Research Article

Systemic epigallocatechin gallate protects against retinal degeneration and hepatic oxidative stress in the P23H-1 rat

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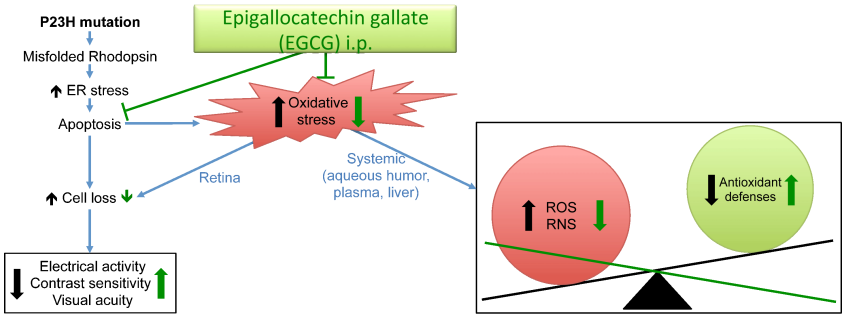
Graphical Abstract *EGCG protects against retinal degeneration*



Abstract

Retinitis pigmentosa (RP) is a group of inherited retinal disorders that lead to photoreceptor loss. RP has been reported to be related to oxidative stress, autophagy, and inflammation. (-)-Epigallocatechin gallate (EGCG), the most abundant catechin-based flavonoid in green tea leaves, has significant antioxidant, anti-carcinogenic, antimicrobial, and neuroprotective properties. EGCG, given its low molecular weight and hydrophilic properties, can cross the blood-retinal barrier and is able to reach different ocular tissues such as the lens, cornea, and retina. EGCG has been shown to provide retinal protection against ischemia; sodium nitroprusside-, N-methyl-D-aspartate-, lipopolysaccharide-, light-, sodium iodate-, or H_2O_2-induced damage and diabetic retinopathy. This suggests that systemic EGCG administration has the potential to protect against retinal degenerative or neurodegenerative diseases such as RP. The aim of this work was to investigate whether EGCG can protect against RP progression in the animal P23H line 1, the model of RP. Albino P23H rats were crossed with pigmented Long Evans rats to produce offspring exhibiting the clinical features of RP. Pigmented P23H rats were treated via intraperitoneal injection with saline or EGCG at a dose of 25 mg/kg every week from P100 to P160 and then compared to wild-type Long Evans rats. Rats treated with EGCG showed better visual and retinal electrical function with increased contrast sensitivity and b-wave values compared with those observed in P23H rats treated with vehicle. EGCG reduced lipid peroxidation and increased total antioxidant capacity and catalase and superoxide dismutase activities. No differences were observed in visual acuity, nitrate levels, nitrite levels or glutathione S-transferase activity. In conclusion, EGCG not only reduced the loss of visual function in P23H rats but also improved the levels of antioxidant enzymes and reduced oxidative damage. This study was approved by the Institutional Animal Care and Use Committee (CEICA) from the University of Zaragoza under project license PI12/14 on July 11, 2014.

Key Words: antioxidants; contrast sensitivity; electroretinogram; green tea; neurodegeneration; oxidative stress; retinal degeneration; retinitis pigmentosa; systemic; visual acuity

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Introduction

Retinitis pigmentosa (RP) is a group of inherited retinal disorders that currently affect approximately two million patients worldwide (Chizzolini et al., 2011). RP is caused by genetic mutations that lead to progressive degeneration of the light-sensing photoreceptors, thus ending in a generalized retinal atrophy.

The main visual symptoms include a progressive decrease in visual acuity (VA) that predominantly affects night vision and the peripheral field (Lax et al., 2011). RP genetic defects predominantly affect rods and induce their degeneration. Subsequently, cones also die, resulting in the loss of central vision (Narayan et al., 2016; Newton and Megaw, 2020). With retinal cell damage, oxidative stress is increased, and apoptosis pathways are activated (Cuenca et al., 2014; Moreno et al., 2018).

The free radicals formed also damage the lysosomes of retinal pigmented epithelial (RPE) cells, which lose their capacity to degrade photoreceptor outer segment material (Kim et al., 2021). This oxidative stress has been related to ischemic retinopathy (Rivera et al., 2017) and other neurodegenerative diseases such as age-related macular degeneration, diabetic retinopathy, and RP (Perdices et al., 2018; Albokhary et al., 2021; Tisi et al., 2021).

One mutation causing RP is the Pro-23-His substitution in the gene encoding rhodopsin (RHO) (Kakavand et al., 2020). The P23H rat model of RP is commonly used due to its phenotypical similarities with the human disease (Pinilla et al., 2016). Vision function loss, retinal degeneration and hepatic oxidative stress have been previously described in the P23H rat (Marc et al., 2003; Perdices et al., 2018).

Although the mutations that cause RP have been identified in different genes, the mechanisms that cause photoreceptor death are still unknown, and currently, no treatment is available (Zhang, 2016). Gene therapy and cell-based therapy are the most promising future options (Mendell et al., 2020; Uyama et al., 2020). However, several alternative strategies are being investigated to reduce or delay RP. (-)-Epigallocatechin gallate (EGCG) is the most abundant catechin-based flavonoid in green tea leaves (Fernando and Soysa, 2016). The multifunctional properties of EGCG include antioxidant, anti-inflammatory, neuroprotective, cardioprotective, anti-carcinogenic and antimicrobial effects (Chakrawarti et al., 2016; He et al., 2018).

EGCG, given its low molecular weight and hydrophilic properties, can cross the blood-retinal barrier and is able to reach different ocular tissues such as the lens, cornea, and retina. This suggests that systemic EGCG administration has the potential to protect against retinal degenerative or neurodegenerative diseases (Chu et al., 2015). EGCG has been shown to provide retinal protection against ischemia (Peng et al., 2008); sodium nitroprusside-, N-methyl-D-aspartate-, lipopolysaccharide-, light-, sodium iodate-, or H_2O_2-induced damage (Zhang and Osborne, 2006; Chen et al., 2012; Cia et al., 2014; Yang et al., 2016; Qi et al., 2017; Ren et al., 2018) and diabetic retinopathy (Silva et al., 2013).

The aim of this work was to study the therapeutic action of EGCG against the progression of RP in the animal model P23H line 1 with a view towards the possible therapeutic application of EGCG for the prevention and treatment of retinal degenerations.

Materials and Methods

Animals

Line 1 albino P23H transgenic rats (SD-Tg(Rho*P23H)1Lav) generously donated by University of California, San Francisco (UCSF) (CA, USA) were bred in a colony at the University of Zaragoza. Homozygous transgenic albino animals were crossed with wild-type pigmented Long Evans (LE) rats (Charles River Laboratories, Barcelona, Spain) to generate transgenic-pigmented offspring (pigmented P23H). The animals were housed under a 12-hour light/dark cycle (light cycle illumination varied from 7 to 30 lux depending on animal position within the respective cages) with food and water available *ad libitum*. All procedures were carried out according to the guidelines on the ethical use of animals from the European Community Council Directive (86/609/EEC) and the Association for Research in Vision and Ophthalmology (ARVO) Statement for the Use of Animals in Ophthalmic and Vision Research and with the authorization and supervision of the Institutional Animal Care and Use Committee (CEICA) from the University of Zaragoza under project license PI12/14 on July 11, 2014. The rats were divided into three groups as follows: (i) LE: LE wild-type animals injected with normal saline (0.9% sodium chloride) at the same volume as EGCG; (ii) P23H-Vehicle: pigmented P23H rats injected intraperitoneally with normal saline; and (iii) P23H-EGCG: pigmented P23H rats injected intraperitoneally with EGCG. Four animals from each group were studied.

Administration of (-)-epigallocatechin gallate

EGCG (Sigma E4268, Madrid, Spain) was dissolved in normal saline injection (10 mg/mL). EGCG was freshly prepared, and the rats were given an IP injection at a dose of 25 mg/kg every week from postnatal day (P) 100 to P160, with a total of 7 doses administered. Vehicle group animals received an equal volume of 0.9% sodium chloride.

Visual acuity and contrast sensitivity evaluation

Visual parameters were measured at P100 and P160. Assessment of VA and contrast sensitivity (CS) was performed using an OptoMotry system (OptoMotry™, CerebralMechanics, Lethbride, Alberta, Canada) as previously described (Segura et al., 2018). The device consisted of four computer monitors that form a square, simulating a virtual cylinder, which is projected in three dimensions giving rise to a vertical sinusoidal grid. In brief, the rat to be examined was placed on a Plexiglass round disc located in the center of the arena and a video camera was placed on the top of the test field. The position of the head in each frame was used to center the hub of the cylinder continually at the rat's viewing position. The cylinder was rotated at a constant speed (12 degrees/second).

During each trial, when the rat moved over the platform, an experimenter followed it, keeping a cross over the head of the animal, which ensured that the frequency of the grid was the adequate one. The spatial frequency threshold, was obtained by incrementally increasing the spatial frequency of the grating at 100% contrast until the animals no longer responded (considered the maximum VA). Thresholds through each eye were measured separately by reversing the rotation of the cylinder (McGill et al., 2007).

A CS curve was produced by identifying the minimum contrast that generated tracking over a range of spatial frequencies (Segura et al., 2015).

Electroretinogram recordings

The rats were dark-adapted overnight at P160 and were then anesthetized in dim red light to keep an absolute darkness with a mixture of ketamine (90 mg/kg i.p.) and xylazine (10 mg/kg i.p.) to avoid the diminution of waves (Nair et al., 2011; Segura et al., 2015). Rats were tested with pupils dilatation using 1% tropicamide (Colircusí Tropicamida; Alcon Labs, Barcelona, Spain). 2% Methocel (OmniVision, Puchheim, Germany) and 0.9% normal saline were used to improve corneal contact and avoid dryness. The electroretinogram (ERG) was recorded using a gold wire loop as electrode and

keeping the rat at 37°C with a heating. Two 25-gauge platinum needles inserted under the scalp and behind the eyes were used as reference electrodes. The ground electrode was placed in the tail. Stimulus presentation and data acquisition were done with the Espion system from Diagnosys LLC (Cambridge, UK).

To characterize the rod and cone pathways (Pinilla et al., 2004), 3 to 8 single-flash presentations 10 µs in duration were displayed with 10 increasing intensities varying from –3.70 to 2.86 log cd·s/m^2. Interstimulus intervals (ISI) were increased to minimize the effects of bleaching on the rods. The results of both wave measurements were averaged for different recordings. The criterion of ERG response amplitude was established at 20 µV for the a- and b-waves.

Isolation of the cone response using a double-flash protocol

The double-flash protocol was used to isolate the cone response as previously described (Pinilla et al., 2004). The probe flash was presented 1 second after a conditioning flash to saturate rods. The intensity of the conditioning flash was equal to the intensity of the probe flash (1.4 log cd·s/m^2) to obtain complete rod bleaching. The results were averaged across three recordings with an ISI of 100 seconds to ensure full recovery of rod responsiveness.

Tissue collection and preparation

The day after the last administration of EGCG, the animals were euthanized by CO_2 inhalation at the end of the experimental period and functional tests. Each animal was placed in the appropriate chamber and 100% CO_2 was introduced at a fill rate of 30–70% of the chamber volume per minute.

Liver tissue homogenates were prepared by dissolving 0.5 g liver tissue in 3.5 mL phosphate buffer 0.2 M in a test tube containing Na_2HPO_4 (dipotassium hydrogen phosphate, Panreac, Barcelona, Spain; A1046), NaH_2PO_4 (potassium dihydrogen phosphate, Panreac; A3559) (pH 7.4), 0.5% Triton X-100 (Panreac; A4975), 5 mM β-mercaptoethanol (Sigma; M6250) and 0.1 mg/mL phenylmethylsulfonyl fluoride (Sigma; P7626). The resulting mixture was homogenized for 10 minutes at 3000 × *g* at 4°C, and the supernatants obtained were used to measure the biomarkers of oxidative stress. Protein concentration was determined according to Bradford method (Bradford, 1976) using Bradford reagent (Sigma, B6916) and bovine serum albumin (Sigma, A7906) as the standard protein.

Oxidative and nitrosative stress markers in liver tissue

Lipid peroxidation is a process in which malondialdehyde (MDA) and 4-hydroxyalkenal (4-HDA) are generated. It is used to assess the degree of lipid breakdown. The assay was performed as previously described (Perdices et al., 2018). The above mentioned products react with the chromogenic reagent N-methyl-2-phenylindole at 45°C to obtain a stable chromophore which can be measured at 586 nm.

Nitric oxide (NO) is a biomolecule that induces a variety of metabolic effects, both beneficial and detrimental, and is considered a free radical due to its high reactivity. Measuring the accumulation of its stable degradation products, nitrate (NO_3) and nitrite (NO_2), it is possible to assess the total amount of NO. In this study, a colorimetric assay kit (Oxford Biomedical Research®, Oxford, UK; NB98) was used to measure the stable degradation products of NO, using the enzyme nitrate reductase. The Griess reagent reacts with total nitrites to form a purple/pink color that can be measured spectrophotometrically at 550 nm (Synergy™ HT Multi-detection microplate reader, Biotek, Winooski, VT, USA).

Antioxidant defenses

The total antioxidant capacity (TAC) was determined using the TAC Assay Kit (BioVision®, K274), which measures total antioxidant capacity based on the reduction of copper (Cu^{2+}) to copper (Cu^{+}) by antioxidant molecules present in the sample. This parameter provides information about the ability of organism to counteract oxidative stress-induced damage.

The antioxidant activities of catalase (CAT), superoxide dismutase (SOD) and glutathione S-transferase were measured as previously described (Perdices et al., 2018). CAT (EC 1.11.1.6) activity was measured according to the decrease in H_2O_2 concentration following its reaction with catalase present in samples. Catalase activity was reversely proportional to the signal obtained.

SOD (EC 1.15.1.1) is responsible for conversion of superoxide radical into hydrogen peroxide and molecular oxygen. This assay measures the inhibition of reduction of cytochrome C (Sigma, C2506) by competing for the superoxide radical ($O_2^{\bullet-}$), produced by the xanthine/xanthine oxidase system. The signal observed at 550 nm was, as in the previous technique, reversely proportional to the amount of SOD. Glutathione S-transferase (GST, EC 2.5.1.18) comprises a family of isoenzymes known by their ability to detoxify xenobiotics. These proteins catalyze the conjugation of 1-chloro-2,4-dinitrobenzene (CDNB), with reduced glutathione (GSH). This reaction produces GSH-CDNB, which can be detected by spectrophotometry at 340 nm. The rate of increase in absorbance is directly proportional to the GST activity in the sample.

Statistical analysis

The data were plotted as the mean ± standard error of the mean for each group. Differential statistical analysis was performed using a non-parametric test, the Kruskal-Wallis test, with subsequent comparison between groups performed using the Mann-Whitney *U* test. *P* values < 0.05 were considered significant for all hypotheses tested. IBM SPSS Statistics 20.0 (IBM Corp, Armonk, NY, USA) was used for the statistical analyses, and graphs were made with GraphPad Prism version 7 (GraphPad Software, San Diego, CA, USA).

Results

VA and CS

Visual parameters VA and CS of both eyes were assessed using an optomotor test. Our results showed a significantly lower VA in the P23H animals (0.461 cycles/degree) compared to the LE wild-type rats (0.535 cycle/degree) (**Figure 1A**). P23H animals treated with EGCG tended to preserve VA (0.470 cycle/degree) than the same untreated group with no statistically significant differences.

Similar results were observed in CS curves (**Figure 1B**) where the maximum frequency peak was obtained for a spatial frequency of 0.089 cycle/degree. CS values significantly decreased in the untreated P23H rats compared with the LE wild-type group (*P* = 0.009); however, EGCG treatment increased the CS values in P23H rats which received active treatment (EGCG) compared to those P23H rats which received normal saline instead (*P* = 0.008).

ERG recordings

The amplitude of ERG waves in P23H animals was significantly lower than that in the wild-type animals (LE rats), but the P23H rats treated with EGCG showed greater wave amplitudes. The maximum a-wave amplitude in

P23H rats was 63 ± 26 µV. This value amounted to only 15% of the corresponding value in wild-type animals (424 ± 39 µV). EGCG treatment did not significantly modify the a-wave amplitude (**Figure 2A**). By contrast, the amplitude of the maximum b-wave in P23H rats treated with ECGC was significantly greater than that in P23H rats treated with

vehicle (630 ± 67 μV *vs*. 528 ± 68 μV; **Figure 2B**). At the maximum stimulus (2.9 log cd·s/m^2), the b-wave amplitude in the EGCG treatment group was 54% higher than that in the vehicle group (480 ± 23 μV *vs*. 312 ± 48 μV). The double-flash-isolated rod-driven response in P23H rats was lower than that in wild-type rats (80 ± 45 μV *vs*. 486 ± 65 μV), and the rod-driven contribution to mixed scotopic b-waves in P23H rats was lower than that in wild-type rats (24% *vs*. 41%; **Figure 2C**). The cone-driven response in P23H rats was also lower than that in wild-type rats (249 ± 32 μV *vs*. 712 ± 51 μV). The cone-driven contribution to mixed scotopic b-waves in P23H rats was higherthan that in wild-type rats (76% *vs*. 59%). EGCG treatment increased the rod-driven response compared with vehicle in P23H rats (163 ± 18 μV *vs*. 80 ± 45 μV), reaching a rod-driven contribution to mixed scotopic b-wave of 39% after EGCG treatment. **Figure 2D** shows an example of the scotopic response with increased intensities in LE, and P23H treated with vehicle or EGCG. Both a- and b-waves amplitude were higher in the treated rats than in the untreated rats and in EGCG-treated P23H rats, the amplitude of b-wave was at –2.8 log cd·s/m^2.

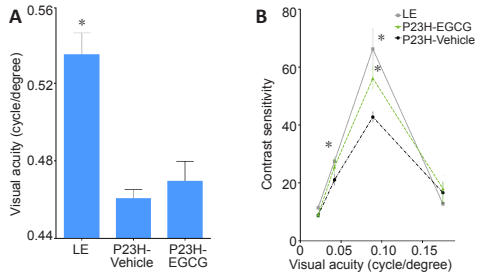

Figure 1 | Visual acuity and contrast sensitivity evaluation in wild-type and EGCG-treated rats.
Values of visual acuity (A) and contrast sensitivity (B) of Long Evans (LE), vehicle-treated transgenic P23H rats (P23H-Vehicle) and P23H rats treated with epigallocatechin gallate (EGCG-P23H) at P160 obtained by an optomotor test. Data are expressed the mean ± SEM (n = 4 per group). *P < 0.05, *vs*. P23H-Vehicle rats (Mann-Whitney U test)

Oxidative stress parameters

Quantification of lipoperoxidation indicators MDA and 4-HAD showed that P23H animals had higher MDA and 4-HAD levels than LE wild-type rats (P = 0.034; **Figure 3A**). EGCG treatment significantly reduced the level of this oxidative stress marker to concentrations lower than those observed in the wild-type rats (P = 0.03). Nitrites provide information about nitrosative damage suffered by the RP retina. Significant differences in MDA and 4-HAD levels were not detected among the wild-type, transgenic P23H and EGCG P23H rats (P > 0.05; **Figure 3B**).

Antioxidant defenses

To evaluate the overall abilities of animals to counteract the action of reactive oxygen species (ROS) and resist oxidative damage, TAC and CAT, SOD and GST levels were determined. The results showed a significantly lower total antioxidant capacity in P23H rats compared with wild-type LE rats (P = 0.004) (**Figure 3C**). Treatment with EGCG increased total antioxidant capacity in P23H rats compared to vehicle-P23H rats (P = 0.005).

Antioxidant enzymes CAT, SOD and GST levels were lower in P23H rats compared to the LE rats (P = 0.048, P = 0.028, P = 0.019 for CAT, SOD, and GST; **Figure 3D–F**). EGCG-treated rats exhibited increased activity levels of both CAT and SOD compared with P23H rats (P = 0.047 and P = 0.028 for CAT and SOD, respectively; **Figure 3D** and **E**). There were no differences in GST levels in LE and P23H-EGCG rats compared to P23H-vehicle rats (P > 0.05; **Figure 3F**).

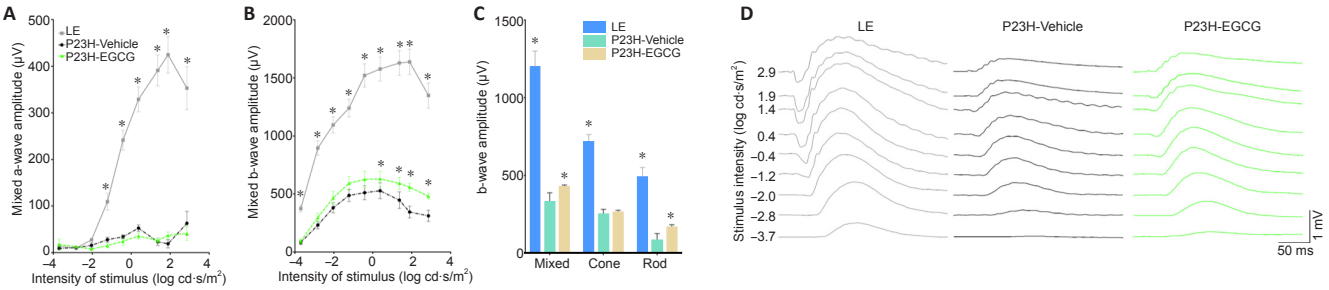

Figure 2 | Scotopic a- and b-waves and double-flash ERG results 1.5 months after first injection.
Scotopic a-wave (A) and b-wave (B) amplitudes obtained by ERG at different intensities of stimulus in Long Evans rats (LE, square), transgenic P23H rats treated with vehicle (P23H-Vehicle, circle) and P23H rats treated with epigallocatechin gallate (P23H-EGCG, triangle). (C) Application of the double-flash protocol. Mixed b-wave: Mixed response obtained with a conditioning flash alone. Cone b-wave: Isolated cone response obtained with a probe flash preceded by the conditioning flash, both set at 1.4 log cd·s/m^2. Rod b-wave: Rod response obtained by subtracting the isolated cone response from the mixed b-wave. Each bar represents the mean ± SEM (n = 8 eyes). *P < 0.05, *vs*. P23H-Vehicle animals (Mann-Whitney U test). (D) Representative examples of ERG curves obtained at different stimulus intensities in Long Evans rats (LE), vehicle-treated transgenic P23H rats (P23H-Vehicle) and P23H rats treated with epigallocatechin gallate (P23H-EGCG). EGCG: (-)-Epigallocatechin gallate; ERG: electroretinogram.

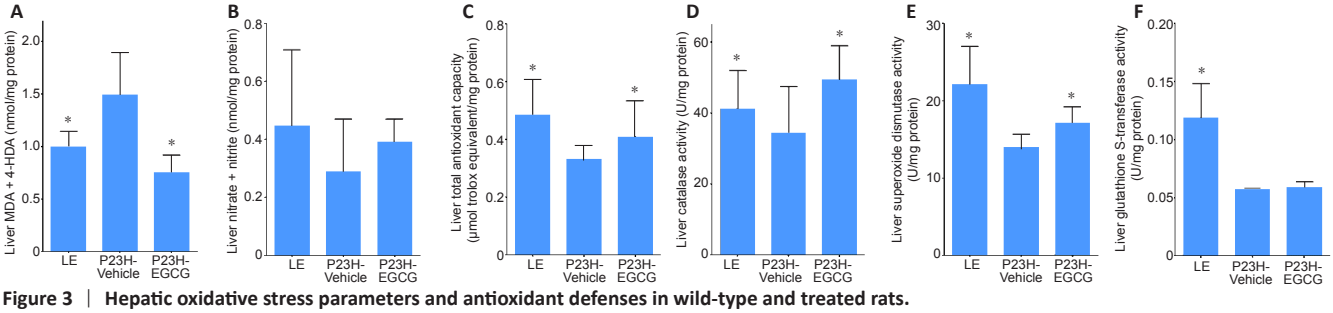

Figure 3 | Hepatic oxidative stress parameters and antioxidant defenses in wild-type and treated rats.
Hepatic lipid peroxidation (A) and nitrosative damage levels (B) of Long Evans rats (LE), transgenic P23H rats treated with vehicle (P23H-Vehicle) and P23H rats treated with epigallocatechin gallate (EGCG-P23H) (n = 4/group). Each bar represents the mean ± SEM (n = 4). *P < 0.05, *vs.* P23H-Vehicle (Mann-Whitney U test). Antioxidant status: TAC (C), CAT (D), SOD (E) and GST (F) activities in livers of LE, P23H-Vehicle and P23H EGCG-P23H. Each bar represents the mean ± SEM (n = 4). *P < 0.05, *vs.* P23H-Vehicle (Mann-Whitney U test). CAT: Catalase; EGCG: (-)-epigallocatechin gallate; GST: glutathione S-transferase; SOD: superoxide dismutase; TAC: total antioxidant capacity.

Discussion

Extensive research has shown that EGCG has significant antioxidant, anti-carcinogenic, and neuroprotective properties and has therapeutic potential against various human diseases (Chakrawarti et al., 2016). In this study, we investigated whether EGCG can protect against the pathophysiological processes of RP in the animal model P23H line. We chose the P23H rat, an animal model of RP that exhibits progressive rod dysfunction but initially shows normal cone function, which is broadly consistent with the clinical findings for patients with RP (Lu et al., 2013).

The assessment of VA and CS was performed by using an optomotor test. Our results showed a reduced visual function in P23H rats but an improved functioning in animals treated with EGCG. VA values decreased 13% and 12% in the vehicle group and EGCG-P23H rats, respectively, compared to wild-type rats; however, there was a significant improvement in CS with EGCG treatment because at the maximum peak, CS was reduced by 35% in transgenic P23H rats compared to wild-type rats but was only reduced by 15% in P23H EGCG-treated rats. There was no significant difference in CS value between wild-type and P23H rats treated with EGCG. CS was recently shown to be affected in patients with inherited retinal degeneration even though VA was relatively unaffected (Alahmadi et al., 2018). Therefore, CS is a more accurate parameter used to evaluate visual function than just VA. Thus, our results showed that due to its physicochemical properties, EGCG is able to reach the retina and improve visual function in EGCG-P23H animals.

Regarding retinal electrical activity, our results clearly showed a marked decrease (64% reduction) in wave amplitude in P23H rats compared to wild-type animals; however, administration of EGCG significantly slowed the loss of wave amplitude in P23H rats. Thus, the preservation of photoreceptors was correlated with the slower loss of b-wave amplitude registered with the ERG in scotopic conditions. The amplitudes observed in animals treated with EGCG were greater than those obtained in vehicle animals (there was an improvement of 24.6% with treatment versus the untreated animals at 1.4 log cd.s/m^2). These results are in accordance with previous studies in which it was demonstrated that the antioxidant, free radical scavenging and anti-inflammatory abilities of EGCG were responsible for a reduction in apoptosis in retinal cells (Qi et al., 2017; Ren et al., 2018; Rivera-Perez et al., 2020).

Oxidative stress is widely linked to the progression of neurodegenerative diseases (Yan et al., 2013; Fang et al., 2017; Islam, 2017; Mashima et al., 2018). Additionally, in patients who suffer from RP, the disorganization that the retina undergoes during the life of the patient is not only due to the mutations but also due to the imbalance in free radical levels (Shen et al., 2005; Tao et al., 2016). Oxidative stress is a common finding in ophthalmic disease; numerous studies support that the oxidative environment that it is created by these radicals contributes to the development of many ocular pathologies such as diabetic retinopathy, glaucoma, and age-related macular degeneration (Kruk et al., 2016). This cannot be solved with the patient's own antioxidant enzymes and systems and generates an increase in radical levels in both the intra- and extracellular spaces. The consequences are that biomolecules such as unsaturated fatty acids in the lipid membrane and numerous structural, plasmatic and membrane proteins and even DNA in the cells are modified and might lead to apoptosis. These processes have a negative feedback loop and are the cause of apoptosis in nearby cells, thus worsening the disease state.

In our study, treatment with EGCG significantly reduced the levels of lipid peroxidation compared to untreated animals, which was supported by other studies in which EGCG also diminished levels of peroxidized lipids (Orsolic et al., 2013; Koonyosying et al., 2018). Although we used adult animals in our study, there were significant differences between the LE and P23H untreated animals; this proved that the mutation in the rhodopsin gene that causes the disease can lead to systemic oxidative stress and general deterioration in health as previously reported (Perdices et al., 2018). In P23H animals treated with EGCG, levels of lipid peroxidation were significantly lower than those in LE animals (wild-type animals); this could be explained because EGCG, as a free radical scavenger, is able to stop radical chain reactions such as those produced during lipid oxidation. All of these results indicate the utility of EGCG in age-related pathologies and are supported by numerous studies linking oxidative stress increases with ageing (Garcia et al., 2011).

In addition to the ability of EGCG to reduce oxidative damage, we studied the ability to reduce nitrosative damage. NO is a messenger molecule synthesized by a group of isoenzymes referred to as nitric oxide synthase (NOS). At physiological level, NO plays a critical role in the eye (Reina-Torres et al., 2020). However, NO derived from nNOS is involved in retinal ganglion cell death by its action as a free radical (Brown and Borutaite, 2002). Previous studies have shown that nNOS inhibition is one mechanism by which EGCG protects retinal ganglion cell against ischemic damage (Peng et al., 2008). In this study, wild-type, transgenic P23H and EGCG-treated P23H animals revealed similar levels of nitrosative damage. Therefore, other studies are necessary to clarify the effect of EGCG on nitrate and nitrite levels in the liver tissue of this animal model.

Antioxidants play an important role in preventing the formation and release of free radicals and other potentially toxic oxidant species. Through the determination of TAC, the combined antioxidant capacity of antioxidants in biological fluids and other samples was measured. These results indicate the full capacity to counteract the action of ROS, the resistance to oxidative damage and the capacity to face diseases related to oxidative stress such as RP. In this work, as in a previous study (Perdices et al., 2018), liver TAC was reduced in transgenic P23H animals. We also showed that treatment with EGCG significantly increased TAC in P23H animals by 23.75% compared to untreated animals, which is in accordance with the observations in other diseases following treatment with EGCG (Skrzydlewska et al., 2002; Yokozawa et al., 2002; Ergen et al., 2017); SOD and CAT were both higher in the treated group. We did not find any differences in GST levels; different GST isoenzymes showed different patterns of expression, and we would need to look in other tissues to further analyze these molecules (de Waziers et al., 1990; Chow et al., 2007). Although previous studies have proven the favorable effect of EGCG on the expression of this enzyme family, its contribution in P23H rats is unclear.

In vitro and *in vivo* studies have demonstrated the antioxidant action of EGCG (Morley et al., 2005; He et al., 2018) and its protection against retinal degeneration promoted by different oxidants (Zhang and Osborne, 2006; Chen et al., 2012; Silva et al., 2013; Cia et al., 2014; Yang et al., 2016; Qi et al., 2017; Ren et al., 2018); however, its mechanism of action remains unclear. The effect of EGCG may be based on its potential antagonizing effect of the NMDA receptor as a glutamate receptor antagonist (Lee et al., 2004).

Recent studies have shown that EGCG also reduces glutamate excitotoxicity, promoting the viability of neuronal cells by inhibiting the ionotropic flow of Ca^{2+}, reducing the production of MDA and decreasing levels of ROS in a dose-dependent manner (Yu et al., 2010). This catechin modulates the interaction between some molecules such as tumor necrosis factor alpha and the activity of transcription factors to

suppress some stress-related signaling pathways as well as the expression of some pro-oxidant and antioxidant enzymes (Chakrawarti et al., 2016). All of these indirect antioxidant effects combined with its direct effects, such as ROS scavenging and metal ion chelating, may explain its effects in reducing oxidative damage and increasing antioxidant defenses in P23H transgenic rats as shown in our study.

A limitation of our study is that we only studied oxidative stress in the liver, knowing its systemic effect. A previous study showed that P23H rats had TAC values decreased more than 50% compared to the wild type animal at P23 (Perdices et al., 2018). The systemic oxidative stress status has an important role in the retinal inherited diseases. Systemic or topical administration should avoid intravitreal injection and we were able to demonstrate that EGCG arrives to the retina to improve functional results when compared to the untreated animals.

Ishizu et al. (2019) demonstrated the relationship between antioxidant status and levels of antioxidant enzymes in RP. They observed differences in serum levels of SOD3 and GPx affected by this disease, suggesting that the severity of the disease could affect these parameters. Measuring serum levels of antioxidant parameters might be an easier and non-invasive method to assess the progression of the disease. Further studies are needed to confirm the usefulness of this analysis. Another limitation of this study was the lack of investigation of retinal histology and the mechanism of photoreceptor death in RP. However, given the size of retinal tissue, more animals would have been needed to perform these analyses. We did not consider greater sacrifice is necessary to study what previous studies have already addressed.

In conclusion, the results obtained in the present study show that EGCG not only reduced the loss of visual function in pigmented P23H rats but also improved the general health of the animals through acting on different antioxidant enzymes and reducing oxidative damage. Further research is necessary to determine the mechanism of action of this catechin in the retina and if this improvement is a consequence of systemic amelioration or is independent. The fact that retinal degeneration could be mitigated by EGCG would provide clinical benefit to patients who suffer from these diseases.

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