Riluzole Improves Functional Recovery after Ischemia in the Rat Retina

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Purpose. Retinal ischemia leads to neuronal death. The effects of riluzole, a drug that protects against the deleterious effect of cerebral ischemia by acting on several types of ion channels and blocking glutamatergic neurotransmission, were investigated in a rat model of retinal ischemic injury.

Methods. Retinal ischemia was induced by increasing intraocular pressure above systolic blood pressure for 30 minutes. Electroretinograms were recorded before ischemia and at different periods of reperfusion. Riluzole was injected or topically applied to the eye before or after ischemia and twice daily during the reperfusion period. Retinas were harvested for histopathology (toluidine blue and silver-impregnation stainings, Tdt-dUTP terminal nick-end labeling [TUNEL] method and immunohistochemistry for cytoskeletal glial fibrillary acid protein and c-jun NH2-terminal kinase (p-JNK).

Results. Ischemia for 30 minutes caused a reduction of a- and b-waves of the electroretinogram. Systemic and topical treatments with riluzole significantly enhanced the recovery of the reduced a- and b-waves after defined reperfusion times. Riluzole also prevented or attenuated ischemia-induced retinal cell death (necrosis and apoptosis) and reduced the activation of p-JNK, c-jun phosphorylation, and the increase of cytoskeletal proteins induced by ischemic injury.

Conclusions. Riluzole acted in vivo as a potent neuroprotective agent against pressure-induced ischemia. Therefore, riluzole may be a major drug for use in protection against retinal injury. (Invest Ophthalmol Vis Sci. 1999;40:729-736)

R etinal ischemia is a leading cause of blindness in developed countries. It can result from such disorders as central artery occlusion, retinopathy of prematurity, and diabetic retinopathy. The mechanisms involved in ischemic retinal degeneration are still poorly understood, and there are no current efficacious therapies. Glutamate serves as the main neurotransmitter in photoreceptors, bipolar cells, and ganglion cells, and glutamate excitotoxicity has been implicated as a pathogenetic mechanism of neuronal cell death in ischemic retinal injury.

Riluzole (2-amino-6-trifluoromethoxy benzothiazole) is a neuroprotective drug that blocks voltage-sensitive Na+ channels and activates a new class of background K+ channels. In addition, riluzole blocks glutamatergic neurotransmission in the central nervous system, probably as a consequence of its action on ion channels. Riluzole has recently been shown in clinical trials to prolong the survival of patients with amyotrophic lateral sclerosis. It protects from neuronal damage in rodent models of global and focal cerebral ischemia and in spinal cord injury. The purpose of the present study was to examine the possible neuroprotective effect of riluzole on ischemic injury in retinal cells by electroretinographic assessment of visual function and by histologic examination of retinal structure.

Materials and Methods

Animals

All investigations involving animals conformed to the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research. Experiments were performed on adult male Brown-Norway rats weighing 200 to 250 g (Charles River, St. Aubin les Elbeuf, France). The animals were housed in a room with controlled temperature and a fixed lighting schedule (lights on from 8 AM to 8 PM). Food and tap water were provided ad libitum. After a 7-day acclimatization to the standard cyclical lighting, the animals underwent a complete eye checkup.

Pressure-Induced Ischemia Model

Rats were anesthetized with an intraperitoneal (IP) injection of 6% pentobarbital (100 μl/100 g) and placed in a stereotaxic frame. After topical instillation of a drop of oxybuprocaine (Laboratoire Chauvin, Montpellier, France), the anterior chamber of the right eye was cannulated with a 30-gauge needle connected to a reservoir containing Hanks' balanced salt solution. Retinal ischemia was induced by raising the saline reservoir to a height of 170 mm, thereby increasing the intraocular pressure for 30 minutes. Electroretinograms were recorded before ischemia and at different periods of reperfusion. Riluzole was injected or topically applied to the eye before or after ischemia and twice daily during the reperfusion period. Retinas were harvested for histopathology (toluidine blue and silver-impregnation stainings, Tdt-dUTP terminal nick-end labeling [TUNEL] method and immunohistochemistry for cytoskeletal glial fibrillary acid protein and c-jun NH2-terminal kinase (p-JNK).
All rats were dark-adapted for 4 hours, their pupils dilated with silver chloride ring recording electrode was placed on the 0.5% tropicamide (Chibret Laboratory, Clermond-Ferrand, France), displayed on an oscilloscope (model 2211; Tektronix, Les Ulis, France), and experimental preparations were performed under dim red light. Silver chloride tip, was connected to the ear. Electroretinography (ERG) responses rapidly disappeared. During the experiment, the animals were kept normothermic with heated jackets (37°C). Animals were killed 2 and 7 days after ischemia.

Drug Treatments
Riluzole (Research Biochemicals, Natick, MA) was first dissolved in 0.1 N hydrochloric acid and then diluted in 0.9% saline. An IP injection of 8 mg/kg riluzole was administered 30 minutes before ischemia or 30 minutes after the end of the ischemic insult and once daily during the recovery period. Control animals received an IP injection of the vehicle solution (acidic saline).

In the topical treatment, a solution of 10 μl 0.02% riluzole dissolved in 0.01% dimethyl sulfoxide and diluted in Hanks’ balanced salt solution was topically applied to the eye 30 minutes before ischemia or immediately after the end of ischemia and was maintained twice daily until the end of the experiment. Eyes of control rats received 10 μl vehicle solution containing 0.01% dimethyl sulfoxide and Hanks’ balanced salt solution.

At each time point during reperfusion (2 and 7 days), six rats, treated as follows, were used in each of the 14 experimental groups: IP and topical control treatment (vehicle solution), IP injection before and after ischemia, and topical application before and after ischemia.

Electroretinography
All rats were dark-adapted for 4 hours, their pupils dilated with 0.5% tropicamide (Chibret Laboratory, Clermond-Ferrand, France) and their body temperatures maintained at 37°C. A silver chloride ring recording electrode was placed on the cornea, which had been anesthetized by a drop of 0.04% oxybuprocaine hydrochloride. The reference electrode, with a silver chloride tip, was connected to the ear. Electroretinograms were recorded in rats under general anesthesia. All experimental preparations were performed under dim red light. Light stimulus (10 μsec) was provided by a stroboscopic flash (model PS-22; Grass Instruments, Quincy, MA) placed 0.2 m in front of the rat with an intensity of 4.7 candela/sec per square meter measured with a digital radiometer-photometer (model 2550; LKC Technologies, Gaithersburg, MD). The electric signals were recorded, amplified (gain:1000) by DC amplifier (model INH; Biological, Claix, France), displayed on an oscilloscope (model 2211; Tektronix, Les Ulis, France), and transmitted to a computer (IBM, Armonk, NY). The photo-stimulator was synchronized with the oscilloscope, and the delivery of the light stimuli initiated the recording of the electric signals. Electroretinograms were recorded at 4-minute intervals before, during, and 2 or 7 days after ischemic insult. At each reperfusion time point, six rats were studied in each experimental group (n = 14). The a-wave amplitude was measured from prestimulus potential to its trough and the b-wave amplitude from the a-wave trough to the b-wave peak. Fifteen consecutive responses were recorded and averaged for each animal (n = 6) per group (n = 14). The amplitude values of a- and b-waves obtained in each animal were expressed as a percentage of control value recorded before ischemia and averaged. Data were expressed as mean ± SEM values of six rats per group. Results from the ERG recordings were not distributed normally and had unequal variance. Therefore, the nonparametric Mann-Whitney U test was used for statistical analysis. The electrophysiologist who recorded the ERGs was blinded to the experimental conditions.

Fixation and Sectioning
The eyes of all rats were enucleated, and a puncture was made at the limbus to permit the infusion of fixative. For frozen retinal sections, the eyes were immediately fixed in ice-cold 4% paraformaldehyde phosphate-buffered saline (PBS) for 4 hours and then cryoprotected in PBS containing 20% sucrose. Frozen sections (7 μm) were cut on a cryostat (Leica, Marseille, France) at −20°C and stored at −70°C until used. Paraffin-embedded retinal sections (7 μm) were prepared by immersing the eyes in Bouin fixative and then embedding the sections in a standard manner in paraffin. All retinal tissues processed for histopathology and immunohistochemistry experiments were taken from animals examined for functional recovery. The neuropathologist, who was blinded to the experimental conditions, performed the histologic assessment using light and fluorescence microscopy. For histopathology (toluidine blue and silver-impregnation stainings) performed on paraffin sections 7 days after ischemia, six retina sections were placed on 3-aminopropylethoxysilane-coated slides, and 10 slides (randomly chosen) per rat (n = 3) and per staining were used in each experimental group (n = 7). For c-jun NH2-terminal kinase (p-JNK) and glial fibrillary acid protein (GFAP) immunohistochemistry performed on frozen sections 2 and 7 days after ischemia, respectively, three retina sections were placed on slides, and 10 slides per rat (n = 3) were used in each experimental group (n = 7). For TdT-DUTP terminal nick-end labeling (TUNEL) performed on paraffin sections 2 days after ischemia, three retina sections were placed on slides, and 10 slides per rat (n = 3) were used in each experimental group (n = 7).

Silver-Impregnation Staining
In addition to toluidine blue staining, argyrophilic staining was used on 7-μm-thick paraffin sections, according to standard procedure.21 Deparaffinized sections were dehydrated for 5 minutes each in 50%, 75%, and 100% 1-propanol. They were esterified at 56°C for 16 hours with 1-propanol containing 4% distilled water and 0.8% sulfuric acid. Sections were then rehydrated in 50% and 25% 1-propanol and two changes of distilled water. They were treated with 3% acetic acid for 3 to 5 minutes and placed in the silicotungstatestate developer until the background turned yellow or light brown (approximately 5 minutes). The developer was always prepared to 3 minutes before use by pouring in 1 part of stock solution B (2.0 g AgNO3, 2.0 g NH4NO3, 20.0 g tungstosilicic acid, and 3.5 ml 40% formaldehyde, dissolved in the order given) to 1 part of stock solution A (100 g Na2CO3 anhydrous in 1 l distilled water) under vigorous stirring until the mixture was completely clear. Sections were washed in 1% acetic acid for 30 minutes, dehydrated with 1-propanol for 5 to 10 minutes, cleared with xylene, and mounted in glycerol.

DNA TUNEL Assay
TUNEL22 was performed with modifications using a commercial kit (In Situ Cell Death Detection kit; Boehringer Mannheim,
Mannheim, Germany) on 7-μm-thick paraffin sections. DNA strand breaks were stained with streptavidin-fluorescein (1:100; Dako).

**Analysis of DNA Fragmentation**

Genomic DNA was extracted from control, ischemic, or treated retina (isolated 2 days after ischemia). Fifty milligrams to 100 mg of tissue was lysed in 1 ml reagent (TRizol; Gibco, Paisley, Scotland, UK). After addition of 0.2 ml chloroform, the samples were centrifuged at 12,000g for 15 minutes at 4°C. After precipitation in ethanol and a series of washes, the DNA was solubilized in 8 mM NaOH. Residual RNA was removed by incubation with 1 μg RNase A at 37°C for 30 minutes. Three micrograms DNA was 3′-end-labeled with [α-32P]deoxycytidine triphosphate and terminal deoxynucleotidyltransferase enzyme, electrophoresed on a 2% agarose gel, and autoradiographed on a phosphoimagier (Fuji, Tokyo, Japan).

**p-JNK and GFAP Immunohistochemistry**

Seven-micrometer-thick frozen sections were blocked with 5% goat serum (Vector, Burlingame, CA) and 0.3% Tween for 1 hour at room temperature followed by a single rinse in PBS. Sections were then incubated with the primary antibody overnight. The antiserum used for the study of cytoskeletal protein expression was rabbit anti-cow GFAP (1:250; Dako, Trappes, France). After the primary incubation and a single rinsing in PBS, the second anti-rabbit GFAP-fluorescein isothiocyanate antibody (1:100, Sigma, Poole, UK) was incubated for 1 hour. The antiserum used for the study of activation of JNK kinases was a monoclonal mouse phospho-cJun antibody, p-JNK (1:200; Santa Cruz Biotechnology, Santa Cruz, CA). The p-JNK expression was visualized by AEC staining (3-amino-9-ethylcarbazole; Sigma) using the ABC kit (VectaStain; Vector). All sections were washed once in PBS, washed once in distilled water, and mounted with glycerol.

**RESULTS**

**Electroretinographic Data**

The electrical function of the retina was monitored by recording the ERG. Alterations of a- and b-waves of the electric response were analyzed to assess retinal damage after an ischemic challenge of 30 minutes (Fig. 1). Electroretinograms were obtained from all animals. During ischemia induced by 30 minutes of increased IOP, the amplitudes of a- and b-waves of the ERG were markedly suppressed, as previously observed. On reperfusion in the absence of riluzole treatment, the a- and b-waves recovered slowly but remained strongly reduced, even 1 week after reperfusion. At a reperfusion time of 2 days, the amplitudes of the a- and b-waves were 38% ± 7% and 34% ± 5%, respectively, of the preocclusion values. At a reperfusion time of 7 days, the amplitudes of a- and b-waves were 47% ± 11% and 54% ± 5% of the preischemia levels (Figs. 1, 2).

Electroretinograms recorded in nonischemic animals treated with riluzole remained unchanged over time, and administration of riluzole had no effect on the amplitude of a- and b-waves (Fig. 1). Intraperitoneal injection of riluzole strongly enhanced the recovery of a- and b-waves, although its effect on the a-wave was more marked in the group treated before ischemia than in the group treated after ischemia. Seven days after ischemia, the amplitude of the a-wave was 96% ± 4% of the control values in the group treated before ischemia and 76.5% ± 8% in the group treated after ischemia (P < 0.006). Seven days after reperfusion, the recovery of the b-wave amplitude was 70% ± 8% and 65% ± 9% of the control values in rats treated before and after ischemia, respectively (Figs. 1, 2).

Topical administration of riluzole strongly enhanced the recovery of the a- and b-waves in the group treated before ischemia (Figs. 1, 2). The amplitude of a- and b-waves reached its maximum value 2 days after reperfusion (93% ± 9% and 89% ± 9% of control values, respectively). Seven days after reperfusion, the amplitude of the a-wave reached 99% ± 9% of control values, and the amplitude of the b-wave was slightly reduced (79% ± 6% of control values). In the group treated after ischemia, the a- and b-wave amplitudes (53% ± 11% and 54% ± 6% of control values, respectively) were partially but not significantly protected after 2 days compared with those in the ischemic group (P > 0.05), and they finally stabilized at 82% ± 14% and 78% ± 6% of control values, 7 days after reperfusion (Figs. 1, 2). The improvement in the a- and b-wave amplitudes after 7 days in the group treated after ischemia was statistically significant (P < 0.02).
Cytoskeleton not expressed in Müller cells, and GFAP immunoreactivity was fore or after ischemia reduced the GFAP response in Müller cell cytoplasm across the full width of the retina from region extending through the inner and the outer retinal layers.

Changes in the Organization of the Retinal Cytoskeleton

Ischemia induced changes in the levels of expression of cytoskeletal proteins such as GFAP and vimentin. A selection of 90 retina sections per group (n = 7) were examined. Figure 3 shows the overall picture of GFAP expression in the different experimental groups. In all control retinas, GFAP was not expressed in Müller cells, and GFAP immunoreactivity was only localized in the retinal astrocytes situated in the nerve fiber-ganglion cell layer (GCL; Fig. 3A). Ischemia caused an increase in GFAP immunoreactivity in the Müller endfoot region extending through the inner and the outer retinal layers. Labeled intermediate filaments were then present within the Müller cell cytoplasm across the full width of the retina from the GCL to the outer nuclear layer (ONL) after 7 days of reperfusion in all ischemic retinas examined (Fig. 3B). In all cases, IP and topical applications of riluzole administered before or after ischemia reduced the GFAP response in Müller cells. Topical treatments provided the best protection against ischemia. All retinas expressed a slight GFAP immunoreactivity. Figure 3C shows the representative retina of a rat topically treated with riluzole at the onset of reperfusion. In contrast, the protection against GFAP immunoreactivity was less efficacious with IP injections of riluzole. GFAP immunolabeling was more pronounced in the Müller cell endfoot and in Müller cell processes with IP injection than with topical application, suggesting that GFAP immunoreactivity was partially affected by IP treatment with riluzole.

Histopathologic Data

Toluidine blue and silver-impregnation stainings were used for histopathology. One thousand eighty retina sections were examined per staining and per group (n = 7). Figure 4 shows the overall picture of the morphology of retina stained with toluidine blue in the different experimental groups. Seven days after reperfusion, histologic examination of ischemic retinas (Fig. 4B) revealed substantial damage compared with control retinas (Fig. 4A). Disruption of the inner nuclear layer (INL), ONL, and outer plexiform layer (OPL) was observed. The inner retinal layers also showed vacuolation, the thickness of the ONL was reduced, and rod outer and inner segments were in a degenerative state. These pathologic changes were globally absent in riluzole-treated groups (Fig. 4C, 4D), which was evidence of riluzole protection against neuronal degeneration.

The retinal layers appeared much better preserved. However, mild cytopathology was observed in some of the sections (20%) of retinas treated before ischemia by IP injection of riluzole. Sometimes, the degree of damage was irregular and difficult to correlate with ERG changes. For this reason, the silver-impregnation staining method, which aids in visualizing degenerating argyrophilic cells, was used to characterize necrosis with better confidence. In Figure 5, the overall histopathologic differences are shown between retina of control rats and saline- and riluzole-treated rats that underwent ischemia and were killed 7 days later. As expected, no argyrophilic staining was observed in control retinas (Fig. 5A). In all ischemic retinas (n = 180 sections) dense argyrophilic staining (dark neurons with shrunken and pyknotic nuclei) appeared in the GCL, INL, and ONL, where 95% of photoreceptors were silver-stained after 7 days of reperfusion (Fig. 5B).

The degeneration of photoreceptors was in accordance with the ERG results (Figs. 1, 2). Again, this approach to analyze retinal degeneration shows the potent effect of riluzole. Before and after injury, riluzole treatments in IP injection or topical application protected the retina against ischemia. Figure 4C shows the overall picture of the qualitative appearance of the riluzole-treated retina. The signs of necrosis were highly reduced, indicated by the absence of dark neurons after topical administration of riluzole after 7 days of reperfusion in the three layers of the retina known to be damaged by ischemia (Fig. 4C).

Apoptosis and c-jun Phosphorylation

Neuronal cell death induced by ischemia occurs through necrosis and apoptosis. Apoptosis has previously been observed in retinal neurons after pressure-induced ischemia, experimental glaucoma, and retinal detachment. One of the specific signaling pathways associated with the induction of programmed cell death involves the activation of the p-JNK-stress-activated protein kinases (JNKs), which phosphorylate c-jun at the N-terminal activation domain and play a role in conveying signals from the cytosol to the nucleus. For this reason, we studied apoptosis and c-jun phosphorylation in parallel. To investigate apoptosis, DNA fragmentation was detected by TUNEL assay and gel electrophoresis. The activation...
FIGURE 3. GFAP immunoreactivity in cross-sections of rat retina after 30 minutes of ischemia-reperfusion and the effect of topical instillation of riluzole administered immediately after ischemia. In control retinas (A), GFAP immunoreactivity was associated with astrocytes in the nerve fiber-ganglion cell layer (GCL; arrows). (B) GFAP immunoreactivity was additionally present in Müller cell processes (arrows') 7 days after ischemia induced by increased IOP. (C) Treatment of retinas with riluzole counteracted the changes in the GFAP immunoreactivity induced in the Müller cells by ischemia. GFAP labeling was present only in the astrocytes of the nerve fiber-GCL (arrows').

of c-jun by JNK was detected with a specific antibody (p-JNK), that recognizes only the phosphorylated form of c-jun at the amino terminal serine regulatory site, Ser-73. Phosphorylation of this site results in transcriptional activation of c-jun.

Ninety retina sections per group (n = 7) were examined for each method (p-JNK immunohistochemistry and TUNEL method). Figure 6 shows the overall picture of the TUNEL labeling and p-JNK expression in the different experimental groups. In control retinas, there were no apoptotic signals (Fig. 6A) or p-JNK expression (Fig. 6D). Two days after ischemia, TUNEL-positive (Fig. 6B) and p-JNK-positive (Fig. 6E) labeling occurred in the GCL. Intraperitoneal and topical administration of riluzole reduced apoptotic cell death and totally prevented the significant p-JNK expression that had been induced by increased IOP in all retinal layers. The topical postischemia treatment with riluzole resulted in an almost total suppression of apoptosis (Fig. 6C) and p-JNK labeling (Fig. 6F) in the GCL 2 days after the ischemic insult. Agarose gel electrophoresis of labeled DNA extracted from retinal tissue (Fig. 7) showed results consistent with the in situ DNA end-labeling. Analysis by electrophoresis confirmed that the TUNEL method reflected nucleosomal DNA fragmentation and again provided evidence for significant protection by riluzole against ischemia-induced apoptosis (Fig. 7). No detectable DNA fragmentation was observed in sham-treated control retinas, whereas there was clear evidence of DNA laddering in extracts from ischemic retinas isolated 2 days after injury. Riluzole totally blocked DNA fragmentation: This protection was seen with all routes of administration and schedules (before or after ischemia).

Riluzole Protects against Retinal Ischemic Damage

FIGURE 4. Morphologic evidence for riluzole protection against retinal degeneration induced by 30 minutes of increased IOP in rat retina analyzed after 7 days of reperfusion and stained with toluidine blue. (A) Control rats. (B) Ischemic saline-treated rats. (C) and (D) Ischemic rats treated after ischemia with IP injection of riluzole or local application of riluzole, respectively. GCL, ganglion cell layer; IPL, inner plexiform layer; INL, inner nuclear layer; OPL, outer plexiform layer; ONL, outer nuclear layer.
DISCUSSION

Intraocular pressure-induced ischemia in mammals is the most frequently used model to investigate mechanisms and possible therapy for retinal ischemia. The degree of insult to the retina is dependent on the animal species, duration, and magnitude of the increase in IOP. In our rat model, an IOP of 130 mm Hg was maintained for 30 minutes to produce ischemia. The electrical response of the retinas was strongly altered, and molecular mechanisms were already engaged to lead retinal cells to their death. For this reason, different procedures including electroretinography, histopathology, and immunocytochemistry were used in this study to observe ischemia-induced changes in the retina and analyze with confidence the neuroprotection after treatment with riluzole. This report shows for the first time the impressive neuroprotective effect of riluzole against retinal ischemia. The alteration of a- and b-waves of the electroretinograms, the necrotic and apoptotic retinal neuronal cell death associated with activation of stress-activated protein kinases, and changes in the expression of the retinal cytoskeleton were analyzed.

In this study, ERGs were used first to assess retinal damage after an ischemic challenge of 30 minutes. After such an ischemic insult the amplitudes of the a-wave and b-wave remained strongly reduced, even 7 days after ischemia. In the absence of any drug treatment, the a-wave, which reflects the activity of photoreceptor cells, showed better recovery than the b-wave, which directly reflects the activity of the Müller cells and indirectly, the activity of neurons in the INL. The observed reduction of the b-wave amplitude is in agreement with results in previous studies,23 which have also shown that 30 minutes
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The present study provides the first demonstration that retinal ischemia evokes a lasting activation of p-JNK and phosphorylation of c-jun at serine 73. Riluzole totally blocked the ischemia-induced increase in p-JNK expression during post-ischemia reperfusion. JNK activation seemed to be involved in the signaling pathway leading to apoptosis in the ischemic retina, as occurs in the ischemic heart.33

The mechanism by which riluzole protects the ischemic tissue is not completely understood in molecular terms, and the remainder of this discussion is therefore rather speculative. An interesting new observation is that riluzole activates a new family of background K⁺ channels, which is highly expressed in the GCL, INL, and ONL and in photoreceptor inner segments of the retina,33 the structures known to be damaged after ischemia. However, riluzole also inhibits voltage-sensitive Na⁺ channels in their inactivated state.11,12 Opening of background K⁺ channels and blockage of voltage-dependent Na⁺ channels that are also highly expressed in GCL, INL, and ONL57,305 may reduce the depolarization triggered by ischemia and consequently may reduce retinal damage. Through its action on ion channels, riluzole may prevent an excessive stimulation of glutamate receptors which is thought to be responsible, as least in part, for retinal damage.7,9 By opening of background K⁺ channels and blockage of voltage-sensitive Na⁺ channels riluzole may inhibit synaptic glutamate release as it has been observed in brain.14,15 At a postsynaptic level, riluzole may block the same Na⁺ channels and activate the same background K⁺ channels and then may prevent some of the postsynaptic effects of glutamate at N-methyl-D-aspartate receptors39-44 by hyperpolarizing cells and thus favoring blockade by Mg²⁺ of N-methyl-D-aspartate receptor-associated ion channels.52 Riluzole would not be active on other types of glutamate receptors because it fails to prevent AMPA and kainate toxicity.45

In this study, the functional, histopathologic, and immunologic analyses of retinas clearly showed for the first time the potent neuroprotective effect of riluzole against retinal ischemia. Riluzole reduced changes in the organization of retinal cytoskeleton and necrotic cell damage, DNA fragmentation, and JNK activation induced by ischemia. A topical application of riluzole provided potent protection for the retina, more potent than a systemic (IP) injection. A topical preischemia and postischemia treatment induced a neuroprotective effect after the short periods of ischemic reperfusion, whereas a topical postischemia treatment was most efficacious in longer periods of reperfusion. The observations reported here (riluzole protection and topical application) show that riluzole has interesting potential for use in ophthalmology.

Acknowledgments

The authors thank Nathalie Vaillant, Frank Aguila, and Yvette Benhamou for technical assistance.

References


