Aqueous Humor Levels of Vascular Endothelial Growth Factor in Retinitis Pigmentosa

David Salom,1 Manuel Diaz-Llopis,1,2,5 Salvador García-Delpech,1 Patricia Udaondo,1 Maria Sancho-Tello,2 and F. Javier Romero2,4

PURPOSE. To determine the level of vascular endothelial growth factor A (VEGF-A) in aqueous humors of patients with retinitis pigmentosa (RP).

METHODS. A prospective, comparative control study. Aqueous humor was collected from 16 eyes of 16 patients with RP. The level of VEGF-A was determined with a commercially available enzyme-linked immunosorbent assay kit. The control group comprised 16 aqueous samples from 16 patients about to undergo cataract surgery and without any other ocular or systemic diseases.

RESULTS. The concentration of VEGF-A in aqueous humor was markedly lower in patients with RP than in control subjects (Mann-Whitney U test, P < 0.001). The level of VEGF-A was 94.9 ± 99.8 (mean ± SD) pg/mL in eyes with RP and 336.5 ± 116.8 pg/mL in the eyes of the control group.

CONCLUSIONS. In patients with RP, the concentration of VEGF-A in aqueous humors is lower than in non-RP subjects. The lack of angiogenic actions attributed to VEGF-A may explain some of the clinical manifestations of this disease, such as narrowing and fibrotic degeneration of retinal blood vessels. (Invest Ophthal Vis Sci. 2008;49:3499–3502) DOI:10.1167/iovs.07-1168

Retinitis pigmentosa (RP) is the most common cause of retinal degeneration, with a prevalence of approximately 1 in 4000.1 Visual impairment in RP is primarily due to loss of photoreceptors, which leads to subsequent damage of the retinal pigment epithelium (RPE) and other layers of the retina.2 RP is a highly variable disorder, with some patients experiencing only sectorial visual field loss and others suffering a profound loss of peripheral visual field, which is, in turn, associated with various degrees of central macular function loss. Changes in retinal vasculature are prominent clinical features, with attenuation of retinal vessels in early stages and fibrotic degeneration in later stages of the disease (Fig. 1).

The group of diseases included under the term RP is genetically heterogeneous but phenotypically similar, with more than 50 genes having been identified (http://www.sph.uth.tmc.edu/Retnet; provided in the public domain by the University of Texas Houston Health Science Center, Houston, TX). Because of the genetic and functional diversity of the proteins involved, the molecular mechanisms underlying the different forms of RP are still unclear. Apoptosis is reported to be the final and common cause of photoreceptor degeneration in all the RP animal models and patients analyzed to date, and the apoptotic pathways engaged in the process have recently been defined.3 Neuroprotective treatments that interfere with apoptosis have the advantage of being less dependent on the disease-causing mutation than are genetic strategies and are therefore widely applicable.1–6 Findings in animal studies have shown that some neurotrophic factors aid photoreceptor survival.7,8

Vascular endothelial growth factor-A (VEGF-A) was initially identified as an endothelial cell mitogen and vascular permeability factor and has recently been shown to influence neuronal growth, differentiation, and survival.9–13 Low VEGF-A levels have been linked to motor neuron degeneration in both animal and human models,14,15 suggesting that VEGF-A plays an important role in neuronal development and maintenance within the central nervous system. Previous reports have shown that the receptors for VEGF-A are present in normal retinal neuron cells,16–18 and a model of ischemia reperfusion injury has demonstrated that VEGF-A exposure results in a dose-dependent reduction in retinal neuron apoptosis, thereby indicating a direct neuroprotective effect for VEGF-A.19

Understanding the role of VEGF-A in the pathogenesis of the disease may aid research into neuroprotective treatments for RP. The objective of this study was to measure quantitatively the levels of VEGF-A in the aqueous humors of patients with RP and compare them with those of healthy control subjects.

METHODS

In this prospective, comparative control study, we investigated the levels of VEGF-A in aqueous humors of patients with RP. Aqueous samples from cataract patients without any other ocular or systemic diseases were collected as control specimens. The study protocol complied with the provisions of the Declaration of Helsinki and was reviewed and approved by the Ethics Committee of the General University Hospital of Valencia, Spain. Informed consent was obtained from each subject.

Patients were enrolled in the Retinitis Pigmentosa Reference Unit of the Valencian Community in the General University Hospital of Valencia between January and June 2007. Undiluted aqueous humor samples were collected from 16 eyes of 16 individuals with typical forms of RP and with no other confounding ocular or systemic disease. The patients were at least 18 years of age and displayed the typical forms of RP, which are characterized by an elevated final dark-adaptation threshold, retinal arteriolar narrowing, and a reduced and delayed electroretinogram. Patients with syndromic forms of RP, such as Laurence-Moon-Bardet-Biedl syndrome and Usher syndrome, were excluded. The age of patients at diagnosis and the clinical characteristics of the retinal vascular degeneration were recorded (see Table 2).

Aqueous humor samples from 16 eyes of 16 age-matched control patients about to undergo surgery for cataracts were obtained as control specimens.
Aqueous samples of patients with RP were collected under sterile conditions by slit lamp, with the aid of one drop of povidone iodine before and after the puncture of the anterior chamber. Antibiotic prophylaxis was subsequently used for several days. The aqueous humors of control patients were collected with a 30-gauge needle before cataract surgery was initiated. Undiluted aqueous samples of at least 0.05 mL were collected from each patient and placed in sterile tubes and were stored immediately at −80°C until use. The specimens were classified and labeled in a masked fashion. All specimens were assayed for VEGF-A in a double-blind arrangement with respect to their group.

An enzyme-linked immunosorbent assay (ELISA) of aqueous humor samples was performed to quantify the levels of VEGF-A using a gene array (Searchlight Human Angiogenesis Array; Pierce Biotechnology, Inc., Woburn, MA), which is designed to detect both the VEGF121 and VEGF165 isoforms of VEGF-A. The sensitivity of the VEGF assay is 4.9 pg/mL. All procedures were performed according to the manufacturer’s manual (http://www.piercenet.com/files/1601463%20arraybrochure.pdf).

Demographic characteristics of the patients were summarized with descriptive statistics (SPSS for Windows; SPSS Inc. Chicago, IL). The Mann-Whitney U test for independent samples was used to compare the VEGF-A levels of the groups. P < 0.05 was accepted as significant.

RESULTS

Thirty-two aqueous humor samples were collected from 16 patients with RP and 16 control patients. All participants were of Spanish nationality. No statistically significant differences were found between the mean ages of the RP (mean ± SD; 51.6 ± 12.5 years, range 35–79) and control (59.2 ± 11.2 years, range 44–77) groups (independent-samples t-test, P = 0.08; Table 1). Table 2 shows detailed information for every patient.

The aqueous humor level of VEGF-A was 94.9 ± 99.8 pg/mL in eyes with RP and 336.5 ± 116.8 pg/mL in control eyes. The VEGF-A levels in the two groups differed significantly (Mann–Whitney U test, P < 0.001), with those of the RP patients proving to be significantly lower than those of the control subjects (Fig. 2; Table 1).

DISCUSSION

The acknowledgment that VEGF-A levels are lower in patients with especially severe forms of RP than in healthy control subjects raises two important questions: the reason for this difference and its consequences for the patients.

We proposed that two aspects exert a combined action, which leads to a reduction of the level of VEGF-A in RP: on the one hand, a loss of RPE cells, which constitute an important source of VEGF in the eye20,21; on the other hand, the relative retinal hyperoxia caused by photoreceptor degeneration.22 Several in vivo studies have revealed that, in a normal eye, VEGF-A is produced constitutively by RPE cells,20,21 whereas VEGF expression in the RPE is known to be essential for the development of choriocapillaris and visual function.23 Retinal degeneration resulting from defects in genes normally expressed in photoreceptors also leads to the degeneration of the

<table>
<thead>
<tr>
<th>Disease Group</th>
<th>Age (y) Mean ± SD (range)</th>
<th>Sex (M:F)</th>
<th>Aqueous Humor Level of VEGF-A Mean ± SD (pg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>RP (n = 16)</td>
<td>51.6 ± 12.5 (35–79)</td>
<td>(9:7)</td>
<td>94.9 ± 99.8</td>
</tr>
<tr>
<td></td>
<td>P = 0.08</td>
<td></td>
<td>P &lt; 0.001*</td>
</tr>
<tr>
<td>Control (n = 16)</td>
<td>59.2 ± 11.2 (44–77)</td>
<td>(10:6)</td>
<td>336.5 ± 116.8</td>
</tr>
</tbody>
</table>

* Compared with control group. The sensitivity of the ELISA test used is 4.9 pg/mL.
The apoptosis of photoreceptors due to the different genetic mutations involved in RP leads to RPE cell death, which may contribute to lower VEGF expression. Adult mice exposed to hyperoxia, which also increases oxygen in the retina, showed a decreased expression of VEGF in the retina. Furthermore, rd mice, an animal model of RP, have been shown to display a decreased expression of VEGF in the retina. The investigators in that study believed that this was because photoreceptor cell death caused a decrease in oxygen usage and thinning of the retina, generating a relative hyperoxia in the inner retina, which, in turn, reduced VEGF expression by retinal cells such as pericytes, endothelial cells, glial cells. Müller cells, and ganglion cells. Of interest, another report demonstrated that neonatal mice with classic inherited retinal degeneration (Pdebrd1/Pdebrd1) failed to mount reactive retinal neovascularization in a mouse model of oxygen-induced proliferative retinopathy associated with an absence of the expected VEGF upregulation in the retina. The same study reported that a patient displayed spontaneous regression of retinal neovascularization, associated with long-standing diabetes mellitus, when RP became clinically evident. Both mouse and human data support the hypothesis that O2 consumption by rod cells is a major driving force in ischemic retinal neovascularization and controls VEGF production.

We believe that the low levels of VEGF-A in RP has two major consequences. The first of these is the degeneration of the retinal blood vessels and choriocapillaris, which is evident even in the early stages of the disease and constitutes one of the most important clinical findings in the clinical evaluation of patients with RP. The second is the undermining of the neuroprotection exerted by VEGF-A over different retinal neuron cells. Recently, a direct neuroprotective effect of VEGF-A over an ex vivo retinal culture has been demonstrated. The isoform responsible for neuroprotection was shown to be VEGF120, which has thus been proposed as the most suitable isoform of VEGF-A for therapeutic neuroprotection. VEGF receptor 2 (VEGFR2) has also been demonstrated to be involved in retinal neuroprotection. Neuronal cells in the ganclion cell layer (GCL) and in the inner nuclear layer (INL) of retinas after ischemia express VEGFR2, whereas photoreceptor cells in the outer nuclear layer (ONL) test negative for VEGFR2 expression. It is logical to assume that patients with RP who have low levels of VEGF-A would not benefit from its neuroprotective effects on the retinal neurons that express VEGFR2.

On the other hand, four of our patients with RP were shown to have almost normal VEGF-levels (patients 6, 7, 8, and 12). These patients had the mildest clinical phenotype, and showed a good preservation of retinal vascularization and macular function. Moreover, their disease became clinically evident, later in their lives, at the ages of 55, 46, 64, and 50, respectively.

We believe that there are many other factors that may contribute to the pathogenesis of RP and that the low levels of VEGF-A levels in the aqueous humors of 16 eyes with retinitis pigmentosa (RP) and 16 control eyes. Arrow: mean VEGF concentration in each group. VEGF levels were significantly different in the two groups (Mann-Whitney U test, P < 0.001), with those of RP patients being significantly lower than those of control subjects.

**Table 2. Detailed Data of Patients**

<table>
<thead>
<tr>
<th>Patients</th>
<th>Age (y)</th>
<th>Sex</th>
<th>Aqueous Humor Level of VEGF-A (pg/mL)</th>
<th>Age at Diagnosis (y)</th>
<th>Retinal Vascular Degeneration</th>
<th>Age (y)</th>
<th>Sex</th>
<th>Aqueous Humor Level of VEGF-A (pg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>40</td>
<td>F</td>
<td>24.98</td>
<td>26</td>
<td>Severe</td>
<td>70</td>
<td>M</td>
<td>231.52</td>
</tr>
<tr>
<td>2</td>
<td>35</td>
<td>M</td>
<td>27.38</td>
<td>8</td>
<td>Severe</td>
<td>70</td>
<td>M</td>
<td>283.77</td>
</tr>
<tr>
<td>3</td>
<td>43</td>
<td>F</td>
<td>51.22</td>
<td>32</td>
<td>Severe</td>
<td>64</td>
<td>M</td>
<td>190.87</td>
</tr>
<tr>
<td>4</td>
<td>38</td>
<td>M</td>
<td>129.00</td>
<td>21</td>
<td>Severe</td>
<td>71</td>
<td>F</td>
<td>213.07</td>
</tr>
<tr>
<td>5</td>
<td>47</td>
<td>F</td>
<td>46.22</td>
<td>27</td>
<td>Severe</td>
<td>55</td>
<td>F</td>
<td>495.27</td>
</tr>
<tr>
<td>6</td>
<td>68</td>
<td>M</td>
<td>277.71</td>
<td>55</td>
<td>Moderate</td>
<td>44</td>
<td>M</td>
<td>320.18</td>
</tr>
<tr>
<td>7</td>
<td>49</td>
<td>F</td>
<td>256.02</td>
<td>46</td>
<td>Mild</td>
<td>55</td>
<td>F</td>
<td>511.53</td>
</tr>
<tr>
<td>8</td>
<td>79</td>
<td>M</td>
<td>260.99</td>
<td>64</td>
<td>Moderate</td>
<td>77</td>
<td>M</td>
<td>623.65</td>
</tr>
<tr>
<td>9</td>
<td>58</td>
<td>M</td>
<td>98.11</td>
<td>35</td>
<td>Moderate</td>
<td>75</td>
<td>F</td>
<td>324.25</td>
</tr>
<tr>
<td>10</td>
<td>45</td>
<td>F</td>
<td>44.29</td>
<td>26</td>
<td>Severe</td>
<td>69</td>
<td>M</td>
<td>383.85</td>
</tr>
<tr>
<td>11</td>
<td>47</td>
<td>M</td>
<td>24.97</td>
<td>30</td>
<td>Severe</td>
<td>52</td>
<td>M</td>
<td>419.52</td>
</tr>
<tr>
<td>12</td>
<td>70</td>
<td>M</td>
<td>216.41</td>
<td>50</td>
<td>Mild</td>
<td>50</td>
<td>M</td>
<td>266.40</td>
</tr>
<tr>
<td>13</td>
<td>40</td>
<td>M</td>
<td>9.80</td>
<td>28</td>
<td>Severe</td>
<td>46</td>
<td>F</td>
<td>234.07</td>
</tr>
<tr>
<td>14</td>
<td>55</td>
<td>F</td>
<td>22.58</td>
<td>14</td>
<td>Severe</td>
<td>52</td>
<td>M</td>
<td>451.95</td>
</tr>
<tr>
<td>15</td>
<td>53</td>
<td>F</td>
<td>19.58</td>
<td>34</td>
<td>Severe</td>
<td>48</td>
<td>M</td>
<td>377.77</td>
</tr>
<tr>
<td>16</td>
<td>59</td>
<td>M</td>
<td>9.80</td>
<td>29</td>
<td>Severe</td>
<td>50</td>
<td>M</td>
<td>256.47</td>
</tr>
</tbody>
</table>
VEGF-A reflects the decreased production of VEGF due to neurodegeneration rather than being the driving force in RP. By demonstrating this presence of low levels of VEGF-A in RP, we have thrown light on areas that, until now, remained obscure, thus helping to comprehend further this devastating disease and aiding research toward the development of neuroprotective treatments.

References