Methylprednisolone Fails to Preserve Retinal Ganglion Cells and Visual Function after Ocular Ischemia in Rats

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PURPOSE. Methylprednisolone (MP) is commonly used to treat traumatic optic neuropathy and optic neuritis, but its benefit in terms of neuronal survival remains controversial. The aim of this study was to investigate the effects of MP on retinal ganglion cell (RGC) survival and visual function after ischemia in rats.

METHODS. Ocular ischemia was induced by elevated intraocular pressure. Rats were treated with NaCl, 1 mg/kg/d, or 30 mg/kg/d intraperitoneal MP over 3 days. RGCs were labeled retrogradely 4 days after ischemia and were quantified 6 days later. Post-ischemic retinal function was assessed by scotopic and photopic electroretinography (ERG). Optic nerve function was investigated on days 4 and 10 after ischemia by visual evoked potentials (VEPs).

RESULTS. Compared with nonischemic eyes, ischemia reduced RGCs with NaCl to 47% ± 3% (mean ± SEM) and to 46% ± 3% and 43% ± 6% with 1 mg/kg/d and 30 mg/kg/d MP. ERG did not differ significantly for any parameter among the three groups. Four days after ischemia, the VEPs of rats receiving any dose of MP were significantly higher than the control. VEPs in both steroid groups fell to control levels 10 days after ischemia.

CONCLUSIONS. Treatment with MP did not improve RGC survival or retinal function. The VEP showed a short-term benefit because of steroids. (Invest Ophthalmol Vis Sci. 2008;49: 5003–5007) DOI:10.1167/iovs.08-1869

Glucocorticoids are natural steroid hormones. Evidence that glucocorticoids are effective in the treatment of central nervous system injury came from different animal models1–3 and from clinical studies for inflammatory4,5 and traumatic diseases.6 Based on those data, glucocorticoids were used in ophthalmology to treat traumatic optic nerve injury7 and optic neuritis.8 However, clinical evidence that glucocorticoids improve the survival of retinal ganglion cells (RGCs) is lacking. The International Optic Nerve Trauma Study did not find a clear benefit after glucocorticoid treatment,9 and optic nerve atrophy does not seem to be affected by glucocorticoids after optic neuritis.10 Doubts about the neuroprotective effects of glucocorticoids were supported by an animal study,11 and recent studies suggested pro-apoptotic effects on RGCs in a model of experimental autoimmune encephalomyelitis12 and after optic nerve crush.13

The aim of the present study was to investigate the hypothesis that methylprednisolone (MP) has neuroprotective properties in the retina. We approached this question using transient ocular ischemia as a standardized damage model for retinal ganglion cells whereby functional and morphologic deficits are induced by interruption of the blood supply. This model was chosen to minimize the confounding beneficial effects of MP, such as the reduction of inflammation and traumatic edema. We assessed the number of vital RGCs, the retinal function by electroretinography (ERG), and the visual pathway by evaluating temporal contrast vision with visual evoked potentials (VEPs).14 MP was injected intraperitoneally in two different dosages directly after ischemia and on the first and second days thereafter: 1 mg/kg/d as a standard anti-inflammatory dosage and 30 mg/kg/d as equivalent to that of the Second National Acute Spinal Cord Injury Study.15

MATERIALS AND METHODS

Animals

Adult male Brown-Norway rats (weight range, 180–200 g) were obtained from Charles River Germany. All animal studies were conducted in accordance with the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research and were approved by the Committee on Animal Care of the University of Freiburg. All manipulations were performed under general anesthesia with isoflurane/O2. Body temperature was maintained at 37°C ± 0.5°C by a heating pad and was monitored with a rectal thermometer probe.

Ocular Ischemia

Rats were anesthetized, and the anterior chamber of the left eye was cannulated with a 30-gauge needle connected to a reservoir containing 0.9% NaCl. Intracocular pressure was increased to 120 mm Hg for 5 minutes, and ischemia was confirmed by retinal edema and stases in retinal arteries. Rats without recovery of retinal perfusion in the first 5 minutes after the ischemic period, or those with lens injuries, were excluded. Twenty-six rats underwent ischemia and were divided into three groups: saline (n = 10) at 1 mg/kg/d, MP (n = 7), and 30 mg/kg/d MP (n = 9) for 3 days.

Visual Evoked Potential

Potentials were evoked by frequency and luminance-modulated flicker stimuli and were recorded in awake, freely moving animals according to the protocol described previously.16 In short, stainless steel screws were implanted 3 mm lateral to the lambda and 5 mm behind the bregma. Reference electrodes were placed over the frontal brain. The electrode assembly was encased in dental acrylic, and the wounds were sutured. For recording, the nonanesthetized rats were placed in a cage surrounded by a Ganzfeld bowl. A patch of aluminum foil in addition to an ointment containing coal particles reversibly blinded the right, nontreated eye (Jehle T, et al. IOVS 2007;48:ARVO EAbstract 3756). Recordings of the left eye were made 1 day before for nonischemic controls and on days 4 and 10 after ischemia.

Electroretinography

Animals were dark adapted overnight and prepared for recording under dim red light using light-emitting diode illumination (>600 nm). After anesthesia, the pupils were dilated with tropicamide (0.5%; Pharma Stulln, Stulln, Germany) and phenylephrine (5%; Ursapharm, Pharma Stulln, Stulln, Germany) and phenylephrine (5%; Ursapharm, None; T. Jehle, None; W.A. Lagra`ze, None; None; C. Dimitriu, None; M. Bach, None; Wolf A. Lagra`ze, None; Thomas Jehle, None.

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Bonn, Germany). The cornea was anesthetized with proparacaine hydrochloride (Dr. Winzer Pharma GmbH, Berlin, Germany). Retinal signals were recorded from the cornea using a DTI electrode, as described.\textsuperscript{14} Recordings were made 2 days before (for nonischemic controls) and on day 7 after ischemia.

Retinal Ganglion Cell Quantification

RGCs were labeled retrogradely by injection of 4 \( \mu \)L fluorescent tracer (Fluoro-Gold; Fluorochrome, Denver, CO) into both superior colliculi using a stereotactic device (Stoelting, Tübingen, Germany) 4 days after ischemia. Rats were killed 6 days after labeling by carbon monoxide. The eyes were removed and fixed. Retinas were then dissected, flattened, and embedded in medium (Vectashield Mounting Media; AXXORA Deutschland, Loerrach, Germany). Fluorescent tracer (Fluoro-Gold; Fluorochrome)-positive RGCs were counted in a blinded fashion under a fluorescence microscope (Axioskop; Carl Zeiss, Jena, Germany) in 12 distinct areas measuring 0.04 mm\(^2\) each. The untreated right eyes of the first 14 rats were used as nonischemic controls.

Data and Statistical Analyses

VEPs and photopic-flicker ERG responses were extracted by Fourier analysis (Igor Pro 5.01; WaveMetrics, Lake Oswego, OR). Spectra magnitudes, including the third harmonic, were added up in VEPs. For the scotopic ERG, the a-wave was calculated as the difference between the baseline and the trough of the first negative deflection. Convolutional b-wave amplitude was the difference between the a-wave’s trough and the b-wave’s peak. Oscillatory potentials were extracted as a bandpass of 75 Hz to 300 Hz from the unattenuated scotopic flash ERG and were quantified as the amplitude between the highest peak and the lowest trough. All averaged data are presented as mean \( \pm \) SEM. Statistical significance was assessed using ANOVA followed by Tukey-Kramer post hoc testing corrected for multiple comparisons. Differences were considered significant at \( P < 0.05 \). Power analysis was performed with a researcher toolkit (DDS Research, Inc., Fort Worth, TX; http://www.ddsresearch.com/toolkit/spcalc/power a2.asp) using the two-tail approach.

RESULTS

MP on RGC Survival

In nonischemic eyes, the mean density of RGCs was 2310 \( \pm \) 24 cells/mm\(^2\) (\( n = 14 \)). Fifty-five minutes of ischemia reduced the RGCs to 47% \( \pm \) 3% (mean \( \pm \) SEM) of control density. The number of surviving RGCs was not altered by intraperitoneal administration of 1 mg/kg/d or 30 mg/kg/d MP (Fig. 1A). ANOVA for the ischemic animals yielded \( P = 0.21 \). To assess the power of these findings, we postulated an effect of 25% when administering 30 mg/kg/d MP. Based on this assumption and the mean and distribution found, the power (1 - \( \beta \)) resulted as 0.81.

MP on Retinal Function

Scotopic and photopic ERGs were recorded to evaluate MP effects on retinal function. Before ischemia, mean (\( \pm \) SEM) amplitudes were 195 \( \pm \) 12 \( \mu \)V for the a-wave, 438 \( \pm \) 13.5 \( \mu \)V for b-wave, 162 \( \pm \) 7 \( \mu \)V for oscillatory potentials, and 57 \( \pm \) 2 \( \mu \)V for photopic-flicker stimulation at 19 Hz. The ischemic insult reduced all amplitudes. Oscillatory potentials and photopic responses were even abolished and could not be extracted from noise (Fig. 2A). Mean amplitudes of the a- and b-waves are shown in Figure 2B. We observed no statistically significant difference between the treatment groups. Peak times of the a-wave were 22.0 \( \pm \) 0.7 ms for nonischemic controls, 22.6 \( \pm \) 1.2 ms for NaCl, 23.3 \( \pm \) 0.9 ms for MP 1 mg/kg, and 21.7 \( \pm \) 0.3 ms for MP 30 mg/kg. Peak times of the b-wave were 39.5 \( \pm \) 2.5 ms for nonischemic controls, 38.5 \( \pm \) 6.0 ms for NaCl, 38.9 \( \pm \) 3.2 ms for MP 1 mg/kg, and 40.8 \( \pm \) 0.9 ms for MP 30 mg/kg. No concentration of MP altered the latencies in comparison with NaCl-treated animals.

MP on Visual Function

As shown in Figure 3, the potentials of rats receiving MP were significantly higher than those of controls on day 4. On day 10 after ischemia, VEPs in both steroid groups decreased to control level.

DISCUSSION

Our aim was to assess whether MP would reveal neuroprotective effects on retinal neurons in an in vivo model independent of inflammation or direct axonal injury. To our knowledge, the effects of MP to protect retinal cells after ischemia have not been published before. We demonstrated that MP did not affect RGC survival, retinal function, or final optic nerve function.

The rationale for choosing two different concentrations of MP was adapted from the commonly used clinical dose. Even if MP is not usually applied after ischemic insult, we chose the transient-ischemia model because it allows functional and morphologic testing. Neuroprotection is typically quantified by the amount of cell loss. We detected no difference in cell count between the MP-injected rats and those injected with NaCl 0.9% solution. This was surprising because MP elicits multiple protective cellular actions, such as inhibiting arachidonic acid metabolism\textsuperscript{15} and lipid peroxidation,\textsuperscript{16,17} stabilizing lysosomal membranes,\textsuperscript{18} reducing basal metabolic energy,\textsuperscript{19} lowering lactate accumulation,\textsuperscript{20} and enhancing the expression of heat-shock proteins.\textsuperscript{21} However, neuroprotection has been demonstrated in the retinal ischemia model with other substances, such as memantine.\textsuperscript{22}
In addition to counting RGCs, we recorded VEPs and ERGs to assess the remaining function after ischemia. Retinal function was markedly reduced, but not abolished, after ischemia, with the b-wave more affected than the a-wave, indicating a different sensitivity of cell populations to ischemia, as shown in earlier publications. MP did not affect the function of inner retinal neurons such as photoreceptors and bipolar cells; it neither damaged nor preserved them, whereas other substances can preserve or improve post-ischemic retinal function.

VEP decreased after retinal ischemia. The relatively small decrease of potentials, especially in the 7.5-Hz stimulation group, is initially surprising given the marked loss of retinal function. However, the loss of function as assessed with the VEP is only loosely correlated with the amount of cell loss in earlier studies. For instance, in monkey experimental glaucoma, Johnson et al. showed that a loss of 91% of the ganglion cells reduces the flash VEP down to only 64%. Four days after ischemia, the VEPs of rats receiving MP were significantly higher than those of controls; 6 days later, the VEPs in both steroid groups fell to control levels. MP may well have delayed cell death or improved the excitation of the surviving cells, leading to the better initial function.

Even considering the RGC, ERG, and VEP results together, MP did not influence cell survival or visual function after ischemia. We do think that the lack of steroid efficacy is not simply a dosage problem because we applied two dosages that differed by a factor of 30. Previous studies found neuroprotective effects of MP on neuronal and retinal tissue after intraperitoneal injection in rats with similar dosages. However, we hypothesize that the neuroprotective and pro-apoptotic effects balanced each other. Thus, treatment with none of the concentrations seemed to influence retinal neurons after ischemia. A similar situation has been found for optic nerve injury in the adult rat.

In summary, we report negative and positive findings with clinical relevance. First, the results suggest that methylprednisolone treatment is not effective in ameliorating retinal injury induced by ischemia. Second, the treatment also did not impair visual function or reduce RGC survival.
FIGURE 3. (A, B) VEP responses of one recording session per column. VEPs were recorded by implanted electrodes in freely moving rats after stimulation of the entire visual field, with the healthy right eye occluded by a patch. (A) Stimuli (7.5-Hz flicker and 19-Hz flicker) with increases in luminance difference from 5% to 80%. VEP traces showed a quasi-sinusoidal waveform. The increase in modulation depth led to a monotonically increase in amplitudes for both stimulation frequencies. (B) Modulation of a full-contrast flicker temporally modulated from 38 to 2.9 Hz. (C) Mean amplitudes with SEM were plotted as a function of modulation depth (7.5 Hz; top), modulation depth (19 Hz; middle), and frequency (bottom). Amplitudes decreased because of ischemia in all recordings. Four days after ischemia, the VEPs of rats receiving any dose of MP were significantly higher than in control. Ten days after ischemia, VEPs in both steroid groups fell to control level.

References


