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Rapid communication

Vitreous levels of vascular endothelial growth factor are not influenced by its serum concentrations in diabetic retinopathy

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Summary Vascular endothelial growth factor (VEGF) plays a major role in the development of neovascularization in proliferative diabetic retinopathy (PDR). The source of intravitreous VEGF is presumably ischaemic retina, but increased levels derived from serum cannot be excluded. The aim of the study is to determine the intravitreous concentrations of VEGF in diabetic patients with PDR and to investigate whether serum VEGF could contribute to the intravitreous concentration. For this purpose, we studied 20 diabetic patients (5 IDDM and 15 NIDDM) with PDR in whom a vitrectomy was performed (group A). Non-diabetic patients ($n = 13$) with other conditions requiring vitrectomy served as a control group (group B). In both groups, VEGF was determined in serum and undiluted vitreous samples obtained simultaneously. Furthermore, serum VEGF was determined in 69 healthy control subjects (group C) and 39 diabetic patients without microvascular complications (group D). Vitreous and serum VEGF was determined by ELISA (R & D Systems, Abingdon, UK); intra-assay CV 3.8%, interassay CV 5.1%. Intravitreous concentrations of VEGF were strikingly higher in group A (median 1.75 ng/ml, range 0.33–6.66) in comparison with group B (median 0.009 ng/ml, range 0.009–0.038); $p < 0.0001$. This difference remained significant after adjusting for intravitreous protein concentration ($p < 0.05$). Differences in serum VEGF among the groups included in the study were not found. We conclude that the high vitreous levels of VEGF observed in diabetic patients with PDR cannot be attributed to serum diffusion across the blood-retinal barrier. Therefore, intraocular synthesis is the main contributing factor for the high vitreous VEGF concentrations observed in PDR. [Diabetologia (1997) 40: 1107–1109]

Keywords Growth factors, vascular endothelial growth factor, diabetic retinopathy, neovascularization, vitreous.

Vascular endothelial growth factor (VEGF) has been identified as an endothelial cell-specific mitogen and angiogenic inducer in vivo. The expression of this growth factor is greatly increased by hypoxia in many retinal cell types [1]. VEGF has been suggested to play a major role in intraocular neovascularization in proliferative diabetic retinopathy (PDR) and other ischaemic retinal diseases. Furthermore, elevated levels of VEGF have been reported in the vitreous of patients with PDR [2–5]. The source of the increased vitreous VEGF concentrations is presumably the ischaemic retina; but the possible contribution of serum VEGF on intravitreous VEGF concentrations has not yet been explored. VEGF is also a potent stimulus of vascular permeability and has been implicated in the breakdown of the blood-retinal barrier in the rat model of PDR [6, 7]. Both, hyperpermeability and breakdown of the blood-retinal barrier are major early functional disorders observed in diabetic retinopathy. The disruption of the retinal barrier could facilitate the access of different serum proteins to ocular fluid. This event could account for the large

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Abbreviations: VEGF, Vascular endothelial growth factor; PDR, proliferative diabetic retinopathy.

variability in the vitreous levels of VEGF found by different authors [2–5]. However, to our knowledge there are no studies focused on the relationship between serum and vitreous levels of VEGF.

The aim of the study is to determine the intravitreous concentration of VEGF in diabetic patients with PDR and to investigate whether serum VEGF could contribute to its intravitreous concentration.

Subjects, materials and methods

We included 20 consecutive diabetic patients (5 IDDM and 15 NIDDM) with PDR in whom a classic three port pars plana vitrectomy was performed (group A, mean age 52 ± SD 14 years). For visualization of the vitreous cavity we used a wide-field system with a precorneal Volk lens of 130° and inversion image system Moeller-Wedel (Hamburg, Germany). Retinopathy was graded intraoperatively in all eyes by the same ophthalmologist using a method previously reported [2]. In summary, neovascularization was considered to be active when perfused preretinal capillaries exist, and to be quiescent if only non-perfused, gliotic vessels or fibrosis were present. Non-diabetic patients (n = 13) with other conditions requiring vitrectomy served as a control group (group B, mean age 63 ± SD 13 years). In these patients the diagnoses included epiretinal membrane (n = 6) and subretinal membrane (n = 7), two disorders in which retina are not directly affected by neovascularization. In order to avoid the massive affluence of serum VEGF into the vitreous, recent vitreous hemorrhage was excluded (less than 1 month) in all cases. In group A and B, undiluted vitreous samples (0.5–1 ml) were obtained at the onset of vitrectomy by aspiration into a 1 ml syringe attached to the vitreous cutter (Alcoon Model, Ten-Thousand Ocutome; Irvine, Calif., USA) before starting the intravitreal infusion of balanced salt solution. The vitreous samples were transferred to a sterile tube, placed immediately on ice and centrifuged at 16000 × g for 5 min at 4 °C. The samples were frozen at –80 °C until assayed.

Serum VEGF concentrations were determined in group A and group B. For this purpose, 5 ml of venous blood sample was collected simultaneously to the vitrectomy. Furthermore, blood samples to determine serum VEGF were obtained in 69 healthy control subjects recruited from hospital staff (group C, age 47 ± 19 years), and 39 diabetic patients without microangiopathic complications (group D, 21 IDDM and 18 NIDDM, age 45 ± 10 years). In all groups, blood samples were centrifuged at 3000 × g for 15 min to obtain serum, then aliquoted and stored at –80 °C until assayed.

The protocol for sample collection was approved by the hospital ethical committee, and informed consent was obtained from patients.

VEGF method assay. Enzyme linked immunosorbent assay (ELISA) of undiluted vitreous and serum samples was performed (R & D Systems, Abingdon, UK), which uses a monoclonal antibody specific for VEGF pre-coated onto a microtitre plate and an enzyme-linked polyclonal antibody specific for VEGF as second antibody. Intraassay coeficient of variation (CV): 3.8 %, interassay CV: 5.1 %. For data processing, we allocated the minimum value detected by ELISA (< 0.009 ng/ml) to all samples with concentrations below the detection threshold. Furthermore, a ratio VEGF (ng/ml) / protein (mg/ml) was performed in order to avoid the influence of higher protein concentrations found in vitreous fluid of diabetic patients. Protein concentrations were determined by a previously validated micro-turbidimetric method with an autoanalyzer (Hitachi 917; Boehringer Mannheim, Mannheim, Germany).

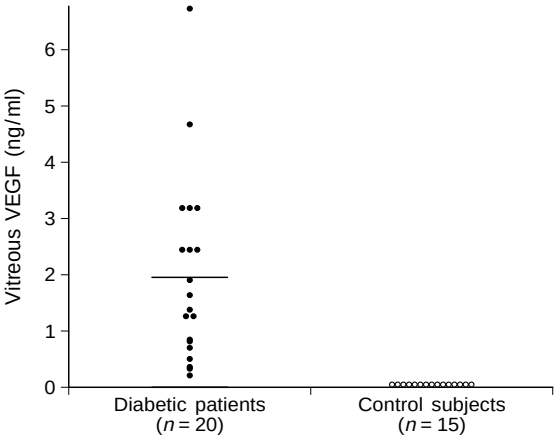

Fig. 1. Vitreous concentrations of VEGF in diabetic patients with proliferative diabetic retinopathy (group A) and non-diabetic patients with other retinal pathologies (group B). Differences between groups were statistically significant ($p < 0.0001$)

Statistical analysis. Kolmogorov-Smirnov and Shapiro-Wilks tests were used to confirm the assumption of normality of variables. Intravitreous VEGF concentrations were displayed as median and range, in view of the skewed distribution. By contrast, serum VEGF concentrations were expressed as the mean ± SD because a normal distribution was obtained. To compare intravitreous concentrations of VEGF, the Mann-Whitney U-test was used. Measurement of serum VEGF among groups was compared by analysis of variance (ANOVA). Correlation between serum and vitreous concentrations of VEGF was studied by linear regression analysis. Levels of statistical significance were set at $p < 0.05$.

Results

Intravitreous concentrations of VEGF were higher in diabetic patients with PDR (group A: median 1.75 ng/ml, range 0.33–6.66), in comparison with control group (group B: median 0.009 ng/ml, range 0.009–0.038), $p < 0.0001$ (Fig. 1). We also detected higher intravitreous protein concentrations in group A (median 297.5 mg/dl, range 61–927 mg/dl) in comparison with group B (median 82.5 mg/dl, range 26–261 mg/dl); $p = 0.0016$. Nevertheless, when the ratio VEGF (ng/ml)/protein (mg/ml) was considered, the differences remain significant (group A: 0.664 ng VEGF/mg protein, group B: 0.011 ng VEGF/mg protein, $p < 0.05$). Furthermore, the vitreous concentrations of VEGF were higher in patients with active proliferative diabetic retinopathy (median 2.13 ng/ml, range 0.5–6.6 ng/ml, n = 16) than in patients with quiescent proliferative diabetic retinopathy (median 0.35, range 0.16–0.69 ng/ml, n = 4), $p < 0.05$) (Fig. 2).

We did not observe statistical differences in serum VEGF among the four groups included in the study (group A: 0.18 ± 0.12 ng/ml; group B: 0.22 ± 0.14 ng/ml; group C: 0.23 ± 0.18 ng/ml, and group D:

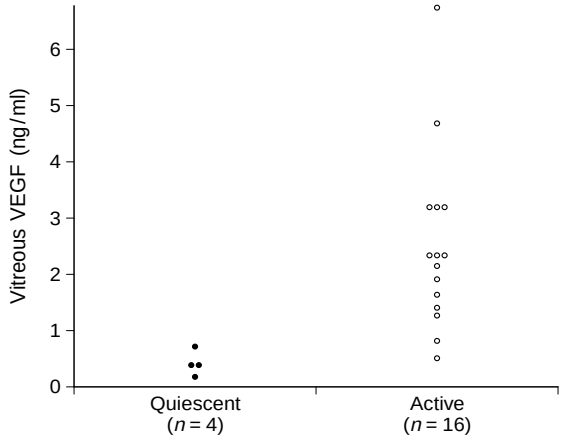

Fig. 2. Vitreous concentrations of VEGF in patients with quiescent proliferative diabetic retinopathy and active proliferative diabetic retinopathy. Differences between groups were statistically significant ($p < 0.03$)

0.21 ± 0.17 ng/ml), p: NS. Moreover, any statistically significant correlation was obtained between serum and vitreous VEGF concentrations in groups A and B ($r = 0.26$ and $r = 0.40$, respectively, p = NS).

Discussion

VEGF has been implicated in the origin of neovascularization, a crucial event for the development of PDR [1]. The expression of VEGF is mainly up-regulated by hypoxia in many retinal cells, including retinal endothelial cells, retinal pigment epithelial cells, pericytes and Müller cells. Recently, experimental studies suggest that expression of VEGF is induced by chronic exposure to a high glucose environment and also by acute glucose deprivation in retinal pigment epithelial cells, retinal endothelial cells and retinal pericytes [8]. These findings suggest that the diabetic milieu favours the expression of VEGF at retinal level. In addition, elevated levels of VEGF have been reported in the vitreous of PDR patients [2–5]. However, it is unknown whether serum VEGF concentrations could contribute to the increased levels detected in the vitreous of these patients.

Serum VEGF has been determined in several conditions [9, 10], but we are unaware of studies in diabetic patients. It could be hypothesized that serum VEGF levels could be increased in diabetic patients due to an endothelial activation. In this regard, elevated serum levels of VEGF have been found in women with pre-eclampsia [9], a condition in which an endothelial activation is involved. Our results suggest that serum VEGF did not reflect the endothelial injury because we did not observe differences among diabetic patients with PDR, diabetic patients without microvascular complications and healthy control subjects. In addition, high sparse values were obtained, reflecting a great interindividual variability. This finding should be considered as a limiting factor in future studies designed to assess the usefulness of VEGF as a serum marker of diabetic microangiopathy.

We have found strikingly higher levels of VEGF in the vitreous of patients with PDR in comparison with other ocular pathologies in which retinal neovascularization is not a predominant finding. It could be speculated that the high intravitreous levels of VEGF obtained in patients with PDR is an unspecific event that reflects the breakdown of the blood-ocular barrier and the subsequently elevated influx of plasma proteins into the vitreous. In fact, we have found about threefold elevated intravitreous protein concentrations in patients with PDR. However, our results argue against this hypothesis because intravitreous VEGF levels remain higher in PDR patients after adjusting for intravitreous protein concentrations. On the other hand, it must be emphasized that we did not find any relationship between serum and vitreous VEGF concentrations. Therefore, intravitreous levels of VEGF in diabetic patients with PDR could not be attributed to serum diffusion. On this basis, we concluded that intraocular synthesis of VEGF but not serum diffusion is the main contributing factor to the high VEGF concentration observed in patients with PDR.

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