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# An antisense oligonucleotide targeting the growth hormone receptor inhibits neovascularization in a mouse model of retinopathy

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**Purpose:** We have demonstrated that a 2'-O-methoxyethyl modified antisense oligonucleotide against the mouse growth hormone (GH) receptor (GHr) reduces GH binding and serum insulin-like growth factor-1 in normal mice. We tested whether this systemically delivered antisense oligonucleotide could inhibit neovascularization in mice with oxygen induced retinopathy (OIR).

**Methods:** OIR was induced in C57BL/6 mice by housing them in 75% oxygen across postnatal days (P)7 to 12 followed by five days in room air. Shams were in room air from P0-17. GHr antisense oligonucleotide, ATL 227446, was administered by early (P7, 8, 9, 11, 13, 15, and 17) or late (P12-16) intervention at doses of 5, 10, 20, and 30 mg/kg. Other mice were treated with either vehicle (saline), the somatostatin analog octreotide (20 mg/kg/bi-daily), or control oligonucleotides ATL 261303 (at 20 mg/kg by late and early intervention) or ATL 260120 (at 20 and 30 mg/kg by early intervention only). Blood vessel profiles were counted in 3 mm paraffin sections of inner retina.

**Results:** OIR increased blood vessel profiles by 2.5 fold compared to shams. In OIR, early intervention GHr antisense oligonucleotide ATL 227446 reduced blood vessel profiles at higher doses including 10 mg/kg, and 30 mg/kg resulted in the greatest reduction (38%). In OIR, late intervention with all doses of GHr antisense oligonucleotide ATL 227446 reduced blood vessel profiles to a similar extent, and the highest dose resulted in a 26% reduction compared to OIR. Octreotide reduced blood vessel profiles in OIR mice by 26%. In OIR, ATL 261303 had no effect on blood vessel profiles, while 30 mg/kg ATL 260120 reduced blood vessel profiles by 18%.

**Conclusions:** Systemically delivered antisense oligonucleotides directed against the GHr are a potential novel treatment for ocular neovascularization related disorders.

Pathological neovascularization is the hallmark feature of both retinopathy of prematurity (ROP) and diabetic retinopathy (DR) [1,2]. In both conditions, retinal neovascularization is associated with vascular leakage, which leads to visual impairment and, in many cases, blindness [1,2]. Growth hormone (GH) may be involved in the development of ROP and DR. The initial association between GH and diabetic retinopathy came from studies in which pituitary ablation was linked to the remission of DR [3-6]. In subsequent studies, DR was found to be approximately three times more prevalent in Type I diabetic patients who are GH sufficient than those who were GH deficient [3]. GH deficient dwarfs with diabetes were free of microvascular complications [7], and GH replacement therapy for patients with GH deficiency induced a diabetic-like retinopathy, which is attenuated after discontinuation of GH treatment [8]. In terms of ROP, Smith and colleagues reported that retinal neovascularization is reduced in transgenic mice expressing a GH antagonist gene that were subjected to experimental ROP [9].

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Therapeutic strategies for DR included early approaches to block the actions of GH such as hypophysectomy and pituitary radiation [10], and more recently, the use of the GH receptor (GHr) antagonist, pegvisomant, or inhibiting the secretion of GH from the pituitary using somatostatin or its analogues such as octreotide [9,11,12]. We recently reported the design and optimization of a “5-10-5” 2'-O-(2-methoxy)ethyl (2' MOE) modified antisense oligonucleotide (ASO) directed to the mouse GHr, which suppresses GHr mRNA levels in vitro and in vivo and reduces binding of GH to liver cells in normal mice [13]. The present study describes the effect of this ASO, ATL 227446, on retinal neovascularization in a mouse model of ROP. In rodents, ROP is induced by exposure of newborns to hyperoxia, which suppresses normal developmental retinal vascularization [9,14,15]. Subsequent exposure to room air results in relative retinal hypoxia and excessive pathological retinal neovascularization known as oxygen induced retinopathy (OIR) [9,14,15]. In the present report, the GHr ASO, ATL 227446, was given systemically to mice with OIR by both early intervention (before and during retinal pathological neovascularization) and late intervention (during retinal pathological neovascularization) in four separate doses. Comparisons were made with the somatostatin analog octreotide and two mismatch control oligonucleotides.

## METHODS

*Animals:* Pregnant female C57BL/6 mice were provided by The Animal Resource Center, Western Australia, and housed in the Biological Research Facility, Department of Physiology, The University of Melbourne. Each litter was randomized to 28 experimental groups (Table 1). All experimental procedures adhered to the guidelines of the Australian National Health and Medical Research Council, which are comparable with the Institute for Laboratory Animal Research Guide for the Care and Use of Laboratory Animals.

The experimental model is summarised in the rest of this paragraph [9,14]. Seven-day-old pups and their nursing dams were exposed to 75% oxygen for five days during which time there is vaso-obliteration and cessation of normal development of the central retinal capillary beds. Medical-grade oxygen was used and controlled by a PROOX oxygen controller model 110 (Reming Bioinstruments, Redfield, NY). The oxygen content of the chambers was checked daily with a MacLab/2E system (Chart v3.5 program on the MacLab/2E System, AD Instruments, Pty Ltd, Bella Vista, New South Wales, Australia). On postnatal day (P) 12, the mice were housed in room air for five days until P17. During this time there is acute retinal ischemia in the avascular regions of the central retina, which is followed by excessive pre-retinal neovascularization. Shams were mouse pups kept with their mother in room air from birth until P17. Throughout the experiment, mothers were provided with water and standard mice chow (GR2, Clark-King and Co., Gladesville, Victoria, Australia) ad libitum and exposed to normal 12 h:12 h light-dark cycle. Pups received nutrition from their mothers.

*Treatments:* ATL 227446 (ACA AAG ATC CAT ACC TGA GA), is an antisense oligonucleotide specific for mouse GHr. It is phosphorothioate throughout with 2'-MOE modifications in the five outer 5' and 3' positions. Control oligonucleotides (ATL 261303; AGA GAG CTA CCT AAC TAA CA, and ATL 260120; TTA CCG TAT GGT TCC TCA CT) with sequences non-specific to the GHr, were prepared with a similar chemical structure [13]. All oligonucleotides were provided by Isis Pharmaceuticals Inc., Carlsbad, CA. The somatostatin analog octreotide was kindly provided as a gift from Bachem AG (Bubendorf, Switzerland). Vehicle treated controls received sterile saline. Doses, administration route, and animal numbers are summarized in Table 1. Agents were administered at a volume of 100 µl. To determine if pathological retinal neovascularization in OIR mice was influenced by treatment administered either prior to or after the hypoxic-induced neovascularization period, we provided therapies by either early or late intervention. Early intervention comprised injections at P7, 8, 9, 11, 13, 15, and 17 (total of seven doses). Late intervention was injections at P12-16 (total of five doses).

*Tissue collection and histology:* Following the 17-day experimental period, mice were sacrificed by an intraperitoneal injection of Nembutal (Rhone Merieux, Queensland, Australia, 120 mg/kg body weight). Both eyes from each mouse pup were removed and fixed in 4% paraformaldehyde in 0.1 M phosphate buffer (0.137 M NaCl, 0.018 M $Na_2HPO_4$, 0.003 M KCl, and 0.001 M $KH_2PO_4$) overnight and processed in graded alcohol baths before being embedded in paraffin wax. Eyes were then serially sectioned at 3 mm, 90 °C to the optic nerve and placed on three aminopropyl-triethoxysilane coated slides (Sigma, St Louis, MO). Approximately 120 sections/eye were collected and incubated overnight at 37 °C.

*Quantitation of blood vessel profiles in the inner retina:* Three sections from one eye from each animal were randomly chosen, deparaffinized, and stained for 5 min each with Mayer's hematoxylin and eosin (5 min; Amber Scientific Laboratories, Belmont, Australia), and coverslipped. Using an established technique [14,16,17] blood vessel profiles (BVP's) were counted in the inner retina and included vessels adherent to the ILM. The inner retina comprised the inner limiting membrane (ILM), ganglion cell layer (GCL), and inner plexiform layer (IPL). Four fields per section were evaluated in a masked manner by two technicians. A BVP was defined as an endothelial cell (stained blue) or a blood vessel with a lumen. Counting was performed on an Olympus BX51 photomicroscope (Olympus, Tokyo, Japan) at a magnification of X40, and images were captured on a Spot digital camera (SciTECH Pty. Ltd., Victoria, Australia) connected to an IBM computer. Quantitation was performed by an investigator, who was masked to the experimental groups.

*Animal numbers and statistics:* Data was analyzed using Statview for Windows version 5.0.1, (SAS Institute Inc, Cary, NC). A two way ANOVA with Fisher's post hoc comparison

**Table 1. Therapies, intervention period, number of animals and body weight at postnatal day 18 in each sham and oxygen induced retinopathy group**

| Group | Intervention | N | Body weight (g) |
|---|---|---|---|
| Controls | | | |
| Sham+vehicle i.p. | early | 9 | 7.02±0.23 |
| OIR+vehicle i.p. | early | 9 | 6.88±0.30 |
| Sham+vehicle i.p. | late | 9 | 6.63±0.22 |
| OIR+vehicle i.p. | late | 9 | 6.79±0.21 |
| ATL 227446 - GHr ASO | | | |
| Sham+5, 10, 20, 30 mg/kg i.p. | early | 9-10 | *6.42±0.25 |
| Sham+5, 10, 20, 30 mg/kg i.p. | late | 8-10 | *6.34±0.21 |
| OIR+5, 10, 20, 30 mg/kg i.p. | early | 9-10 | *6.93±0.18 |
| OIR+5, 10, 20, 30 mg/kg i.p. | late | 9-10 | *6.55±0.28 |
| Octreotide | | | |
| Sham+20 mg/kg/bi-daily s.c. | late | 10 | 6.79±0.17 |
| OIR+20 mg/kg/bi-daily s.c. | late | 10 | 6.48±0.39 |
| ATL 261303 - control oligonucleotide | | | |
| Sham+20 mg/kg i.p. | early | 9 | 6.64±0.23 |
| Sham+20 mg/kg i.p. | late | 9 | 6.33±0.25 |
| OIR+20 mg/kg i.p. | early | 10 | 6.71±0.19 |
| OIR+20 mg/kg i.p. | late | 9 | 7.12±0.34 |
| ATL 260120 control oligonucleotide | | | |
| Sham+20 mg/kg i.p. | early | 9 | 6.58±0.28 |
| Sham+30 mg/kg i.p. | early | 9 | 6.81±0.22 |
| OIR+20 mg/kg i.p. | early | 7 | 6.36±0.18 |
| OIR+30 mg/kg i.p. | early | 9 | 7.12±0.21 |

The following abbreviations were used: growth hormone receptor (GHr), antisense oligonucleotide (ASO), intraperitoneal (i.p.), and subcutaneous (s.c.). Early intervention is administration at P7, 8, 9, 11, 13, 15, and 17 (total of seven doses). Late intervention is administration at P12-16 (total of five doses). Values are mean±sem. There were no differences in body weights between all groups. Asterisk indicates *mean for four treatment groups.

was applied, with $p<0.05$ considered to be statistically significant.

## RESULTS

*Body weight:* The results are summarized in Table 1. Body weights were similar between sham+vehicle and OIR+vehicle groups. Treatments had no effect on body weights in either sham or OIR animals. Treatments did not affect the health and growth of mouse pups.

*Early intervention treatment is more effective than late intervention treatment with growth hormone receptor antisense oligonucleotide ATL 227446 in reducing neovascularization in oxygen induced retinopathy:* In both early and late intervention sham control groups, retinas appeared normal and had similar numbers of BVPs in the inner retina (Figure 1 and Figure 2). In contrast, in both early and late intervention OIR control groups, numerous blood vessels were observed in the inner retina, which often penetrated into the vitreous cavity (Figure 1 and Figure 2).

In sham mice treated with GHr ASO ATL 227446 by either early or late intervention, retinas were similar to sham vehicle controls (Figure 1 and Figure 2). In OIR mice treated with early intervention GHr ASO ATL 227446, BVPs in the inner retina were reduced with 10, 20, and 30 mg/kg but not the 5 mg/kg dose when compared to all OIR controls. Early intervention with 30 mg/kg GHr ASO ATL 227446 was associated with a 38% reduction in BVPs, which represented the greatest reduction across all OIR groups (Figure 1). In OIR mice treated with late intervention ATL 227446, BVPs in the inner retina were reduced to a similar extent with all doses, and the highest dose of 30 mg/kg resulted in a 26% reduction compared to all OIR vehicle controls (Figure 1 and Figure 2). When comparing early and late interventions in OIR, 10 mg/kg ATL 227446 reduced BVPs to a similar extent in both protocols, while early intervention with 20 and 30 mg/kg ATL 227446 resulted in a greater reduction in BVPs than all doses of late intervention ATL 227446 ($p<0.05$ and $p<0.0005$, respectively).

*Late intervention treatment with growth hormone receptor antisense oligonucleotide ATL 227446 is equally effective as late intervention treatment with octreotide in reducing neovascularization in oxygen induced retinopathy:* The inner retina of sham vehicle controls and sham mice treated with octreotide appeared normal and had similar numbers of BVPs (Figure 3). In OIR mice, late intervention with octreotide to OIR mice resulted in a similar decrease (26%) in BVPs in the inner retina as late intervention with GHr ASO ATL 227446 (Figure 3). However, octreotide was less effective in reducing

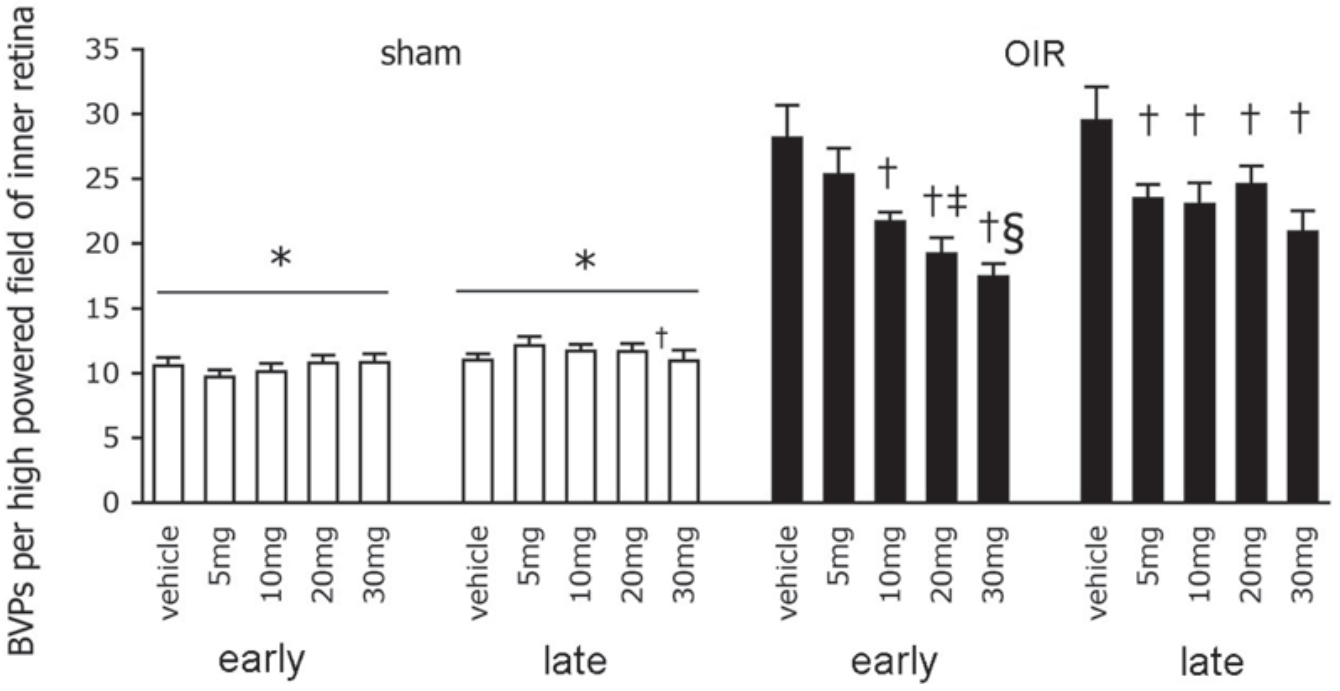



Figure 1. Quantitation of blood vessel profiles in the inner retina from mice with oxygen induced retinopathy and treated with mouse GHR ASO ATL 227446 using an early or late intervention protocol. ATL 227446 was associated with a reduction in pathological angiogenesis in mice with oxygen induced retinopathy that was most effective when administered by early intervention. ATL 227446 had no effect on normal physiological angiogenesis in retina of sham mice. The following abbreviations are used: early intervention (EI); late intervention (LI); oxygen induced retinopathy (OIR); growth hormone receptor (GHR) and antisense oligonucleotide (ASO). EI represents treatment between postnatal days 7 and 17. Late intervention represents treatment between postnatal days 12 and 16. Values are mean±sem. N=9 to 10 mice per group. Asterisk denotes $p<0.0001$ compared to all OIR groups. Cross denotes $p<0.001$ compared to OIR+vehicle (EI and LI). Double cross denotes $p<0.05$ compared to all OIR+ATL 227446 late intervention. Double squiggle denotes $p<0.0005$ compared to all OIR+ATL 227446 late intervention.

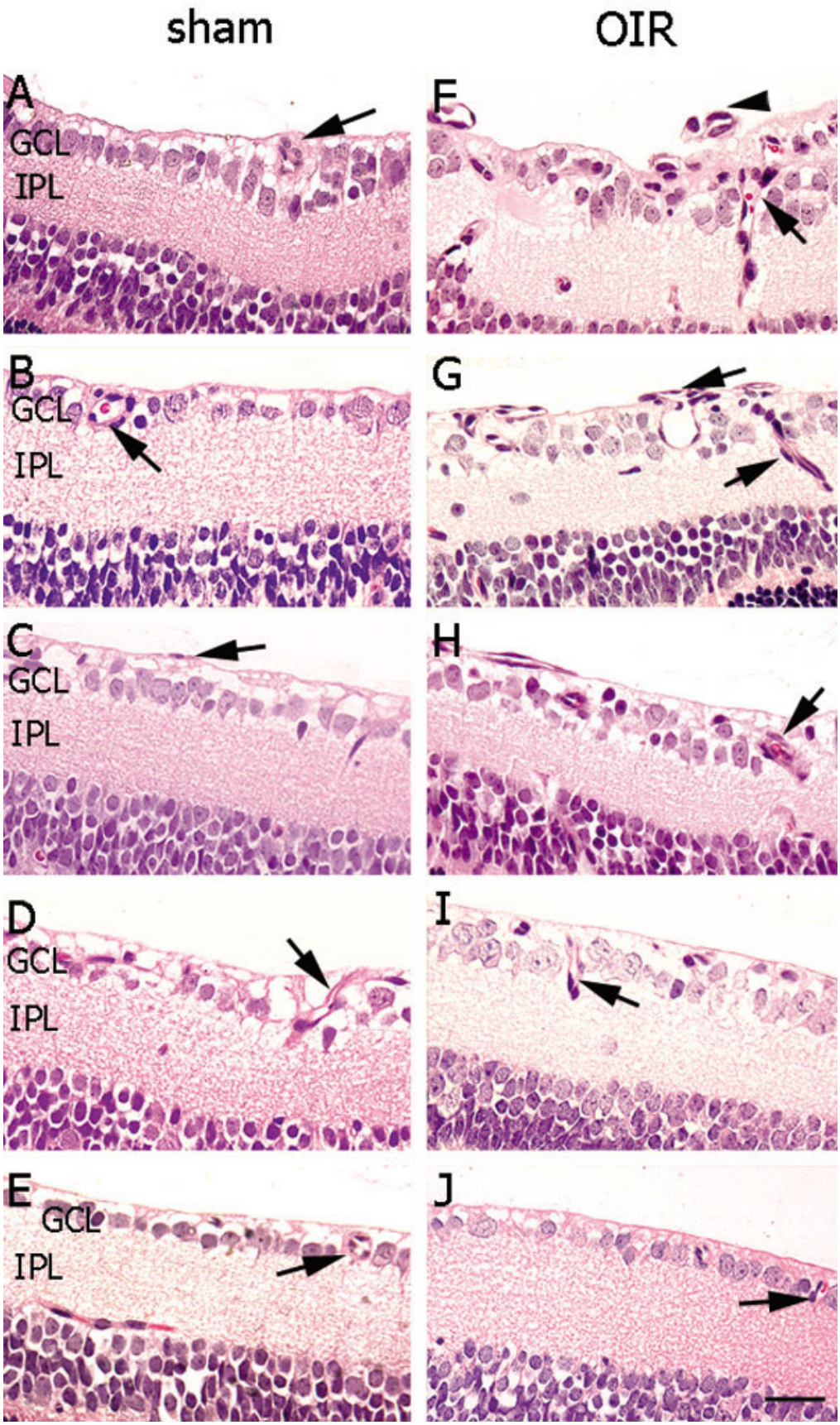
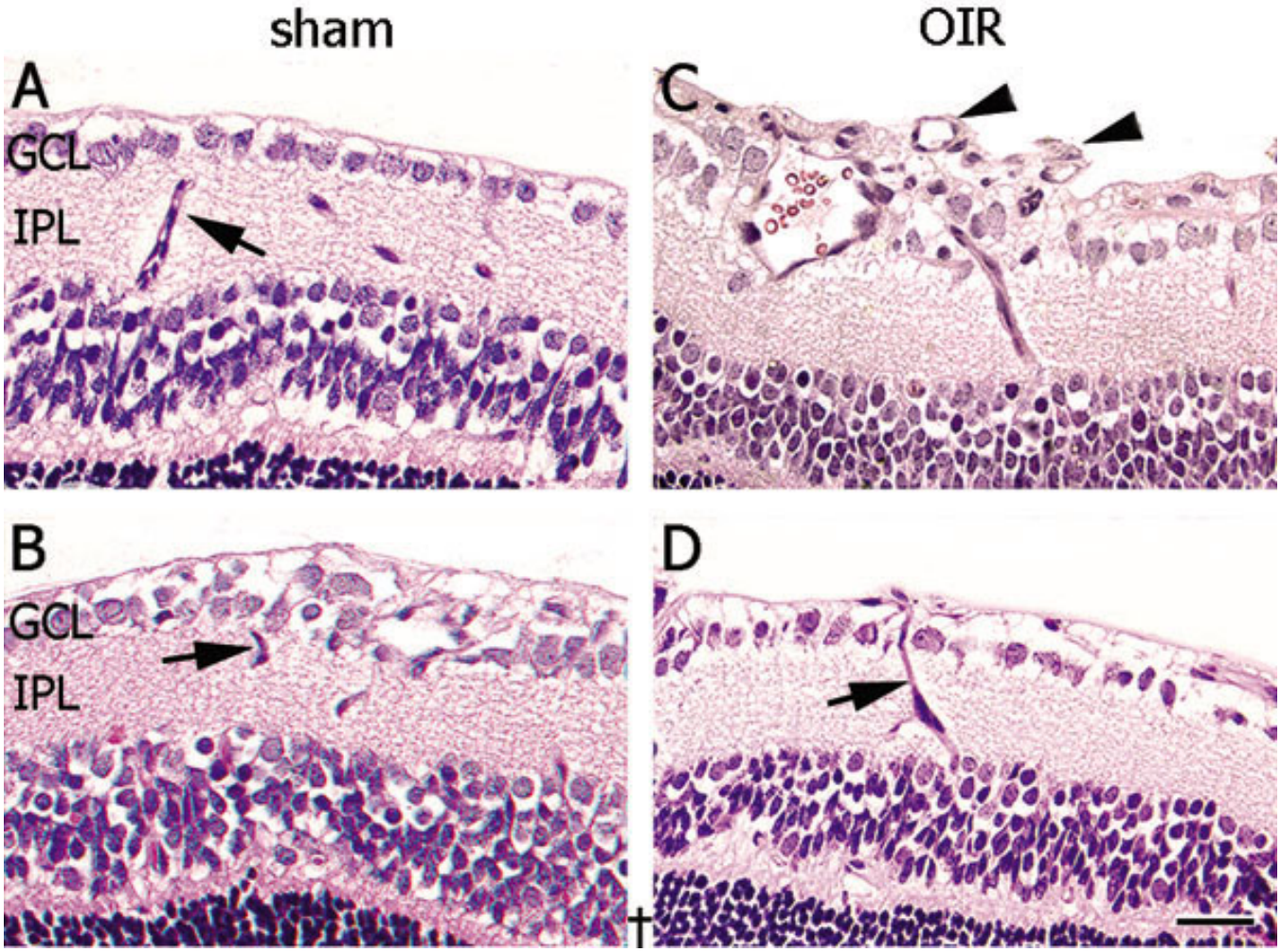



Figure 2. Three μm paraffin sections of inner retina from mice with oxygen induced retinopathy and treated with mouse GHr ASO, ATL 227446, using an early intervention protocol. Oxygen induced retinopathy is associated with pathological angiogenesis in the inner retina compared to sham mice, which is reduced with ATL 227446. The following abbreviations are used: oxygen induced retinopathy (OIR); growth hormone receptor (GHR); antisense oligonucleotide (ASO); ganglion cell layer (GCL); inner plexiform layer (IPL) and blood vessel profiles (BVPs). The sections are stained with hematoxylin and eosin. Magnification x150. Scale bar equals 50 μm. **A** is sham+vehicle, **B** is sham+5 mg/kg ATL 227446, **C** is sham+10 mg/kg ATL 227446, **D** is sham+20 mg/kg ATL 227446, **E** is sham+30 mg/kg ATL 227446, **F** is OIR+vehicle, **G** is OIR+5 mg/kg ATL 227446, **H** is OIR+10 mg/kg ATL 227446, **I** is OIR+20 mg/kg ATL 227446, and **J** is OIR+30 mg/kg ATL 227446. Retina in all sham groups (**A** to **E**) appeared normal with BVPs (arrows) in the inner retina. In OIR vehicle controls (**F**), BVPs (arrows) were present in the inner retina and also adherent to the retinal surface (arrowhead). In OIR, 5 mg/kg ATL 227446 (**G**) did not alter BVPs, however higher doses reduced BVPs (**H**, **I**, **J**) to a greater extent than late intervention (Figure 1 and Figure 2).

BVPs in OIR mice than early intervention with either 20 or 30 mg/kg ATL 227446.

*Control oligonucleotides and pathological retinal neovascularization in oxygen induced retinopathy:* Sham mice treated with control oligonucleotides ATL 261303 or ATL 260120 had a similar number of BVPs in the inner retina compared to all sham control groups (Figure 4 and Figure 5). In OIR mice, ATL 261303 when administered by either an early or late intervention protocol (20 mg/kg) did not alter the number of BVPs in the inner retina compared to all OIR controls (Figure 4). In OIR mice, ATL 260120 when administered by early intervention at 20 mg/kg did not alter the number of BVPs in the inner retina when compared to all OIR controls (Figure 5). However, the highest dose of 30 mg/kg reduced



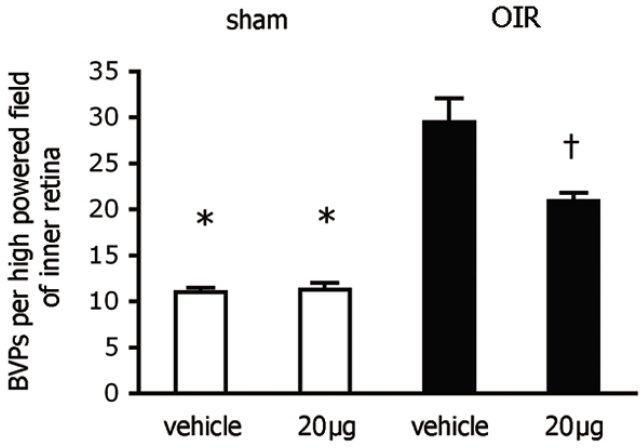
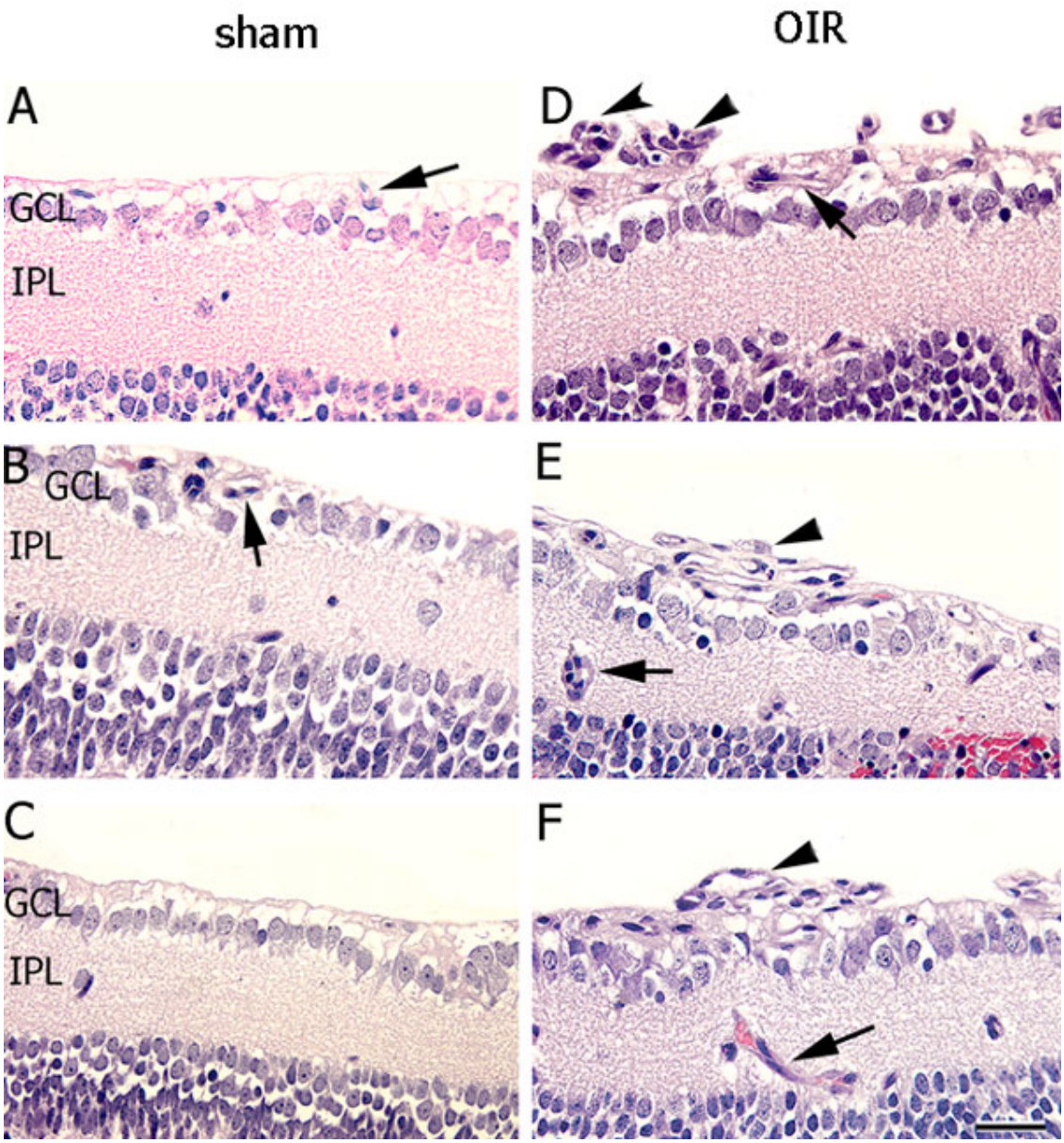




Figure 3. Three µm paraffin sections and quantitation of blood vessel profiles in the inner retina from mice with oxygen induced retinopathy and treated with the somatostatin analog octreotide using a late intervention protocol. Octreotide reduced pathological angiogenesis in the retina of mice with oxygen induced retinopathy and had no effect on normal physiological angiogenesis in sham mice. The following abbreviations are used: oxygen induced retinopathy (OIR); ganglion cell layer (GCL); inner plexiform layer (IPL) and blood vessel profiles (BVPs). The sections are stained with hematoxylin and eosin. Magnification x150. Scale bar equals 50 µm. **A** is sham+vehicle, **B** is sham+20 mg/kg octreotide, **C** is OIR+vehicle, and **D** is OIR+20 mg/kg octreotide. Retina in all sham groups appeared normal with BVPs (arrows) evident in the GCL and IPL. In OIR vehicle controls (**C**), numerous BVPs (arrows) were present in the inner retina and also adherent to the retinal surface (arrowhead). Values are mean±sem. N=9 to 10 mice per group. Asterisk denotes $p<0.0001$ compared to all OIR groups. Cross indicates $p<0.0001$ compared to OIR+vehicle.



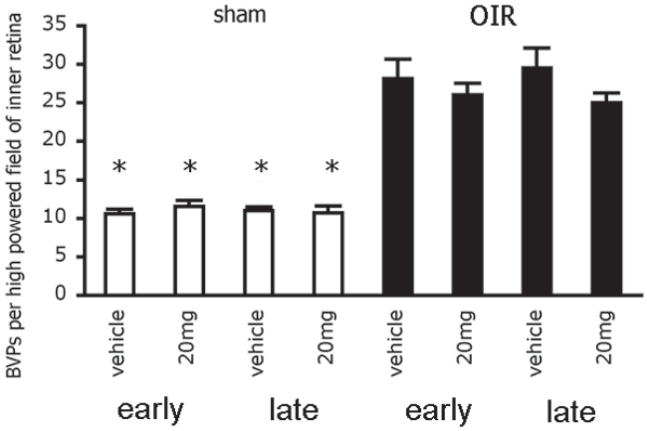




Figure 4. Three μm paraffin sections and quantitation of blood vessel profiles in the inner retina from mice with oxygen induced retinopathy and treated with the control oligonucleotide ATL 261303 using either an early or late intervention protocol. ATL 261303 had no effect on pathological angiogenesis in mice with oxygen induced retinopathy, or on normal physiological angiogenesis in sham mice. The following abbreviations are used: oxygen induced retinopathy (OIR); ganglion cell layer (GCL); inner plexiform layer (IPL); blood vessel profiles (BVPs); early intervention (EI) and late intervention (LI). The sections are stained with hematoxylin and eosin. Magnification x150. Scale bar equals 50 μm. **A** is sham+vehicle, **B** is sham+20 mg/kg ATL 261303 and EI, **C** is sham+20 mg/kg ATL 261303 and LI, **D** is OIR+vehicle, **E** is OIR+20 mg/kg ATL 261303 and EI, and **F** is OIR+20 mg/kg ATL 261303 and LI. Retina in all shams appeared normal with BVPs (arrows) in the GCL and IPL. In untreated OIR mice (**D**), numerous BVPs (arrows) were present and also adherent to the retinal surface (arrowhead). Retina from OIR mice treated with ATL 261303 either early (**E**) or late (**F**), appeared similar to untreated OIR (D) with numerous BVPs (arrows) in the inner retina and penetrating into the vitreous (arrowhead). Values are mean±sem. N=9 to 10 mice per group. Asterisk denotes p<0.0001 compared to all OIR groups.

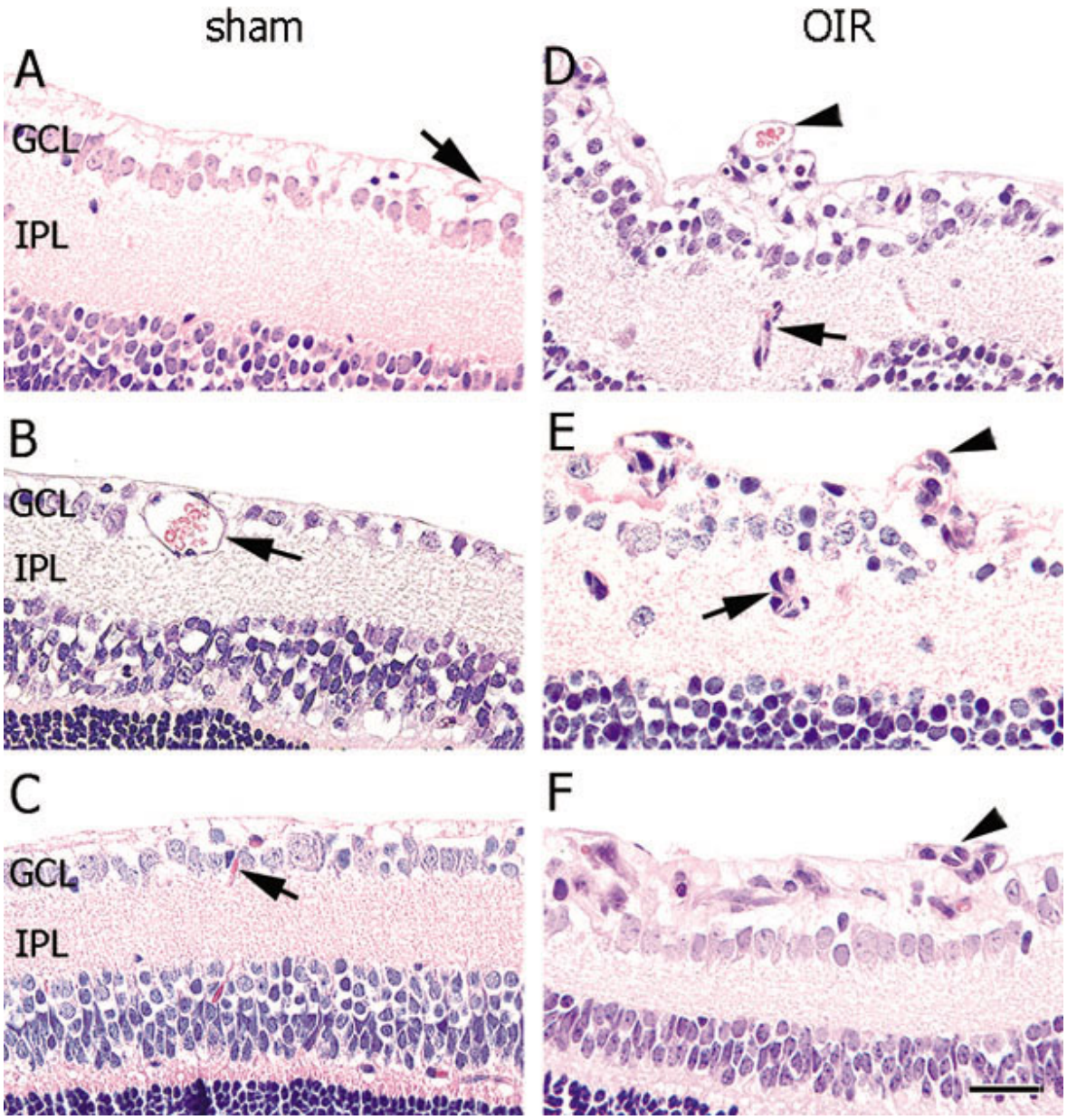



Figure 5. Three μm paraffin sections and quantitation of blood vessel profiles in the inner retina from mice with oxygen induced retinopathy and treated with the control oligonucleotide ATL 260120 using an early intervention protocol. At a dose of 20 mg/kg, ATL 260120 reduced pathological angiogenesis in mice with oxygen induced retinopathy, but had no effect on physiological angiogenesis in sham mice. The following abbreviations are used: oxygen induced retinopathy (OIR); ganglion cell layer (GCL); inner plexiform layer (IPL) and blood vessel profiles (BVPs). The sections are stained with hematoxylin and eosin. Magnification x150. Scale bar equals 50 μm. **A** is sham+vehicle, **B** is sham+20 mg/kg ATL 260120, **C** is sham+30 mg/kg ATL 260120, **D** is OIR+vehicle, **E** is OIR+20 mg/kg ATL 260120, and **F** is OIR+30 mg/kg ATL 260120. In all shams, BVPs (arrows) were evident in the GCL and IPL. In OIR+vehicle (**D**), numerous BVPs (arrows) were in the inner retina and adherent to the retinal surface (arrowhead). Retina from OIR+20 mg/kg ATL 260120 (**E**) appeared similar to OIR+vehicle (**D**). Retina from OIR+30 mg/kg ATL 260120 (**F**) had fewer BVPs (arrows) in the inner retina than OIR+vehicle (**D**), however BVPs occasionally penetrated into the vitreous (arrowhead). **G**: Values are mean±sem. N=7 to 9 mice per group. Asterisk denotes p<0.0001 compared to all OIR groups. Cross denotes p<0.005 compared to OIR+vehicle.

BVPs in OIR mice by 18% compared to all OIR controls. This reduction in BVPs was not statistically significant from the reduction that occurred with late intervention GHr ASO or octreotide, but was not as great as early intervention with GHr ASO at 20 and 30 mg/kg ($p<0.001$).

## DISCUSSION

Improvements in antisense technology have resulted in the successful use of ASOs in animals [18,19] and the clinic [20], including the second-generation 2-MOE ASOs of the type described in this study. The ASO used for targeting the GHr was selected based on its ability to knockdown the target mRNA in a dose dependent manner in cultured mouse brain endothelial cells [13]. In vivo efficacy was demonstrated by both reductions in GHr mRNA in normal mouse liver and the binding of GH to mouse liver cells [13]. Based on previous findings that mice transgenic for a GH antagonist gene have reduced retinal neovascularization when subjected to OIR [9], we tested the GHr ASO, ATL 227446, in a mouse model of OIR. Our major finding is that ATL 227446 reduced pathological neovascularization in OIR but had no effect on normal vascularization of the developing retina. ATL 227446 was most effective when administered by early intervention at the highest dose of 30 mg/kg (38% reduction) rather than late intervention (26% reduction), and was more retinoprotective than late intervention octreotide or control oligonucleotides.

Antisense drugs in the same class as ATL 227446 are designed to direct the RNaseH-mediated cleavage of RNA targets. When an RNaseH-directing ASO like ATL 227446 enters the cell, it hybridises to the complementary sequence of its target RNA in the cell nucleus. The resulting heteroduplex is recognised by the endogenous nuclear enzyme RNaseH, which then cleaves the RNA strand of the duplex at the ASO target site. RNaseH-directing ASOs have been demonstrated to be effective in vivo pharmacological agents [18,19]. This mechanism of action can necessitate a slower onset of action than conventional drugs, so for this reason, ATL 227446 was administered by early intervention over the hyperoxic period when normal developmental retinal vascularization was suppressed and continued through the hypoxic period when pathological retinal neovascularization was stimulated. This treatment regimen resulted in the highest doses of ATL 227446 (20 and 30 mg/kg), conferring a greater reduction in retinal neovascularization in OIR than equivalent doses administered by late intervention during just the hypoxic period. However, it should be noted that all doses of late intervention ATL 227446 reduced retinal neovascularization in OIR. Few studies have evaluated GH as a therapeutic target for pathological neovascularization in ROP and DR. Smith and colleagues reported that in transgenic mice expressing a GH antagonist gene and subjected to OIR, the experimental equivalent of ROP, retinal neovascularization was reduced by 34% [9]. In contrast, mice with the GH agonist gene and OIR did not exhibit a decrease in retinal neovascularization [9]. A role for GH therapy in DR has been evaluated in a small clinical trial of Type I or Type II diabetic patients. The GHr antagonist pegvisomant did not affect retinopathy in 16 patients, while nine patients showed progression [11]. It is possible that the study size and 12-week treatment period were too small for definitive effects of GHr antagonism to be determined.

Another reason for the effectiveness of early rather than late GHr ASO treatment may relate to the known actions of GH on insulin-like growth factor-1 (IGF-I) [21,22]. ROP in pre-term babies occurs in two phases, and involves differential changes in IGF-I and vascular endothelial growth factor (VEGF) [23]. In phase I, serum IGF-I is reduced due to decreased availability from maternal and placental sources [24,25]. If this is sustained, normal developmental retinal vascularization is incomplete. High oxygen exposure (as occurs in animal models and some pre-term infants) may also suppress VEGF, further contributing to inhibition of retinal vascularization. As the infant grows, IGF-I levels increase and are accompanied by increased VEGF. This may also be stimulated by exposure of animal models or pre-term infants to room air, which represents relative retinal hypoxia subsequent to high oxygen exposure [23]. In phase II of ROP, IGF-I and VEGF co-operate to promote extensive pathological neovascularization of the inner retina [23]. In the present report, the advantage of the early over the late intervention protocol may be due to a reduction in IGF-I not only during the hypoxic-induced neovascularization period, but also during the hyperoxic period. Although serum IGF-I was not measured in this study, we reported in a previous study that 30 mg/kg GHr ASO reduced serum IGF-I in mice by 44%, and this reduction was sustained over a 10-week period [13].

Both GH and IGF-I are involved in normal development of the retinal vasculature. In children born at term and with congenital deficiencies in GH, fewer branching points occur in retinal vessels when retina are examined between 3.8 and 18.7 years of age [26]. This was found to occur regardless of treatment with GH [26]. Few studies have examined the effect of GHr antagonism on normal retinal neovascularization in rodents. In rodents, retinal maturity occurs by approximately P14 [27-29]. In the present study, GHr ASO had no effect on the number of BVPs in the inner retina of sham mice regardless of the timing of treatment during the period of retinal maturation. These findings would suggest that GH is not a major factor in normal retinal vascular development, at least in the mouse.

Somatostatin analogues, such as octreotide, have shown potential as a treatment for DR, with reports that treatment halts progression in patients with either long-term proliferative DR (PDR) [30,31] or severe non-PDR or non-high-risk early PDR [32]. In terms of vessel growth in OIR, we found octreotide reduced retinal neovascularization, a finding that is consistent with a similar mouse OIR study, which used a similar concentration and dosing regimen of octreotide [12]. In the present study, octreotide reduced retinal neovascularization in OIR to a similar extent as late intervention with GHr ASO, but was less effective than early treatment with 20 and 30 mg/kg GHr ASO. It is possible that if octreotide was administered by an early intervention protocol to OIR mice that further benefits may have occurred.

As reported previously, when adolescent mice were dosed subcutaneously with the mismatch oligonucleotides ATL 261303 and ATL 260120 at doses similar to those reported here, neither GH receptor mRNA in liver nor the binding of GH to liver cells was affected [13]. In the present study, while ATL 261303 had no effect on either developmental or pathological retinal neovascularization in neonatal mice, ATL 260120 when dosed at the maximal 30 mg/kg in early intervention mode caused a reduction in pathological neovascularization in OIR mice that reached statistical significance if compared with vehicle-treated OIR mice. To place this in context, it is important to note that based on its effect on BVP, which in the early intervention mode was clearly dose-dependant, the GH receptor-specific oligonucleotide was significantly more potent than the control oligonucleotides when compared with control effects in the same dosing mode (early or late intervention).

An important consideration when interpreting the findings of the present study is the method used to quantitate BVPs. A variety of techniques are currently used to distinguish blood vessels in OIR including histochemical markers such as adenosine diphosphate (ADPase), immunolabeling with isolectins and fluorescein-perfused wholemounts or cross-sections of retina [12,33-35]. Quantitation methods are also variable, including counting vessels in the pre-ILM of serially sectioned whole retina [34], the retina partitioned into clock hours [36], and retina that has been divided into avascular and neovascular regions [35]. In this and some of our previous studies, we used hematoxylin and eosin stained cross-sections of retina to identify and count both inner retinal vessels and pre-retinal vessels [14,16,17]. Similar approaches have produced reproducible results even when compared to some other methods [34]. In the present study, we were able to detect a dose-dependent effect on BVPs in OIR with early treatment suggesting that the BVP technique was able to distinguish changes in vessel density, however, it is quite possible that identification of vessels with specific markers in particular regions of the retina may have highlighted more subtle differences between our experimental groups.

In conclusion, treatment with the GHr ASO, ATL 227446, reduced pathological retinal neovascularization in OIR to a greater extent than octreotide or control oligonucleotides. Systemically delivered GHr ASO may be a potential treatment for ischemic retinopathies such as ROP and DR.

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