Erythropoietin Protects the Developing Retina in an Ovine Model of Endotoxin-Induced Retinal Injury

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PURPOSE. Intrauterine infection is a common antecedent of preterm birth. Infants born very preterm are at increased risk for neurologic dysfunction, including visual deficits. With increasing survival of very preterm infants, there is a need for therapies that prevent adverse neurologic outcomes. Using an ovine model, the authors investigated the neuroprotective potential of recombinant human erythropoietin (rhEPO) on retinal injury induced by intrauterine inflammation.

METHODS. At 107 ± 1 days of gestational age (DGA), chronically catheterized fetal sheep received either of the following on 3 consecutive days: intravenous (IV) bolus dose of lipopolysaccharide (LPS; ~0.9 μg/kg; n = 8); IV bolus dose of LPS, followed at 1 hour by 5000 IU/kg rhEPO (LPS + rhEPO; n = 8); rhEPO alone (n = 5). Untreated fetuses (n = 8) were used for comparison with the three treatment groups. Fetal physiological parameters were monitored. At 116 ± 1 DGA, fetal retinas were assessed quantitatively for morphologic and neurochemical alterations.

RESULTS. Exposure to LPS alone, but not to rhEPO alone, resulted in fetal hypoxemia and hypotension (P < 0.05). Exposure to LPS alone caused retinal changes, including reductions in thickness of the inner nuclear layer (INL), somal areas of INL neurons, process growth in the plexiform layers, and numbers of ganglion and tyrosine hydroxylase immunoreactive (TH-IR) dopaminergic amacrine cells. Treatment of LPS-exposed fetuses with rhEPO did not alter the physiological effects of LPS but significantly reduced alterations in retinal layers and ganglion and TH-IR cell numbers.

CONCLUSIONS. rhEPO treatment was beneficial in protecting the developing retina after LPS-induced inflammation. Retinal protection could occur by the antiapoptotic or anti-inflammatory actions of EPO. (Invest Ophthalmol Vis Sci. 2011;52:2656–2661) DOI:10.1167/iovs.10-6455

Prematurity affects 8% to 12% of all live births, and the incidence is increasing.1 Because of advances in neonatal care, many preterm infants survive, even those born as early as 25 weeks’ gestation.2 Intrauterine infection and inflammation have been causally linked to preterm birth3 and have been shown to increase the risk for major neurologic disorders such as cerebral palsy in preterm infants.4–5 Prenatal exposure to infection and inflammation is also associated with specific abnormalities of the central nervous system (CNS), including alterations to the visual system.6–8 Preterm infants are at increased risk for long-term visual impairments, among them reduced visual acuity,9,10 refractive errors,9,11 and subtle changes such as abnormalities in color vision9 and contrast sensitivity.9,10,12 Fetal and neonatal infection have recently been implicated as antecedents to the development of retinopathy of prematurity (ROP).7,8 ROP is a vasoproliferative disorder of the developing retina that may cause secondary retinal dysfunction and, in its most severe form, blindness in infancy.

Prevention or protection from neural injury in survivors of very preterm birth remains one of the most important challenges in perinatal medicine. It is therefore important to identify treatments that may be neuroprotective to the developing CNS after prenatal compromise. The cytokine erythropoietin (EPO), which is used in human infants as a safe treatment for anemia, is now being investigated for its neuroprotective properties. Recent randomized controlled studies of small groups of very preterm13 and extremely low-birth-weight14 infants have shown that there are no significant adverse effects with early high-dose recombinant EPO treatment, paving the way for further trials. Initial studies have already found that early EPO treatment improves neurodevelopmental outcomes15,16 and reduces the systemic inflammatory response in preterm infants.17

The ovine fetus is an ideal model in which to examine clinically relevant experimental paradigms, such as the effects of lipopolysaccharide (LPS)-induced intrauterine inflammation on CNS development, because the sheep has a long gestation period and a CNS development sequence similar to that of humans. We have recently shown that recombinant human (rh)EPO reduces brain injury and protects against alterations to myelination in the corticospinal tract and optic nerve in our ovine model of LPS-induced inflammation.18 The aim of the present study was to determine whether rhEPO treatment is also successful in ameliorating the retinal damage we previously described in this model.19 We previously showed that, in the developing retina, tyrosine hydroxylase immunoreactive (TH-IR) dopaminergic amacrine cells, which are believed to play a role in contrast sensitivity,20 are particularly sensitive to LPS-induced inflammation, whereas other retinal amacrine cells (cholinergic, nitrergic, and substance P-containing) are not affected. In the present study, we tested the hypothesis that rhEPO prevents or ameliorates LPS-induced alterations to the developing retina. Exposure of the developing prenatal CNS to LPS is a widely used and clinically relevant model of fetal inflammation.21 LPS was administered at approximately 0.72 of gestation, followed by early high-dose rhEPO treatment; the retina was examined histologically 1 week later. This
gestational age was chosen because it equates to approximately 27 to 28 weeks in humans; birth at this age is regarded as very preterm.

METHODS

All procedures received institutional approval and conformed to guidelines of the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research and the National Health and Medical Research Council of Australia.

Surgery and Experimental Protocol

Date-mated pregnant Merino × Border Leicester ewes (n = 26) underwent aseptic surgery at 102 ± 1 days of gestational age (DGA; term ~147 days), and a fetal artery and vein in each were chronically catheterized. After recovery from surgery, at 107 ± 1 DGA, fetuses were randomly assigned to 1 of 4 treatment groups: group 1, LPS followed 1 hour later by saline (LPS alone, n = 8); group 2, LPS followed 1 hour later by rhEPO (LPS + rhEPO, n = 8); group 3, saline followed by rhEPO (rhEPO alone, n = 5); group 4, saline followed by saline (saline control, n = 5). In groups 1 and 2, LPS was administered intravenously as a bolus (~0.9 μg/kg estimated fetal weight; Escherichia coli, 055:B5; Sigma Chemical, St. Louis, MO). One hour after the initial injection of LPS (group 2) or saline (group 3), we administered rhEPO (5000 IU/kg fetal body weight, intravenously, epoetin-α; Janssen-Cilag, Sydney, Australia). Each treatment protocol was repeated for 3 consecutive days. Physiological data were monitored, and arterial blood samples (0.3 mL) were collected throughout the experimental period to measure blood gases and cytokines, as previously described.18 Eyes were collected from three additional noncatheterized fetuses (unoperated controls) for histologic analysis for comparative purposes. The unoperated and saline fetuses served as the control group.

Tissue Preparation

At 116 ± 1 DGA, ewes and fetuses were euthanized with an overdose of sodium pentobarbitone (Lethobarb; 130 mg/kg, intravenously; Vibrac Animal Health, Peakhurst, NSW, Australia) administered to the ewe. Fetuses were weighed, and fetal eyes were perfused in situ with 4% paraformaldehyde (PFA) in 0.1 M phosphate buffer. Before incubation with antibodies to Iba1, sections were pretreated with citrate buffer (pH 6.0) for 7 minutes in a microwave oven. Retinas were dissected and processed as previously described.22 The left retinas were prepared as whole-mounts for TH-IR. From the right retinas, small blocks of tissue (2 x 5 mm) were collected both centrally (immediately adjacent to the optic nerve head, temporal central) and peripherally (from the inferior temporal quadrant, temporal peripheral) and embedded in paraffin for ionized calcium-binding adapter molecule 1 (Iba1) immunohistochemistry or Epon-Araldite sections (1 μm) of the temporal peripheral and temporal central retina, thickness was measured in the following: total retina, ganglion cell layer (GCL), inner plexiform layer (IPL), inner nuclear layer (INL), outer nuclear layer (ONL), outer plexiform layer (OPL), total photoreceptor layer, inner and outer segments of the photoreceptors (5 sections/block, 10 measurements/section; 50 measurements/animal in total; ×300) using an image analysis (IA) system (Image Pro v4.1; Media Cybernetics, Bethesda, MD; ×600). Somal areas of INL and GCL areas were assessed23 using an IA system (10 cells each section, 5 sections each animal, 50 cells/animal in total; ×1300).

Ganglion Cell Number. The number of ganglion cells was assessed in semithin sections of 300-μm lengths of central and peripheral retinas in five sections from each animal (2 measurements/section, 10 measurements/animal in total; ×1300).24 Counts were expressed as the average number of ganglion cells per millimeter length of retina for each animal; the mean was determined for each group.

Vasculature. The proportion of retina occupied by blood vessels was assessed in semithin sections using a point counting technique.25 Ten sections each of central and peripheral retinas were examined, and 50 regions were randomly sampled, in total, per animal (×600). Vessel diameters were determined by measuring the smallest diameter of transected vessel profiles, thus accounting for vessels cut tangentially25. 100 randomly selected measurements were made per animal (×1300). Qualitative assessment was made of neovascularizations, specifically capillary sprouts composed of putative endothelial tubes surrounded by pericytes.26

Retinal Areas. The total area of each left retina was measured in TH-IR whole-mount preparations using a computerized digitizing program (Sigma Scan Pro v4.0; SPSS Science, Chicago, IL).

TH-IR Amacrine Cells. The mean density of TH-IR amacrine cells was determined with a stereology system (Stereo Investigator; MBF Biosciences, Williston, VT) set to randomly sample 100 fields (each measuring 0.17 mm²) per retina. The total number of cells was calculated from the mean density and retinal area measurements. To analyze the somal area, 50 to 100 randomly selected somata were sampled throughout each retina (×1000, oil immersion).25 The number of TH-IR dendrites per soma (50–100 cells per retina; ×1000, oil immersion) was also assessed and expressed as average dendrite number per animal. Additionally, retinas from the temporal inferior quadrant of the right eye of one control, one LPS, and one LPS + rhEPO fetus were reacted for TH-IR to assess whether the immunohistochemical procedure resulted in tissue shrinkage. Retinal areas were measured before and after processing. Given that shrinkage was <5%, it was not taken into account during the assessment of total cell numbers.

Microglia/Macrophages. For each animal, three transverse sections of both the central and peripheral retinas were examined (10 regions/section, 60 measurements in total); the number of Iba-IR microglia/macrophages was counted and expressed as cells per square millimeter.

Statistical Analysis

Differences between groups for physiological analyses (LPS alone, LPS + rhEPO, rhEPO alone, saline controls) or for histologic analyses (LPS alone, LPS + rhEPO, rhEPO alone, controls [saline and unoperated controls]) were analyzed by one-way ANOVA. Animals in both control groups (noncatheterized controls and saline-infused catheterized controls) were pooled for all structural analyses. When both control groups were compared with respect to quantitative parameters, no statistical differences were identified; this combined group is referred to as controls (n = 8). Bonferroni’s post hoc analysis was used.

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where appropriate and corrected for multiple comparisons. Data are presented as mean ± SEM. Linear regression analysis was undertaken to determine whether there was a correlation between fetal blood gas parameters (maximal change on day 1, 2, or 3 and mean combined maximal change over the 3 days18) and retinal parameters (total number and density of TH-IR amacrine cells, retinal thickness, GCL and INL somal areas, ganglion cell number) and between the area of microglial invasion in the cerebral hemispheres16 and retinal parameters. Differences were considered significant at \( P < 0.05 \).

RESULTS

Fetal Physiology

Exposure to LPS, but not to rhEPO alone or saline, resulted in transient fetal hypoxemia, hypotension, and transiently elevated blood levels of tumor necrosis factor-alpha (TNF-α) and interleukin-6 (IL-6; \( P < 0.05 \)). Treatment of LPS-exposed fetuses with rhEPO did not alter the physiological effects of LPS in the fetus; we have reported this in detail previously.18 At necropsy, there were no differences (\( P > 0.05 \)) between groups in fetal body weight (control, 2.0 ± 0.1 kg; LPS, 2.1 ± 0.1 kg; LPS + rhEPO, 2.2 ± 0.1 kg), brain weight (control, 33.0 ± 0.8 g; LPS, 35.3 ± 1.1 g; LPS + rhEPO, 34.7 ± 1.5 g), or eye weight (control, 3.64 ± 0.05 g; LPS, 3.75 ± 0.36 g; LPS + rhEPO, 3.10 ± 0.32 g).

Retinal Morphology

EPOR-IR. Staining was localized most prominently to membranes of ganglion cells, ganglion cell axons projecting into theNFL, neurons at the inner (vitreal) aspect of the INL, and processes in the IPL and OPL (Fig. 1). Therefore, at the gestational age of the study (116 DGA), EPO receptors were present in the fetal ovine retina.

Retinal Layers. There were reductions in the total thickness of the central (\( P < 0.05 \)) and peripheral retinas (\( P < 0.05 \)) in LPS fetuses compared with both LPS + rhEPO and control fetuses (Table 1; Figs. 2A–C). Specifically, in the central retina of LPS fetuses, there were reductions in the thickness of the OPL, INL, IPL, and GCL (\( P < 0.05 \)) compared with LPS + rhEPO fetuses and/or controls. In the peripheral retina the OPL was thinner (\( P < 0.05 \)) than in both LPS + rhEPO and control fetuses, and the INL, IPL, and GCL were thinner (\( P < 0.05 \)) than in control fetuses.

Ganglion Cell Number. The number of ganglion cells (cells/mm) was lower (\( P < 0.05 \)) in LPS fetuses than in LPS + rhEPO and control fetuses in the central and peripheral regions (Table 2).

Somal Areas. The somal areas of cells in the INL were lower in LPS fetuses than in both LPS + rhEPO and control fetuses (\( P < 0.05 \); Figs. 2D–F). There was no difference (\( P > 0.05 \)) between groups (Table 2).

Vasculature. There was no difference in the total proportion of retinas occupied by blood vessels (\( P > 0.05 \)) or in the width of blood vessels (\( P > 0.05 \)) between groups (Table 2). There was no evidence of neovascularization or alteration to the blood vessel morphology in any fetus.

Retinal Area. There was no difference in the total area of the retina between control, LPS, or LPS + rhEPO fetuses (control, 664 ± 12 mm²; LPS, 632 ± 35 mm²; LPS + rhEPO, 621 ± 18 mm²).

TH-IR Amacrine Cells. There was a lower total number (\( P < 0.01 \)) of dopaminergic amacrine cells in LPS compared with LPS + rhEPO and control fetuses (Table 2; Figs. 2G–I). There was no difference in dendrite number between groups (\( P > 0.05 \)).

Table 1. Effects of LPS and rhEPO Treatment on Retinal Thickness

<table>
<thead>
<tr>
<th>Thickness Parameter (μm)</th>
<th>TC Control (n = 8)</th>
<th>LPS (n = 8)</th>
<th>LPS + rhEPO (n = 8)</th>
<th>TP Control (n = 8)</th>
<th>LPS (n = 8)</th>
<th>LPS + rhEPO (n = 8)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total</td>
<td>212 ± 7</td>
<td>166 ± 7†</td>
<td>210 ± 5</td>
<td>194 ± 4</td>
<td>145 ± 8†</td>
<td>182 ± 1</td>
</tr>
<tr>
<td>PR layer</td>
<td>15 ± 2</td>
<td>13 ± 1</td>
<td>15 ± 1</td>
<td>14 ± 3</td>
<td>13 ± 1</td>
<td>17 ± 1</td>
</tr>
<tr>
<td>ONL</td>
<td>61 ± 8</td>
<td>58 ± 5</td>
<td>63 ± 3</td>
<td>57 ± 4</td>
<td>53 ± 5</td>
<td>58 ± 2</td>
</tr>
<tr>
<td>OPL</td>
<td>14 ± 1</td>
<td>8 ± 1†</td>
<td>14 ± 1</td>
<td>13 ± 1</td>
<td>7 ± 1†</td>
<td>13 ± 1</td>
</tr>
<tr>
<td>INL</td>
<td>44 ± 2</td>
<td>31 ± 3§</td>
<td>42 ± 2</td>
<td>41 ± 3</td>
<td>25 ± 2§</td>
<td>34 ± 2</td>
</tr>
<tr>
<td>IPL</td>
<td>52 ± 3</td>
<td>56 ± 5§</td>
<td>50 ± 3</td>
<td>44 ± 3</td>
<td>50 ± 4§</td>
<td>41 ± 1</td>
</tr>
<tr>
<td>GCL</td>
<td>29 ± 2</td>
<td>18 ± 2§</td>
<td>28 ± 2</td>
<td>27 ± 2</td>
<td>16 ± 3§</td>
<td>21 ± 1</td>
</tr>
</tbody>
</table>

TC, temporal central; TP, temporal peripheral.

\( * P < 0.001 \) vs. control; \( † P < 0.001 \) vs. LPS + rhEPO; \( ‡ P < 0.01 \) vs. LPS + rhEPO; \( § P < 0.01 \) vs. control; \( ‖ P < 0.05 \) vs. control; \( ¶ P < 0.05 \) vs. LPS + rhEPO.
Microglia/Macrophages. There was no difference \( (P > 0.05) \) between groups in the number of Iba1-IR cells across the retina (Table 2). Activated microglia/macrophages (round morphology and attenuated processes) were observed infrequently, and the incidence did not differ between groups.

**Correlations**

There were no correlations \( (P > 0.05) \) between any of the measured physiological parameters and structural or neurochemical alterations in the retina. There were also no correlations \( (P < 0.05) \) between cerebral white matter damage from our previous study\(^{18} \) and retinal parameters.

**DISCUSSION**

We have unequivocally demonstrated that rhEPO reduces LPS-induced injury in the developing ovine retina, restoring gan-

**FIGURE 2.** (A–C) Methylene blue-stained (1-µm) transverse sections of peripheral retina showing arrangement of neuronal and plexiform layers. There was a reduction in the total thickness of the retina in LPS (B) compared with control (A) and LPS + rhEPO fetuses (C). (D–F) Methylene blue-stained (1-µm) transverse sections of the INL. There was a reduction in the INL cell somal area (arrow) in LPS (E) compared with control (D) and LPS + rhEPO fetuses (F). (G–I) Photomicrographs of THR retinal whole-mounts showing a reduction in the number of dopaminergic amacrine cells (arrow) in LPS-exposed (H) compared with control (G) and LPS + rhEPO fetuses (I). Scale bars: 150 µm (A–C); 9 µm (D–F); 28 µm (G–I).

**TABLE 2.** Effects of LPS and rhEPO Treatment on Retinal Parameters

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control (n = 8)</th>
<th>LPS (n = 8)</th>
<th>LPS + rhEPO (n = 8)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ganglion cells/mm (peripheral region)</td>
<td>33.0 ± 1.6</td>
<td>27.5 ± 1.4†</td>
<td>33.4 ± 1.1</td>
</tr>
<tr>
<td>Ganglion cells/mm (central region)</td>
<td>33.1 ± 1.6</td>
<td>25.5 ± 1.9‡</td>
<td>34.3 ± 1.1</td>
</tr>
<tr>
<td>Somal area of INL cells, µm(^2)</td>
<td>35.1 ± 1.2</td>
<td>31.0 ± 1.3†</td>
<td>35.5 ± 1.1</td>
</tr>
<tr>
<td>Somal area of ganglion cells, µm(^2)</td>
<td>216 ± 14</td>
<td>175 ± 12</td>
<td>187 ± 10</td>
</tr>
<tr>
<td>Proportion of retina occupied by blood vessels, %</td>
<td>1.1 ± 0.2</td>
<td>1.7 ± 0.7</td>
<td>1.1 ± 0.4</td>
</tr>
<tr>
<td>Blood vessel width, µm</td>
<td>7.1 ± 1.0</td>
<td>11.8 ± 3.2</td>
<td>9.7 ± 2.0</td>
</tr>
<tr>
<td>Total number of THHR dopaminergic amacrine cells</td>
<td>10923 ± 573</td>
<td>7985 ± 7984§</td>
<td>9423 ± 553</td>
</tr>
<tr>
<td>Total number of dendrites/THHR dopaminergic amacrine cell</td>
<td>2.2 ± 0.2</td>
<td>2.3 ± 0.3</td>
<td>2.5 ± 0.1</td>
</tr>
<tr>
<td>Iba1-IR macrophages/macrophia in total retina, cells/mm(^2)</td>
<td>75 ± 8</td>
<td>85 ± 19</td>
<td>120 ± 26</td>
</tr>
</tbody>
</table>

\( * P < 0.05 \) vs. control; † \( P < 0.05 \) vs. LPS + rhEPO; ‡ \( P < 0.01 \) vs. LPS + rhEPO; § \( P < 0.01 \) vs. control.
glion and dopaminergic cell numbers and protecting against alterations to somal size and process growth of neurons in the INL. These findings support and extend our recent findings on the neuroprotective potential of rhEPO treatment in reducing LPS-induced brain injury and protecting against alterations to myelination in the optic nerve and corticospinal tract.18

Restoration of Retinal Structure

We have shown that fetal LPS exposure caused reductions in both neuronal (GCL and INL) and plexiform (IPL and OPL) layers, indicating that both neurons and the growth of their processes were affected. Although we were unable to count total numbers of ganglion cells in the present study, the reduction in the number of cells per length of the GCL, combined with the observation that the area of the retina was significantly reduced, suggested that LPS exposure led to an overall loss of ganglion cells. Axonal and ganglion cell death could occur simultaneously because of inflammatory and excitotoxic processes induced by LPS; alternatively, axonal injury could lead to retrograde damage of ganglion cells through the inhibition of axoplasmic transport and reduced supply of neurotrophic factors (see Ref. 27 for review).

Although there was no evidence of changes to photoreceptors during the present study, it is possible that damage was sustained in the long term. It has been established that photoreceptors are vulnerable to hypoxic-ischemic insults and are affected by chronic hypoxemia in the ovine fetus.22 Retinal neurons not assessed in the present study, including horizontal cells, might also have been affected by LPS because there was a decrease in the somal areas of retinal INL cells and in the thickness of this layer; however, investigation of these cells was outside the scope of the present study. Although there was no difference in the number of Iba1-IR microglia between groups in our study, it is possible that microglial invasion might have occurred transiently, before the end point of the experiment; in the rat, it has been reported that LPS exposure causes an acute inflammatory response within 48 to 72 hours, attenuating by 6 days after insult.40 In our earlier study19 on the effects of LPS on the fetal retina, inflammation was induced 10 days earlier in gestation, and retinal morphology was not affected. It is possible that the retina is more vulnerable to inflammatory processes later in development or that the effects on retinal development become more pronounced with retinal growth.

Perinatal inflammation has been implicated in the pathogenesis of ROP in preterm human neonates.7,8 In a recent study, alterations in the levels of eight cytokines were observed, including IL-6, which was elevated within 1 day of delivery; it is not yet certain how these alterations cause vasoproliferation in the developing retina. It is relevant that plasma concentrations of IL-6 were elevated within 6 hours of exposure to LPS in our ovine model of intrauterine inflammation.51 In the present study no alterations were observed in the proportion of the retina occupied by blood vessels, nor was there any evidence of neovascularization or alteration to blood vessel morphology such as occurs in ROP. Given that the vasoproliferative phase of ROP is not generally recognized in humans until ophthalmic examination 4 to 6 weeks after birth, the short time frame of our experiments in fetal sheep might not have allowed for the development of such a pathologic process. We note that EPO supplementation, at least in the mouse, appears to have a time-dependent effect on angiogenesis with early administration,52 as in the present study, preventing hypoxia-induced neovascularization, whereas later administration enhances neovascularization.52

For rhEPO therapy to be effective, it was important to establish that EPOR was present in the ovine retina at the time of treatment. It is already known that EPOR is present during fetal development in human,53 mouse,54 and guinea pig55 retinas. We localized EPORs to ganglion cells and their axons, to neurons at the vitreal aspect of the INL (possibly including dopaminergic amacrine cells), and to processes in the IPL and OPL. High-dose rhEPO (5000 U/kg) administered systemically has been shown to penetrate the neonatal rat blood-eye barrier and likely does so in the ovine fetus at an equivalent developmental age. We propose that, in our study, rhEPO entered the eye and preserved retinal integrity by activating EPOR-dependent signaling pathways, initiating downstream mechanisms to protect neurons and their processes during the inflammatory insult.

We have previously reported that rhEPO administration in the fetus does not affect any measured physiological variable, nor does it significantly exacerbate or ameliorate the transient fetal hypoxemia and hypotension that are caused by LPS exposure.18 Furthermore, it did not alter any of the other physiological responses that occur after fetal LPS exposure, namely a tendency for transient acidemia, hypercapnia, and polycythemia.51 These results suggest that rhEPO does not act by ameliorating any potential adverse effects of hypoxia; rather, it acts by inhibiting the inflammatory effects of LPS that induce injury.18 It is known that EPO can decrease glutamate toxicity,57 induce antiapoptotic factors including Bcl-x and Bcl-2,58 decrease nitric oxide–mediated injury,29 and induce direct antioxidant effects.40

Restoration of Dopaminergic Amacrine Cell Numbers

rhEPO treatment ameliorated the LPS-induced reduction in dopaminergic amacrine cells. It is not possible to determine whether LPS induces a loss of cells or downregulates TH; similarly, we were unable to determine the specific mechanisms of protection of these cells by rhEPO. However, protection by EPO of striatal and midbrain dopaminergic cells has been studied extensively in animal models of Parkinson’s disease.41,42 It has been suggested that EPO exerts its neuroprotective effects on these cells by several means, including EPOR-mediated mechanisms,41,42 as EPORs are located on midbrain dopaminergic cells43 and through anti-inflammatory or antioxidant mechanisms.42 Furthermore, because EPO has trophic effects on dopaminergic neurons,44 such effects could contribute to the survival of these cells after LPS-induced inflammation.

Conclusion

This is the first study in a long-gestation species to demonstrate the neuroprotective potential of rhEPO treatment in LPS-induced inflammatory damage to the fetal retina. Treatment with rhEPO soon after LPS exposure clearly reduced the extent of retinal injury while not altering the physiological effects of LPS. This study provides evidence supporting the therapeutic potential of rhEPO in the treatment of retinal pathology caused by prenatal inflammation and has implications for the improvement of visual impairments in infants born prematurity. It is of interest that a dosing regimen similar to the one used by us did not increase retinal angiogenesis or exacerbate retinopathy of prematurity in the neonatal rat.56

Acknowledgments

The authors thank Megan Probyn for assistance with surgery, Mary Tolcos and Nadia Hale for preparation of the eyes, and Veronica Martin for assistance with immunohistochemistry.
References


