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Regeneration of zebrafish retina following toxic injury

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ABSTRACT

The structure of the zebrafish retina appears to be very similar to that of mammals, that is why it is used as a model for studying the eye. Indeed, the zebrafish retina can regenerate itself through mechanisms of Müller cell reprogramming. In this research, adult zebrafish were exposed to aluminum to cause damage in the retina and thus evaluate the regenerative capacity of the damaged tissue. Histological and histochemical analyses assessed the retinal structure and the neurodegenerative process, respectively. An expression analysis of PARPs was carried out to verify whether a potential oxidative DNA damage happens. In addition, some genes involved in the regeneration process (*pax6a*, *pax2a*, *ngn1*, and *notch1a*) were analyzed. The data confirmed the toxicity of aluminum which caused retinal neurodegeneration, but also highlighted the ability of zebrafish to regenerate the retinal structure, repairing the damage and confirming its use as a good model for translational studies.

1. Introduction

Danio rerio, commonly referred to as zebrafish, is known to be one of the most widely used model organisms in basic and clinical research. Its transparent eggs, ease of reproduction, rapid development, and low maintenance cost have made it useful for many ecotoxicological studies (Avallone et al., 2015; Shankar et al., 2021; La Pietra et al., 2024). In addition, its 70 % homology with humans allows studies on various human diseases to be carried out (Howe et al., 2013; Shams et al., 2018). Some organs of the zebrafish have similar development and functioning to those of humans, as in the case of the eye (Richardson et al., 2017). The zebrafish and the human eyes originate from three different embryological tissues, the neuroectoderm, ectoderm, and mesenchyme. In particular, the retina originates from neuroectoderm (Richardson et al., 2017). Comparable to the human retina, the zebrafish retina eye is composed of three nuclear layers separated by two plexiform layers where synapses between nuclear layers occur (Richardson et al., 2017). Müller cells are glial cells that participate in the structure, homeostasis, and neuronal protection of the retina (Wan and Goldman, 2016). However, unlike humans, zebrafish can regenerate the retina (Jui and Goldman, 2024). The regeneration process begins with Müller cells, which following damage, reprogram themselves and repair it (Wan and Goldman, 2016).

It is currently known that Aluminum (Al) poses a threat to the environment and humans. It is the third most abundant metal on the planet and its increase is caused by anthropogenic sources (Igbokwe et al., 2019). It is toxic for terrestrial organisms that live in the soil such as snails and earthworms (Coleman et al., 2010; Guglielmi et al., 2024), and for aquatic organisms altering biochemical processes, growth and gill-breathing (Botté et al., 2022).

Aluminum is used for cooking utensils, packaging, cosmetics, and medicine, and its presence has been found in food, water, and air, so humans are inevitably exposed to it (Rahimzadeh et al., 2022). Once entered the human body Al can compete with other ions altering different metabolic processes (Mahieu et al., 2004; Kaur and Gill, 2005) and bind to proteins causing alterations in autogenous epitopes which are recognized as foreign by T cells with subsequent production of autoantibodies (Galiciolli et al., 2023). Aluminum was involved in several human diseases such as osteomalacia, anaemia, and dialysis encephalopathy (Malluche, 2002). It can pass the blood-brain barrier (Bryliński et al. 2023), and its storage in the brain was associated with Alzheimer's disease (AD), Parkinson's disease (PD), and amyotrophic lateral sclerosis (SLA) (Zatta et al., 2003; Kawahara and Kato-Negishi, 2011). In fact, Al promoted oxidative stress in the nervous tissue resulting in neurodegeneration that is the basis of neurological diseases (Exley, 2006). In our previous studies, we have demonstrated that Al altered redox homeostasis and caused apoptosis in zebrafish embryos (Capriello et al., 2021a). In the muscle and gills of zebrafish, the Al exposure

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induced histological and redox homeostasis alterations (Ferrandino et al., 2022; Napolitano et al., 2023). Furthermore, this metal caused oxidative stress, neurodegeneration (Capriello et al., 2021b), and DNA damage in the brain, with consequent poly(ADP-ribosyl)ation system alteration (Capriello et al., 2022). Poly(ADP-ribosyl)ation is a post-translational modification catalyzed by poly(ADP-ribose) polymerase (PARP) enzymes. PARPs use NAD^+ as a substrate to produce linear and/or branched ADP-ribose polymers (PAR), which are rapidly hydrolyzed by poly(ADP-ribose) glycohydrolase (PARG), an enzyme with both exo- and endoglycosidase activities (Barkauskaite et al., 2013). PARP can be considered a marker of pollution, and its activity seems to be related to the ageing process (Arena et al., 2019). Significant variations of enzyme activity in plants (Arena et al., 2014) and animals (Guerriero et al., 2018) tissues were also measured after exposure to xenobiotic agents such as metals. In addition, a balance between PARP activity/ PAR synthesis and PARG activity/ PAR degradation was demonstrated in zebrafish brain at 10 and 15 days of Al exposure. The presence of a complete poly(ADPribosyl)ation system ensured the cell survival, preventing death by parthanatos (Bianchi et al., 2023). Data collected so far on zebrafish have all shown the same trend. Aluminum caused damage at early times of exposure, while at longer times it returned to control-like conditions. Therefore, this work aimed to test if the zebrafish eye regenerates following toxic damage caused by aluminum. Adult zebrafish were exposed to 11 mg/L of Al, a concentration of polluted surface water (Agarwal et al., 2016) for 10, 15, and 20 days. We evaluated the general morphology of the retina, and the neurodegeneration process through histological and histochemical analyses. Biochemical analysis was performed to identify the presence of one or more PARP isoforms and to verify a possible alteration of their expression after exposure to aluminum for different days. Finally, the expression of *pax6a, pax2a, ngn1*, and *notch1a*, genes involved in the eye formation and regeneration phenomena (Thummel et al., 2008, 2010; Campbell et al., 2022) were also analyzed by quantitative Real-Time PCR (qRT-PCR).

2. Materials and methods

2.1. Zebrafish breeding

Adult zebrafish were housed in glass tanks with a photoperiod of 12:12 h light/dark, at a temperature of 28 °C and pH of 7.6, at the Department of Biology of the University of Naples "Federico II" (Capriello et al., 2022). Zebrafish were fed with a commercial diet (TetraMin Tropical Flake Fish®) supplemented with *Artemia* sp. Nauplii (Westerfield, 2000) and water parameters were monitored daily. All experiments were performed following the guidelines dictated by European regulations on animal welfare (Directive, 2010/63/EU) and approved by the Italian Ministry of Health (licence 147/2019-PR).

2.2. Treatment and sample collection

Three groups of 11 zebrafish each, were exposed to 11 mg/L of Al, prepared as previously described by Capriello et al. (2022), in three different tanks of 25 L for 10 (T10), 15 (T15), and 20 (T20) days respectively. Another group of 11 zebrafish was exposed to water only (1/3 distilled water, 2/3 tap water) and used as control group (Ctrl). Once a day the solution was renewed, and food debris was removed. Each experiment was repeated in triplicate and animal welfare was maximized by reducing according to the 3Rs principle (Replacement, Reduction and Refinement). At the end of each exposure period, animals were sacrificed by an overdose of ethyl 3-aminobenzoate methane sulfonate (MS-222 solution, Sigma Aldrich, Germany, Cas No. 886–86–2) (300 mg/L), and the eyes were collected for analyses.

2.3. Hematoxylin-eosin staining

Three eyes for each group were processed for light microscopy. The samples were fixed in Bouin's solution for 48 h, dehydrated, and included in paraffin. Sections of 5 μm were deparaffinized, hydrated, and stained with hematoxylin-eosin. The images were acquired using light Axioskop 50 microscope (Carl Zeiss, Germany) equipped with Axiocam 305 color camera (Carl Zeiss, Germany) and Axiovision 4.8 Software (Carl Zeiss, Germany). The thickness of each layer was measured using Image J software by setting the scale bar of 5 μm. Three measurements were taken for each layer and repeated in triplicate.

2.4. Fluoro-Jade B

Fluoro-Jade B (FJB) (AG310 EMD, Millipore, Billerica, MA, USA) is an anionic fluorochrome derived from fluorescein that was used to reveal neuronal degeneration. After deparaffinization, sections of 5 μm were incubated for 1 h at 4 °C with FJB, in the dark with gently orbital shaken, and then were dried at room temperature in the dark overnight. Lastly, they were clarified and mounted. The images were acquired with Axioskop 50 microscope (Carl Zeiss, Germany) equipped with fluorescence, using the FITC channel, Axiocam 305 color camera (Carl Zeiss, Germany) and Axiovision 4.8 Software (Carl Zeiss, Germany). Semi-quantitative analysis of fluorescence spots was performed by ImageJ Software on binary image using the "threshold" tool. Positive Fluoro Jade B cells were counted using the "analyze particles" tool on the total area of the single image. Finally, the total cells counted was reported as ratio (Monaco et al., 2017).

2.5. Homogenate preparation

Eye samples (10 mg) exposed and not exposed (control) for 10, 15, and 20 days to 11 mg/L Al from adult zebrafish were homogenized in Buffer A containing 10 mM Tris-HCl (pH 7.5), 1 mM EDTA, 1 mM EGTA (pH 8), 1 mM β-mercaptoethanol, 0.15 mM spermine, 0.75 mM spermidine, 1 mM PMSF, and 2 μg/mL protease inhibitor cocktail, as described in Bianchi et al. (2023). Protein concentration was determined by the Bradford assay (#5000006, BioRad, Hercules, CA, USA).

2.6. Electrophoresis and western blotting of PARP

Electrophoresis and the subsequent western blotting were performed according to Bianchi et al. (2023). Two aliquots of each homogenate (20 μg) were analyzed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis on 12 % gel di polyacrylamide (SDS-PAGE) in 0.025 M Tris-0.192 M glycine-0.1 % SDS buffer, pH 8.3 at 18 mA. One part of gel was stained with 0.1 % Coomassie G in 10 % acetic acid and 30 % methanol, to evidence protein pattern, while the other part was transferred onto a polyvinylidene fluoride (PVDF) filter (0.45 μm; IPVH00010, Merck Millipore, Milano, Italy) at 200 mA in buffer containing 0.025 M Tris, 0.192 M glycine and 0.025 % SDS for 2 h at 4 °C. The membrane was blocked for 2 h at room temperature with 3 % gelatine in Tris-buffered saline (50 mM Tris-HCl and 150 mM NaCl, pH 8.0) containing 0.05 % Tween-20 (TBS-T). After repeated washings in TBS-T, the immunoblotting was carried out using monoclonal anti-poly (ADP-ribose) polymerase (sc-8007, Santa Cruz Biotechnology, Inc., Texas, USA, 1:500) and horseradish peroxidase (HRP)-conjugated anti-mouse secondary (sc-525409, Santa Cruz Biotechnology, Inc., Texas, USA, 1:2000).

The detection of peroxidase activity was determined using a chemiluminescence kit (ECL, 32106, Thermo Fisher Scientific Inc., Waltham, MA, USA), and the images were acquired by the ChemiDoc XRS system (Bio-Rad). The immunopositive signals to anti-PARP antibodies, corresponding to proteins of about 113 kDa and 70 kDa were quantified by densitometry analysis using the Image Lab software (BioRad, Hercules, CA, USA). The measured intensity/mm^2 values were expressed as ratio

to the control samples.

2.7. Quantitative real-time PCR

To analyze the expression gene of *pax6a*, *pax2a*, *ngn1*, and *notch1a*, qRT-PCR was performed. Total RNA from zebrafish eyes of each experimental group was extracted using Direct-zolTM RNA Miniprep Plus Kit (R2050, ZYMO RESEARCH, Irvine, CA, USA). The concentration and purity of RNA were verified using Nanodrop® spectrophotometer 2000 (Thermo Scientific Inc., Waltham, MA USA) and then 1000 ng of total RNA were retrotranscribed in cDNA with All-In-One 5X RT MasterMix (G592, Applied Biological Materials, Richmond, BC, Canada). An aliquote of 2 μL of cDNA was used in a reaction containing 0.5 μL of each primer (Table 1) (Eurofins Genomics, Ebersberg, Germany) at 10.0 μM for qRT-PCR using BlastTaqTM 2X qPCR MasterMix (G891, Applied Biological Materials, Richmond, BC, Canada). The reaction was carried out with StepOnePlus Real-Time PCR System (Thermo Fisher Scientific, Waltham, MA, USA) and the thermocycling conditions were: one cycle for enzyme activation (95 °C for 3 min), 40 cycles for denaturation and annealing/extension (95 °C for 15 s, 60 °C for 1 min) and melting curve analysis following the instrument instructions. The expression gene levels were analyzed by the Ct value and normalized to *β-actin*, using the REST software (Relative Expression Software Tool, Weihenstephan, Germany, version 1.9.12) based on Pfaffl's method (Pfaffl, 2001; Pfaffl et al., 2002).

2.8. Statistical analyses

Statistical analysis was performed using GraphPad Prism Software (version 8.02 for Windows, GraphPad Software, La Jolla, CA, USA). One-way analysis of variance (ANOVA) method following by Tukey's test was used for layers thickness measurements, semiquantitative analysis of FJB and expression gene levels between treated groups. The data were statistically significant at $p < 0.05$. Statistically significant differences between the intensity values of aluminum exposed samples compared to the control were analyzed using ANOVA followed by the Holm-Sidak multiple comparison test and the minimum acceptable level of significance was set at $p < 0.05$. All experiments were repeated at least in triplicate and data were expressed as mean ± SEM.

3. Results

3.1. Hematoxylin-eosin

The zebrafish control retina showed the typical organization with different cell layers (Fig. 1A). In the retina at 10 days of aluminum exposure (Fig. 1B), the ganglion cell layer (GCL) was altered showing

Table 1
Name, primer sequences, accession No. and references of genes used for qRT-PCR: **F** (forward); **R** (reverse).

Gene	Primers		Accession No.	Reference
β-actin	F	CGAGCAGGAGATGGGAACC	NM_131031.1	Babich and Van Beneden, (2019)
	R	CAACGGAAACGCTCATTGC		
pax6a	F	CTGACGTTTTTGCACGAGAA	NM_131304.1	Babich and Van Beneden, (2019)
	R	AAAGGATACTGGCGTTGTGG		
pax2a	F	TCTCACCCGCAGTACACAAC	NM_131184.2	Babich and Van Beneden, (2019)
	R	CTAGTGGCGGTCATAGGCAG		
ngn1	F	CAGAAGCAGGGCAAGTCAAG	NM_131041.1	Babich and Van Beneden, (2019)
	R	CACTACGTCGGTTTGCAAGT		
notch1a	F	ACATCACCCTTCCAGCAGTC	NM_131441.1	Uribe et al., (2012)
	R	AGGCTTCCCTAAACCCTGAA		

cellular disorganization compared to the control retina. At 15 days of exposure, the thickness of INL, IPL and NFL was reduced compared with control and other treated groups (Fig. 1C) and an alteration in the cellular organization of PRL layer was observed. This alteration appeared to return to a condition like that of the control at 20 days of exposure (Fig. 1C, D). At 20 days of exposure the NFL thickness, although it was still reduced compared with the control, showed a recovery respect to 15 days of exposure (Fig. 1D). The quantification of layer thickness was reported in Fig. 1E and confirmed what was observed in the histological images. The values of thickness layer were reported in supplementary materials (Table S1).

3.2. Fluoro-Jade B

Using Fluoro-Jade B it was possible to identify degeneration phenomena in the retina through fluorescence signals. The fluorescence spots were most evident in retina of zebrafish at 15 days of exposure (Fig. 2C), while no difference emerged in retina at 10 and 20 days of exposure (Fig. 2B, D) compared with control retina (Fig. 2A). Semi-quantitative analysis confirmed an increase of fluorescence cells in retina at 15 days of exposure compared with control retina and with retina at 20 days of exposure (Fig. 2E). The ratio shown in the graph represents the number of cells counted in treated compared to those counted in the control and represented the average of three experiments. Values of semi-quantitative analysis were reported in supplementary materials (Table S2).

3.3. PARP Expression

Electrophoretic analysis conducted on eye homogenates of zebrafish control and exposed to Al for 10, 15, and 20 days showed no significant qualitative and quantitative differences in the protein patterns (Fig. 3 A).

Immunoblotting, performed with anti-PARP antibody, able to cross react with the highly conserved catalytic site of PARPs, allowed to identify several enzyme isoforms with different molecular weight (Fig. 3B). Among them, PARP1(~113 kDa) and PARP2 (~70 kDa), two nuclear enzymes were recognized. At 10 days of exposure, the intensity of both bands was higher than that measured in the control homogenates (Fig. 3 C, D). Densitometric quantification values of the ~113 kDa and ~70 kDa bands are reported in supplementary materials (Table S3). At longer exposure, instead, their intensity returned comparable to that measured in the control (Fig. 3 C, D). Finally, an immunoreactive signal of about 18 kDa was detected too. It could correspond to Gig2, a protein with a catalytically active PARP domain, known for its antiviral properties.

3.4. Gene expression

The graph (Fig. 4) showed the fold changes of gene expression analyzed through qRT-PCR. Values of fold change were reported in Table S4. *Pax6a* was up-regulated at T10 and mostly at T15 compared to the control group, but there was a decrease of up-regulation at T20 compared to other groups. *Pax2a* and *ngn1* were up-regulated only at T15 while at T20 of exposure returned in conditions similar to control. Instead, the *notch1a* gene was up-regulated at all exposure times compared to the control group. In addition, at T15 and T20 were up-regulated to a greater extent than T10.

4. Discussion

The zebrafish retina regeneration appears to occur from 10 days of exposure, when an increase in the expression of PARPs, which are activated to repair DNA damage, is observed. At 15 days of exposure an up-regulation of genes involved in the regeneration process is observed, and at 20 days of exposure, repair and recovery of damage is observed at
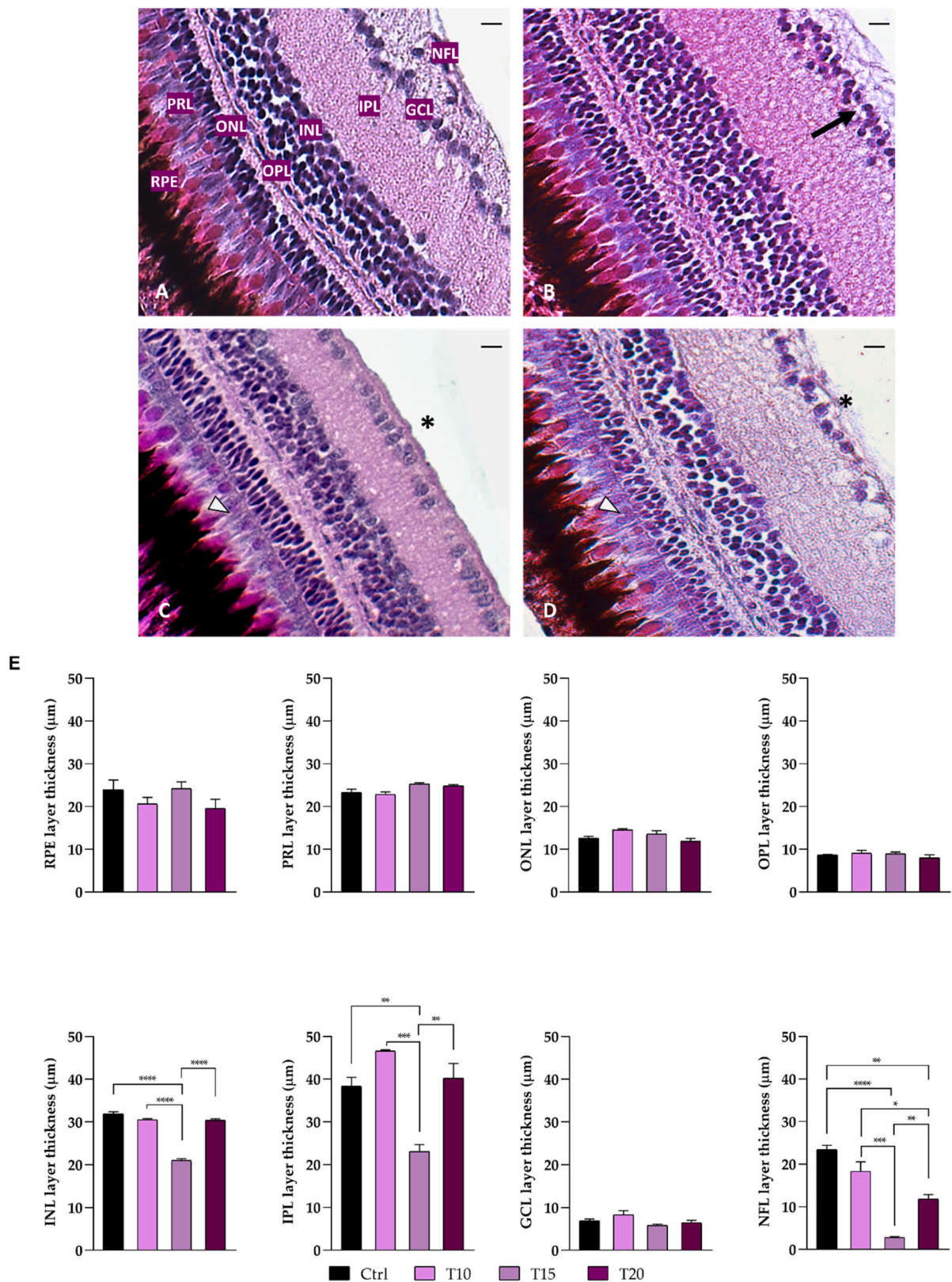
Fig. 1. Histological cross section of zebrafish retina with hematoxylin-eosin staining. Typical layers of the retina: (RPE) retinal pigmented epithelium; (PRL) photoreceptors layer; (ONL) outer nuclear layer; (OPL) outer plexiform layer; (INL) inner nuclear layer; (IPL) inner plexiform layer; (GCL) ganglion cell layer; (NFL) nerve fiber layer. (A) control retina (Ctrl); (B) retina at 10 days of exposure (T10); (C) retina at 15 days of exposure (T15); (D) retina at 20 days of exposure (T20). (Black arrow) disorder in GCL (B); (White arrowheads) alterations of photoreceptor layers (C, D); (*) alterations of NFL (C, D). Scale bar: 5 μm. (E) Layer thickness measurements (n=3). Statistical differences were determined by ANOVA followed by Tukey's test (* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$; **** $p < 0.0001$).
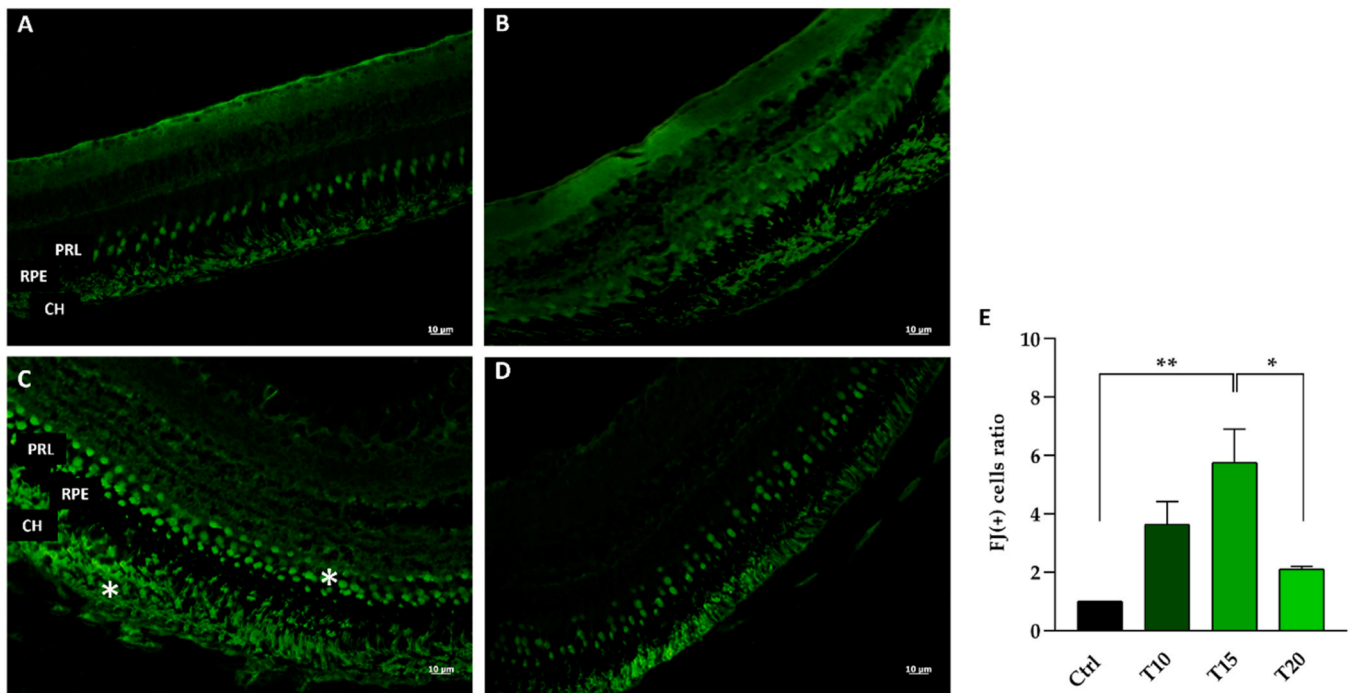
Fig. 2. Fluoro-Jade B staining on zebrafish retina. (CH) choroid; (RPE) retinal pigmented epithelium; (PRL) photoreceptors layer. (A) control retina; (B) retina at 10 days of exposure (T10); (C) retina at 15 days of exposure (T15); (D) retina at 20 days of exposure (T20). (*) increased fluorescence (C). Scale bars: 10 μm. (E) Semi-quantitative analysis (n=3) was reported as the ratio of fluoro-jade positive cells. Statistical differences were determined by ANOVA followed by Tukey's test (* $p < 0.05$; ** $p < 0.01$).

the histological level.

Other studies have shown the regeneration of zebrafish retina following neurotoxin injections (Fimbel et al., 2007; Sherpa et al., 2008). In this study, aluminum was used to induce damage in the zebrafish eye. Hematoxylin-eosin staining revealed a reduction in photoreceptors and nerve fiber layers and disorganization of ganglion cell layers at 10 and 15 days of exposure while at 20 days of exposure, recovery was observed in all retina layers. Damage to the nerve fiber layer observed in the zebrafish retina may be related to neurodegeneration found previously in the brain (Capriello et al., 2022). The explanation of how Al interacts with human neurons was provided by Cirovic et al. (2023). Transferrin binds aluminum, and subsequently neurons pick up transferrin thus interacting with aluminum (Cirovic et al., 2023). In the cells, aluminum could compete with other ions involved in normal physiological processes, could interact with proteins causing uncontrolled phosphorylation, inducing conformational changes in peptides, and altering entire signaling cascade systems (Kaizer et al., 2008). The damage shown by our data at the nerve fiber layer level in zebrafish could be due to the uptake of aluminum by neurons, which could change cellular metabolism as previously explained, resulting in the alteration of the whole nerve structure with complete absence at 15 days of exposure. At this time of exposure, a reduction in retinal thickness and an alteration of photoreceptors layer were observed, as also by Lu et al. (2002) in rats at 16 weeks of exposure with disappearance in the plexiform layers (outer and inner) and photoreceptors layer. Aluminum may interact with the chromatin of the neurons or dendrites of plexiform and photoreceptor layers causing their changes (Lu et al., 2002).

Fluoro-Jade B has been shown to have affinity for retinal degenerating cells (Fernandes et al., 2004). Our data showed neurodegeneration mainly in the layers of the pigmented epithelium and photoreceptors at 15 days of exposure that returned in normal conditions at 20 days of exposure. The neurodegeneration in the retina could be compared to that previously observed in the brain (Capriello et al., 2022). The neurodegeneration phenomena in these two anatomical structures (retina

and brain) could be related, although the mechanisms of this correlation are still uncertain (Mancino et al., 2019). One hypothesis for neurodegeneration could be correlated to apoptotic processes induced by aluminum as previously demonstrated by Lukiw et al. (2005). The hypothesis that explained the mechanisms by which aluminum induced apoptosis, suggests that it may interfere with the regulation of pro-apoptotic and anti-apoptotic gene expression. Aluminum may replace magnesium in the bond between DNA and protein and alter the transcription of genes involved in apoptosis processes (Suárez-Fernández et al., 1999). In addition, it was demonstrated that pigmented epithelium absorbs the metals through fenestrated capillaries in the choriocapillaris and retains them trying to protect the innermost layers of the retina (Pamphlett et al., 2020). Probably, at 15 days of exposure, the pigmented epithelium did not retain aluminum, which acted at the level of photoreceptors inducing apoptosis and then neurodegeneration.

When damage occurs to the zebrafish retina, the dying cells send signals to Müller cells for activation (Wan and Goldman, 2016) which re-enter the cell cycle, and Müller-associated retinal progenitors can dedifferentiate and function as multipotent retinal stem cells to regenerate other types of retinal neurons (Bernardos et al., 2007; Campbell et al., 2022). In this case, the cells damaged by aluminum toxicity send signals to Müller cells to activate the regeneration process. The acquisition of stem cell properties by Müller cells is associated with changes in gene expression that precede the entire regeneration process. Changes in gene expression are associated with altered DNA methylation and thus activation of some genes (Powell et al., 2013). *Pax6a*, *pax2a*, and *ngn1* genes are involved in retinogenesis and development of zebrafish eye (Babich and Van Beneden, 2019), but these genes have also been shown to be expressed during regeneration events both in zebrafish and other organisms (Thummel et al., 2008, 2010; Parrilla et al., 2012). In fact, the inhibition of *pax6a* blocks the division of neuronal progenitor cells (Brockerhoff and Fadool, 2011). The up-regulation of *pax6a*, *pax2a*, and *ngn1* mainly at 15 days of exposure could indicate that Müller cells were proliferating to regenerate the retina. The return to conditions similar to
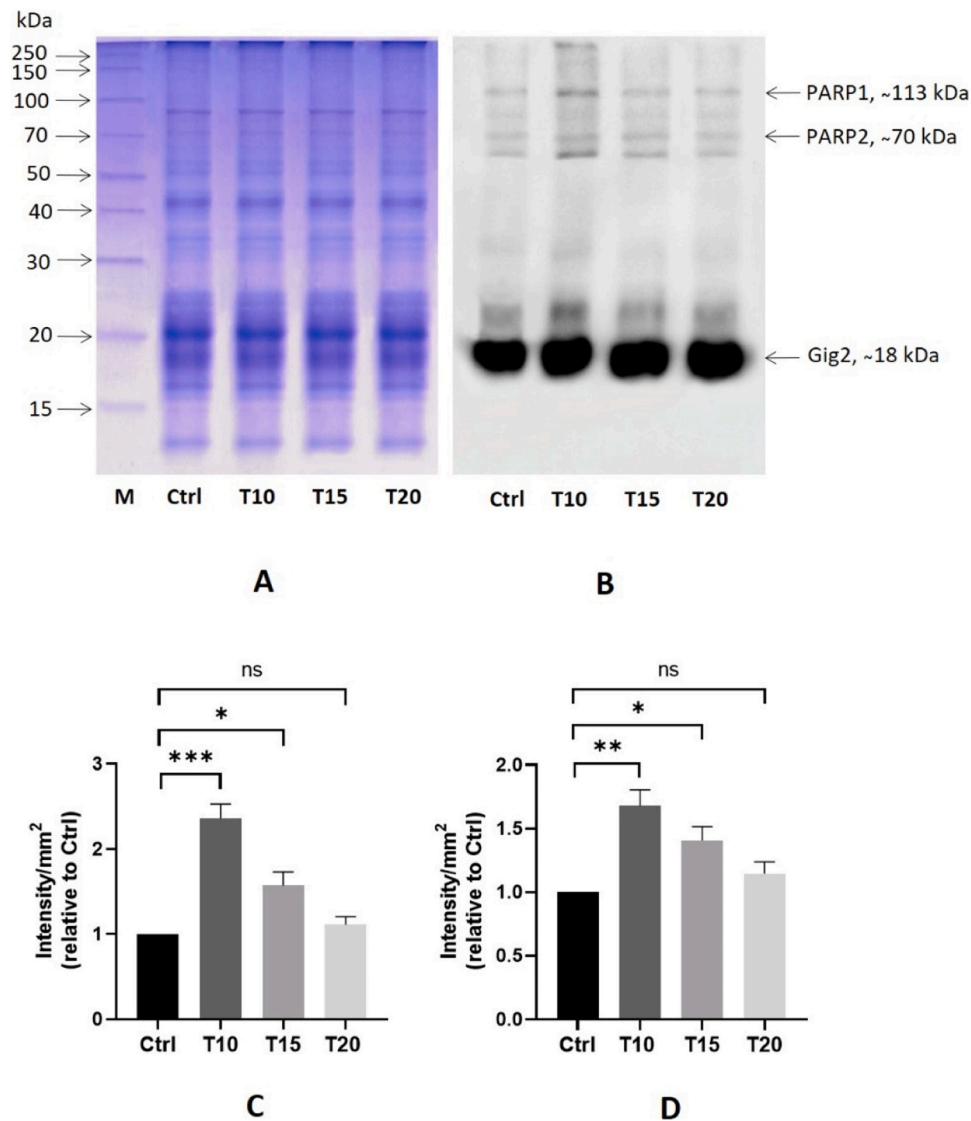
Fig. 3. 12 % SDS-PAGE (A), immunoblotting with anti-PARP antibodies (B) of zebrafish eyes control (Ctrl) and exposed to Al for 10 (T10), 15 (T15) and 20 (T20) days. Densitometric analysis of ~113 kDa (C) and ~70 kDa (D) bands. Data are presented as mean (n=4) ± SEM (error bars). Asterisks (* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$) indicate significant differences in the samples exposed to Al compared to the control; n.s., not significant.
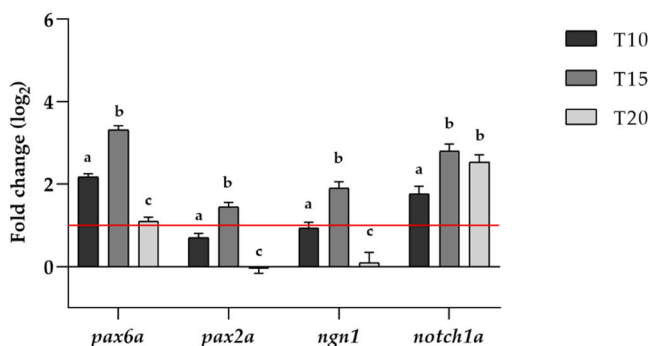

Fig. 4. Gene expression analysis (n=4). Fold changes are calculated according to the formula fold change $= 2^{-\Delta\Delta Ct}$. The red line indicates fold change thresholds of 2. Fold changes greater than 2 were considered significant compared to control. For each gene, different lowercase letters indicate significant differences between treated groups and same letters indicate no significant differences (Tukey's test).

control at 20 days of exposure could indicate that the damage was re-entered and that retinal function has been restored. Regarding *notch1a*, it is involved in the differentiation of retinoblasts during zebrafish retinogenesis (Uribe et al., 2012). However, Campbell et al. (2021) demonstrated the involvement of *notch1a* during retinal regeneration. Notch signaling plays a key role during retinal regeneration, regulating Müller cells proliferation during dedifferentiation, and inhibiting it when it is not needed (Wan et al., 2012). The expression of *notch3* is reduced during retinal proliferation, while other components of notch signaling, such as *notch1a* or *Delta*, are required for the continuous proliferation of neuronal progenitor cells (Campbell et al., 2022). In fact, suppression of *notch1a* during retinal injury caused decreased production of proliferating Müller cells and progenitors (Campbell et al., 2021). The up-regulation of *notch1a* that emerged from our data is in line with what has been reported in the literature indicating that neuronal progenitor cells are in proliferation. In addition, since *notch1a* is involved in the differentiation of glial cells, its up-regulation at 20 days could be related to progenitor cells that are still undergoing differentiation into glial cells in agreement with what has already been observed by Scheer et al. (2001) which showed Müller cells continue to express activated *notch1a*.

Indirect evidence of aluminum-induced DNA damage is provided by the increased expression of PARP1 and PARP2, the two nuclear enzymes that, for the first time, were identified in the zebrafish eye. In detail, the expression of these two nuclear proteins increased already after 10 days of aluminum exposure. This data suggests that genotoxic DNA damage occurs before histological damage and seems to confirm the fundamental role of PARPs in maintaining genomic integrity (Eisemann and Pascal, 2020). The re-entered of both histological and molecular damage, confirmed by the PARP expression decrease at longer exposure times (20 days) is probably due to an adaptive mechanism already observed in our previous studies on gills, muscle, and brain of zebrafish (Capriello et al., 2022; Ferrandino et al., 2022; Bianchi et al., 2023; Napolitano et al., 2023). The presence of Gig2, a protein with catalytically active PARP domains, was identified in all experimental groups. The detection of this protein has been previously demonstrated in healthy zebrafish, but its mechanism has not yet been clarified (Zhang et al., 2013; Madushani et al., 2021). Further studies are needed to explain the presence of Gig 2 in the zebrafish eye.

5. Conclusion

The aluminum-damaged retina has proven to be a good experimental approach for studying regeneration in zebrafish. Regeneration begins by sending signals to Müller cells, which are activated and dedifferentiate retinal progenitor cells. Our data showed that genes involved in regeneration were up-regulated and that a DNA repair system was activated when aluminum damage is present. However, both genes and the repair system returned to physiological conditions when the damage was repaired. The zebrafish retina is ideal for studying the mechanism of regeneration, and the physiological and structural similarity to the human retina lays the foundation for understanding whether it is possible to stimulate a regeneration process even in mammals in which these mechanisms are not present.

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CRediT authorship contribution statement

Anna Rita Bianchi: Writing – original draft, Methodology, Investigation, Data curation, Conceptualization. **Alessandra La Pietra:** Writing – original draft, Methodology, Investigation, Data curation, Conceptualization. **Teresa Mobilio:** Methodology, Investigation. **Teresa Capriello:** Methodology, Investigation. **Anna De Maio:** Writing – review & editing, Validation, Supervision, Formal analysis, Conceptualization. **Annamaria Guagliardi:** Writing – original draft, Investigation. **Ida Ferrandino:** Writing – review & editing, Validation, Supervision, Project administration, Formal analysis, Conceptualization.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.etap.2024.104582.

Data availability

Data will be made available on request.

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