Retinal detachment: visual acuity and subretinal immunoreactive endothelin-1

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INTRODUCTION

At present, surgery is the only effective treatment for retinal detachment (RD). However, an early and ophthalmoscopically successful retinal reattachment often fails to restore normal visual capacity [1]. Even in cases of successful reattachment surgery, patients often describe permanent defects in color vision and a decline in visual acuity [2]. Macular function has been found to be depressed even in cases of purely peripheral detachment [1, 3].

Experimental detachment of the neural retina from the pigment epithelium causes complex alterations and remodeling of the retinal tissue, in addition to the initial damage to the outer segments and the apoptotic death of photoreceptor cells [4]. There are morphologic and biochemical alterations of the inner retinal neurons [5] as well as fast activation of pigment epithelial cells and macro- and microglial cells [6, 7]. Reactive gliosis on detachment has been suggested as a clinically significant limiting factor in the recovery of vision after reattachment [8, 9].

We recently described endothelin-1 (ET-1) immunoreactivity in the photoreceptor inner segments [10] — where ET-1 binding sites have been also localized [11] — of the human retina, suggesting that ET-1 in the photoreceptor terminal may play a role in neuromodulation or neurotransmission.

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Purpose. To analyze whether subretinal (SRF) endothelin-1 (ET-1) — a vasoactive, mitogenic, and pro-apoptotic peptide — levels are related to visual acuity (VA) in rhegmatogenous retinal detachment (RD).

Patients and methods. Sixty-six healthy patients between 42 and 70 years of age with unilateral RD, all candidates for scleral buckling surgery (PVR < C3) as a first and single surgical procedure, were prospectively selected. Using radioimmunoassay, immunoreactive ET-1 (IR-ET-
We reported ET-1 immunoreactivity on the cell body of the astrocytes and both ET-1 mRNA and ET-1 immunoreactivity on the RPE cells [10]. Elevated subretinal fluid (SRF) [12] and vitreous [13] immunoreactive ET-1 (IR-ET-1) levels were associated with RD and proliferative vitreoretinopathy (PVR). IR-ET-1 was localized in the cellular and stromal components of PVR membranes, and furthermore, ETA and ETB receptor expression has been demonstrated in these membranes [13].

ET-1 can modulate anterograde fast axonal transport, which is essential for maintaining synaptic function and neuronal survival [14]. Recently, experimental evidence that stimulation of endothelinergic receptors may modulate photoreceptor survival and glial activation has been provided [15].

The aim of the current study was to analyze whether SRF and/or plasma IR-ET-1 (a vasoactive, mitogenic, and pro-apoptotic peptide) levels are related to visual acuity (preoperative VA, postoperative VA, and the postoperative minus preoperative difference) in RD with PVR (and without PVR).

**PATIENTS AND METHODS**

A prospective, consecutive, nonrandomized, intervention comparative, clinical cross-sectional study was conducted after approval from our institutional clinical research committee, in accordance with the principles embodied by the Declaration of Helsinki. Informed consent was obtained from patients after the nature of the study was explained.

**Patient population**

Based on our earlier plasma IR-ET-1 values (No-PVR group: 2.7±0.06 pg/ml; PVR group: 2.95±0.73 pg/ml) [13], at least 32 eyes (α=0.01 and β=0.86) had to be included in each group of the study to obtain significant differences. Therefore, 70 patients with RD — 35 with PVR (PVR group) and 35 with no PVR (No-PVR group) — were consecutively selected.

Inclusion criteria were age between 42 and 70 years, mean arterial blood pressure (MAP) ≤105 mmHg, unilateral rhegmatogenous retinal detachment, best corrected Snellen VA ≥20/200 (≥0.1), IOP ≤16 mmHg (and no known IOP alterations), refraction defect ≤3 D (spheric and/or cylinder), no systemic or other ocular diseases, and being a candidate for scleral buckling surgery (PVR ≤C3) as a first and single surgical procedure. Patients were prospectively selected for the study from July 1st 1998 to January 31st 2005. During the same time period, a total of 1,976 cases arrived at our hospital as emergencies and/or referred. Preoperative PVR was graded according to the Retinal Society Classification [16]. The retina of the patients included in the study had to remain attached during the study follow-up period (8 months).

Exclusion criteria were PVR>C3 (in these cases we indicate vitrectomy), MAP>105 mmHg; best corrected Snellen VA <20/200 (<0.1); IOP >16 mmHg, refractive defect >3 D; blunt trauma affecting the eye within the last 6 months; concurrent eye conditions such as infection, intraocular eye surgery, aphakia, pseudophakia, or previous retinal detachment; and/or current topical therapy. Patients with cardiovascular, hematological, metabolic disorders, and/or current systemic treatment were also excluded. Finally, if retina redetachment occurred during the follow-up period (8 months), the case was excluded.

At the end, four of the selected (n=70) RD patients had to be excluded for two reasons: blood contamination of subretinal fluid samples (during the surgical drainage procedure) in two of them and not showing at the last follow-up visit in other two patients.

A total of 66 healthy patients (42-70 years) with unilateral RD (33 with PVR and 33 with no PVR) with preoperative best corrected Snellen VA ≥20/200 (≥0.1), preoperative IOP ≤16 mmHg, MAP ≤105 mmHg, preoperative refractive defect ≤3 D, retina reattached after scleral buckling surgery — always performed by the same surgeon — with 8 months of follow-up and recorded final (8 months after surgery) best corrected Snellen VA were studied.

**Observation procedures and outcome measures**

The patients were given a complete ophthalmological examination before retinal reattachment surgery and their usual follow-up at 1 week, and at 1, 3 and 6 months after surgery. MAP was calculated by the mean of systolic and diastolic blood pressures measured...
on the upper arm between 7 am and 9 am on the day of surgery. Best corrected Snellen VA (decimal values) and retinal status were recorded before and 8 months after surgery for our study.

Venous blood samples (10 ml) were obtained prior to the anesthetic procedure for the scleral buckling surgery. SRF was obtained during the drainage procedure of retinal reattachment surgery [17], as previously reported [12]. Briefly, after 1 mm sclerotomy and local diathermic coagulation of the choroid at the site of maximum prominence of the detached retina, a plastic disposable fire tip transfer pipet (Life Scan Inc., Milpitas, CA, USA) was used to obtain the SRF. Both SRF and blood samples were collected in chilled tubes containing protease inhibitors [12, 13]. Blood samples were centrifuged at 3,000 rpm for 20 min at 4º C plasma. SRF samples were centrifuged at 13,000 rpm for 15 min at 4º C. The supernatants were stored at —70º C for less than 3 months before testing.

The ET-1 extraction with Sep-Pack C18 cartridge (Waters Associates, Milford, MA, USA) and IR-ET-1 measurements were carried out in plasma and SRF samples, as previously reported [12, 13]. Briefly, IR-ET-1 was assayed in the laboratory by RIA, using polyclonal antibody against synthetic ET-1 (Peninsula Laboratories, Merseyside, UK) at a 1:90,000 final dilution. The antibody fully reacted in the lab with ET-1 (100%) and it also cross-reacted with ET-2 (7%), ET-3 (7%), porcine big-endothelin (35%), and human big-endothelin (17%). However it did not show any cross-reactivity with somatostatin, β-endorphin, angiotensin I, II, III, vasopressin, or atrial natriuretic factor (ANF). The sensitivity of the RIA was 1 pg/ml. The 50% intercept was 50 pg/ml. The interassay variation was 13% and the intraassay variation was 10%. Recoveries of 1.5 and 3 pg of ET-1 added to pooled vitreous were 78±3.2% (n=10).

### Statistical evaluation

The statistical evaluation consisted of correlation coefficients and t-tests. ANOVA, chi-square, and Fisher exact tests were used to analyze the influence of age, blood pressure, and sex. The results are presented as mean ± standard deviation of the mean (SD).

### RESULTS

Sixty-six patients were studied. Their mean age was 56.8±6.4 years (range, 42-70 years); 34 (51.5%) were men and 32 (48.5%) were women. The age of the patients did not influence any of the VA measurements (Pearson correlation coefficients not significant). No statistically significant differences in age (ANOVA), MAP (ANOVA), or sex (chi-square and Fisher exact test) were found between the two groups of RD patients (No-PVR group and PVR group), and age did not influence VA measurements in either of them (Pearson correlations not significant). The average interval (days) from the start of symptoms to surgery was greater for cases with PVR (table I).

Mean IR-ET-1 values were plasma 2.91±0.44 pg/ml and SRF 10.71±7.95 pg/ml. Mean VA values were preoperative VA 0.31±0.16, postoperative VA 0.66±0.29, and VA difference 0.35±0.18 (table I).

Plasma IR-ET-1 showed significant (p<0.0001) negative linear correlations with preoperative VA (r=-0.52), postoperative VA (r=-0.69), and VA difference (r=-0.65). SRF IR-ET-1 also showed significant (p<0.0001) negative linear correlations with preoperative VA (r=-0.48), postoperative VA (r=-0.61), and VA difference (r=-0.54) (table II).

SRF IR-ET1, plasma IR-ET-1, and the difference between SRF-plasma IR-ET-1 levels were higher, and VA values (preoperative, postoperative, and postoperative minus preoperative difference) were lower in the PVR group than in the No-PVR group, each statistically significant (table I).

The SRF IR-ET1 and plasma IR-ET-1 values showed a significant positive correlation in RD (r=0.792, p=0.0001) and also in the No-PVR group (r=0.687, p=0.0001) and in the PVR group (r=0.742, p=0.0001), which suggests intraocular IR-ET-1 stems in part from systemic circulation.

### Table I

Mean values and standard deviation of the variables considered in the study by group of patients.

<table>
<thead>
<tr>
<th>Variables</th>
<th>RD Total (n=66)</th>
<th>No PVR group (n=33)</th>
<th>PVR group (n=33)</th>
<th>t test</th>
</tr>
</thead>
<tbody>
<tr>
<td>Preoperative VA</td>
<td>0.31±0.17</td>
<td>0.36±0.17</td>
<td>0.24±0.13</td>
<td>0.0003</td>
</tr>
<tr>
<td>Postoperative VA</td>
<td>0.67±0.29</td>
<td>0.81±0.24</td>
<td>0.53±0.27</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>VA difference</td>
<td>0.35±0.18</td>
<td>0.42±0.13</td>
<td>0.28±0.20</td>
<td>0.0021</td>
</tr>
<tr>
<td>Plasma IR-ET1 (pg/ml)</td>
<td>2.92±0.44</td>
<td>2.62±0.31</td>
<td>3.22±0.34</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>SRF IR-ET1 (pg/ml)</td>
<td>10.71±7.95</td>
<td>3.21±0.72</td>
<td>18.21±3.46</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>SRF/Plasma IR-ET1 difference (pg/ml)</td>
<td>7.79±7.60</td>
<td>0.59±0.55</td>
<td>14.98±3.22</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Interval from the start of symptoms to surgery (days)</td>
<td>5.94±3.62</td>
<td>4.27±2.34</td>
<td>7.61±3.88</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>

ET-1: endothelin 1, IR: immunoreactive, PVR: proliferative vitreoretinopathy, RD: retinal detachment, SRF: subretinal fluid, VA: visual acuity, VA difference=postoperative minus preoperative VA. Values are presented as means±standard deviation.
The SRF IR-ET1, plasma IR-ET1, and the SRF-plasma IR-ET1 difference showed significant negative linear correlations with preoperative VA in the No-PVR group, but these correlations were not significant in the PVR group (table I).

There were significant negative linear correlations between SRF IR-ET1, plasma IR-ET1, and the SRF-plasma IR-ET1 difference, respectively, and postoperative VA both in the No-PVR group and in the PVR group (fig. 1 and 2, table II).

Ultimately, SRF IR-ET1, plasma IR-ET1, and the SRF-plasma IR-ET1 difference showed significant negative linear correlations with VA difference (postoperative minus preoperative) in the PVR group, but these correlations were not significant in the No-PVR group (table II). The highest negative linear correlations were found between the three IR-ET-1 values studied and VA difference in the PVR group (table II).

**DISCUSSION**

Correlations between IR-ET-1 levels and VA were always negative and higher for postoperative VA. When these correlations were analyzed by groups, the PVR group showed the highest negative correlations between each of the three IR-ET-1 measurements of the study and postoperative VA and VA difference. Therefore, the higher the IR-ET-1 levels the lower the postoperative VA and the lower the VA difference.

Previously we reported the relationship between increased IR-ET-1 levels in the eye (in the vitreous and in SRF) and the proliferative complications of retinal detachment [12, 13]. The significant correlation found between SRF IR-ET1 and plasma IR-ET1 suggests that intraocular IR-ET1 derives in part from systemic circulation, but the slope of the correlation, always greater for the PVR group, is consistent with enhanced access of plasma proteins in the eye due to a disruption of the blood-ocular barrier. The increased difference of SRF-plasma IR-ET1 in the PVR group may also suggest a local ET-1 production in PVR.

Endothelins could play a role in photoreceptor synaptic transmission, and this would require tight control of the endothelin extracellular concentration. Modulation of synaptic transmission might affect photoreceptor survival, perhaps by regulating glutamate release [18]. The RPE playing a role in endothelin-mediated photoreceptor survival cannot be excluded because this retinal
layer contains ET-1, preproET-1, and ETA immunoreactivities [19]. RPE and glial cells are the main contributors to membrane formation and contraction in PVR. ET-1 may also act as a growth factor for astrocytes, inducing DNA synthesis and proliferation [20]. Astrocytic proliferation together with an excessive secretion of ET-1 have been reported in vivo in cerebral focal ischemia [21]. Sasaki et al. [22] demonstrated that ET-1 specifically stimulated the efflux of glutamate via ETB receptors from cultured rat astrocytes, suggesting that ET-1 may exacerbate neurodegeneration. Infusion of ETB selective antagonists attenuates the increase in astrocytes after injury of the brain cortex, indicating that induction of reactive astrocytes depends on the activation of ETB receptors [23]. Reactive gliosis has been suggested before as a clinically significant limiting factor in the recovery of vision [8, 9] after RD. Excess of ET-1 released by injured glial cells can be compensated by scavenging ETB receptors [24], and it has been suggested that blockade of these receptors after central nervous system injury might modulate glial scar formation, providing a more permissive substrate for neural survival and regeneration [25].

We can conclude that VA was related (negative correlation) to SRF IR-ET1 levels in RD. Nevertheless, the highest negative correlations between postoperative VA and VA difference and the SRF IR-ET1 levels were found in the PVR group.

Our findings support the idea of doing prompt primary vitrectomy in RD to eliminate the intraocular peptide and perhaps coadjuvant pharmacologic therapy in RD and, more importantly, in PVR.

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REFERENCES


