Angiogenesis is the major feature in the pathogenesis of proliferative diabetic retinopathy (PDR).\(^1\) In this condition, retinal neovascularisation has a catastrophic effect on vision by causing vitreous haemorrhage, retinal detachment with formation of a fibrovascular membrane, and eventual blindness.\(^2\) The factors that stimulate the growth of retinal blood vessels have not been fully defined, but circumstantial evidence indicates that this not only involves angiogenic cytokines such as vascular endothelial growth factor (VEGF) but also vasoactive hormones such as angiotensin II.\(^3\)

Angiotensin II has a proliferative effect and has been reported to regulate the growth of vascular smooth muscle cells\(^4\) and to stimulate the induction of various growth factors.\(^5\) Recent studies have suggested that abnormalities of the renin-angiotensin system (RAS) may also play a part in the progression of diabetic retinopathy.\(^6\) Inhibition of angiotensin converting enzyme (ACE) has been reported to be associated with a reduction of PDR,\(^7\) suggesting that suppression of the RAS may be of value for preventing and treating retinal neovascularisation. The hypothesis that an ocular RAS is involved in the development of PDR is supported by evidence that all components of the RAS are present in the retina\(^8\) and that angiotensin II, the effector molecule of this system, has angiogenic activity.\(^9\)\(^10\)

These findings prompted us to examine whether angiotensin II plays a part in the development of PDR in combination with VEGF, which is considered to be the most potent factor in promoting angiogenesis. Therefore, we investigated the relation between the levels of angiotensin II and VEGF in the vitreous fluid of diabetic patients as well as the correlation between these factors and the severity of PDR. The present study revealed that angiotensin II and VEGF levels in the vitreous fluid were correlated with the severity of PDR and that the vitreous levels of these two molecules were also correlated with each other. Furthermore, both angiotensin II and VEGF were elevated in the active stage of PDR. Angiotensin II may induce neovascularisation via a paracrine effect on VEGF in diabetic patients with PDR.

**MATERIALS AND METHODS**

**Patients**

Undiluted vitreous fluid samples were harvested at the start of vitrectomy after informed consent was obtained from each subject following an explanation of the purpose and potential adverse effects of the procedure. This study was performed in accordance with the 1975 Declaration of Helsinki, as revised in 1983. Vitreous fluid samples were obtained from 51 patients with PDR, six diabetic patients without diabetic retinopathy, and 16 non-diabetic patients with ocular disease. Vitrectomy was performed on the 51 patients with PDR for the following reason: 27 had vitreous and/or preretinal haemorrhage, 17 had retinal detachment, and seven had macular heterotropia with proliferative tissues. The cases with macular heterotropia hoped to undergo the surgery because of the disturbed vision. The six diabetic patients without diabetic retinopathy included four with macular hole and two with epiretinal membrane, while the 16 non-diabetic patients included 12 with macular hole and four with epiretinal membrane (none of these 16 patients had proliferative vitreoretinopathy). Exclusion criteria for this study were: (1) treatment with an ACE inhibitor or an angiotensin II receptor antagonist (ARA), (2) major ocular surgery within the previous 3 months, (3) previous vitrectomy, (4) treatment with a systemic steroid, (5) age less than 18 years, (6) pregnancy, (7) any other ocular disease except PDR, and (8) squint.

**Methods**

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**Results**

The vitreous fluid levels of VEGF and angiotensin II were significantly higher in patients with PDR than in diabetic patients without retinopathy (all p<0.0001). The vitreous fluid level of angiotensin II was significantly correlated with that of VEGF (p<0.0001), and the vitreous concentrations of both VEGF and angiotensin II were significantly higher in patients with active PDR than in those with quiescent PDR (p<0.001 and p=0.0005, respectively).

**Conclusion**

The authors found that both angiotensin II and VEGF levels were significantly higher in the vitreous fluid of patients with PDR than in those of non-diabetic patients or diabetic patients without retinopathy, and that the levels of both angiotensin II and VEGF were elevated in the active stage of PDR. These findings suggest that angiotensin II contributes to the development and progression of PDR in combination with VEGF.
Plasma was rapidly frozen at −80°C. All samples were obtained at the time of vitreoretinal surgery, with the protocol for sample collection being approved by the institutional review board and with all patients giving informed consent.

Statistical analysis

All analyses were performed with SAS System 6.12 software (SAS Institute Inc, Cary, NC, USA). Data are presented as the frequency or mean (SD). Data with a skewed distribution were transformed to a logarithmic scale, and the geometric mean was calculated together with 1 SD below and 1 SD above the mean on that scale. Analysis of variance (ANOVA) was used to test for statistically significant differences among the groups and the Turkey-Kramer multiple comparison test was also applied when appropriate. Correlations were tested using Spearman’s rank correlation coefficients. A two tailed p value of less than 0.05 was considered to indicate statistical significance.

RESULTS

Vitreous levels of VEGF and angiotensin II

The diabetic patients included 31 men and 26 women, mean age 60.7 (SD 10.5) years, with a diabetes duration of 16.2 (6.3) years. The PDR patients included 31 men and 26 women, mean age 60.7 (SD 10.5) years, with a diabetes duration of 16.2 (6.3) years. The NDR patients included 31 men and 26 women, mean age 60.7 (SD 10.5) years, with a diabetes duration of 16.2 (6.3) years.

Integrating these findings, we observed a significant correlation between vitreous VEGF levels and the severity of diabetic retinopathy, particularly in patients with PDR. This suggests a potential role for VEGF in the pathogenesis of diabetic retinopathy.

Figure 1  [A] VEGF concentrations in the vitreous fluid of non-diabetic patients (NDM), diabetic patients without retinopathy (NDR), and PDR patients (PDR) (*p<0.0001). [B] Angiotensin II concentrations in the vitreous fluid of non-diabetic patients (NDM), diabetic patients without retinopathy (NDR), and PDR patients (PDR) (*p<0.0001).
years and an Hba1c level of 7.6% (2.3%). The 16 patients with non-diabetic ocular disease included eight men and eight women aged 63.4 (6.5) years. There was no significant difference in age between the diabetic and non-diabetic patients (p=0.2364). Vitreous fluid concentrations of VEGF were significantly elevated in the samples from patients with PDR (1135.2 pg/ml (75.6 to 3280.0)) when compared with the samples from non-diabetic patients (19.3 pg/ml (15.6 to 40.6)) (p<0.0001) or diabetic patients without retinopathy (49.9 pg/ml (23.4 to 105.0)) (p<0.0001) (Fig 1A). There was a significant relation between the vitreous fluid concentration of VEGF and that of angiotensin II (ρ=0.702, p<0.0001) (Fig 2). Furthermore, the vitreous fluid concentrations of VEGF and angiotensin II in the patients with active PDR (VEGF: 1697.3 pg/ml (107.0 to 3280.0); angiotensin II: 30.4 pg/ml (6.0 to 76.0)) were significantly higher (p<0.0001) than in the patients with quiescent PDR (VEGF: 332.2 pg/ml (75.6 to 968.0); angiotensin II: 17.4 pg/ml (4.0 to 48.0)) (Fig 3A and B).

Vitreous and plasma levels of VEGF, angiotensin II, and ACE

The vitreous fluid concentration of VEGF was significantly higher than the plasma VEGF level (50.9 pg/ml (15.6 to 396.0)) in the patients with PDR (p<0.0001) (Table 1). The vitreous fluid concentration of angiotensin II was also significantly higher than the plasma angiotensin II level (17.5 pg/ml (4.0 to 46.0)) in the PDR patients (p =0.0106) (Table 1). Plasma angiotensin II levels showed a significant correlation with the vitreous fluid levels of both VEGF and angiotensin II (p=0.596, p<0.0001 and p=0.755, p<0.0001, respectively). The plasma ACE level (14.2 pg/ml (2.5 to 26.0)) was significantly correlated with the vitreous fluid level of angiotensin II (p=0.372, p=0.0013), but was not significantly correlated with that of VEGF (p=0.223, p=0.0594). There was no significant relation between plasma and vitreous fluid VEGF levels (p=0.128, p=0.1490). There was also no significant relation between Hba1c (7.6 % (4.8 to 12.3)) and vitreous fluid levels of VEGF or angiotensin II (p=0.220, p=0.1712 and p=0.253, p=0.0626, respectively).

**DISCUSSION**

The present study showed that both angiotensin II and VEGF levels were increased in the vitreous fluid of patients with PDR and were correlated with the severity of diabetic retinopathy. In addition, angiotensin II and VEGF showed a statistically significant correlation with each other and the vitreous fluid level of angiotensin II was elevated in the active stage of PDR. We showed that not only the vitreous level of VEGF but also that of angiotensin II was significantly elevated in PDR patients when compared with non-diabetic patients or diabetic patients without retinopathy. Angiotensin II has been shown to promote the growth of capillary vessels in the chorioallantoic membrane and to stimulate new vessel formation in the rabbit cornea. A protective effect of an ACE inhibitor and of an ARA AT1 receptor on hyperoxia induced and normoxia induced neovascularisation has been demonstrated in newborn mice. However, continuous transvitreal infusion of angiotensin II alone produced retinal artery constriction, but not new vessel formation from the retina to the vitreous in the cat eye. Angiotensin II not only has a growth promoting effect, but also stimulates the induction of many cytokines and growth factors. Therefore, angiotensin II may affect neovascularisation in combination with other cytokines or growth factors.

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Comparison of VEGF and angiotensin II concentrations in vitreous fluid and plasma in diabetic patients with PDR</th>
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<tbody>
<tr>
<td></td>
<td>Vitreous fluid</td>
</tr>
<tr>
<td>VEGF</td>
<td>1135.2 (837.7)</td>
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<tr>
<td>Angiotensin II</td>
<td>25.0 (14.3)</td>
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**Figure 3** | A) VEGF levels in the vitreous fluid of patients with active PDR and quiescent PDR (*p<0.0001).  
   | B) Angiotensin II levels in the vitreous fluid of patients with active PDR and quiescent PDR (*p=0.0005).
In the present study, the vitreous level of angiotensin II was statistically correlated with that of VEGF. It has been suggested that induction of VEGF mRNA most probably occurs through transcriptional regulation. Receptors for angiotensin II are present on endothelial cells, and angiotensin II acts to stimulate endothelial cell growth and upregulate VEGF mRNA expression. Moreover, angiotensin II may potentiate VEGF induced angiogenic activity in the retina through increased expression of the VEGF receptor Flk-1/KDR. The effect of angiotensin II on VEGF expression was completely inhibited by an ARA. There is a possibility that angiotensin II might influence VEGF elicited signal transduction or post-transcriptional regulation of KDR. The functioning of the ocular RAS is not yet clear. However, our results and previous studies have suggested that an autocrine-paracrine relation may exist between angiotensin II and VEGF in ocular tissues. The capacity of VEGF to act as a potent angiogenic agent suggests that an angiotensin II induced increase of VEGF production could have a key role in the occurrence of neovascularisation in PDR. Further investigations will be needed to clarify the ocular interactions between angiotensin II and VEGF as well as the role of angiotensin II during neovascularisation in PDR.

It remains unclear for the vitreous fluid levels of angiotensin II and VEGF to vary with the severity of PDR. In fact, we found that the vitreous fluid levels of both angiotensin II and VEGF were significantly higher in active PDR than in quiescent PDR. The levels of both angiotensin II and VEGF in the vitreous fluid seem to increase during active neovascularisation and to decrease in the absence of neovascularisation, because we classified the severity of PDR according to the activity of neovascularisation in this study. It was previously reported that the vitreous fluid level of VEGF was higher in active PDR than in quiescent PDR and that VEGF played a major part in mediating intraocular neovascularisation in diabetic retinopathy. However, to our knowledge, the present study provides the first evidence that the vitreous fluid levels of angiotensin II are elevated in the active stage of PDR.

It is still unclear whether production of angiotensin II can occur in ocular tissues. From our results, it cannot be said whether ocular angiotensin II is located intracellularly or extracellularly and it is also impossible to determine whether angiotensin II is synthesised locally in the eye or sequestered from the plasma. Sequestration is not very likely since that would imply a specific uptake process. The local concentration of angiotensin II in the retinal microvasculature is reported to be higher than the serum and vitreous fluid levels. In the present study, the vitreous fluid level of angiotensin II was significantly higher than the plasma level, but the statistical difference was small. Furthermore, the plasma levels of angiotensin II and ACE were significantly correlated with the vitreous fluid level of angiotensin II. The level of angiotensin II in the ocular fluid from normal porcine eyes is low to undetectable, in contrast with the relatively high levels in surrounding ocular tissues such as the RPE and choroids. Breakdown of the blood-retinal barrier (BRB) may facilitate diffusion of angiotensin II from the blood into the vitreous fluid. Since the vitreous can be considered the repository for products originating from the retina, a high level of angiotensin II might well be explained by its production and secretion from the retina. Accordingly, angiotensin II may be produced locally in ocular tissues, but little of this angiotensin II may leak into the ocular fluid under normal conditions and only when the BRB is disrupted will angiotensin II reach the vitreous fluid in high concentrations. The patients with active PDR in the present study had a hyperfluorescein pattern on FAG just before surgery. These results and previous reports suggest that disruption of the BRB may lead to elevation of the vitreous fluid concentration of angiotensin II. ACE inhibitors have been reported to maintain the BRB in diabetic patients and to have a favourable effect on diabetic retinopathy.
Angiotensin II and VEGF in vitreous fluid


