Erythropoietin Is Highly Elevated in Vitreous Fluid of Patients With Proliferative Diabetic Retinopathy

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Diabetic retinopathy is characterized by increased vascular permeability, ischemia, and neovascularization. Ischemia in the retina can induce neovascularization via induction of vascular endothelial growth factor (VEGF) expression by the transcription factor hypoxia-inducible factor-1 (1). Hypoxia is also the main stimulus for erythropoietin (EPO) gene expression. It has been reported that EPO may have not only erythropoietic (2) but also neuroprotective (3) and angiogenic (4) effects. EPO is expressed at a number of sites including kidney, liver, brain, uterus (5), and the adult mammalian retina, which also expresses EPO receptors (6). The aim of this study was to investigate whether EPO is elevated in the vitreous fluid of patients with proliferative diabetic retinopathy (PDR) and whether vitreous EPO levels are related to systemic levels of EPO, anemia, or nephropathy.

RESEARCH DESIGN AND METHODS

— Vitreous fluid samples were obtained from 59 PDR patients and 16 macular hole patients at the start of the vitrectomy procedure. Concentrations of EPO in vitreous fluid and serum samples were measured by an enzyme-linked immunosorbent assay for EPO (Toyobouseki, Osaka, Japan). Concentrations of VEGF were measured by an enzyme-linked immunosorbent assay for human VEGF (R & D Systems, Minneapolis, MN). Protein in vitreous fluid was determined using the method of Bradford (Bio-Rad, Hercules, CA). The Mann-Whitney *U* test was used to compare intravitreous concentrations of protein, VEGF, and EPO. Correlations between intravitreous concentrations of EPO and VEGF and intravitreous and serum concentrations of EPO were examined by Spearman's rank correlation test.

RESULTS

— Patients with PDR had significantly higher intravitreous concentrations of EPO (median 366.6 mIU/ml [range 44.8–2,023.1]) than patients with a macular hole (11.0 mIU/ml [0–38.2]; $P < 0.001$) (Fig. 1*A*). When the intravitreous EPO concentration was normalized to total protein concentration, there was still significantly more EPO in the vitreous fluid of PDR patients (12.4 mIU $\cdot$ ml^{-1} $\cdot$ μg^{-1} protein [1.5–331.9] vs. 1.5 mIU $\cdot$ ml^{-1} $\cdot$ μg^{-1} protein [0–27.5], respectively; $P < 0.0001$) (Fig. 1*B*). Intravitreous concentrations of VEGF were also significantly elevated in PDR patients (2.34 ng/ml [0.1–20.6]) compared with macular hole patients (0.09 ng/ml [0.07–0.12]; $P < 0.0001$).

When PDR patients were focused upon, we found no significant correlation between intravitreous and serum concentrations of EPO ($r = 0.18$, $P = 0.18$) and weak correlation between intravitreous concentrations of EPO and VEGF ($r = 0.3$, $P = 0.0003$).

To determine whether EPO levels vary with degree of nephropathy, PDR patients were divided into three groups: Group A ($n = 24$) had no proteinuria (negative albustix reading), group B ($n = 13$) had proteinuria (albustix reading 1+ or more) and serum creatinine levels <1.2 mg/dl, and group C ($n = 22$) had proteinuria and elevated serum creatinine levels (<1.2 mg/dl). There was a significant decrease in Hb with progression of nephropathy from a mean of 13.2 g/dl in group A to 11.1 g/dl in group C ($P = 0.0003$). There were no significant differences in intravitreous EPO concentrations between the three subgroups (group A 578.0 mIU/ml [58.1–1,705.8], group B 460.9 mIU/ml [125.8–2,023.1], and group C 249.5 mIU/ml [44.8–1,684.7]), and serum EPO levels were not different between the three groups (group A 2.87 mIU/ml [0.8–8.2], group B 2.7 mIU/ml [1.0–5.6], and group C 2.87 mIU/ml [0.3–24.3]).

In a second analysis to examine whether EPO concentrations are related to anemia, we divided the PDR patients according to the presence of anemia, defined as Hb <13 g/dl in men and Hb <12 g/dl in women. However, there was no difference in serum EPO concentrations between anemic (2.87 mIU/ml [0.3–24.3) and nonanemic patients (2.4 mIU/ml [0.8–5.6]) nor in intravitreal EPO concentrations between anemic (412.6 mIU/ml [44.8–1705.8]) and nonanemic patients (282.3 mIU/ml [58.1–2,023.1]).

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Abbreviations: EPO, erythropoietin; PDR, proliferative diabetic retinopathy; rhEPO, recombinant human EPO; VEGF, vascular endothelial growth factor.

A table elsewhere in this issue shows conventional and Système International (SI) units and conversion factors for many substances.

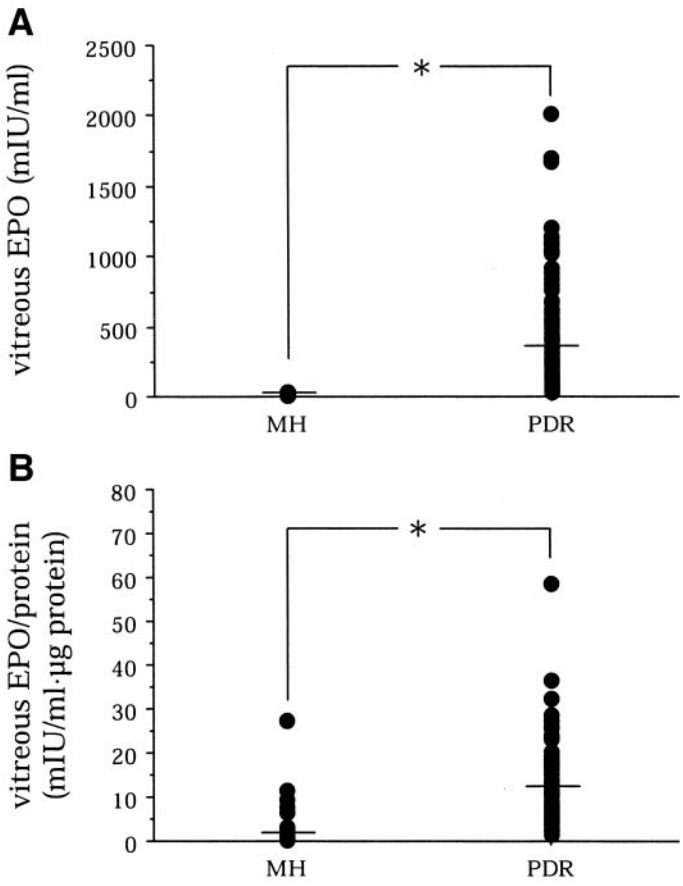

Figure 1—A: *EPO concentrations in the vitreous fluid of PDR and MH patients. The horizontal line represents the median.* *P < *0.001.* B: *EPO concentrations per microgram of protein in the vitreous fluid of PDR and MH patients. The horizontal line represents the median.* P < *0.0001. MH, macular hole.*

CONCLUSIONS — In this study, we found that vitreous EPO concentrations were markedly higher in PDR patients than in macular hole patients who had no retinopathy. These results are consistent with those of Inomata et al. (7) who also reported that vitreous EPO concentrations are higher in PDR than in other ocular diseases. They also found, in contrast to our observations, a significant and strong correlation between EPO and VEGF concentrations in vitreous fluid. This difference may be explained by the fact that they examined all patients with all eye diseases, whereas we only included diabetic patients who had the markedly increased intravitreous EPO levels in our analysis.

Because systemic hypoxia may aggravate diabetic retinopathy, we examined the relation of EPO and anemia. Anemia induces systemic hypoxia and may increase hypoxia in the retina, but it is a poor prognostic factor for PDR (8). However, we found no significant difference between the vitreous EPO concentrations of anemic patients and those of nonanemic patients, suggesting that that vitreous EPO is not induced by systemic anemia.

What is the role of EPO in the vitreous fluid of diabetic patients? EPO promotes the proliferation and differentiation of erythroid precursors through the induction of antiapoptotic proteins (2) and inhibition of apoptosis (9). Jaquet et al. (4) reported that EPO has the same angiogenic potential on endothelial cells as VEGF. Some diabetic patients with severe renal anemia may be treated with recombinant human EPO (rhEPO), and if as our results suggest, EPO can induce intraocular angiogenesis, we should reconsider the therapeutic use of rhEPO, which can cross the blood-retinal barriers (4). However, one study has actually reported that EPO treatment had a favorable impact on retinopathy in diabetic subjects (10), suggesting that the increased blood Hb level induced by rhEPO improved oxygen carriage to retinal tissue and ameliorated diabetic retinopathy. Recently, EPO has been shown to be neuroprotective following ischemic and hypoxic stress in the nervous system (3). EPO also protects retinal ganglion cells from ischemia and reperfusion injury through an antiapoptotic mechanism (11). Retinas of diabetic patients have significantly more apoptotic neurons than control subjects (12), and apoptosis plays some role in diabetic retinopathy. Therefore, while EPO levels are elevated in the eyes of patients with PDR, there is evidence to suggest that treatment with EPO may benefit patients with retinal disease, and there is a clear need for further study to elucidate the role of this important factor in diabetic retinopathy.

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