Positive association of pigment epithelium-derived factor (PEDF) with total anti-oxidant capacity in the vitreous fluid of patients with proliferative diabetic retinopathy

Masahiko Yokoi¹², Sho-ichi Yamagishi³, Akari Saito², Yumiko Yoshida³, Takanori Matsui³, Wataru Saito², Shigeki Hirose², Kazuhiro Ohgami², Manabu Kase¹², Shigeaki Ohno⁴

From ¹Department of Ophthalmology, Teine-Keijinkai Hospital, Sapporo, Japan, ²Department of Ophthalmology, Hokkaido University Graduate School of Medicine, Sapporo, Japan, ³Department of Medicine, Division of Cardiovascular Medicine, Kurume University School of Medicine, Kurume, Japan

Corresponding author: M. Yokoi, MD, Department of Ophthalmology, Teine-Keijinkai Hospital, Sapporo, 006-8555, Japan (Tel.: +81-11-681-8111; Fax: +81-11-685-2196; Email: zoyokoi.tdr@keijinkai.or.jp)

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Abstract
Background: We have recently found that pigment epithelium-derived factor (PEDF), a glycoprotein with potent neuronal differentiating activity, inhibits advanced glycation end product-induced retinal hyperpermeability and angiogenesis through its anti-oxidative properties, suggesting that PEDF may exert beneficial effects on diabetic retinopathy by acting as an endogenous anti-oxidant. However, the interrelationship between PEDF and total anti-oxidant capacity in the eye remains to be elucidated. In this study, we determined vitreous PEDF and total anti-oxidant levels in patients with proliferative diabetic retinopathy (PDR), and investigated the relationship between them.

Methods: Vitreous levels of PEDF and total anti-oxidant capacity were measured by an ELISA in 39 eyes of 36 diabetic patients with PDR and in 29 eyes of 29 non-diabetic control subjects.

Results: Vitreous levels of total anti-oxidant capacity were significantly lower in diabetic patients with PDR than in control subjects (0.16±0.05 mmol/l vs 0.24±0.09 mmol/l, p<0.001). PEDF levels were positively correlated with total anti-oxidant status in the vitreous of patients with PDR (r=0.37, p<0.05) and control subjects (r=0.41, p<0.05). Further, vitreous levels of PEDF in PDR patients without vitreous hemorrhages (VH(-)) were significantly (p<0.05) decreased, compared with those in the control or PDR patients with vitreous hemorrhage (VH(+)) (PDR VH(-), 4.5±1.1 µg/ml; control, 7.4±4.1 µg/ml; PDR VH(+) 8.5±3.6 µg/ml)

Conclusion: This study demonstrates that PEDF levels are associated with total anti-oxidant capacity of vitreous in humans. Our observations suggest that PEDF may act as an endogenous anti-oxidant in the eye and could play a protective role against PDR.
Introduction

Pigment epithelium-derived factor (PEDF) is a glycoprotein that belongs to the superfamily of serine protease inhibitors. It was first purified from the conditioned media of human retinal pigment epithelial cells as a factor with potent neuronal differentiating activity.[1] Recently, PEDF has been shown to be a highly effective inhibitor of angiogenesis in cell culture and animal models. PEDF inhibits the growth and migration of cultured endothelial cells (ECs), and it potently suppresses ischemia-induced retinal neovascularization.[2,3] PEDF levels in aqueous humor or vitreous are decreased in diabetic patients, especially with proliferative retinopathy (PDR).[4-6] These observations suggest that the loss of PEDF activity in the eye may contribute to the pathogenesis of PDR.

We have previously shown that advanced glycation end products (AGEs), senescent macromolecular derivatives formed at an accelerated rate under diabetes, elicit oxidative stress and induce vascular inflammation in the eye, thereby being involved in the pathogenesis of PDR.[7-9] Recently, PEDF is reported to inhibit the AGE-signaling pathway to retinal hyperpermeability and angiogenesis by suppressing reactive oxygen species (ROS) generation and subsequent vascular endothelial growth factor (VEGF) overexpression.[7-9] In addition, we have found that vitreous levels of both AGEs and VEGF are significantly higher in diabetic patients than in control subjects, and that their levels are elevated in association with the severity of neovascularization in diabetic retinopathy.[10] We also found in the previous study that vitreous levels of AGEs and VEGF were correlated each other and that both factors were inversely associated with the total anti-oxidant status in the vitreous fluid of non-diabetic controls and patients with diabetic retinopathy.[10] These observations suggest the active participation of oxidative stress in the pathogenesis of PDR and that PEDF may exert beneficial effects on PDR by acting as an endogenous anti-oxidant in the eye. However, the interrelationships between vitreous levels of PEDF and anti-oxidant status in diabetic patients with retinopathy remain to be elucidated.

In this study, we used an enzyme-linked immunosorbent assay (ELISA) to determine PEDF and total anti-oxidant levels in the vitreous fluid of patients with diabetic retinopathy, and investigated the relationship between them.

Patients and methods

Subjects. This study involved 36 diabetic patients (21 men and 15 women) with PDR with a mean age of 52±12 years. Their known duration of diabetes was 10±7.7 years and a current level of HbA1c was 6.7±1.4 % (mean±SD). The diagnosis of diabetes was
made by the criteria of the ADA reported in 1997. According to the previous reports published by Guidry et al [11], we divided PDR patients into two groups; PDR patients with vitreous hemorrhage (VH (+)) (n=32 eyes) and PDR patients without vitreous hemorrhage (VH(-)) (n=7 eyes). Twenty nine non-diabetic patients (6 men and 23 women, mean age of 63±8.4 years) with idiopathic macular hole or epiretinal membrane served as control subjects. The study protocol was approved by our institutional ethics committees. Informed consent was obtained from all subjects.

Measurement of vitreous levels of PEDF and anti-oxidant capacity. Undiluted vitreous samples were obtained during therapeutic vitrectomy from 39 eyes with PDR and 29 eyes as controls. These specimens were collected in sterile tube and stored at –80°C until use. Vitreous levels of PEDF and anti-oxidant capacity were measured with ELISA as described previously.[10,12] Inter- (n=17) and intra-assay (n=14) coefficient of variations of the ELISA were 4.7 and 7.3 %, respectively. Recovery of the added recombinant PEDF in serum samples was 94.2±1.7 % (mean±SD). The assay linearity was shown intact with serial dilution of serum. We also confirmed the specific interaction between the PEDF antibody used for the ELISA and PEDF in the samples with western blot analysis.[12]

Statistical analysis. Data were expressed as mean ± SD. Statistical significance was evaluated using Mann-Whitney U test for paired comparison. Pearson’s correlation coefficient test was used for analysis of correlation between PEDF levels and total anti-oxidant capacity in the vitreous fluid of patients with PDR, and Spearman’s correlation coefficient by rank test for the analysis of the data in controls. A p value of less than 0.05 was considered to be significant.

Results
Total anti-oxidant status in the vitreous was decreased in patients with PDR compared with control subjects (0.16±0.05 mmol/l vs 0.24±0.09 mmol/l, p<0.001) (Figure 1A). There was no significant difference of total anti-oxidant capacity in the vitreous fluid between PDR patients with vitreous hemorrhage and without hemorrhage (0.17±0.05 mmol/l vs 0.13±0.05 mmol/l). Further, when we classified the PDR patients into two groups, sufficiently treated group with panretinal photocoagulation (PRP) (n=16) and insufficiently treated group (no or focal retinal photocoagulation) (n=23), there was no significant difference of vitreous total anti-oxidant capacity between them (0.17±0.05 mmol/l vs 0.15±0.05 mmol/l). In addition, there was no significant correlation between
the vitreous total anti-oxidant status and HbA1c levels (data not shown). As shown in Figure 1B and 1C, total anti-oxidant status in the vitreous fluid was positively correlated with vitreous levels of PEDF in patients with PDR (r=0.37, p<0.05) and in control subjects (r=0.41, p<0.05).

Vitreous PEDF levels in PDR patients were not different from those in the controls (7.8±3.7 µg/ml vs 7.4±4.1 µg/ml). However, vitreous levels of PEDF in PDR patients without vitreous hemorrhage were significantly decreased, compared with those in the controls or PDR patients with vitreous hemorrhage (PDR VH(-), 4.5±1.1 µg/ml; control, 7.4±4.1 µg/ml; PDR VH(+) 8.5±3.6 µg/ml) (Figure 2).

Discussion
In the present study, we demonstrated for the first time that total anti-oxidant status in the vitreous fluid in patients with PDR was significantly decreased, compared with non-diabetic controls and that vitreous levels of PEDF and total anti-oxidant capacity were positively correlated each other both in patients with PDR and in non-diabetic controls. We have previously found that vitreous levels of AGEs and VEGF are correlated each other, both of which are inversely associated with vitreous total anti-oxidant capacity in patients with diabetic retinopathy.[10] Further, PEDF is reported to inhibit the AGE-signaling pathway to VEGF overexpression through its anti-oxidant properties.[8] These findings suggest that PEDF may act as an endogenous anti-oxidant in the eye and could play a protective role against PDR by counteracting the AGE-VEGF axis.

In this study, although PEDF levels in the vitreous fluid of PDR patients with vitreous hemorrhage were not decreased, vitreous levels of PEDF in PDR without vitreous hemorrhage were significantly lower than those in the controls. These findings were consistent with the previous reports to show that vitreous levels of PEDF were decreased in diabetic retinopathy, especially in PDR.[4,5] The contamination of PEDF from the circulating blood may affect the vitreous levels of PEDF in PDR patients with vitreous hemorrhage. Oxidative stress generation may be involved in retinal PEDF down-regulation in diabetic retinopathy because AGEs or H2O2 suppresses PEDF gene expression in microvascular ECs and that an anti-oxidant N-acetylcysteine restores high glucose-induced decrease in PEDF gene expression in cultured retinal pericytes. [8,13,14] Moreover, intravenous administration of AGEs induces oxidative stress and subsequently decreases retinal PEDF expression as well.[8] These observations suggest that decreased retinal PEDF levels in diabetic retinopathy further enhance oxidative stress generation in the eye, thus exacerbating diabetic retinopathy. Taken together, our
present study suggests that vitreous levels of PEDF may be one of the biomarkers of oxidative stress in the eye and pharmacological up-regulation or substitution of PEDF may offer a novel therapeutic strategy for the treatment of PDR.
Figure legend

Figure 1. (A) Total anti-oxidant capacity of the vitreous fluid of patients with PDR and non-diabetic controls. Bars show mean values. Vitreous total anti-oxidant capacity were significantly lower in diabetic patients with PDR than in control subjects (0.16±0.05 mmol/l vs 0.24±0.09 mmol/l, p<0.001). Scatter plots of vitreous PEDF levels versus total anti-oxidant capacity in patients with PDR (B) and in non-diabetic controls (C). PEDF levels were positively correlated with total anti-oxidant status in the vitreous of patients with PDR (r=0.37, p<0.05) (B) and the controls (r=0.41, p<0.05) (C).

Figure 2. Vitreous levels of PEDF in the control, PDR patients without vitreous hemorrhage (VH(-)) and PDR patients with vitreous hemorrhage (VH(+)). Vitreous PEDF levels in PDR with VH (-) was significantly lower than those in the control (4.5±1.1 µg/ml vs 7.4±4.1 µg/ml, p<0.05) or PDR patients with VH(+) (4.5±1.1 µg/ml vs 8.5±3.6 µg/ml, p<0.01)
References


Figure 1

A

Total antioxidant capacity

mmol/l

0.4

0.3

0.2

0.1

0

control

PDR

p < 0.001

B

Total antioxidant capacity

mmol/l

0.4

0.3

0.2

0.1

0

PEDF

μ g/ml

C

Total antioxidant capacity

mmol/l

0.4

0.3

0.2

0.1

0

PEDF

μ g/ml
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