ORIGINAL ARTICLE

3,6'-Dithiothalidomide Reduces Tinnitus Phenotype in Two Different Mouse Preclinical Models of Tinnitus

Sergio Gonzalez-Gonzalez[1], Chantal Cazevieille[2]

[1]In Vivex SAS, Incubator Via Innova, 177b Avenue Louis Lumière, 34400 Lunel, France, [2]COMET, Institute for Neurosciences of Montpellier, Hopital Saint Eloi, 80, Rue Augustin Fliche, 34091 Montpellier, France

ABSTRACT

Background: Tinnitus is the perception of a sound in the absence of external stimulation. Tinnitus is a common consequence of damage to the auditory periphery, affecting around 5–12% of the population and inducing intolerable discomfort. Today, no treatment exists to cure tinnitus. From an electrophysiology point of view, tinnitus leads to auditory brainstem responses (ABRs) wave modifications and spontaneous activity changes of the primary auditory cortex (PAC) in human and rodents. Moreover, plasma brain-derived neurotrophic factor (BDNF) levels vary with the severity of tinnitus, suggesting that plasma BDNF level can be used as a biomarker to objectively evaluate tinnitus in rodent and humans. Several publications suggested that tinnitus is closely related to auditory system inflammation and proinflammatory cytokine TNF-α. **Results:** we evaluated the efficacy of a novel thalidomide-based TNF-α lowering agent, the 3,6'-dithiothalidomide (TLD) in two different preclinical models of tinnitus. We observed that TLD treatment increased the % of startle GAP suppression, increased ABR wave I amplitude and decreased wave IV latency, decreased spontaneous activity of PAC, and significantly decrease plasma BDNF concentration in the salicylate-induced and noise-induced tinnitus mouse models. **Conclusions:** Our data confirm that proinflammatory cytokines are directly linked to tinnitus phenotype suggesting that inflammatory pharmacological modulators could be a promising therapy targeting tinnitus disorder.

Key words: ABR wave, acoustic trauma, auditory cortex, biomarker, dithiothalidomide, salicylate, tinnitus

INTRODUCTION

Tinnitus is a common consequence of damage to the auditory peripheral system, affecting around 5–12% of the population, and inducing intolerable discomfort,[1] while often described as a ringing, it may also sound such as a clicking, hiss, or roaring. Tinnitus may mainly occur following a single exposure to high-intensity impulse noise, long-term exposure to repetitive impulses, long-term exposure to continuous noise, or exposure to a combination of impulses and continuous noise. Other reasons such as ototoxic compounds administration (such as aspirin also called salicylate),[2] diseases, or external traumatic factors could also lead to tinnitus disorder. Persistent tinnitus causes anxiety and depression. Moreover, psychological problems, such as depression, anxiety, sleep disturbances, and concentration difficulties, are directly linked to tinnitus disorder.

Auditory evoked potentials are used to examine the synchronous discharge of fibers in the auditory pathway and identify the presence of abnormal neuronal activity such as tinnitus. The waveforms that occur in the first milliseconds of an auditory evoked potential are called auditory brainstem response (ABR). ABR is the test of choice when patients present with symptoms that suggest a cochlear or retrocochlear lesion site. ABR is indicated in the evaluation of tinnitus for a number of reasons, including the fact that it is an objective electrophysiological measure of the functioning of the cochlea and the brainstem auditory

Address for correspondence:
Sergio Gonzalez-Gonzalez, In Vivex SAS, Incubator Via Innova, 177b Avenue Louis Lumière, 34400 Lunel, France.

pathways. In addition, ABR may assist in the differentiation of central versus peripheral tinnitus. Thus, ABR may contribute to the clarification of the origin of tinnitus in normal listeners.

An increasing number of publications demonstrated significant changes in ABR wave amplitudes and latency during tinnitus disorder in humans and rodents.[3-5] These publications demonstrated a decrease of the wave I amplitude and latency and also electrophysiological profile changes of wave IV, strongly suggesting that ABR wave analysis can be used as an objective and reproductive readout to determine the efficacy of new pharmacological candidates targeting tinnitus.

On the other hand, some publications confirm that tinnitus disorder induces electrophysiological changes of the primary auditory cortex (PAC). Modifications of the central frequency, Q10dB value, and significant increase of spontaneous activity of the auditory cortex have been reported in tinnitus human and salicylate- and noise-induce tinnitus mouse model.[6] Taken together, these data suggest that unicellular electrophysiology recording is a validated preclinical readout allowing to determine the efficacy of pharmacological candidates targeting tinnitus disorder and auditory cortex electrophysiological changes induced by tinnitus disorder.

Brain-derived neurotrophic factor (BDNF) is a prosurvival factor induced by cortical neurons that is necessary for the survival of striatal neurons in the brain. It is known to promote neuronal survival and differentiation. mRNA products of the BDNF and neurotrophin-3 (NT)-3 genes are detected in the adult human brain, suggesting that these proteins are involved in the maintenance of the adult nervous system. BDNF and other NTs are critically involved in long-term potentiation (LTP). BDNF-mediated LTP is induced postsynaptically. BDNF has trophic effects on serotonergic (5-HT) neurons in the central nervous system. BDNF has an essential maintenance function in the regulation of anxiety-related behavior and food intake through central mediators in both the basal and fasted state.

There is a close relationship between tinnitus and depression, in which BDNF has a pathophysiological role. To determine, whether BDNF levels could be used to evaluate tinnitus severity, Goto and collaborators evaluated plasma BDNF levels in patients with tinnitus. They showed for the 1st time that plasma BDNF levels vary with the severity of tinnitus, suggesting that plasma BDNF level is a useful biomarker for objective evaluation of tinnitus.[7] Moreover, Yi and collaborators demonstrated that BDNF and p-CREB were upregulated in plasma of tinnitus rodent and they observed structural changes of BDNF at the synapses in the auditory cortex of rats treated chronically with salicylate.[8]

The proinflammatory cytokine TNF-α, which plays a central role in initiating and regulating the cytokine cascade during an inflammatory response,[9,10] has been found upregulated in tinnitus. In this study, we investigated the efficacy of a novel thalidomide-based TNF-α lowering agent, 3,6′-dithiothalidomide (TLD) in tinnitus. This compound, similar to but more potent than thalidomide,[11] readily enters the brain and lowers the rate of synthesis of TNF-α post-transcriptionally through the 3′-untranslated region of TNF-α mRNA.[12-14] Importantly, Wang and collaborators described for the 1st time in 2019 the effect of TLD administration in pro-inflammation induced by noise trauma and the efficacy of this compound reducing tinnitus phenotype in mice.[15]

Corroborating this publication, we observed that TLD treatment increased the % of startle GAP suppression, increased ABR wave I amplitude and decreased wave IV latency, decreased spontaneous activity of PAC, and significantly decrease plasma BDNF concentration in the salicylate-induced and noise-induced tinnitus mouse models. Taken together, our data suggest that inflammatory modulators could be a promising therapy targeting tinnitus disorder in rodent and humans.

MATERIALS AND METHODS

Animal housing and drug administration

Two-month-old male C57BL/6J mice were kept in the A1 animal house facility. Mice were housed in ventilated and clear plastic boxes and subjected to standard light cycles (12 h in 90-lux light and 12 h in the dark). The animals were treated with vehicle (vhc) or (3,6′-dithiothalidomide) TLD (ACME Bioscience) once a day at 50 mg/kg. Treatments were performed before salicylate administration and acoustic trauma. For the salicylate treated group, a sodium salicylate solution at 10 mg/mL was administrated by intraperitoneal injection at 250 mg/kg 2 h before tinnitus analysis. All animal experiments were approved by the Comité d'Ethique en Expérimentation Animale du Languedoc-Roussillon, Montpellier, France, and the Ministère de l'Enseignement Supérieur et de la Recherche, Paris, France (D-3417223).

Acoustic trauma induction

For noise-induced tinnitus groups, bilateral acoustic trauma was induced individually by a continuous sound at 16 kHz at 116 dB for 1 h. The stimuli were delivered bilaterally in anesthetized animals using earphones previously calibrated in an acoustic box allowing homogeneous sound administration. Control group animals were anesthetized, but no acoustic trauma was administrated. This method was developed by Turner and collaborators in 2012.

Behavioral GPIAS test

Gap detection testing uses background sounds presented through one speaker and startle stimuli presented through a second speaker (Ugo Basile. Ref #48000). The floor of

the chamber is attached to a piezo transducer and provided a measure of startle force applied to the floor. Each test session begins with a 3 min acclimation period followed by three trials consisting of an abrupt startle-eliciting noise burst (115 dB SPL 50 ms duration), which serves to habituate the startle response to a more stable baseline. Each session consists of startle-only trials presented before or after gap trials, and then, startle-only trials presented before or after PPI trials, in a counter-balanced manner throughout the session. The stimuli that warn from the arrival of the startle stimulus ("Gap" for gap trials or "Prepulse" for PPI trials) begin 50 ms before the startle stimulus are 50 ms duration and are shaped with a 1ms rise/fall and at 16 kHz of frequency. Each trial is repeated 5 times during the GPIAS test (4 times per session). The interval between trials is randomized by the software to avoid the habituation of the animal and a decrease of the startle response. The maximum of the intensity of the startle response (in Newton) after the startle sound is considered.

ABR waves' analysis

ABRs are electric potentials recorded from scalp electrodes, and the first ABR wave represents the summed activity of the auditory nerve fibers contacting the inner hair cells. For ABR studies, mice were anesthetized using isoflurane, and body temperature is regulated using a heating pad at 37°C. Then, earphones were placed in the left ear of each mouse, an active electrode was placed in the vertex of the skull, a reference electrode under the skin of the mastoid bone, and a ground electrode was placed in the neck skin. The stimuli consisted of tone pips at 16 kHz and at 80 dB. ABR measures of each animal were performed individually. Evoked potentials were extracted by the signal averaging technique for each frequency, and the amplitude and latency of each wave were determined using a software and manual file confirmation. The left ears were analyzed in this study.

PAC spontaneous activity analysis

Electrophysiology recording of spontaneous spikes of the PAC neurons allows to study the increase of spontaneous activity induced by tinnitus disorder. For PAC spontaneous spikes quantification, mice were anesthetized using ketamine/xylazine mixture, and body temperature is regulated using a heating pad at 37°C. Then, the head is fixed using a stereotaxic system and three points at labeled at –1 mm, –2 mm, and –3 mm from the bregma. Three holes are performed using a microdrill of 0.9 mm on the auditory cortex area, and the dura matter is removed using small forceps. Finally, a microelectrode of 27 μm is introduced into the layer 3/4 of the PAC, and the spontaneous spikes are recorded during 40 s. The mid-frequency region was analyzed.

BDNF quantification

In ketamine/xylazine anesthetized animals, 2 mL of blood was sampled by cardiac puncture and collected in a tube containing EDTA as an anticoagulant. Samples were centrifuged for 15 min at 1000× *g* (or 3000 rpm) at 2–8°C within 30 min of collection. The supernatant (plasma) was store at –20°C. Plasma was diluted at 1/10, and BDNF quantification for each animal was performed in duplicated by ELISA method.

Cytocochleogram using succinate dehydrogenase staining

Animals were sacrificed by cervical dislocation. Then, three cochleae par group were dissected out and a succinate dehydrogenase solution containing 2.5 ml 0.2 M sodium succinate, 2.5 ml 0.2 M phosphate buffer (pH 7.6), and 5 ml blue 0.1% tetrazolium was infused through the cochlear windows. The cochleae were placed in this solution at 37°C for 1 h and then fixed with 4% paraformaldehyde solution for 24 h. The membranous and sensory spiral containing the organ of Cortis were extracted and mounted on a histological slide in glycerin and observed under light microscopy (×400).

Statistical analysis

Descriptive statistics by groups were expressed as mean ± SEM for continuous variables. Statistical significances were determined using a 2-tailed Student's *t*-test or two-way ANOVA, followed by a Bonferroni multiple comparisons *post hoc* test, allowing comparisons between groups versus control, assuming the normal distribution of the variable, and the variance homoscedasticity. Statistical analyses were performed using GraphPad Prism version 5.02 for Windows, GraphPad Software, La Jolla California USA. $P < 0.05$ was considered significant $n = 6$ animals/group.

RESULTS

TLD reduces behavioral and electrophysiological tinnitus phenotype in salicylate-induced tinnitus model

Behavioral and electrophysiological measures were performed before and 2 h after salicylate administration.

Behavioral GPIAS analysis was performed to validate the presence of tinnitus in salicylate treated mice. All salicylate-treated animals presented significant tinnitus at 16 kHz 2 h after treatment. A significant decrease in the % of startle GAP suppression was observed in the salicylate+vhc group compared to the control group. Moreover, the salicylate+TLD group also presented a significant decrease in the % of startle GAP suppression, but this decrease was statistically different from the salicylate+vhc group [Figure 1a].

Then, ABR measures were performed to analyze the amplitude and latency of the waves. At 16 kHz, salicylate+vhc animals presented a significant decrease of the wave I amplitude at 2.35 ± 0.33 μV compared to the amplitude of control animals. However, the salicylate+TLD group also presented a significant decrease of the wave I amplitude at 4.11 ± 0.42 μV compared to the control group, but this decrease was

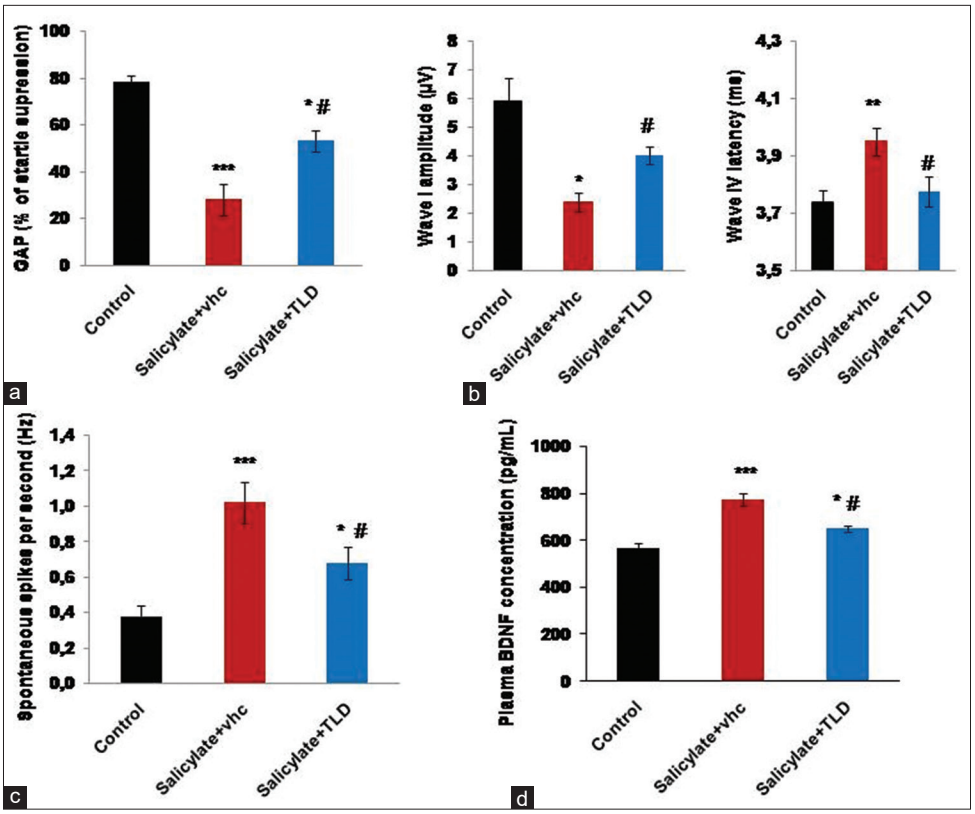

Figure 1: Electrophysiological and biochemical changes observed in the salicylate-induced tinnitus mouse model. (a) % of startle GAP suppression at 16 kHz, (b) auditory brainstem responses wave I amplitude (left panel) and wave IV latency (right panel), (c) spontaneous activity of the mid-region of the PAC, and (d) plasma brain-derived neurotrophic factor concentration of control (black bars), salicylate+vehicle mice (red bars), and salicylate+TLD (blue bars) 2 h after salicylate administration. Error bars indicate SEM. Statistical tests are repeated measures one-way ANOVA test comparing groups to control (*) or vehicle ($^{\#}$) values. $^{\#*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$. $n = 6$

statistically different compared to the salicylate+vhc group [Figure 1b left panel].

Concerning the wave IV latency, salicylate+vhc animals presented a slight, but significant increase of wave IV latency at 3.95 ± 0.05 ms compared to the latency of control animals (3.73 ± 0.05 ms). Moreover, no significant changes in wave IV latency were observed in the salicylate+TLD group compared to the control group [Figure 1b right panel].

Taken together, these results suggest that salicylate treatment induces important changes in the ABR at 16 kHz probably due to the presence of tinnitus and that TLD attenuates these electrophysiological ABR changes.

TLD decreases the spontaneous activity of the PAC and tinnitus biomarker in salicylate-induced tinnitus model

Because several publications demonstrated an increase of spontaneous spikes of the PAC in tinnitus rodent, we analyzed the spontaneous activity of the mid-region of the PAC 2 h after salicylate administration.

In this way, we observed a significant increase in the number of spontaneous spikes per second (Hertz) in the salicylate+vhc group at 1.03 spikes/s, whereas the control group presented a spontaneous activity of 0.38 spikes/s after salicylate administration. Interestingly, TLD treatment significantly reduced the number of spontaneous spikes per second in the salicylate+TLD group at 0.71 spikes/s confirming the protective role of TLD observed in our previous electrophysiological ABR data [Figure 1c].

Recent publications demonstrated an increase of BDNF marker in tinnitus patients and rodents.[8,16] In this way, ELISA analysis of plasma demonstrated a significant increase of the BDNF tinnitus biomarker at 774.6 ± 26.5 pg/mL of plasma in the salicylate+TLD group, whereas the BDNF concentration in control mice was 568.1 ± 20.2 pg/mL of plasma 2 h after salicylate administration. Moreover, TLD treatment significantly reduced BDNF plasma concentration at 610.2 ± 12.1 pg/mL in the salicylate+TLD group. Even if the BDNF concentration of the salicylate+TLD group was significantly different compared to the control group, a statistical difference was also observed compared to the vehicle group [Figure 1d].

Taken together, these data confirm that salicylate administration induced an increase of spontaneous activity of PAC and plasma BDNF biomarker, and the TLD treatment significantly attenuates these electrophysiological and biochemical changes in the salicylated-induced tinnitus model.

TLD reduces behavioral and electrophysiological tinnitus phenotype in noise-induced tinnitus model

Twenty-four h after noise trauma, ABR was analyzed to determine if the animals were sensitive to the trauma. Animals with an ABR threshold shift <20 dB (Delta = post-trauma – baseline) at 16 kHz were considered as not-sensitive to acoustic trauma and were excluded from the study. Around 20% of animals were excluded from the study.

Fourteen days, after behavioral GPIAS analysis was performed to validate the presence of tinnitus in noise traumatized mice. Our experience demonstrated that around 25% of the animals do not develop tinnitus after noise trauma. However, because we started TLD treatment before GPIAS analysis, not behavioral test exclusion was performed.

A significant decrease in the % of startle GAP suppression was observed in the noise trauma+vhc group compared to the control group. Moreover, the noise trauma+TLD group also presented a significant decrease in the % of startle GAP suppression, but this decrease was statistically different from the noise trauma+vhc group [Figure 2a].

Then, ABR measures were performed to analyze the amplitude and latency of the waves. At 16 kHz, noise trauma+vhc animals presented a significant decrease of the wave I amplitude at 2.05 ± 0.46 μV compared to the amplitude of control animals (4.97 ± 0.65 μV). However, the noise trauma +TLD group also presented a significant decrease of the wave I amplitude compared to the control group, but this decrease was statistically different compared to the noise trauma +vhc group [Figure 2b left panel].

Concerning the wave IV latency, noise trauma+vhc animals presented a significant increase of wave IV latency at 4.05 ± 0.08 ms compared to the latency of control animals (3.683 ± 0.049 ms). The TLD treated group presented a significant decrease in the latency compared to the vehicle-treated

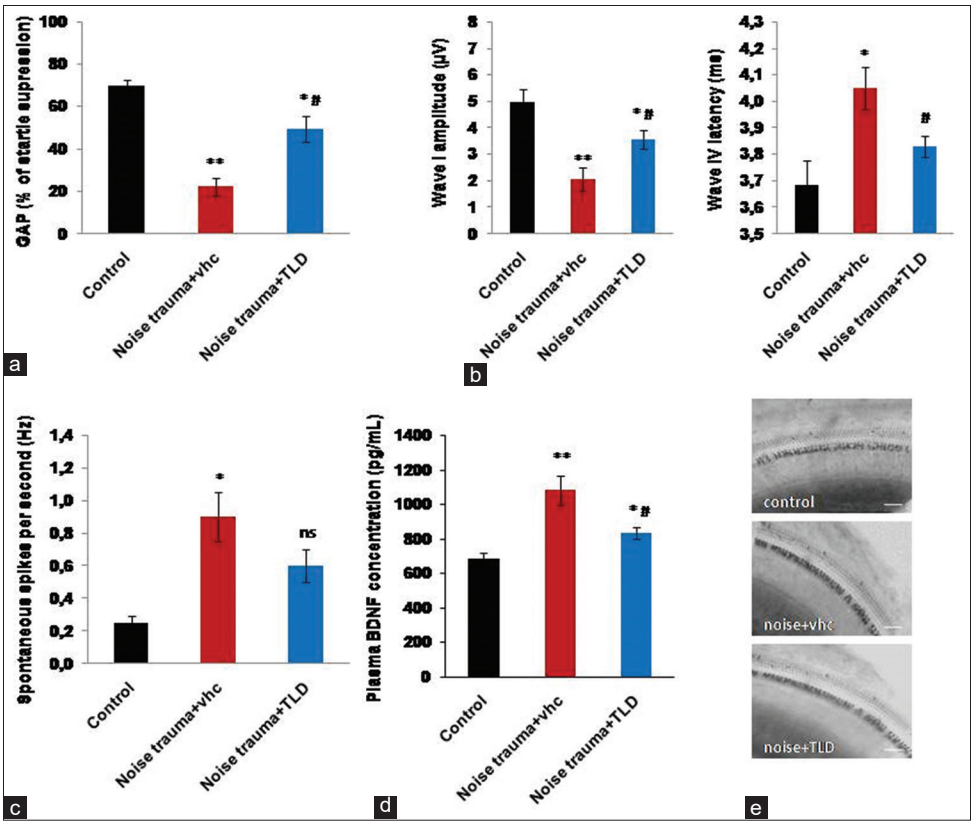

Figure 2: Electrophysiological and biochemical changes observed in the noise-induced tinnitus mouse model. (a) % of startle GAP suppression at 16 kHz, (b) auditory brainstem responses wave I amplitude (left panel) and wave IV latency (right panel), (c) spontaneous activity of the mid-region of the PAC, and (d) plasma brain-derived neurotrophic factor concentration of control (black bars), noise trauma+vehicle mice (red bars), and noise trauma+TLD (blue bars) fourteen after acoustic trauma. (e) Cytocochleogram of control, noise trauma+vehicle, and noise trauma+TLD groups 14 days after acoustic trauma. Scale bar: 100 μm. Error bars indicate SEM. Statistical tests are repeated measures one-way ANOVA test comparing groups to control (*) or vehicle (#) values. #*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$, ns: No-significant. $n = 6$

group. Moreover, no significant changes in wave IV latency were observed in the noise trauma +TLD group compared to the control group [Figure 2b right panel]. Taken together, these results suggest that acoustic trauma induces important changes in the ABR at 16 kHz probably due to the presence of tinnitus and that TLD attenuates these electrophysiological changes.

TLD decreases the spontaneous activity of the PAC and tinnitus biomarker in the acoustic trauma-induced tinnitus model

Spontaneous activity of the PAC was analyzed 14 days after acoustic trauma. We observed a significant increase in the number of spontaneous spikes per second (Hertz) in the noise trauma+vhc group at 0.9 spikes/s, whereas the control group presented a spontaneous activity of 0.25 spikes/s after salicylate administration. Moreover, as observed in the salicylate model, the TLD treatment significantly reduced the number of spontaneous spikes per second in the noise trauma+TLD group at 0.61 spikes/s [Figure 2c]. No significant differences were observed between control and noise trauma+TLD groups.

As observed in the salicylate model, the ELISA analysis of plasma also demonstrated a significant increase of the BDNF tinnitus biomarker in the noise trauma+TLD group compared to the control group 14 days after noise trauma. Moreover, TLD treatment significantly reduced BDNF plasma concentration in the noise trauma+TLD group. Even if the BDNF concentration of the noise trauma+TLD group was significantly different compared to the control group, a statistical difference was also observed compared to the vehicle group [Figure 2d].

Because noise trauma can induce cochlear hair cell damage, we performed a histological analysis of the cochlea 14 days after trauma using succinate dehydrogenase staining.

No significant differences in the number of cochlear outer and inner hair cells were observed between noise and control groups suggesting that the noise conditions did not have a deleterious effect on the hair cell and cochlear tissue [Figure 2e].

Taken together, these data confirm that acoustic trauma-induced an increase of spontaneous activity of PAC and plasma BDNF biomarker, and the TLD treatment significantly attenuates this electrophysiological and biochemical changes in the noise-induced tinnitus model.

DISCUSSION

In this study, we described the changes in ABR wave amplitude and latency, the increase of PAC spontaneous activity, and the increase of plasma BDNF biomarker presented in a salicylated-induced and acoustic trauma-induced tinnitus models. Because similar results were observed in salicylate and noise-induced tinnitus models, our data suggest that these electrophysiological and biochemical changes are directly related to tinnitus disorder.

Because it is well established that sodium salicylate administration leads to transient tinnitus in rodent and humans at 16 kHz, these results confirm that electrophysiological recording changes and plasma biomarker can be used to determine the presence of tinnitus in mouse and humans. On the other hand, several publications confirm that noise acoustic trauma leads to semi-permanent tinnitus in rodent and humans[17] confirming also that electrophysiological recording changes and plasma biomarker can be used to determine the presence of tinnitus in mouse and humans. These readouts become a robust and reproducible method to determine the efficacy of new pharmacological candidates targeting tinnitus disorder.

Wang and collaborators described that TLD administration reduced noise-induced tinnitus phenotype in mice by pharmacologically blocking TNF-α expression and preventing neuroinflammation. In this study, we reproduced Wang *et al*., publication, we observed that TLD treatment increased the % of startle GAP suppression, increased ABR wave I amplitude and decreased wave IV latency, decreased spontaneous activity of PAC, and significantly decrease plasma BDNF concentration in the salicylate-induced and noise-induced tinnitus mouse models.

Increasing evidence indicates that tinnitus and conductive hearing loss can lead to inflammatory responses, such as the activation of microglia and the release of proinflammatory cytokines,[18-20] in the early stages of the central auditory pathway. Neuroinflammation is the central nervous system's response to external and internal insults, such as infection, injury, diseases, and abnormal neural activity.[21] It mobilizes microglia to remove invading pathogens and damaged brain cells through phagocytosis.[22,23] Microglia can present target antigens from pathogens and damaged brain cells to cytotoxic T cells, which then further attack the targets.[24] Responding to insult, microglia will release proinflammatory cytokines that are involved in neural repair as well as cell death.[25] In addition to interacting with pathogens and injured cells, microglia and their released cytokines modulate the functions of normal neurons. For example, microglia play an important role in neural development, maturation, plasticity, and aging.[26-28] Proinflammatory cytokines also modulate neuronal functions such as synaptic transmission, plasticity, and membrane excitability.[29-32]

Although neuroinflammation is important in maintaining homeostasis of the central nervous system against external and internal insults, it can be detrimental if it becomes overactive or chronic.[22] Persistent neuroinflammation is a major pathological component of brain diseases such as autism,

schizophrenia, Alzheimer's disease, Parkinson's disease, multiple sclerosis, traumatic brain injury, and ischemia.[33,34] Chronic neuroinflammation resulting from noise exposure and hearing loss has been reported in the early stages of the central auditory pathway.[20] However, the impact of hearing loss-induced inflammation on neuronal function and its role in tinnitus, hyperacusis, and central auditory processing disorder has not yet been explored.

At present, there is no established treatment for patients, and it is limited to prevention and follow-up. However, some clinical trials have been carried out for the temporary threshold shift to study tinnitus, in which administration of antioxidant nutritional supplements and anti-inflammatory compounds, before moderate noise exposure showed some beneficial effects. Accumulating evidence demonstrated that free radical scavengers and inflammatory modulators may serve as effective therapeutic agents to block the activation of electrophysiological and biochemical mechanisms, leading to tinnitus disorder.

CONCLUSION

Changes in ABR wave amplitude and latency, an increase of PAC spontaneous activity, and increase of plasma BDNF biomarker were observed 2 h after salicylate administration and 15 days after acoustic trauma. Moreover, because it is well established that salicylate administration and noise acoustic trauma lead to transient and semi-permanent tinnitus, respectively, our results confirm that electrophysiological recording changes and plasma biomarker can be used to determine the presence of tinnitus in mouse and humans. These readouts become a robust and reproducible method to determine the efficacy of new pharmacological candidates targeting tinnitus disorder.

Finally, corroborating Wang *et al.* publication,[15] we demonstrated that TLD treatment attenuated tinnitus phenotype in both salicylate and noise-induced tinnitus models, suggesting that inflammatory modulators could be a promising therapy targeting tinnitus disorder in rodent and humans.

REFERENCES

1. Esmaili AA, Renton J. A review of tinnitus. Aust J Gen Pract 2018;47:205-8.
2. Puel JL, Guitton MJ. Salicylate-induced tinnitus: Molecular mechanisms and modulation by anxiety. Prog Brain Res 2007;166:141-6.
3. Sawka B, Wei S. The effects of salicylate on auditory evoked potential amplitwde from the auditory cortex and auditory brainstem. J Otol 2014;9:30-5.
4. Lowe AS, Walton JP. Alterations in peripheral and central components of the auditory brainstem response: A neural assay of tinnitus. PLoS One 2015;10:e0117228.
5. Wood NJ, Lowe AS, Walton JP. Sodium salicylate alters temporal integration measured through increasing stimulus presentation rates. Int J Audiol 2019;58:141-50.
6. Stolzberg D, Chen GD, Allman BL, Salvi RJ. Salicylate-induced peripheral auditory changes and tonotopic reorganization of auditory cortex. Neuroscience 2011;180:157-64.
7. Goto F, Saruta J, Kanzaki S, To M, Tsutsumi T, Tsukinoki K, *et al*. Various levels of plasma brain-derived neurotrophic factor in patients with tinnitus. Neurosci Lett 2012;510:73-7.
8. Yi B, Wu C, Shi R, Han K, Sheng H, Li B, *et al*. Long-term administration of salicylate-induced changes in BDNF expression and CREB phosphorylation in the auditory cortex of rats. Otol Neurotol 2018;39:e173-80.
9. Makhatadze NJ. Tumor necrosis factor locus: Genetic organisation and biological implications. Hum Immunol 1998;59:571-9.
10. Sutton ET, Thomas T, Bryant MW, Landon CS, Newton CA, Rhodin JA. Amyloid-beta peptide induced inflammatory reaction is mediated by the cytokines tumor necrosis factor and interleukin-1. J Submicrosc Cytol Pathol 1999;31:313-23.
11. Baratz R, Tweedie D, Rubovitch V, Luo W, Yoon JS, Hoffer BJ, *et al*. Tumor necrosis factor-α synthesis inhibitor, 3,6'-dithiothalidomide, reverses behavioral impairments induced by minimal traumatic brain injury in mice. J Neurochem 2011;118:1032-42.
12. Tweedie DR, Fishman K, Frankola KA, van Praag H, Holloway HW, Luo W, *et al*. Tumor necrosis factor-α synthesis inhibitor 3,6'-dithiothalidomide attenuates markers of inflammation, Alzheimer pathology and behavioral deficits in animal models of neuroinflammation and Alzheimer's disease. J Neuroinflammation 2012;9:106.
13. Greig NH, Giordano T, Zhu X, Yu QS, Perry TA, Holloway HW, *et al*. Thalidomide-based TNF-alpha inhibitors for neurodegenerative diseases. Acta Neurobiol Exp (Wars) 2004;64:1-9.
14. Tweedie D, Luo W, Short RG, Brossi A, Holloway HW, Li Y, *et al*. A cellular model of inflammation for identifying TNF-α synthesis inhibitors. J Neurosci Methods 2009;183:182-7.
15. Wang W, Zhang LS, Zinsmaier AK, Patterson G, Leptich EJ, Shoemaker SL, *et al*. Neuroinflammation mediates noise-induced synaptic imbalance and tinnitus in rodent models. PLoS Biol 2019;17:e3000307.
16. Xiong H, Yang H, Liang M, Ou Y, Huang X, Cai T, *et al*. Plasma brain-derived neurotrophic factor levels are increased in patients with tinnitus and correlated with therapeutic effects. Neurosci Lett 2016;622:15-8.
17. Galazyuk A, Hébert S. Gap-prepulse inhibition of the acoustic startle reflex (GPIAS) for tinnitus assessment: Current status and future directions. Front Neurol 2015;6:88.
18. Fuentes-Santamaria V, Alvarado JC, Lopez-Munoz DF, Melgar-Rojas P, Gabaldon-Ull MC, Juiz JM. Glia-related mechanisms in the anteroventral cochlear nucleus of the adult rat in response to unilateral conductive hearing loss. Front Neurosci 2014;8:319.
19. Baizer JS, Wong KM, Manohar S, Hayes SH, Ding D, Dingman R, *et al*. Effects of acoustic trauma on the auditory system of the rat: The role of microglia. Neuroscience 2015;303:299-311.
20. Fuentes-Santamaria V, Alvarado JC, Melgar-Rojas P,

Gabaldon-Ull MC, Miller JM, Juiz JM. The role of glia in the peripheral and central auditory system following noise overexposure: Contribution of TNF-α and IL-1β to the pathogenesis of hearing loss. Front Neuroanat 2017;11:9.
21. Graeber MB, Li W, Rodriguez ML. Role of microglia in CNS inflammation. FEBS Lett 2011;585:3798-805.
22. Brown GC, Neher JJ. Microglial phagocytosis of live neurons. Nat Rev Neurosci 2014;15:209-16.
23. Diaz-Aparicio I, Beccari S, Abiega O, Sierra A. Clearing the corpses: Regulatory mechanisms, novel tools, and therapeutic potential of harnessing microglial phagocytosis in the diseased brain. Neural Regen Res 2016;11:1533-9.
24. Gehrmann J, Matsumoto Y, Kreutzberg GW. Microglia: Intrinsic immuneffector cell of the brain. Brain Res Brain Res Rev 1995;20:269-87.
25. Simon DW, McGeachy MJ, Bayir H, Clark RS, Loane DJ, Kochanek PM. The far-reaching scope of neuroinflammation after traumatic brain injury. Nat Rev Neurol 2017;13:572.
26. Wolf SA, Boddeke HW, Kettenmann H. Microglia in physiology and disease. Annu Rev Physiol 2017;79:619-43.
27. Salter MW, Beggs S. Sublime microglia: Expanding roles for the guardians of the CNS. Cell 2014;158:15-24.
28. Allen NJ, Barres BA. Signaling between glia and neurons: Focus on synaptic plasticity. Curr Opin Neurobiol 2005;15:542-8.
29. di Filippo M, Sarchielli P, Picconi B, Calabresi P. Neuroinflammation and synaptic plasticity: Theoretical basis for a novel, immune-centred, therapeutic approach to neurological disorders. Trends Pharmacol Sci 2008;29:402-12.
30. Stellwagen D, Malenka RC. Synaptic scaling mediated by glial TNF-alpha. Nature 2006;440:1054-9.
31. Steinmetz CC, Turrigiano GG. Tumor necrosis factor-α signaling maintains the ability of cortical synapses to express synaptic scaling. J Neurosci 2010;30:14685-90.
32. Bellinger FP, Madamba S, Siggins GR. Interleukin 1 beta inhibits synaptic strength and long-term potentiation in the rat CA1 hippocampus. Brain Res 1993;628:227-34.
33. Kemanetzoglou E, Andreadou E. CNS demyelination with TNF-α blockers. Curr Neurol Neurosci Rep 2017;17:36.
34. Karpenko MN, Vasilishina AA, Gromova EA, Muruzheva ZM, Bernadotte A. Interleukin-1β, interleukin-1 receptor antagonist, interleukin-6, interleukin-10, and tumor necrosis factor-α levels in CSF and serum in relation to the clinical diversity of Parkinson's disease. Cell Immunol 20183;27:77-82.

How to cite this article: Gonzalez-Gonzalez S, Cazevieille C. 3,6'-Dithiothalidomide Reduces Tinnitus Phenotype in Two Different Mouse Preclinical Models of Tinnitus. J Community Prev Med 2020;3(1):1-8.