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Aqueous humor levels of vascular endothelial growth factor and adiponectin in patients with type 2 diabetes before and after intravitreal bevacizumab injection

Ciro Costagliola [a,*], Aurora Daniele [b], Roberto dell'Omo [a], Mario R. Romano [a], Fabiana Aceto [a], Luca Agnifili [c], Francesco Semeraro [d], Antonio Porcellini [e]

[a] Dpt di Medicina e di Scienze per la Salute, Università degli Studi del Molise, Via F. De Sanctis, snc, 86100 Campobasso, Italy
[b] Dpt di Scienze Ambientali, Seconda Università degli Studi di Napoli, Caserta, Italy
[c] Dpt di Medicina e Scienze dell'Invecchiamento, Università G. D'Annunzio, Chieti, Italy
[d] Dpt di Chirurgia e Medicina Legale, Università di Brescia, Brescia, Italy
[e] Dpt di Biologia Strutturale e Funzionale, Università degli Studi "Federico II", Napoli, Italy

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ABSTRACT

To determine the levels of vascular endothelial growth factor (VEGF) and adiponectin (APN) in the aqueous humor of patients with type 2 diabetes before and after injection of bevacizumab (IVB). Twenty eyes of twenty consecutive patients with type 2 diabetes with PDR and clinically significant macular edema were enrolled in this study. Aqueous samples were collected at baseline and one month after IVB to evaluate VEGF and APN levels. Twenty age-matched patients undergoing cataract surgery were used as control. Best-corrected visual acuity (BCVA) and foveal thickness (FT) changes after IVB were also measured. Safety was assessed by recording the incidence of ocular and non-ocular adverse events. At baseline APN and VEGF levels were significantly lower in controls than in PDR patients (APN: 3.6 ± 1.1 vs 18.7 ± 4.5 ng/ml; VEGF: 22.6 ± 16.1 vs 146.2 ± 38.71 pg/ml). After IVB, both compounds significantly decreased. FT and BCVA at baseline were significantly different between controls and patients (FT: 215.6 ± 34.8 vs 532.7 ± 112.4 μm; BCVA: 23.6 ± 4.2 vs 18.4 ± 7.3 letters). After IVB a significant decrease of FT with a concomitant improvement of BCVA occurred. Neither ocular nor systemic adverse events were reported. Our findings demonstrate that patients with type 2 diabetes, PDR and macular edema show VEGF and APN levels in aqueous humor higher than those found in control subjects. IVB significantly reduced the levels of both compounds, which remained anyway at concentrations higher than those recorded in control subjects.

1. Introduction

Diabetic retinopathy (DR), the most frequent diabetic microvascular complication, affects 30%–50% of all diabetic patients and represents the main cause of legal blindness in developed countries (Semeraro et al., 2011). It is caused by changes in the retina microvasculature and hyperglycemia itself seems to be the key factor in the etiology of DR although, recently, focus has been directed to the molecular basis of the disease, and several biochemical factors other than hyperglycemia, have been considered (Cai and Boulton, 2002). These mechanisms act affecting cellular metabolites and inducing release of cytokines (Caldwell et al., 2003); among these, vascular endothelial growth factor (VEGF) is the most representative, and its role in angiogenesis and microvascular permeability is well known (Aiello et al., 1994; Caldwell et al., 2003).

Current evidence indicates that VEGF plays a central role in the development of choroidal neovascularisation (CNV). In fact, vitreous levels of VEGF were found to be significantly higher in patients with CNV compared to those found in healthy controls (Aiello et al., 1994; Kvanta et al., 1996; Wells et al., 1996), as well as intravitreous injection of VEGF is able to induce proliferation of choroidal endothelial cells in experimental animal models (Tolentino et al., 1996). Since VEGF plays a key role in the pathogenesis of CNV, targeting VEGF has been an attractive strategy in the treatment of CNV, initiating extensive research in recent years (Ferrara et al., 2007). Anti-VEGF therapy can arrest choroidal angiogenesis and also reduce vascular permeability, frequently the main cause of visual acuity deterioration. These findings provide the rationale for anti-VEGF therapy in retinal vascular diseases associated with new vessel formation such as diabetic retinopathy (Sawada et al., 2007; Matsuyama et al., 2009).

* Corresponding author. Tel.: +39 (0)8744041.
E-mail address: ciro.costagliola@unimol.it (C. Costagliola).

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Adiponectin (APN) is a polypeptide hormone produced exclusively in adipocytes that circulates at very high levels in the bloodstream. In experimental studies, APN has been shown to exert anti-inflammatory and anti-atherosclerotic effects, and to inhibit neo-intimal thickening and vascular smooth muscle cell proliferation in mechanically injured arteries (Kubota et al., 2002). Plasma APN concentrations are decreased in obesity, insulin resistance, type 2 diabetes, coronary disease and hypertension (Frystyk et al., 2005). Several studies have indicated that APN possesses anti-inflammatory properties and thus may negatively modulate the process of atherogenesis (Goldstein and Scalia, 2004). The role of APN in the development of microvascular disease (such as diabetic retinopathy and nephropathy) is largely unknown. Clinically, patients with type 2 diabetes suffering from diabetic retinopathy (proliferative as well as non-proliferative) are reported to have lower levels of APN than matched patients without retinopathy (Yilmaz et al., 2004).

The aim of this study was to determine the level of VEGF and APN in the aqueous humor of patients with diabetic proliferative retinopathy (DPR) and to evaluate the effects of intravitreal bevacizumab (IVB) on the concentration of these compounds.

2. Materials and methods

2.1. Subjects

Twenty eyes of twenty consecutive patients with type 2 diabetes mellitus (DM), PDR and clinically significant macular edema were enrolled in this study. Diabetes mellitus was diagnosed based on the American Diabetes Association criteria (Expert Committee on the Diagnosis and Classification of Diabetes Mellitus, 2003). Diagnosis of PDR was made based on an international standard (Watkins, 2003). Patients in the PDR group had been diagnosed with type 2 DM an average of 15 (±2.5) years earlier. Clinically significant macular edema was defined according to the EDTRS criteria (ETDRS report no. 19. Early Treatment Diabetic Retinopathy Study Research Group, 1995). Twenty age-matched patients undergoing cataract surgery constituted the control group. The procedures used in this study were conformed to the tenets of the Declaration of Helsinki and were performed after receiving institutional review board approval. Informed consent was obtained from all patients.

2.2. Diagnostic procedures

At baseline all the patients underwent BCVA measurement using an early treatment diabetic retinopathy study (ETDRS) chart at 4 m, fundus biomicroscopy, tonometry, fluorescein and indocyanine green angiographies (FA and ICGA), and spectral domains optical coherence tomography (SD-OCT). All examinations, with the exception of angiographic tests, were repeated one month after bevacizumab intravitreal injection (IVB). Angiographic tests and OCT scans were recorded using Spectralis SD-OCT (Heidelberg Engineering, Heidelberg, Germany).

2.3. Inclusion and exclusion criteria

In the PDR group patients were included if they presented one or more of the following abnormalities: new vessels on the disc and new vessels elsewhere. Eyes with vitreous hemorrhage obscuring retina details or evidence of tractional retinal detachment on OCT were excluded from the study group. All diagnoses were confirmed by at least 2 doctors independently at the time of admission (C.C. and R.d.O.).

Subjects were excluded if they had type 1 diabetes, were younger than 18, or were older than 90 years of age. To minimize the compounding complications of data interpretation from other risk factors, we excluded all subjects and patients with hypertension, hyperlipidemia, nephropathy, coronary heart disease, heart failure or renal failure and those who had ocular surgery within 3 months preceding inclusion.

In the control group exclusion criteria were age younger than 18 and older than 90 years, arterial hypertension, hyperlipidemia, nephropathy, coronary heart disease, heart failure or renal failure; any type of retinal disease, glaucoma, previous vitrectomy, laser coagulation, diabetes mellitus, use of immunosuppressive drugs, malignant tumors at any location, and participation in any study of investigational drugs within 3 months before recruitment.

2.4. Aqueous sampling and bevacizumab injections

All patients with DPR received intravitreal injections of 1.25 mg/0.05 mL of bevacizumab (Avastin; Genentech Inc, South San Francisco, California, USA). Immediately before the intravitreal injection, aqueous samples were obtained by aspirating 0.05–0.1 mL of aqueous using a 30-gage needle connected to a tuberculin syringe at the temporal limbus. IVB injection was then performed using a 30-gage needle in the inferotemporal quadrant at 3.5–4 mm posterior to the limbus. The undiluted aqueous samples were transferred into sterile containers and immediately stored in a −80C freezer until analysis. The same procedure was performed one month later, when PDR patients underwent to the second bevacizumab intravitreal injection.

In controls aqueous humor samples were collected in the same fashion described above for eyes with PDR, before performing surgery. The undiluted aqueous samples were transferred into sterile containers and immediately stored in a −80C freezer until analysis.

2.5. Vascular endothelial growth factor and adiponectin assay

Collected samples were gradually equilibrated to room temperature before beginning the assay and diluted up to 500 μL with the sample diluent provided by the manufacturer and the dilution factor calculated for each sample. The VEGF content was determined in 50 μL of diluted sample with a human VEGF ELISA kit (EHVEGF, Pierce Biotechnology, Rockford, Illinois, USA) according to the manufacturer's instruction. VEGF concentration in the AH was assessed against an *x*-point standard curve, extending from 3.750 to 500 pg/mL. The minimum detectable level was 3.5 pg/mL. Values inferior to 3.5 pg/mL were considered equal to 1 for statistical analysis. VEGF was measured in triplicate.

APN concentrations were measured with an ELISA using a polyclonal antibody produced in-house vs a human APN amino acid fragment (H_2N-ETTTQGPGVLLPLPKG-COOH) as previously described in detail (Daniele et al., 2008). APN was measured in triplicate.

2.6. Statistical analysis

According to sample size calculation a sample size of 20 patient-control pairs had 80% power at the 5% significance level to detect a 10% difference in selected parameters between control subjects and patients. Twenty eyes of 20 patients (8 male and 12 female) with bilateral PDR and 20 eyes of 20 patients (12 male and 8 female) undergoing cataract surgery were included in this study.

All statistical analyzes were carried out using SPSS 12.0 for Windows (SPSS, Inc, Chicago, Illinois, USA). The aqueous levels of VEGF and APN were expressed as a mean with standard deviation (SD). To analyze the statistical differences, the Wilcoxon signed rank test was used between pre-injection and post-injection