Comparative Effects of Early Postnatal Ibuprofen and Indomethacin on VEGF, IGF-I, and GH during Rat Ocular Development

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PURPOSE. Ibuprofen and indomethacin are nonselective prostaglandin synthetase inhibitors that have been shown to improve oxygen-induced retinopathy in mice. Vascular endothelial growth factor (VEGF), insulin-like growth factor (IGF)-I, and growth hormone (GH) are potent growth factors involved in retinal development. This study was conducted to examine and compare the effects of early postnatal ibuprofen and indomethacin on ocular and systemic VEGF, IGF-I, and GH during rat ocular development.

METHODS. Newborn rats were treated with intraperitoneal injections of low and high doses of ibuprofen or indomethacin at birth (postnatal day [P]1) and on P2 and P3. A control group received equivalent volumes of saline. At P14, vitreous fluid, retinal homogenates, and serum were analyzed for VEGF, IGF-I, and GH protein levels. Retinal mRNA expression of VEGF splice variants (VEGF188, VEGF164, VEGF120), VEGF receptors (VEGFR-1, VEGFR-2, Npn-1, Npn-2), and pigment epithelium-derived factor (PEDF) were also examined.

RESULTS. Animals treated with high-dose ibuprofen had significantly lower somatic growth and higher serum and vitreous IGF-I levels. High-dose ibuprofen decreased retinal VEGF levels and retinal VEGF164, VEGF120, and VEGFR-2 transcripts, resulting in a significant increase in the cecal period in 87% of rats at P14. Both indomethacin doses suppressed retinal VEGF164 transcripts without affecting VEGF receptors.

CONCLUSIONS. Ibuprofen may be more effective than indomethacin for suppression of retinal VEGF signaling, suggesting a possible therapy for retinal neovascularization. However, deficits in somatic growth concurrent with higher systemic IGF-I levels suggests decreased IGF-I bioactivity. These adverse effects should be considered. (Invest Ophthalmol Vis Sci. 2006; 47:3036–3043) DOI:10.1167/iovs.06-0057

Indomethacin is a nonselective cyclooxygenase (COX) inhibitor that leads to decreased prostaglandin (PG) synthesis. It is widely used in the neonatal intensive care unit during the first few days of postnatal life, to treat symptomatic patent ductus arteriosus (PDA) in premature newborns. Despite its effectiveness, indomethacin has significant maternal, fetal and neonatal adverse effects such as impaired platelet and neutrophil function1 and cerebral, gastrointestinal, and renal hemodynamics.2–4 Indomethacin is also associated with neonatal complications such as intraventricular hemorrhage (IVH), renal dysfunction, and necrotizing enterocolitis (NEC).5–10 Similar to indomethacin, ibuprofen inhibits PG synthesis via its effects on COX. However, it has been shown to be as effective as indomethacin for closure of the ductus arteriosus with less cerebral and renal side effects.11–13 The differences between these two nonsteroidal anti-inflammatory drugs (NSAIDs) may well be due to differences in their pharmacologic characteristics in the neonate.14 Despite their differences, animal studies have shown that both ibuprofen and indomethacin appear to improve oxygen-induced retinopathy without affecting normal retinal development,15,16 perhaps because of their suppressive effects on HIF-1α7 and VEGF.18 Whereas we have shown that ibuprofen enhances retinal and choroidal blood flow autoregulation in newborn piglets exposed to hypertension and hypotension,19 other studies have demonstrated that infants treated with indomethacin appear to be more than 1.5 times more likely to have retinopathy of prematurity (ROP) than untreated infants.20 The mechanism(s) by which ibuprofen and indomethacin exert their effects on retinal neovascularization have not been previously studied, but may in part be related to their effects on growth factors such as vascular endothelial growth factor (VEGF), insulin-like growth factor (IGF)-I, and/or growth hormone (GH).

VEGF is a potent endothelial cell mitogen and angiogenic factor involved in embryonic development.21 Alternative splicing of a single gene yields three murine isoforms: VEGF188, VEGF164, and VEGF120. VEGF164 and VEGF120 are highly involved in angiogenesis and may constitute up to 98% of total VEGF overexpression.22 There is overwhelming evidence of the involvement of VEGF in oxygen-induced retinopathy.23–27 Pigment epithelium–derived factor (PEDF) is a potent naturally occurring angiogenic antagonist that regulates endothelial cell proliferation and blood vessel growth in the eye. Evidence suggests that PEDF may be responsible for regulation of VEGF.28 IGF-I, a somatic growth factor involved in ocular development and physiology, has also been implicated in abnormal retinal angiogenesis.29–31 The role of GH in ROP has not been well studied; however, GH deficiency in children has been reported to be associated with reduced retinal vascular-
Effect of Prostaglandin Synthase Inhibitors on Growth Factors

Hypothesis was that there were no significant differences between the two drugs. Data emerging from our laboratory have shown that during the course of postnatal retinal development in the rat, VEGF and IGF-I act synergistically to promote retinal development and that the vitreous fluid may be a reservoir for these growth factors. Based on these data, as well as previously published observations, we examined and compared the effects of early postnatal administration of ibuprofen and indomethacin on systemic and ocular VEGF, IGF-I, and GH in the normal, but immature retina of the newborn rat. The null hypothesis was that there were no significant differences between the two drugs.

### Materials and Methods

All experiments were approved by the Memorial Health Services Institutional Animal Care and Use Committee (Long Beach, CA). Animals were managed according to the ARVO Statement for the Use of Animals in Ophthalmic and Visual Research and were treated humanely, according to the guidelines outlined by the United States Department of Agriculture (USDA), and the Guide for the Care and Use of Laboratory Animals (National Research Council). Euthanasia was conducted according to the ARVO Statement for the Use of Animals in Ophthalmic and Visual Research and were treated humanely, according to the guidelines of the American Veterinary Medical Association.

### Experimental Design

Certified infection-free, timed-pregnant Sprague-Dawley rats carrying fetuses (n = 10–14) of known gestational age (19 days) were purchased from Charles River Laboratories (Wilmington, MA). The pregnant rats were placed in USDA-approved cages and remained undisturbed until delivery (22 days gestation). The animals were housed in an animal facility with a 12-hour day–night cycle and provided standard laboratory diet and water ad libitum. Within 24 hours of birth, newborn rat pups delivering on the same day were pooled and randomly assigned to expanded litters of 15. One dam remained with the same litter for the entire study. Each pup was weighed and measured for linear growth (nose to tail length). Litters were randomly assigned to receive intraperitoneal injections of (1) low-dose ibuprofen (10 mg/kg) on P1 followed by 5 mg/kg on P2 and P3; (2) high-dose ibuprofen (50 mg/kg) on P1 followed by 25 mg/kg on P2 and P3; and (3) low-dose indomethacin (0.2 mg/kg) on day 1 (P1), followed by 0.1 mg/kg on P2 and P3; (4) high-dose indomethacin (1.0 mg/kg) on P1 followed by 0.5 mg/kg on P2 and P3; or (5) equivalent volume saline injected intraperitoneally (n = 2 litters/group). Indomethacin (Indocin IV; Merck & Co., Inc., West Point, PA) was diluted in 0.9% saline before injection. Ibuprofen IV solution was provided by Farmacia Laboratories (Westport, CT) and diluted in 0.9% saline before injection. At P14 (the time of retinal vascular maturity) the rat pups were examined for eye opening (eyes were considered opened if at least one eye was open), weighed, measured, and killed by decapitation. Blood samples were pooled for a total of 15 samples, collected in sterile tubes (Eppendorf, Fremont, CA), and placed on ice before processing.

Immediately after death, both eyes from each pup were enucleated and placed in ice-cold phosphate-buffered saline (PBS, pH 7.4). Enucleation was performed with the use of iris forceps and scissors for separation of the eyes from the surrounding connective tissue, nerve, and muscles. The eyes were dried on sterile gauze, and the vitreous fluid was aspirated with a 0.5-mL insulin syringe and placed on ice in sterile tubes (Eppendorf). Vitreous fluid was pooled for a total of six samples per group. None of the vitreous fluid samples were contaminated with blood. After removal of the vitreous fluid, the corneas were removed, and the eye cups were placed in ice-cold phosphate-buffered saline (PBS, pH 7.4). The retinas were excised under a dissecting microscope and placed in a sterile tube containing ice-cold PBS on ice, before homogenization for VEGF, IGF-I, and GH protein determination. For total RNA extraction, retinas were rinsed in ice-cold PBS and placed in TRIzol (Invitrogen, Grand Island, NY) and stored at −80°C for RNA extraction.

### Table 1. Eye Opening at P14

<table>
<thead>
<tr>
<th></th>
<th>Ibuprofen</th>
<th>Indomethacin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saline</td>
<td>Low Dose</td>
<td>High Dose</td>
</tr>
<tr>
<td>Male</td>
<td>12/16 (75)</td>
<td>14/16 (88)</td>
</tr>
<tr>
<td>Female</td>
<td>8/14 (57)</td>
<td>8/14 (57)</td>
</tr>
<tr>
<td>Total</td>
<td>20/30 (67)</td>
<td>22/30 (73)</td>
</tr>
</tbody>
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Low-dose ibuprofen (10 mg/kg IP on P1 followed by 5.0 mg/kg on P2 and P3); high-dose ibuprofen (50 mg/kg IP on P1 followed by 25 mg/kg on P2 and P3); low-dose indomethacin (0.2 mg/kg IP on P1 followed by 0.1 mg/kg on P2 and P3); and high-dose indomethacin (1.0 mg/kg IP followed by 0.5 mg/kg on P2 and P3). The control group received equivalent volume saline IP on P1, P2 and P3. Data are the number of rats with open eye(s) at P14/total group (percentage).

### Table 2. Effect on Somatic and Linear Growth, and Serum VEGF, IGF-I, and GH Levels at P14

<table>
<thead>
<tr>
<th></th>
<th>Ibuprofen</th>
<th>Indomethacin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saline</td>
<td>Low Dose</td>
<td>High Dose</td>
</tr>
<tr>
<td>Male</td>
<td>25.0 ± 0.4</td>
<td>25.0 ± 0.5</td>
</tr>
<tr>
<td>Female</td>
<td>13.2 ± 0.2</td>
<td>13.0 ± 0.1</td>
</tr>
<tr>
<td>Total</td>
<td>14.7 ± 13.0</td>
<td>12.7 ± 12.2</td>
</tr>
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</table>

Groups are as described in Table 1.

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**P < 0.01; ***P < 0.001 vs. saline, ibuprofen (low dose), indomethacin (low dose and high dose).  
† P < 0.05 vs. saline and indomethacin (low dose); †† P < 0.01 vs. saline.

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in reagent (TRIZol; Invitrogen, Gaithersburg, MD) before extraction of total RNA. Retinas were pooled for a total of five samples/group for retinal homogenates and five samples/group for total RNA extraction.

Assay for VEGF

VEGF levels in serum, undiluted vitreous fluid, and retinal homogenates were assayed using commercially available sandwich immunoassay kits (BioSource International, Inc., Camarillo, CA). The assay predominantly binds the monomeric VEGF165 but also detects the VEGF121 isoform. The assay recognizes the 164-amino acid splice variant of mouse VEGF and has a 98% affinity for the rat sequence. The assay uses a monoclonal anti-VEGF detection antibody conjugated to horseradish peroxidase and color development with tetramethylbenzidine/hydrogen peroxide (TMB solution). All assays were performed on a 1.5% agarose gel stained with ethidium bromide. RT-PCR was conducted with the use of one-step RT-PCR kits (SuperScript; Invitrogen). Amplification of cDNA was performed using specific sense and antisense primers for GAPDH, ribosomal RNA bands (18S and 28S) was confirmed on 1% agarose gel stained with ethidium bromide. The data are expressed as the mean transcript/GAPDH ratio ± SEM.

RT-PCR

Total RNA was isolated from the retinas (TRIZol reagent; Invitrogen) according to the manufacturer’s recommendations. Integrity of the ribosomal RNA bands (18S and 28S) was confirmed on 1% agarose gel stained with ethidium bromide. RT-PCR was conducted with the use of one-step RT-PCR kits (SuperScript; Invitrogen). Amplification of cDNA was performed using specific sense and antisense primers for GAPDH, VEGF isoforms, PEDF, VEGFR-1, VEGFR-2, Npn-1, and Npn-2 as previously described. 24-35 Gel electrophoresis of the PCR products was performed on a 1.5% agarose gel containing five or fewer observations. One-way analysis of variance was then normalized to the density of the GAPDH bands. Identification of the DNA fragments was accomplished with the use of a 100-base-pair DNA ladder, which consisted of 15 blunt-ended fragments between 100 and 1500 bp. The data are expressed as the mean ± SEM.

Statistical Analysis

Categorical data were analyzed by $\chi^2$ or Fisher exact tests for cells that contained five or fewer observations. One-way analysis of variance

Assay of GH

GH levels in serum, undiluted vitreous fluid, and retinal homogenates were determined using active ultrasensitive rat GH enzyme immunoassay kits (Diagnostic Systems Laboratories, Webster, TX). The sensitivities of the GH assay was 0.66 pg/mL and the intra- and interassay coefficient of variations were <10%. GH levels in the retinal homogenates were standardized using total cellular protein levels.

Assay of IGF-I

IGF-I levels in serum, undiluted vitreous fluid, and retinal homogenates were determined by using commercially available, nonextraction, rat IGF-I enzyme immunoassay (EIA) kits (Diagnostic Systems Laboratories, Webster, TX). This kit uses a highly sensitive antibody method, which allows detection of extremely low levels of immunoreactive free IGF-I. The assay measures the true free IGF-I fraction plus the fraction readily dissociated from insulin-like growth factor binding proteins (IGFBPs), which together form the biologically active pool. Samples, standards, and controls were incubated with a free IGF-I antibody in microtitration wells. After incubation and washing, the wells were treated with an anti-free IGF-I detection antibody labeled with horseradish peroxidase. After the plate was washed, it was developed and the absorbance measured at 450 nm. The absorbance measured is directly proportional to the free IGF-I levels. The sensitivity of the assay was 0.015 ng/mL and intra- and interassay coefficients of variation were <10%. IGF-I levels in the retinal homogenates were standardized using total cellular protein levels.

Figure 1. Comparative effects of early postnatal ibuprofen and indomethacin on vitreous fluid (A) and retinal (B) VEGF protein levels in neonatal rats at P14 (the time of retinal maturation). VEGF protein in the retinal homogenates was standardized by total cellular protein levels. The litter size per group was 30 pups. Blood samples were pooled for a total of 15 samples. Undiluted vitreous fluid samples were pooled for a total of six samples per group. Retinas were pooled for a total of 30 samples per group. The saline group received intraperitoneal injections of saline on days P1, P2, and P3, low dose ibuprofen received 10.0 mg/kg on P1 followed by 5.0 mg/kg on P2 and P3 (IB-10), high dose ibuprofen received 50.0 mg/kg on P1 followed by 25.0 mg/kg on P2 and P3 (IB-50), low-dose indomethacin received 0.2 mg/kg on P1, followed by 0.1 mg/kg on P2 and P3 (IN-0.2), and high dose indomethacin received 1.0 mg/kg on P1 followed by 0.5 mg/kg on P2 and P3 (IN-1.0). Data are presented as the mean ± SEM.

Figure 2. Effects of ibuprofen and indomethacin on mRNA expression of VEGF188 (A) and VEGF164 (B) splice variants. RT-PCR analysis was performed on whole retinas pooled for a total of five samples per group. The groups and litter size are as described in Figure 1. Data are presented as the mean ratio of VEGF188 or VEGF164/GAPDH ± SEM.
RESULTS

Eye Opening and Somatic Growth

Pooling and randomization resulted in equal distribution of runts among the groups. No more than 15 pups were assigned to each dam, which accepted all the pups as her own, despite pooling from other litters. There were no differences in total body weight (in grams) or linear growth before treatment at P1. To determine the comparative effects of ibuprofen and indomethacin on the cecal period in rats, we documented the number of rat pups with at least one eye open at P14 (the time of retinal maturation). As shown in Table 1, only 13% (P < 0.001) of pups randomized to receive the high dose of ibuprofen had at least one eye open compared with 93% in the group treated with the high dose of indomethacin. Animals randomized to receive high-dose ibuprofen had decreased total body weight and linear growth. In contrast, high-dose indomethacin resulted in significantly higher total body weights and linear growth concurrent with lower serum VEGF serum levels.

Ocular and Systemic VEGF Levels

To determine the effects of ibuprofen and indomethacin on VEGF levels in the vitreous fluid and retinal homogenates, normal vitreous fluid and retinal VEGF levels as determined by VEGFR-1, VEGFR-2, and PEDF mRNA. (ANOVA) was used to determine differences among the groups for normally distributed data, and the Kruskal-Wallis test was used for non-normally distributed data after Bartlett’s test for equality of variances. Post hoc analysis was performed with the Tukey and Student-Newman-Keuls tests for significance. Significance was set at P < 0.05, and data are reported as the mean ± SEM, where applicable. All analyses were two-tailed and performed on computer (SPSS; SPSS, Inc. Chicago, IL, and Prism; GraphPad Software Inc., San Diego CA).

Retinal VEGF Signaling

To determine the effects of ibuprofen and indomethacin on rat retinal VEGF signaling, we examined the mRNA expression of three VEGF splice variants (Figs. 2, 3) as well as the mRNA expression of VEGF receptors-1 and 2 (Fig. 4) and Npn-1 and -2 (data not shown). Both ibuprofen and indomethacin had no significant effect on retinal VEGF188 mRNA expression, although there was a nonsignificant trend for lower expression with the high dose of indomethacin (Fig. 2A). In contrast, retinal VEGF164 mRNA expression was suppressed with the high dose of ibuprofen (P < 0.05) and both doses of indomethacin (P < 0.01) compared with the control (Fig. 2B). Retinal VEGF120 mRNA expression was suppressed with both ibuprofen doses, although significance was achieved only with the high dose (P < 0.01; Fig. 3A). Retinal PEDF mRNA expression was unchanged with ibuprofen treatment; however, both doses of indomethacin suppressed PEDF mRNA expression (P < 0.01) compared with the control (Fig. 3B). Retinal VEGFR1 (Flk-1) mRNA expression did not significantly change in response to ibuprofen or indomethacin treatment, despite a nonsignificant trend for higher expression with ibuprofen (Fig. 4A). High-dose ibuprofen suppressed VEGFR2 (Flk-1) mRNA expression (P < 0.01) compared with the control (Fig. 4B). Retinal Npn-1 and -2 remained comparable among the groups (data not shown). Figure 5 is a representative gel from a single sample of RT-PCR amplification of rat retinal GAPDH, VEGF isoforms, VEGFR-1, VEGFR-2, and PEDF mRNA.
Ocular and Systemic IGF-I Levels

In the vitreous fluid, IGF-I levels (ng/mL) were higher in the group that received high-dose ibuprofen (121.9 ± 10.00, P < 0.05) compared with saline (80.4 ± 4.0). In contrast, high-dose indomethacin suppressed vitreous fluid IGF-I levels (21.8 ± 14.1, P < 0.05) compared with saline (Fig. 6A). Retinal IGF-I levels remained relatively unchanged among the groups (Fig. 6B). A similar response to ibuprofen treatment was noted in the serum (Table 2). Both low (P < 0.05) and high (P < 0.01) doses of ibuprofen increased serum IGF-I levels compared with saline and indomethacin.

Ocular and Systemic GH Levels

Vitreous fluid GH levels (ng/mL) were suppressed only with the high dose of ibuprofen (2.0 ± 0.39, P < 0.05) compared with the control (3.53 ± 0.37; Fig. 7A). No significant changes in retinal GH levels were detected among the groups (Fig. 7B). Similarly, ibuprofen and indomethacin had no appreciable effect on GH levels in the serum (Table 2).

DISCUSSION

To our knowledge, the present study is the first to examine and compare the effects of early postnatal administration of ibuprofen and indomethacin on VEGF, IGF-I, and GH in systemic and ocular compartments during normal rat retinal development. The dosage and timing of ibuprofen and indomethacin were based on previous reports that demonstrated efficacy of the drugs for closure of the ductus in preterm infants and on pharmacokinetic data. Based on reported data, we examined whether the beneficial effects of ibuprofen and indomethacin on retinal neovascularization are in part due to its effects on VEGF, IGF-I, and GH. Our data showed that VEGF levels tended to be higher in the vitreous fluid and lower in the retina with ibuprofen treatment, whereas an opposite response was noted with indomethacin treatment. Examination of retinal VEGF signaling showed that high-dose ibuprofen suppressed VEGF164, VEGF120, and VEGFR-2 transcripts, resulting in a significant increase in the cecal period in 87% of rats at P14. Indomethacin treatment suppressed retinal VEGF164 and PEDF but had no effect on VEGF receptor transcripts. Of note, 93% rats treated with high-dose indomethacin had at least one eye open at P14 compared with only 13% with high-dose ibuprofen. These findings suggest that ibuprofen may be more effective than indomethacin for the suppression of VEGF signaling if administered during the vasoproliferative phase of ROP.

The rat model is valid for the study of retinal vascular development because, as in premature newborn infants, the rat retina is immature at birth, and the maturation process continues until the time of eye opening. During rat retinal development, undifferentiated retinal cells are evident by 14 days of gestation. By postnatal day 15, the vessels are fully developed. The ontogeny of the rat retina is similar to that of humans, and when newborn rats are maintained in room air after hypoxia, their retinas show characteristics of human plus disease; abnormal vessel development, vascular leaks, vitreal hemorrhage, and vascular tufts. There is a common timetable for the development of the visual system related to the time of eye-opening in mammals.

The cecal period (the

![Figure 5](https://example.com/figure5.png)

**Figure 5.** RT-PCR analysis was performed on whole retinas pooled for a total of five samples per group. A single representative gel is shown for each group. The groups and litter size are as described in Figure 1. Lane 1: saline; lane 2: low-dose ibuprofen; lane 3: high-dose ibuprofen; lane 4: low-dose indomethacin; lane 5: high-dose indomethacin. Data were standardized using GAPDH.

![Figure 6](https://example.com/figure6.png)

**Figure 6.** Comparative effects of early postnatal ibuprofen and indomethacin on vitreous fluid (A) and retinal (B) IGF-I protein levels in neonatal rats at P14 (the time of retinal maturation). IGF-I protein in the retinal homogenates was standardized by total cellular protein levels. The groups and litter size are as described in Figure 1. Undiluted vitreous fluid samples were pooled for a total of six samples per group. Retinas were pooled for a total of five samples per group. Data are presented as the mean ± SEM.
time of conception to eye opening) corresponds to retinal maturity. The novel finding that high-dose indomethacin shortened the cecal period in 93% of animals compared with only 13% with high-dose ibuprofen, suggests increased retinal vessel growth and may explain at least in part, why premature newborn infants treated with indomethacin are 1.5 times more prone to development of ROP. Of note is the higher number of female than male rat pups with closed eyes at P14, suggesting that male rats are more likely to have a shorter cecal period resulting from exposure to NSAIDs. The significance of this finding remains to be determined.

VEGF is an angiogenic and permeability-enhancing factor that is linked to retinal ischemia-associated neovascularization. In the human, increased vitreous VEGF correlated with retinal ischemia-related neovascularization in diabetic retinopathy, retinal vein occlusion, and ROP. VEGF<sub>164</sub> (VEGF<sub>165</sub> in rats) appears to be the dominant isoform induced by IGF-I and is a major retinal isoform. This isoform is bioactive, freely soluble, and a major mediator of angiogenesis. All the murine VEGF isoforms were present in our rat retinas with equivalent abundance. Whereas VEGF<sub>164</sub> remained unaffected by ibuprofen and indomethacin, VEGF<sub>120</sub> was downregulated with the high dose of ibuprofen and both doses of indomethacin. However, only ibuprofen suppressed VEGF<sub>120</sub> mRNA expression. Because VEGF<sub>120</sub> is the dominant isoform induced by IGF-I, this finding suggests that the high dose of ibuprofen suppresses VEGF<sub>120</sub> through decreased uptake of retinal IGF-I and provides further evidence that ibuprofen is more effective than indomethacin for suppression of retinal VEGF. The cellular effects of VEGF are mediated by VEGFR-1 and -2, which are expressed exclusively by endothelial cells. Knockout studies in mice have shown that the VEGFR-1 receptor mediates organization of the vasculature, whereas VEGFR-2 mediates endothelial cell differentiation and proliferation. Our data showed that ibuprofen suppressed VEGFR-2, suggesting decreased VEGF signaling and retinal vascular growth. It should be noted that this inhibition was observed only at higher concentrations, which are needed to inhibit prostaglandin synthesis. Despite the ability of both ibuprofen and indomethacin to inhibit COX, the differences between the two drugs were remarkable. These differences may be due to their selectivity for COX-1 and COX-2. Using murine COX-null cell systems, Kirtikara et al. demonstrated that ibuprofen was more selective for COX-2 than indomethacin based on COX-2 IC<sub>50</sub>/COX-1 IC<sub>50</sub> ratio ranking. A similar finding was reported by Uzan. The interaction between COX-2 and VEGF is well-documented. Treatment with VEGF increased COX-2 but not COX-1 protein in a dose-dependent manner in endothelial cells. Therefore, in our study, it seems plausible to speculate that ibuprofen at high doses may exert its effect on VEGF through COX-2. However, further studies are needed to confirm or refute this speculation.

Considering the role of IGF-I and GH as important regulators of linear and somatic growth in mammals, we measured serum levels in our animals as well as their somatic and linear growth. A decline in body weight and linear growth started to emerge by 4 days after the last treatment (P<sup>7</sup>) in the group that received 50 mg/kg ibuprofen, an effect that persisted until P14, the time of retinal vascular maturation in rats. We were surprised to find that serum IGF-I levels in the same group were significantly elevated. At first, the elevated serum IGF-I levels was difficult to reconcile with decreased somatic and linear growth because it is well documented that IGF-1 is the major stimulus for pre- and postnatal growth, and its deficiency has been shown to cause marked fetal growth retardation. One possible hypothesis is that the high dose of ibuprofen may act by cleaving IGF-I from its binding proteins thereby decreasing its bioactivity. Further studies are needed to confirm this hypothesis. It was interesting to note that despite reductions in serum VEGF levels with high-dose indomethacin, there was adverse impact on body weight and linear growth. Previous studies have shown that indomethacin treatment stimulates GH levels in humans. Although there was a trend for higher GH levels in that group, it was not sufficient to achieve statistical significance. Therefore, the increased weight accretion is not attributable to GH, but may be due to increased uptake of VEGF. IGF-I has been shown to act synergistically with VEGF to increase angiogenesis. IGF-I may promote retinal neovascularization through its effects on retinal VEGF gene expression. There are abundant data to support a pathologic link between IGF-I and neovascularization. Data from our laboratory as well as others have shown that the vitreous fluid may act as a reservoir for retinal growth factors. Our data showed that VEGF levels in the vitreous were several times higher than IGF-I and GH, with GH being close to subnanomolar levels. Lower VEGF and IGF-I levels in the vitreous with indomethacin suggest increased uptake by the retina and may explain the shorter cecal period with indomethacin treatment.

In summary, we have demonstrated for the first time that the effects of ibuprofen and indomethacin on systemic and ocular growth factors are different, and therefore we reject the null hypothesis. High doses of ibuprofen delay the cecal period in rats, suggesting suppression of retinal vascular growth. This finding is corroborated by high IGF-I levels in the vitreous fluid, suggesting decreased uptake by the retina. In contrast, indomethacin increased somatic and linear growth, possibly by increased uptake of VEGF. High-dose ibuprofen suppressed retinal VEGF<sub>164</sub>, VEGF<sub>120</sub>, and VEGFR-2, suggesting decreased VEGF signaling, whereas, low-dose ibuprofen had no appreciable effects. These data imply that ibuprofen may be beneficial if administered during the vasoproliferative phase of ROP. However, the adverse effects of high doses on somatic and linear growth should be considered.
Acknowledgments

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References