

Functional rescue of experimental ischemic optic neuropathy with α B-crystallin

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LABORATORY STUDY

Abstract

Purpose Anterior ischemic optic neuropathy (AION) is an important cause of acute vision loss in adults, and there is no effective treatment. We studied early changes following experimental AION and tested the benefit of a potential treatment.

Materials and Methods We induced experimental AION in adult mice and tested the effects of short-term (daily for 3 days) and long-term (every other day for 3 weeks) α B-crystallin (α BC) treatment using histological and serial intracranial flash visual evoked potential recordings.

Results One day after experimental AION, there was swelling at the optic nerve (ON) head and increased expression of α BC, a small heat shock protein important in ischemia and inflammation. This upregulation coincided with microglial and astrocytic activation. Our hypothesis was that α BC may be part of the endogenous protective mechanism against injury, thus we tested the effects of α BC on experimental AION. Daily intravenous or intravitreal α BC injections did not improve visual evoked potential amplitude or latency at days 1–2. However, α BC treatment decreased swelling and dampened the microglial and astrocytic activation on day 3. Longer treatment with intravenous α BC led to acceleration of visual evoked potential latency over 3 weeks, without improving amplitude. This latency acceleration did not correlate with increased retinal ganglion cell survival but did correlate with complete rescue of the ON oligodendrocytes, which are important for myelination.

Conclusions We identified α BC as an early marker following experimental AION. Treatment with α BC enhanced this endogenous, post-ischemic response by

decreasing microglial activation and promoting ON oligodendrocyte survival.

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Introduction

Anterior ischemic optic neuropathy (AION) is the most common acute optic neuropathy in patients over 50-years-old and typically leads to permanent vision loss.^{1–2} Although experts agree that non-arteritic AION results from ischemia of the optic nerve (ON) head,^{1,3} there is still significant controversy regarding the mechanism of disease⁴ and there is currently no effective treatment. Photochemical thrombosis animal models of AION have been instrumental in elucidating the events following ON head ischemia.^{5–9} In experimental AION, there is early glial and axonal dysfunction, with ON oligodendrocyte apoptosis occurring by day 6 and progressive nerve demyelination within 2 weeks.^{5,8,9} Retinal ganglion cell (RGC) loss peaks during weeks 2 and 3.^{7,8,10,11} During these 3 weeks of critical period for treatment considerations, there are important signs of self repair, such as the upregulation of retinal heat shock proteins HSP 70, 84, and 86.^{5,8} Heat shock proteins act as molecular chaperones and are thought to have important roles in optic neuropathies, retinal diseases, and central nervous system inflammatory and neurodegenerative diseases.^{12–16}

In this study, we used a mouse model for AION via photochemical thrombosis^{5–8,10,11,17} to examine the early changes following ON head ischemia. We then assessed the effectiveness of a small heat shock protein named α B-crystallin

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(α BC) in the treatment of experimental AION, using functional and histological approaches.

Materials and methods

Animals, care, and sedation

All animal care and experiments were carried out in accordance with the ARVO guideline for the Use of Animals in Ophthalmic and Vision Research and the Stanford Administrative Panel on Laboratory Animal Care. Adult wild-type 129S2/Sv mice were purchased (Charles River, Wilmington, MA, USA; Taconic Farm, Hudson, NY, USA) and housed in a 12-h light/12-h dark cycle with food and water ad libitum. All procedures were performed under sedation, achieved with ketamine 50–100 mg/kg, xylazine 2–5 mg/kg, and buprenorphine 0.05 mg/kg.

Experimental AION

Our model of experimental AION is a modification of previously published studies.^{5–8,10,11,17} After rose bengal (1.25 mM in PBS, 2 μ l per g body weight) injection via tail vein, the ON head was exposed to 20 light spots (frequency doubled Nd:YAG laser, Pascal, OptiMedica, Santa Clara, CA, USA), each with 100 μ m diameter (equivalent to 39 μ m on the nerve), at a power of 35 mW and duration of 500 ms. We delivered small laser spots at low power and shorter duration circumferentially around the ON head in order to focus more precisely while covering the entire ON head. The degree and extent of whitening due to ischemia¹⁸ are similar to that of the Bernstein model in our direct comparison. As reported previously,^{5,8} application of the laser in the absence of rose bengal did not cause significant ischemia.

Western blotting

Protein was prepared from the ON head and sensory retina of three pairs of eyes from adult mice 1 day post-AION per routine protocol in the presence of protease inhibitors. Protein concentrations were determined using micro BCA kit (Pierce, Rockford, IL, USA). Equal amount of protein per lane was loaded onto a 10% mini-SDS-PAGE gel (Invitrogen, Carlsbad, CA, USA) and run as per the recommended protocol. Western blotting was carried out with anti- α BC rabbit polyclonal antibody (1 : 200 dilution, Abcam, Cambridge, MA, USA) and anti-class III β -tubulin monoclonal antibody (1 : 500 dilution, Abcam) and developed per the ECL protocol (Pierce). Four experiments for each antibody were scanned under equal conditions and quantified with Image J (<http://imagej.nih.gov/>). Student's paired *t*-test was used for statistical analysis, and all error bars are in SEM.

Immunohistochemistry and morphometric analyses

At each time point, we performed intracardiac perfusion (India ink for angiogram, 4% paraformaldehyde in PBS for fixation) and whole mount neuroretinal dissection. For immunofluorescence microscopy, primary antibody with anti-Iba-1 rabbit polyclonal antibody (1 : 500–1000 dilutions, WAKO, Richmond, VA, USA) or anti-glial fibrillary acidic protein (GFAP) monoclonal antibody (1 : 500–1000 dilutions, Sigma, St Louis, MO, USA) and goat anti-rabbit or anti-mouse IgG secondary antibodies (1 : 200–400 dilutions, Invitrogen) were used. The retinas were mounted with Vectashield containing DAPI (Vector Labs, Burlingame, CA, USA). For paraffin-embedded or frozen horizontal sections, the eyes and ONs were dissected, and 10 μ m horizontal sections were prepared and H and E stained. All morphometric analysis was performed under masked condition so the identity of the data was not known. A circular area (300 pixel radius) centered at the ON head was quantified with Image J. Following background subtraction, regions of interest were multiplied by the intensity to calculate the intensity*pixel². For each pair of eyes, the AION eye was normalized to the unlasered eye, which served as control for every step of the experiment. Statistical significance between the lasered and unlasered eyes was determined using paired Student's *t*-test and between saline and α BC-treated groups, unpaired *t*-test.

α BC treatment

For intravenous treatment, 10 μ g of recombinant α BC (U.S. Biological, Swampscott, MA, USA) vs saline alone (final volume 100 μ l) was injected into the tail vein under masked conditions within 5 h of AION. For short-term treatment, the animals were injected once per day for 3 days. For longer treatments, the animals were injected every other day for 3 weeks. Animals in the experimental and control groups received same number of injections. To see if intravitreal injection of α BC led to additional benefit compared with intravenous injections alone, we performed intravitreal injection of 2 μ g α BC or saline (final volume 1 μ l) given to each eye within 5 h of AION in addition to intravenous injections.

Intracranial electrode implantation

Small burr holes were drilled in the skull overlying the visual cortex on each side (2.5–3 mm parasagittal and 2–3 mm anterior to the lambdoid suture^{19,20}) for placement of small stainless steel screw electrodes (JI Morris, Southbridge, MA, USA). Dental cement secured the electrodes over the skull, with several sutures to close the scalp. Implanted mice behaved like unimplanted mice, with normal response to stimuli, locomotion and weight gain over months.

Visual evoked potential recording

We used serial intracranial photopic flash visual evoked potential (fVEP) recordings to monitor ON function, assuming no changes elsewhere. Recordings were conducted under standardized conditions (sedation, body temperature, circadian rhythm, hydration status). A reference electrode was inserted near the nose, and a ground electrode, near the tail. Impedance was monitored before each trial. In the Ganzfeld dome (Espion E², Diagnosys, Westford, MA, USA), implanted mice (10 mice per group, 4–5 months old) were exposed to white light flashes at 2 Hz in background luminance of 20 cd/m². Data were acquired at 1 kHz and band pass filtered at 0.5–100 Hz. Each fVEP response trial was the average of 50 traces, with multiple trials recorded per luminance intensities (0.001–20 cd s/m²) per day. A fVEP of relatively large amplitude is shown in Figure 3a to illustrate responses over a large range of luminance intensities. There were no fVEP responses when both eyes were occluded. The recordings were conducted while masked to the treatment given.

The amplitude and latency of N₁, the dominant fVEP waveform, were averaged from 30–50 trials (minimum of three trials/mouse/time point) from six animals per treatment group, where good recording was performed in every time point. Data analysis was restricted to luminance intensities between 2.15–20 cd s/m². Raw amplitude was calculated as difference between baseline and N₁ peak ranged between 3–50 μ V. The latency was calculated as time to N₁ peak from stimulus onset and ranged between 30–80 ms. Although latencies for N₁ were extremely stable across several examinations in each mouse, their raw amplitudes showed more variability. Data were analyzed with Excel and Origin, Grubbs' test was used to eliminate outliers (0–3 per data point), and statistical differences were determined using Student's *t*-test and one-way ANOVA.

RGC quantification

H and E stained horizontal sections of the posterior pole were photographed with a $\times 40$ objective. Under masked conditions, the number of cells in the RGC layer per high power field was quantified using Image J. Following color deconvolution, the entire RGC layer was counted with nucleus counter, using a size threshold of 500 pixels, and then visually confirmed. Statistical significance was determined using unpaired Student's *t*-test.

ON oligodendrocyte quantification

We stained for oligodendrocytes with anti-CC1 mAb antibody (Calbiochem, Gibbstown, NJ, USA), goat anti-mouse secondary antibody, and mounted with

DAPI-containing Vectorshield medium (Vector Labs). We photographed the first 500 μ m of the myelinated ONs and used image J to quantify the number of cells that were CC1-negative and DAPI-positive, which represented oligodendrocytes. CC1 antibody staining did not overlap with that of GFAP expression in astrocytes. Statistical significance was determined using unpaired Student's *t*-test.

Statement of ethics

We certify that all applicable institutional and governmental regulations concerning the ethical use of animals were followed during this research.

Results

Early post-AION upregulation of α BC

Immediately following experimental AION, there was loss of peripapillary microvasculature, swelling, and leakage at the ON head (Figure 1a). At day-1 following AION, western blot analysis (Figure 1b-c) revealed an upregulation of α BC protein in the ON head and neuroretina compared with control eyes ($n = 4$ experiments, $P < 0.03$), but no significant change in the expression of class-III β -tubulin, a major component of the neuronal microtubule that is highly expressed in RGCs. Consistent with the idea that α BC is important in inflammation,^{15,21} we found that on post-ischemia day 3, there was increased ON head Iba-1 staining (data not shown, $n = 5$, see Figure 2), which suggested microglial activation, and elevated GFAP expression (data not shown, $n = 5$, see Figure 2), which correlated with astrocytic activation. These findings resembled changes after laser-induced photochemical thrombosis in the retina²² and axonal ischemia near the retina-ON junction²³ and the brain.²⁴ Our data indicated that α BC was an early marker following experimental AION, which may serve as part of the endogenous protective mechanism.

Short-term α BC treatment

To test this hypothesis, we performed short-term and long-term α BC treatment following experimental AION in adult mice. For short-term treatment, animals received daily intravenous injections of α BC (10 μ g per injection) vs phosphate-buffered saline (PBS) alone for 3 days, and we performed immunohistochemistry and analyzed whole mount retina for ON head staining with Iba-1 antibody for microglia and with anti-GFAP antibody for astrocytes. In the PBS-treated group, like in non-treated eyes, the Iba-1 expression following AION was significantly increased compared with that of the control

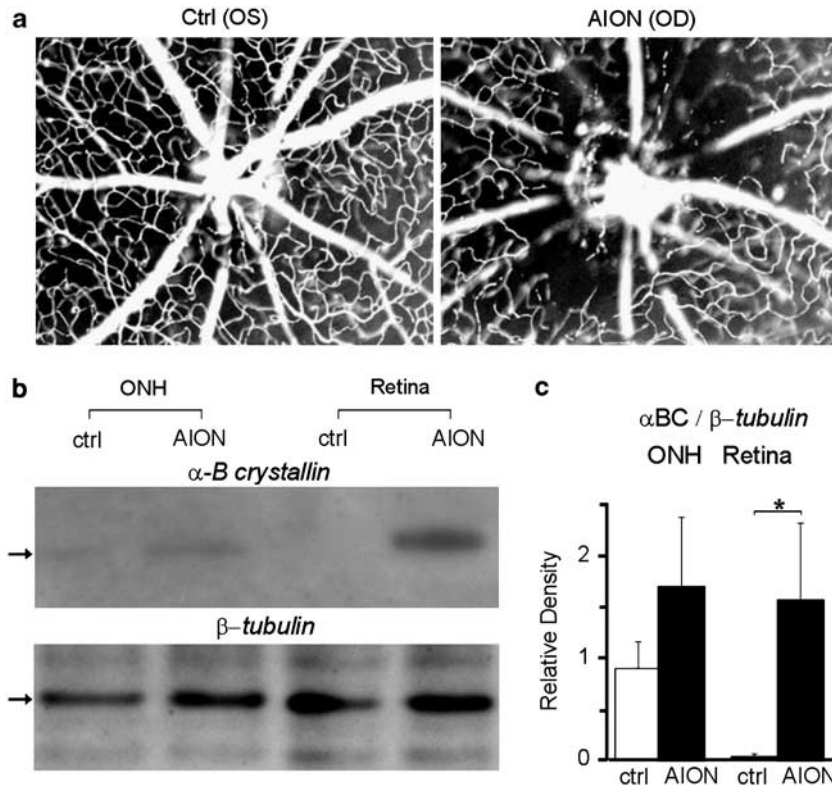


Figure 1 Experimental AION and early upregulation of α BC. (a) Retinal angiography following experimental AION showed prominent loss of peri-papillary small vessels OD. Ctrl: control, OD: right eye, OS: left eye. (b) Western blotting of control vs AION ON head (ONH) and retina 1 day following experimental AION showed increased α BC protein expression in the AION eyes compared with control eyes. There was no significant change in the expression of class-III β -tubulin. (c) Quantification of western blot data ($n = 4$ experiments) revealed elevated α BC expression compared with β -tubulin following AION. $*P < 0.03$.

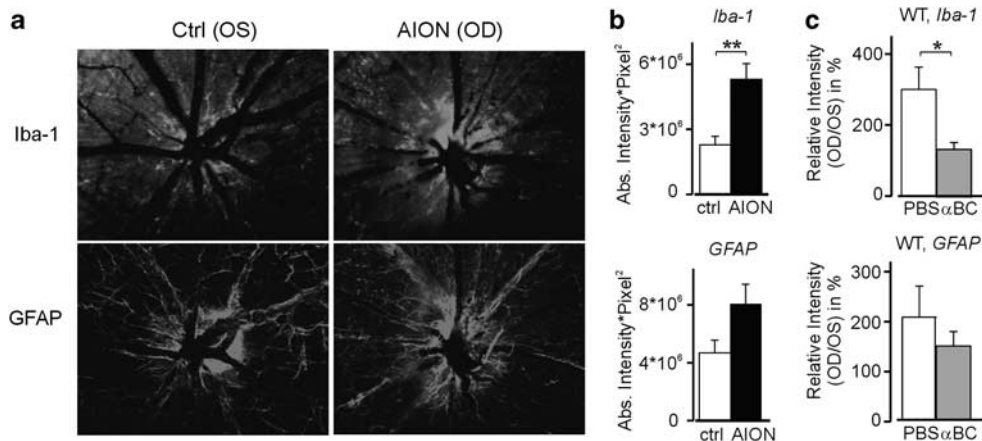


Figure 2 Acute post-AION microglial and astrocytic activation. (a) Increased ON head staining with Iba-1 and GFAP antibodies following AION. Eyes shown are the same as that of Figure 1a pictures. (b) Quantified, pooled data of PBS-treated mice showed increased Iba-1 and GFAP antibody staining following AION. $**P < 0.002$. (c) Compared with PBS-treated animals, α BC-treated mice exhibited dampened post-AION microglial activation. There was a similar but not significant trend in the GFAP data. Relative intensity was calculated as the AION eyes normalized to paired control eyes. $*P < 0.05$.

eyes (AION/control: $298 \pm 62\%$, $n = 8$ pairs of eyes, $P < 0.002$) (Figures 2 a and b). This was consistent with post-AION microglial activation. The α BC treatment

effectively suppressed this microglial activation ($P < 0.05$) (Figure 2c), suggesting that α BC decreased post-AION inflammation. Similar to Iba-1 antibody staining, the ON

head GFAP antibody staining showed a trend of increased expression in the AION eyes compared with that of the control eyes ($n = 5$ pairs of eyes, $P = 0.09$) (Figures 2a and b). The α BC treatment showed a trend of decreased GFAP upregulation (Figure 2c) (PBS: $202 \pm 62\%$, $n = 4$ pairs of eyes; α BC: $147 \pm 29\%$, $n = 3$ pairs of eyes, $P > 0.5$).

To monitor visual function, we recorded serial photopic flash VEP responses using electrodes implanted in the occipital lobes. Monocular light stimulation led to prominent contralateral occipital cortical responses,²⁰ with increasing amplitudes and decreasing latencies of the N_1 dominant peak in response to increasing

luminance intensities (Figure 3a). We compared responses with 20 cd s/m^2 , the brightest light stimuli used, between the α BC-treated and the control groups. Compared with baseline, at days 1–2 post-AION, there was a significant decrease of fVEP amplitudes in both saline- (baseline: $14.2 \pm 1.3 \mu\text{V}$; days 1–2: $9.8 \pm 0.2 \mu\text{V}$; $P = 0.02$, $n = 6$) and α BC-treated (baseline: $15.1 \pm 1.0 \mu\text{V}$; days 1–2: $11.7 \pm 1.3 \mu\text{V}$; $P = 0.04$, $n = 6$) groups (Figure 3b-c). There was no change in the latency of responses in both groups (saline: $P = 0.7$; α BC: $P = 0.3$) (Figure 3b-c). These findings suggested that short-term α BC treatment did not improve ON function early after experimental AION.

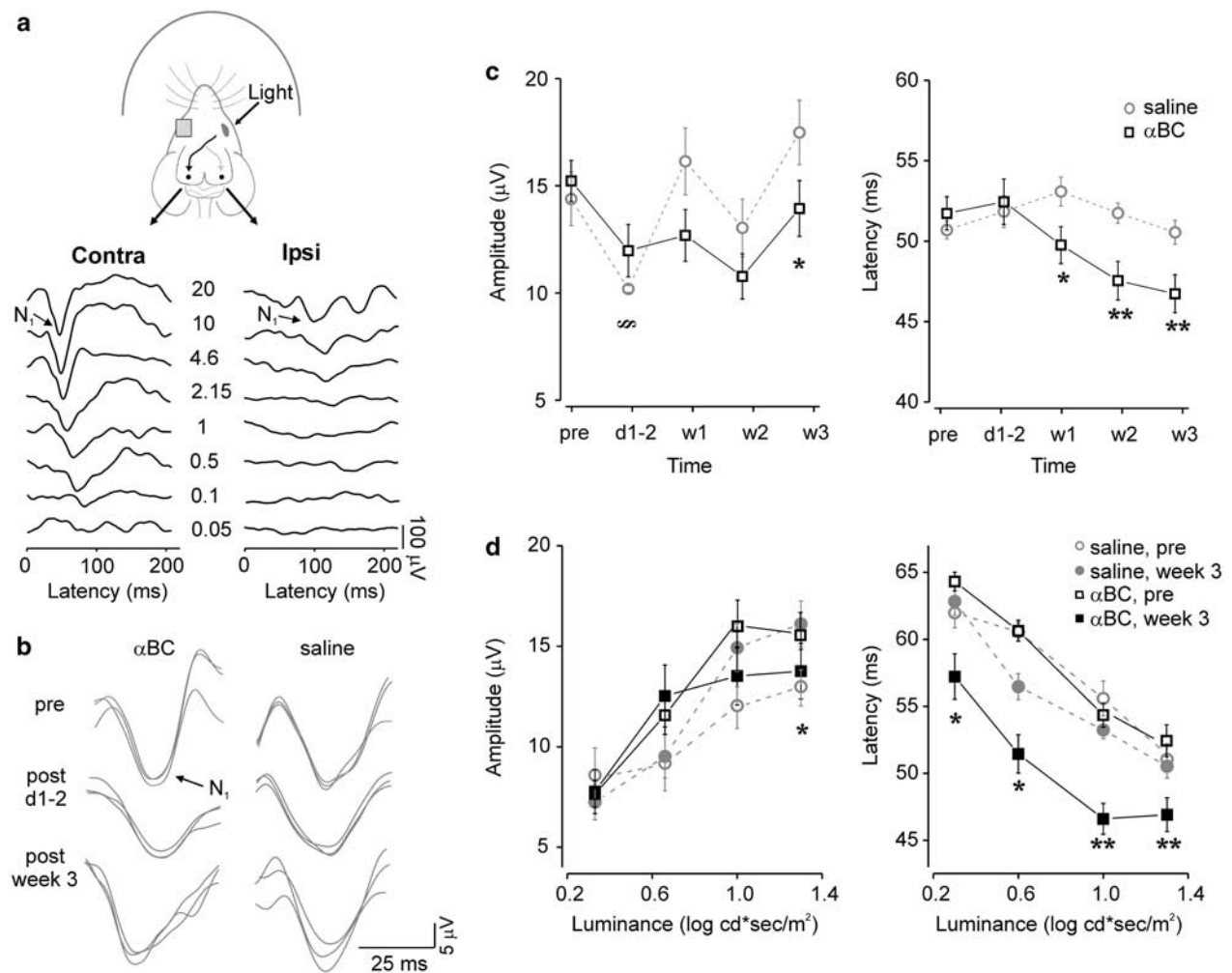


Figure 3 The α BC treatment significantly improved the latencies of the intracranial photopic flash fVEPs. (a) Recording setup and visual evoked responses (stimuli in cd s/m^2). Please note that an animal with relatively large fVEP response amplitudes were chosen to demonstrate luminance-dependent changes. (b) fVEPs from α BC-treated (α BC) and control (saline) groups. (c) Population fVEP data showed variability of fVEP amplitudes with no improvement in the α BC-treated group and significant acceleration of fVEP latencies from weeks 1 to 3 in the α BC-treated groups. $^{\S}P < 0.05$ (within each treatment group), $^*P < 0.05$, $^{**}P < 0.002$ (saline vs α BC groups). (d) Luminance intensity series at baseline vs at 3 weeks showed variability and no differences of fVEP amplitudes across different luminance intensities. In contrast, there was improvement of fVEP latencies across all luminance intensities in the α BC-treated group. Note y axis labels for (c) and (d) do not start at 0. $^*P < 0.03$, $^{**}P < 0.009$.

Long-term α BC treatment

For long-term treatment, we injected adult mice intravenously with α BC (10 μ g per injection) every other day for 3 weeks. Control group was injected with saline alone, and injections were performed under masked condition. We used fVEP recording to monitor visual function weekly over 3 weeks. Compared with days 1–2, at week 1, the fVEP amplitudes in the control group improved back to baseline (compared with days 1–2, $P < 0.001$; compared with baseline, $P = 0.2$) whereas the α BC-treated group had persistently low amplitudes (compared with days 1–2, $P > 0.5$; compared with baseline, $P = 0.07$). There was variability in the fVEP amplitudes throughout the 3 weeks, with overall improvement in both control and α BC-treated groups at 3 weeks compared with immediately post-AION.

The most striking finding in the long-term α BC experiment was the fVEP latencies, which typically showed much less variability compared with the fVEP amplitudes. Compared with days 1–2, by week 1, the α BC-treated group demonstrated decreased response latencies (baseline: 52.2 ± 1.1 ms; week 1: 50.1 ± 1.2 ms; $P < 0.05$, $n = 6$) while the latencies in the saline-treated group actually increased (baseline: 51.1 ± 0.6 ms; week 1: 53.7 ± 0.9 ms; $P < 0.05$, $n = 6$). This acceleration of fVEP latencies in the α BC-treated group continued at weeks 2 and 3 (Figure 3c, right). Comparing the two treatment groups, the latency improvement in the α BC-treated group was significant at week 1 ($P = 0.02$), week 2 ($P = 0.002$) and week 3 ($P = 0.009$). By the third week of α BC treatment, fVEP latencies at 20 cd s/m² were faster by 5.2 ms compared with days 1–2 (d1-2: 52.1 ± 1.3 ms,

week 3: 46.9 ± 1.3 ms, $P = 0.02$), whereas the latencies in the saline-treated group remained unchanged ($P = 0.3$).

Compared with baseline, the improvement of fVEP latency in the α BC-treated group at week 3 was evident across multiple luminance intensities (Figure 3d, right) (2.15 cd s/m²: $P = 0.01$, 4.6 cd s/m²: $P = 0.03$, 10 cd s/m²: $P = 0.001$, 20 cd s/m²: $P = 0.009$), unlike the changes in the amplitudes, which were more variable and showed smaller fVEP amplitudes at 20 cd s/m² in the α BC-treated group compared with the control group ($P < 0.05$) but no difference at 10 cd s/m² (Figure 3d, left). These findings supported a significant functional improvement of the speed but not the amplitude of the visually evoked responses during the 3-week α BC treatment.

α BC rescued ON oligodendrocytes but not RGCs

We looked to see if the fVEP improvement in the α BC-treated group correlated with greater RGC survival. A total of 52 ± 5 fields were counted for the AION and control eyes in both treatment groups. At week 3, there was a significant loss of RGC layer cells^{5,8} in the AION eyes compared with the controlled eyes in both the α BC-treated group and in the control group ($P < 0.001$ between AION and controlled eyes for both treatment groups) and no difference between the 2 treatment groups (Figure 4).

As fVEP latency is affected by myelination and the number and function of the ON oligodendrocytes, we analyzed if long-term intravenous α BC (10 μ g, every other day) vs saline treatment differentially affected ON oligodendrocyte survival. This is particularly important since weeks 1–2, when the fVEP latency started to differ

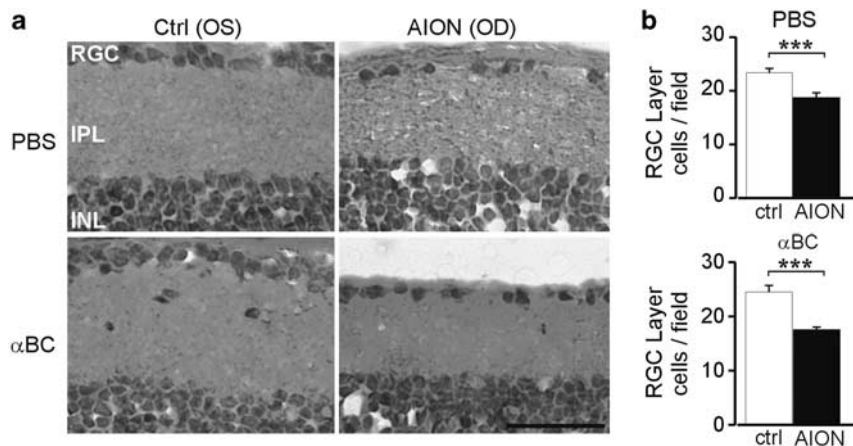


Figure 4 The α BC treatment did not increase RGC survival. (a) Horizontal thin sections of mouse retina 3 weeks post-AION showed loss of cells in the RGC layer in both α BC-treated (α BC) and control (PBS) groups. (b) Quantification of RGC layer in AION and control eyes showed significant loss of RGC layer cells in the AION eyes in both treatment groups. A total of 52 ± 5 fields were counted for the AION and control eyes in both treatment groups. *** $P < 0.001$, within group. Scale bar: 0.05 mm.

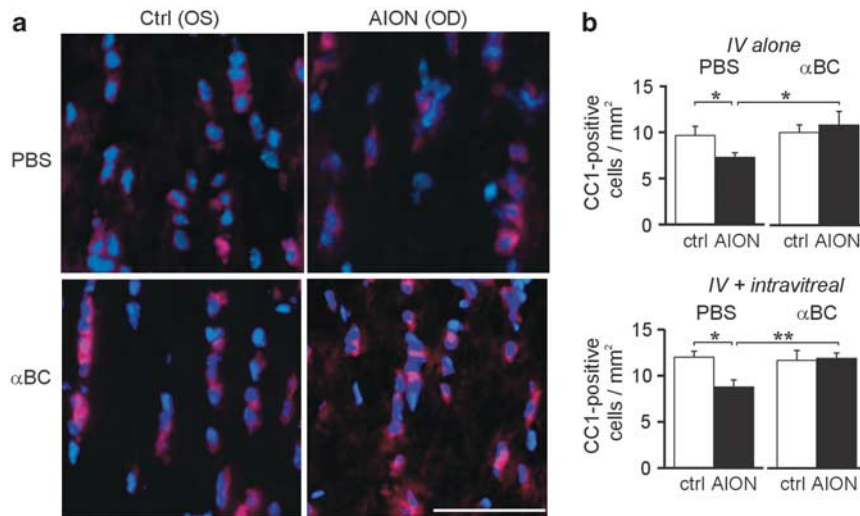


Figure 5 The α BC treatment salvaged the ON oligodendrocytes. (a) Thin, horizontal sections of ONs at day 9 stained with antibody against CC1 (red, cytoplasmic stain), a marker of oligodendrocytes, and DAPI (blue, nuclear stain) showed fewer CC1⁺, DAPI⁺ oligodendrocytes in the AION, saline-treated (PBS) group compared with the AION, α BC-treated group (α BC). There was no difference between the control eyes (Ctrl). OD, right eye; OS, left eye. (b) Top: quantification of CC1⁺, DAPI⁺ cells in all animals showed complete rescue of ON oligodendrocytes after intravenous (IV) α BC treatment. Bottom: population data of CC1⁺, DAPI⁺ cells in the intravenous plus intravitreal α BC treatment group compared with the saline-treated group. There was significant rescue of oligodendrocytes in the α BC-treated group. * $P < 0.05$, ** $P \leq 0.005$. Scale bar: 0.05 mm.

between the α BC-treated group compared with the control group, is known to be a peak period of ON oligodendrocytes loss following AION.⁸ In case the intravenously delivered α BC did not reach the ON head due to vascular compromise, we also looked at the effect of additional intravitreal α BC injection, which should deliver sufficient α BC to the ON head. On day 9 after treatment, during time of peak post-AION oligodendrocyte loss,⁸ we counted the number of cells that stained positive for CC1 and DAPI, which corresponded to the number of oligodendrocytes. In the intravenous saline-treated group, there was 25% fewer oligodendrocytes in the post-AION ONs compared with the control ONs (control: 9.7 ± 1 cells/mm², AION: 7.3 ± 0.5 cells/mm², $n = 6-14$ fields per condition, $P < 0.03$) (Figure 5). In contrast, in the intravenous α BC treated group, there was a complete rescue of the ON oligodendrocytes following AION (control: 10.0 ± 0.9 cells/mm², AION: 10.8 ± 1.5 cells/mm², $n = 6-14$ fields per condition) and significant improvement compared with the intravenous saline group ($P = 0.02$). A combination of intravenous and intravitreal α BC treatment also resulted in complete rescue of the ON oligodendrocytes following AION and significant improvement compared with the saline-treated group ($n = 5-11$ fields per condition per group, $P = 0.005$). These data showed that α BC treatment promoted survival of ON oligodendrocytes, which correlated in timing with the significant fVEP latency improvement at weeks 1–2.

Discussion

Experimental AION led to early ON head swelling, loss of peripapillary vessels,^{1,2} upregulation of micro- and macroglia, and decreased fVEP responses.^{25,26} The upregulation of α BC, a molecular chaperone important in retinal ischemia, experimental glaucoma, and central nervous system inflammatory disease,^{14–16,27} following experimental AION was consistent with its role as an endogenous protective mechanism.^{28–30} Consistent with this idea, early, 3-day intravenous α BC treatment dampened post-AION microglial activation, although it did not impact fVEP responses on days 1–2. Three-week α BC treatment, however, led to significant improvement of the latency of the fVEP responses as early as week 1, which was maintained at weeks 2 and 3. This acceleration of the visually evoked responses correlated with the rescue of the ON oligodendrocytes on day 9 but not survival of RGCs at week 3. Intravenous treatment was as efficacious as intravenous plus intravitreal treatment. Taken together, our data suggested an ON protective effect of α BC following ON head ischemia by dampening inflammation and salvaging oligodendrocytes.

Expressed in the RGCs³⁴ and ON glia,¹⁶ α BC is poised to have a role in optic neuropathies. The upregulation of α BC immediately following ON head ischemia is consistent with its function as a small heat shock protein with both protective and anti-apoptotic actions.^{14–16}

Historically known as the predominant structural proteins in the lens, the α -crystallins have been shown to act as molecular chaperones like Hsp70 and GroE,¹⁴ and α BC protects against UVA-induced apoptosis in cultured human lens epithelium. An upregulation of α BC has also been seen in response to hypoxia in cultured human ON head astrocytes,³⁵ oxidative stress in cultured trabecular meshwork,³⁶ chemical hypoxia in retina,³⁷ and oxidative stress in cultured retinal pigment epithelium.³⁸ Furthermore, α BC upregulation may prevent apoptosis from oxidative stress in photoreceptors in uveitis.³⁹ In the absence of α BC, retinal pigment epithelium is more susceptible to H₂O₂-induced apoptosis³⁷ and exhibits greater post-ischemic inflammatory changes at the ON head, supporting α BC's role as a negative regulator of inflammation (this report). In contrast, progressive RGC degeneration observed in the ocular hypertension rat model for glaucomatous neuropathy is associated with a temporary downregulation of α BC after 2 weeks and recovery after 5 weeks,^{31–33} suggesting a transient negative effect of ocular hypertension on α BC gene expression.³²

The improvement in fVEPs responses following α BC treatment developed over 3 weeks, during a period of known axonal and oligodendrocyte loss.⁸ The rescue of ON oligodendrocytes but not RGCs following experimental AION suggested that α BC acted by protecting the ON but not neurons, presumably associated with an increased ratio of oligodendrocytes to axons in the ON. Curiously, fVEP latencies in the α BC-treated group improved beyond that of the baseline, which may be related to the direct effects of α BC or from α BC-facilitated developmental acceleration of VEP latency.⁴⁰ Aside from the effects on ON oligodendrocytes, we cannot rule out that α BC treatment may have led to the selective survival of faster conducting RGCs, such as the parasol cells, over those of the slower conducting cells, such as the midget cells,⁴¹ which could explain the decreased latencies observed in the α BC treated mice. Although the melanopsin-containing RGCs are known to be more resistant to ischemia, they do not contribute to evoked responses in the occipital lobe and therefore cannot account for the VEP latency changes.

Interestingly, α BC treatment significantly improved paralysis and reduced inflammation in the central nervous system in an animal model of multiple sclerosis,¹⁵ a disease, which is also associated with VEP prolongation due to axonal and oligodendrocyte dysfunction. Injection of α BC intravitreally has also been shown to improve ON axon density following crush injury.⁴² Here we provided compelling histological and functional evidence for the potential therapeutic value of a novel treatment α BC in AION.

Summary

What was known before

- The α B-crystallin is an important molecule in inflammation and ischemia and is upregulated following different brain and eye injury models.
- Intravitreal treatment with α B-crystallin improved optic nerve crush model.
- Treatment with α B-crystallin improved animal model of multiple sclerosis.

What this study adds

- The α B-crystallin is upregulated rapidly following experimental anterior ischemic optic neuropathy.
- Treatment with α B-crystallin enhanced optic nerve function as shown by improvement in the latency of visually evoked responses. This was done with serial intracranial visual evoked potentials.
- The α B-crystallin improved optic nerve function by complete rescue of optic nerve oligodendrocytes following experimental anterior ischemic optic neuropathy.

Conflict of interest

The authors declare no conflict of interest.

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