Comparing the otoprotective effects of two polyphenols, ferulic acid and caffeic acid, in the experimental model of noise-induced hearing loss.

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Abstract
Noise-induced hearing loss is one of the most common acquired sensorineural hearing loss, and it is due to metabolic damage in cochlear structures, leading to increased oxidative stress and cell death with permanent loss of function. Thus, addressing the possibility to counteract redox imbalance by using exogenous antioxidant, such as dietary polyphenols, is challenging. In this study we compared the otoprotective effect of two polyphenol compounds, caffeic acid and ferulic acid, against noise-induced hearing loss cochlear damage.
Thus, we used an experimental model of acoustic trauma by exposing Wistar rats to acute noise. Animals were treated with 30 mg/kg of CA and 300 mg/kg of FA in a peri-traumatic window before and after noise exposure. Electrophysiological recording of auditory brainstem nuclei was used to evaluate hearing sensitivity. Morphological and immunofluorescence analyses were used to study the effect of antioxidant supplementation in cochlear structures. Our data demonstrated that both caffeic acid and ferulic acid showed a similar otoprotective effect at functional and morphological level, by attenuating threshold shift values and reducing hair cell death after noise exposure. However, analyses of lipid peroxidation in cochlear specimens indicate a best neuroprotective effect of ferulic acid against lipid peroxidation induced by noise, especially in spiral ganglion neurons. Collectively, our data suggest that antioxidant supplementation with polyphenols can be effective against noise and that comparing caffeic acid and ferulic acid, the last molecule can be more efficient in counteracting neuronal damage, with the possibility to prevent synaptopathy or neuropathy consequences of noise-induced hearing loss.

Keywords: *acoustic trauma, antioxidants, oxidative stress, lipid peroxidation*

1. INTRODUCTION

Among acquired hearing disabilities, Noise-Induced Hearing Loss (NIHL) represents the most common form of acquired sensorineural hearing loss in adult population worldwide (Nelson et al., 2005; Fetoni et al., 2019; Paciello et al., 2023). Although occupational noise has been the most frequently studied type of noise exposure, research focus has extended to social and environmental noise and the hearing disability concern has been widened to a range of no-auditory health effects (sleep disorders, impairment of cognitive performance, cardiovascular diseases etc.) (Basner et al., 2014). Experimental studies allowed to detect the main characteristic features of NIHL, including hearing threshold

elevation and loss of the not re-generable auditory sensory cells in the cochlea, evaluated by using non-invasive electrophysiology techniques (i.e., otoacoustic emissions and auditory brainstem responses-ABR) as well as by morphological studies. About molecular mechanisms underlying cochlear damage induced by noise exposure, consensus has been gained on the relevant role played by failure of redox homeostasis (excess of mitochondrial reactive oxygen species-ROS), inflammatory processes (Frie et al., 2019; Kalinec et al., 2017) and hypoxia/blood flow reduction (Henderson et al 2006, Fetoni et al., 2019; Paciello et al., 2023), similarly to what occurs in ototoxicity (Fetoni et al., 2022) and age-related hearing loss (Bielefeld et al., 2010; Fetoni et al., 2011; Fujimoto and Yamasoba, 2014, Alvarado et al., 2015).

The maintenance of intracellular redox homeostasis, that is crucial to counteract increased ROS amount, is dependent on the activity of the endogenous antioxidant system (Birben et al., 2012). However, because of the imbalance in production of free radicals and endogenous antioxidant enzyme activity, ROS concentrations may increase and become toxic, causing oxidative stress-induced cell damage (Halliwell, 2006; Sena and Chandel, 2012; Böttger and Schacht, 2013; Fujimoto and Yamasoba, 2014). Specifically, the hair cells are particularly vulnerable to the damage induced by ROS, due to their high metabolic activity (Maulucci et al., 2014; Paciello et al., 2023).

In line with these considerations, finding molecules able to potentiate the endogenous antioxidant system to counteract ROS overexpression in NIHL is challenging, also considering that no FDA approved drugs are available for treating this hearing impairment. Currently, there is a growing interest on the potential benefits of natural products or nutraceuticals and a significant body of evidence has indicated that polyphenols may exert antioxidant properties, attenuating oxidative stress and inflammation (Fetoni et al., 2014; Fetoni et al., 2015; Hussain et al., 2016; Fetoni et al., 2019). Polyphenols are natural products, found in plant-based foods, including several compounds with phenolic structural features (Hussain et al., 2016; Fraga et al., 2019). Among polyphenol compounds, we focused on two antioxidant molecules, ferulic acid (FA) and caffeic acid (CA). These compounds showed physiological and pharmacological properties that include antiviral, antioxidant, anti-inflammatory, anticarcinogenic, immunomodulatory, antidiabetic, cardioprotective, antiproliferative and hepatoprotective activity (Gulcin et al., 2006; Fetoni et al., 2010; Kumar and Pruthi, 2014; Espíndola et al., 2019). The aim of this study was to compare the otoprotective efficacy of these two phenolic compounds in an experimental animal model of NIHL, based on their ability to counteract cochlear oxidative stress, in order to gain more insights on the effectiveness of antioxidant therapy, in a translational point of view.

2. MATERIAL AND METHODS

2.1. Animals

Male adult Wistar rats of 3 months of age were used. The auditory function of each animal was tested for the presence of Preyer's reflex. The experiments were performed on a total of 35 animals, randomized and assigned to six experimental groups as follows: 1) normal hearing rats used as control (Ctrl; n = 5); 2) control animals treated with FA (CtrlFA; n = 5); 3) control animals treated with CA (CtrlFA; n = 5); 4) animals exposed to noise (Noise; n = 5); 5) animals exposed to noise and treated with FA (Noise+FA; n = 5); 6) animals exposed to noise and treated with CA (Noise+CA; n = 5); 6) noise+DMSO (Noise+DMSO; n = 5). In a preliminary study, 15 additional animals were used to determine the most protective dose of FA and CA (dose/response curve). All animals were sacrificed under deep anesthesia (Ketamine at dose 20 mg/kg and Medetomidine-Domitor at dose of 20 mg/kg) at day 7 after noise exposure. For the whole experimental period, the animals were housed two per cage at controlled temperature (22-23°C) and constant humidity (60 ± 5%), under a 12-h light/dark cycle, with food (Mucedola 4RF21, Italy) and water *ad libitum*. All efforts were made to minimize animal suffering and to reduce their number, in accordance with the European Community Council Directive of 24 November 1986 (86/609/EEC). All procedures were performed in compliance with the Labo-

ratory of Animal Care and Use Committee of the Catholic University, School of Medicine of Rome and were approved by the Italian Department of Health (*Ministero della Salute*).

2.2. Drug administration

Caffeic Acid (CA) (Cod.205546-500MG, Merk Millipore) was dissolved in Dimethyl-Sulfoxide (DMSO), which was used as a vehicle. The stock solution was diluted to 0.02 mg/ml in DMSO. The diluted solution was prepared freshly daily and administered intraperitoneally (i.p.). Ferulic acid (FA) (Cat. No. 12,870-8, Sigma-Aldrich, St. Louis, MO, USA) was diluted in DMSO, prepared freshly daily, and administered i.p.

Antioxidant solutions (containing FA or CA) were injected in a peri-traumatic window, such as 1 hour before noise exposure and once daily for the following three days. The animals of Ctrl+CA and Ctrl+FA group received a similar drug treatment for a total of 4 days with no noise exposure, while the Ctrl+DMSO animals were treated with 0.3 ml DMSO with the same experimental procedure. In all experiments, rats exposed to vehicle (Ctrl+DMSO) and animals treated with CA or FA (Ctrl+CA or Ctrl+-FA) alone, did not show any significant change in ABR evaluations compared to Ctrl animals. Similarly, no ABR differences were detected between Noise and Noise+DMSO groups.

2.3. Noise exposure

As described in previous papers (Fetoni et al., 2013; Paciello et al., 2018; Paciello et al., 2021), the acoustic trauma was induced by a continuous pure tone of 10 kHz generated by a waveform generator (LAG-120B, Leader, NY, USA) and amplified by an audio amplifier (A-307R, Pioneer, CA, USA). Under anesthesia, all animals were placed in a sound-proof room in a fixation cradle with their head gently maintained in a fixed position by a neck and a nose ring and exposed for 60 min to a 120 dB SPL sound, which was presented to the ears in free field via loudspeakers (TW034X0, Audax, France) positioned at a distance of 10 cm in front of the animal's head. Sound level was measured using a calibrated 1/4 in. microphone (Model 7017, ACO Pacific Inc., Belmont, CA, USA) and a calibrated preamplifier (Acoustic Interface System, ACO Pacific Inc).

2.4. Electrophysiological measurements of auditory function.

Hearing function was evaluated in all animals by measuring auditory brainstem responses at low (6 kHz), mid (12, 16, 20 kHz) and high (24, 32 kHz) frequencies. ABRs were assessed mono-laterally prior to noise exposure to assure normal hearing and reassessed 1, 3 and 7 days after noise exposure. Animals were mildly anesthetized (Ketamine, 10 mg/Kg body weight and Medetomidine-Dormitor 10 mg/Kg body weight) and placed in the anechoic room. As described previously (Fetoni et al., 2016; Paciello et al., 2018) three electrodes were subcutaneously inserted into the right mastoid (active), vertex (reference) and left mastoid (ground). A PC-controlled TDT System 3 (Tucker– Davis Technologies, Alachua, Florida, USA) data acquisition system with real time digital signal processing was used for ABR recording and auditory stimulus generation. Tone bursts of pure tones from 6 to 32 kHz (1 ms rise/fall time, 10 ms total duration, 20/s repetition rate) were presented monaurally in open field. Responses were filtered (0.3–3 kHz), digitized, and averaged across 500 discrete samples at each frequency-level combination. The threshold value was defined as the lowest intensity able to evoke an appropriate ABR response (Fetoni et al., 2013), whereas the threshold shift was obtained calculating the difference between ABR measurement before and after noise exposure. Animals were sacrified after the last ABR recording (day 7).

2.5. Morphological analysis: F-actin staining.

F–Actin staining was used to visualize the stereociliary arrays and cuticular plates of hair cells at day 7. This immunofluorescence staining allows as to detect hair cells in whole mount preparations of the organ of Corti to estimate cell survival. As described previously (Paciello et al., 2020), the removed cochleae were fixed with 10% buffered Formalin for 4 h. After removal of the bony capsule and the lateral wall tissues, the epithelium of the organ of Corti was separated from the bony modiolus and dissected in half-turns in 0.1 M PBS under a dissecting microscope. Surface preparations of the organ of Corti were incu-

bated with a solution containing ActinGreen 488 Ready Probes Reagent (Cat. No. R37110, Thermo Fisher, Waltham, MA, USA) in 0.1 M PBS for 30 min at room temperature protected from light. Positive cells were counted in all cochlear turns (apical, middle and basal). Hair cells were considered missing if both the stereocilia bundles and the cuticular plates were absent, and outer hair cell (OHC) loss was calculated as percentage with respect to controls. All morphologic observations were performed with the aid of the confocal laser scanning system (Nikon Ti-E, Confocal Head A1 MP, Tokyo, Japan).

2.6. Immunofluorescence analyses

The expression of 4-HNE was detected in order to evaluate lipid peroxidation, a well-known consequence of increased oxidative stress (Ayala et al., 2014). As described in a previous works (Fetoni et al., 2013; Fetoni et al., 2015), cochlear cryosections of 12 mm, obtained by using a cryostat (SLEE) were incubated with a blocking solution containing 1% fatty acid-free bovine serum albumin (BSA), 0.5% Triton X-100 and 10% rabbit serum in PBS for 1 h at room temperature. The specimens were incubated overnight at 4 °C with a solution containing rabbit polyclonal anti-4-HNE primary antibody (rabbit anti 4-HNE antiserum, Cat#HNE11s, Alpha Diagnostics, TX, USA) diluted 1:100 in PBS. The rabbit anti-4-HNE antibody cross-reacted with the rat tissue. At the end of incubation, all specimens were washed twice in PBS, and incubated at room temperature for 2 h, protected from light, in labelled conjugated goat anti-rabbit secondary antibody (Alexa Fluor 488, IgG, Invitrogen) diluted 1:400 in 0.1 M PBS and then washed in PBS. The specimens were double stained with DAPI (blue fluorescence) diluted 1:1000 in PBS for 20 min at room temperature and protected from light. DAPI labelling was used to identify condensed hair cell nuclei. The slides were cover slipped with an antifade medium (ProLong Gold, Invitrogen P36930). Images of immunolabelled specimen were taken by a confocal laser scanning system (Nikon Ti-E, Confocal Head A1 MP, Tokyo, Japan). 4-HNE positive cells were identified by green fluorescence scattered over the length of the organ of Corti. As negative controls, the primary antibodies were omitted during processing of tissues randomly selected across experimental groups. Stain was absent from the negative control specimens, indicating absence of spontaneous fluorescence and non-specificity of the secondary antibody. Tissues from experimental groups were always processed together in pairs during immunostaining procedures to limit variables relate to antibody penetration, incubation time, and post sectioning age/condition of tissue. Analysis of fluorescence intensity signals were obtained by measuring optical density in the main cochlear regions (organ of Corti, spiral ganglion neurons and stria vascularis) by using Image J software.

2.6. Statistical analyses

Results are presented as means ± standard error of the mean (SEM) and differences were assessed by using variance analysis (ANOVA). Post-hoc comparisons were assessed with Tukey's test (Statistica, Statsoft, Tulsa, OK, USA). A p value <0.05 was considered significant.

3. RESULTS

3.1. CA and FA attenuate hearing loss induced by noise exposure.

To determine the protective effect of polyphenol administration against cochlear damage induced by noise exposure, ABR recordings were measured before and after 1, 3 and 7 days from acoustic trauma. In the Noise group, the average threshold shift increased remarkably, reaching about 45-50 dB at 12-24 kHz at day 1 (Fig. 1A). Progressively an attenuation of about 5-10 dB was observed at day 3 at all frequencies (Fig. 1A). At day 7, we observed a further attenuation of about 5-10 dB (Fig. 1A). Namely, at day 7 finally threshold shift in the Noise group was 30-35 dB at all frequencies.

Regarding the study of CA otoprotection, we obtained a dose-response curve by testing different doses of CA administration (15, 30 and 50 mg/kg) at 1, 3 and 7 days after noise exposure (Fig. 1B). We found that the best auditory protection was reached at the dose of 30 mg/kg in the Noise+CA group. Indeed,

compared to noise-exposed animals, ABRs values was about 30-35 dB at day 1 (Fig.1D) and decrease of about 5-10 dB at day 3 (Fig. 1E). Lastly, at day 7, finally threshold shift values reaching about 20-25 dB (Fig. 1F).

Animals treated with different doses of FA showed a similar trend. Our dose/response curve (Fig. 1C) indicates that the best protection was achieved when FA was used at a concentration of 300 mg/kg. Indeed, Noise+-FA animals showed a threshold shift of about 35-40 dB at day 1 (Fig. 1G), 25-30 dB at day 3 (Fig. 1H) and of 20-25 dB at day 7 (Fig. 1I). Thus, overall, the antioxidant protection of FA at day 7 was of about 20 dB.

In all experiments, Ctrl+DMSO, Ctrl+FA and Ctrl+CA animals did not show any significant change at any ABR frequencies with respect to Ctrl animals. Similarly, rats exposed to Noise+DMSO did not show any significant changes in threshold values with respect to Noise group (data not shown). Taken together, these results that both CA and FA significantly decrease threshold shift values induced by noise exposure, with an overall protection of about 20 dB at all frequencies analyzed.

3.2. Antioxidant effect of FA and CA supplementation on hair cell survival.

In order to evaluate inner hair cell (IHC) and outer hair cell (OHC) survival, F-actin staining was performed (Fig. 2). In the Ctrl animals, was shown the normal structural organization of surface preparation of organ of Corti, which was characterized by an orderly arrangement of the three rows of OHCs and one row of IHCs (Fig. 2E). In Noise group cell loss was characterized by dark spots (asterisks), damage to stereocilia hair bundles, phalangeal scars, and disappearance of cuticular plate (Fig. 2F) indicating that the hearing loss is likely related to functional impairment of these cells than their disappearance. Moreover, quantitative analysis suggests that noise exposure damage involved mainly OHCs of the outer row and only few IHCs were lost (Fig. 2A-D). Specifically, OHCs percentage of survivor was about 70% in the middle turn, 80% in the basal turn and 95% in the apical turn (Fig. 2A-D). In the Noise+CA group, OHC loss was significantly reduced in middle and basal turn as compared to Noise group (Fig. 1C,G). Similar results were obtained in Noise+FA group (Fig. 2D,H).

3.3. FA and CA ability to counteract increased lipid peroxidation after noise exposure.

4-HNE immunofluorescence was used, in order to quantify lipid peroxidation production in the main cochlear structures: organ of Corti, spiral ganglion neurons (SGNs) and stria vascularis (Fig. 3). In control slices we observed a very faint green fluorescence, indicating very low level of lipid peroxidation, as expected (Fig. 3A-C). Otherwise, 4-HNE expression increased markedly 7 days after noise trauma, and high level of green fluorescence was found in the stria vascularis (Fig. 3D), SGNs (Fig. 3E) and in the organ of Corti (Fig. Fig.3F). CA administration significantly reduced lipid peroxidation enhancement in all cochlear structures (Fig. 3G-I), although a best protective effect was found in the stria vascularis and in the organ of Corti (Fig. 3G,I), and a lower protective effect was observed in SGNs (Fig. 3H). FA treatment showed the same tend, counteracting lipid peroxidation (Fig. 3J-L). However, FA protective effects seems to involve all cochlear structures, including SGNs, where 4HNE expression was significantly reduced with respect to both Noise and Noise+CA groups (compare panels E, H and K in Figure 3). These results were also confirmed by fluorescence signal quantification (Fig. 3M).

4. Discussion

The aim of our study was to compare the otoprotective effect of two polyphenol compounds, CA and FA, in order to gain more insights on the effectiveness of antioxidant therapy that represent a still challenge in clinical practice. Our results demonstrate that: 1) acute noise exposure induced a high increase of threshold shift; 2) this damage involves specifically OHCs, and caused an increase of lipid peroxidation in all cochlear structures; 3) antioxidant treatment with CA attenuated threshold shift and hair cell damage, counteracting cochlear lipid peroxidation, specifically in the organ of Corti and stria vascularis; 4) FA

showed a protective effect in reducing auditory thresholds and hair cell death and it had antioxidant ability in attenuating lipid peroxidative damage in all cochlear structures (organ of Corti, stria vascularis and SGNs).

Collectively, our data indicate that both CA and FA are effective in counteracting NIHL, however, FA showed a greatest neuronal protective property, ensuring a best protection of SGNs against lipid peroxidation.

It is known that preserving neuronal cochlear compartment is crucial to attenuate hearing loss. Indeed, despite the loss of hair cell, that is an irreversible event, is responsible for a permanent loss of the hearing sensitivity (Spoendlin, 1985), the damage of SGNs is also a key factor, which can occur slower after noise insult (Johnsson, 1974). Moreover, several studies demonstrated that synapses between neuronal afferent fibers and hair cells are damaged before the hair cells were affected in the acute phase of noise exposure (Kujawa and Liberman, 2009; Liberman, 2017). This can lead to a condition named "hidden hearing loss", characterized by synaptopathy (loss of hair cell/afferent fibers synaptic contacts), associated with increased Ca2+ influx and glutamate excitotoxicity (Moser et al., 2013; Fetoni et al., 2019). Clinically, the consequence of synaptopathy is a poor speech recognition ability, usually observed in patients exposed to noise (Bharadwaj et al., 2015).

The "free radical theory" is currently recognized by the scientific community to be the main mechanism underlying cochlear damage after noise exposure. Indeed, due to their high oxygen intake, high lipid content, high number of mitochondria, and high energy necessity, both neurons and hair cells are particularly vulnerable to damage caused by ROS. These is also the reason why oxidative stress has been proposed to be the pathogenic mechanism of sensorineural hearing loss (Henderson et al., 2006), as well as of diseases involving neuronal damage, also in the central nervous system (Uttara et al., 2009; Paciello et al., 2023). In our study we found, as expected, an increase of lipid peroxidation in noise samples, indicating a high level of oxidative stress. Antioxidant supplementation with both CA and FA reduced significantly the expression of the marker of lipid peroxidation, 4-HNE. Thus these data are in line with our previous findings demonstrating that the two selected polyphenols attenuated hearing threshold elevation and in parallel, interacting with ROS signaling pathways, induced adaptive stress responses by up-regulating nuclear translocation of Nrf-2 and also exhibited anti-inflammatory properties decreasing expression of typical markers of inflammatory pathways NF-κB and IL-1β. (Paciello et al., 2020; Paciello et al., 2020b).

However, interestingly, FA showed a best protective effect against neuronal oxidative stress, whit a greater antioxidant efficacy in SGNs. In line with the above considerations, our results suggest that the use of FA can be more effective in counteracting synaptopathy and neuropathy that are key consequences of hearing loss. We know that increased peroxidative damage in hair cells starts very early after acute noise exposure, also causing lipid membrane destructuration and decreasing membrane fluidity, thus impairing the electromotility properties of OHC membrane (Maulucci et al., 2014). Our data demonstrate that both CA and FA exert a significant effect in reducing lipid peroxidation in the organ of Corti. However, the kinetic of lipid peroxidation in neuronal compartment can be slower, in association with the slower damage that is usually observed in SGNs, compared to hair cells, after noise damage (Johnsson, 1974). Thus, we can speculate that the greater antioxidant effect of FA on neurons can be related to a different kinetic of action of this drug, ensuring a best protection over time. Indeed, the neuroprotective effect of FA and its ability to attenuate lipid peroxidation has been widely supported by both *in vitro* and *in vivo* studies in several models of neuronal damage (Kanski et al., 2022; Catino et al., 2016; Ren et al., 2017; Davis et al., 2018; Meng et al., 2018).

Accordingly, with our previous results in the model of cisplatin ototoxicity, polyphenols can act as a double-edge sword in supplement use exhibiting both anti-oxidant and pro-oxidant effects; also showing different mechanisms of action depending on cell context and dosage. Herein, we demonstrate that in this context of cochlear toxicity FA responded more linearly at different dosage as shown in the dose response curve with respect to the U-shaped model exhibited by CA. Thus, more

studies are desirable to better understand this critical point for clinical translation (Paciello et al 2020).

Taken together, our data document a similar efficacy of the use of both CA and FA in counteracting NIHL, supporting the idea of the use of polyphenols is useful in treating hearing impairment. However, our molecular analyses revealed a best neuronal protection of FA compared with CA, suggesting that choosing this polyphenol could be more effective in counteracting synaptopathy and neuropathy conditions occurring in NIHL.

On a translational point of view, more studies are needed to better understand the kinetic of action of antioxidants and to better understand how to overcome the limit of antioxidant use in clinical practice, including dosage and bioavailability.

Conflict of interest: No other relationships/conditions/circumstances that present potential conflict of interest

Figures

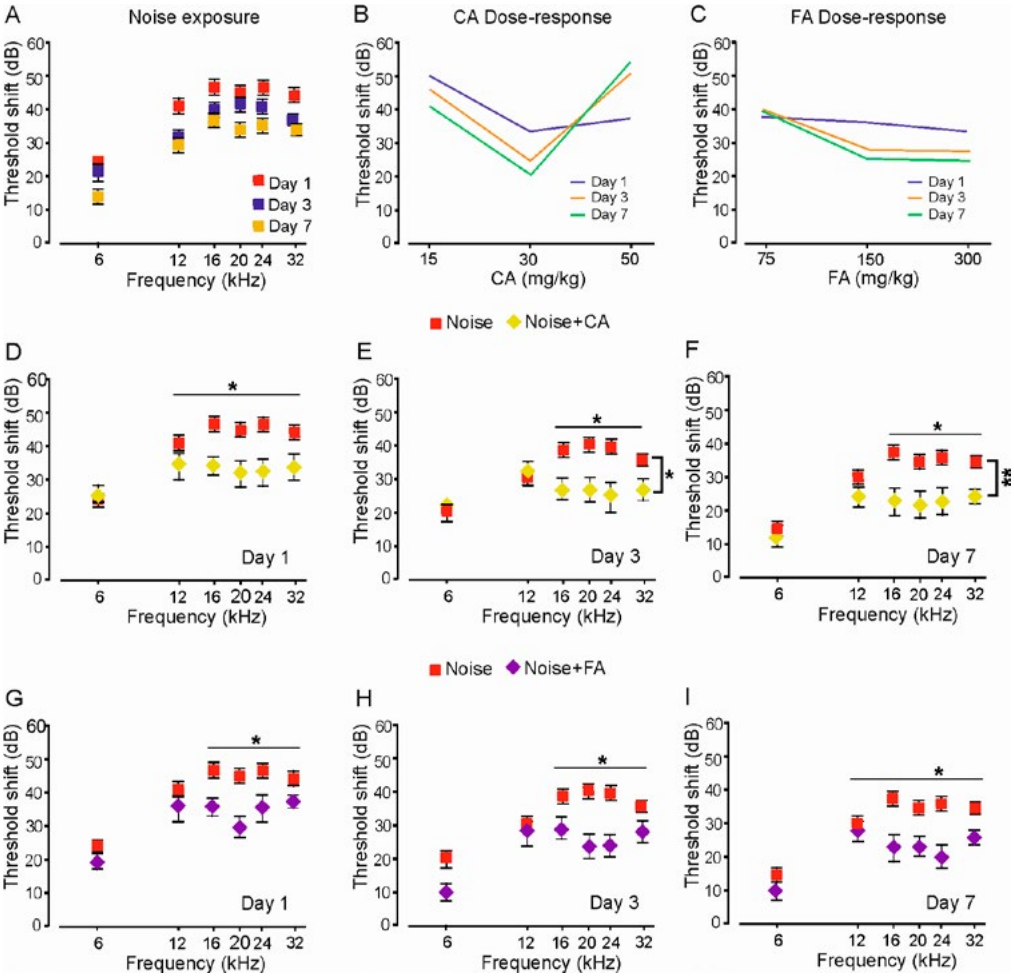

*Figure 1. Polyphenol administration attenuates hearing loss in noise exposed-animals. A: Graph (mean ±SEM) represent threshold shift (the difference between threshold values before and after noise exposure) in noise-exposed animals at different time points. B-C: Dose-response curves showing CA (B) and FA (C) best dosage in counteracting noise-induced hearing loss. D-F: Graphs (mean ±SEM) showing threshold shift of noise-exposed animals compared to animals exposed to noise and treated with CA. Threshold shift of animals supplemented with the antioxidant (Noise+CA) was significantly lower compared to Noise group at all time points analyzed. G-I: Graph (mean ±SEM) showing threshold shift of noise-exposed animals compared to animals exposed to noise and treated with FA. Animals of Noise+FA group showed a lower threshold shift with respect to noise-group, indicating a significant protection at all time points. * p< 0.01*

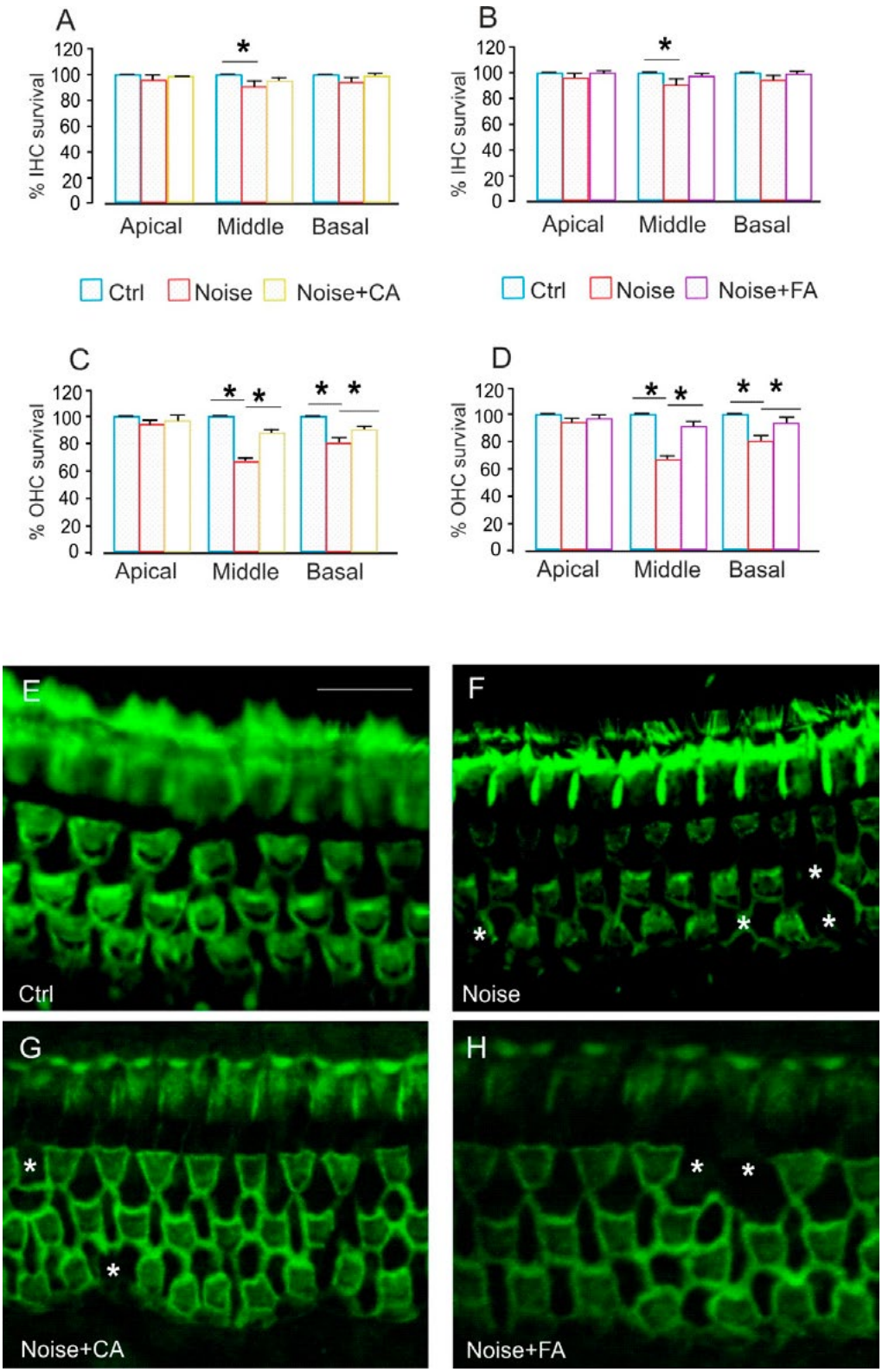

Figure 2. Antioxidant supplementation of CA and FA counteract hair cell damage.
*A-D: Histograms (mean ±SEM) show inner hair cells (IHCs; A, B) and outer hair cells (OHCs; C, D) survival at day 7 in all experimental groups. E-H: Representative images of surface preparations of the organ of Corti stained with F-Actin, showing the basilar membrane with one row of IHC and the three rows of OHCs in Ctrl (E), Noise (F), Noise+CA (G) and Noise+ FA (H) groups. Asterisks indicate missing cells. Scale bar: 20 mm. * p< 0.01.*

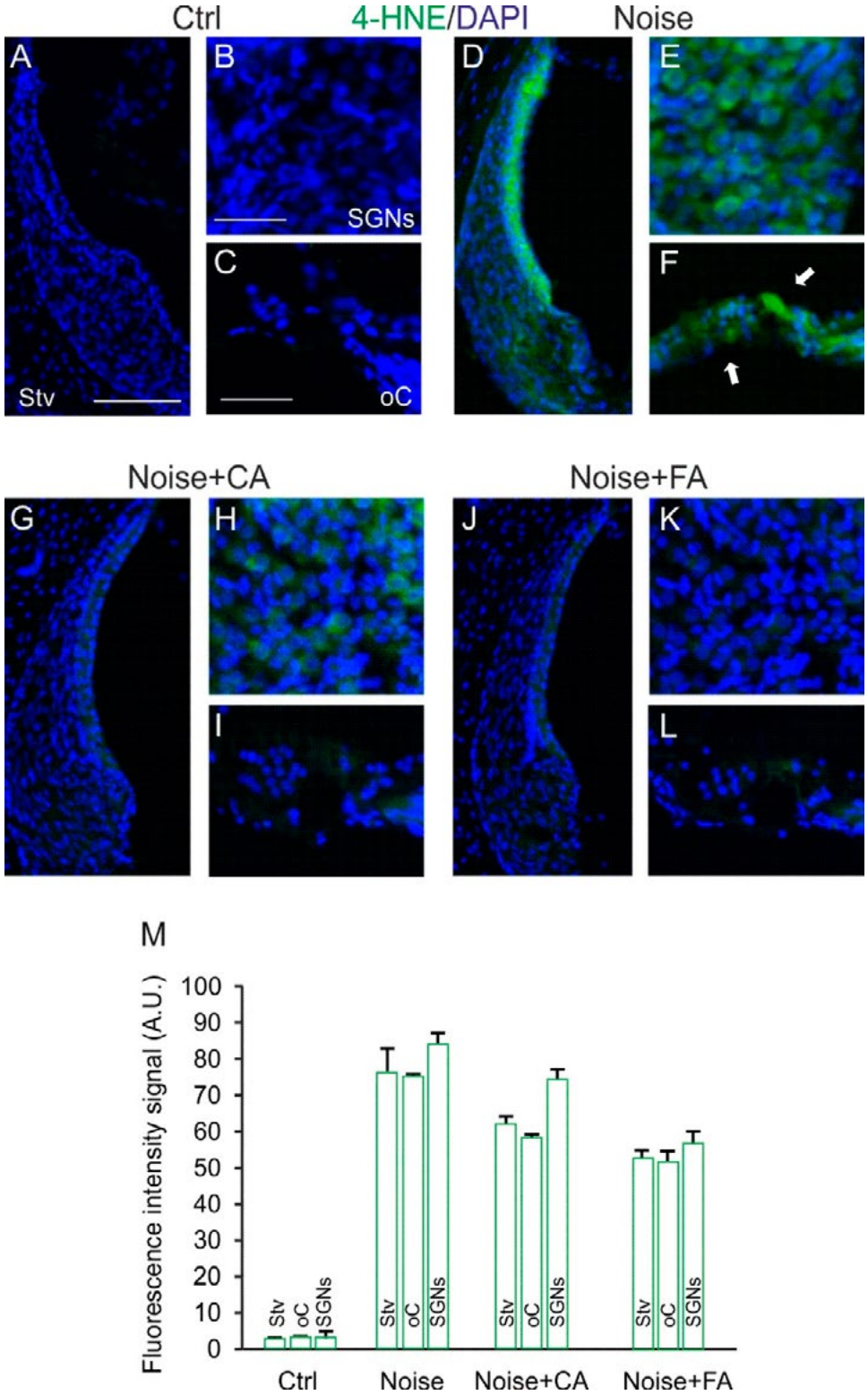

Figure 3. CA and FA protective effect against lipid peroxidative damage in cochlear structures. A-L: Representative images of cochlear cryosections showing the principal cochlear structures: Stria vascularis (Stv; A,D,G,J), spiral ganglion neurons (SGNs; B,E,H,K) and the organ of Corti (oC; C,F,I,L) immunolabeled with an antibody against 4-HNE in green to detect lipid peroxidation and DAPI stained in blue to visualize cell nuclei in all experimental groups. M: Histograms (mean ±SEM) show a semi-quantitative analyses of fluorescence intensity signals in the three cochlear structures in all experimental groups. Scale bar: A, 100 mm, B-C, 20 mm.

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