Müller glial cells (MGC) and astrocytes participate in the formation and maintenance of the inner blood–retinal barrier (iBRB), which is required for a normal vision. Transport processes and tight junctions between the vascular endothelial cells composing the iBRB regulate the flow of ions, nutrients, water, and toxic molecules between the retina and vessels to preserve the proper balance necessary for neuronal function. The loss of iBRB alters the balance of fluids between retinal tissues and retinal vessels. It may cause retinal edema with lasting toxicity of neurotransmitters. These phenomena are associated with the continuous fluid reabsorption by MGC and the selective water transport protein, aquaporin-4 (AQP4). In normal mouse retina, AQP4 and Kir4.1 are strongly expressed in MGC and mainly localized at MGC endfeet and in processes surrounding the blood vessels. In many retinal diseases, including diabetic retinopathy, retinal vein occlusion, and retinal detachment with iBRB breakdown, the expression and distribution of these channels are altered in MGC. Another important function of MGC is the uptake of neurotransmitters supporting the synaptic activity. A MGC is a neurotransmitter recycler whose neuroprotective effect prevents retinal neurons from the long-lasting toxicity of neurotransmitters.
Protection of Glial Müller Cells by Dexamethasone

particularly important to maintain the retinal homeostatic balance in case of retinal disorder.

Dystrophin Dp71, a membrane-associated cytoskeletal protein that forms the core of the dystrophin-associated protein complex, is predominantly localized in retinal MGC, mainly at their endfeet and around the retinal vessels,14,15 and is responsible for the clustering and anchoring of AQP4 and Kir4.1.8,14,16 We have previously shown in mice that a retinal detachment induces reactive MGC gliosis and changes in AQP4, Kir4.1, and Dp71 expression or localization.15 Moreover, the genetic inactivation of Dp71 (in Dp71-null mice)17 is associated with iBRB breakdown.14 The osmotic swelling capacity of MGC exposed to a hypoosmotic solution is exceeded in the absence of Dp71,14 increasing the vulnerability of retinal nerve cells to transient ischemia in Dp71-null mice.8,14 Moreover, in the absence of Dp71, AQP4 is downregulated, Kir4.1 distribution in MGC is modified, the retina is in a proinflammatory state,18 and the morphology of retinal astrocytes is altered.19

Dexamethasone is currently injected intravitreally in clinical practice to treat retinal edema because of its anti-inflammatory and antiedematous properties on the retina. Furthermore, in the rat retina, dexamethasone induces alterations in the expression of the potassium channel Kir4.1 in MGC.20 Intravitreal injections of dexamethasone in a rat model of streptozotocin-induced diabetes inhibit significantly the iBRB breakdown.21,22 Moreover, as suggested by Zhao et al.20 a lower dose of dexamethasone than the doses used in clinical practice may have therapeutic effects.

The aim of this study was to determine the pathway leading to AQP4, Kir4.1, and Dp71 impairments over the postoperative course of a surgically induced iBRB breakdown in a mouse model and to explore the effects of a low dose of dexamethasone on altered AQP4, Kir4.1, Dp71, and iBRB in this model.

Materials and Methods

Animal Care and Surgical Procedure

For this study, 8-week-old C57BL/6j mice (Janvier, St Bethervin, France) were used in accordance with the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research. Surgical procedure was performed using isoflurane; pupils were diluted with topical phenylephrine (2.5%) and tropicamide (1%). Under the operating microscope, a 30-gauge needle was inserted into the posterior part of the lens to remove it (Fig. 1A), allowing a direct contact between remaining lens fragments and the vitreous to create vitreous inflammation and iBRB breakdown in the context of an Irvine Gass syndrome model.

Quantification of Blood–Retinal Barrier Permeability

Vascular permeability was quantified using the Evans blue (EB) method.14,25 Evans blue (Sigma-Aldrich Corp., St. Louis, MO, USA) was injected (6 mg/mL) through the penile vein under isoflurane anesthesia. Blood samples were taken 3 hours after dye injection. Mice were perfused for 2 minutes with citrate buffer (0.05 M, pH 3.5) warmed at 37°C. Under isoflurane, a single dose (1 μL) of 10 mg/mL dexamethasone (Sigma-Aldrich, Saint-Quentin, France) diluted in balanced salt solution (BSS) (Alcon Laboratories, Inc., Rueil-Malmaison, France) was injected intravitreally with a 33-gauge microinjector (Hamilton, Bonaduz, Switzerland). A control group received an equal volume of vehicle (BSS). Lens surgery was performed at day 0. Twenty-four hours post surgery, mice received a dexamethasone or vehicle injection (n = 22). Inner BRB permeability was measured 24 hours (n = 5 per group) and 48 hours (n = 5 per group) after injection, and at 48 hours, the total proteins were extracted (n = 12 per group). A timeline illustrating experimentation chronology is presented in Supplementary Figure S1.

Intravitreal Dexamethasone Injection and Experimental Procedure

Under isoflurane, a single dose (1 μL) of 10 mg/mL dexamethasone (Sigma-Aldrich, Saint-Quentin, France) diluted in balanced salt solution (BSS) (Alcon Laboratories, Inc., Rueil-Malmaison, France) was injected intravitreally with a 33-gauge microinjector (Hamilton, Bonaduz, Switzerland). A control group received an equal volume of vehicle (BSS). Lens surgery was performed at day 0. Twenty-four hours post surgery, mice received a dexamethasone or vehicle injection (n = 22). Inner BRB permeability was measured 24 hours (n = 5 per group) and 48 hours (n = 5 per group) after injection, and at 48 hours, the total proteins were extracted (n = 12 per group). A timeline illustrating experimentation chronology is presented in Supplementary Figure S1.

Quantitative RT-PCR Analysis of Retinal RNA

Total RNA was extracted using Trizol reagent (Invitrogen, Waltham, MA, USA); n = 4 at 24, 48, and 72 hours after lens surgery. Reverse transcription was performed on 5 μg total RNA using Superscript III (Thermo Fisher Scientific) and random hexamers (Invitrogen). Complementary DNA were amplified using power SYBR Green (Applied Biosystems, Waltham, MA, USA) on a LightCycler instrument (Applied Biosystems) following the manufacturer’s instructions. Polymerase chain reaction primers were designed using Primer3 software24 and are listed in the Table. The Ct values of real-time PCR products were compared using the dCt method. The amount of Dp71 cDNA was normalized to β-actin used as the standard internal control.

Western Blot Analysis

Western blot analysis25 was performed on total proteins extracted 24, 48, and 72 hours post surgery. Retinal proteins were separated on NuPAGE Tris-Acetate 4–12% gradient gels (Invitrogen) and electrotransferred onto polyvinylidene difluoride (PVDF; Millipore, Billerica, MA, USA) membranes. Polyvinylidene difluoride membranes were blocked in PBS containing 0.1% Tween 20, 5% dry milk (Bio-Rad, Marnes-la-Coquette, France) for 1 hour at room temperature, then incubated with the primary antibody at 4°C for the night in the same blocking buffer. Polyclonal antibodies directed against dystrophins (H4) were previously characterized,26 whereas antibodies directed against Kir4.1 and AQP4 were from Alomone Labs (Souffelweyersheim, France), a rabbit polyclonal anti-Kir4.1 (APC-035) at the dilution of 1:100, and a rabbit polyclonal anti-AQP4 (AQP-004) at the dilution of 1:100. A rabbit polyclonal anti-HSF1 (HSF1-4556) from Cell Signaling Technology (Danvers, MA, USA) at the dilution of 1:1000 was also used. Blots were washed and incubated with the secondary anti-rabbit antibody (Interchim, Montluçon, France) conjugated to horseradish peroxidase (Jackson Immunoresearch Laboratories, West Grove, PA, USA). A chemiluminescence substrate (ECL plus Western blotting detection system, using X-ray) was added and chemiluminescence was detected using X-ray films (GE Healthcare, Little Chalfont, UK).

Immunohistochemistry

Dissected eyes were fixed in 4% paraformaldehyde for 1 hour, cryoprotected in 30% sucrose, frozen, embedded in Cryomatrix
(Thermo Scientific, Strasbourg, France), cut into 10-μm cryostat sections, and mounted on SuperFrost/Plus slides (Microm, Francheville, France). Sections were prepared for hematoxylin and eosin (HE) staining or immunohistology. Sections were permeabilized for 10 minutes with 0.1% PBS (phosphate-buffered saline)–Triton X-100 and blocked for 1 hour in 1% bovine serum albumin, 0.1% Tween 20, and PBS, then incubated with the primary antibody at 4°C overnight. Primary antibodies used were a mouse monoclonal anti-glutamine synthetase (MAB302; Millipore), dilution 1:500, a rabbit polyclonal anti-Kir4.1 (APC-035 from Alomone Labs) at the dilution of 1:500, a rabbit polyclonal anti-AQP4 (AQP-004, Dystrophin Dp71

### Table. List of PCR Primers

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<th>Primer Name</th>
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<td>Dystrophin Dp71</td>
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<tr>
<td>AQP4 Sense</td>
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<td>Aquaporin-4</td>
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<td>Anti-sense</td>
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<td>Inwardly rectifying K channel Kir4.1</td>
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</table>
Protection of Glial Müller Cells by Dexamethasone

Alomone Labs) at the dilution of 1:500, and a mouse monoclonal anti-GFAP (glial fibrillary acidic protein) (Sigma-Aldrich) at 1:500. Secondary antibodies (Interchim) coupled to Alexa Fluor (Invitrogen) were used diluted 1:800 in 1% bovine serum albumin, 0.1% Tween 20, and PBS for 1 hour. Sections were mounted with Fluorosave reagent (Millipore) and observed by confocal microscopy. ImageJ software (http://imagej.nih.gov/ij/; provided in the public domain by the National Institutes of Health, Bethesda, MD, USA) was used for pixel quantification. For Kir4.1 immunohistochemistry we used six retinas and 10 sections per retina. For AQP4 immunohistochemistry, we used six retinas, 44 sections for vessel quantification, and an average of 20 sections at the inner limiting membrane (ILM). For H4 staining, we used six retinas, six sections per retina. For each section, two images were taken.

Semi-quantification of Dystrophins, AQP4, Kir4.1

Retinal section preparations were captured using a Zeiss LSM 700 Meta Axioplan 2 laser scanning confocal microscope (Carl Zeiss, Fougères, France) on the same area, using the same objective and intensity. Glial fibrillary acidic protein, dystrophins, AQP4, and Kir4.1 were then immunolocalized on retinal sections. Staining was quantified using a semi-quantitative measurement with ImageJ software. Density and mean gray value were integrated after subtraction of the unspecific signal. The corrected total cell fluorescence (CTCF) was obtained using the following formula: CTCF = Integrated density – (Area of selected cells × Mean fluorescence of background readings).

Retinal Explants

In a sterile six-well plate containing 500 µL/well of isotonic solution (136 mM NaCl, 3 mM KCl, 2 mM CaCl2, 1 mM MgCl2, 10 mM HEPES, and 11 mM glucose, adjusted to pH 7.4 with Tris) or hypoosmotic solution (60% of isotonic solution) containing 1 mM of ions Ba2+, which are potassium channel blockers, three Millicell insert (Millipore) membranes were placed into each well with the hydrophilic side facing the well bottom in contact with the culture medium.27 After enucleation, eyes were maintained in CO2-independent medium (Gibco, Waltham, MA, USA). Retinas were placed with the photoreceptors facing the membrane, and 1 mL solution was added to each well. Dexamethasone (10 mg/mL) was added to the retina top. The plates were incubated at 35°C in a humidified incubator with 5% CO2 for 8 hours. After retinal explant incubation, the retinas were processed to obtain RNA as previously described or were treated with papain (0.5 mg/mL) and immunostained using anti-GS antibody and DAPI (Sigma-Aldrich) to identified MGC soma. Images were captured using the confocal microscope (Carl Zeiss). The quantification of soma size was performed selecting the soma area of individual cells and using the area measure tool of ImageJ software (n = 30 cells); the values are expressed in percentages.

Statistical Analysis

All data were analyzed using the nonparametric Mann-Whitney test and expressed as means ± SEM. GraphPad Prism 6 software (La Jolla, CA, USA) was used for all analyses, and statistical significance was set at P < 0.05.

RESULTS

Partial Lens Surgery Induces iBRB Permeability

Posterior capsular lens discontinuation was confirmed post surgery on cryosections stained with HE (Fig. 1Bb, black arrow). In C57BL6/J control mice, the EB extravasation rate was of 4.03 ± 0.5 µL EB/g dry retina/h, and it was increased to 13.78 ± 0.5 µL EB/g dry retina/h (± 2.5) and to 13.23 ± 0.5 µL EB/g dry retina/h (± 2.5) at 24 hours (P < 0.001) and 48 hours (P < 0.001) post surgery, respectively. After 72 hours, iBRB impermeability was restored with an extravasation rate of 5.65 ± 0.5 µL EB/g dry retina/h (± 1.1) (Fig. 1C).

Partial Lens Surgery Causes Dystrophin Dp71, AQP4, and Kir4.1 Alterations

Dp71 is mainly localized at MGC endfeet in the retina.15,17 Dp71 mRNA was quantified by real-time PCR 24 and 48 hours post surgery and was downregulated by 30% and 45%, respectively, compared to control retinas (Fig. 2A). After 72 hours, Dp71 mRNA level returned close to normal, as it was downregulated by only 15% compared to control retinas. Western blot analysis showed a downregulation of Dp71 72 hours post surgery (Fig. 2B). Immunostaining on control retinal sections with H4, a pan-specific dystrophin antibody, showed the previously reported dystrophin expression pattern15,18 mainly around the blood vessels (arrow, Fig. 2Cb) and at MGC endfeet at the ILM (arrowhead, Fig. 2Cb). In these areas, a dramatic decrease (50%) in H4 staining was observed 72 hours post surgery, assessed by measuring pixel intensity both at the ILM and around the blood vessels (Figs. 2Cc, 2D). In control retinas, as previously described4,7 (Fig. 3Ab), Kir4.1 was localized at MGC endfeet at the ILM and around the blood vessels. Kir4.1 was distributed all along MGC membranes 72 hours post surgery (Fig. 3Ac), mainly at the inner plexiform layer (IPL) (Fig. 3B) without change in overall protein expression as shown by Western blot (Fig. 3D).

In control retinas, AQP4 was localized at MGC endfeet (Fig. 3Ac). However, 72 hours post surgery, an overall downregulation of AQP4 was observed (Fig. 3Ad) by 50% at the ILM and by 15% around the vessels (Fig. 3C). Despite this finding, AQP4 was still mainly localized around the vessels and at the ILM (Fig. 3Ad). A downregulation of AQP4 was confirmed by Western blot (Fig. 3E). Thus, Dp71 mRNA and protein levels were strongly reduced post surgery, possibly leading to the mislocalization of Kir4.1 and the downregulation of AQP4.

Moreover, as previously described by our team in a model of retinal detachment,13 MGC gliosis may occur in cases of retinal injury. Here, in control retinas, GFAP was expressed only in astrocytes (Fig. 3Aa, arrow), but 72 hours post surgery, GFAP was unequivocally upregulated in MGC (Fig. 3Ad, arrowhead).

Partial Lens Surgery Does Not Alter Astrocyte Morphology and Causes No Retinal Inflammation

As shown previously, the constitutive lack of Dp71 induces a change in astrocyte morphology19 and retinal inflammation.18 Here, we explored if similar phenomena could be observed after downregulation of Dp71 induced by partial lens surgery and associated with a transient iBRB breakdown. No change in astrocyte morphology (Supplementary Fig. S2) and no significant change in the expression of retinal inflammation biomarkers were observed post surgery with the exception of the TNFα gene 48 hours after surgery (Supplementary Table S1).

Dexamethasone Injections In Vivo Prevent Dp71, AQP4, and Kir4.1 Alterations Without Preventing iBRB Breakdown

A low dose of dexamethasone (23 nM) was injected intravitreally 24 hours post surgery and the iBRB permeability was assessed 48 and 72 hours post surgery: iBRB permeability was
respectively 5- and 6-fold higher than in control retinas (Fig. 4A) and was not reduced compared to vehicle injection. This showed that, at this concentration, dexamethasone was unable to restore the increased iBRB permeability observed post surgery in mice.

However, using the same experimental conditions, Western blot analysis showed that dexamethasone significantly prevented the downregulation of Dp71 and AQP4 observed 72 hours post surgery (Figs. 4B, 4D, respectively) but had no effect on Kir4.1 expression (Fig. 4C). Moreover, immunofluorescence of dystrophins and AQP4 showed that the intravitreal injection of dexamethasone not only prevented their downregulation but also unequivocally preserved Dp71 immunoreactivity (Figs. 5Ab, 5D) and AQP4 localization mainly at MGC endfeet and around the blood vessels (Figs. 5Ch, 5F). Likewise, the distribution of Kir4.1 around the blood vessels and at the ILM was also preserved (Figs. 5Be, 5E) compared to retinas injected only with the vehicle (Fig. 5B).

Ex Vivo Dexamethasone Prevents MGC Swelling and the Downregulation of Dp71, Kir4.1, and AQP4

To explore the toxicity of intraretinal fluid resulting from iBRB breakdown on MGC and the effect of dexamethasone, retinal explants27 embedded in a controllable fluid environment capable of inducing MGC swelling28 were used. As previously described,28 we used a fluid composed of a hypoosmotic solution containing Ba$^{2+}$ in the presence or absence of a low dose of dexamethasone.

In retinal explants incubated in the hypoosmotic solution containing Ba$^{2+}$, a significant increase in MGC soma area size was observed (Fig. 6A). Under these conditions, the hypoosmotic treatment led to a significant downregulation of Dp71, Kir4.1, and AQP4 mRNA (Figs. 6B–D). Moreover, dexamethasone administration also prevented the downregulation of Dp71, Kir4.1, and AQP4 mRNA (Figs. 6B–D).

Dexamethasone Regulates HSF1 Expression

Heat shock factor 1 (HSF1) has recently been identified as a regulator of Dp71 expression,29 acting as a transcription factor that can bind the heat shock–responsive element (HSE) on Dp71 promoter. To further investigate the role of dexamethasone in the prevention of the downregulation of Dp71, Kir4.1, and AQP4 associated with MGC swelling, the level of HSF1 mRNA was quantified. In the retinal explants incubated with the hypoosmotic solution containing Ba$^{2+}$, the expression level of HSF1 was similar to that found in control explants. However, in retinas incubated with dexamethasone, HSF1 expression level was significantly increased (Fig. 6E), while Dp71 was not.
downregulated and no MGC swelling was induced after exposure to the hypoosmotic solution containing Ba\(^{2+}\).

In order to explore the effect of dexamethasone on HSF1 expression, in vivo, we quantified HSF1 retinal protein expression by Western blot analysis 72 hours after lens surgery, in eyes treated or not by intravitreal injection of dexamethasone. We showed a downregulation of HSF1 72 hours post surgery (Fig. 6F) while dexamethasone intravitreal injection...
after surgery significantly prevented the downregulation of HSF1.

DISCUSSION

Using an experimental mouse model of partial lens surgery, we (1) confirmed the subsequent occurrence of a transient iBRB breakdown; (2) showed that reactive MGC were strongly altered with a downregulation of Dp71 and AQP4 and a delocalization of Kir4.1; (3) reversed the impairments of Dp71, AQP4, and Kir4.1 using low-dose dexamethasone both in vivo and ex vivo while no effect was observed on iBRB; and, finally, (4) observed that using dexamethasone led to the overexpression of HSF1, one of the transcription factors of Dp71.

Inner BRB breakdown is one of the factors leading to the formation of macular edema, one of the leading causes of vision loss worldwide in diabetic patients and patients with other retinal diseases. In clinical practice, the loss of visual acuity usually poorly correlates with the retinal thickness and iBRB breakdown. Our results suggest that retinal homeostatic alterations could be independent of iBRB breakdown since the injection of a low dose of dexamethasone prevented retinal homeostatic alterations without preventing iBRB breakdown.

An adapted model of iBRB breakdown induced by partial lens surgery and confirmed using the EB method was used in this study.25 Inner BRB breakdown due to partial lens surgery appeared similar to the Irvine Gass syndrome observed in humans, which is a rare situation in which an iBRB breakdown and a retinal edema appear after cataract surgery. The localization and proper clustering of AQP4/Kir4.1 seem to be crucial to control the osmotic balance of the retina. In healthy retina, the proteins Kir4.1 and AQP4 are strongly expressed in MGC and mainly localized at MGC endfeet or in the processes surrounding the retinal blood vessels. After partial lens surgery, AQP4 was downregulated and Kir4.1 had lost its polarization. We also showed that dystrophin Dp71, a protein that plays a crucial role in MGC functions such as retinal water and potassium homeostasis and in iBRB maintenance that is the core of a complex responsible for anchoring and proper clustering of the channels Kir4.1 and AQP4, was also downregulated. Chronologically after lens surgery, the iBRB was restored while the downregulation of Dp71 and AQP4 and delocalization of Kir4.1 were not, suggesting that iBRB...
breakdown could be an important factor altering Dp71, AQP4, and Kir4.1. In previous work from our laboratory, it was suggested that the constitutional lack of Dp71 in Dp71-null mice could be at the origin of the downregulation of AQP4, changes in Kir4.1 localization, and increase in iBRB permeability. However, conversely here, we showed that the transient iBRB breakdown model induced by partial lens surgery preceded the downregulation of Dp71, which could be responsible for the changes observed in AQP4 and Kir4.1 expression in MGC. We could assume that one of the mechanisms responsible for this phenomenon could be the effect of retinal fluid toxicity on MGC and the resulting MGC gliosis (Fig. 3), as previously suggested in a model of retinal detachment,14 or we can also suggest that after breakdown of the iBRB, blood-derived factors may induce alterations in Müller cells.

We chose to inject dexamethasone in this model because its efficacy as a treatment for retinal edema has previously been shown in humans, and its anti-inflammatory effect is higher than that of other corticosteroids. Previous works have demonstrated that intravitreal dexamethasone restored an effective iBRB in diabetic rats31 and inhibited VEGF-induced vascular leakage in rabbits.32 At a high dose (1 μM), in vivo, dexamethasone increased Kir4.1 level in a normal rat retina while a low dose (100 nM) had no effect.20 However in a pathological model of endotoxin-induced uveitis (EIU), at low dose (100 nM), dexamethasone was able to regulate the expression of Kir4.1.
To the best of our knowledge, we were the first to use dexamethasone injections in mice to restore iBRB permeability or altered AQP4/Kir4.1 in MGC during iBRB breakdown. We adapted the intravitreal dose previously used in vivo in rats \(^{31}\) (50 \(\mu g\) or 115 nM) to the weight of the mice, so 10 \(\mu g\) was injected. Using this low dose of dexamethasone (23 nM), we observed no effect on iBRB permeability, although Dp71 and AQP4 downregulation and Kir4.1 delocalization were prevented. These results are coherent with those reported by Zhao et al.\(^{20}\) who have demonstrated that dexamethasone could regulate the expression of AQP4/Kir4.1 via its effect on the glucocorticoid receptor. Dexamethasone acts also on Dp71, independently of its effect on the iBRB, suggesting that dexamethasone could have a differential effect depending on the dose used. Based on these observations, we hypothesized that at high dose, dexamethasone could help restore iBRB while at a lower dose, it could act on MGC without restoring iBRB; and as a consequence iBRB permeability, in this model, seemed to be independent of Dp71, Kir4.1, and AQP4 expression. We hypothesized that the paradoxical increase of

![Figure 6](https://example.com/figure6.png)

**Figure 6.** Protective effect of dexamethasone tested on retinal explants. The Müller glial cell soma size (A), measured by ImageJ software and expressed as a percentage (considering that control retinas, i.e., explants exposed to an isotonic solution, were 100%), was considerably reduced after dexamethasone exposure while a swelling was observed after exposure to a hypoosmotic solution containing Ba\(^{2+}\). The decreased expression of Dp71 (B), Kir4.1 (C), and AQP4 (D) was prevented by dexamethasone exposure compared to control. An increase in HSF1 relative expression (E) was observed after dexamethasone exposure compared to control. (F) Western blot of HSF1 72 hours post surgery. Injecting dexamethasone prevented the downregulation of HSF1 72 hours post surgery. Data are expressed as means ± SEM, \(n=4\) for the Western blot. Values were normalized to controls. *\(P<0.05\) **\(P<0.01\) ***\(P<0.001\) considered significant versus control retinas.
the iBBR permeability in comparison to control observed when surgery was followed by intravitreal injection, was due to the effect of intravitreal injection per se in a small eye even without new lens trauma.

Noteworthy in the explant model, we also observed that MGC swelling, Dp71, AQP4, and Kir4.1 downregulation after exposure to a hypoosmotic solution containing Ba2+ could be prevented using a low dose of dexamethasone. The regulation of Dp71 remains unclear. However, HSF1 has recently been identified as a regulator of Dp71 expression, acting as a transcription factor. Previous reports have shown that dexamethasone induces and activates HSF1 in mice. Here, we observed the upregulation of HSF1 in retinas incubated with dexamethasone. It is conceivable to propose that dexamethasone could prevent the downregulation of Dp71, AQP4, and Kir4.1 and MGC swelling via the upregulation of HSF1. Zhao et al. have previously shown that dexamethasone could regulate the expression of AQP4 and Kir4.1 in MGC. Based on our observations, we could assume that dexamethasone could act on these proteins through the regulation of Dp71 after overexpression of HSF1.

The iBBR-independent regulation of such MGC functions could explain, at least partially, the dissociation between the visual acuity and the edema severity observed in clinical practice. These mechanisms need to be understood. Indeed, in clinical practice, the way to eliminate fluid accumulated in the retina is known most of the time; however, how to protect the retina during iBBR breakdown and macular edema remains unknown, and our findings suggest that the use of corticoids but at a very low dose could be useful. In this study, we showed that dexamethasone was able to prevent MGC swelling ex vivo, suggesting a possible protective role of dexamethasone in MGC. Moreover, since MGC produce trophic substances, remove metabolic waste, recycle neurotransmitters, and modulate the synaptic transmission releasing neurotransmitter precursors, dexamethasone’s effect on MGC could be useful in a neuroprotective therapeutic approach.

It could help to eliminate potentially toxic neurotransmitters and prevent alterations in water and ionic channels occurring during iBBR breakdown and thus to maintain retinal osmotic balance.

In conclusion, in this study we observed alterations of dystrophin Dp71, AQP4, and Kir4.1 in a mouse model of surgically induced iBBR breakdown. A single injection of low-dose dexamethasone prevented the occurrence of molecular changes without effect on the iBBR. Based on the results of the ex vivo model, we suggest that dexamethasone could upregulate HSF1, one of the transcription factors of Dp71 and a protein stabilizing AQP4/Kir4.1 in MGC. Considering these effects that are independent of its action on the iBBR, low-dose dexamethasone could be a neuroprotective agent with potential utility in clinical practice, probably associated with less complication than when it is used at higher doses.

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References


