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# Oxygen-Induced Retinopathy: Lack of Adverse Heparin Effect

ROSEMARY D. HIGGINS AND DALE L. PHELPS

*University of Rochester Medical Center, Departments of Pediatrics and Ophthalmology, Rochester, New York 14642*

**ABSTRACT. Retinopathy of prematurity is a disorder of abnormal retinal vascular proliferation, and one hypothesis for its pathogenesis involves abnormal activity of angiogenic growth factors in the retina. One of these, acidic fibroblast growth factor, is found primarily in retina and brain tissues. Its mitogenic effect is greatly potentiated *in vitro* by heparin. Because retinopathy of prematurity occurs most often in premature infants who receive the greatest amount of heparin, we tested the hypothesis that heparin may adversely affect the retinopathy observed in kittens after hyperoxic (80% oxygen) exposure. Seventeen litters of kittens were randomly assigned to receive either saline or heparin s.c. injections from d 2 through recovery to 28 d of age; 65 h of high oxygen exposure was started on d 3 to induce a standard retinal injury in our model. There were no differences in the degree of retinopathy between the heparin-treated group [severity score 5.9 ± 2.2 (mean ± SD)], and the saline-treated group (severity score 7.1 ± 1.7, $p > 0.20$, 80% power to detect a 2-point difference in score at $\alpha = 0.05$). These findings do not support a concern that clinical doses of heparin potentiate retinopathy of prematurity. (*Pediatr Res* 27: 580–582, 1990)**

### Abbreviations

**OIR, oxygen-induced retinopathy**
**ROP, retinopathy of prematurity**
**FGF, fibroblast growth factor**
**aFGF, acidic fibroblast growth factor**
**bFGF, basic fibroblast growth factor**

ROP is a vasoproliferative disorder of immature retinal vessels initially described in 1942 (1) and subsequently found to be increased in frequency by prolonged routine supplemental oxygen (2–6). Animal models of a similar retinopathy have been described (7, 8). Despite the early restriction of oxygen use (6) and subsequent strict monitoring of blood oxygen (9, 10), the problem of ROP persists, albeit at a lower frequency, and the complete pathophysiology remains unclear.

Gestational age correlates with the degree of retinal vessel maturity and is the major risk factor for the development of ROP (10–12). Case-matched studies controlled for gestational age show that the sickest infants are at greatest risk for ROP (11, 12). These infants have multiple procedures including i.v. cannulations in greater numbers for longer periods of time. This is associated with the long-term need for i.v. access and heparin to preserve these lines (13). Therefore, the sickest infants are exposed to more heparin for longer periods of time, and these are the infants at highest risk for ROP.

ROP results when aberrant retinal vessel regrowth occurs after an initial injury. It has been thought to be driven by ischemic avascular tissue releasing angiogenic growth factors as has recently been demonstrated in the kitten model of OIR (14). Presumably, the angiogenic growth factors, initially called retinal-derived growth factors and later found to be largely aFGF and bFGF (15), are contributing to the development of ROP. Observations in infants and experimental animals suggest that retinal ischemia is positively correlated with angiogenesis in OIR (16) and other retinopathies as well (17). The retinopathies develop in unregulated pathologic manners, possibly because of excessive signals for capillary growth.

Capillary growth and development has been demonstrated to be influenced by several growth factors (18, 19). Acidic FGF, found mainly in neural tissue, is relatively abundant in the retina (15), and is mitogenic for a variety of cell types including endothelial cells (20). Significantly, many of the effects of aFGF *in vitro* are potentiated by heparin or heparin-like molecules (21–23). Acidic FGF activity is potentiated by heparin *in vitro* (24, 25); the importance of size, sulfation, and anticoagulant activity has been described (26). Furthermore, heparin copper-complex has been shown to be angiogenic *in vivo* (27). Basic FGF is also angiogenic, and has been shown to positively influence capillary growth *in vitro* but has not been shown to be potentiated by heparin. Other unknown factors may also contribute to abnormal vessel growth in ROP.

A logical concern is that widespread heparin use for maintaining central lines and for use in hyperalimentation might exacerbate ROP by potentiating aFGF *in vivo*. We therefore undertook a study to test the hypothesis that heparin may adversely affect OIR in the kitten model of OIR.

## MATERIALS AND METHODS

Seventeen pathogen-free pregnant queens were obtained from a commercial vendor (Liberty Laboratories, Liberty Corner, NJ). The day that kittens were first observed was defined as d 1. Within each litter, the kittens were randomly assigned to receive heparin (150 U/kg/d) or saline (equal volume) s.c. divided every 12 h beginning on d 2 (both solutions with benzyl alcohol as a preservative). On d 3, the queen and her kittens were placed in an infant incubator (Air Shields Isolette, Hatboro, PA) at 26°C with oxygen concentration adjusted to 80 ± 1% for a total 65 h to produce a standard OIR (16, 28–30). The study design is depicted in Figure 1. Oxygen concentrations were measured at least twice daily with a Beckman oxygen analyzer (Beckman Instruments, Inc., Fullerton, CA) and were consistently stable. Kittens subsequently recovered in room air, continuing to receive either heparin ($n = 28$) or saline ($n = 32$) injections until 28 ± 2 d of age (Fig. 1). The dose of heparin (Heparin lock flush solution, USP, 10 U/mL, Elkins-Sinn, Inc., Cherry Hill, NJ) was based

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Correspondence: Rosemary D. Higgins, M.D., University of Rochester School of Medicine and Dentistry, Box 651, Departments of Pediatrics (Neonatology) and Ophthalmology Rochester, NY 14642.
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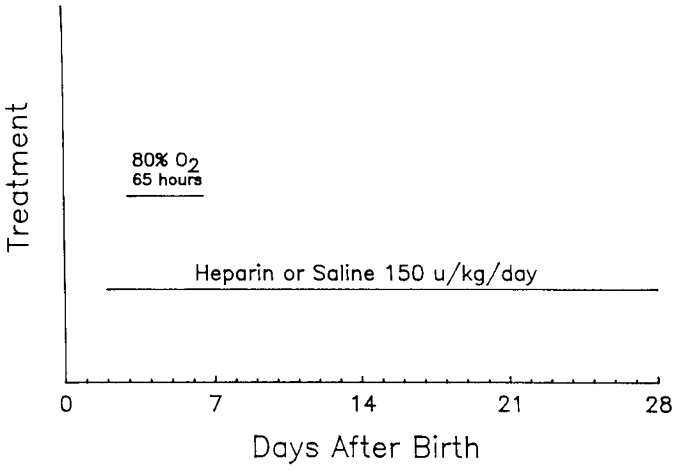


Fig. 1. Experimental design. Time in days on the x-axis and various therapies (oxygen exposure, heparin, saline) on the y-axis of the experimental design.

| SCORE CATEGORY | 0 | 1 | 2 | 3 | 4 |
|---|---|---|---|---|---|
| VESSEL GROWTH A | EQUAL TO CONTROL | MILDLY RETARDED ESPECIALLY TEMPORALLY | RETARDED GROWTH ALL AROUND | SMALL CIRCLE OF VESSELS ONLY | |
| MAJOR VESSEL PATTERN B | 3 PAIRS OF VESSELS, DELICATE PATTERN | 4 TO 7 PAIRS OF DELICATE POSTERIOR VESSELS WITHOUT PERIPHERAL "BRUSH BORDER" | PILED ON VESSELS PERIPHERALLY (BRUSH BORDER) | UNIFORM PILED ON VESSELS; BASIC PATTERN STILL DISCERNABLE | HEAVILY PILED ON VESSELS; NO BASIC 3 PAIRS VISIBLE |
| PERI-ARTERIOLAR CAPILLARY FREE ZONE C | ZONE APPARENT AROUND EACH ARTERIOLE TO THE PERIPHERY | PATCHY LOSS OF CLEAR ZONE | UNIFORM LOSS OF CLEAR ZONE | | |
| CAPILLARY TUFT FORMATION D | NONE | 1 OR 2 LARGE OR SMALL AT THE PERIPHERY | MORE THAN 3 SCATTERED NEAR THE PERIPHERY | SMALL OR LARGE EXTENDING TOWARD THE DISK | EXTENSIVE TUFT FORMATION WITH A MASK OF VESSELS OVER THE DISK |

Fig. 2. Retinal scoring system. Total score for retina is equal to sum of scores in each of four categories. Reprinted with permission from *Pediatrics*, Vol. 73, p. 3, © 1984 (ref. 16).

Table 1. *Experimental retinopathy (mean ± SEM)*

| | Scores (heparin treatment *vs* saline treatment) | | | | |
|---|---|---|---|---|---|
| | Vessel growth | Vessel pattern | Capillary free zone | Vitreal vessels | Total |
| Heparin | 1.1 ± 0.4 | 1.8 ± 0.7 | 1.0 ± 0.3 | 2.1 ± 1.1 | 5.9 ± 2.2 |
| Saline | 1.2 ± 0.4 | 2.1 ± 0.5 | 1.1 ± 0.3 | 2.6 ± 0.7 | 7.1 ± 1.7 |
| *p* | NS | NS | NS | NS | NS |

on a clinical survey of actual heparin use in sick infants under 1 kg birth wt. On average, these infants received 0.5 U of heparin per mL of hyperalimentation fluids in addition to fluids in other lines and periodic flushes. At an average of 150 mL/kg/d, they received approximately 75 U/kg/d. The dose was doubled in the kitten model, but not increased further because of concerns for hemorrhage.

At 28 d, kittens were killed, the retinal vasculature perfused with warmed Higgins india ink (Faber-Castell, Lewisburg, TN) diluted 50% in saline via aortic cannulation, and retinal flat mounts prepared (28). The retinas were coded and scored independently in a masked fashion by three individuals. Four components of the retinopathy were scored separately yielding a total score of 13 for the worst cases and 0–1 for normal retinal vascularization (16). The score categories (Fig. 2) included *A*), vessel growth or extent of vascularization in the retina (score 0–3); *B*), the major vessel pattern including number of vessels and patterns of vessel growth (score 0–4); *C*), the periarteriolar capillary free zone (score 0–2); and *D*), the amount of extraretinal neovascularization, specifically vitreal vessels (score 0–4). A binocular dissecting microscope (Bausch & Lomb, Inc., Rochester, NY) at 0.7–3X was used to score the retinas. The final score for each kitten was taken as the arithmetic mean of the scores from the left and right eyes across all three sets of scores. There were two final scores for each litter, the average of the saline-treated kitten scores and the average of the heparin-treated kitten scores.

Table 2. *Mean total retinopathy scores for heparin- and saline-treated kittens in each litter*

| Litter | Heparin | Saline | Sign |
|---|---|---|---|
| 1 | 8.75 | 3.40 | + |
| 2 | 4.51 | 7.87 | − |
| 3 | | 11 | |
| 4 | 6.13 | 7.17 | − |
| 5 | 2.58 | 6.75 | − |
| 6 | 4.13 | 7.87 | − |
| 7 | 8.25 | 7.18 | + |
| 8 | 3.33 | 5.35 | − |
| 9 | 4.06 | 7.75 | − |
| 10 | 7.33 | 6.25 | + |
| 11 | 10.20 | 6.67 | + |
| 12 | 7.79 | | |
| 13 | 6.91 | 7.61 | − |
| 14 | 6.38 | 5.63 | + |
| 15 | 6.38 | 6 | + |
| 16 | 3.69 | 7.94 | − |
| 17 | 4.38 | 9.06 | − |
| Mean ± SD | 5.9 ± 2.2 | 7.1 ± 1.7 | 6/15 |
| | $p > 0.20$ *t* test | | $p > 0.25$ sign test |

The sample size was based on previous experience with this model where the total retinopathy score after a room-air recovery to 4 wk after 65 h of 80% oxygen at d 3 has been 5.3 ± 2.5 (mean ± SD). Seventeen litters permit the detection of at least a 2-point change in the total score, within litters, at an $\alpha$-error of 0.05 with a power of 80%. This protocol was reviewed and approved by the University of Rochester, Committee on Animal Research.

## RESULTS

All 17 litters completed the study and appeared healthy. Each group (saline and heparin) grew equally well (no statistically significant differences in their wt at birth and weekly intervals to 4 wk), and no hemorrhagic complications arose.

There were no statistically significant differences in either the total retinopathy score or any of the four subcategory scores when the heparin group was compared with the saline group by *t* test on the aggregate scores, or the paired *t* test on the differences between control and heparin groups within litters. Table 1 shows the retinopathy scores for each of the four subcategories and the total scores. Table 2 shows the mean total retinopathy score values by litter and analysis using the sign test on the direction of differences within each litter, which also confirms the paired *t* test analysis ($p > 0.25$).

## DISCUSSION

The degree of retinopathy in kittens treated with saline (control group) was consistent with observations in other studies (16, 28–30). Heparin did not affect the OIR in the kitten in the selected dose, 150 U/kg/d given s.c.

Previous investigators have indicated that FGF are involved in eye development and vision. They have been shown to bind basement membranes of the eye, specifically the inner limiting membrane of the retina (31). Basement membranes play an important role in eye morphogenesis (32), and it has been speculated that their developmental modification could be involved in regulating their affinity for FGF (31). Further, *in vitro* experiments have shown that the basement membrane composition can be modified by treatment with FGF (33, 34). It has therefore been postulated that the FGF and extracellular matrix of the eye are involved jointly in proliferation of endothelial cells and differentiation in normal and pathologic states in the eye (33).

Several factors could be responsible for the lack of observed effect of heparin on OIR. Although *in vitro* experiments have shown that heparin potentiates the activity of aFGF in cultured cells (22–25) and in human endothelial cell proliferation (35), perhaps the *in vivo* model of OIR involves an aFGF effect that is already maximal and cannot be further potentiated. Alternatively, OIR might be driven by bFGF or other growth factors rather than aFGF. A third possibility may be that endogenous cell surface heparin is already sufficient to maximize the effect of aFGF. Conversely, the dose of heparin used may not have been high enough, although this dose was twice that in common clinical use. Finally, the s.c. route may not have presented the heparin to the tissues affected in a sufficiently high dose to have an effect. Premature infants are given heparin without established studies of its safety, but based on extrapolation downward between age and size from older children. Because the smallest survivors are so immature at birth now (36), we must heighten our awareness of the physiologic differences in the still-differentiating premature infant and his older counterpart. Despite this caution, these data do not support a basis for changing the current use of heparin in neonatal intensive care units.

In summary, we found no difference in the degree of retinopathy in the OIR kitten model when kittens were treated with heparin *versus* saline (control). This finding suggests that heparin administration to premature infants is not a significant factor in the incidence or severity of ROP.

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