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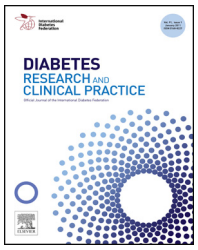

Suppressive effect of aqueous humor from person with Type 2 diabetes with or without retinopathy on reactive oxygen species generation

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ABSTRACT

Aim: To evaluate the antioxidant capacity and concentrations of vascular endothelial growth factor (VEGF) and interleukin 6 (IL-6) in aqueous humor from patients with type 2 diabetes mellitus (T2DM) with or without retinopathy.

Methods: Aqueous humor was obtained during elective cataract surgery from T2DM patients with or without retinopathy and from healthy subjects. Reducing response was evaluated by MTT dye reduction and the generation of reactive oxygen species (ROS) was determined by chemiluminescence assay. Granulocytes were treated with phorbol dibutyrate (PDB)-stimulated. Cytokines were quantified by ELISA.

Results: Antioxidant capacity of aqueous humor from patients with retinopathy was greater ($P < 0.05$) than that of healthy controls or persons with diabetes without retinopathy. ROS production in PDB (protein kinase C activator)-stimulated granulocytes from T2DM patients with or without retinopathy was inhibited by autologous aqueous humor. Concentrations of VEGF and IL-6 were similar in aqueous humor from healthy controls and from patients without retinopathy, but lower ($P < 0.05$) than those from T2DM patients with retinopathy. Plasma levels of VEGF and IL-6 were similar ($P > 0.05$) in healthy controls and in T2DM patients with and without retinopathy.

Conclusion: Aqueous humor from T2DM patients with retinopathy exhibits elevated antioxidant activity with significant suppressive effect on ROS production and enhanced levels of locally secreted VEGF and IL-6 in comparison with T2DM patients without retinopathy. These results suggest an inflammatory profile in the absence of typical oxidative stress for T2DM patients with retinopathy, possibly resulting from the compensatory antioxidant response detected in the aqueous humor improving the ocular redox state.

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Abbreviations: AGEs, advanced glycation end-products; VEGF, vascular endothelial growth factor; T2DM, type 2 diabetes mellitus; MTT, [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; PDB, phorbol dibutyrate; PKC, protein kinase C; DG, diacylglycerol.

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1. Introduction

Aqueous humor is located inside the anterior and posterior chambers of the eye and supplies nutrients to the non-vascularized cornea, lens and trabecular meshwork. Certain physiological or pathological states of the retina can affect the composition of the aqueous humor [1,2] and biomarkers associated with a number of pathological processes, including vascular diseases, arteriosclerosis, ischemia, necrosis and inflammation, can be detected in this fluid. It is assumed, therefore, that the profile of the aqueous humor can afford useful information relating to inflammatory processes in the eyes [3].

Retinopathy is the most common microvascular complication in patients with diabetes, and its onset and progression are associated with chronic hyperglycemia, increased oxidative stress and elevated inflammatory cytokines. Enhanced levels of vascular endothelial growth factor (VEGF) and interleukin 6 (IL-6) in the aqueous humor from patients with retinal vein occlusion have been reported, and an association between the upregulation of these proinflammatory cytokines and serous retinal detachment has been proposed [4–6]. Moreover, it has been suggested that patients with retinopathy exhibit increased generation of reactive oxygen species (ROS) in aqueous humor and plasma [7]. Interestingly, the oxidative damage to the lens that occurs in cataract formation can be reversed *in vitro* by treatment with an antioxidant (ROS scavenger) such as pyruvate or ethyl pyruvate, suggesting that the prophylactic intake of antioxidants may be beneficial in delaying the onset of manifestations of aging such as cataract development [8].

Oxidative stress is defined as an increase in the oxidative metabolic response in the absence of a concomitant increase in the reductive metabolic response. An oxidative profile (redox imbalance in which the oxidative response is greater than the reductive response) may induce tissue damage, while a reductive profile may be associated with tissue protection. Information concerning redox imbalance and the inflammatory cytokine profile in the aqueous humor may provide an indication of the severity of pathological response in the retina of patients with diabetes.

The aim of the present study was to evaluate the capacity of aqueous humor to neutralize ROS generation in autologous granulocytes from type 2 diabetes mellitus (T2DM) patients with and without retinopathy, and to determine the levels of cytokines VEGF and IL-6 in the plasma and aqueous humor from patients with diabetes and healthy controls.

2. Material and methods

2.1. Ethical approval

The Ethical Committee from Santa Casa Hospital of Belo Horizonte – Brazil approved this study and the informed consent was obtained of all participants. Patients suffering from type 2 diabetes (diagnosed according to the criteria of the American Diabetes Association).

2.2. Study population

Participants were recruited from patients scheduled to undergo cataract surgery at the Ophthalmology Center of the Santa Casa Hospital, Belo Horizonte, Brazil. Volunteers were within the age range of 30–80 years and included individuals presenting T2DM as well as healthy individuals. Prior to the study, all volunteers were submitted to full physical examinations, and detailed evaluations of medical histories and laboratory data were carried out. Subjects presenting dementia, inflammation, infection or malignant disease were excluded from the study, as were pregnant women and individuals with alcohol or tobacco dependency. The final study population comprised 15 T2DM patients presenting retinopathy, 15 T2DM patients without retinopathy, and 15 healthy control. Patients with diabetes were under statins, beta-blockers, besides hypoglycemic drugs. A blood sample and a 100 μL aliquot of aqueous humor were collected from each patient at the time of the cataract surgery.

2.3. Separation of granulocytes

A modified version of the Ficoll-Hypaque gradient method described by Bicalho et al. [9] was employed to purify granulocytes from heparinized venous blood. Briefly, blood samples (10 mL) were subjected to double Ficoll-Hypaque gradients of different densities in order to generate three interfaces after centrifugation. The fraction at the first interface from the top was rich in peripheral blood mononuclear cells while that at the second interface comprised granulocytes. The cellular viability of each sample was determined using the trypan blue exclusion test and was found to be >90% in all cases.

2.4. Reductive response

The MTT dye [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] method [10] was employed to estimate reductive response. Assay mixtures containing 50 μL of aqueous humor and 300 μL of phosphate buffered saline (PBS; pH 7.3) were incubated with 25 μL of an MTT solution (5.0 mg/mL in PBS) for 3 h at 37 °C. Reactions were stopped by the addition of 1.5 mL of 0.04 M hydrochloric acid in isopropanol, the mixtures centrifuged for 10 min at 200 × *g*, the supernatants collected and their absorbencies measured at 570 nm.

2.5. Oxidative response

A luminol-based chemiluminescence method was employed to determine oxidative response. In each assay, 200 μL of luminol dissolved in 0.4 M dimethyl sulfoxide was mixed with a 100 μL aliquot of a suspension of granulocytes (1 × 105/100 μL) in PBS. In order to determine the activation of ROS production, some assays were carried out in the presence of the protein kinase C (PKC) activator phorbol dibutyrate (10^{-4} M). In other experiments, autologous aqueous humor (50 μL) was added to the assay mixture together with phorbol dibutyrate. In all cases, the final volume of the assay mixture was adjusted to 700 μL with PBS, and chemiluminescence was measured over a 30 min period using a Turner Biosystems model 20/20n Luminometer.