Vitreous Levels of IGF-I, IGF Binding Protein 1, and IGF Binding Protein 3 in Proliferative Diabetic Retinopathy

A case-control study

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OBJECTIVE — To evaluate vitreous levels of IGF-I and its binding proteins IGFBP-1 and IGFBP-3 in patients with proliferative diabetic retinopathy (PDR). Because intravitreal proteins are elevated in patients with PDR due to the disruption of the blood-retinal barrier, we have corrected vitreal IGF-I and IGFBPs by total vitreal proteins to avoid this confounding factor.

RESEARCH DESIGN AND METHODS — We compared 21 diabetic patients with proliferative retinopathy (group A) and 13 nondiabetic patients (group B) in whom a vitrectomy was performed. Both groups were matched by age, serum IGF-I, IGFBP-1, and IGFBP-3 levels. Serum and vitreous levels of IGF-I, IGFBP-1, and IGFBP-3 were measured by immunological methods. Vitreal proteins were assessed by turbidimetric method.

RESULTS — Vitreal levels of IGF-I were elevated in group A (median 1.35 ng/ml [range 0.3–8.7]) in comparison with group B (median 0.25 ng/ml [range 0–1.38], P < 0.001. After adjusting by vitreal proteins [ratio IGF-I (ng/ml)/protein (mg/ml)], the differences remain significant (P < 0.005). Vitreous levels of IGFBP-1 and IGFBP-3 were also elevated in diabetic patients (IGFBP-1: group A, median 1.6 ng/ml [range 0.6–20.7]; group B, median 0.4 ng/ml [range 0.3–1.9], P < 0.001. IGFBP-3: group A, median 102.6 ng/ml [range 33.9–360.8]; group B, median 29.0 ng/ml [range 3.2–87.8], P < 0.001). However, when the ratio IGF-protein was considered, the differences were not significant.

CONCLUSIONS — Intraocular synthesis contributes to elevated vitreous concentrations of IGF-I found in PDR. By contrast, unspecific increase of intravitreal proteins is the main factor explaining the elevated vitreous levels of IGFBP-1 and IGFBP-3 found in diabetic patients.


IGF-I is a polypeptide structurally homologous to insulin that regulates the proliferation and differentiation of several cell types (1,2). In vivo, IGF-I acts in a paracrine/autocrine manner to mediate many of the physiological actions of growth hormone, and its activity in extracellular fluids is regulated by insulin-like growth factor binding proteins (IGFBPs) that prolong IGF-I half-life in the circulation and serve as a reservoir of IGFs. Moreover, IGFBPs have been shown to modulate IGF action on target cells (3,4).

Several clinical studies have suggested the role of growth hormone/IGF-I in the development of diabetic retinopathy. Growth hormone-deficient dwarfs with diabetes and diabetic patients with hemochromatosis and infiltrative disease of the pituitary gland have little evidence of retinopathy (5,6). Conversely, proliferative diabetic retinopathy could improve after hypophysectomy (7–10). IGF-I has been involved in the impairment of diabetic retinopathy observed in puberty and pregnancy. Two physiological conditions associated with serum IGF-I increasing (11–13). Furthermore, IGF-I has been implicated in the worsening of preexisting diabetic retinopathy observed after improved glycemic control by intensive insulin therapy (14–16). Interestingly, a transient elevation of serum IGF-I levels has been observed at the time of retinal vessel formation in a prospective 2-year follow-up study (17). Nevertheless, regional IGF-I concentrations in the retina may be more important than systemic levels. Vitreous fluid obtained from diabetic patients with proliferative diabetic retinopathy (PDR) submitted to a vitrectomy is a unique material to explore indirectly the synthesis of growth factors in the retina, and elevated vitreal levels of both IGF-I and IGFBPs have been reported (17–27). These proteins could be enhanced in vitreous fluid as a consequence of the increased synthesis by the retina. By contrast, the increase of vitreous levels of IGF-I and IGFBPs could be due to the disruption of the blood-retinal barrier, reflecting the leakage of proteins that occurs in diabetic microangiopathy. In fact, we have observed that intravitreal protein levels are elevated threefold in diabetic patients with PDR in comparison with control subjects (28). Therefore, the results of previous studies that have found higher intravitreal IGF-I and IGFBP concentrations could be due to this unspecific increase of proteins.

In the present study vitreous levels of IGF-I, IGFBP-1, and IGFBP-3 were determined in diabetic patients with PDR and compared with a nondiabetic control group matched by serum IGF-I, IGFBP-1, and IGFBP-3. Furthermore, the results were adjusted by total vitreal proteins. This design permits one to analyze more accurately the results obtained in diabetic patients.
RESEARCH DESIGN AND METHODS

Subjects
We included in the study 21 diabetic patients (6 type 1 and 15 type 2) with proliferative diabetic retinopathy in whom a classic three-port pars plana vitrectomy was performed (group A), and 13 nondiabetic patients (group B) with other conditions requiring vitrectomy (epiretinal or subretinal membrane). In all cases, a recent vitreous hemorrhage was excluded (less than 2 months). Both groups were matched by sex, age, and levels of serum IGF-I, IGFBP-1, and IGFBP-3 (Table 1).

Undiluted vitreous samples were obtained at the onset of vitrectomy by aspiration into a syringe attached to the vitreous cutter, transferred to a sterile tub, placed immediately on ice, and centrifuged at 16,000g for 5 min at 4°C. The samples were frozen at −80°C until assayed. A venous blood sample was collected simultaneously with the vitrectomy, then centrifuged at 3,000g for 15 min to obtain serum, aliquoted, and stored at −80°C until assayed.

The protocol was approved by the hospital ethical committee and all patients were fully informed before they gave their consent.

IGF-I method assay
IGF-I was measured by radioimmunoassay in which IGFBPs are dissociated by acidification and the addition of an excess of IGF-II (Mediagnost, Tübingen, Germany). The IGF-I antibodies have an extremely low cross-reactivity with IGF-II, and excess IGF-II does not disturb the interaction of the first antibody with IGF-I. This radioimmunoassay is validated to detect IGF-I in organic fluids other than serum. The minimum value detectable was 0.02 ng/ml (coefficient of variation [CV] intra-assay 3.8%, CV interassay 6.1%).

IGFBP-1 method assay
IGFBP-1 was measured by immunoenzymometric assay, which uses a monoclonal antibody specific to human IGFBP-1 precoated in microwell plates, and another monoclonal antibody specific for IGFBP-1 conjugated with horseradish peroxidase (Medix Biochemicals, Kaunaien, Finland). The minimum value detectable was 0.3 ng/ml (CV intra-assay 2.7%, CV interassay 6.2%).

IGFBP-3 method assay
IGFBP-3 was measured by radioimmunoassay (Nichols Institute Diagnostics, Wijchen, the Netherlands). The lowest measurable concentration was 0.25 ng/ml (CV intra-assay 5.6%, CV interassay 5.8%).

Protein assay
Vitreal proteins were measured by a previously validated microturbidimetric method with an autoanalyzer (Hitachi 917; Boehringer Mannheim, Mannheim, Germany). This method, based in the benzotriolium chloride reaction, is a highly specific method for detection of proteins and has a higher sensibility and reproducibility than the classic method of Lowry. The lowest level of proteins detected is 0.02 mg/ml. The coefficients of variation intra-assay and interassay were 2.9 and 3.7%, respectively.

Data are means ± SD or medians (range). Statistical analysis was performed with the Mann-Whitney U test.

Table 1—Age and serum levels of IGF-I, IGFBP-1, and IGFBP-3 in diabetic patients with PDR (group A) and control subjects (group B)

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<th>Group A</th>
<th>Group B</th>
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<tr>
<td>n</td>
<td>21</td>
<td>13</td>
<td>—</td>
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<tr>
<td>Age (years)</td>
<td>62.2 ± 14.8</td>
<td>63.6 ± 13.5</td>
<td>0.72</td>
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<tr>
<td>Serum IGF-I (ng/ml)</td>
<td>136.7 (66.2–302.3)</td>
<td>123.3 (43–212)</td>
<td>0.88</td>
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<tr>
<td>Serum IGFBP-1 (ng/ml)</td>
<td>3.6 (0.7–17.2)</td>
<td>2.3 (0.6–12.5)</td>
<td>0.18</td>
</tr>
<tr>
<td>Serum IGFBP-3 (ng/ml)</td>
<td>3,212 (1,530–4,840)</td>
<td>3,141 (764–5,126)</td>
<td>0.80</td>
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Statistical analysis
IGF-I, IGFBPs, and total intravitreal proteins were displayed as a median and range, because of their skewed distribution. A nonparametric test (Mann-Whitney U test) was used to compare serum and vitreous levels of IGF-I and IGFBPs. The Spearman rank test was performed to explore the correlation between total vitreal proteins and intravitreal IGF-I, and it has been graphically represented by means of a Pearson’s correlation test after a log transformation. Levels of statistical significance were set at P < 0.05.

RESULTS — Vitreal levels of IGF-I were elevated in group A in comparison with group B (Table 1). Total vitreal proteins were also elevated in diabetic patients in comparison with control subjects (group A, median 2.99 mg/ml [range 0.62–9.29]; group B, median 0.82 mg/ml [range 0.28–2.63], P < 0.005). A positive correlation was observed between vitreous IGF-I and total vitreous proteins in group A (Fig. 1), but it was not detected in group B (r = 0.44; P = 0.13). When a correction considering vitreal proteins was performed (ratio IGF-I/total vitreous proteins), the differences between group A and group B were not so evident, but remained significant (Table 2). In addition, intravitreal levels of IGF-I were higher in the subgroup of diabetic patients who had similar intravitreal proteins than in control subjects (Table 3).

Vitreal levels of IGFBP-1 and IGFBP-3 were also elevated in group A in comparison with group B. However, after correcting for total vitreal proteins, the differences were not significant (Table 2).

Correlation between total vitreal proteins and vitreal IGF-I in diabetic patients (group A). A logarithmic transformation was performed because of the skewed distribution of both parameters.
Vitreous IGF-I and IGFBPs in diabetic retinopathy

Table 2—Vitreous concentrations of IGF-I, IGFBP-1, and IGFBP-3 in absolute terms and after correction by total vitreal proteins in patients with PDR (group A) and control subjects (group B)

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<th>P</th>
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<tbody>
<tr>
<td>n</td>
<td>21</td>
<td>13</td>
<td></td>
</tr>
<tr>
<td>IGF-I (ng/ml)</td>
<td>1.35 (0.3–8.7)</td>
<td>0.25 (0.02–1.38)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>IGF-I/proteins (ng/mg)</td>
<td>0.44 (0.17–1.87)</td>
<td>0.25 (0.00–0.71)</td>
<td>&lt;0.005</td>
</tr>
<tr>
<td>IGFBP-1 (ng/ml)</td>
<td>1.6 (0.6–20.7)</td>
<td>0.4 (0.3–1.9)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>IGFBP-1/proteins (ng/mg)</td>
<td>0.6 (0.1–4.8)</td>
<td>0.4 (0.1–0.9)</td>
<td>0.4</td>
</tr>
<tr>
<td>IGFBP-3 (ng/ml)</td>
<td>102.6 (53.9–350.8)</td>
<td>29.0 (3.2–87.8)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>IGFBP-3/proteins (ng/mg)</td>
<td>43.5 (15.9–88.5)</td>
<td>33.3 (6.8–50.8)</td>
<td>0.2</td>
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Data are medians (range). Statistical analysis was performed with the Mann-Whitney U test.

CONCLUSIONS—There is increasing experimental evidence regarding the role of IGF-I in diabetic retinopathy. Intravitreous injections of IGF-I in rabbits (29) and pigs (30) cause a breakdown of the blood-retinal barrier and neovascularization that progresses in a similar manner to PDR. IGF-I is expressed constitutively by human retinal endothelial cells (31,32) as well as by retinal pigment epithelium (33,34), and participates in each step for neovascularization (2). Interestingly, IGF-I has been involved in the degradation of basement membranes and extracellular matrix proteolysis, a capital event to initiate the angiogenesis (35–37). Furthermore, other angiogenic agents, such as the basic fibroblast growth factor and vascular endothelial growth factor, have an additive effect with IGF-I on endothelial cell growth (38).

Vitrectomy fluid samples obtained from diabetic patients with PDR are currently used to explore indirectly the synthesis of growth factors by the retina. Several authors have obtained elevated IGF-I levels in the vitreous of diabetic patients with PDR (19,23–27). However, some caveats should be kept in mind: First, serum levels of IGF-I could influence its vitreal concentration and it should be considered in the evaluation of the results. This problem has been solved in the present study because diabetic patients and control subjects were matched by serum IGF-I. Second, the elevated IGF-I levels in the vitreous of diabetic patients with PDR could only reflect the increase of total vitreal proteins observed in these patients due to the disruption of the blood-retinal barrier. Our results underscore the importance of this concept because a positive correlation was found between intravitreous IGF-I and total vitreal proteins in diabetic patients with PDR. However, in the present study, vitreal levels of IGF-I in diabetic patients with PDR were higher than those in control subjects, not only in absolute terms, but also after adjusting for total vitreal proteins. Furthermore, intravitreous levels of IGF-I were higher in the subgroup of diabetic patients who had similar intravitreous IGF-I than in control subjects. These findings suggest that intraocular synthesis of IGF-I also contributes to its intravitreal increase. Locally synthesized IGF-I could be more pathogenic for PDR development due to its ability to act as an autocrine-paracrine factor. In this regard, it has been demonstrated that microvascular endothelial cells and retinal pigment epithelial cells express IGF-I mRNA as well as receptors for IGF-I, and the autocrine/paracrine actions of IGF-I in PDR are supported by several studies (2,23,31–33). Nevertheless, it must be noted that the median of vitreous IGF-I concentrations obtained in our study was 5.4-fold higher in diabetic patients than in control subjects in absolute terms, but the ratio decreased to 1.7 after correction by vitreal proteins. Therefore, the main contributing factor to vitreal IGF-I levels in diabetic patients with PDR seems to be serum diffusion.

IGFBPs regulate bioavailability of IGF. IGFBPs can extend the half-life of the IGFs and, in addition, are capable of modulating IGF activity by either enhancing or inhibiting ligand receptor interactions. These proteins are intimately involved in the mechanisms of IGF action (1,3). Like other authors (23–25,27), we have found elevated vitreous levels of IGFBP-1 and IGFBP-3 in patients with PDR. However, our results suggest that serum diffusion is the main contributing factor explaining this finding, thus reflecting the unspecific increase of proteins found in PDR. This finding is in agreement with the lack of evidence of the expression in vivo of IGFBPs in the retina. Finally, it could be speculated that the imbalance between vitreal IGF-I and IGFBP-1/IGFBP-3 increases the bioavailability of vitreal IGF-I quantitatively and qualitatively, thus promoting neovascularization. Further studies with free IGF-I immunoassays are required to prove this hypothesis.

In summary, our results suggest that although serum diffusion is the main factor accounting for the increase of IGF-I observed in diabetic patients with PDR, intraocular synthesis also contributes to this enhancement. By contrast, the elevated vitreous levels of IGFBP-1 and IGFBP-3 could be only the consequence of the unspecific leakage of serum proteins.

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