

# Somatostatin Analogs Inhibit Neonatal Retinal Neovascularization

ROSEMARY D. HIGGINS<sup>a\*</sup>, YUN YAN<sup>a</sup> AND BRUCE K. SCHRIER<sup>b</sup>

<sup>a</sup>Department of Pediatrics, Division of Neonatology, Georgetown University Children's Medical Center, 3800 Reservoir Road, NW M3400, Washington, DC, 20007, U.S.A. and <sup>b</sup>Oakwood Laboratories, L.L.C., Oakwood Village, OH, U.S.A.

(Received Cleveland 4 May 2001 and accepted in revised form 6 October 2001)

The goal of this study was to determine the effect of two somatostatin analogs, Woc4D and octreotide, on oxygen induced retinopathy in the mouse. Oxygen induced retinopathy was produced in C57BL6 mice. Octreotide and Woc4D were administered from post-natal day 12–16. Retinopathy was assessed by a retinal scoring system utilizing fluorescein perfused retinal whole mounts. Animals treated with Woc4D and octreotide, respectively, had median retinopathy scores of 4(3,5) [median(25th, 75th quartile)] with  $P = 0.01$  and 3.5(2.9,4.3) with  $P = 0.01$  compared to oxygen and sham treated oxygen animals with scores of 6.6(5.3,8.5) and 7.4(5.8,8.6), respectively. Woc4D and octreotide treated animals had decreased blood vessel tufts and decreased extra-retinal neovascularization when compared to oxygen treated animals. Pituitary growth hormone (GH) mRNA expression was increased 8.3-fold by Woc4D treatment and 106-fold by oxygen exposure, and GH and mRNA was markedly reduced by Woc4D as well as octreotide. Growth as measured by animal weight was unaffected by either treatment. Woc4D and octreotide inhibited retinal neovascularization in an equally effective manner in the mouse model of oxygen induced retinopathy.

© 2002 Elsevier Science Ltd.

*Key words:* angiogenesis; growth hormone; insulin-like growth factor-1; retinopathy; somatostatin.

## 1. Introduction

Somatostatin is a natural peptide hormone that has modulating activity on the release of a number of other hormones, such as growth hormone (GH), glucagon, insulin, and gastrin. As the half-life of the native hormone in the blood stream is only 1–3 min, the effects are best seen when analogues with longer half-lives are used, and when the release mechanism for one of the affected hormones is markedly elevated, such as a pituitary adenoma with increased secretion of GH in acromegaly. Somatostatin agonist analogs have also been shown to be anti-angiogenic in vitro (Grant et al., 1993; Danesi and Del Tacca, 1996; Danesi et al., 1997) and in vivo (Woltering et al., 1991; Barrie et al., 1993; Patel et al., 1994; Baudouin et al., 1995; Demir et al., 1999). This activity involves inhibition of endothelial cell proliferation (Grant et al., 1993; Danesi and Del Tacca, 1996; Danesi et al., 1997) and may result in the inhibition of tumor growth via inhibition of blood vessel growth (Woltering et al., 1991).

Inhibition of angiogenesis in proliferative retinopathies using somatostatin analogues has been investigated. The rationale extends to the likelihood that the elevations of circulating GH in patients with progressive diabetic retinopathy (Alzaid et al., 1994) may affect its progression, and that controlling the excess of GH might slow this progression. Increased GH and

insulin-like growth factor-1 (IGF-1) have been implicated in retinopathy. Diabetic patients with progressive retinopathy have been shown to have higher serum levels of GH and IGF-1. Poor glycemic control in the type I diabetic patient has been associated with increased serum IGF-1 and increased GH which can result in a reduction of IGF-1. IGF-1 has been postulated to have protective role in diabetic retinopathy (Janssen and Lamberts, 2000).

Recently, the relationship between vascular endothelial growth factor (VEGF) and IGF-1 has been described in retinopathy of prematurity (Hellstrom et al., 2001). When preterm birth occurs, IGF-1 levels fall below in utero levels and vessel growth ceases despite the presence of VEGF at the vessel front. Higher oxygen levels may also decrease VEGF levels causing further lack of vessel growth. As the preterm retina grows in thickness, vessel growth does not always occur concomitantly, and the retina may become hypoxic. With concurrent hypoxia, VEGF and IGF-1 levels increase and neovascularization occurs.

These potential targets may be avenues for treatment of retinopathy as shown in animal studies (Smith et al., 1997; Smith et al., 1999). Octreotide has recently been reported to retard progression of retinopathy in patients with diabetes mellitus and delay time to laser treatment (Grant et al., 2000). It is possible that somatostatin analogues may protect from the development of other ischemic retinopathies as well. Similarities in pathology between angiogenesis or

\* Author for correspondence. E-mail: [higginsr1@gunet.georgetown.edu](mailto:higginsr1@gunet.georgetown.edu)

neovascularization in diabetic retinopathy and in retinopathy of prematurity promoted a study of somatostatin analog activity to determine the effect of Woc4D, a novel somatostatin analog, and octreotide on oxygen induced retinopathy in the mouse.

## 2. Materials and Methods

### *Animal Model of Oxygen Induced Retinopathy*

This protocol was approved by the Georgetown University Animal Care and Use committee and adhered to the Association for Research in Vision and Ophthalmology Statement for the Use of Animals in Ophthalmic and Vision Research. C57BL6 mice were obtained from Taconic Laboratories (Germantown, New York). Neonatal mice were placed in an infant incubator with their nursing mother with 75% oxygen at P7 and remained in oxygen until removal to room air at P12 (Smith et al., 1994). Oxygen was delivered at  $75 \pm 2\%$ , measured with an oxygen analyser (Hudson Ventronics, Temecula, CA, U.S.A.), and checked at least twice daily. Individual litters were either oxygen or room air reared. Within litters, differential treatment was performed including no treatment, octreotide, Woc4D, and sham injection. Animals were treated with injections for 5 days from P12 to P16. Animal killing was performed using a lethal dose of pentobarbital (120 mg/kg i.p.). Animals were perfused with fluorescein conjugated dextran as previously described (D'Amato, Wesolowski and Smith, 1993) and retinopathy was assessed by a retinal scoring system shown in Table I (Higgins et al., 1999).

### *Administration of Woc4D and Octreotide*

Woc4D was synthesized by California Peptide research with following sequence: D-Tyr-D-Tyr-D-Tyr-D-Tyr-cyclo(Cys-Phe-D-Trp-Lys-Thr-Cys)-Thr-NH<sub>2</sub>. It was received and used as the acetate salt. Octreotide acetate was purchased from Polypeptide Laboratories. Woc4D was initially used at  $20 \mu\text{g kg}^{-1} \text{day}^{-1}$  in a single injection subcutaneously and octreotide was used at  $10 \mu\text{g kg}^{-1} \text{dose}^{-1}$  given twice daily subcutaneously from P12–P16. The doses were increased to  $50 \mu\text{g kg}^{-1} \text{day}^{-1}$  of Woc4D and  $20 \mu\text{g kg}^{-1} \text{bid}$  of octreotide following initial experiments. The analogs were dissolved in 33 mM acetate buffer (pH 5) with 135 mM NaCl. Sham injections were performed with that vehicle.

### *Growth Hormone Reverse Transcriptase Polymerase Chain Reaction*

In order to assess the effect of oxygen, octreotide, and Woc4D on growth hormone expression, pituitary tissue was obtained for GH expression. Three to four animals per group (room air, room air + Woc4D, room air + octreotide, oxygen, oxygen + Woc4D, and oxygen + octreotide) were killed on day 16 following a 5 day treatment (P12–P16) with no drug, octreotide ( $20 \mu\text{g kg}^{-1} \text{bid}$ ) or Woc4D ( $50 \mu\text{g kg}^{-1} \text{day}^{-1}$ ) and pituitary glands were obtained from them. RNA was extracted using TRIzol reagent (Life Technologies, Rockville, MD, U.S.A.) as described by the manufacturer and reverse transcriptase polymerase chain reaction (RT-PCR) was performed. Ten  $\mu\text{g}$  of RNA and 2  $\mu\text{M}$  oligo(dT) 16 (total volume 22  $\mu\text{l}$ ) were heated at 68°C for 2 min and cooled on ice. First strand synthesis was performed by incubating

TABLE I  
*Retinopathy scoring system*

	Score				
	0	1	2	3	4
Blood vessel growth	Complete	Incomplete outer third	Incomplete middle third	Incomplete inner third	
Blood vessel tufts	None	Few, scattered < 3 clock hours	3–5 clock hours	6–8 clock hours	9–12 clock hours
Extra retinal neovascularization	None	Mild < 3 clock hours	Moderate 3–6 clock hours	Severe > 6 clock hours	
Central vasoconstriction	None	Mild early zone 1 (inner 50% of zone 1)	Moderate throughout zone 1 (outer 50% of zone 1)	Severe extending to zone 2	
Retinal hemorrhage	Absent	Present			
Blood vessel tortuosity	None	Mild < 3 clock hours	Moderate 3–6 clock hours	Severe > 6 clock hours	

the RNA and oligo(dT) in a reaction mixture (total volume, 50  $\mu$ l) containing 50 mM Tris-HCl, pH 8.5, 40 mM KCl, 8 mM MgCl<sub>2</sub>, 2 mM DTT, 50 U reverse transcriptase, and 0.8 mM each of dATP, dCTP, dGTP, and dTTP. The mixture was incubated at 42°C for 1 hr, then 99°C for 5 min, and 4°C for 5 min. The resultant cDNA was diluted with 100  $\mu$ l of water and stored at -20°C until PCR was performed. PCR reaction mixture (25  $\mu$ l total volume) was prepared with 0.2 mM each of dATP, dCTP, dGTP, and dTTP; 50 mM KCl; 10 mM Tris-HCl, pH 8.3; 1.5 mM MgCl<sub>2</sub>; 100 ng each of the mouse GH forward and reverse primers (see sequence below, Life Technologies, Rockville, MD, U.S.A.); 0.625 U of Taq DNA polymerase (Perkin-Elmer, Branchburg, NJ, U.S.A.); and 3  $\mu$ l of the diluted cDNA. The mixture was incubated for 4 min at 94°C, followed by 28 cycles of 45 s at 94°C, 45 s at 58°C, and 45 s at 72°C, followed by 7 min at 72°C, in a PCR apparatus (model 2400, Perkin-Elmer, Branchburg, NJ, U.S.A.). To verify equal amounts of RNA were used in the PCR reaction and to verify uniform amplification process,  $\beta$ -actin mRNA was also amplified from the sample as an internal control. PCR products were separated on a 1.2% agarose gel and were visualized by staining with ethidium bromide. A 100 bp DNA ladder was used as a size marker. The number of cycles versus intensity of the PCR band was evaluated to determine the optimum number of cycles to be in the linear range. Gels were photographed and scanned for density using the Quantiscan program (Biosoft, Ferguson, MO, U.S.A.). The RT-PCR was repeated three times for the experiment. The PCR products were sequenced for confirmation. The oligonucleotide primer sets used to amplify GH (Linzer and Talamantes, 1985) and  $\beta$ -actin (R&D Systems, Minneapolis, MN, U.S.A.) were: (forward) 5'CTGCTGACACCTACAAAGAG3' and (reverse) 5'GCGTCAAACCTTGTCATAGG3'; and (forward) 5'-CTACAATGAGCTGCGTGTGG-3' and (reverse) 5'-AAGGAAGGCTGGAAGAGTGC-3'. The result PCR products were 385 bp (GH) and 528 bp ( $\beta$ -actin).

### Statistical Analyses

Retinopathy scores were evaluated by the Kruskal-Wallis test for the overall group and by the Mann-Whitney test to determine differences between groups. Animals weights were evaluated using Student's *t*-test. Statistical significance was determined at the  $P < 0.05$  level.

### 3. Results

Initial studies were performed using Woc4D at dose of 20  $\mu$ g kg<sup>-1</sup> day<sup>-1</sup> from P12 to P16 octreotide at a dose of 10  $\mu$ g kg<sup>-1</sup> bid from P12 to P16. Woc4D treated animals had a median total retinopathy score of 6(3,7); octreotide treated animals also had score of

6(3,7). Oxygen reared animals had a score of 7(5,8). Based on these results doses were adjusted upwards.

Animals treated with higher concentrations of Woc4D (50  $\mu$ g kg<sup>-1</sup> day<sup>-1</sup>) had a significant improvement in their retinopathy scores when compared to oxygen and oxygen and sham treated animals as shown in Fig. 1. Woc4D improved retinopathy ( $P = 0.01$ ) when compared to oxygen alone. Pups treated with higher dose octreotide also had a significant improvement in their retinopathy scores as shown in Fig. 1 ( $P = 0.01$ ). Specifically, Woc4D and octreotide provided improvement in blood vessel tufts and in extra-retinal neovascularization as shown in Table II. Representative retinal whole mounts are shown in Fig. 2. When Woc4D and octreotide were compared at the higher doses, they were equally effective at reducing retinopathy ( $P = 0.93$ ).

Because the analogs may have their effect in part, by suppression of serum GH levels and subsequent reduction of serum IGF-1 levels, the authors examined pituitary GH mRNA expression and growth. There was no effect (Table III) on growth by any of the treatment regimens. The data indicate that use of somatostatin analogs to control retinopathy in the mouse model was not detrimental to overall growth rate. GH expression as measured by RT-PCR was increased 8.3-fold (4–16-fold range) by Woc4D treatment in room air reared animals and increased 106-fold (27–227-fold range) by oxygen exposure (Figs. 3 and 4). It should be noted that this altered

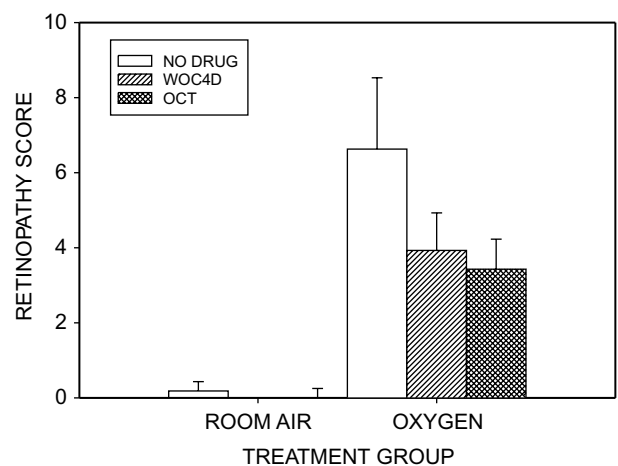


FIG. 1. Median total retinopathy score (error bar denotes 75th quartile) for Woc4D and octreotide experiment. By Kruskal Wallis test  $P < 0.01$  for entire group; by Mann-Whitney test,  $P = 0.01$  for oxygen versus control and oxygen versus oxygen + Woc4D; and by Mann-Whitney test,  $P = 0.01$  for oxygen versus control and oxygen versus + octreotide. The number of animals in each group were as follows: room air, no drug,  $n = 11$ ; room air, Woc4D,  $n = 7$ ; room air, octreotide,  $n = 7$ ; oxygen, no drug,  $n = 20$ ; oxygen, Woc4D,  $n = 11$ ; and oxygen, octreotide,  $n = 12$ ; Sham treated animals had retinopathy scores of 0.375(0,0.5) in the room air reared group and 7.375(5.8,8.6) in the oxygen group.

TABLE II  
Retinopathy subscores [expressed as median (25th, 75th quartile); oct, octreotide]

	Room air	Room air + Woc4D 50	Room air + oct 20 bid	Oxygen	Oxygen + Woc4D 50	Oxygen + oct 20 bid
Number of animals ( <i>n</i> )	11	7	7	20	11	12
Blood vessel growth	0(0,0)	0(0,0)	0(0,0)	0.25(0,0.5)	0(0,0.375)	0(0,0)
Blood vessel tufts	0(0,0)	0(0,0)	0(0,0)	1.25(0.69,2.3)	0.5(0.5,0.63)*	0.5(0,1)*
Extra retinal neovascularization	0(0,0)	0(0,0)	0(0,0)	0.75(0.5,1)	0(0,0.125)†	0.125(0,0.5)†
Central vasoconstriction	0(0,0)	0(0,0)	0(0,0)	1.88(1,2.5)	1.5(1.125,2.25)	1(1,1.25)
Hemorrhage	0(0,0)	0(0,0)	0(0,0)	1(0.5,1)	1(0.5,1)	0.5(0.38,0.63)
Blood vessel tortuosity	0(0,0)	0(0,0)	0(0,0)	1.5(1,1.18)	1(0.75,1.38)	1.13(0.75,1.5)

\* Denotes  $P < 0.05$  when compared to oxygen treated group.

† Denotes  $P < 0.001$  when compared to oxygen treated group.

pituitary GH expression did not appear to be translated to an effect on growth rate per se.

#### 4. Discussion

This study describes a decrease in retinal neovascularization in mice treated with Woc4D and octreotide in a mouse model of oxygen induced retinopathy. Inhibition of the growth hormone axis has been postulated to improve diabetic retinopathy (Smith et al., 1997). A systematic evaluation in the mouse model to assess the effects of Woc4D and octreotide on retinopathy adds to the pre-existing evidence that the growth hormone and IGF1 pathways are important in the evolution of retinopathy. In addition, that is supported by a recent clinical study (Grant et al., 2000) which suggests that octreotide treatment retards progression of diabetic retinopathy and may delay time to laser surgery in such patients.

The effects of Woc4D and octreotide on improving retinal neovascularization were similar in this study. Total daily doses were  $50 \mu\text{g kg}^{-1}$  in the Woc4D group and  $40 \mu\text{g kg}^{-1}$  in the octreotide treated animals. Woc4D was given as a single dose as opposed to octreotide which was given in a twice daily dose regimen. Thus, the two somatostatin analogues appear equally effective in reducing retinal neovascularization in the mouse model of oxygen induced retinopathy.

It has been shown that native somatostatin exerts its effects through as many as five different receptors, designated SSTR1–5 (Reisine and Bell, 1995). The US-marketed agonist analog, octreotide, is effective in stimulating SSTR2 to the virtual exclusion of the other receptors, and this interaction is thought to be the basis of its activity in the many disease states in which it is being employed. It has been difficult to determine the natural activities of the other receptors due partly to the dearth of agonists and antagonists that are specific to them. Among the analogs developed

(Woltering et al., 1999) with N-terminal tyrosine residues and designed to be used for radioactive imaging is an agonist, designated Woc4D, that exhibits high affinity interaction with SSTR5 as well as SSTR2. The analogs Woc4D and octreotide are similar in other regards except that the blood half-life of Woc4D in rats primates is approximately five times longer than of octreotide.

Somatostatin analogues could be postulated to inhibit growth via inhibition of growth hormone release. The data in the mouse model suggest that 5 day treatment (either following oxygen exposure or in room air reared animals) does not affect growth as measured by body weight. Similar results were also seen with Woc4D and octreotide agents with respect to animal growth. Oxygen exposure followed by room air exposure caused an increased in pituitary GH expression. A prior study (Averbukh et al., 1998) showed an increase in serum GH levels in rats during a state of relative hypoxia, perhaps comparable to the animals at P16. It is unclear as to why Woc4D caused an increase in pituitary GH expression in the absence of hypoxic stimulus. The authors speculate that since Woc4D acts through both SSTR2 and SSTR5, and octreotide acts mainly through SSTR2, that receptor modulation at the level of the pituitary may be different with the two somatostatin analogs. Both Woc4D and octreotide markedly diminished oxygen-induced pituitary GH expression at day 16 suggesting these drugs have a central effects as well as an effect on decreasing retinal neovascularization.

Somatostatin analogs have antiproliferative effects which may explain their observed actions on decreasing retinal neovascularization in this study. SSTR receptor signaling can result in apoptosis or cell cycle arrest. This has been shown to be mediated by decreasing intracellular cAMP levels by inactivation of adenylyl cyclase activity (Medina, Toro and Santisteban, 2000). A second signaling even mediated by SSTR activation leads to an increased activation of the protein tyrosine phosphatases SHP-1 and SHP-2

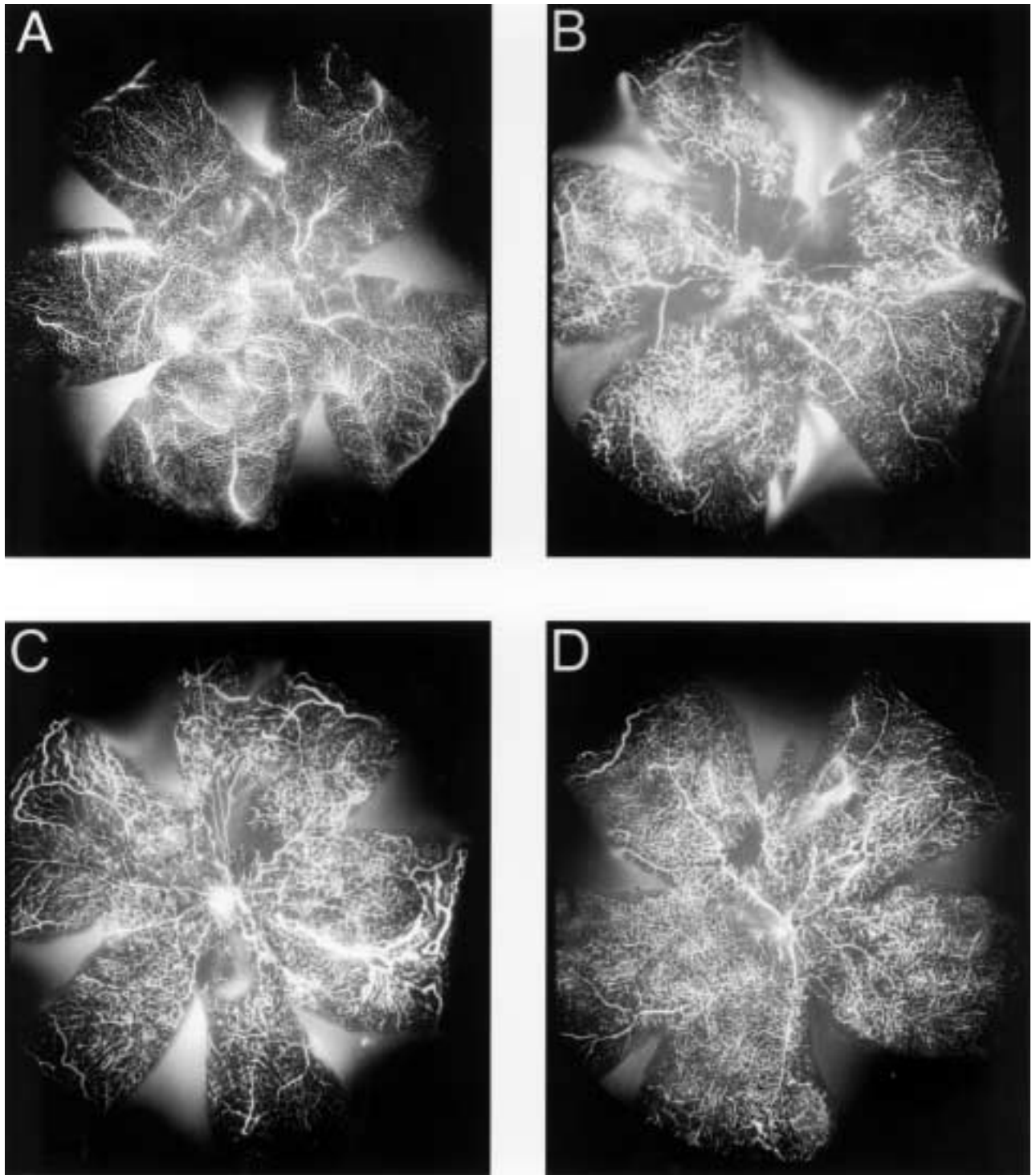


FIG. 2. Representative retinal whole mounts. (A) depicts a fluorescein perfused retina from a room air reared animal. (B) shows an oxygen treated retina. Note the central loss of blood vessels and blood vessel tufts. (C) shows a retinal whole mount from an octreotide treated animal. Similarly, (D) shows a retina from a Woc4D treated animal with less retinal vascular pathology. (B–D) show larger, more engorged vessels characteristic of oxygen exposed retinas. 4–5 $\times$  magnification.

(Reardon et al., 1997) thus potentially blocking the stimulation effect of any growth factor whose receptor signaling occurs through tyrosine kinase activity. Octreotide or Woc4D may be acting to block cellular proliferation mediated via activation of tyrosine kinase activity.

Previous work has shown that systemic inhibition of GH or IGF-1, or both may have potential in

preventing some forms of retinopathy (Smith et al., 1997). Further work has shown that IGF-1 receptor regulation of VEGF action is mediated through VEGF activation of p44/42 mitogen activated protein kinase (Smith et al., 1999). In addition, blockade of VEGF signaling by inhibition of receptor kinase activity can block retinal neovascularization (Ozaki et al., 2000). IGF-1 and VEGF are inter related in

TABLE III  
Weight data for various treatment groups

	Number of animals (n)	P7 Weight	P12 Weight	PSAC Weight	Weight change P12-Psac (g day <sup>-1</sup> )
Room air	13	4.03 ± 0.69	6.44 ± 0.98	8.59 ± 0.86	0.33 ± 0.05
Room air + Woc4D 20 µg	2	4.02 ± 0.66	6.76 ± 0.77	9.08 ± 1.07	0.29 ± 0.04
Room air + oct 10 µg bid	4	4.25 ± 0.74	7.02 ± 0.67	8.69 ± 0.39	0.30 ± 0.07
Room air + Woc4D 50 µg	7	4.10 ± 0.39	6.54 ± 0.38	8.64 ± 0.57	0.35 ± 0.04
Room air + oct 20 µg bid	7	4.08 ± 0.12	6.47 ± 0.59	8.83 ± 0.71	0.37 ± 0.05
Room air sham	5	4.42 ± 0.40	7.25 ± 0.61	8.94 ± 0.61	0.29 ± 0.08
Oxygen	21	3.81 ± 0.92	5.91 ± 1.15	8.12 ± 1.36	0.31 ± 0.14
Oxygen + Woc4D 20 µg	12	4.36 ± 0.77	6.53 ± 0.97	8.67 ± 1.12	0.32 ± 0.09
Oxygen + oct 10 µg bid	6	4.97 ± 1.29	7.06 ± 1.21	9.44 ± 1.17	0.35 ± 0.15
Oxygen + Woc4D 50 µg	11	3.95 ± 0.66	6.13 ± 0.60	8.10 ± 1.00	0.28 ± 0.17
Oxygen + oct 20 µg bid	12	4.09 ± 0.43	6.45 ± 0.50	8.59 ± 0.94	0.30 ± 0.12
Oxygen sham	15	4.06 ± 0.83	6.29 ± 1.26	8.58 ± 1.45	0.29 ± 0.14

Values are shown as mean ± SD (g). Psac, post natal day of sacrifice; oct, octreotide.

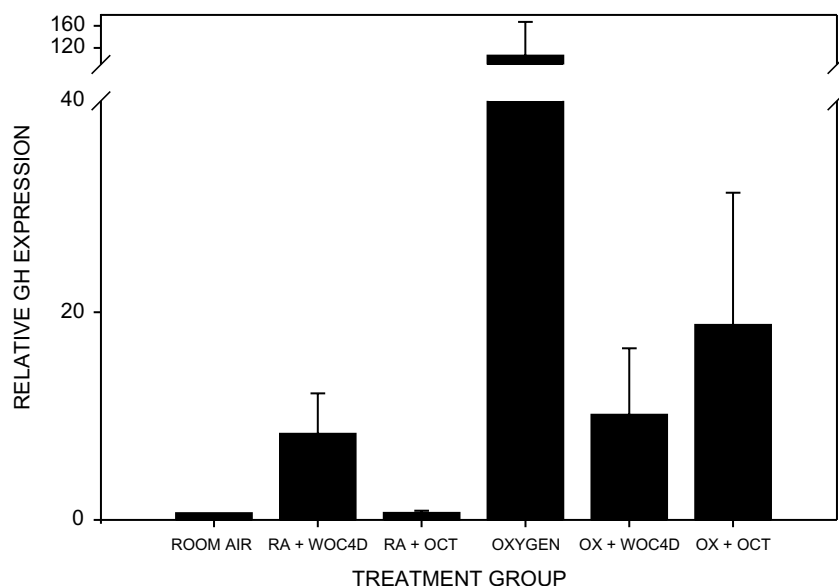


FIG. 3. Pituitary growth hormone expression. Relative GH expression is shown on the *y*-axis and the various treatment groups are shown on the *x*-axis. Values are expressed as mean ± S.E.M. with room air being normalized to one.

retinopathy of prematurity (Hellstrom et al., 2001). Somatostatin analogues may interfere with such a retinopathy-producing cascade at one or several of these levels.

A prior study (Averbukh et al., 2000) showed that octreotide failed to inhibit hypoxia-induced retinal neovascularization in the rat. Results show an improvement in the mouse model of oxygen induced retinopathy. Several aspects of the two studies could explain differing results. The dose used in the Averbukh study was 0.7 µg g<sup>-1</sup> birth weight versus 0.02 µg g<sup>-1</sup> of weight at the time of experiment in the study. Timing of dose may also account for discrepant results. The models are somewhat different. The mouse model uses as 5 day exposure of 75% oxygen with return to room air. The rat model used by Averbukh

alternates hypoxia and hyperoxia (which may result in a different injury) of 10 and 50% oxygen, respectively. Finally, the two species are different (rat versus mouse).

In summary, these data further support the possibility of use of somatostatin analogues in the treatment of retinopathy. Octreotide and Woc4D are



FIG. 4. Gel photograph of GH expression. Lane 1 shows a DNA 100 bp ladder, lane 2 shows room air; lane 3, room air plus Woc4D; lane 4, room air + octreotide; lane 5, oxygen; lane 6, oxygen + Woc4D; lane 7, oxygen + octreotide.

effective at reducing retinal neovascularization in a mouse model of oxygen induced retinopathy. Further, they have no effect on animal weight gain during the course of exposure to the drugs, although pituitary GH expression is altered. Speculation can be made that Woc4D, in addition to octreotide, may be useful for treatment of retinal neovascularization in retinopathy.

### Acknowledgements

Funded by Oakwood Laboratories, L.L.C.

### References

- Alzaid, A. A., Dinneen, S. F., Melton, L. J. and Rizza, R. A. (1994). The role of growth hormone in the development of diabetic retinopathy. *Diab. Care* **17**, 531–4.
- Averbukh, E., Weiss, O., Halpert, M., Yanko, R., Moshe, R., Nephesh, I., Flyvbjerg, A., Yanko, L. and Raz, I. (1998). Gene expression of insulin-like growth factor-1, its receptor and binding proteins in retina under hypoxic conditions. *Metabolism* **47**, 1331–6.
- Averbukh, E., Halpert, M., Yanko, R., Peer, J., Levinger, S., Flyvbjerg, A. and Raz, I. (2000). Octreotide, a somatostatin analogue, fails to inhibit hypoxia-induced retinal neovascularization in the neonatal rat. *Int. J. Exp. Diab. Res.* **1**, 39–47.
- Barrie, R., Woltering, E. A., Hajarizadeh, H., Mueller, C., Ure, T. and Fletcher, W. S. (1993). Inhibition of angiogenesis by somatostatin and somatostatin-like compounds is structurally dependent. *J. Surg. Res.* **55**, 446–50.
- Baudouin, C., Imbert, F., Ettaiche, M. and Gastaud, P. (1995). Evaluation of antiproliferative effects of the somatostatin analogue somatoline in a rabbit model of traction retinal detachment. *Fund. Clin. Pharmacol.* **9**, 357–65.
- D'Amato, R., Wesolowski, E. and Smith, L. E. (1993). Microscopic visualization of the retina by angiography with high-molecular-weight fluorescein-labeled dextrans in the mouse. *Microvasc. Res.* **46**, 135–42.
- Danesi, R. and Del Tacca, M. (1996). The effect of the somatostatin analog octreotide on angiogenesis in vitro. *Metabolism* **45**, 49–50.
- Danesi, R., Agen, C., Benelli, U., Paolo, A. D., Nardini, D., Bocci, G., Basolo, F., Campagni, A. and Tacca, M. D. (1997). Inhibition of experimental angiogenesis by the somatostatin analogue octreotide acetate (SMS 201–995). *Clin. Cancer Res.* **3**, 265–72.
- Demir, T., Celiker, U. O., Kukner, A., Mogulkoc, R., Celebi, S. and Celiker, H. (1999). Effect of octreotide on experimental corneal neovascularization. *Acta Ophthalmol. Scand.* **77**, 386–90.
- Grant, M. B., Caballero, S. and Millard, W. J. (1993). Inhibition of IGF-1 and bFGF stimulated growth of human retinal endothelial cells by the somatostatin analogue, octreotide: a potential treatment for ocular neovascularization. *Regul. Pept.* **20**, 267–78.
- Grant, M. B., Mames, R. N., Fitzgerald, C., Hazariwala, K. M., Cooper-DeHoff, R., Caballero, S. and Estes, K. S. (2000). The efficacy of octreotide in the therapy of severe nonproliferative and early proliferative diabetic retinopathy. *Diab. Care* **23**, 504–9.
- Hellstrom, A., Perruzzi, C., Ju, M., Engstrom, E., Hard, A. L., Liu, J. L., Albertsson-Wikland, K., Calsson, B., Niklas-son, A., Sjodell, L., LeRoith, D., Senger, D. R. and Smith, L. E. H. (2001). Low IGF-1 suppresses VEGF-survival signaling in retinal endothelial cells: direct correlation with clinical retinopathy of prematurity. *Proc. Nat. Acad. Sci.* **98**, 5804–8.
- Higgins, R. D., Yu, K., Sanders, R. J., Nandgaonkar, B., Rotschild, T. and Rifkin, D. B. (1999). Diltiazem reduces neovascularization in a mouse model of oxygen induced retinopathy. *Curr. Eye Res.* **18**, 20–7.
- Janssen, J. A. M. J. L. and Lamberts, S. W. J. (2000). Circulating IGF-1 and its protective role in the pathogenesis of diabetic angiopathy. *Clin. Endocrinol.* **52**, 1–9.
- Linzer, D. I. H. and Talamantes, F. (1985). Nucleotide sequence of mouse prolactin and growth hormone mRNAs and expression of these mRNAs during pregnancy. *J. Biol. Chem.* **260**, 9574–9.
- Medina, D. L., Toro, M. J. and Santisteban, P. (2000). Somatostatin interferes with thyrotropin-induced G<sub>1</sub>-S transition mediated by cAMP-dependent protein kinase and phosphatidylinositol 3-kinase. *J. Biol. Chem.* **275**, 15549–56.
- Ozaki, H., Seo, M. S., Ozaki, K., Yamada, H., Yamada, E., Okamoto, N., Hofmann, F., Wood, J. M. and Campochiaro, P. A. (2000). Blockade of vascular endothelial cell growth factor receptor signaling is sufficient to completely prevent retinal neovascularization. *Am. J. Pathol.* **156**, 697–707.
- Patel, P. C., Barrie, R., Hill, N., Landeck, S., Kurozawa, D. and Woltering, E. A. (1994). Postreceptor signal transduction mechanisms involved in octreotide-induced inhibition of angiogenesis. *Surgery* **116**, 1148–52.
- Reardon, D. B., Dent, P., Wood, S. L., Kong, T. and Sturgill, T. W. (1997). Activation in vitro of somatostatin receptor subtypes 2, 3, or 4 stimulates protein tyrosine phosphatase activity in membranes from transfected ras-transformed NIH 3T3 cells: coexpression with catalytically inactive SHP-2 blocks responsiveness. *Mol. Endocrinol.* **11**, 1062–9.
- Reisine, T. and Bell, T. I. (1995). Molecular biology of somatostatin receptors. *Endocrinol. Rev.* **16**, 427–42.
- Smith, L. E. H., Wesolowski, E., McLellan, A., Kostyk, S. K., D'Amato, R., Sullivan, R. and D'Amore, P. A. (1994). Oxygen-induced retinopathy in the mouse. *Invest. Ophthalmol. Vis. Sci.* **35**, 101–11.
- Smith, L. E. H., Kopchick, J. J., Chen, W., Knapp, J., Kinose, F., Daley, D., Foley, E., Smith, R. G. and Schaeffer, J. M. (1997). Essential role of growth hormone in ischemia-induced retinal neovascularization. *Science* **276**, 1706–9.
- Smith, L. E. H., Shen, W., Perruzzi, C., Soker, S., Kinose, F., Xu, X., Robinson, G., Bischoff, J., Zhang, B., Schaeffer, J. M. and Senger, D. R. (1999). Regulation of vascular endothelial growth factor-dependent retinal neovascularization by insulin-like growth factor-1 receptor. *Nat. Med.* **5**, 1390–5.
- Woltering, E. A., Barrie, R., O'Dorisio, T. M., Arce, A., Ure, T., Cramer, A., Holmes, D., Robertson, J. and Fassler, J. (1991). Somatostatin analogues inhibit angiogenesis in the chick chorioallantoic membrane. *J. Surg. Res.* **50**, 245–51.
- Woltering, E. A., O'Dorisio, M. S., Murphy, W. A., Chen, F., Drouant, G. J., Espenan, G. D., Fisher, D. R., Sharma, C., Diaco, D. S., Maloney, T. M., Fuselier, J. A., Nelson, J. A., O'Dorisio, T. M. and Coy, D. H. (1999). Synthesis and characterization of multiply-tyrosinated, multiply-iodinated somatostatin analogues. *J. Pept. Res.* **53**, 201–13.