Ketorolac Administration Attenuates Retinal Ganglion Cell Death After Axonal Injury

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Purpose. To assess the neuroprotective effects of ketorolac administration, in solution or delivered from biodegradable microspheres, on the survival of axotomized retinal ganglion cells (RGCs).

Methods. Retinas were treated intravitreally with a single injection of tromethamine ketorolac solution and/or with ketorolac-loaded poly(D,L-lactide-co-glycolide) (PLGA) microspheres. Ketorolac treatments were administered either 1 week before optic nerve crush (pre-ONC) or right after the ONC (simultaneous). In all cases, animals were euthanized 7 days after the ONC. As control, nonloaded microspheres or vehicle (balanced salt solution, BSS) were administered in parallel groups. All retinas were dissected as flat mounts; RGCs were immunodetected with brain-specific homeobox/POU domain protein 3A (Brn3a), and their number was automatically quantified.

Results. The percentage of Brn3a$^+$RGCs was 36% to 41% in all control groups (ONC with or without BSS or nonloaded microparticles). Ketorolac solution administered pre-ONC resulted in 63% survival of RGCs, while simultaneous administration promoted a 53% survival. Ketorolac-loaded microspheres were not as efficient as ketorolac solution (43% and 42% of RGC survival pre-ONC or simultaneous, respectively). The combination of ketorolac solution and ketorolac-loaded microspheres did not have an additive effect (54% and 55% survival pre-ONC and simultaneous delivery, respectively).

Conclusions. Treatment with the nonsteroidal anti-inflammatory drug ketorolac delays RGC death triggered by a traumatic axonal insult. Pretreatment seems to elicit a better output than simultaneous administration of ketorolac solution. This may be taken into account when performing procedures resulting in RGC axonal injury.

Keywords: NSAIDS, neuroprotection, PLGA, microspheres, ketorolac, Brn3

The rodent retina is often used to study the toxicity of different treatments or pharmacologic formulations on central nervous system (CNS) neurons. The retina is easily reached for experimental manipulations, and injury to it is less detrimental for the animal than injury to other CNS structures. Thus, retinal populations have been well characterized, and a number of injury models have been developed and characterized for the retina, such as ischemia,[1,2] ocular hypertension,[3–9] or axotomy.[10–15] Optic nerve axotomy is a well-established model to study the neuroprotective effects of a given treatment on the survival of injured CNS neurons.[16–22] Optic nerve axons arise from retinal ganglion cells (RGCs), the only efferent neurons of the retina. Upon optic nerve axotomy, either crush or transection, the majority of RGCs die within the first 2 weeks[13,23] without concomitant death of other retinal neurons. Thus, optic nerve axotomy is an excellent model to carry out in vivo proof-of-concept assays aimed to test the efficacy of a drug, compound, or drug delivery system with potential neuroprotective efficacy.

It is well known that a common physiological response to injury is an inflammatory response. Inflammation is needed to resolve the insult, but in the nervous system this event has a dual role, exacerbating the neuronal loss or impeding regeneration. Corticosteroids present high anti-inflammatory properties and have been tested in animal models of CNS injury[24–29]; they are often used in patients with ocular diseases such as optic nerve neuropathy or diabetes.[30–32] Besides the side effects of corticosteroids,[33,34] it has been reported that these therapeutic agents increase neuronal apoptosis and axonal loss.[35–37] In ophthalmology, nonsteroidal anti-inflammatory drugs (NSAIDs) have a modulating effect on ocular inflammation and pain through the prevention of prostaglandin synthesis by nonspecific cyclooxygenase enzyme (COX) inhibition.[38] This has prompted exploration of the use of NSAIDs in patients and animal models.[39–50]

Ketorolac belongs to the group of NSAIDs, being a nonselective inhibitor of the COX enzyme. Cyclooxygenase-1 and COX-2 are expressed by RGCs, among other cells, in the

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rodent retina,[51] and they are upregulated in the retina after ischemia[52] and optic nerve injury.[53] The safety, pharmacokinetics, and anti-inflammatory efficacy of intravitreal administration of ketorolac have been studied in rabbits.[40,54,55] However, there are no studies related to the neuroprotective effect of ketorolac after optic nerve axotomy.

Intraocular drug delivery systems (IDDS) have been developed to provide sustained concentrations of therapeutic molecules in the target site during the long term. Depending on the size, they are classified as implants (>1 mm), microparticles (1–1000 μm), and nanoparticles (1–1000 nm).[56] Among them, implants and microparticles are able to deliver the drug for months, avoiding repeated administrations. Both drug delivery systems have been loaded with different drugs for the treatment of retinal diseases.[57] The inherent advantages over larger devices are that microsized IDDS can be administered as a conventional injection and that different amounts of particles can be administered depending on patient needs. According to their structure, microparticles are referred to as microcapsules (reservoir) or microspheres (matrix).[58] Biodegradable microspheres release the drug and then undergo degradation at the site of administration.[57] The most frequently used polymers to prepare biodegradable drug delivery devices belong to the group of polyesters, mainly poly (lactic) and poly (glycolic) acid and their derivatives.[59,60] These biomaterials degrade to metabolic products and are easily eliminated from the body. The use of biodegradable microspheres for intraocular delivery has become more popular over the last few decades. Biodegradable microspheres have been loaded with different active substances and intended for posterior segment diseases such as diabetic retinopathy, uveitis, glaucoma, macular edema, or age-related macular degeneration.[56] Once in contact with aqueous media, an initial release due to the penetration of water on the matrix pores is typically observed. After that, the controlled release is governed by a combination of polymer diffusion and erosion due to hydrolysis of ester bonds. These long-acting injectable drug formulations represent an effective alternative to repeated intraocular injections.

The question arises as to the potential advantage of using ketorolac in solution as a single injection or delivered in low amounts from a biodegradable drug delivery system (microspheres) in an acute injury model of axonal trauma. In the present work we have tested the effect of an intravitreal solution of ketorolac and/or delivered from poly(D,L-lactide-co-glycolide) (PLGA) microspheres on RGC survival after optic nerve crush in rats. The two treatments have been evaluated after administration simultaneous with the optic injury or before the injury.

Materials and Methods

Materials

Ketorolac was supplied by Chemos GmbH (Regentstauf, Germany). Poly(D,L-lactide-co-glycolide) (PLGA) 85:15 (MWt 87,000 g/mol, inherent viscosity = 0.62 dL/g in $CHCl_3$) was purchased from Lactel (Durect Corporation, Birminghan Division, Pelham, UK). Polyvinyl alcohol 67,000 g/mol (PVA) was supplied by Merck KGaA (Darmstadt, Germany). Tetrahydrofurane, HPLC grade (THF; Romil, Teknokroma, Barcelona, Spain), and ammonium dihydrogen-phosphate crystalin extrapure ($NH_4H_2PO_4$; Merck KGaA) were used to prepare the mobile phase in HPLC determinations.

Microsphere Preparation

Microspheres (MP) were prepared according to the O/W emulsion solvent extraction-evaporation technique.[61] Briefly, 400 mg PLGA were dissolved in 1400 μL of a mixture of methylene and acetonitrile (3:1). After dissolution, 60 mg ketorolac were suspended in the organic solution by ultrasonication in a water/ice bath for 5 minutes (Transsonic 460; Elma, Singen, Germany). Then the suspension was gently sonicated at low temperature (Sonicator XL; Heat Systems, Inc., Farmingdale, NY, USA) for 1 minute. This prepared organic phase was emulsified with 5 mL PVA MilliQ water solution (1% wt/vol). Emulsification was performed in a homogenizer (Polytron PT 3000, RECO; Kinematica GmbH, Lucerna, Switzerland) at 9500 rpm for 2 minutes. Subsequently, 10 mL PVA solution (0.1%) was added to the emulsion and kept at the same homogenization conditions for an extra minute. The formed emulsion was finally poured onto 90 mL aqueous PVA solution (0.1%) and maintained under agitation for 3 hours to allow the evaporation of the organic solvents. Once formed, MP were washed with MilliQ water to eliminate PVA and sieved according to their particle size (40, 10, and 1 μm). The mature MP were then frozen and freeze-dried. Lyophilized MP were kept at −20°C under dry conditions until use.

Microsphere Characterization

The production yield percentage (PY%) of each batch was calculated from the following equation:

$$\mathrm{PY}(\%) = \frac{M_T(\text{microspheres})}{M_T(\mathrm{PLGA}) + M_T(K)} \times 100$$

where M_T is the mass of each component in the formulation.

Morphologic evaluation of MP was performed by scanning electron microscopy (SEM) (JSM-6335F; JEOL, Tokyo, Japan). The samples were gold sputter-coated before observation.

Mean particle size and particle size distribution were measured by light scattering (S3500; Microtrac, York, PA, USA). Samples were prepared by suspending MP in distilled water.

Encapsulation Efficiency

The content of the encapsulated anti-inflammatory agent in the MP was determined as follows: Briefly, an amount (10 mg) of ketorolac-loaded MP was placed in methylene chloride (5 mL). After dissolution of the PLGA, the drug was extracted with 12 mL ethanol that promoted polymer precipitation. After vortex mixing, the samples were centrifuged at 5000 rpm for 5 minutes and the ethanolic supernatant was recovered and filtered (0.45 μm). The ketorolac content in ethanol was quantified by HPLC as described below.

In Vitro Release Studies

Release experiments were performed by suspending an amount (10 mg) of MP in 1.5 mL phosphate buffer saline (PBS). Then the samples were placed in a water shaker bath with a constant agitation of 100 rpm at 37°C (Clifton Shaking Bath NE5; Nikel Electro Ltd., Avon, UK). At defined time intervals (1 hour, 24 hours, 3 days, and once a week until the end of the assay, 42 days), MP suspensions were centrifuged at 5000 rpm for 5 minutes, and the supernatants were removed and replaced by the same volume of fresh medium to continue the release study. After filtration (0.45 μm), the concentration of the released ketorolac from the MP was measured by HPLC as described below.

Ketorolac Quantification by High-Performance Liquid Chromatography (HPLC)

Ketorolac HPLC quantification was performed according to the 1755 monograph of the European Pharmacopoeia 8.5 (2015). The

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Table 1. Experimental Design

	No Lesion to Optic Nerve: Toxicity Assay	ONC and Treatment	Treatment→7 d Later ONC	ONC
Treatment	Analysis at 14 d	7 d therapy and lesion	14 d therapy, 7 d lesion	7 d lesion
BSS, vehicle		6		
Ketorolac solution, KET-SOL	4	8	7	
Nonloaded microspheres, NL-MP	6	9	7	
Ketorolac microspheres, KET-MP	6	8	8	
Ketorolac microspheres + ketorolac solution, KET-MP+KET-SOL		7	6	
None	6* (naive)			5

Number of retinas analyzed per group.
* Naive intact animals were used as control of the RGC population.

analysis was carried out on a Waters 717 Plus system composed of 600E pump, an autosampler Waters 717 plus (Waters, Barcelona, Spain). The reverse-phase liquid chromatography analyses were performed with a Mediterranean Sea 18 column (15 cm × 0.4 mm, 5-µm particle size; Teknokroma) preceded by a guard cartridge SEA 18 (100 × 4.0 mm; Teknokroma). The column was maintained at 40°C using a heating column system (Waters TCM/CMM). The mobile phase was a mixture of an aqueous solution of ammonium dihydrogen-phosphate 0.05 M (pH = 3): tetrahydrofurane (70:30, vol/vol). The flow rate was set at 1 mL/min, and the absorbance was monitored at 313 nm (Waters 486 UV/Vis detector). The column was eluted over minutes and peak areas were determined with the Empower Login software (Waters, Milford, MA, USA). The concentration of ketorolac was calculated from a calibration curve.

Animal Handling, Anesthesia, and Analgesia

Two-month-old female albino Sprague-Dawley (180–220 g body weight) rats were obtained from the University of Murcia breeding colony. All experimental procedures were carried out in accordance with the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research and the European Union guidelines for the use of animals in research and were approved by the Ethical and Animal Studies Committee of the University of Murcia (Spain).

For anesthesia, a mixture of xylazine (10 mg/kg body weight; Rompun; Bayer, Kiel, Germany) and ketamine (60 mg/kg body weight; Ketolar; Pfizer, Alcobendas, Madrid, Spain) was used intraperitoneally (IP). After surgery, an ointment containing tobramicin (Tobrex; Alcon S.A., Barcelona, Spain) was applied on the cornea to prevent its desiccation. Rats were given oral analgesia (Buprex; buprenorphine 0.3 mg/mL; Schering-Plough, Madrid, Spain) at 0.5 mg/kg (prepared in strawberry-flavored gelatin) the day of the surgery and during the next 3 days.

All animals were euthanized with an IP injection of an overdose of pentobarbital (Dolethal, Vetoquinol; Especialidades Veterinarias, S.A., Alcobendas, Madrid, Spain).

Table 2. Ketorolac Dose in the Different Formulations Tested in This Work

Formulation	Total Dose
KET-SOL	84.35 µg ketorolac tromethamine: equivalent to 57.15 µg ketorolac
KET-MP	57.15 µg ketorolac
KET-SOL+KET-MP	114.3 µg ketorolac: 84.35 µg ketorolac tromethamine + 57.15 µg ketorolac in MP

Experimental Design

Animals were divided into several groups (Table 1). To assess whether ketorolac protected RGCs from axotomy-induced death, retinas were intravitreally treated with a single injection of ketorolac-tromethamine solution and/or with ketorolac-loaded MP. Two experimental designs were carried out: intravitreal administration 1 week before the ON lesion and intravitreal administration right after the ON lesion. In either case, all animals were euthanized 7 days after the optic nerve (ON) injury. Nonloaded MP or ketorolac vehicle (balanced salt solution, BSS) was administered to animals subjected to the same procedures as the animals treated with ketorolac. As negative control of the RGC population, naive animals were used, while the injury control (positive) was a group of animals undergoing optic nerve crush (ONC) without treatment.

To investigate the possible toxic effect of ketorolac and MP, three groups of animals were used: intravitreal injection of ketorolac-tromethamine solution, intravitreal injection of non-loaded MP, and intravitreal injection of ketorolac-loaded MP. Rats were euthanized 14 days later to match the longest time of treatment in the injured groups.

Surgery

For ONC, the left ON was intraorbitally crushed at 0.5 mm from the optic disc following standard procedures in our laboratory.[12,14,20,62] Right after the injury, the eye fundus was checked to verify that the retinal blood supply was intact. Animals were euthanized 7 days later.

Animals received an intravitreal injection (5 µL) of either vehicle (BSS), ketorolac-tromethamine solution (16.87 mg/mL corresponding to 57.15 µg ketorolac base), nonloaded MP, ketorolac-loaded MP (10% wt/vol), or simultaneous ketorolac-loaded MP and ketorolac solution. For the treatments, an initial suspension of MP in BSS (10% wt/vol) was prepared and briefly vortexed immediately before each injection to ensure a uniform dispersion of MP in the injected fluid. Five microliters of each sample was then injected into the vitreous using a Hamilton Syringe (Sigma Aldrich, Madrid, Spain) with a 30-gauge needle. Rats received 0.5 mg MP containing 57.15 µg ketorolac and the same dose in solution (Table 2).

Retinal Dissection and RGC Immunodetection

Animals were perfused transcardially with 4% paraformaldehyde in phosphate buffer after a saline rinse. Eyes were enucleated and all retinas dissected as flat mounts as previously described.[12,14,15,63,64]

To identify RGCs, brain-specific homeobox/POU domain protein 3A (Brn3a) was immunodetected in all retinas as

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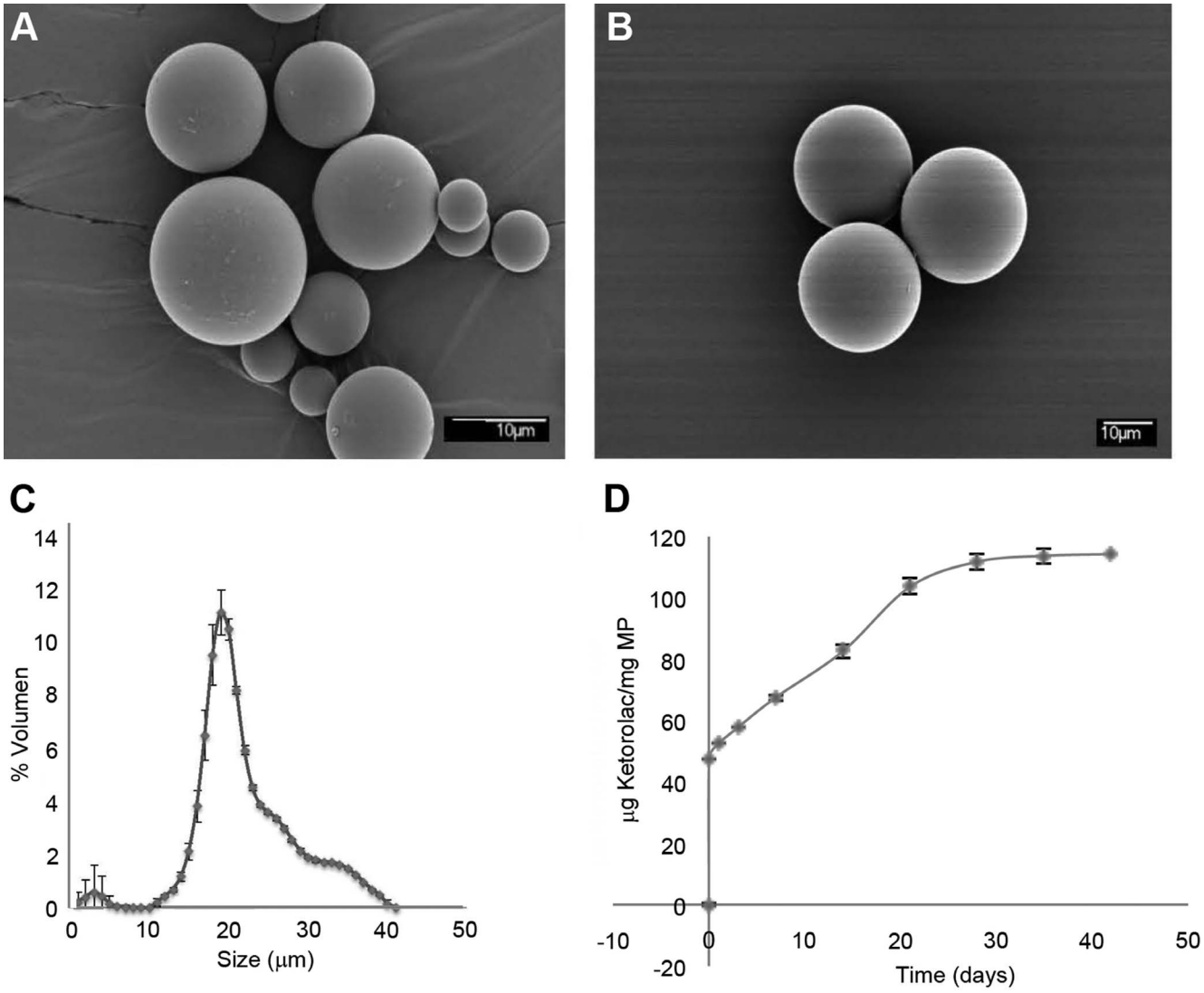


FIGURE 1. SEM micrograph of ketorolac microspheres prepared by O/W solvent evaporation technique. (**A**) Nonloaded microspheres, (**B**) ketorolac microspheres, (**C**) particle size distribution of ketorolac microspheres (10–40 µm) (mean ± SD). (**D**) Cumulative ketorolac release (µg ketorolac/mg) from microspheres (114.3 µg ketorolac/mg MP). During the first 24 hours a burst release effect was observed (47.25 ± 0.45 µg ketorolac/mg MP). After that, ketorolac was delivered for 30 days according to a zero order release kinetic (2.338 µg ketorolac/mg MP/day).

previously reported[12,14,15,63,64] using goat-anti Brn3a (C-20) (1:750; Santa Cruz Biotechnologies. Heidelberg, Germany) and as secondary detection donkey anti-goat IgG(H+L)-Alexa 594 (1:500; Molecular Probes, Life Technologies, Madrid, Spain).

Image Acquisition

All retinas were photographed with an epifluorescence microscope (Axioscop 2 Plus; Zeiss Mikroskopie, Jena, Germany) equipped with a computer-driven motorized stage (ProScan H128 Series; Prior Scientific Instruments, Cambridge, UK), controlled by the Image Pro Plus software (IPP 5.1 for Windows; Media Cybernetics, Silver Spring, MD, USA) as previously described.[12,14,15] To make reconstructions of retinal whole mounts, retinal multiframe acquisitions were taken in a raster scan pattern. The 154 frames/retina were combined automatically into a single tiled high-resolution photomontage using IPP for Windows.

Automated Quantification of RGCs and Isodensity Maps

Brn3a$^+$RGCs were automatically quantified and their topographical distribution assessed by isodensity maps as reported before.[12,14,15,63,64]

Statistical Analysis

Data were analyzed with SigmaStat for Windows version 3.11 (Systat Software, Inc., Richmond, CA, USA). Differences were considered significant when $P \leq 0.05$, and tests are detailed in Results.

RESULTS

Microsphere Characterization

The MP prepared by the O/W solvent evaporation technique were spherical and exhibited smooth surfaces with the

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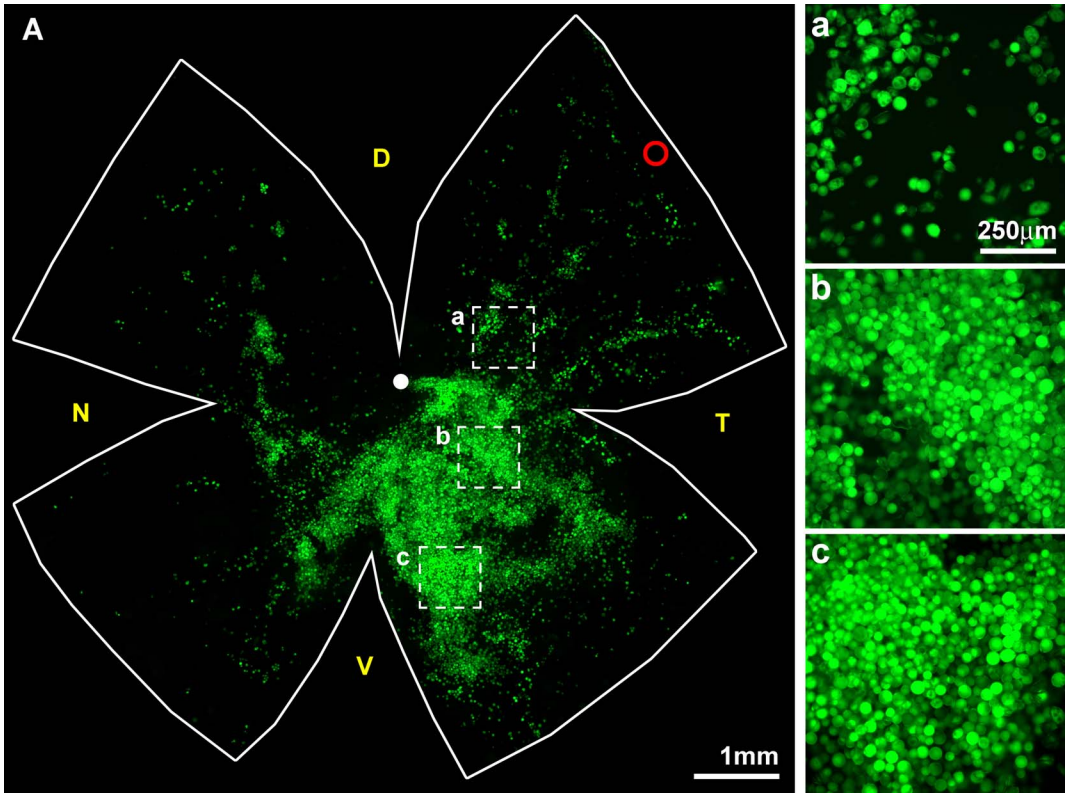


FIGURE 2. In vivo administration of microspheres. Photomontage from a retina dissected 14 days after intravitreal administration of microspheres (*green*). The *red circle* marks the place where the needle was inserted (dorsotemporal quadrant). (**a–c**) Magnifications taken from their respective squared areas in the photomontage showing the microspheres in detail.

absence of drug crystals on the MP surface (Figs. 1A, 1B). The encapsulation method led to a production yield of 47.6%. The 10- to 40-μm size fraction, with a mean particle size of 26.99 ± 3.46 μm, was selected for intravitreal injection in animals (Fig. 1C). The ketorolac loading in the MP resulted in 114.3 μg ketorolac/mg MP (encapsulation efficiency of 87.69% ± 1.92%).

According to the in vitro release profile, MP were able to release the active molecule for 30 days in a controlled fashion (Fig. 1D). During the first 24 hours, a burst release effect was observed (47.25 ± 0.45 μg ketorolac/mg MP) that resulted in 44% ± 0.39% of the encapsulated drug. After that, ketorolac was delivered for 30 days according to a zero order release kinetic (release rate: 2.338 μg ketorolac/mg MP/day). At the end of the release study (42 days), MP had delivered almost all the encapsulated drug.

Toxicity Assay

First we studied the fate of MP after intravitreal administration. Microspheres remained in the vitreous during the time of the study (7 or 14 days) (Fig. 2). They were brushed away when cleaning the vitreous; thus, the retina in Figure 2 was photographed before vitreous cleansing. Fourteen days after administration, many MP were still observed across the retina. Most of them were found on the ventral retina, where they sunk after being injected through the dorsotemporal quadrant (red circle in Fig. 2).

In a second round of experiments, the toxicity on RGCs of all formulations was tested. Thus, ketorolac solution (KET-SOL), nonloaded microspheres (NL-MP), or ketorolac-loaded microspheres (KET-MP) were administered to uninjured retinas (Table 3) and analyzed 14 days later to match the longest treatment time (see Table 1).

In all groups, the mean number of RGCs did not differ from the number of RGCs quantified in naive animals (Table 3). This is also observed in the isodensity maps shown in Figures 3A through 3E.

TABLE 3. Toxicity Assay

	No. of RGCs
Intact (naive) retinas	79,646 ± 998
Ketorolac solution	78,083 ± 1,181
Nonloaded microspheres	79,621 ± 3,247
Ketorolac microspheres	80,068 ± 3,218

Number ± SD of RGCs in intact retinas and in uninjured retinas analyzed 14 days after administration of ketorolac, nonloaded microspheres, or ketorolac microspheres.

Effect of Ketorolac Treatment on RGC Survival After ONC

As previously described, in order to evaluate the neuroprotective effect of ketorolac, the molecule was delivered in two formulations: in solution, dissolved in BSS and/or in MP for a slow and continuous release (see above). In a series of experiments, each formulation and the respective controls were administered 1 week before the injury. In a second series, the treatment was administered simultaneously with the lesion. Quantitative results are shown in Table 4 and Figure 4.

As control of the RGC population, naive intact retinas were used because it has been described as a contralateral response to unilateral injury.[16] However, in this work the number of

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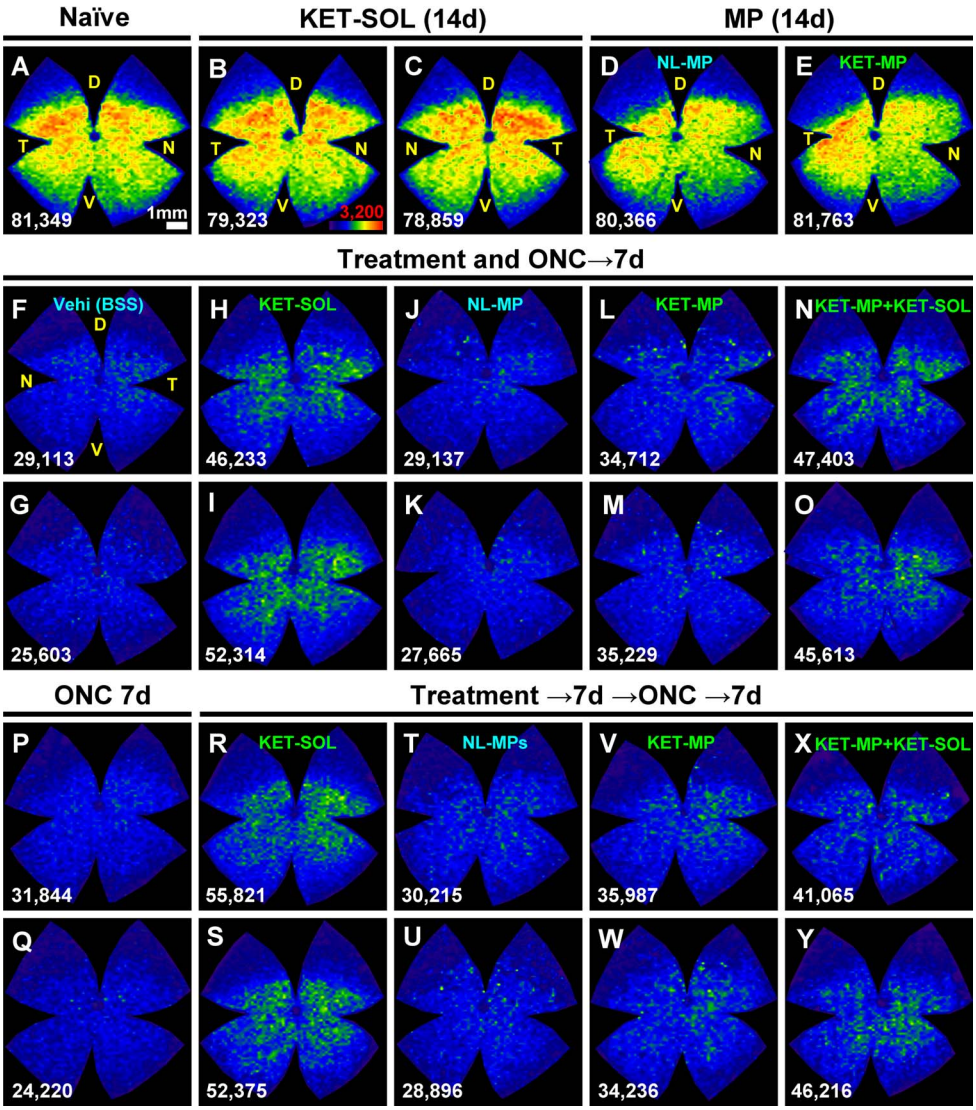


Figure 3. Distribution of surviving RGCs in naive, injured, and treated retinas. (**A**) Distribution of RGCs in naive retinas. (**P**, **Q**) Distribution of surviving RGCs 7 days after ONC alone. (**B**–**E**) Toxicity assay, RGC isodensity maps from uninjured retinas treated with ketorolac solution (**B**, **C**), nonloaded microspheres (**D**), or ketorolac microspheres (**E**). (**F**–**O**) Isodensity maps from groups in which the treatment and the injury were simultaneous: vehicle (**F**, **G**), ketorolac solution (**H**, **I**), ketorolac microspheres (**L**, **M**), and combination of ketorolac microspheres and ketorolac solution (**N**, **O**). (**R**–**Y**) Isodensity maps from groups in which the treatment was administered 7 days before the injury: ketorolac solution (**R**, **S**), nonloaded microspheres (**T**, **U**), ketorolac microspheres (**V**, **W**), and combination of ketorolac microspheres and ketorolac solution (**X**, **Y**). These maps show the density of RGCs in the retina with a *color scale* (**B**, *bottom right*) that goes from 0 RGCs/mm^2 (*purple*) to 3200 or more RGCs/mm^2 (*red*). At the *bottom left* of each map is shown the number of RGCs quantified in the retina from which the map was created.

RGCs in the right eyes, contralateral to the lesion and treatment, was within normality.

There was no difference between ONC alone, ONC and vehicle, and NL-MP treatment (prelesion or simultaneous). In these groups the percentage of surviving RGCs was 36% to 41% (Fig. 4).

In all groups in which ketorolac was administered in solution, there was a significantly higher number of RGCs compared to the values in all control groups. The best neuroprotection was observed after intravitreal injection of KET-SOL 1 week before the injury, where 63% of the RGCs were still present at 7 days after the injury. Retinal ganglion cell survival was similar between KET-SOL injected at the time of the injury and KET-SOL+KET-MP either simultaneous or preinjury (53% or 55% of survival, respectively). Ketorolac delivered by MP alone (KET-MP) was not as efficient as in solution (42% simultaneous with ONC, 43% pre-ONC); and although the number of surviving RGCs was higher than after ONC alone, it did not reach statistical significance. However, compared to NL-MP, KET-MP delayed RGC death in significant numbers.

In Figure 3 are shown representative isodensity maps from each group. These images support the quantitative data and document that ketorolac protection occurs across the retina, in spite of the fact that ketorolac solution was injected in the dorsotemporal quadrant.

Discussion

Here we show for the first time that a single administration of ketorolac, a NSAID, delays neuronal death in an acute model of axonal injury. The elicited neuroprotection is better when the drug is administered before the injury and when it is delivered

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Table 4. Ketorolac Administration Attenuates RGC Death After ONC

	No. ± SD of RGCs	
	No Lesion	**7 d After ONC**
No treatment		
Right retinas: contralateral to injured ones	80,193 ± 1,066	
Injured		30,543 ± 6,742
Vehicle: BSS		
Treatment→ONC: 14 d therapy		
ONC+treatment: 7 d therapy		32,333 ± 6,130
NL-MP		
Treatment→ONC: 14 d therapy		29,022 ± 4,808
ONC+treatment: 7 d therapy		29,772 ± 3,686
KET-SOL		
Treatment→ONC: 14 d therapy		50,173 ± 3,266*†
ONC+treatment: 7 d therapy		43,249 ± 4,460*†
KET-MP		
Treatment→ONC: 14 d therapy		34,142 ± 2,711*‡
ONC+treatment: 7 d therapy		33,568 ± 1,646*‡
KET-MP+KET-SOL		
Treatment→ONC: 14 d therapy		43,915 ± 5,140*‡
ONC+treatment: 7 d therapy		42,412 ± 5,028*‡

Treatment→ONC, treatment 7 days before injury; ONC+treatment, simultaneous treatment and injury.

* *t*-test $P \leq 0.03$ compared to ONC.

† *t*-test $P \leq 0.002$ compared to ONC+BSS.

‡ *t*-test $P \leq 0.023$ compared to their respective nonloaded MP (NL-MP) group. Right retinas, retinas contralateral to the experimental ones.

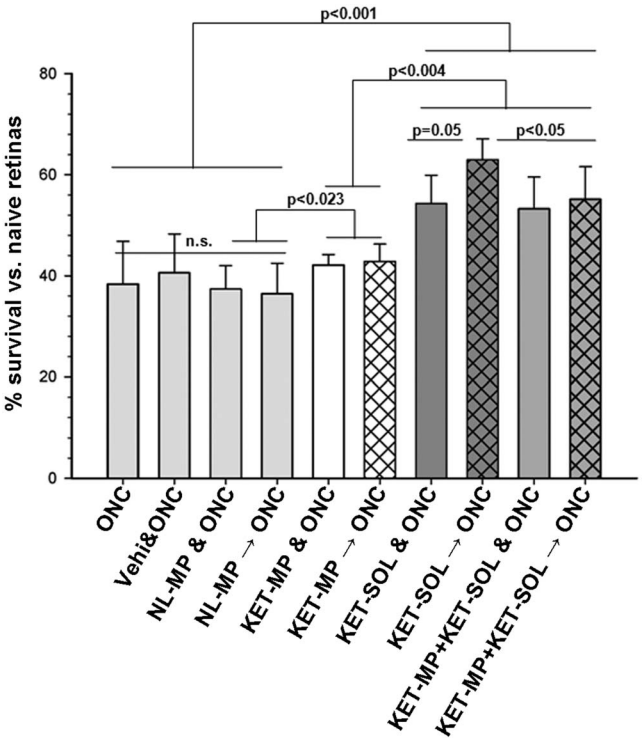


Figure 4. Ketorolac delays RGC death after optic nerve crush. Bar graph showing the percentage of surviving RGCs in all the analyzed groups compared to naive retinas (100%). n.s., not significant. Statistical analysis: one-way ANOVA all pairwise multiple comparison procedures.

in aqueous solution. Furthermore, as has been reported in rabbits,[54] intravitreal administration of ketorolac did not show toxic effects on RGC survival for the period of study of 1 week.

Corticoids are widely used to treat injuries of the CNS, and also as therapy in patients with ocular diseases.[30–32] It has been reported that cortisol administered intravitreally in rats neuroprotects RGCs after axotomy.[26] The authors showed that 50% of RGCs survive at 15 days post axotomy when treated, while without treatment only 17% of the original RGC population is still alive. In the present work KET solution administered prior to axonal injury (pre-ONC) rescued 63% of RGCs 7 days after the lesion. While this neuroprotection may appear lower than that produced by cortisol, it is important to have in mind that steroidal drugs may be toxic and induce a number of undesirable side effects.[33]

The use of NSAIDs as neuroprotectors has gained a lot of interest during the last decade. The neuroprotective effect of several NSAIDs has been demonstrated in different axonal injuries. Their effect is mainly based on the suppression of microglial activation occurring by different pathways. The two isoforms of cyclooxygenase (COX), the constitutive (COX-1) and inducible (COX-2) forms responsible for tissue prostaglandin synthesis, are clearly involved in the inflammatory process. Subsequently, their inhibition has been reported to suppress toxic actions of microglia.[65] In fact, some authors have described COX inhibition as a primary mechanism in the neuroprotective effect of NSAIDs.[43] The neuroprotective effect of ketorolac has been previously reported in spinal cord ischemic injury, with reduced neuronal death observed after intrathecal pretreatment with the anti-inflammatory agent; this effect was attributed to COX inhibition. In the present work, the effect displayed for ketorolac could be attributed to the reduction of damage during the inflammatory phase after the injury probably through COX inhibition. A neuroprotective effect has been attributed also to other nonspecific COX inhibitors. Ibuprofen has been reported to affect multiple target cells in the nervous system such as astroglia, microglia, and neurons, with its main action the unspecific inhibition of COX enzyme and the inhibition of RhoA and modulation of glial activity.[66] The neuroprotective effect of ibuprofen has been observed after axotomy in the peripheral nervous system. The authors identified this NSAID as a molecule with the ability to block cytoskeleton rearrangement inhibitor Rho.[44] Additionally, this anti-inflammatory agent has also been shown to attenuate injury in rats exposed to transient forebrain ischemia.[45]

Sustained intraocular delivery of therapeutic molecules is an emerging tool for the treatment of retinal diseases. Drug delivery devices have been developed to avoid repeated injections mainly into the vitreous cavity.[57] Biodegradable MP loaded with different drugs have been developed to treat disorders associated with inflammation. This is the case with the anti-inflammatory drug dexamethasone delivered from PLGA MP. Intravitreal injection of the microparticle formulation has been demonstrated to be useful in the inhibition of intraocular inflammation generated by lipopolysaccharide (LPS) in rabbits.[67]

In this work, neither the initial drug release from MP (47.25 μg in the first 24 hours) nor the continuous and slow release of ketorolac from the MP (1.169 μg ketorolac/day released from 0.5 mg of MP) observed in vitro promoted a moderate rescue of RGCs; it did not show an additive effect with ketorolac in solution, indicating that low ketorolac doses are not enough to protect RGC in an acute injury. In this study, a bolus ketorolac concentration of 57.15 μg was needed to observe a neuroprotective effect in RGC cells in this animal model

All these findings suggest that the injury produced by axotomy probably requires higher doses of the active

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substance at early stages after damage compared to chronic diseases in which a sustained delivery of the active substance seems to be more effective than a single injection of the therapeutic molecule in solution. In fact, contrary to the results presented here, we have previously demonstrated that sustained delivery of another neuroprotective agent (the glial-derived neurotrophic factor, GDNF) in low amounts (0.8 pg/day) was effective in an animal model of glaucoma in which the damage to RGCs is less acute (episcleral injection of a hypertonic solution in rats)—with a 72.68% RGC survival compared to 30.65% observed after injection of GDNF in solution[68] during long-term (11 weeks after administration).

One of the most relevant results observed in the present study is the survival of the RGCs present at 14 days after KET solution injection (57.15 μg), indicating that the anti-inflammatory agent in solution administered 1 week before the injury protects RGCs better than simultaneous administration. It has been reported that intraocular ketorolac was able to reduce the inflammation induced by LPS and showed a good penetration into the retina and choroid, but it was rapidly cleared, being detected in the vitreous up to 48 hours and in the retina up to 24 hours.[40,55] Presumably, the ketorolac has disappeared from the vitreous after 7 days of administration, although the half-life in the rat vitreous has not been reported. According to our results, it is possible that the therapeutic action of ketorolac was longer than its half-life. In fact, this study presents clear evidence that a bolus administration of ketorolac in solution is necessary to promote RGC survival. However, contrary to what was expected, the combination therapy did not provide better neuroprotection (Fig. 4). Reasons for these findings might be related to a different ketorolac tromethamine biodistribution caused by the presence of the MP in the vitreous cavity or may be that higher doses of the NSAID are not able to promote an increase of RGC survival. Further studies in chronic neurodegenerative animal models and dose–response experiments will help to elucidate the mechanism of neuroprotection associated with the nonspecific COX inhibitor ketorolac and to establish the most adequate dosage regimen in acute injury and chronic degeneration.

In conclusion, we show here for the first time that the nonspecific COX inhibitor NSAID ketorolac prevents neuronal death triggered by a traumatic insult to the eye. Future studies with selective inhibitors of COX-1 and COX-2 in axonal injury might be helpful to clarify the effect of ketorolac on enhancing RGC survival.

Although controlled ketorolac drug delivery systems (biodegradable MP) were not able to promote RGC survival in the acute model used in this work (axotomy), further studies should be performed to determine the real usefulness of these IDDS in chronic neurodegenerative animal models.

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References

1. Lafuente Lopez-Herrera MP, Mayor-Torroglosa S, Miralles de Imperial J, Villegas-Perez MP, Vidal-Sanz M. Transient ischemia of the retina results in altered retrograde axoplasmic transport: neuroprotection with brimonidine. *Exp Neurol*. 2002;178:243–258.
2. Selles-Navarro I, Villegas-Perez MP, Salvador-Silva M, Ruiz-Gomez JM, Vidal-Sanz M. Retinal ganglion cell death after different transient periods of pressure-induced ischemia and survival intervals. A quantitative in vivo study. *Invest Ophthalmol Vis Sci*. 1996;37:2002–2014.
3. Cuenca N, Pinilla I, Fernandez-Sanchez L, et al. Changes in the inner and outer retinal layers after acute increase of the intraocular pressure in adult albino Swiss mice. *Exp Eye Res*. 2010;91:273–285.
4. Salinas-Navarro M, Alarcon-Martinez L, Valiente-Soriano FJ, et al. Functional and morphological effects of laser-induced ocular hypertension in retinas of adult albino Swiss mice. *Mol Vis*. 2009;15:2578–2598.
5. Salinas-Navarro M, Alarcon-Martinez L, Valiente-Soriano FJ, et al. Ocular hypertension impairs optic nerve axonal transport leading to progressive retinal ganglion cell degeneration. *Exp Eye Res*. 2010;90:168–183.
6. Valiente-Soriano FJ, Salinas-Navarro M, Jimenez-Lopez M, et al. Effects of ocular hypertension in the visual system of pigmented mice. *PLoS One*. 2015;10:e0121134.
7. Valiente-Soriano FJ, Nadal-Nicolas FM, Salinas-Navarro M, et al. BDNF rescues RGCs but not intrinsically photosensitive RGCs in ocular hypertensive albino rat retinas. *Invest Ophthalmol Vis Sci*. 2015;56:1924–1936.
8. Vidal-Sanz M, Salinas-Navarro M, Nadal-Nicolas FM, et al. Understanding glaucomatous damage: anatomical and functional data from ocular hypertensive rodent retinas. *Prog Retin Eye Res*. 2012;31:1–27.
9. Vidal-Sanz M, Valiente-Soriano FJ, Ortin-Martinez A, et al. Retinal neurodegeneration in experimental glaucoma. *Prog Brain Res*. 2015;220:1–35.
10. Agudo-Barriuso M, Lahoz A, Nadal-Nicolas FM, et al. Metabolomic changes in the rat retina after optic nerve crush. *Invest Ophthalmol Vis Sci*. 2013;54:4249–4259.
11. Agudo M, Perez-Marin MC, Lonngren U, et al. Time course profiling of the retinal transcriptome after optic nerve transection and optic nerve crush. *Mol Vis*. 2008;14:1050–1063.
12. Nadal-Nicolas FM, Jimenez-Lopez M, Sobrado-Calvo P, et al. Brn3a as a marker of retinal ganglion cells: qualitative and quantitative time course studies in naive and optic nerve-injured retinas. *Invest Ophthalmol Vis Sci*. 2009;50:3860–3868.
13. Villegas-Perez MP, Vidal-Sanz M, Rasminsky M, Bray GM, Aguayo AJ. Rapid and protracted phases of retinal ganglion cell loss follow axotomy in the optic nerve of adult rats. *J Neurobiol*. 1993;24:23–36.
14. Nadal-Nicolas FM, Jimenez-Lopez M, Salinas-Navarro M, et al. Whole number, distribution and co-expression of brn3 transcription factors in retinal ganglion cells of adult albino and pigmented rats. *PLoS One*. 2012;7:e49830.
15. Nadal-Nicolas FM, Salinas-Navarro M, Jimenez-Lopez M, et al. Displaced retinal ganglion cells in albino and pigmented rats. *Front Neuroanat*. 2014;8:99.
16. Galindo-Romero C, Valiente-Soriano FJ, Jimenez-Lopez M, et al. Effect of brain-derived neurotrophic factor on mouse axotomized retinal ganglion cells and phagocytic microglia. *Invest Ophthalmol Vis Sci*. 2013;54:974–985.
17. Jehle T, Dimitriu C, Auer S, et al. The neuropeptide NAP provides neuroprotection against retinal ganglion cell damage

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after retinal ischemia and optic nerve crush. *Graefes Arch Clin Exp Ophthalmol*. 2008;246:1255–1263.

18. Lindqvist N, Peinado-Ramonn P, Vidal-Sanz M, Hallbook F. GDNF, Ret, GFRalpha1 and 2 in the adult rat retino-tectal system after optic nerve transection. *Exp Neurol*. 2004;187:487–499.
19. Mansour-Robaey S, Clarke DB, Wang YC, Bray GM, Aguayo AJ. Effects of ocular injury and administration of brain-derived neurotrophic factor on survival and regrowth of axotomized retinal ganglion cells. *Proc Natl Acad Sci U S A*. 1994;91:1632–1636.
20. Parrilla-Reverter G, Agudo M, Sobrado-Calvo P, Salinas-Navarro M, Villegas-Perez MP, Vidal-Sanz M. Effects of different neurotrophic factors on the survival of retinal ganglion cells after a complete intraorbital nerve crush injury: a quantitative in vivo study. *Exp Eye Res*. 2009;89:32–41.
21. Peinado-Ramon P, Salvador M, Villegas-Perez MP, Vidal-Sanz M. Effects of axotomy and intraocular administration of NT-4, NT-3, and brain-derived neurotrophic factor on the survival of adult rat retinal ganglion cells. A quantitative in vivo study. *Invest Ophthalmol Vis Sci*. 1996;37:489–500.
22. Sanchez-Migallon MC, Nadal-Nicolas FM, Jimenez-Lopez M, Sobrado-Calvo P, Vidal-Sanz M, Agudo-Barriuso M. Brain derived neurotrophic factor maintains Brn3a expression in axotomized rat retinal ganglion cells. *Exp Eye Res*. 2011;92:260–267.
23. Berkelaar M, Clarke DB, Wang YC, Bray GM, Aguayo AJ. Axotomy results in delayed death and apoptosis of retinal ganglion cells in adult rats. *J Neurosci*. 1994;14:4368–4374.
24. Brown J Jr, Hacker H, Schuschereba ST, Zwick H, Lund DJ, Stuck BE. Steroidal and nonsteroidal antiinflammatory medications can improve photoreceptor survival after laser retinal photocoagulation. *Ophthalmology*. 2007;114:1876–1883.
25. Dimitriu C, Bach M, Lagreze WA, Jehle T. Methylprednisolone fails to preserve retinal ganglion cells and visual function after ocular ischemia in rats. *Invest Ophthalmol Vis Sci*. 2008;49:5003–5007.
26. Heiduschka P, Thanos S. Cortisol promotes survival and regeneration of axotomised retinal ganglion cells and enhances effects of aurintricarboxylic acid. *Graefes Arch Clin Exp Ophthalmol*. 2006;244:1512–1521.
27. Jiang S, Khan MI, Middlemiss PJ, et al. AIT-082 and methylprednisolone singly, but not in combination, enhance functional and histological improvement after acute spinal cord injury in rats. *Int J Immunopathol Pharmacol*. 2004;17:353–366.
28. Mihalcea O, Arnold AC. Side effect of head and neck radiotherapy: optic neuropathy. *Oftalmologia*. 2008;52:36–40.
29. Min DI, Monaco AP. Complications associated with immunosuppressive therapy and their management. *Pharmacotherapy*. 1991;11:119S–125S.
30. Al Dhibi HA, Arevalo JF. Clinical trials on corticosteroids for diabetic macular edema. *World J Diabetes*. 2013;4:295–302.
31. Guy WM, Soparkar CN, Alford EL, Patrinely JR, Sami MS, Parke RB. Traumatic optic neuropathy and second optic nerve injuries. *JAMA Ophthalmol*. 2014;132:567–571.
32. Montero JA, Ruiz-Moreno JM. Intravitreal inserts of steroids to treat diabetic macular edema. *Curr Diabetes Rev*. 2009;5:26–32.
33. Berthelot JM, Le GB, Maugars Y. Side effects of corticosteroid injections: what's new? *Joint Bone Spine*. 2013;80:363–367.
34. Conti SM, Kertes PJ. The use of intravitreal corticosteroids, evidence-based and otherwise. *Curr Opin Ophthalmol*. 2006;17:235–244.
35. Diem R, Hobom M, Maier K, et al. Methylprednisolone increases neuronal apoptosis during autoimmune CNS inflammation by inhibition of an endogenous neuroprotective pathway. *J Neurosci*. 2003;23:6993–7000.
36. Prodanov D, Mantchev G, Iliev A, et al. Effects of dexamethasone in rat neonatal model of axotomy-induced motoneuronal cell death. *Arch Physiol Biochem*. 1998;106:355–361.
37. Steinsapir KD, Goldberg RA, Sinha S, Hovda DA. Methylprednisolone exacerbates axonal loss following optic nerve trauma in rats. *Restor Neurol Neurosci*. 2000;17:157–163.
38. Rodrigues EB, Farah ME, Bottós MJ, Aggio BF. Nonsteroidal anti-inflammatory drugs (NSAIDs) in the treatment of retinal diseases. *Dev Ophthalmol*. 2016;55:212–220.
39. Bagriyanik HA, Ozogul C, Alaygut E, et al. Neuroprotective effects of ketorolac tromethamine after spinal cord injury in rats: an ultrastructural study. *Adv Ther*. 2008;25:152–158.
40. Baranano DE, Kim SJ, Edelhauser HF, Durairaj C, Kompella UB, Handa JT. Efficacy and pharmacokinetics of intravitreal nonsteroidal anti-inflammatory drugs for intraocular inflammation. *Br J Ophthalmol*. 2009;93:1387–1390.
41. Bucolo C, Marrazzo G, Platania CB, Romano GL, Drago F, Salomone S. Effects of topical indomethacin, bromfenac and nepafenac on lipopolysaccharide-induced ocular inflammation. *J Pharm Pharmacol*. 2014;66:954–960.
42. Kapin MA, Yanni JM, Brady MT, et al. Inflammation-mediated retinal edema in the rabbit is inhibited by topical nepafenac. *Inflammation*. 2003;27:281–291.
43. Lopez-Villodres JA, De La Cruz JP, Munoz-Marin J, Guerrero A, Reyes JJ, Gonzalez-Correa JA. Cytoprotective effect of nonsteroidal antiinflammatory drugs in rat brain slices subjected to reoxygenation after oxygen-glucose deprivation. *Eur J Pharm Sci*. 2012;45:624–631.
44. Madura T, Tomita K, Terenghi G. Ibuprofen improves functional outcome after axotomy and immediate repair in the peripheral nervous system. *J Plast Reconstr Aesthet Surg*. 2011;64:1641–1646.
45. Park EM, Cho BP, Volpe BT, Cruz MO, Joh TH, Cho S. Ibuprofen protects ischemia-induced neuronal injury via up-regulating interleukin-1 receptor antagonist expression. *Neuroscience*. 2005;132:625–631.
46. Sharma J, Barr SM, Geng Y, Yun Y, Higgins RD. Ibuprofen improves oxygen-induced retinopathy in a mouse model. *Curr Eye Res*. 2003;27:309–314.
47. Sharp KG, Yee KM, Stiles TL, Aguilar RM, Steward O. A reassessment of the effects of treatment with a non-steroidal anti-inflammatory (ibuprofen) on promoting axon regeneration via RhoA inhibition after spinal cord injury. *Exp Neurol*. 2013;248:321–337.
48. Turan-Vural E, Halili E, Serin D. Assessing the effects of ketorolac and acetazolamide on macular thickness by optical coherence tomography following cataract surgery. *Int Ophthalmol*. 2014;34:525–531.
49. Wang QW, Yao K, Xu W, et al. Bromfenac sodium 0.1%, fluorometholone 0.1% and dexamethasone 0.1% for control of ocular inflammation and prevention of cystoid macular edema after phacoemulsification. *Ophthalmologica*. 2013;229:187–194.
50. Wang X, Budel S, Baughman K, Gould G, Song KH, Strittmatter SM. Ibuprofen enhances recovery from spinal cord injury by limiting tissue loss and stimulating axonal growth. *J Neurotrauma*. 2009;26:81–95.
51. Ju WK, Neufeld AH. Cellular localization of cyclooxygenase-1 and cyclooxygenase-2 in the normal mouse, rat, and human retina. *J Comp Neurol*. 2002;452:392–399.
52. Ju WK, Kim KY, Neufeld AH. Increased activity of cyclooxygenase-2 signals early neurodegenerative events in the rat retina following transient ischemia. *Exp Eye Res*. 2003;77:137–145.

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53. Wang AG, Lee CM, Wang YC, Lin CH, Fann MJ. Up-regulation of cytochrome oxidase in the retina following optic nerve injury. *Exp Eye Res*. 2002;74:651–659.
54. Margalit E, Kugler LJ, Brumm MV, et al. The safety of intraocular ketorolac in rabbits. *Invest Ophthalmol Vis Sci*. 2006;47:2093–2099.
55. Wang M, Liu W, Lu Q, et al. Pharmacokinetic comparison of ketorolac after intracameral, intravitreal, and suprachoroidal administration in rabbits. *Retina*. 2012;32:2158–2164.
56. Herrero-Vanrell R, Bravo-Osuna I, Andres-Guerrero V, Vicario-de-la-Torre M, Molina-Martinez IT. The potential of using biodegradable microspheres in retinal diseases and other intraocular pathologies. *Prog Retin Eye Res*. 2014;42:27–43.
57. Yasukawa T, Ogura Y, Tabata Y, Kimura H, Wiedemann P, Honda Y. Drug delivery systems for vitreoretinal diseases. *Prog Retin Eye Res*. 2004;23:253–281.
58. Herrero-Vanrell R, Cardillo JA. Ocular pharmacokinetic drug, bioavailability and intraocular drug delivery systems. In: Nguyen Q, Rodriguez EB, Farah ME, Mieler WF, eds. *Retinal Pharmacotherapy*. China: Saunders Elsevier; 2010:60–65.
59. Yasukawa T, Tabata Y, Kimura H, Ogura Y. Recent advances in intraocular drug delivery systems. *Recent Pat Drug Deliv Formul*. 2011;5:1–10.
60. Zimmer A, Kreuter J. Microspheres and nanoparticles used in ocular delivery systems. *Adv Drug Deliv Rev*. 1995;16:61–73.
61. Martinez-Sancho C, Herrero-Vanrell R, Negro S. Study of gamma-irradiation effects on aciclovir poly(D,L-lactic-co-glycolic) acid microspheres for intravitreal administration. *J Control Release*. 2004;99:41–52.
62. Parrilla-Reverter G, Agudo M, Nadal-Nicolas F, et al. Time-course of the retinal nerve fibre layer degeneration after complete intra-orbital optic nerve transection or crush: a comparative study. *Vision Res*. 2009;49:2808–2825.
63. Nadal-Nicolas FM, Madeira MH, Salinas-Navarro M, et al. Transient downregulation of melanopsin expression after retrograde tracing or optic nerve injury in adult rats. *Invest Ophthalmol Vis Sci*. 2015;56:4309–4323.
64. Rovere G, Nadal-Nicolas FM, Agudo-Barriuso M, et al. Comparison of retinal nerve fiber layer thinning and retinal ganglion cell loss after optic nerve transection in adult albino rats. *Invest Ophthalmol Vis Sci*. 2015;56:4487–4498.
65. Klegeris A, McGeer PL. Cyclooxygenase and 5-lipoxygenase inhibitors protect against mononuclear phagocyte neurotoxicity. *Neurobiol Aging*. 2002;23:787–794.
66. Redondo-Castro E, Navarro X. Chronic ibuprofen administration reduces neuropathic pain but does not exert neuroprotection after spinal cord injury in adult rats. *Exp Neurol*. 2014;252:95–103.
67. Barcia E, Herrero-Vanrell R, Diez A, Alvarez-Santiago C, Lopez I, Calonge M. Downregulation of endotoxin-induced uveitis by intravitreal injection of polylactic-glycolic acid (PLGA) microspheres loaded with dexamethasone. *Exp Eye Res*. 2009;89:238–245.
68. Checa-Casalengua P, Jiang C, Bravo-Osuna I, et al. Retinal ganglion cells survival in a glaucoma model by GDNF/Vit E PLGA microspheres prepared according to a novel microencapsulation procedure. *J Control Release*. 2011;156:92–100.

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