EXPERIMENTAL STUDY OF OTOTOXIC INTERACTION OF STYRENE AND FUROSEMIDE IN THE GUINEA PIG

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Abstract

The effect of styrene or furosemide alone and the interaction between both substances on the auditory function of the guinea pig were studied by auditory brainstem response (ABR) and scanning electron microscopy (SEM). In the first and second experiments, the animals were exposed to 700 or 900 ppm styrene alone (8 h/day) for 21 days and to 60, 80 or 100 mg/kg/day furosemide alone for 14 days. In the third experiment, the animals were exposed to 900 ppm styrene alone (8 h/day) for 21 days and in combination with furosemide at 60 or 80 mg/kg/day for 14 days, beginning with the eighth day of the exposure to styrene. In the first experiment, ABR results showed significant threshold shifts at 4 and 16 kHz frequencies, and SEM showed that some outer hair cells were disrupted in the group of 900 ppm styrene exposure. In the second experiment, there was a significant threshold shift at the 8 kHz frequency in ABR in the group of 100 mg/kg furosemide exposure, but SEM results showed no cochlear damage in any of the groups. In the third experiment, there were interactions between exposure dose and time at 4 and 8 kHz frequencies in ABR and SEM findings indicated that the combined exposure caused more severe cochlear damage than the exposure to styrene alone. The results suggested that styrene and furosemide synergistically impair auditory function.

INTRODUCTION

Styrene, an organic solvent, is the most common chemical agent which is used as a precursor for polystyrene plastics at many industrial sites. Some studies have shown that long-term exposure to styrene can cause irreversible auditory deficits both in animals [3,5] and humans [7]. Exposure to styrene combined with other ototoxic agents, can cause synergistic auditory deficits [6]. Since furosemide was introduced for clinical use, many clinical studies [4] and animal experiments [10] have proven that furosemide can temporarily disrupt auditory function with just one injection and that a main cause is the increased osmosis of the stria vascularis. Synergistic interactions

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between furosemide and other ototoxic agents [8] have also been reported. Many workers exposed to styrene have the possibility to take loop diuretics represented by furosemide for congestive heart failure, hypertension and other conditions. The combined exposure to styrene and furosemide may reinforce auditory dysfunction, and it could be an important health problem. Few studies, however, have focused on the combined effects of organic solvents and loop diuretics.

We, therefore, designed a study to clarify the ototoxic interaction between styrene and furosemide in guinea pigs. In this study, three experiments were carried out. The first and second experiments were designed to confirm the effects of styrene and furosemide alone on the auditory function. Based on the results of the first and second experiments, the third experiment was designed to confirm the combined effects of styrene and furosemide.

MATERIALS AND METHODS

Experimental Animals

Materials were male guinea pigs who had normal Preyer pinna reflexes. Four guinea pigs were housed in each standard stainless cage for two weeks before the experiment so that they could adjust to the environment.

The experiments were carried out in compliance with the Ethical Committee for Animal Experiments, Wakayama Medical University. The animals were housed and handled in accordance with the Helsinki Declaration.

Exposure System

The exposure to styrene was conducted with a servo-mechanized system for solvent vapor exposure in which four parallel chambers were built. Liquid styrene was vaporized in a flask, and the generated vapor was mixed with room air at the inflow orifice at the top of the chamber.

Study Design

First Experiment: Exposure to Styrene Alone

Twelve guinea pigs were divided into three groups: two groups for styrene exposure (4 guinea pigs/group) and one for control (4 guinea pigs). The guinea pigs in the exposure groups were exposed to styrene in quantities of 700 and 900 ppm (S700 group and S900 group, respectively) from 9:00 to 17:00 for 21 consecutive days.

Second Experiment: Exposure to Furosemide Alone

Sixteen guinea pigs were divided into four groups: three groups for furosemide exposure (4 guinea pigs/group) and one for control (4 guinea pigs). They were intraperitoneally administered furosemide in doses of 60, 80 and 100 mg/kg (F60 group,

F80 group and F100 group, respectively). The guinea pigs in the control group were administered 8 ml/kg saline. The administration was continued for 14 days.

Third Experiment: Combined Exposure to Styrene and Furosemide

Fifteen guinea pigs were divided into four groups: 1) The first group (4 guinea pigs) was exposed to styrene in quantity of 900 ppm from 9:00 to 17:00 for 21 days (S900 group). 2) The second and third groups (4 guinea pigs/group) were exposed to styrene in quantities of 900 ppm from 9:00 to 17:00 for 21 days, and they were intraperitoneally administered furosemide in doses of 60 and 80 mg/kg for 14 days, beginning with the eighth day of the experiment (S900 + F60 group and S900 + F80 group, respectively). 3) The last group (3 guinea pigs/group) was administered 8 ml/kg saline for 14 days, beginning with the eighth day of the experiment (control group).

Auditory Brainstem Response Measurement

An auditory brainstem response (ABR) activity was recorded in a soundproof room by an evoked response recording system (Nihon Koden, MEB-5304) in order to evaluate the auditory function of the guinea pigs before the exposure and every two weeks from the first day after the end of exposure to the last day of the fourth week. Acoustic stimuli of 2, 4 and 8 kHz tone bursts were given through the evoked response recording system, and a 16 kHz tone burst stimulus was generated by an acoustic stimulator system (Nihon Koden, SSS-3200). All of the stimuli were transduced by a loudspeaker (BOSE, 301AVM) positioned at 20 cm from the center of both pinnas. The ABR activities collected with 1000 responses were averaged.

The stimulus intensity was decreased in 5 dB steps to the detection threshold from the initial intensity at 70 dB SPL. The detection threshold was defined as the lowest intensity level where ABR activity (the presence of a clear wave I or II, the last wave to disappear) could be visually detected.

Morphological Examination of the Cochlea

Under deep anesthesia with sodium pentobarbital (50 mg/kg), cardiac perfusion was carried out with a fixative (2.5% glutaraldehyde, 2% paraformaldehyde in 0.1 M phosphate buffer, pH 7.4), and the temporal bony capsules were removed after the ABR measurement on the last day of the fourth week after the end of exposure. Cochleae were exposed and perfused with 2.5% glutaraldehyde in 0.1 M phosphate buffer through the oval and round windows. The specimens were then prepared for SEM (HITACH S-2300).

Statistical Analysis

The mean threshold shifts (TSs), the difference between the pre- and the post-exposure detection threshold on ABR, among the groups on the first day after the end of exposure were analyzed by one-way analysis of variance. Multiple comparisons were used to make pair-wise comparisons between groups.

The changes in TSs among groups with the passage of the time were analyzed by repeated measures of two-way analysis of variance. Dunnett's multiple comparison was used to make pair-wise comparisons between groups in the first and second experiments, and Tukey's multiple comparison was used in the third experiment. Statistical significance was assumed for p values of less than 0.05. All statistic analyses were performed by SPSS 11.0J for Windows (SPSS Japan Inc.).

RESULTS

Effects of Styrene Alone

Figure 1a shows the mean TSs on the first day after the end of exposure in response to 2, 4, 8 and 16 kHz tone bursts in the first experiment. The mean TSs in the S900 group were significantly greater than those in the control and the S700 groups in response to the 4 kHz tone burst.

In response to all frequencies of tone bursts, there was no significant increase in the mean TSs of the S700 and the control groups throughout the experimental period. In response to 4 and 16 kHz tone bursts, there were interactions between exposure concentration and time throughout the experimental period. Consequently, the change in the TSs in the S900 group was statistically different from those in the other groups. No significant difference was found in the mean TSs in response to 2 and 8 kHz tone bursts.

Figure 2a, b shows representative examples of the SEM photographs in the first experiment. Neither inner hair cells (IHCs) nor outer hair cells (OHCs) were damaged in the control and S700 groups, whereas some OHCs were disrupted in the S900 group. Approximately 3.3% (1/30/visual field) disruptions of OHCs were found in the second turn.

Effects of Furosemide Alone

The mean TSs on the first day after the end of exposure were significantly greater in the F100 group than those in another groups in response to the 8 kHz tone burst (Figure 1b).

In response to the 8 kHz tone burst, there was an interaction between exposure dose and time throughout the experimental period. Thus, the change in the TSs in the F100 group was statistically different from those in other groups, but there was no significant difference among all groups at four weeks after the end of exposure. No significant difference was found in the mean TSs among all groups in response to 2, 4 and 16 kHz tone bursts.

The SEM photographs showed that neither IHCs nor OHCs had any damage in all groups.

Combined Effects of Styrene and Furosemide

In response to all frequencies of tone bursts, the mean TSs on the first day after the end of exposure increased in the following order: the S900 group, the S900 + F60 group and the S900 + F80 group (Figure 1c). In response to the 4 kHz tone burst, the mean TSs in the S900 + F60 and the S900 + F80 groups were significantly greater than that in the control group. In response to the 8 kHz tone burst, the mean TSs in the S900 + F80 group were significantly greater than that in the control group.

In response to all frequencies of tone bursts, there was no significant increase in the mean TSs in the S900 and the control groups throughout the experimental period. There were interactions between exposure dose and time throughout the experimental period in response to the 4 and 8 kHz tone bursts. Consequently, the changes in the TSs in the S900 + F80 and S900 + F60 groups were statistically different from those in the other groups. In response to the 2 kHz tone burst, there was no interaction between exposure dose and time throughout the experimental period, but the mean TSs in the S900 + F80 and S900 + F60 groups were significantly greater than those in the control group. The increased detection threshold in the combined exposure groups had slower recovery than that in the S900 and the control groups in response to all frequencies of tone bursts. This slower recovery was likely to continue, especially in response to the 2 and 8 kHz tone bursts.

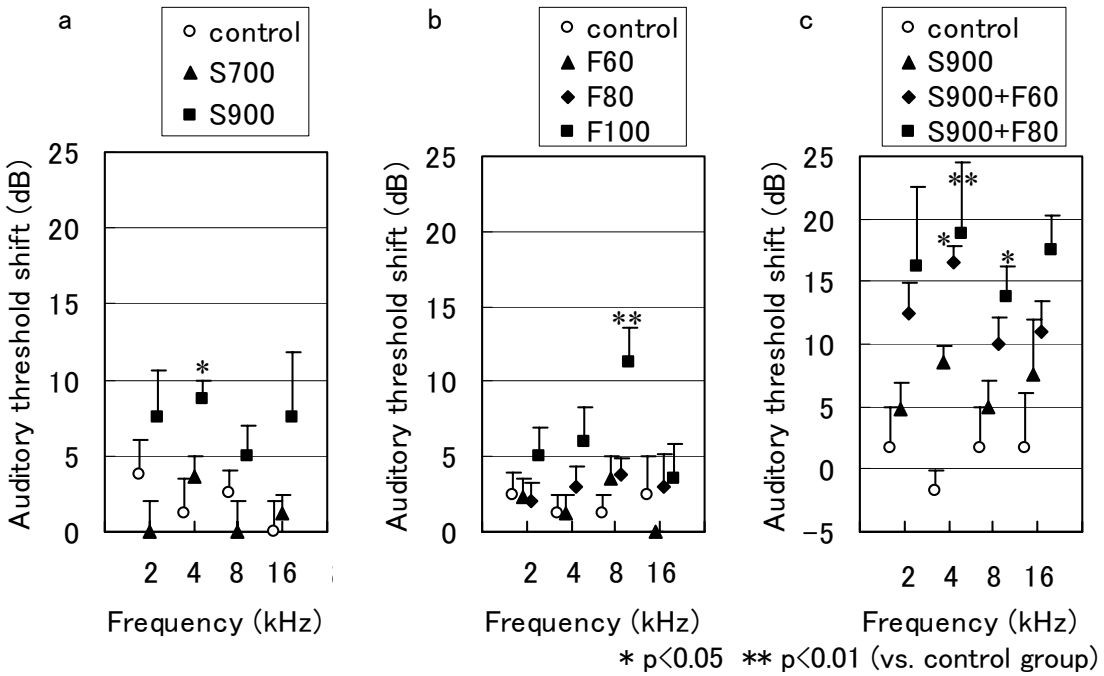

Figure 1 Mean threshold shift, in dB, on the first day after the end of exposure in response to 2, 4, 8 and 16 kHz tone bursts in the first experiment (a), in the second experiment (b) and in the third experiment (c). Vertical bar means the standard error.

The SEM photographs in Figure 2c, d show representative examples in the third experiment. In the S900 + F60 group, there were approximately 5.2% (1.5/29/visual field) disruptions of OHCs in the upper basal turn and approximately 6.7% (2.4/36/visual field) disruptions of OHCs in the lower and upper second turn. Deformities of IHCs were slight. In the S900 + F80 group, approximately 3.3% (1/30/visual field) disruptions of OHCs were found in the upper basal turn, but in the lower and upper second turn, there were approximately 15.8% (6/38/visual field) disruptions of OHCs and deformities of IHCs. The damage of the hair cells in the S900 + F80 group was greater than that in the S900 + F60 group.

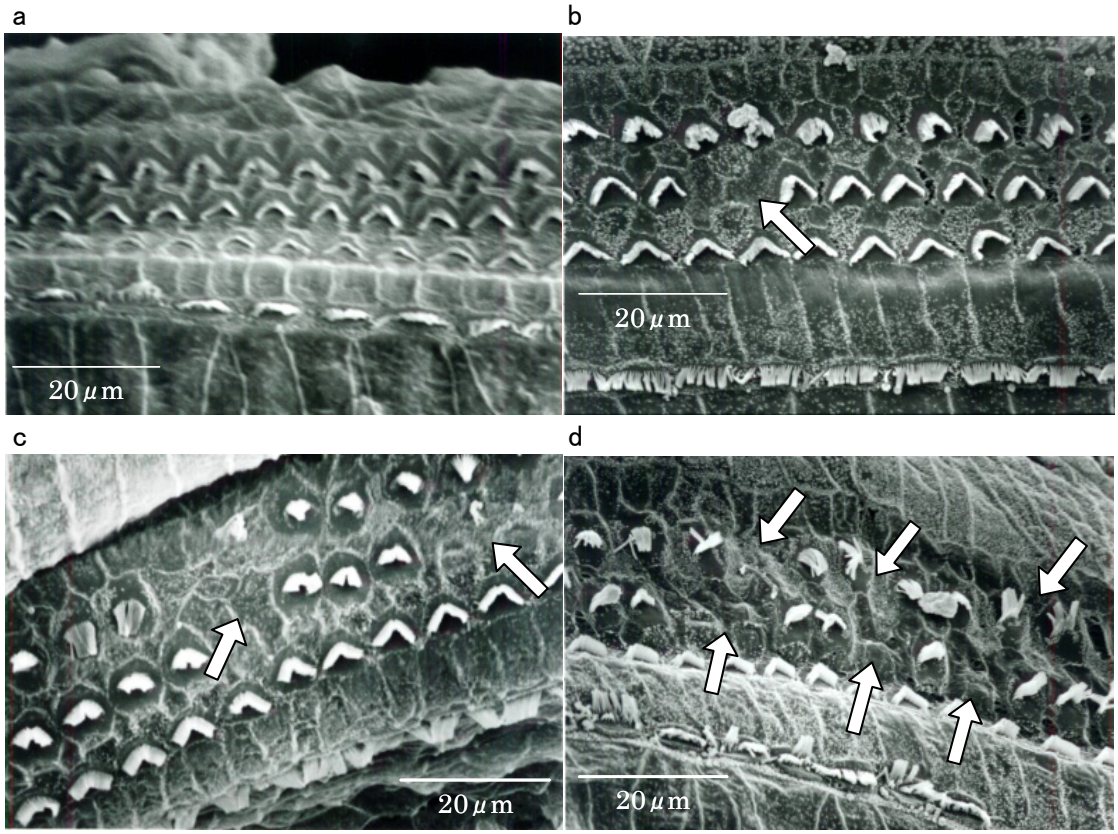

Figure 2 Scanning electron micrographs (×1500) of the guinea pigs organ of Corti at the second turn from S700 (a), S900 (b), S900+F60 (c) and S900+F80 (d) groups. Arrows in the photographs show OHC damages.

DISCCUSION

This study was undertaken to explore the possible interaction between styrene and furosemide on the auditory function of the guinea pig.

In the first experiment, the mean TSs in the S900 group were significantly different from those in other groups in response to 4 and 16 kHz tone bursts, and some OHCs were disrupted. Exposure to 700 ppm styrene caused no change in either ABR or

SEM results. Consequently, in this experimental condition, 900 ppm styrene was regarded as the critical dose at which an influence on the auditory function began to be exerted.

In the second experiment, the mean TSs in the F100 group were significantly different from those in other groups in response to the 8 kHz tone burst, whereas histopathological results showed no damage in all groups. These findings are in agreement with those of previous studies which indicated that furosemide administration caused no permanent damage to hair cells [2, 4]. Administration of 60 and 80 mg/kg furosemide caused no change based on ABR and SEM. Thus these doses were regarded as subcritical, not exerting an influence on the auditory function under this experimental condition.

In the third experiment, the mean TSs in the combined exposure groups were significantly higher than those in the group exposed to styrene alone, and the recovery tended to be worse. This suggests a clear synergistic interaction between styrene and furosemide on auditory function. On the first day after the end of exposure, the mean TSs increased in the following order in response to all frequencies of tone bursts: the S900 group, the SF900+60 group and the SF900+80 group. This result suggests the existence of a dose-effect relationship.

The most obvious change in the SEM was the damage of the OHCs. Exposure to styrene alone caused only an occasional loss of OHCs (approximately 3.3%), but combined exposure to 900 ppm styrene and 60 mg/kg furosemide caused severe OHC damage and combined exposure to 900 ppm styrene and 80 mg/kg furosemide caused more severe damage (approximately 6.7 and 15.8%, respectively). The site of maximum damage was found in the second turn. This corresponds with frequencies at which significant differences were identified by ABR (2, 4 and 8 kHz).

Combined administration of aminoglycosides and furosemide results in increased ototoxicity in animals [1, 4, 8, 9]. However, combined exposure to noise and furosemide could not increase ototoxicity compared to only noise exposure in animals [11]. In other words, furosemide cannot reinforce the physical ototoxicity such as noise. If a similar mechanism exists in the combined exposure to styrene and furosemide, the concentration of styrene in serum and perilymph may have been increased, and it can be concluded that the ototoxicity of styrene exposure is reinforced by furosemide.

We have clearly demonstrated that exposure to styrene combined with furosemide could cause greater auditory deficits. At clinical site, when patients in the recipe of loop diuretics complain of hearing loss, it may also be necessary to ask them if or not work with aromatic solvents such as a styrene.

CONCLUSIONS

We carried out animal experiments by means of ABR and SEM to confirm the effects of combined exposure to styrene and furosemide on auditory function. The results demonstrated are; (1) A dose of 900 ppm styrene is the critical dose; i.e., it is the dose which began to cause both electrophysiological and morphological changes. (2) Doses of 60 and 80 mg/kg furosemide are the subcritical doses, the doses which could

not exert an influence on auditory function. (3) Combined exposure to styrene and furosemide resulted in increased ototoxicity both on ABR and SEM. This suggests clear synergistic interaction between styrene and furosemide on auditory function.

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