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Cochlear injuries induced by the combined exposure to noise and styrene

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ABSTRACT

Workers exposed to industrial solvents are also frequently exposed to mechanical noise. In this study, a combination of a continuous noise (100 dB SPL) and an impact noise (110 dB SPL) was used to mimic the noise exposure in the workplace. A noise band of 10–20 kHz was used to induce a cochlear injury in the same cochlear region in the rat as styrene exposure. Styrene levels of 300 and 400 mg/kg were applied to induce outer hair cell (OHC) loss limited to the third row of the middle turn, but without significant cochlear functional loss. The combined exposures of the noise and styrene for 3 weeks caused greater threshold shifts than the noise alone, although the styrene alone did not induce significant threshold shift. Correspondingly, the combined exposures induced OHC losses that were greater than the summated OHC losses induced by the noise and styrene exposure alone. Apoptosis in Deiters cells was also examined after a short-term exposure (7 days) to a combined exposure of a high-level styrene (800 mg/kg) and the noise. The styrene-noise synergistic interaction was also observed in the Deiters cells. The synergistic interaction between the noise and styrene suggests that each of the exposures alone (noise or styrene) may cause stress, temporary alteration, or nonlethal injury in cochlear cells and the combined exposure strengthens the stress leading to cell death.

1. Introduction

Styrene, an aromatic hydrocarbon, is used extensively in the production of plastics, fiberglass, synthetic rubbers, resins, insulators and protective surface coatings (Rybak, 1992; Johnson and Nylén, 1995). Hearing loss from styrene occupational exposure has been reported repeatedly (Morioka et al., 1999; Morata et al., 2002; Sliwinska-Kowalska et al., 2003). However, the occupational styrene exposure-related hearing loss was not observed in a few early reports (Muijser et al., 1988; Möller et al., 1990; Sass-Kortsak et al., 1995; Calabrese et al., 1996). Evaluation of cochlear damages in the styrene-exposed workers is not available. In a recent animal experiment, we have found that styrene-induced cochlear injury may appear prior to the functional loss (Chen et al., 2008). It appears that a low-level styrene exposure may cause a significant injury in the cochlea without detectable functional loss.

In the workplace, workers are frequently, sometimes heavily, exposed to the combination of machine noise and solvents (Miller et al., 1994; Morata et al., 1994). Several studies have suggested a synergistic interaction between noise and solvents (Barregard and Axelsson, 1984; Morata et al., 1994), whereas other studies showed that the effects of noise dominated (Jacobsen et al., 1993; Sass-Kortsak et al., 1995).

Animal experiments have shown a synergistic interaction between noise and toluene, another industrial solvent (Johnson et al., 1988; Lataye and Campo, 1997). However, the noise–styrene synergistic interaction was not seen in a report using a single time exposure (styrene at 500 ppm, noise at 95 dBA for 7 h, Fechter, 1993). Repeated exposures to styrene and noise in two recent studies in rats did show the synergistic interaction (Lataye et al., 2000; Makitie et al., 2003). However, Makitie et al. (2003) only measured hearing loss at frequencies lower than 8 kHz, probably because they used a low-frequency noise (31.5–10 kHz). Styrene is known to cause hearing loss in the middle-frequency region around 10–20 kHz in the rat (e.g. Lataye et al., 2000; Chen et al., 2007). In the two studies, missing OHCs were counted and the remaining OHCs were examined (Lataye et al., 2000; Makitie et al., 2003). Loss of pillar cells was also reported (Makitie et al., 2003). However, influences of the combined exposure to noise and styrene on Deiters cells were not studied. Deiters cells are the most vulnerable target of styrene exposure (Chen et al., 2007, 2008).

In the current study, a combination of a continuous noise (110 dB SPL) and an impact noise (110 dB SPL) was used to mimic noise exposure in the workplace, although the noise level is higher than that in the workplace. A band of 10–20 kHz was used to produce damage and stress on the styrene-affected cochlear region in

Abbreviations: CAP, compound action potential; FITC, fluorescein isothiocyanate; I/O, input/output; IHC, inner hair cell; NIHL, noise-induced hearing loss; OHC, outer hair cell; PBS, phosphate buffered saline; PBSB, PBS containing bovine serum albumin; PI, propidium iodide; SGN, spiral ganglion neuron

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the rat. Styrene levels of 300–800 mg/kg were used, which may produce blood levels of 8–20 mg/l (Chen et al., 2007), which is higher than the blood levels measured in the workers occupationally exposed to styrene (up to 2 mg/l, Mizunuma et al., 1993). Hearing losses and OHC losses were compared between experimental groups exposed to styrene, noise, and their combinations. Apoptotic Deiters cells were also counted after a short-term combined exposure to a high-level styrene and noise and compared between groups.

2. Methods

2.1. Subjects

Long Evans pigmented rats (male, about 2 months of age) were acquired from Harlan Sprague Dawley and housed in the University at Buffalo animal facility after delivery. All animal facilities are registered with the US Department of Agriculture and are inspected semiannually by the members of the Institutional Animal Care and Use Committee (IACUC) serving the Research Foundation of State University of New York. Background noise level in the colony room was 45 dBA. Temperature was maintained at 71°F. Lights were on from 6:00 am to 6:00 pm. All procedures regarding the use and handling of animals and styrene/noise exposures were reviewed and approved by the IACUC.

2.2. Noise exposure

Rats were exposed to a kurtotic noise, which consisted of a combination of a continuous noise (100 dB SPL) and an impact noise (110 dB SPL) for 6 h per day for 5 days per week for 3 weeks ($n = 8$) or 6 h per day for 7 days ($n = 5$) (see Table 1, #1 and #6). The continuous noise was measured at the level of the animals' ears by using a linear weighting and adjusted to 100 dB SPL. The impact noise was added to the continuous noise signal electronically and the peak amplitude of the impact noise was set at a level of 3.16 times (10 dB) higher than the continuous noise. The band of the noise was 10–20 kHz. Duration of the impacts was 30 ms with 1 ms of rise and 29 ms of fall time (Fig. 1). The rate of the impacts was 1/s. The noise was generated using a TDT RP2 Real time signal processor (TDT, Gainesville, FL), amplified by AMP 300 power amplifier (AudioSource Inc., Portland, OR) and then delivered to speakers (Vifa D25AG35 1″ Dome Tweeter, Madisound Speaker Components, Inc., Middleton, WI) in the glass exposure chamber (24″ × 24″ × 36″). The noise level was calibrated at the level of the animals' heads utilizing a calibrated Model 800B sound level meter (Larson Davis Inc., Depew, New York) and a 1/2″ condenser microphone. Each animal was restricted in a small wire cage (8″ × 6″ × 6.5″) in the exposure chamber. For each wire cage, a speaker was sited on the top of the cage, which was 3.25″ above the level of the animal's ear. The animals were conscious and food and water were available during the noise exposure.

Table 1
Noise and styrene exposure.

Exposure	Duration	Animal number
1. Noise	3 weeks	8
2. ST300	3 weeks	6
3. ST400	3 weeks	4
4. Noise + ST300	3 weeks	6
5. Noise + ST400	3 weeks	4
6. Noise	3 weeks	5
7. ST800	7 days	4
8. Noise + STSOO	7 days	3
9. No exposure	7 days	10

2.3. Styrene exposure

Six rats were exposed to styrene (⩾99%, S4972, Sigma) mixed in olive oil (0.4 ml/kg) by gavage at a dose of 300 mg/kg once per day for 5 days per week for 3 weeks (see Table 1, #2). Four rats were exposed to styrene at a dose of 400 mg/kg once per day for 5 days per week for 3 weeks (see Table 1, #3). Four rats were exposed to styrene at a dose of 800 mg/kg once per day for 7 days (see Table 1, #7).

2.4. Combined exposure to noise and styrene

Six rats were exposed to the kurtotic noise and styrene at 300 mg/kg for 5 days per week for 3 weeks (see Table 1, #4). Four rats were exposed to the kurtotic noise and styrene at 400 mg/kg for 5 days per week for 3 weeks (see Table 1, #5). Three rats were exposed to the kurtotic noise and styrene at 800 mg/kg for 7 days (see Table 1, #8). The styrene was given 10–30 min before the 6-h noise exposure each day.

Ten rats were used as controls (see Table 1, #9).

2.5. Cochlear compound action potential (CAP) recording

Three to five days after the 3-week exposures, rats were deeply anesthetized with ketamine (50 mg/kg, i.m.) and xylazine (6 mg/kg, i.m.). The right cochlea was surgically exposed using a ventrolateral approach and a silver wire electrode was carefully placed on the round window membrane for CAP recording. A silver chloride reference electrode was placed beneath the neck skin. Tone bursts at 2, 6, 8, 12, 16, 20, 24, 30 and 40 kHz for eliciting CAP were generated in a real time processor (RP2.1, system3, TDT, Gainesville, FL). The signals (duration: 10 ms; rise/fall time: 1 ms) were amplified and delivered to an earphone (rebuilt using an ACO 1/2″ microphone, 7013) placed within a speculum that was opened to the ear drum. Stimulation intensities were controlled with a TDT PA5 programmable attenuator. Sound levels at the eardrum were calibrated with a 1/8″ microphone (4138 B&K) using a coupler that mimics the rat ear canal. Cochlear responses in a time window of 20 ms were recorded and amplified with the DAM50 Bio-amplifier (World Precision Instruments, Sarasota, FL). The gain of the preamplifier was set at 1000, and the band of the filter was set from 0.1 to 3 kHz. The amplified cochlear responses were averaged 50 times in the TDT RP2.1 real time processor using custom-written data acquisition/analysis software (D. Sinex on Matlab 6.1) and stored on a disk in a personal computer. CAP amplitudes were measured using the custom-written data acquisition/analysis software and plotted as a function of stimulation intensity, known as the CAP input/output function (I/O function). CAP thresholds were measured on the CAP I/O function. The threshold was defined as the stimulation intensity that produced a CAP of 5 μV amplitude.

2.6. Histochemical staining for hair cell counting and examination

2.6.1. Staining based on activity of succinate dehydrogenese (SDH)

After CAP recording, the cochleae were removed immediately. The round and oval windows and the apex of the cochlea were opened to facilitate perfusion. The cochleae were perfused with an incubation solution containing 0.05% nitrotetrazolium blue chloride (cat#N6876, Sigma), 0.05 M sodium succinate, and 0.05 M phosphate buffer and then incubated in the solution for 1 h (37 °C). Nitrotetrazolium blue is an electron acceptor that, on reduction, precipitates as an insoluble and highly colored formazan. SDH in the cell oxidizes sodium succinate and provides electrons for the reduction of the electron acceptor. The stained cochleae were fixed in 10% buffered formalin for 2 days. After fixation, the cochleae were decalcified in 7% EDTA (ethylenediamine

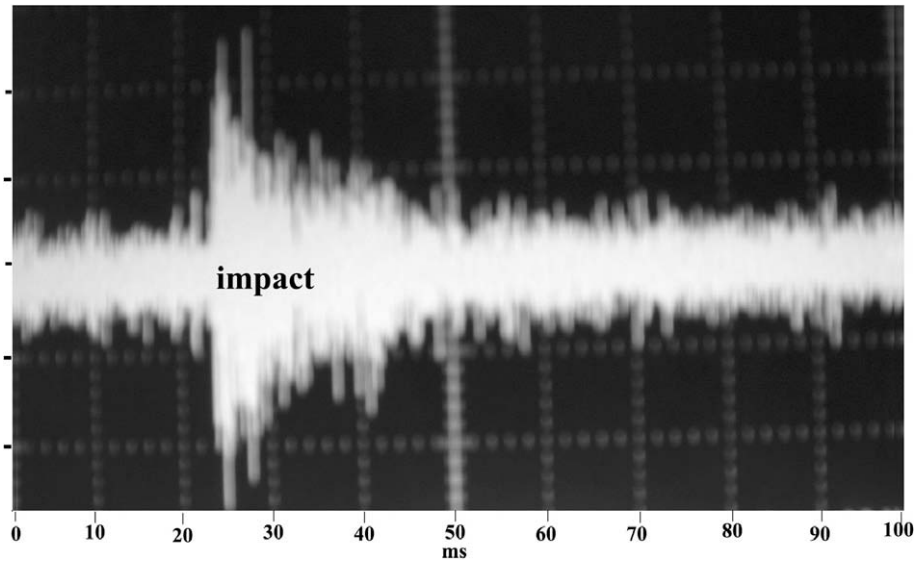

Fig. 1. Waveform of the kurtotic noise . Noise band: 10–20 kHz; Impact: 30-ms duration, 1-ms rise time, 29-ms fall time, 1/s; Steady part of the noise: 100 dB SPL; Peak level of the impact: 110 dB SPL.

tetraacetic acid) solution for 3 days or longer as needed. Cochlear micro-dissection was accomplished under a light microscope. Hair cells within each segment of 250 μM on the basilar membrane were counted under a light microscope (DMBA300 Digital Microscope, Microscope World). The counted cell numbers were compared to the normal numbers obtained in the control rats and cell losses were expressed as percentages. Cell losses were plotted as a function of % distance from the cochlear apex (cochleograms). Representative photomicrographs were captured using the microscope's built-in camera (DMBA300 Digital Microscope, 400×) with the provided software (Motic Image Plus 2.0 ML).

2.6.2. Staining for F-actin and nuclei in the cochlear cells

Cochleae in the rats exposed to the high-dose styrene (800 mg/kg) and noise were removed after the 7-day exposures and fixed in 10% buffered formalin for 12 h and then dissected in PBS (phosphate buffered saline). The specimen (the basilar membrane with the organ of Corti) was incubated in the solution of PBSB (PBS containing bovine serum albumin, 5 mg/ml) containing FITC (fluorescein isothiocyanate) labeled phalloidin (cat#P5282, Sigma) at a concentration of 5 μg/ml for 30–40 min for F-actin. Propidium iodide (PI) is a fluorescent dye that binds specifically to DNA. After incubation in the FITC-labeled phalloidin, the specimen was stained in the solution of PI (cat#P-3566, molecular probes) at a concentration of 5 μg/ml for 10 min. The stained specimen was mounted on a slide with ProLong Gold antifade reagent (cat#P-36934, molecular probes) and examined using the Carl Zeiss Laser Scanning Systems LSM 510. Hair cells were counted. Stereocilia of the remaining OHCs were examined, and nuclei of OHCs and Deiters cells were examined. Condensed nuclei of Deiters cells were also counted. Representative images were captured and analyzed using Zeiss LSM Image Examiner (v. 4.0.0.91).

2.7. Statistical analysis

Differences between exposure groups with respect to CAP hearing threshold changes, cochleograms, and CAP I/O functions were analyzed using two-way ANOVA. Differences in each individual point between groups were analyzed using *t*-tests. A *p*-value <0.05 was considered to be statistically significant.

3. Results

Fig. 2 presents CAP I/O functions at 12 kHz after 3 weeks of exposures (5 days/week). The styrene exposure by gavage at a dose of 300 mg/kg (ST300) only caused slight CAP reductions at low stimulus levels <30 dB SPL compared to the control group (open triangles). The styrene exposure by gavage at a dose of 400 mg/kg (ST400) caused slight CAP reductions at all stimulus levels from 0 to 90 dB SPL compared to the control group (inverse open triangles) and the differences reached statistical significance by two-way ANOVA ($p = 0.04$). However, the differences did not reach significance at any individual stimulus level ($p > 0.05$). The kurtotic noise exposure caused a 'low-stimulation-level-bias' CAP reduction (open circles), resulting in a shift of CAP I/O function towards the linear line (dashed line) from the control position (asterisks), indicating a reduction of cochlear amplification. CAP amplitudes at each stimulus level below 60 dB SPL were significantly different

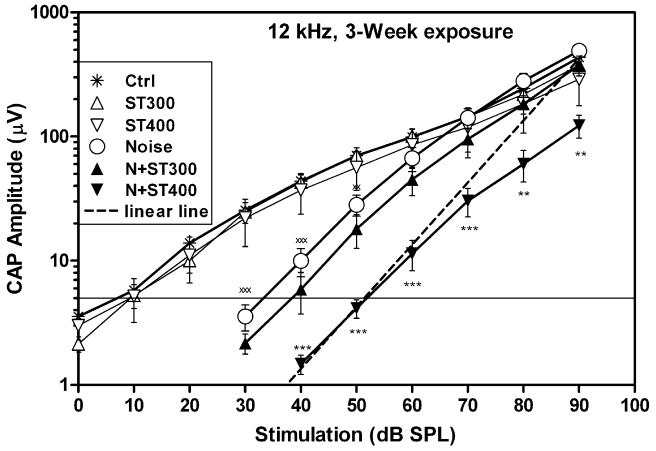

Fig. 2. CAP I/O functions at 12 kHz after the exposures. Noise: 10–20 kHz, 100/110 dB SPL, 6 h/day for 5 days/week for 3 weeks; styrene exposures: by gavage, 1/day, 5 days/week for 3 weeks; combined exposures: styrene was given 10–30 ms prior to the starting of the noise exposure; N: noise; ST300: styrene at 300 mg/kg; ST400: styrene at 400 mg/kg; linear line: 1 dB/dB; ** or xx: $p < 0.01$; *** or xxx: $p < 0.001$. Vertical bars represent SE (standard error).

from the control levels ($^{xx}p < 0.01$; $^{xxx}p < 0.001$). The combined exposure to the noise and styrene at 300 mg/kg (N+ST300) showed further reduction of CAP compared to the noise-group. The differences between the noise-group and the N+ST300-group were statistically significant by two-way ANOVA ($p < 0.01$), but were not significant at any individual stimulus level by t-test ($p > 0.05$). The combined exposure to the noise and styrene at 400 mg/kg (N + ST400) caused significant CAP reductions not only at low stimulus levels ($^{***}p < 0.001$) but also at high stimulus levels ($^{**}p < 0.01$), likely indicating injury expanding from OHCs to inner hair cell (IHC)/nerve-fiber/spiral ganglion neuron (SGC) levels.

Fig. 3 presents CAP threshold shifts as a function of frequency after 3 weeks of exposure to styrene, noise, or their combinations. Fig. 3A shows exposures to the noise and styrene at 300 mg/kg and Fig. 3B shows exposures to the noise and styrene at 400 mg/kg. The styrene exposures induced either no or only slight threshold shifts (open triangles). The noise exposure caused threshold shifts at frequencies in the noise band and high frequencies (open circles). The combined exposure to the noise and styrene at 300 mg/kg caused greater threshold shifts (see filled circles in Fig. 3A) than the noise exposure alone, although the styrene exposure did not cause threshold shift except a slight shift at 12 kHz. The differences between the noise-group and the N+ST300-group were statistically significant by two-way ANOVA ($p < 0.001$). The differences at 8 and 16 kHz also reached significance on t-testing ($p < 0.001$ and $p < 0.05$ respectively). The combined exposure to the noise and styrene at 400 mg/kg caused threshold shifts at and above 40 dB in the middle-frequency region (see filled circles in Fig. 3B). The differences between the noise-group and the N + ST400-group were significant by two-way ANOVA ($p < 0.0001$), and at all frequencies, except 40 kHz, were significant by t-test ($^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$). The threshold shifts induced by the combined exposure were also greater than the summated threshold shifts induced by the noise and the styrene (400 mg/kg) alone (thick lines). The data indicated that the styrene exposures potentiated noise-induced hearing loss (NIHL).

Fig. 4 presents representative surface preparations showing hair cells in the middle turn (55% from the apex) after 3-week exposure to styrene at 400 mg/kg (A), the kurtotic noise at 100/110 dB SPL (B), and the combination of the styrene and the noise (C). The styrene exposure caused loss of OHCs in the third row (marked with asterisks in A), although only about 5 dB of threshold shift was observed at the related frequency (16 kHz). The noise exposure only caused occasional loss of OHCs in the first row (marked with 'x' in B), although an 18-dB threshold shift was observed at 16 kHz. The combined exposure caused a severe OHC loss in all three rows (marked with asterisks in C), which was related to a >40 dB of threshold shift at 16 kHz, indicating a complete loss of cochlear amplification.

Fig. 5 presents OHC losses as a function of cochlear length (cochleogram) after 3 weeks of exposures. Although the noise exposure induced threshold shifts up to about 30 dB at 12 kHz, the exposure

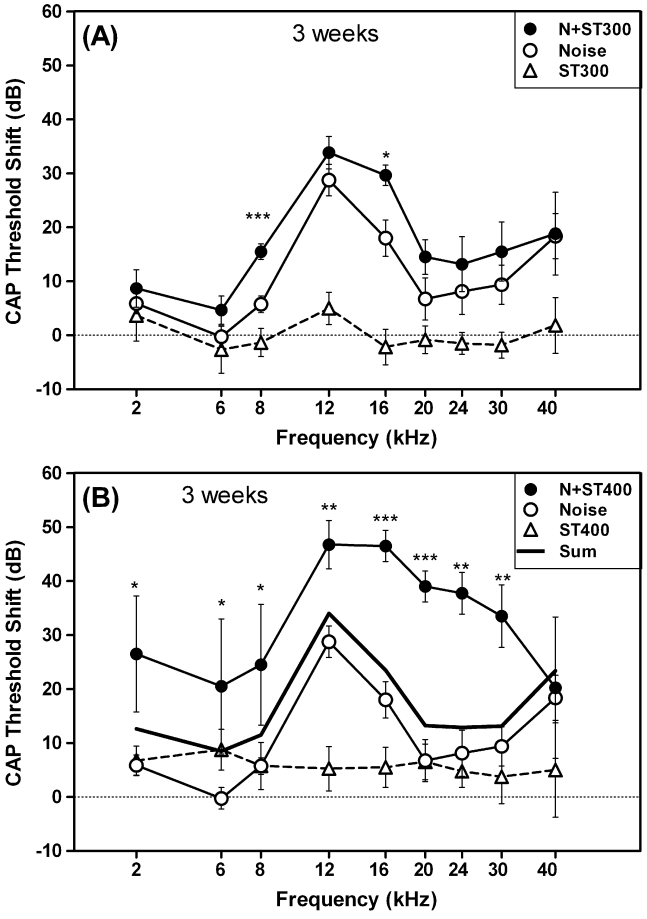

Fig. 3. CAP threshold shifts as a function of frequency. (A) styrene gavage at a dose of 300 mg/kg; (B) styrene gavage at a dose of 400 mg/kg; exposures are as described in Fig. 2. Sum: summated threshold shifts induced by the noise and the styrene alone. *: $p < 0.05$; **: $p < 0.01$; ***: $p < 0.001$. Vertical bars represent SE.

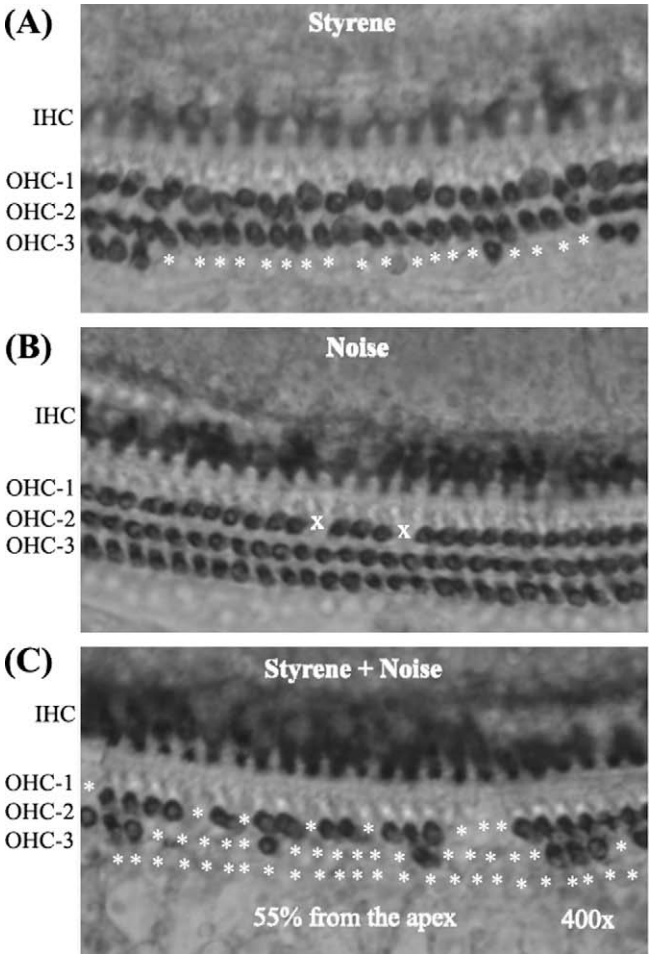

Fig. 4. Representative images showing hair cells after 3-week exposure to styrene at 400 mg/kg (A), to the noise (B), and to their combination (C). The exposures are as described in Fig. 2. Asterisks or crosses mark the missing OHCs. The location of the images in the cochleae is about 55% from the apex.

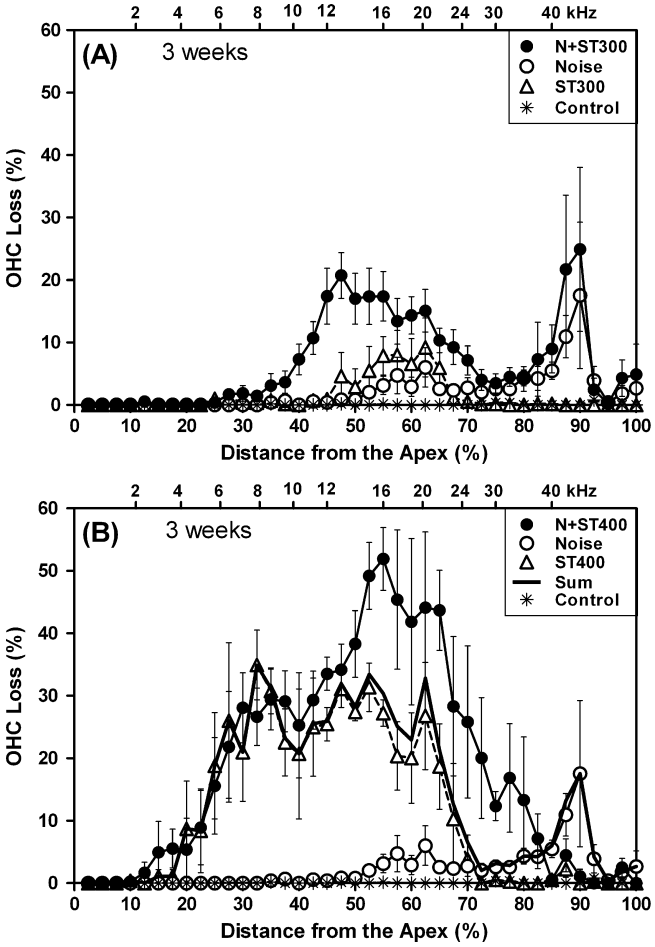

Fig. 5. OHC losses as a function of cochlear length. (A) styrene gavage at a dose of 300 mg/kg; (B) styrene gavage at a dose of 400 mg/kg; exposures are the same as described in Fig. 2. Sum: summated OHC losses induced by the noise and the styrene alone. Vertical bars represent SE.

only caused slight OHC losses with 2 peaks: one was 6% loss in the middle turn and another was 18% loss in the basal turn (open circles). The styrene exposure at 300 mg/kg caused OHC losses (see open triangles in Fig. 5A) similar to the noise-induced OHC loss in the middle-frequency region, although it did not cause threshold shift. The combined exposure to the noise and styrene at 300 mg/kg (N-ST300) caused OHC losses significantly greater than those caused by the noise or styrene ($p < 0.0001$ by two-way ANOVA). The styrene exposure at 400 mg/kg caused about 20–30% OHC losses in the middle turn (see open triangles in Fig. 5B), due to missing of OHCs in the third row (see Fig. 4A). The combined exposure to the noise and styrene at 400 mg/kg caused significantly greater OHC loss than the styrene or the noise ($p < 0.0001$ by two-way ANOVA). The loss of OHCs induced by the combined exposure was also greater than the sum of OHC losses induced by the noise and styrene alone (thick lines), indicating a synergistic interaction between styrene and noise exposures.

To study the noise–styrene interaction during the cochlear cell death process, short-term exposures with a high level of styrene (800 mg/kg) and the noise were used. Fig. 6 presents representative images showing morphological features of auditory hair cells after exposure to the kurtotic noise at 100/110 dB SPL for 6 h per day for 7 days (A), to styrene at 800 mg/kg/day for 7 days (B), and to the combination of the exposures for 7 days (C). The noise exposure caused death of 3 OHCs in the first row in the segment 57% from the apex (marked with 'arrows' in A). Nuclei of the three OHCs were missing and the stereocilia and cuticular plate of the cells were disrupted. Fusion of stereocilia was observed in one OHC in the first row (marked with arrowheads in A and in the insert focused on the stereocilia), which had a normal nucleus. No morphological disruption was observed in other remaining OHCs at this examination level. The styrene exposure induced death of 7 OHCs in the third row and 3 OHCs in the second row in the segment from the similar region (57% from the apex) showed in B (pointed with arrows). Eight of 10 disrupted OHCs were completely gone and two had small pieces of the nuclei (marked with arrowheads) remaining. In the similar region in a rat exposed to the combination of the noise and styrene (54% from the apex), the majority of OHCs were destroyed and only 14 OHCs were present in the segment (C). One of the remaining OHCs had a condensed nucleus (marked with arrowhead), indicating apoptotic cell death.

Fig. 7 presents OHC losses as a function of cochlear length after 7 days of exposures. Exposure to the noise (100/110 dB SPL) and styrene (800 mg/kg) alone for 7 days only caused slight OHC losses (open symbols). The combined exposure to the noise and styrene caused OHC losses up to 90% in the middle-frequency region (filled circles).

Our previous study revealed that the most sensitive target of styrene in the cochlea was the Deiters cells instead of the OHCs (Chen et al., 2007). However, Deiters cell loss is hard to count precisely. In this report, we counted condensed nuclei of Deiters cells (dying Deiters cells) after the 7-day exposures.

Fig. 8 presents representative images as shown in Fig. 6, but were focused on the Deiters cell layer. The noise exposure did not cause damage to Deiters cells (A). Nuclei of the Deiters cells, including those with the related OHCs being absent (marked by arrows), were intact. However, many Deiters cells were absent or had condensed nuclei (apoptosis) after the styrene exposure (marked with arrows in B). For comparison, three intact nuclei of Deiters cells were marked with arrowheads. The majority of Deiters cells in the rat exposed to the combination of the noise and styrene were absent. Only a few normal (marked with arrowhead) and condensed nuclei (marked with arrows) were recognized.

Fig. 9 presents condensed nuclei of Deiters cells as a function of cochlear length after the 7-day exposures. No condensed nuclei were found in the Deiters cell layer in the cochleae exposed to the noise (open circles). The styrene exposure (800 mg/kg/day) induced condensation of nuclei of Deiters cells in the middle turn (open triangles). The combined exposure to the noise and styrene induced more condensed nuclei of Deiters cells in cochlear regions of <45% and >65% (filled circles). However, in the region of 45–65% from the apex, there were fewer condensed nuclei of Deiters cells than induced by the styrene exposure alone. This was because the majority of Deiters cells in this area had already died. The data indicated that the noise exposure potentiated styrene-induced apoptosis in Deiters cells.

4. Discussion

4.1. Noise trauma in the rat cochlea

In the rat, noise exposure may induce a certain level of hearing loss with only occasional missing hair cells. Noise-induced permanent threshold shift (PTS) up to 40 dB in the low- and middle-frequency regions (<24 kHz) was often observed without significant OHC loss (Chen and Fechter, 2003). The occasional OHC death or injury in the stereocilia was usually seen first in the first row (Chen and Zhao, 2007; Lataye et al., 2000). The majority of the surviving OHCs were morphologically intact, even in the cochlea with a

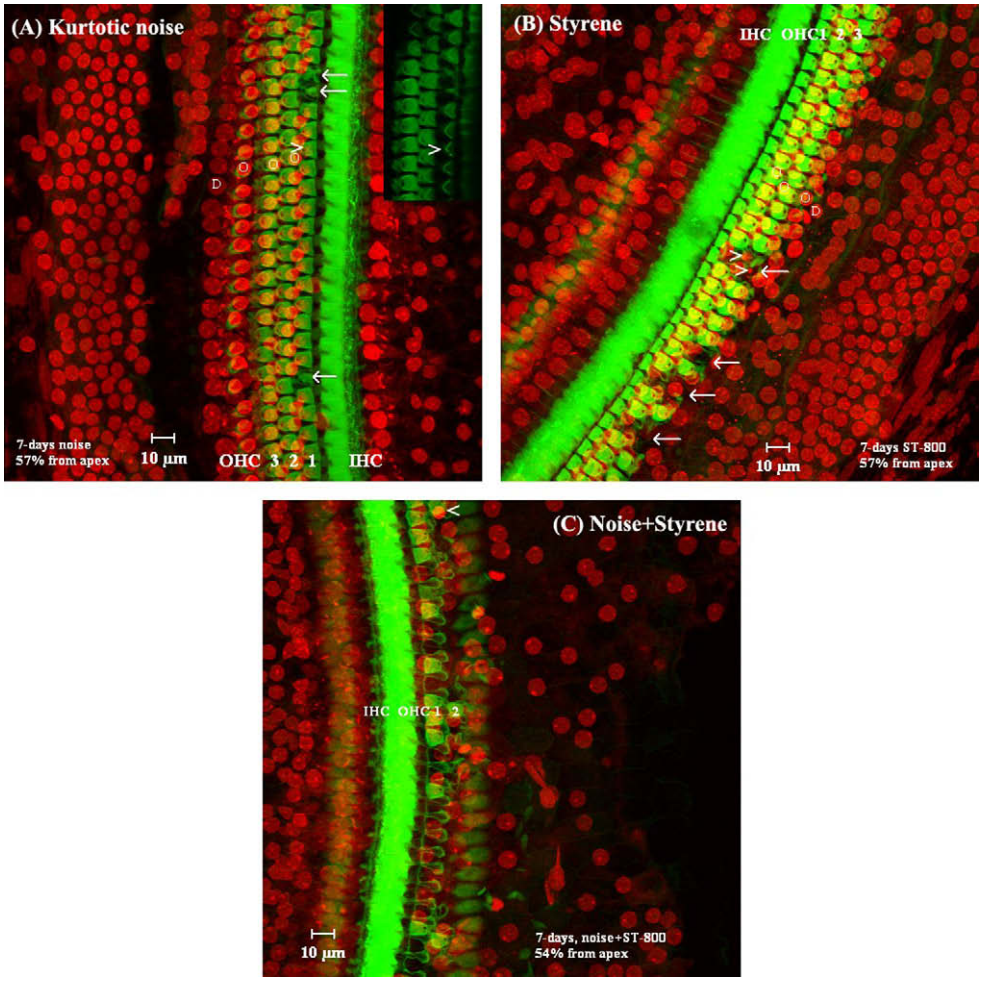

Fig. 6. Representative images showing stereocilia, cuticular plate, and nuclei of OHCs in the cochleae exposed to the kurtotic noise at 100/110 dB SPL for 6 h/day (A), styrene at 800 mg/kg once per day (B), and their combination (C) for 7 days. Location of the images was 55–60% from the apex. Arrows point to missing OHCs. Arrowheads point to (A) an OHC with fusion in its stereocilia or (B and C) condensed nuclei of OHCs. Horizontal bars represent 10 μm.

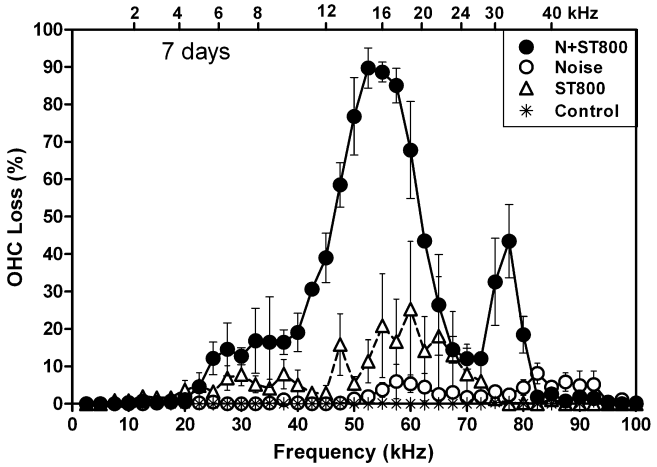

Fig. 7. OHC losses after 7 days of exposures to the noise at 100/110 dB SPL for 6 h/day, styrene at 800 mg/kg/day, and their combination as a function of cochlear length. Vertical bars represent SE.

linear cochlear I/O function, indicating a complete loss of cochlear amplification suggesting dysfunction of the surviving OHCs (Chen and Zhao, 2007). Dysfunction of the noise-exposed OHCs may mainly result from the disrupted lateral wall, such as reduced membrane fluidity or injured motor protein, since loss of cochlear amplification may be observed without reduction of the OHC receptor potential (Chen, 2006; Chen and Zhao, 2007).

Reactive oxygen species (ROS) are commonly involved in various traumatic processes. ROS accumulation has been directly detected in noise-exposed cochlea (Liu, 1992; Ohinata et al., 2000; Ohlemiller et al., 1999a; Rao et al., 2001; Yamane et al., 1995a,b). Protective effect of antioxidants against NIHL also indirectly suggest existence of ROS in the cochlea after noise exposure (e.g. Rao and Fechter, 2000; Henderson et al., 1999; Kopke et al., 2000; Ohlemiller et al., 1999b; Seidman et al., 1993).

In the current study, rats were exposed to a combination of a continuous noise (100 dB SPL for 6 h/day) and an impact noise (110 dB SPL, 1/s), called kurtotic noise. The 3-week exposure (5 days/week) to the noise caused an about 29-dB threshold shift at 12 kHz and 7–19 dB threshold shifts at higher frequencies The threshold shifts were smaller than those induced by a 4-h exposure of the continuous noise with the same band (10–20 kHz) at 110 dB SPL (Chen, 2006). The noise exposure only caused slight OHC losses in the middle turn around the location of 60% from the apex and some OHC losses in the basal turn. About 98% of OHCs were still surviving in the cochlea and no detectable morphologically abnormality has been observed in the stereocilia, cuticular plate, and nuclei (data not shown). The noise did not induce Deiters cell loss. Even those Deiters cells with the related OHCs missing were still present with normal nuclei.

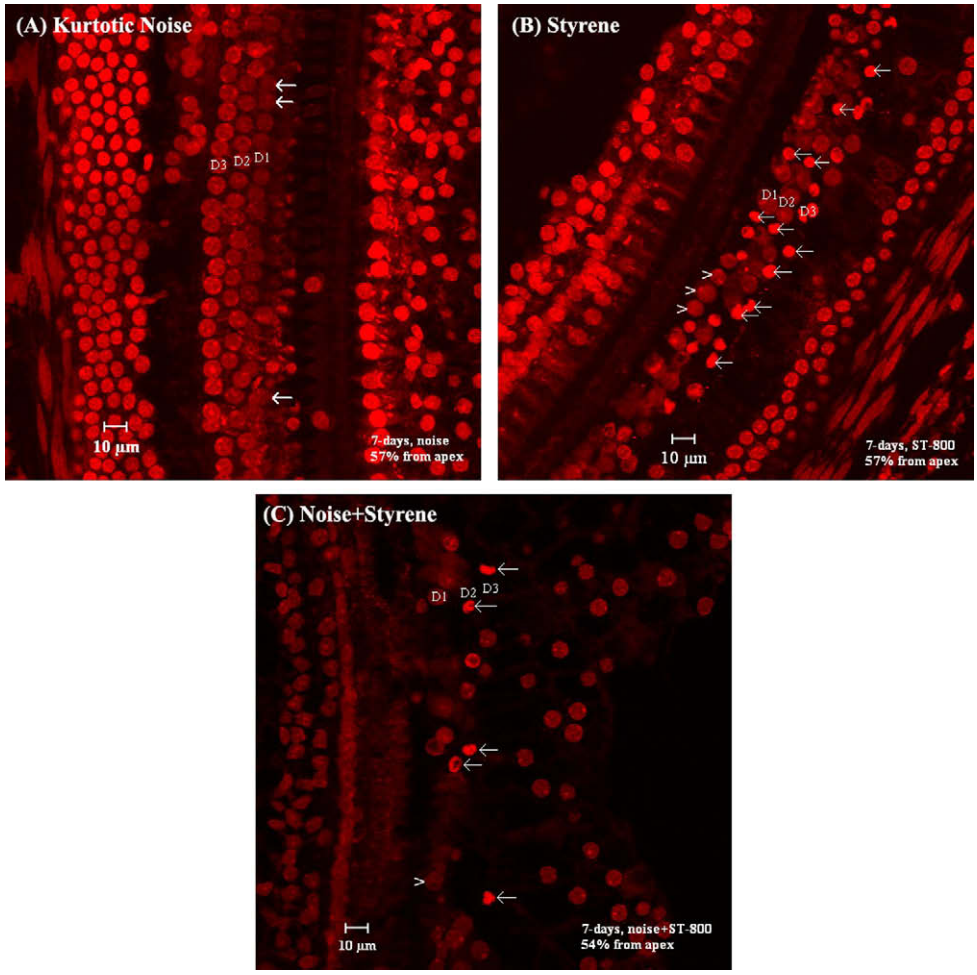

Fig. 8. Representative images showing nuclei of Deiters cells in the cochleae exposed to the noise (A), styrene at 800 mg/kg (B), and their combination (C) for 7 days. The images were obtained from the same places as those shown in Fig. 6, but focused on the layer of Deiters cells. Thick arrows in A point to nuclei of the Deiters cells with missing OHCs. Thin arrows in B and C point to condensed nuclei of Deiters cells. Horizontal bars represent 10 μm.

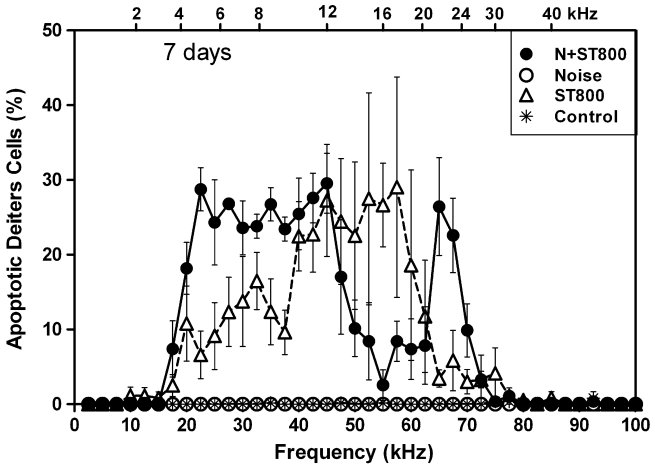

Fig. 9. Deiters cells with condensed nuclei as a function of cochlear length. The animals were exposed to the kurtotic noise, styrene at 800 mg/kg, and their combination for 7 days. Vertical bars represent SE.

4.2. The ototoxic effect of styrene in the rat cochlea

Styrene exposure disrupts cochlear cells starting from the middle turn. The location-dependency of the disruption was explained with distribution gradients of styrene measured in our previous report (Chen et al., 2007) and antioxidants in the cochlea. Another characteristic of the styrene-induced disruption is that the degeneration starts from the third row of OHCs (Campo et al., 2001; Chen et al., 2007; Gagnaire and Langlais, 2005; Lataye et al., 2000, 2001, 2003; Loquet et al., 1999, 2000; Makitie et al., 2003), instead of the first row as seen in the noise-exposed cochlea. Our recent study revealed that Deiters cells were the most vulnerable target of styrene (Chen et al., 2007, 2008). Death of OHC may result from styrene insult directly or is due to the death of the related Deiters cell. Campo et al. (1999) postulated an intoxication route from the lateral side from the blood supply in the stria vascularis. Because normal cochlear responses were observed in some styrene-exposed cochleae with only the first and the second row of OHCs remaining (Chen et al., 2008), it would seem that styrene destroys the encountered OHCs and Deiters cells from the lateral side in the organ of Corti, but does not severely affect the remaining cells. Styrene-induced death of cochlear cells has been reported to be mainly through caspase-dependent apoptosis pathways (Chen et al., 2007; Yang et al., 2008). It is still unclear how the styrene exposure triggers apoptotic processes. Styrene in the body is mainly metabolized to styrene 7,8-oxide (SO), which may form covalent adducts to DNA, RNA, and proteins (Koskinen et al., 2001a,b; Liu et al., 2001; Vodicka et al., 2001). SO has also been proposed to have a direct oxidative stress effect on cells (Marczynski et al., 2000).

In this study, the styrene exposure by gavage at 400 mg/kg caused a loss of OHCs mainly in the third row in the middle turn. However, the styrene-induced cochlear injury only resulted in a threshold shift of about 5 dB, consistent with our previous finding that styrene-induced loss of OHCs less than 30% may not affect cochlear function (Chen et al., 2008). The data indicated that the remaining OHCs in the first and the second rows were still functioning and sufficient to maintain the cochlear amplification.

4.3. Interaction between the noise and styrene exposures

Consistent with previous reports (Lataye et al., 2000; Makitie et al., 2003), the combined exposure to noise and styrene in this experiment induced greater cochlear functional loss and hair cell loss than the summated losses caused by the styrene and the noise alone. The combined exposure also had greater effect on Deiters cells than the styrene exposure. The styrene exposure targeted Deiters cells. However, the noise exposure did not show any effects on Deiters cells.

Although the remaining OHCs in the first and the second rows in the styrene-exposed cochleae in this experiment appeared to be sufficient to maintain the cochlear amplification, the combined noise exposure severely destroyed the cells. The data suggest that the styrene exposure may have induced nonlethal injuries in the remaining OHCs and the added stress by the noise exposure in the same region eventually kill the cells. Noise exposure at an extremely high level may cause direct mechanical damage, including clefts between the third row OHCs and Deiters cells (Henderson et al., 1994). However, noise exposure usually does not show effects on Deiters cells. In this study, the noise did not cause Deiters cell loss. However, the noise exposure potentiated the styrene-induced degeneration in the Deiters cells. The data indicated that the noise exposure had caused some stress on the Deiters cells, although no Deiters cells were missing after the noise exposure.

The combined exposure to the noise and styrene at 400 mg/kg caused CAP reduction not only at low stimulus levels but also at high stimulus levels, suggesting some damage involving IHCs and SGNs. Both the noise and the styrene exposure alone did not show effects on CAP at high stimulus levels. However, the exposures (the noise and the styrene) may have already caused a certain degree of stress on the IHC/SGN level. The combined exposure increases the stress to a critical level leading to functional loss of the cells. We did not find death of IHCs. The SGNs were not examined in this study.

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References

Barregard, L., Axelsson, A., 1984. Is there an ototraumatic interaction between noise and solvents? Scand. Audiol. 13, 151–155.
Calabrese, G., Martini, A., Sessa, G., Cellini, M., Bartolucci, G.B., Marcuzzo, G., De Rosa, E., 1996. Otoneurological study of workers exposed to styrene in the fiberglass industry. Int. Arch. Occup. Environ. Health. 68, 219–223.
Campo, P., Loquet, G., Blachere, V., Roure, M., 1999. Toluene and styrene intoxication route in the rat cochlea. Neurotoxicol. Teratol. 21, 427–434.
Campo, P., Lataye, R., Loquet, G., Bonnet, P., 2001. Styrene-induced hearing loss: a membrane insult. Hear. Res. 154, 170–180.
Chen, G.D., 2006. Prestin gene expression in the rat cochlea following intense noise exposure. Hear. Res. 222, 54–61.
Chen, G.D., Fechter, L.D., 2003. The relationship between noise-induced hearing loss and hair cell loss in rats. Hear. Res. 177, 81–90.
Chen, G.D., Zhao, H.B., 2007. Effects of intense noise exposure on the outer hair cell plasma membrane fluidity. Hear. Res. 226, 14–21.
Chen, G.D., Chi, L.H., Kostyniak, P.J., Henderson, D., 2007. Styrene induced alterations in biomarkers of exposure and effects in the cochlea: mechanisms of hearing loss. Toxicol. Sci. 98, 167–177.
Chen, G.D., Tanaka, C., Henderson, D., 2008. Relationship between outer hair cell loss and hearing loss in rats exposed to styrene. Hear. Res. 243, 28–34.
Fechter, L.D., 1993. Effects of acute styrene and simultaneous noise exposure on auditory function in the guinea pig. Neurotoxicol. Teratol. 15, 151–155.
Gagnaire, F., Langlais, C., 2005. Relative ototoxicity of 21 aromatic solvents. Arch. Toxicol. 79, 346–354.
Henderson, D., Spongr, V., Subramaniam, M., Campo, P., 1994. Anatomical effects of impact noise. Hear. Res. 76, 101–117.
Henderson, D., McFadden, S.L., Liu, C.C., Hight, N., Zheng, X.Y., 1999. The role of antioxidants in protection from impulse noise. Ann. NY Acad. Sci. 884, 368–380.
Jacobsen, P., Hein, H.O., Suadicani, P., Parving, A., Gyntelberg, F., 1993. Mixed solvent exposure and hearing impairment: an epidemiological study of 3284 men. The Copenhagen male study. Occup. Med. 43, 180–184.
Johnson, A.C., Juntunen, L., Nylen, P., Borg, E., Hoglund, G., 1988. Effect of interaction between noise and toluene on auditory function in the rat. Acta Otolaryngol. (Stockh.) 105, 56–63.
Johnson, A.C., Nylén, P.R., 1995. Effects of industrial solvents on hearing. Occup. Med. 10, 623–640.
Kopke, R.D., Weisskopf, P.A., Boone, J.L., Jackson, R.L., Wester, D.C., Hoffer, M.E., Lambert, D.C., Charon, C.C., Ding, D.L., McBride, D., 2000. Reduction of noise-induced hearing loss using L-NAC and salicylate in the chinchilla. Hear. Res. 149, 138–146.
Koskinen, M., Vodicka, P., Hemminki, K., 2001a. Identification of 1-adenine DNA adducts in workers occupationally exposed to styrene. Occup. Environ. Med. 43, 694–700.
Koskinen, M., Vodickova, L., Vodicka, P., Warner, S., Hemminki, K., 2001b. Kinetics of formation of specific styrene oxide adducts in double-stranded DNA. Chem. Biol. Interact. 138, 111–124.
Lataye, R., Campo, P., 1997. Combined eÀects of a simultaneous exposure to noise and toluene on hearing function. Neurotoxicol. Teratol. 19, 373–382.
Lataye, R., Campo, P., Loquet, G., 2000. Combined effects of noise and styrene exposure on hearing function in the rat. Hear. Res. 139, 86–96.
Lataye, R., Campo, P., Barthelemy, C., Loquet, G., Bonnet, P., 2001. Cochlear pathology induced by styrene. Neurotoxicol. Teratol. 23, 71–79.
Lataye, R., Campo, P., Pouyatos, B., Cossec, B., Blachere, V., Morel, G., 2003. Solvent ototoxicity in the rat and guinea pig. Neurotoxicol. Teratol. 25, 39–50.
Liu, Z., 1992. Experimental study on the mechanism of free radical in blast trauma induced hearing loss. Chung Hua Erh Pi Yen Hou Ko Tsa Chih. 27, 24–26.
Liu, S.F., Fang, Q.M., Jin, Z.L., Rappaport, M.S., 2001. Investigation of protein-styrene oxide adducts as a molecular biomarker of human exposed to styrene. J. Environ. Sci. (China) 13, 391–397.
Loquet, G., Campo, P., Lataye, R., 1999. Comparison of toluene-induced and styrene-induced hearing losses. Neurotoxicol. Teratol. 21, 689–697.
Loquet, G., Campo, P., Lataye, R., Cossec, B., Bonnet, P., 2000. Combined effects of exposure to styrene and ethanol on the auditory function in the rat. Hear. Res. 148, 173–180.
Makitie, A., Pirvola, U., Pyykko, I., Sakakibara, H., Riihimaki, V., Ylikoski, J., 2003. The ototoxic interaction of styrene and noise. Hear. Res. 179, 9–20.
Marczynski, B., Peel, M., Baur, X., 2000. New aspects in genotoxic risk assessment of styrene exposure–a working hypothesis. Med. Hypotheses 54, 619–623.
Miller, R.R., Newhook, R., Poole, A., 1994. Styrene production, use, and human exposure. Crit. Rev. Toxicol. 24 (Suppl. 1), S1–S10.
Mizunuma, K., Yasugi, T., Kawai, T., Horiguchi, S., Ikeda, M., 1993. Exposure–excretion relationship of styrene and acetone in factory workers: a comparison of a lipophilic solvent and a hydrophilic solvent. Arch. Environ. Contam. Toxicol. 25, 129–133.
Möller, C., Odkvist, L., Larsby, B., Tham, R., Ledin, T., Bergholtz, L., 1990. Otoneurological findings in workers exposed to styrene. Scand. J. Work. Environ. Health. 16, 189–194.
Morata, T.C., Dunn, D.E., Sieber, W.K., 1994. Occupational exposure to noise and ototoxic organic solvents. Arch. Environ. Health 49, 359–365.
Morata, T.C., Johnson, A.C., Nylen, P., Svensson, E.B., Cheng, J., Krieg, E.F., Lindblad, A.C., Ernstgard, L., Franks, J., 2002. Audiometric findings in workers exposed to low levels of styrene and noise. J. Occup. Environ. Med. 44, 806–814.
Morioka, I., Kuroda, M., Miyashita, K., Takeda, S., 1999. Evaluation of organic solvent ototoxicity by the upper limit of hearing. Arch. Environ. Health. 54, 341–346.
Muijser, H., Hoogendijk, E.M.G., Hooisma, J., 1988. The effects of occupational exposure to styrene on high-frequency hearing thresholds. Toxicology 49, 331–340.
Ohinata, Y., Miller, J.M., Altschuler, R.A., Schacht, J., 2000. Intense noise induces formation of vasoactive lipid peroxidation products in the cochlea. Brain Res. 878, 163–173.
Ohlemiller, K.K., Wright, J.S., Dugan, L.L., 1999a. Early elevation of cochlear reactive oxygen species following noise exposure. Audiol. Neurootol. 4, 229–236.
Ohlemiller, K.K., McFadden, S.L., Ding, D.L., Flood, D.G., Reaume, A.G., Hoffman, E.K., Scott, R.W., Wright, J.S., Putcha, G.V., Salvi, R.J., 1999b. Targeted deletion of the cytosolic Cu/Zn-superoxide dismutase gene (Sod1) increases susceptibility to noise-induced hearing loss. Audiol. Neurootol. 4, 237–246.
Rao, D., Fechter, L.D., 2000. Protective effects of phenyl-*N*-tert-butylnitrone on the potentiation of noise-induced hearing loss by carbon monoxide. Toxicol. Appl. Pharmacol. 167 (2), 125–131.

Rao, D.B., Moore, D.R., Reinke, L.A., Fechter, L.D., 2001. Free radical generation in the cochlea during combined exposure to noise and carbon monoxide: an electrophysiological and an EPR study. Hear. Res. 161, 113–122.

Rybak, L.P., 1992. Hearing: the effects of chemicals. Otolaryngol. Head. Neck. Surg. 106, 677–686.

Sass-Kortsak, A.M., Corey, P.N., Robertson, J.M., 1995. An investigation of the association between exposure to styrene and hearing loss. Ann. Epidemiol. 5, 15–24.

Seidman, M.D., Shivapuja, B.G., Quirk, W.S., 1993. The protective effects of allopurinol and superoxide dismutase on noise-induced cochlear damage. Otolaryngol. Head Neck Surg. 109, 1052–1056.

Sliwinska-Kowalska, M., Zamyslowska-Szmytke, E., Szymczak, W., Kotylo, P., Fiszer, M., Wesolowski, W., Pawlaczyk-Luszczynska, M., 2003. Ototoxic effects of occupational exposure to styrene and co-exposure to styrene and noise. J. Occup. Environ. Med. 45, 15–24.

Vodicka, P., Koskinen, M., Vodickova, L., Stetina, R., Smerak, P., Barta, I., Hemminki, K., 2001. DNA adducts, strand breaks and micronuclei in mice exposed to styrene by inhalation. Chem. Biol. Interact. 137, 213–227.

Yamane, H., Nakai, Y., Takayama, M., Iguchi, H., Nakagawa, T., Kojima, A., 1995a. Appearance of free radicals in the guinea pig inner ear after noise-induced acoustic trauma. Eur. Arch. Otorhinolaryngol. 252, 504–508.

Yamane, H., Nakai, Y., Takayama, M., Konishi, K., Iguchi, H., Nakagawa, T., Shibata, S., Kato, A., Sunami, K., Kawakatsu, C., 1995b. The emergence of free radicals after acoustic trauma and strial blood flow. Acta. Otolaryngol. Suppl. (Stockh). 519, 87–92.

Yang, W.P., Hu, B.H., Chen, G.D., Bielefeld, E.C., Henderson, D., 2008. Protective effect of *N*-acety-L-cysteine (L-NAC) against styrene-induced cochlear injuries. Acta Otolaryngol. 1–8 (First article).