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Original Article

Dexamethasone Effect on Sudden Hearing Loss is Validated in Stress-induced Animal Models: Hypothetical Study

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BACKGROUND: Stress could be a contributing cause of sudden hearing loss. This study intended to develop an animal model of stress-induced sudden hearing loss and to evaluate the effects of dexamethasone.

METHODS: Two stress models (I and II) for rats were designed using various stressors and modified by adjusting the stress protocol to increase the threshold significantly. For the stress model with a significant increase in threshold after stress exposure, changes in cortisol levels according to stress exposure were measured. The threshold shift and the change in the cellular structure associated with stress exposure and dexamethasone administration were analyzed.

RESULTS: While hearing thresholds increased only at 16 kHz in rats of stress model I (n = 10), the thresholds increased at 16 and 32 kHz in rats of stress model II (n = 16). Cortisol level increased after stress exposure ($P = .015$) in stress model II. Among stress model II rats (stress only and stress + dexamethasone groups), the threshold shift at 16 kHz significantly decreased 1 day after dexamethasone injection in the stress + dexamethasone group (n = 8). Histologically, the cochlear cellularity of the stress + dexamethasone group was more compact than that of the stress-only group (n = 8).

CONCLUSION: Our preliminary study presented the development of an animal model of stress-induced sudden hearing loss and the positive results of steroids in terms of hearing recovery.

KEYWORDS: Animal models, stress, sudden sensorineural hearing loss, dexamethasone, auditory brainstem response

INTRODUCTION

Exposure to various types of stress, whether physical, mental, psychosocial, or psycho-emotional, is a common feature of everyday life. Selye and Fortier[1] defined the stress response as a nonspecific response of the body when a person is subjected to psychosocial, physical, and biological stimuli. Chronic stress has been linked to dermatitis, depression, cardiovascular disease, immune suppression, insulin resistance, and other diseases. The common pathophysiology is thought to include vasoconstriction, hemoconcentration, and reduced blood flow throughout the body.[2]

Sudden hearing loss (SHL) is defined as sensorineural hearing loss > 30 dB over at least 3 contiguous frequencies within 72 hours. Although SHL in most patients is classified as idiopathic, it has become clear that stress is a contributing cause of hearing disabilities or SHL.[3-6] Moreover, SHL itself can be a cause of stress that further worsens the quality of life. The mechanism of SHL has been explained through cellular response to cell-level stress in animal studies.[7] During this cellular response, stimuli such as ischemia, hypoxia, and heat shock induce the expression of several genes regulating cell survival and apoptosis. However, there are few

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animal models of SHL caused by lifestyle stress, such that the causal relationship between stress exposure and accompanying cellular changes remains poorly understood and drug targets for therapeutic intervention are largely unknown.

Stress-induced SHL can be managed using various therapeutic approaches, including stress elimination (relaxation techniques and sedative medications), re-establishment of blood flow (hemodilution, blocking the cervical sympathetic chain, steroids, and vasodilators), and stress management (cognitive behavioral therapy).[8-10] Steroids have been widely used to treat inner ear diseases, including SHL, tinnitus, and Meniere's disease, by a mechanism involving the reduction of inflammation and edema in the inner ear.

The aim of this study was to develop an animal model of stress-induced SHL and then use it in a preliminary evaluation of the effect of dexamethasone (DEX) on stress-induced SHL.

METHODS

All procedures performed in studies involving animals were in accordance with the ethical standards of the Internal Animal Care and Use Committee of Ajou University Hospital Biomedical Research Institute (protocol no. 2017-0027).

The experiment was carried out in 2 stages as follows: identification of stress protocol causing SHL and validation of the effect of steroids on SHL.

Identification of Stress Protocol Causing Sudden Hearing Loss

Male Sprague-Dawley (SD) rats (8 weeks of age, 250-300 g) were used in the study and were managed in compliance with the Guidelines for the Care and Use of Laboratory Animals of the National Institutes of Health. The animals were maintained under controlled conditions of temperature (22 ± 2°C), humidity (70%-75%), and a regulated 12-hour dark/12-hour light cycle. In the cage, stress was minimized by providing the rats with free access to food and water.

The stress conditions applied were as follows: (a) swimming for 1 hour in a water tank with a height longer than the length of the rats and a water temperature of 22°C; (b) cold stimuli: a 4°C environment for 4 hours while fastened in a restraining cage (50 × 170 cm); (c) overcrowding: placement in a narrow case (220 × 270 × 130 cm) without food or water and exposed to a 22-hour light environment; (d) saline injection with 200 μL of 0.09% saline 4 times in 1 hour subcutaneously; (e) shaking for 1 hour at 250 rpm using a shaking incubator (K.M.C.-8480SF; Vision Scientific Co., Ltd., Daejeon, Korea), and maintenance at 40°C when heating was included; (f) social isolation in a small box under a silent environment without food, water, or a distinction between day and night (22 hours of light or dark).

We designed 2 stress models with different stress combinations and stress schedules using 6 stress conditions. A 12-day protocol was repeated twice for a total of 24 days in the stress model I (SM-I) rats (n = 10) and a 6-day schedule was applied in the stress model II (SM-II) rats (n = 16). All experiments were conducted according to the following time schedule: morning stress activities started at 9 AM and afternoon stress activities at 2:00 PM (Table 1).

Auditory brainstem responses (ABRs) were recorded in all rats just prior to their exposure to the stress protocol, 1 day after the completion of the stress protocol (Figure 1). For auditory evaluation, each rat was anesthetized by intraperitoneal injection with Zoletil 50 (Virbac Laboratories, Carros, France) at a dose of 0.1 cc/100 g and with Rompun 2% (Bayer Korea, Ansan, Korea) at a dose of 0.02 cc/100 g. Auditory brainstem responses were recorded in a soundproof and electrically shielded room. An auditory evaluation was conducted based on ABRs using the Biosig 32 system (Tucker-Davis Technologies, Gainesville, Fla, USA). Recording needle electrodes were inserted subcutaneously into the vertex (+) of the scalp and in the postauricular area (−). Ground needle electrodes were inserted in the neck region contralateral to the recording electrodes. The stimuli were tone bursts 5 ms in duration with 1-ms rise/fall times. Sound frequencies of 16 and 32 kHz were delivered to the left ear of the rat at a rate of 11.1 per second. Responses were averaged over at least 512 stimulus repetitions, with the stimulus decreased by 5 dB at subthreshold levels. The hearing thresholds were determined by the lowest sound threshold showing wave I. We analyzed the change in hearing thresholds according to stress exposure.

Enzyme-Linked Immunosorbent Assay

When a significant threshold shift was confirmed after stress exposure, cortisol level was measured using enzyme-linked immunosorbent assay (ELISA). At baseline, SD rats of the stress + DEX group were placed under deep anesthesia, and 1 mL of blood was obtained from the jugular vein of each one. Additional samples were collected 1 day after the completion of the stress protocol. The samples were centrifuged (2000 × *g*) for 10 minutes, and the resulting supernatant (plasma) was collected and stored frozen at −70°C. The cortisol level in the supernatant was measured using a rat cortisol ELISA kit (EK0509; Signalway Antibody, College Park, Md, USA) as described in the manufacturer's instructions. The optical density at 450 nm was measured in each sample using an ELISA microplate reader.

Validation of the Effect of Steroid on Sudden Hearing Loss

Sprague-Dawley rats with SHL after stress exposure were evaluated for the effect of steroids in hearing recovery in comparison with 3 groups: a control group without stress exposure, a stress-only group, and a stress + DEX group. Sprague-Dawley rats in the stress + DEX group received an intraperitoneal injection of DEX (1.25 mg/kg)

MAIN POINTS

- Compared with stress model I (n = 10), the thresholds significantly increased at 16 and 32 kHz at 1 and 2 weeks after stress exposure in rats of redesigned stress model II (n = 16).
- The mean cortisol levels measured using a rat cortisol enzyme-linked immunosorbent assay were 0.166 ± 0.037 ng/mL before stress exposure and 0.200 ± 0.048 ng/mL after stress exposure in the rats of stress model II (n = 11) ($P = .015$)
- Among stress model II rats, the mean thresholds and threshold shifts after dexamethasone (DEX) administration at 16 and 32 kHz were overall different between the stress + DEX group and the stress-only group ($P > .05$). In the case of an intra-group comparison, the stress + DEX group showed a significant decrease in the mean threshold at 16 kHz at post-DEX day 1 compared with the value of post-stress day 1 in the stress + DEX group ($P = .038$).

Table 1. Stress Conditions and Schedules of SM-I* (n = 10) and SM-II (n = 16)

SM-I	Time	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6
	AM	Overcrowding	Swimming	Saline injection	Social isolation (light)	Shaking	Overcrowding
	PM		Shaking	Cold stimuli		Cold stimuli	
	Time	Day 7	Day 8	Day 9	Day 10	Day 11	Day 12
	AM	Swimming	Social isolation (Dark)	Saline injection	Overcrowding	Swimming	Social isolation (Light)
	PM	Saline injection		Cold stimuli		Shaking	
SM-II	Time	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6
	AM	Swimming	Overcrowding	Swimming	Swimming	Overcrowding	Swimming
	PM	Cold stimuli		Shaking + Heating	Cold stimuli		Shaking

SM, stress model.
*A 2-week protocol was repeated twice for 4 weeks.

daily for 1 week after completion of the stress protocol (post-stress days 2-8, Figure 1). Auditory brainstem responses were recorded in all rats 1 day after DEX administration and 1 week after DEX administration (Figure 1).

Histological Analysis

Following the ABR recordings, the rats were euthanized. The extracted cochleae were placed in 4% (v/v) paraformaldehyde in phosphate-buffered saline overnight, decalcified in Calci-Clear Rapid (National Diagnostics, Atlanta, Ga, USA) for 3 days, and embedded in paraffin blocks. Sections (5 μm thick) were cut from the blocks along the cochlear axis and stained with hematoxylin and eosin. Hair cells and spiral ganglion cells in the cochlea were observed by microscopy.

Data Analysis

All data are presented as the mean ± standard error. Hearing status was analyzed using a one-way analysis of variance, Tukey's HSD post-hoc test, and the Mann–Whitney *U*-test. A *P*-value < .05 was considered to indicate statistical significance.

RESULTS

Stress-Induced Sudden Hearing Loss

The ABR thresholds were compared before and after the exposure to the stress of rats in the SM-I. At 16 kHz, the mean threshold before stress exposure was 17.5 ± 2.6 dB HL, while 2 and 4 weeks after stress exposure, it was 18.5 ± 3.4 and 23.5 ± 4.1 dB HL, respectively. The mean threshold at 32 kHz was 18.5 ± 2.4, 18.5 ± 3.4, and 21.5 ± 4.1 dB HL, respectively. The mean threshold at 16 kHz differed significantly before versus 2 and 4 weeks after stress exposure. By contrast, the mean thresholds at 32 kHz were similar at all 3 time points.

In SM-II rats, the mean threshold at 16 kHz was 11.0 ± 3.0 dB HL before stress exposure, 45.6 ± 20.8 dB HL at post-stress day 9, and

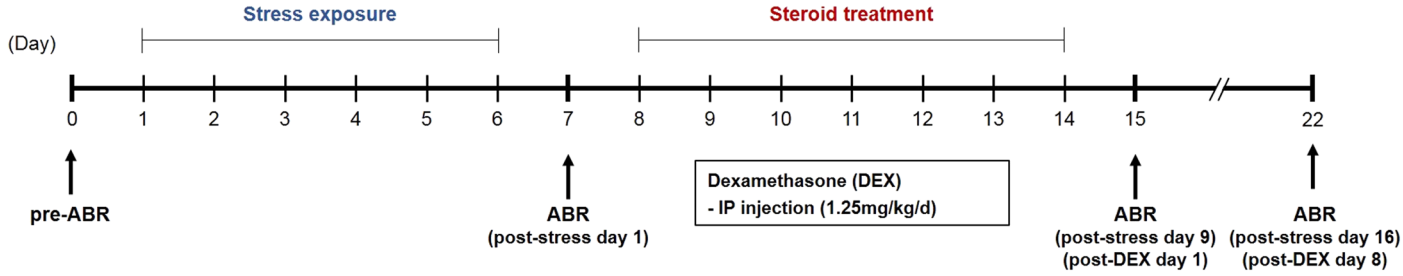

Figure 1. Experimental schedules in SM-II. Rats in the SM-II (n = 16) were divided into a control group without stress exposure (n = 4), a stress-only group (n = 8), and a stress + DEX group (n = 8) injected intraperitoneally with 1.25 mg of DEX/kg daily for 1 week after completion of the stress protocol. The ABRs were recorded just prior to conducting the stress protocol, 1 day after the completion of the stress protocol, 1 day after DEX administration, and 1 week after DEX administration.ABR, auditory brainstem responses; DEX, dexamethasone; SM, stress model.

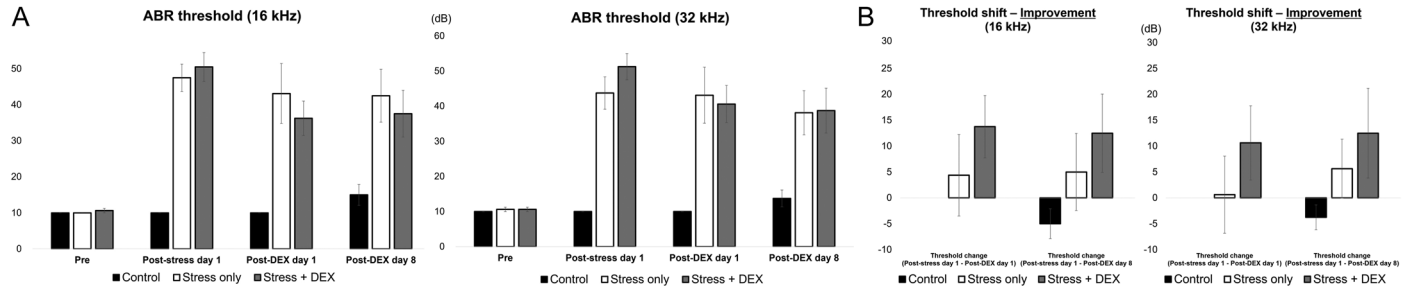

Figure 2. The therapeutic effect of DEX in stress-exposed rats in the SM-II. (A) The mean ABR thresholds at 16 and 32 kHz after completion of the stress protocol and DEX administration. (B) The threshold shift at 16 and 32 kHz after completion of the stress protocol and DEX administration. ABR, auditory brainstem response; DEX, dexamethasone; SM, stress model.

49.6 ± 23.1 dB HL at post-stress day 16. The mean threshold at 32 kHz was 11.3 ± 3.2, 45.0 ± 20.5, and 46.8 ± 20.5 dB HL, respectively. The differences before versus post-stress days 9 and 16 after stress exposure were significant in both frequencies. The mean cortisol levels in the rats were 0.166 ± 0.037 ng/mL before stress exposure and 0.200 ± 0.048 ng/mL after stress exposure ($P = .015$).

Effect of Dexamethasone After Stress Exposure in Stress Model II

The 16 SD rats in the SM-II were randomly divided into 3 subgroups including the control group: a control group without stress exposure (n = 4), a stress-only group (n = 8), and a stress + DEX group (n = 8). The rats were weighed before and after the stress protocol to confirm sufficient stress exposure. In the SM-II, the change in body weight (g) was 42.2 ± 17.0 in the control group and 5.9 ± 4.1 in the stress-only and stress + DEX groups ($P = .001$).

Figure 2(A) shows the mean ABR thresholds at 16 and 32 kHz in the control, the stress-only, and the stress + DEX groups. The mean thresholds at 16 and 32 kHz tended to be higher in the stress + DEX group than in the stress-only group at "post-stress day 1," but there was no significant difference. The threshold shifts after DEX administration in each frequency are shown in Figure 2(B). The threshold shifts of both

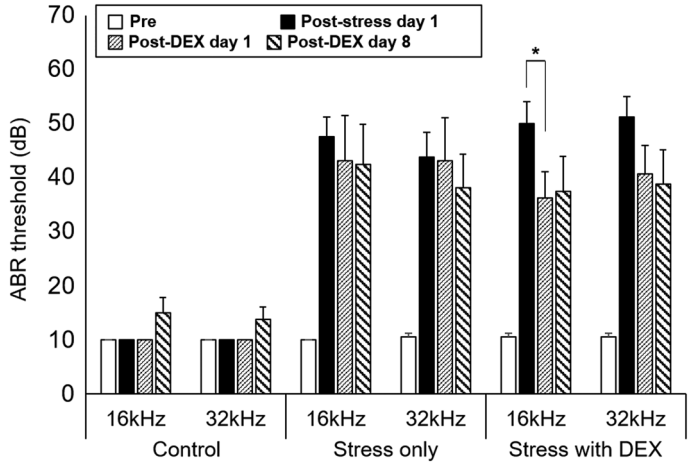

Figure 3. The therapeutic effect of DEX in stress-exposed rats in the SM-II group. The change in the ABR after completion of the stress protocol and DEX administration. *$P < .05$. ABR, auditory brainstem response; DEX, dexamethasone; SM, stress model.

frequencies in the stress + DEX group tended to be larger than those in the stress-only group at "post-DEX day 8," which were not significant. From another perspective, when analyzing changes in hearing

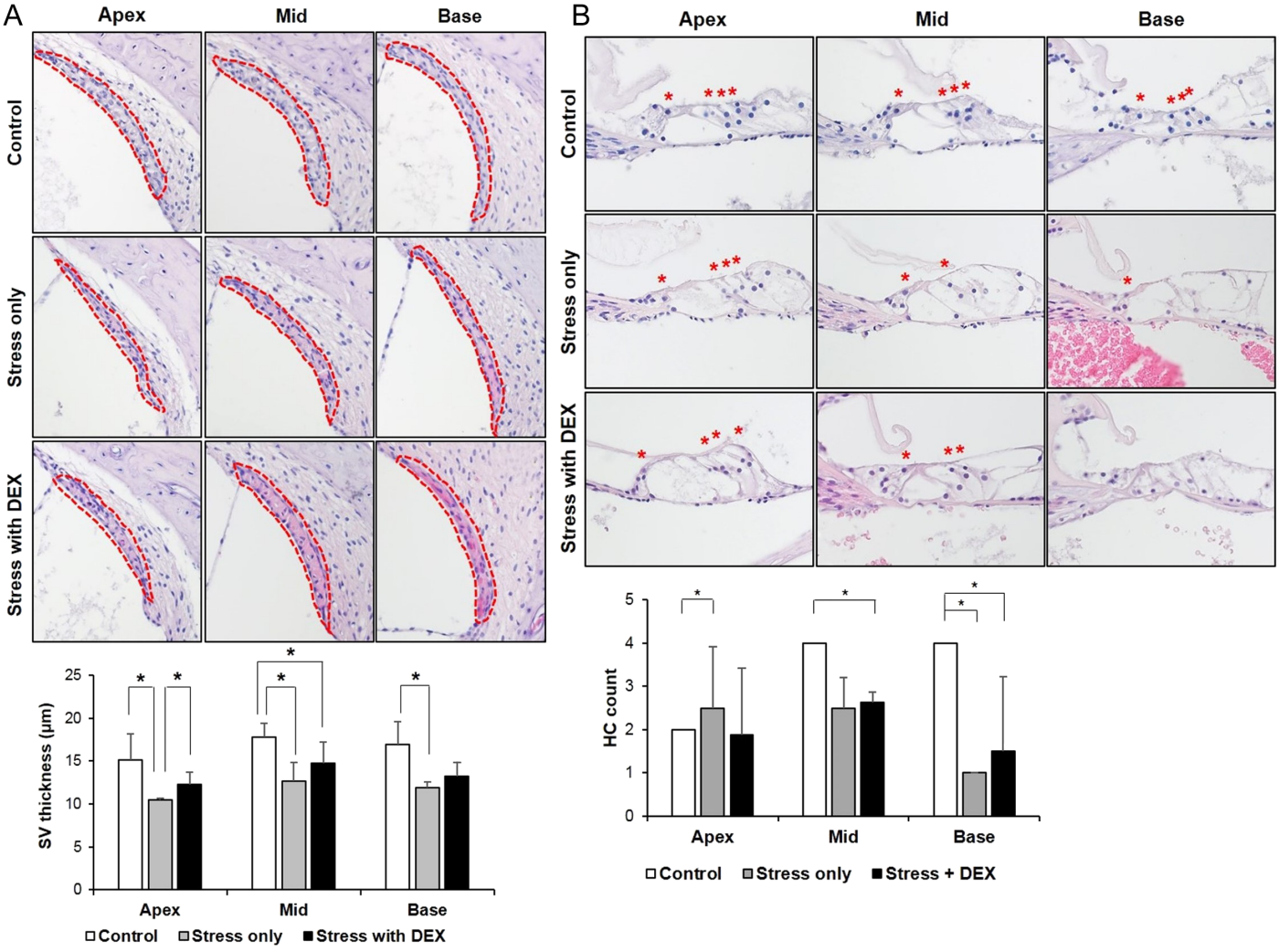

Figure 4. Microscopic images of hematoxylin and eosin-stained sections of the (A) stria vascularis (SV), (B) organ of Corti, (magnification, ×40; scale bar, 25 μm). *$P < .05$. DEX, dexamethasone; HC, hair cell.

thresholds within a group, the mean threshold at 16 kHz decreased significantly at "post-DEX day 1" (50.0 ± 11.3 dB HL) compared with the value at "post-stress day 1" (36.3 ± 13.6 dB HL; $P = .038$) in the stress + DEX group (Figure 3).

After the threshold in the rats had been measured, all animals in each group were euthanized and their cochleae were isolated for histological examination. The stria vascularis (SV) of the control group was significantly thicker than that of the stress-only group ($P < .05$) but was similar to that of the stress + DEX group ($P > .05$). The cellular density of hair cells, spiral ganglion cells, and spiral ligament cells, although not significant, tended to be denser in the stress + DEX group than in the stress-only group. (Figure 4).

DISCUSSION

Stress factors and an inability to cope with such factors are important psychological factors mediating SHL.[3,4] Influence of stress on the hearing status has been using physical acute stress. Severe pain such as tracheostomy without general anesthesia caused auditory threshold shift.[11] In another study, SD rats subjected to restraint for 10 days, 2 hours per day showed auditory impairment and atrophy of inferior colliculs, auditory tissue-specific degeneration.[12] Psychosocial stress was related to auditory impairments. Wistar rats exposed to 24 hours of stress (non-harmful sonic stress) developed temporary reduction of ABR thresholds.[13]

There have been few reports suggesting animal models of stress condition inducing hearing loss. Yamaguchi et al[14] reported that chronic stress was irrelevant to SHL in mice. In their study, 3 stress conditions, water-immersion restraint, social defeat, and social isolation, were applied individually, but the changes in hearing threshold after stress exposure were not significant. However, the stress-induced physical responses might not have been sufficient as stress models and single stress may not be a necessary and sufficient condition to affect hearing. This study was performed to determine whether the intensity of stress burden and exposure to a combination of stresses over a long period are important factors in the onset of SHL rather than stress itself.

Since there have been few reports of psychologic or physical stresses inducing SHL, we designed our stress-induced SHL protocol using stress conditions based on various studies.[14-17] We controlled the intensity of the stress by using various combinations of physical and psychosocial stressors. Comparisons of the 2 groups in this study showed that the threshold change was more prominent in SM-II than in SM-I rats. Although the stressors were similar between the 2 groups, the relatively weak stressors such as saline injection and social isolation were combined in the SM-I. The several sessions of exposure to these weak stressors could give the rats time to recover. In the SM-II, the stress conditions inducing an abrupt change in body temperature were intensified and the relatively weak stresses of saline injection and social isolation were excluded. Body weight differed significantly according to stress exposure. In addition, the cortisol level increased significantly after stress exposure in SM-II rats. These observations demonstrate that the SM-II stress protocol was powerful enough to cause stress in experimental animals. The standard deviations at all time points after stress exposure in the SM-II were huge due to the insufficient sample size. Therefore, further study is needed to acquire the reproducibility of this protocol.

Among the mechanisms thought to underlie the relationship between stress and SHL, the most prominent is impairment of the inner and outer hair cells induced by a lack of oxygen, which in turn is caused by a diminished blood supply to the inner ear. This change in blood flow induced by the cardiovascular stress response is regulated by the immune system and cytokine network, through activation of the sympathetic nervous system and the hypothalamic–pituitary–adrenal axis. Thus, psychosocial, physical, and biological stresses from the external environment may cause a nonspecific response in the body that activates the hypothalamic–pituitary–adrenal axis and thus activates the cardiovascular stress response.

The clinical characteristics of SHL can be explained by the stress response theory, which proposes the activation of nuclear factor κ-light-chain-enhancer of activated B cells (NF-κB) as a stress sensor. Nuclear factor κ-light-chain-enhancer of activated B cells has been shown in many studies to play an essential role in regulating the immune system and inflammatory responses.[18] Idiopathic SHL may reflect the stress response of the cochlear lateral wall through NF-κB activation subsequent to systemic stress. A change in the organ of Corti cells may occur secondary to lateral wall dysfunction. Therefore, synergistic interactions of multiple stressors may be necessary for NF-κB activation in the lateral wall, leading to HL.

Steroids are the most common initial treatment for SHL. They are thought to reduce the inflammatory response, improve cochlear blood flow, and protect against cochlear ischemia. It has also been reported that steroids play a role in restoring endocochlear potential, by improving the function and morphology of the SV.[19] The synthetic glucocorticoid DEX has a high affinity for glucocorticoid receptors (GRs). It selectively activates pituitary GRs, which downregulate cortisol production and thereby inactivate the hypothalamus–pituitary–adrenal axis in the adrenal cortex. Thus, DEX administration not only reduces cortisol production but also stabilizes inner ear function, where it prevents the downregulation of GRs.

Since a threshold change was clearly observed in the SM-II rats in this study, the steroid was administered only to this group. The efficacy of steroids on hearing preservation was prominent at 16 kHz. Although not significant, the threshold was decreased after steroid administration at 32 kHz, but the demonstration of a significant difference may require a larger sample size. The effect of DEX on hearing preservation was indirectly supported by the change in cellularity seen in the histological analysis.

In this study, stress-induced SHL and the effect of steroids on SHL were evaluated through the histopathologic change in cochlear structure. The spiral ganglion, organ of Corti, or SV are regarded as 3 major cochlear structures that can independently degenerate and influence the degree of hearing loss.[20,21] Similarly, the difference in histological degenerative changes between the stress + DEX group and the stress group is related to the difference in the degree of hearing loss and indirectly explains the effect of DEX administration.

The stress conditions applied in this study would not be experienced by patients in real life. However, the pathophysiology of SHL after stress exposure is similar regardless of the type of stressor. In

addition, there have been several clinical reports linking stress with hearing loss.[22,23] Therefore, this animal model of stress-induced SHL may have sufficient power to explain the causal relationship between stress and SHL under clinical conditions.

Clinically, the schedule of steroid administration is prednisolone (Solondo; Yuhan, Seoul, Korea) for 10 days consisting of 60 mg/day for 5 days, 40 mg/day for 2 days, 20 mg/day for 2 days, and 10 mg/day for 1 day. We modified the dose and duration for intraperitoneal administration to rats in the present study. We calculated the appropriate DEX dose for SD rats using animal equivalent dose calculation based on body surface area.[24]

Despite interesting data, our study has several limitations that need to be addressed in future studies. First, our results were limited by the small number in each group, which might have weakened the statistical power. For instance, a weak correlation between threshold shift and DEX administration might be diluted by the small sample size. Thus, a well-designed comparison study with a larger number of animals is warranted to confirm the current hypothesis. Second, it could be questionable whether the physical stress conditions applied in this study could be the causative factor for SHL, not a confounder or associated factor. It has been known that virus infection, acoustic trauma, ototoxicity, and cochlear ischemia are the main etiologies for SHL. A further study evaluating the effect of stress on hearing loss caused by noise exposure and ototoxicity is needed for additional information on this relationship. Furthermore, studies elucidating the mechanism of cochlear ischemia at the cellular level due to physical stress can be a challenging but interesting research topic.

CONCLUSION

In summary, we successfully designed an animal model of stress-induced SHL. In a comparison of two stress models, the level of HL was affected by the extent of stress condition, and the optimal experimental condition achieved with the SM-II protocol was suggested. Furthermore, our preliminary evaluation of the effects of DEX demonstrated the utility of steroids in hearing recovery. However, these results remain to be confirmed in longer studies testing different steroid doses and modes of administration.

Ethics Committee Approval: Ethical committee approval was received from the Ethics Committee of Ajou University (Approval no: 2017-0027).

Informed Consent: N/A

Peer-review: Externally peer-reviewed.

Author Contributions: Concept – Y.H.C.; Design – Y.H.C., Y.C.K.; Supervision – Y.J.K.; Materials – Y.C.K., J-S.L.; Data Collection and/or Processing – Y.C.K., J.H.J.; Analysis and/or Interpretation – O-S.C., Y.Y.L.; Literature Review – Lee.JS., Y.C.K.; Writing Manuscript – J.H.J., Y.C.K.; Critical Review – Y.J.K., Y-H.C.

Declaration of Interests: The authors declare that they have no conflict of interest.

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