RESEARCH WORK

Functional, Histological and Molecular Characterization of a Preclinical Mouse Model of Age-Related Neuropathy, Age-Related Hearing Loss and Age-Related Blindness

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ABSTRACT

Introduction and aim: Hearing loss, blindness and neuropathy are common sensory impairment in aging population. The senescence-accelerated prone strain 8 (SAMP8) mouse model have been identified as suitable for use as models in senescence research. This strain has been widely used in aging research to study phenotypes such as peripheral neuropathy, hearing loss, immune dysfunction, osteoporosis, blindness and brain atrophy. The aim of this study was to perform a functional and histological characterization of the SAMP8 mice at different ages in order to validate this mouse as a robust and reproducible model for preclinical research. **Material and methods:** We applied behavioral tests, electromyogram, ABR, DPOAE and electroretinogram to determine the sensory and neuromuscular performances at 1, 3 and 5 months old. Moreover, we performed histological analysis of sciatic nerve, cochlea and retina to determine the degeneration of the sensory system from a histological point of view. **Results:** We observed hearing impairment, cochlear hair cells loss, neuromotor disorders, visual dysfunction, retinopathy and increase of inflammatory biomarkers observed in SAMP8 mice at 5 months old. Moreover, N-acetylcysteine treatment allows delaying the senescence process by slowing the neuromotor impairment, the age-related hearing loss, protecting the cochlear hair cells and improving visual acuity. **Conclusion:** Here we demonstrated that SAMP8 mice present accelerated senescence characterized by peripheral neuropathy, hearing loss and blindness from 3 months old and we show that chronic administration of N-acetylcystine (NAC) reduced the analyzed sensory age-related disorders in SAMP8 mice.

Key words: Neuropathy, Aging, Hearing loss, Retinopathy, Senescence.

INTRODUCTION

Age-related peripheral neuropathy, age-related hearing loss and age-related retinopathy are common sensory disorders in the elderly population (12, 3).

Age-related neuropathy (ARN) is one of the challenging diagnoses encountered by a neurologist with an estimated prevalence of 2–8% in the general population. The incidence of peripheral neuropathy increases with age, commensurate with 'ageing' of the peripheral nervous system and the high prevalence of systemic disorders like diabetes mellitus. Briefly, age-related peripheral neuropathy, a result of damage to the nerves located outside of the brain and spinal cord (peripheral nerves) induced by senescence, often causes weakness, numbness and pain, usually in the hands and feet. It can also affect other areas and body functions including digestion, urination and circulation. In humans, the etiology of neuropathy

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DOI: 10.33309/2638-7719.040203

in elderly is varied, and the leading causes include vasculitis, diabetes, alcohol and nutritional deficiencies. The diagnostic work up needs to be tailored to each individual patient based on the bedside history and examination, topographic pattern and evolution of clinical symptoms and signs.

Concerning the hearing impairment, Age-related hearing loss (ARHL), or presbycusis, is the most common sensory impairment (1). According to the National Institute on Deafness and Other Communication Disorders (NIDCD), age-related hearing loss affects 1 in 3 people between the ages of 64 and 74. By age 75, that figure rises to approximately 50%. Moreover, several publications suggest that ARHL affect communication leading to social isolation, depression, anxiety and reduced physical and psychological wellbeing. Moreover, even if several studies established an association between deafness induced by aging and oxidative stress strongly suggesting a role of the antioxidants in age-related hearing loss.

The senescence-accelerated prone strain 8 (SAMP8) mouse model is a strain derived from AKR/J mice, selected for a phenotype toward either accelerated senescence allowing to study gerontological disorders (22, 8, 23). This strain presents many of the hallmarks found in neurodegenerative processes, as impairments in learning tasks, altered emotions, abnormality of the circadian rhythm (18), spongy degeneration (24), neuronal cell loss (9), and gliosis in the brain (19). Moreover, SAMP8 mice have been widely used in aging research to study phenotypes such as peripheral neuropathy, hearing loss, immune dysfunction, osteoporosis, blindness and brain atrophy (16). Moreover, it has been reported that the premature SAMP8 senescence causing early presbycusis was linked to altered levels of antioxidant enzymes and decreased activity of complexes I, II, and IV, which in turn lead to chronic inflammation and triggering of apoptotic cell death pathways. This molecular mechanism displays sequential degeneration of outer hair cells (OHCs), spiral ganglion neurons, striavascularis and ultimately inner hair cells (IHCs) which mimic human ARHL (17, 14, and 15). In this article, we describe the functional, histological and molecular characterization of the SAMP8 mouse model and we shown that oral administration of the antioxidant N-acetylcystine (NAC) reduced the age- related neuropathy, age-related deafness and age-related retinopathy in SAMP8 when administrated chronically in the drinking water during 5 months.

MATERIAL AND METHODS

Animal housing and drug administration

Male SAMR1 (control) and SAMP8 (Envigo, France) were kept in the A1 animal house facility. Animals were housed in ventilated and clear plastic boxes and subjected to standard light cycles (12 hours in 90-lux light, 12 hours in the dark). N-acetyl-Lcystine (NAC) (Sigma Aldrich, A7250-50G) was diluted in drinking water at 0.1 g/mL and treatment was performed from 1 month old to 5 months old. All animal experiments were approved by the CEEALR, Montpellier, France.

Balance beam test

Balance beam test is a narrow "walking bridge" that rodents can cross to test balance and neurosensory coordination. The beam (thickness 6 cm) was elevated with the help of two feet with platforms to hold mice. The time required to cross the beam from side to side was quantified for each mouse. Each animal underwent 3 trials a day at 5minutes intervals. For each day, values from the 3 trials were averaged for each animal, normalized according to animal weight, and then averaged for each treated group.

Grip strength test

Neuromuscular strengths of all mice were assessed in standardized grip strength tests for front limbs, hind limbs, and all limbs. All limbs grip strength was measured by supporting each rodent on a metal grid and pulling the animal's tail toward a horizontal grid connected to a gauge. Front and hind limb grip strengths were measured by supporting the forelimbs or hind limbs, respectively, and pulling the animal's tail toward a horizontal T-bar connected to a gauge. The maximum force (measured in newtons) exerted on the T-bar or grid before the animal lost its grip was recorded, and the mean of 3 repeated measurements was calculated. All data were normalized according to animal weight.

Sciatic nerve electrophysiology

Standard electromyography was performed on mice anesthetized with ketamine/xylazine mixture following SOP-A15-V1. A pair of steel needle electrodes (AD Instruments. MLA1302) was placed subcutaneously along the nerve at the sciatic notch (proximal stimulation). A second pair of electrodes was placed along the tibial nerve above the ankle (distal stimulation). Supramaximal square-wave pulses, lasting 10 ms at 1 mA for mice were delivered using a PowerLab 26T (AD Instruments). CMAP was recorded from the intrinsic foot muscles using steel electrodes. Both amplitudes and latencies of CMAP were determined. The distance between the 2 sites of stimulation was measured alongside the skin surface with fully extended legs, and NCVs were calculated automatically from sciatic nerve latency measurements. Only the left sciatic nerves were analyzed in this study.

Auditory brainstem responses (ABR)

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 anesthetizedusingketamine/xylazine

mixture following in vivex procedure SOP-A15-V1, and body temperature is regulated using a heatingpad 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 of five frequencies (4 kHz, 8 kHz, 16 kHz, 24 kHz and 32 kHz) at various sound levels (from 0 to 90 dB) ranging to cover the mouse auditory frequency range. ABR measures of each animal were performed individually and using OtoPhyLab system. Evoked potentials were extracted by the signal averaging technique foreach noise level and ABR thresholds for each frequency were determined using OtoPhyLab software. Only the left ears were analyzed in this study.

Distortion product otoacoustic emission (DPOAE)

DPOAEs are acoustic signals created and amplified bythe cochlear epithelium, offering an index of cochlearfunctions. They are linked to outer hair cell (OHCs) healthwhich amplifies sound-evoked cochlearvibrations. They do not depend on IHCs or auditorynerve fibers. For DPOAEmeasures, mice were anesthetized using ketamine/xylazine mixture following SOP-A15-V1 and aprobe (OtoPhyLab) was inserted into the external leftear canal. The primary tone F2 was set at five frequencies (4 kHz, 8 kHz, 16 kHz, 24 kHz and 32 kHz) at 58 dB. The frequency ratio F2/F1 was set at1.2. At all frequencies (F2), the input of DPOAE systems were received, digitized and evaluated using the outputof the microphone. The amplitudes of the frequencycomponent at the distortion product frequency were determined and represented.

Electroretinogram

Mice were anesthetized using ketamine/xylazine mixture following in vivex procedure SOP-A15-V1. Both eyes were treated with 1% atropine sulfate, 2.5% phenylephrine hydrochloride and 0.5% proparacaine hydrochloride, allowing drops to sit on the eyes for ~2 min before wicking with a cotton swab and applying the next drop. Then, the mouse was positioned on the heated platform. The ground needle electrode was placed in the base of the tail, reference needle electrode subdermally between the eyes and the contact lens electrodes onto the corneas. The scotopic (dark-adapted) ERG measures were performed at 0.001 cd s/m^2;0.1cd s/m^2; 1 cd s/m^2; and 10 cd s/m^2 with 5 trials by step, pulse frequency: 0.2 Hz, sample frequency: 1.000 Hz. trial pre-trigger time: 20 msec and trial post-trigger time: 250 msec. At the end of the measure, electrodes were removed from the mouse and the animal was placed in a clean cage on top of a heat pad until recovering.

Optometry test

Optokinetic responses constitute a fast and robust method to quantitatively assess visual function in mouse. The animals were individually placed on a platform in the center of a cylinder. Animals were adapted to the environment for 5 min. The mouse number and Condition was selected on the software. The step size of the stimulus was selected manually. The stimulation conditions were: 0.05 c/d sinusoidal for the spatial frequency, speed 12 d/s and temporal frequency 0.6 Hz. The reflexive head and neck movements were recorded for each animal. The improvement of the spatial frequency measured in cycles/degree (c/d) was measured. Each animal underwent 3 trials a day at 5 minutes intervals. For each day, values from the 3 trials were averaged for each animal and then averaged for each treated group.

Cytocochleogram

Cocheae of all animals were dissected out and fixed with paraformaldehyde solution overnight and then, decalcified for 7 days in EDTA. The membranous and sensory spiral containing the organ of Cortis was extracted and the hair cells were immune labeled for anti-Myosin-VIIa. The medial region of the organ of Corti was mounted on glass slides and images were acquired with a confocal microscope.

Retina histology

The left eye of all animal was dissected out using a scalpel to cut the connective tissue in the orbital cavity surrounding the eye. Then, the retina was dissected and fixed with paraformaldehyde solution overnight at 4 °C. After washing for 30 minutes in 0.2 M PBS buffer, the samples were dehydrated using ethanol gradient solutions, and embedded in epoxy resin. Semi thin cross sections were cut and stained with Mayer's hematoxylin solutionbefore to be observed using a light microscope.

TNF-α quantification

For each animal, 2 mL of blood was sampled by cardiac puncture following in vivex procedure SOP-A06-V1 and collected in a tube containing EDTA as anticoagulant. Samples were centrifuge for 15 minutes at 1000 × g (or 3000 rpm) at 2 - 8°C within 30 minutes of collection. The supernatant (plasma) was store at -20°C. Plasma was diluted at 1/10 and TNF-α quantification for each animal was performed in duplicated by ELISA method (Sigma Aldrich. Ref. RAB0477).

IL-6 quantification

For each animal, 2 mL of blood was sampled by cardiac puncture following in vivex procedure SOP-A06-V1 and collected in a tube containing EDTA as anticoagulant. Samples were centrifuge for 15 minutes at 1000 × g (or 3000 rpm) at 2 - 8°C within 30 minutes of collection. The supernatant (plasma) was store at -20°C. IL-6 plasma quantification for each animal was performed in duplicated by ELISA method (Sigma Aldrich. Ref. RAB0308).

Western blot

Plasma samples were denaturalized and separated in 10% SDS-polyacrylamide gel and transferred onto nitrocellulose

membrane. Membrane was blocked for 60 min with Licor Blocking Buffer. The membrane was incubated overnight with rabbit anti-β-tubuline (1:100) and mouse anti-prestin (1:100). The following day the membrane was washed in TBS Tween-20 (0.1% V/V) and the incubated with the secondary fluorescence antibodies: donkey anti-rabbit IRDye 800 (1:10.000) and Goat anti-mouse IRDye 680 (1:10.000). Then, the membrane was washed in TBS Tween-20 (0.1% V/V) and visualization of the bands was performed using Licor scanning devise.

Statistics

Data are represented as mean ± SEM. Statistical significance was determined using 2-way ANOVA, followed by a Dunnett's multiple comparison post-hoc test. A P value of less than 0.05 was considered significant. n= 8 animals/group.

RESULTS

SAMP8 mouse present neuromuscular and neuromotor impairment from 3 months old

Walking performances were analyzed using balance beam test. Similar balance beam cross times were observed in SAMR1, SAMP8 and SAMP8+NAC groups at 1 months old (5.0 ± 0.3 s; 5.8 ± 1.0 s; 5.9 ± 0.4 s respectively).

At 3 months old, the beam cross time was increased at 10.0 ± 1.6 s in the SAMP8 group mice whereas the cross time of SAMR1 and SAMP8+NAC animals remained at 6.5 ±0.3s and 7.2 ± 1.1 s respectively. However, this increase of beam cross time of the SAMP8 animals was not significant compared to SAMR1 and SAMP8+NAC groups at 3 months old (Figure 1B). No statistical differences were observed between SAMP8+NAC and SAMR1 at this time point.

At 5 months old, the beam cross time of the SAMP8group was significantly increased at 14.1 ± 1.0 s whereas theSAMR1 and SAMP8+NAC animals presented a beam cross time of 7.0 ± 0.5 s and 9.5 ± 0.6 s respectively (Figure1B). Even if the SAMP8+NAC group presented a slight increase of the cross time, no statistical differences were observed between SAMP8+NAC and SAMR1 animals at 5 months old.

Then, neuromuscular strength was analyzed using grip test. Similar grip strengths were observed in SAMR1, SAMP8 and SAMP8+NAC groups for all paws at 1 months old (11.0 ± 1.1 N; 9.8 ± 2.6 N; 11.6 ± 1.7 N respectively).

At 3 months old, the grip strength of all paws was decreased at 9.2 ± 1.6N in the SAMP8group mice whereas the grip strength of SAMR1 and SAMP8+NAC animals remained at 13.3 ± 0.5N and 10.6 ± 1.3 N respectively. However, this decrease of grip strength of the SAMP8 animals was not significant compared to SAMR1 and SAMP8+NAC groups at 3 months old for all paws. Moreover, no statistical differences were observed between SAMP8+NAC and SAMR1 at this time point (Figure 1A).

At 5 months old, the grip strength of the SAMP8group was significantly decreased at 5.6 ± 0.7N whereas theSAMR1 and SAMP8+NAC animals presented a grip strength of14.2 ± 1.8 s and 10.3 ± 1.6 N respectively for all paws. Even if the SAMP8+NAC group presented a slight decrease of the grip strength, no statistical differences were observed between SAMP8+NAC and SAMR1 animals for all paws at 5 months old (Figure 1A).Similar results were observed for front and hind paws separately confirming the data observed for all paws.

Taken together, these results suggest that NAC treatment allows reducing of the age-related neuromotor impairment in SAMP8 mice.

To validate the behavioral tests, the peripheral nervous system was analyzed using sciatic nerve electrophysiology (electromyography). Concerning the CMAP amplitude, similar values were observed in SAMR1, SAMP8 and SAMP8+NAC groups at 1 months old (4.58 ± 0.10 mV; 4.89 ± 0.58 mV; 4.26 ± 0.50 mV respectively).

At 5 months old, the CMAP amplitude was significantly decreased at 2.20 ± 0.42mV in the SAMP8group mice whereas theSAMR1 and SAMP8+NAC mice presented a CMAP amplitude of4.70 ± 0.53mV and 3.64 ± 0.41mV respectively. Even if the SAMP8+NAC group presented a slight decrease of CMAP amplitude, no statistical differences were observed between SAMP8+NAC and SAMR1 animals at 5 months old (Figure 1D).

Concerning the nerve conduction velocity (NCV), similar values were observed in SAMR1, SAMP8 and SAMP8+NAC groups at 1 months old (13.75 ± 1.17 m/s; 12.42 ± 2.59 m/s; 14.27 ± 2.98 m/s respectively).

At 5 months old, the NCV was significantly decreased at 4.32 ± 0.72m/s in the SAMP8group mice whereas theSAMR1 and SAMP8+NAC mice presented a NCV of13.70 ± 1.54m/s and 3.64 ± 0.41 mV respectively. Even if the SAMP8+NAC group presented a slight decrease of the NCV, no statistical differences were observed between SAMP8+NAC and SAMR1 animals at 5 months old (Figure 1C).

These results confirm that NAC treatment allows reducing of the age-related neuropathy in SAMP8 mice.

Inflammation is widely observed in aging people and TNF-α and IL-6 is recognized as inflammatory biomarker directly linked to peripheral neuropathy. ELISA analysis of plasma demonstrated a significant increase of the inflammatory

biomarker TNF-α at 280.0 ± 28.2 pg/mL of plasma in the SAMP8 mice whereas the TNF-α concentration in SAMR1 and SAMP8+NAC mice were 153.0 ± 18.6 pg/mL and 194.7 ± 15.9pg/mL of plasma respectively at 5 months old (Figure1F).

Moreover, ELISA analysis of plasma also demonstrated a significant increase of the inflammatory biomarker IL-6 at 73.7 ± 8.8 pg/mL of plasma in the SAMP8 mice whereas the IL-6 concentration in SAMR1 and SAMP8+NAC mice were22.8± 3.2 pg/mL and 37.5 ± 2.9 pg/mL of plasma respectively at 5 months old (Figure 1E).

Finally, the histological analysis of the sciatic demonstrated a degeneration in the SAMP8 group compared to the SAMR1 group at 5 months oldconfirming the neuropathic phenotype previously characterized by behavioral and electrophysiological readouts. In particular, a decrease of the number of axons and axonal diameter is observed in SAMP8 group (Figure 1G) whereas the animals treated with NAC presented an increase of the number of axons and axonal diameter at this time point.

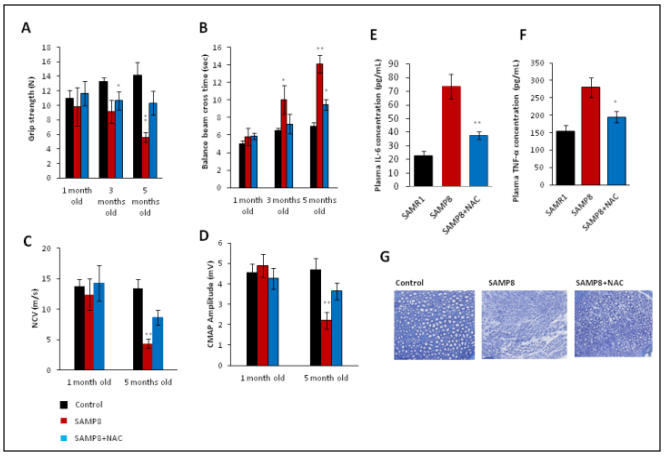

Figure 1. Neuromuscular impairment observed in SAMP8 mice. (A) Grip strength, (B) Balance beam cross time, (C) nerve conduction velocity of sciatic nerve (D) Compound muscle action potential amplitude of sciatic nerve (E) plasma IL-6 concentration, (F) plasma TNF-α concentration and (G) toluidine blue staining semithin sciatic nerve cross sections of control (SAMR1), SAMP8 and SAMP8+NAC. Error bars indicate SEM. Statistical tests are repeated measures two-way ANOVA test comparing CMT to control mouse values. * p<0.05, ** p<0.01, *** p<0.001, no significant (ns). n=8

SAMP8 mouse presents hearing impairment from 3 months old

ABR analysis was performed to determine the hearing capability of the animals. Similar ABR thresholds were observed in SAMR1, SAMP8 and SAMP8+NAC groups at 1 months old (Figure 2A).

At 3 months old, the ABR threshold was significantly increased at 32 kHz in the SAMP8 group mice compared to theSAMR1 and SAMP8+NAC. Moreover, no statistical differences were observed between SAMP8+NAC and SAMR1 at this time point at the analyzed frequencies (Figure 2B).

At 5 months old, the ABR thresholds of the SAMP8 group were significantly increased at 4 kHz, 8 kHz, 16 kHz, 24 kHz and 32 kHz compared totheSAMR1 group. Moreover, ABR thresholds of the SAMP8+NACgroup were also significantly increased at 4 kHz, 8 kHz, 16 kHz and 32 kHz compared totheSAMR1 group (Figure 2C). No statistical differences were observed between SAMP8 and SAMP8+NAC animals at 5 months old.

These results suggest that NAC treatment allows delaying of the age-related hearing impairment in SAMP8 mice.

Then, distortion product otoacoustic emission (DPOAE) analysis was performed to determine the functionality of cochlear hair cells. Similar DPOAE amplitudes were observed in SAMR1, SAMP8 and SAMP8+NAC groups at 1 month old (Figure 2D).

At 3 months old, the DPOAE amplitudes were significantly decreased at 24 kHz in the SAMP8group mice compared to theSAMR1 and SAMP8+NAC. Moreover, no statistical differences were observed between SAMP8+NAC and SAMR1 at this time point at the analyzed frequencies (Figure 2E).

At 5 months old, the DPOAE amplitude of the SAMP8group were significantly decreased at 8 kHz, 16 kHz and 24 kHz compared totheSAMR1 group. Moreover, DPOAE amplitudes of the SAMP8+NACgroup were also decreased at all analyzed frequencies, but this decrease was not statistically significant compare to the SAMR1 group (Figure 2F). Finally, no statistical differences were observed between SAMP8 and SAMP8+NAC animals at 5 months old.

Taken together these results suggest that NAC treatment allows to delay, but not to prevent, the age-related hearing impairment in SAMP8 mice.

Using immune histochemistry, the cytocochleogram analysis demonstrated a strong loss of outer hair cells (OHC) in the SAMP8 group whereas the three rows of OHCs were intact in the SAMR1 (control) and SAMP8+NAC groups at 5 months old (Figure 2G).

Finally, prestin has been described as a new hearing loss plasma biomarker (20, 6). For this reason, we studied plasma

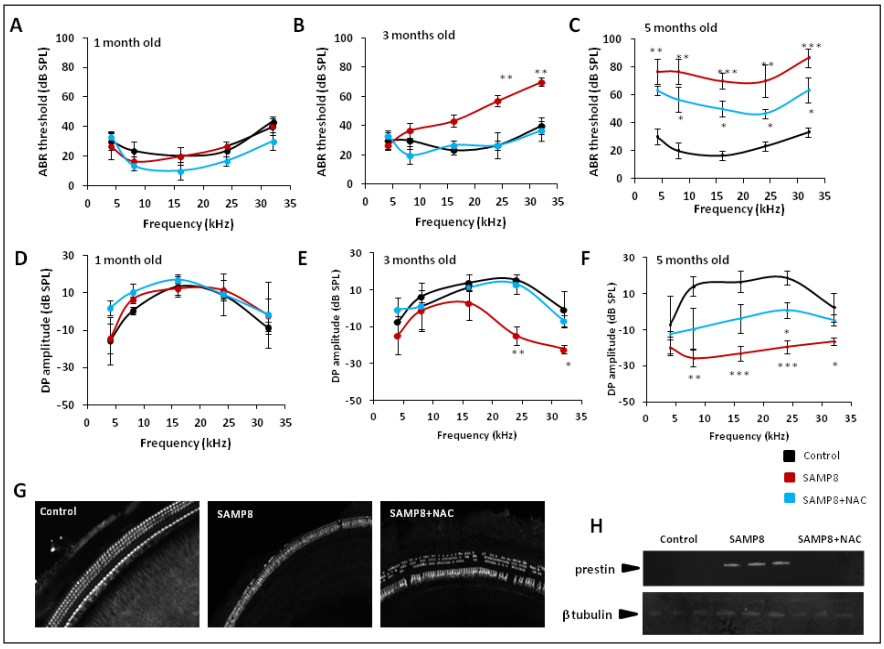

Figure 2. Hearing impairment observed in SAMP8 mice. ABR threshold at 1 month old (A), 3 months old (B) and 5 months old (C) at the stated frequencies. DPOAE amplitude at 1 month old (D), 3 months old (E) and 5 months old (F) at the stated frequencies. (G) representative images of cytocochleogram of the mid segment of the cochlea of control (SAMR1), SAMP8 and SAMP8+NAC animals. (H) western blot analysis of the hearing loss biomarker prestin is plasma of control (SAMR1), SAMP8 and SAMP8+NAC animals. B-tubulin was used as housekeeping leading control protein. Error bars indicate SEM. Statistical tests are repeated measures two-way ANOVA test comparing CMT to control mouse values. * $p<0.05$, ** $p<0.01$, *** $p<0.001$, no significant (ns). n=8

prestinin control, SAMP8 and SAMP8 mice at 5 months old. The western blot analysis of prestin demonstrated an increase of the prestin biomarker in SAMP8 mice whereas no prestin was detected in control and NAC treated group (Figure 2H) confirming a protective effect of NAC treatment in the hearing impairment induced by aging.

SAMP8 mouse presents visual impairment from 3 months old

Optometry test was applied to determine the visual acuity of the animals. Similar visual acuity was observed in SAMR1, SAMP8 and SAMP8+NAC groups at 1 month old (0.32 ± 0.04c/d; 0.32 ± 0.07c/d; 0.36 ± 0.05c/d respectively) (Figure3A).

At 3 and 5 months old, the spatial frequency of the SAMP8group was significantly decreased compared totheSAMR1 and SAMP8+NAC spatial frequencies (Figure 3B and 3C). Even if the SAMP8+NAC group presented a slight decrease of the spatial frequency, no statistical differences were observed between SAMP8+NAC and SAMR1 animals at 3 and 5 months old.

The behavioral test was then validated by an electroretinogram analysis at 1 month old, 3 months old and 5 months old. Similar scotopic a-wave amplitudes were observed in SAMR1, SAMP8 and SAMP8+NAC groups at 1 month old at all analyzed intensities.

At 3 and 5 months old, the scotopic a-wave amplitudes of the SAMP8group were significantly decreased at1 cd s/m^{2}and 10 cd s/m^{2}compared totheSAMR1 groups. Moreover, scotopic a-wave amplitude of the SAMP8+NACgroup was also significantly decreased at 10 cd s/m^{2}compared to the SAMR1 group (Figure 3D). No statistical differences were observed between SAMP8 and SAMP8+NAC animals at 5 months old at any analyzed intensity.

These results suggest that NAC treatment allows delaying of the age-related retinopathy in SAMP8 mice.

Finally, the histological analysis of the retina demonstrated a degeneration in the SAMP8 group compared to the SAMR1 group at 5 months old (Figure 3E). In particular, a decrease of the number and thickness of the outer nuclear layer is observed in all SAMP8 retinas. Decrease of degeneration is observed in the SAMP8 animals treated with NAC confirming the behavioral and electrophysiological data previously showed.

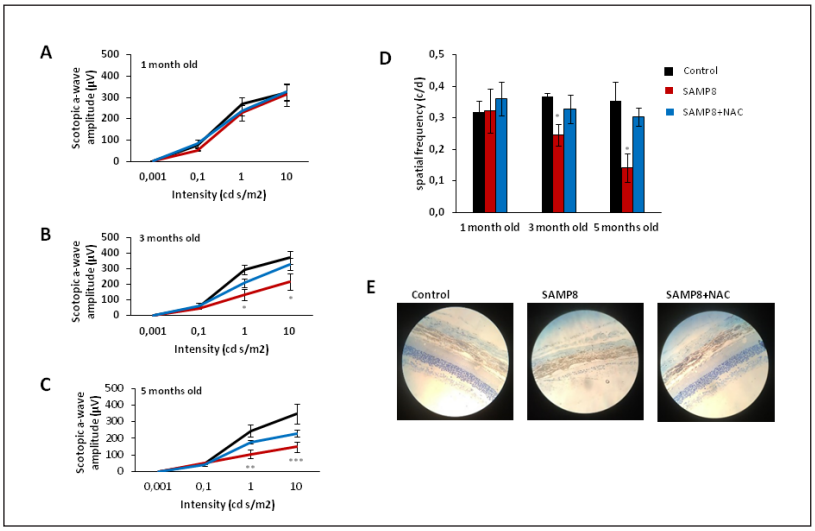

Figure 3. Progressive blindness observed in SAMP8 mice. Scotopic a-wave analysis measured by electroretinogram at 1 month old (A), 3 months old (B) and 5 months old (C). (D) Visual acuity at 1 months old, 3 months old and 5 months old. (E) Representative images toluidine blue staining semithinretina sections of control (SAMR1), SAMP8 and SAMP8+NAC.Error bars indicate SEM. Statistical tests are repeated measures two-way ANOVA test comparing CMT to control mouse values. * $p<0.05$, ** $p<0.01$, *** $p<0.001$, no significant (ns). n=8

DISCUSSION

Hearing impairment, cochlear hair cells loss, neuromotor disorders, visual dysfunction, retinopathy and increase of inflammatory biomarkers observed in SAMP8 mice at 3 and 5 months old. Moreover, NAC treatment allows delaying the senescence process by slowing the neuromotor impairment, the age-related hearing loss, protecting the cochlear hair cells and improving visual acuity.

Taken together, these results confirm that SAMP8 mouse is a homogenous and fast model to analyze the neuropathy disorders, hearing disorder and retina degeneration induced by senescence. This model allows determining the efficacy of new pharmacological candidates targeting age-related disorders.

Because several studies suggest that increase and imbalance of oxidative stress is the first step of age-related sensory disorders, we hypothesize that NAC reduces the overexpression of ROS produced by mitochondria leading to a reduction of cellular damage and preventing sensory and neuromotor system degeneration. Taken together, these data confirm that NAC induces neuro-protective effect on peripheral nerves, cochlea and retinal tissue in SAMP8 mouse model and open the door to the development of new antioxidant strategies targeting ROS imbalance.

Peripheral neuropathy and ARHL is directly associated with an increase of reactive oxygen species and a decrease in endogenous antioxidants (17, 7, 13). Moreover, a genetic predisposition to oxidative damage could also play a role in the imbalance between cellular production of reactive oxygen species and antioxidant defenses. It can result in cell damage by oxidation of cellular components such as membrane lipids, proteins, and DNA (21) leading to cellular dysfunctions and progressive sensory systemdegeneration and inflammation(4).

Moreover, age-related retinopathy is also directly linked to ROS imbalance. During senescence, the increase of ROS result in retinal metabolic abnormalities, and these metabolic abnormalities can also produce ROS. Sustained exposure to ROS damages the mitochondria and compromises the electron transport system and, ultimately, the mitochondrial DNA of retinal cells is damaged. Damaged mtDNA impairs its transcription, and the vicious cycle of ROS continues to propagate leading to a progressive retina degeneration and age-related blindness (11).

Several publications demonstrated that antioxidants have generated beneficial effects in ameliorating neuropathy (5), retinopathy and hearing loss (6, 15) in several preclinical rodent models, however limited clinical studies have not been encouraging(2) and some clinical trials using NAC remain presently controversialand inconclusive (10). With the ongoing use of antioxidants for other chronic diseases, there is a need for a controlled trial to recognize their potential in ameliorating the development of age-related sensory disorders.

CONCLUSION

Here, we describe the hearing impairment, neuromotor disorders, visual dysfunction, and retinopathy and inflammation phenotype in the accelerated senescence mouse model SAMP8 at 3 and 5 months old. Moreover, we show that chronic administration of NACallows to delay the senescence process by slowing the neuromotor impairment, the age-related hearing loss, protecting the cochlear hair cells and improving visual acuity leading to a decrease of the age-related disorders in SAMP8 mice and demonstrating than antioxidants are promising pharmacological compounds for sensory disorders induced by senescence.

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How to cite this article: Gonzalez S G, Caumes B, Asin A R. Functional, Histological and Molecular Characterization of a Preclinical Mouse Model of Age-Related Neuropathy, Age-Related Hearing Loss and Age-Related Blindness. J Community Prev Med 2022;4(2):10-17.
DOI: 10.33309/2638-7719.040203