Postnatal Serum Insulin-Like Growth Factor I Deficiency Is Associated With Retinopathy of Prematurity and Other Complications of Premature Birth

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ABSTRACT. Objective. Insulin-like growth factor I (IGF-I) is necessary for normal development of retinal blood vessels in mice and humans. Because retinopathy of prematurity (ROP) is initiated by abnormal postnatal retinal development, we hypothesized that prolonged low IGF-I in premature infants might be a risk factor for ROP.

Design. We conducted a prospective, longitudinal study measuring serum IGF-I concentrations weekly in 84 premature infants from birth (postmenstrual ages: 24–32 weeks) until discharge from the hospital. Infants were evaluated for ROP and other morbidity of prematurity: bronchopulmonary dysplasia (BPD), intraventricular hemorrhage (IVH), and necrotizing enterocolitis (NEC).

Results. Low serum IGF-I values correlated with later development of ROP. The mean IGF-I ± SEM level during postmenstrual ages 30–33 weeks was lowest with severe ROP (25 ± 2.41 μg/L), 29 ± 1.76 μg/L with moderate ROP, and 33 ± 1.72 μg/L with no ROP. The duration of low IGF-I also correlated strongly with the severity of ROP. The interval from birth until serum IGF-I levels reached >33 μg/L was 23 ± 2.6 days for no ROP, 44 ± 4.8 days for moderate ROP, and 52 ± 7.5 days for severe ROP. Each adjusted stepwise increase of 5 μg/L in mean IGF-I during postmenstrual ages 30 to 33 weeks decreased the risk of proliferative ROP by 45%. Other complications (NEC, BPD, IVH) were correlated with ROP and low IGF-I levels. The relative risk for any morbidity (ROP, BPD, IVH, or NEC) was increased 2.2-fold (95% confidence interval: 1.41–3.43) if IGF-I was ≤33 μg/L at 33 weeks’ postmenstrual age.

Conclusions. These results indicate that persistent low serum concentrations of IGF-I after premature birth are associated with later development of ROP and other complications of prematurity. IGF-I is at least as strong a determinant of risk for ROP as postmenstrual age at birth and birth weight. Pediatrics 2003;112:1016–1020; preterm birth, retinopathy of prematurity, insulin-like growth factor I, intraventricular hemorrhage, bronchopulmonary dysplasia, necrotizing enterocolitis, vascular endothelial growth factor.

Retinopathy of prematurity is a blinding disease, initiated by lack of retinal vascular growth after preterm birth. We have established that lack of insulin-like growth factor I (IGF-I) in mice prevents normal retinal vascular growth despite the presence of vascular endothelial growth factor (VEGF), a hypoxia-stimulated growth factor critical to vessel development because a minimum level of IGF-I is required for VEGF signaling.1,2 Our previous studies suggested that early lack of IGF-I in the first phase of retinopathy of prematurity (ROP) followed by a slow increase in IGF-I to a level critical to permit neovascularization could precipitate the disease.1,2 The greater the duration of low IGF-I, the less the vessel growth, the greater the retinal hypoxia and elevation of hypoxia-induced vasoproliferative factors such as VEGF,1 and the more severe the late stage of ROP.

Preterm birth is associated with a rapid fall in serum IGF-I levels as maternal sources of IGF-I are lost. This is particularly true at postmenstrual ages corresponding to the third trimester,3 because IGF-I levels in the fetus rise rapidly during the third trimester of pregnancy, in conjunction with the development of fetal tissue.4 In premature infants, IGF-I may be reduced further by conditions such as poor nutrition, acidosis, hypothyroxinemia, and sepsis. IGF-I levels rise slowly after preterm birth. These data suggested that low IGF-I levels perinatally might predict the subsequent final stage of ROP.
METHODS

We hypothesized that preterm infants with serum IGF-I concentrations below \( \sim 33 \mu g/L \) for a prolonged period are at increased risk for later development of ROP. We therefore undertook a prospective, longitudinal study examining the relationship between serum IGF-I and ophthalmologic findings in premature infants with a postmenstrual age of \(<32\) weeks at birth. The Ethics Committees of the Medical Faculties at Göteborg and Uppsala Universities approved the study, and the parents of the infants gave informed consent.

Study Participants

Infants born at a postmenstrual age of \(<32\) weeks at the Queen Silvia Children’s Hospital in Göteborg between December 1999 and April 2002 and at Uppsala Akademiska Hospital in Uppsala between February 2001 and April 2002 were recruited for the study. Exclusion criteria were inability to complete postnatal clinical follow-up until an age corresponding to 40 postmenstrual weeks and any conspicuous congenital anomaly.

At the Queen Silvia Children’s Hospital, 71 infants were identified as potential participants in the study and survived until a postmenstrual age of 36 weeks. The parents of all 71 infants gave permission for participation. Later, the parents of 1 infant withdrew permission, leaving 70. At Uppsala University Hospital, 15 infants were identified and recruited as potential participants. During data collection 1 infant was moved to another hospital, leaving 14 study subjects. The 84 infants included 11 twin pairs. The median postmenstrual age at birth (based on fetal ultrasonography) performed at 16–18 postmenstrual weeks) was 27.2 weeks (range: 23.0–31.8 weeks).

Twenty-seven of the infants were included in an earlier basic science study of mechanism of ROP to highlight the translation of the basic work into the clinic. Based on the same hypothesis, a larger purely clinical study with a new analysis was performed. When the 27 infants were excluded from the present analyses, the statistical significance remained, as it did when twin pairs were removed.

All infants were hospitalized in a neonatal intensive care unit and nourished according to the routines for premature infants at the neonatal units. Enteral feeding with increasing amounts of breast milk was introduced early (2–48 hours after birth). If full enteral feeding was not achieved, supplementary parenteral nutrition with glucose, amino acids, and fat was given. The breast milk given to infants with a birth weight below 1500 g was fortified with 0.8 g of protein/100 mL (gradually introduced over 1 week) from 10 days of age until the infant weighed 2000 g.

Study Plan

IGF-I Analysis

Venous blood samples (0.5 mL) were taken weekly, and the serum was stored at \(-20^\circ C\) to \(-80^\circ C\) until assayed. All samples from each infant were analyzed in the same assay. Serum was diluted 1:50, and IGF-I was measured in duplicate by an IGF binding protein-blocked radioimmunoassay, without extraction and in the presence of \(\sim 250\)-fold excess of IGF-II (Mediagnost GmbH, Tübingen, Germany). The intra-assay coefficient of variation at 10.2 and 34.5 \(\mu g/L\) was 15.7% and 9.6%, respectively. The interassay coefficient of variation at 10.2 and 34.5 \(\mu g/L\) was 23.9% and 12.1%, respectively.

Morbidity Evaluation: ROP Evaluation

ROP was classified according to the International Classification\(^a\) and subdivided into stage 1 (demarcation line), stage 2 (ridge), stage 3 (ridge with extraretinal fibrovascular proliferations), stage 4 (subtotal retinal detachment), and stage 5 (total retinal detachment). In all gestational weeks each child was classified according to the most advanced ROP stage observed. Proliferative retinopathy was defined as stage 3, and moderate ROP as stage 1 and stage 2. The infants were examined according to a routine protocol, which consisted of dilated eye fundus examinations once or twice a week, depending on the severity of the disease, from the chronological age of 5 to 6 weeks until the eyes were fully vascularized or until the condition was considered stable. After pupillary dilatation with 1% cyclopentolate, the eyes were examined by indirect ophthalmoscopy by a trained pediatric ophthalmologist, who had no knowledge of IGF-I status. Care was taken to minimize pain and stress during the examinations. The eyelids were gently held apart with cotton-tipped applicators or by a lid speculum, and the child’s head was tilted to visualize the periphery. The fundus was visualized in all 4 quadrants to the edge of the vascularized retina to fully assess the stage of ROP.

Other Morbidity Evaluation

The diagnosis bronchopulmonary dysplasia (BPD) was based on the typical appearance of BPD on serial chest radiographs and on the need for oxygen supplementation at postmenstrual week 36. The hospital record of each child was reviewed for information on the occurrence of intraventricular hemorrhage (IVH; grade 2–4), diagnosed by perinatal cerebral ultrasonography,\(^b\) and necrotizing enterocolitis (NEC) with gut perforation leading to surgery.

Statistical Analysis

The length of time between birth and the achievement of serum IGF-I \(>33 \mu g/L\) and the mean level of IGF-I during postmenstrual weeks 30 to 33 were analyzed with first Kruskal–Wallis and, subsequently, with the Wilcoxon–Mann–Whitney U test. A multiple logistic regression, performed in the statistical program SAS (SAS Institute Inc., Cary, NC), was used for analysis of no ROP as compared with proliferative ROP. The parameters in logistic regression are estimated by maximum likelihood, which are asymptotically normally distributed. This means that the estimates might have a small bias when the number of observations is small. The potential explanatory variables in the model were postmenstrual age at birth (gestational age [GA]), birth weight (BW), and the individual mean level of IGF-I during postmenstrual weeks 30 to 33. The model used was logit (proliferative ROP = ROP ? 3, non-ROP = 0) = \(\alpha + \beta_1 \times \text{mean IGF-I}\) weeks 30–33 (\(\mu g/L\)) + \(\beta_2 \times \text{GA (days)} + \beta_3 \times \text{BW (100 g)}\). This equation was the basis for estimates of probability in Fig 2. Individual longitudinal serum IGF-I levels were used in the evaluation of the IGF-I pattern. Postnatal morbidity was dichotomized as no morbidity (ROP stage 0, no BPD, no IVH, and no NEC) or postnatal morbidity (ROP, BPD, IVH, or NEC). P values of <.05 were considered significant, and all P values were 2-sided. Because there were 11 dizygote twin pairs in the study, the variability may be slightly greater than the ones given by the statistical program. However, to avoid losing precision and to be representative, they were included in the estimates.

RESULTS

Mean Perinatal Serum IGF-I Correlates With the Severity of Late-Stage ROP

The longitudinal pattern of mean serum IGF-I values among the infants with no ROP (stage 0), moderate ROP (stages 1 and 2), and proliferative ROP (stage 3) showed lower mean values of IGF-I with increasing severity of ROP at almost every time point. (Fig 1; Table 1). Those without ROP had an increase in mean serum IGF-I at postmenstrual ages 30 to 33 weeks (Fig 1) to levels within the 95% confidence interval of IGF-I concentrations found in utero. In contrast, infants with moderate ROP had mean values of IGF-I that were just in or outside the 95% confidence range of values found in utero, and mean IGF-I values of infants with proliferative ROP were never within the 95% confidence interval of IGF-I values found in utero (Fig 1). The mean \(\pm\) SEM level of IGF-I at 30–33 weeks postmenstrual age for infants with proliferative ROP was \(25 \pm 2.41 \mu g/L\) (range: 14–46 \(\mu g/L\)); for infants with moderate ROP, it was \(29 \pm 1.76 \mu g/L\) (range: 15–51 \(\mu g/L\)); and for infants without ROP, it was \(33 \pm 1.72 \mu g/L\) (range: 16–57 \(\mu g/L\)). The Kruskal–Wallis test yielded a P value of .023, indicating a significant difference between the three groups in “mean IGF-I.” Multiple comparisons for
means, with use of the sequentially rejective Bonferroni procedure, showed a significant difference between no ROP and proliferative ROP on an overall significance level of $\alpha = 0.05$.

We hypothesized that the duration of low IGF-I correlates with severity of ROP, because low IGF-I prevents normal retinal vascular development, thereby causing increasing hypoxia. These studies indicated a very strong association between the duration of low IGF-I levels and severity of ROP. The mean interval from birth to the time that IGF-I reached 33 $\mu$g/L was 23 ± 3 days (range, 1–47 days) in infants with no ROP, 44 ± 5 days for infants with moderate ROP (range, 0–123 days), and 52 ± 7 days for proliferative ROP (range, 1–101 days). The Kruskal–Wallis test yielded a $P$ value of .001, indicating a significant difference between the three groups. Multiple comparisons for means, with use of the sequentially rejective Bonferroni procedure, showed a significant difference between no ROP and moderate ROP as well as between no ROP and proliferative ROP on an overall significance level of $\alpha = 0.01$.

The baseline characteristics of the 84 infants with different ROP stages (0–3) indicate that the higher the ROP stage the lower the gestational age and birth weight, (Table 2). No infant developed ROP stages 4 to 5.

There was a strong association between the occurrence of ROP stages 1 and other significant morbidity (Table 2). Only 1 infant had morbidity (IVH) without ROP. Of 84 infants, 36 had no ROP (stage 0) and no other morbidity, and 8 had ROP stage 1 without other morbidity. Twenty-six infants had ROP stage 2 to 3 as well as some other morbidity (22 BPD, 4 NEC leading to surgery, and 6 IVH), whereas 13 children had ROP stage 2 to 3 without other morbidity.

### Table 1. Longitudinal Mean IGF-I With Respect to ROP Severity

<table>
<thead>
<tr>
<th>Postmenstrual Age (weeks)</th>
<th>Mean IGF-I ± SEM (µg/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No ROP</td>
</tr>
<tr>
<td>29</td>
<td>24 ± 2.4</td>
</tr>
<tr>
<td>30</td>
<td>29 ± 3.9</td>
</tr>
<tr>
<td>31</td>
<td>29 ± 1.9</td>
</tr>
<tr>
<td>32</td>
<td>37 ± 2.4</td>
</tr>
<tr>
<td>33</td>
<td>40 ± 2.6</td>
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<tr>
<td>34</td>
<td>39 ± 2.6</td>
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<tr>
<td>35</td>
<td>39 ± 2.6</td>
</tr>
<tr>
<td>36</td>
<td>40 ± 3.3</td>
</tr>
<tr>
<td>37</td>
<td>36 ± 4.2</td>
</tr>
<tr>
<td>38</td>
<td>42 ± 7.5</td>
</tr>
<tr>
<td>39</td>
<td>46 ± 8.9</td>
</tr>
<tr>
<td>40</td>
<td>54 ± 4.5</td>
</tr>
</tbody>
</table>

### Table 2. Baseline Characteristics of Infants With Available Outcome Data

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>No ROP ($n = 37$)</th>
<th>Nonproliferative ROP ($n = 34$)</th>
<th>Proliferative ROP ($n = 13$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PMA, weeks (mean ± SD)</td>
<td>28 ± 2</td>
<td>26.7 ± 2</td>
<td>25 ± 1</td>
</tr>
<tr>
<td>Birth weight, g (mean ± SD)</td>
<td>1207 ± 350</td>
<td>943 ± 278</td>
<td>738 ± 90</td>
</tr>
<tr>
<td>Male sex, n (%)</td>
<td>17 (46)</td>
<td>18 (53)</td>
<td>5 (38)</td>
</tr>
<tr>
<td>Singleton, n (%)</td>
<td>28 (77)</td>
<td>27 (79)</td>
<td>10 (77)</td>
</tr>
<tr>
<td>Other morbidity</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BPD, n (%)</td>
<td>0</td>
<td>14 (54)</td>
<td>8 (61)</td>
</tr>
<tr>
<td>NEC, n (%)</td>
<td>0</td>
<td>0</td>
<td>4 (31)</td>
</tr>
<tr>
<td>IVH, n (%)</td>
<td>1 (3)</td>
<td>5 (19)</td>
<td>1 (8)</td>
</tr>
</tbody>
</table>

PMA indicates postmenstrual age.
Because of rounding, percentages may not total 100.
morbidity (Table 2). At a postmenstrual age of 33 weeks, 15 (6 of whom had ROP stage 1 and no other morbidity) of the 48 children with ROP or other postnatal morbidity had IGF-I values >33 µg/L (31%), whereas 33 children had IGF-I values ≤33 µg/L (69%). Among the 36 infants with no postnatal morbidity, 27 infants had IGF-I values >33 µg/L (75%), whereas 9 infants had values <33 µg/L (26%). Thus, preterm infants with IGF-I ≤33 µg/L at 33 weeks' postmenstrual age had a relative risk of 2.2 (95% confidence interval: 1.41–3.43) to develop ROP or other postnatal morbidity. Among 10 of 11 dizygotic twin pairs in the study, the twin with more morbidity had the lowest IGF-I values (data not shown).

**Multiple Regression Analysis (Mean IGF-I Versus Postmenstrual Age at Birth and Birth Weight)**

The results of multiple logistic regression analysis, taking into account IGF-I and postmenstrual age (GA), was logit (proliferative ROP) = 29.91 – 0.12(mean IGF-I weeks 30–33/µg/L) – 0.14(GA/days), for n = 50 children. The R² value is 0.43, by the statistical program SAS, in the multiple logistic regression. The relative risk of proliferative ROP associated with a 5 µg/L increase of mean IGF-I during postmenstrual weeks 30–33 was −0.60, when adjusting for postmenstrual age and birth weight. Thus, an increase of 5 µg/L in mean IGF-I during postmenstrual weeks 30 to 33 decreased the risk of having proliferative ROP by 45% (Fig. 2). Taking into account only GA, the R² value is 0.34. Thus, the unexplained uncertainty of developing ROP is reduced by including both mean IGF-I (weeks 30–33) as well as GA in the model. However, the further inclusion of BW in the model did not increase R² (0.43) and was thus not significant as an added risk factor (P = .67).

**DISCUSSION**

The greatest known risk factor for development of ROP has been low birth weight⁹ (and gestational age). In this study we found that the mean serum IGF-I concentration at postmenstrual weeks 30 to 33 was at least as important a predictive factor for ROP as the degree of prematurity.

IGF-I is an important somatic growth factor that is associated with birth weight¹⁰,¹¹ and gestational age. Preterm infants have a significant reduction in IGF-I levels compared with infants that remain in utero due to loss of fetal sources of IGF-I, the placenta, and perhaps ingested amniotic fluid.¹³ Our results show that in preterm infants who develop ROP and other severe postnatal morbidities (BPD, IVH, and NEC), low serum levels of IGF-I persist after birth, never reaching comparable age-matched fetal in utero levels. However, in contrast, IGF-I levels in infants with no ROP and no other postnatal morbidity tend to rise more rapidly, reaching peak levels closer to those seen in utero, at an age corresponding to gestational weeks 30 to 33 (Fig 1). This is a critical developmental period, during which significant maturation of the blood vessels and eyes, as well as brain and other organs normally takes place.¹⁴ IGF-I is required for late prenatal as well as postnatal development.¹⁴ In particular, we have previously shown that IGF-I is critical for normal retinal vascular development in mice and in humans,¹⁶ and that cessation of normal vascular development initiates ROP. IGF-I−/− mice have retarded retinal vascular growth compared with normal controls. Minimal levels of IGF-I are required for VEGF, activation of pathways promoting retinal vascular endothelial cell proliferation and survival (MAPK and Akt).²,⁴ The level of IGF-I required for maximum VEGF activation of the Akt pathway corresponds to the level observed in those premature infants who had no or only minimal ROP. The critical role of the IGF-I system in retinal vascular development has been confirmed in a clinical study where patients with defects in the IGF-I or IGF-I receptor gene were found to have a reduced number of retinal vascular branching points.¹⁶ Therefore, lack of IGF-I after preterm birth in those infants unable to produce sufficient IGF-I may impede normal development of the retinal vasculature, causing retinal hypoxia and later triggering proliferative retinopathy as IGF-I levels rise to a critical point.¹ Thus, although the association between IGF-I and ROP could be simply an association between ROP and general illness in premature infants, there is much evidence that IGF-I is specifi-
cally required for vascular development and that low IGF-I contributes to the development of ROP.

Our results suggest that, in premature infants, early restoration of IGF-I to levels similar to those present in utero might help prevent ROP by promoting normal vascular development. This perhaps could be accomplished by assuring sufficient nutrient intake. Because IGF-I is a nutrition-dependent factor, insufficient intake of nutrients will result in a further decline of this peptide and adequate nutrition will increase IGF-I. Early breast milk feedings may be particularly beneficial as it has been shown that breast milk contains available IGF-I, and that supplementation with human milk increases circulating IGF-I more than supplementation with formula. However, in preterm infants, gastrointestinal development is incomplete at birth, and as a result, enteral nutrition may not be tolerated. Administering IGF-I enhances gastrointestinal development in fetal sheep. Thus, careful further IGF-I supplementation to in utero levels may be beneficial for development of the gastrointestinal tract as well as for the retinal vasculature. It is important to note, however, that delay in raising IGF-I until the time that the nonvascularized retina becomes hypoxic may promote the late neovascular, destructive phase of ROP. IGF-I administration (particularly if in excess of in utero levels) could also have other untoward effects on organ development.

Several clinical studies have shown that children born after intrauterine growth retardation have reduced serum IGF-I levels. In addition, other studies have shown that intrauterine growth retardation implies an increased risk for ROP development. It was recently demonstrated that exacerbation of ROP was found by inducing postnatal growth retardation by raising newborn rats in expanded litters. This finding would support the theory that sufficient nutrient intake is important to reduce the risk for ROP.

CONCLUSION

Low levels of serum IGF-I in preterm infants appear to predict an increased risk of ROP, as well as other severe perinatal morbidity associated with preterm birth. Whether this relationship is causal is not established by this study. Longitudinal measurement of IGF-I might facilitate the identification of infants at risk so that preventive measures could be taken. Restoration of IGF-I levels to those normally found in utero may help prevent ROP and other morbidity in preterm infants.

ACKNOWLEDGMENTS

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