 1 k and

4 k Hz, but not those at 8 k and 12 k Hz, in tannery workers with low and high levels of t-Cr in hair and toenails were significantly decreased compared with those in non-tannery workers, suggesting that excess exposure to Cr decreases hearing levels at 1 k and 4 k Hz in humans (Figure 1).

In the multivariate analysis, hearing levels at 1 k and 4 k Hz, but not those at 8 k and 12 k Hz, in tannery workers with low and high levels of t-Cr in hair and toenails were significantly decreased compared with those in non-tannery workers after adjustments for age, BMI, smoking, occupational noise exposure and levels of other DHL-related elements including arsenic (As), barium (Ba), manganese (Mn) and iron (Fe) in hair and toenails (Figure 2). Calculations of Nagelkerke's R^2 and Psuedo R^2 values shows that Cr levels in hair and toenails were the strongest contributor to hearing levels at 1 k (hair: 12.5%; toenail: 5.5%) and 4 k Hz (hair: 12.5; toenail: 4.5%).

In the animal experiment, hearing level of mice at the lower frequency 4 k Hz (Figure 3A), but not that at the higher frequency 32 k Hz (Figure 3B), in the intervention mice was significantly lower than that in the control mice. Increased damage of the eardrum (Figure 4A, B) externally treated with 19 μg of Cr(III) (Figure 4C, D) was observed by analyses of SEM (Figure 4A, B) and SEM-EDX (Figure 4C, D) and was confirmed by semi-quantitative analysis (Figure 4E), suggesting the DHL with a damaged eardrum at a lower frequency is caused by external exposure to Cr(III).

【Discussion】

External exposure to Cr(III) in tannery workers occurs via wastewater on the floor and large droplets in the air because tannery workers suffer from disorders of external organs including otorhinolaryngologic, dermatological, ophthalmologic and oral disorders. An otorhinolaryngologic disorder has been reported to be the most frequent disorder in tannery workers despite limited information on the disorder. Therefore, the present study was carried out to clarify the effects of Cr(III) exposure on ear function in tannery workers.

This cross-sectional study first demonstrated that there were significantly decreased hearing levels at lower frequencies (1 k and 4 k Hz) but not at higher frequencies (8 k and 12 k Hz) in the tannery workers compared to those in the non-tannery workers. Clinical characteristics of the DHL caused by exposure to Cr(III) in the tannery workers were then considered. The characteristics of decreased hearing levels in the tannery workers are quite different from the previously reported characteristics of sensorineural DHL caused by aging, noise and internal exposure to elements such as As, Ba, Mn and Fe. Correspondingly, the correlation between t-Cr levels in hair and toenails and DHLs was maintained after considering confounding factors such as age, noise and elements that affect hearing level.

Cr(III) can directly reach the eardrums of tannery workers via droplets in the air and also indirectly reach the eardrum via picking an ear canal with a finger contaminated with

wastewater including Cr(III). The animal study showed that external exposure to BCS containing the mean level of Cr(III) in wastewater inside tanneries through eardrops decreased the hearing level at a lower frequency but not that at a higher frequency with damage of eardrums in mice. On the other hand, there was a limited effect of internal exposure to BCS containing the same amount of Cr(III) through intraperitoneal injection on hearing levels at lower and higher frequencies in mice. These results suggest that DHL can occur more easily by external exposure to Cr(III) than by internal exposure to Cr(III). Consideration should be given to the use of ear protectors for preventing Cr(III)-mediated conductive DHL in workplaces in which there might be high levels of external exposure to Cr(III).

【Conclusion】

In this study, our epidemiological investigation showed for the first time diagnostic DHLs caused by Cr(III) exposure in tannery workers. Then the animal experiments confirmed the development of Cr(III)-mediated DHL and proposed the mechanism of DHL via eardrum damage caused by external exposure to Cr(III).