Original article

Morphometric analysis of corneal endothelium after intravitreal ranibizumab (Lucentis®) in age-related macular degeneration treatment

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ABSTRACT

Purpose: To determine the effect of intravitreal injection of 0.5mg ranibizumab on the corneal endothelium in patients with age-related macular degeneration (AMD).

Methods: Observational, prospective case series pilot study. Twenty-six eyes of 26 consecutive patients with AMD were evaluated. All participants received one monthly intravitreal injections of 0.5mg ranibizumab for three consecutive months. The follow-up period was 6 months. Central corneal specular microscopy was performed before injection and at 7 days and 6 months after the first intravitreal injection. The endothelial cell density, coefficient of variation of cell size, and percentage of hexagonal cells were analyzed and the central corneal thickness was measured.

Results: There were no significant differences in the endothelial cell densities, coefficients of variation of cell size and percentages of hexagonal cells before injection and at 7 days and 6 months after the first intravitreal ranibizumab injection (P>0.5). There was also no significant difference in central corneal thickness measurements through the follow-up period (P>0.5).

Conclusions: Repeated intravitreal injections of 0.5mg ranibizumab do not seem to cause substantial changes in the corneal endothelium.

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Palabras clave:
Degeneración macular asociada a la edad
Endotelio corneal
Ranibizumab

Análisis morfométrico del endotelio corneal tras ranibizumab intravítreo en la degeneración macular asociada a la edad

RESUMEN

Objetivo: Evaluar el efecto de la inyección intravítreo de 0,5 mg ranibizumab sobre el endotelio corneal en pacientes con degeneración macular asociada a la edad (DMAE).

Método: Estudio piloto de serie de casos, observacional y prospectivo con observador enmascarado. Veintiséis ojos (26 pacientes) con DMAE fueron evaluados. Todos los pacientes recibieron 1 inyección intravítreo mensual de 0,5 mg de ranibizumab durante 3 meses consecuti-

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Introduction

The inhibition of the vascular endothelial growth factor A (VEGF-A) is an effective strategy for treating age-related macular degeneration (AMD).\textsuperscript{1-3} Ranibizumab (rhuFabV2. Lucentis; Genentech, San Francisco, CA, USA) is a humanized monoclonal antibody fragment which neutralizes all the biologically active forms of VEGF, including VEGF110, VEGF121 and VEGF165 by preventing the interaction of VEGF-A with its VEGFR-1 (Flt-1) and VEGFR-2 receptors (Flk-1/KDR).\textsuperscript{4,5} Intravitreal injections of ranibizumab have demonstrated high degrees of efficacy for treating choroidal neovascularization secondary to AMD.\textsuperscript{2,3}

Various immunohistochemical studies\textsuperscript{6-9} have revealed the expression of VEGF and its receptors in the corneal endothelium. Although the pharmacokinetic profile of intravitreous ranibizumab in humans is not fully determined, in animal models\textsuperscript{10,11} ranibizumab was detected in the aqueous humor, after standard intravitreal injections. Therefore, it seems reasonable to consider whether ranibizumab could be potentially cytotoxic for the corneal endothelium. Morphometric analysis of endothelium obtained with mirror microscopy is a sensitive indicator of endothelial function.\textsuperscript{12,13} Accordingly, the aim of this study is to analyze with mirror microscopy the possible toxicity induced by intravitreous ranibizumab over the corneal endothelium in the treatment of AMD.

Material and methods

26 eyes of 26 patients with AMD were included in this pilot, observational and prospective series of cases. The study protocol was approved by the Ethical Committee of the Príncipe de Asturias University Hospital. All the patients signed an informed consent. The inclusion criteria were: a) clinical and angiographic signs of subfoveal choroidal neovascular membrane with best corrected visual acuity (BCVA) of 20/100 or less, b) no indication of photocoagulation with laser or photodynamic therapy, c) age ≥ 50 years, d) no history of use of contact lenses, and e) no ocular and/or systemic diseases such as diabetes or collagen diseases that could alter the morphology of the corneal endothelium. All the patients received one intravitreous injection of 0.05 ml, 0.5 mg ranibizumab per month for three consecutive months in accordance with the study protocol. The initial exploration and those made prior to the administration of each monthly ranibizumab injection comprised BCVA measurement, biomicroscopy, applanation tonometry, indirect ophthalmoscopy and optic coherence tomography (OCT Stratus 3000, Carl Zeiss Meditec Inc). the effect of the treatment was monitored on the basis of the BEST CORRECTED VISUAL ACUITY improvement and OCT measurements.

Endothelial cell analysis

No-contact mirror microscopy was performed in the central cornea with a Topcon SP-3000P (Topcon, Corp, Tokyo, Japan) mirror microscope. Before the ranibizumab injection and seven days and six months after the first injection, a masked observer (JBH) captured three images of the central cornea, keeping only the best for analysis. The Mirror microscope automatically assessed the endothelial cell density, the cell size variation coefficient (an objective measurement of polymegatism), and the cell hexagonality percentage (pleomorphism index). In addition, said microscope also measured automatically the corneal thickness. The same masked observer performed a manual analysis of the endothelial cells. At least, 75 cells with well defined edges were manually counted in a defined square of the photograph, which corresponds to 0.03 mm\textsuperscript{2} of the actual cornea.

Statistical analysis

All of the data were expressed as mean values and standard deviation (SD). The differences between the pre- and post injection data were statistically assessed with the variance test and P values lower than 0.01 were considered to be significant. The Tukey test was utilized to correct the effect of multiple comparisons. The statistical analysis was made with the GraphPad InStat software version 3.00 for Windows 95, GraphPad Software (San Diego, CA).
Results

The study group included 12 males (46.16%) and 14 females (53.84%). The mean age of these patients was of 68.26 SD 5.03 years (range: 62 to 80 years). Fifteen eyes (57.69%) were phakic and 11 eyes (42.31%) were pseudoaphakic. All patients completed the six month follow-up period. No adverse effects to treatment were found.

Endothelial cell density and morphology

All the measurements were made before the injection and seven days and six months after the first intravitreous injection of ranibizumab. The mean endothelial cell density prior to the injection and after seven days and six months were of 2.339.73 SD 367.08 cells/mm² (range: 1.020 to 2.862), 2.322.88 SD 366.83 cells/mm² (range: 1.030 to 2.844) and 2.298.42 SD 387.60 cells/mm² (range: 1.001 to 2.840) respectively (table 1). The mean variation coefficients before the injection and after seven days and 6 months post-injection were 35.19 SD 6.35 (range: 27 to 49), 36.50 SD 7.66 (range: 23 to 55) and 37.53 SD 8.33 (range: 28 to 55) respectively (table 1). The mean pre- and post-injection (day 7, month 6) hexagonality percentages were 51.15 SD 7.30 (range: 32 to 60), 51.57 SD 7.74 (range: 38 to 66) and 49.84 SD 8.82 (range: 32 to 67) respectively (table 1). The differences were not statistically significant in any of the variables in the various periods of the analysis (p>0.5).

In what concerns the effect of intravitreal ranibizumab on cornea thickness, no statistically significant differences were found between the pre-injection measurements (0.501 SD 0.038 µm) and month 6 (0.507 SD 0.044 µm) (p>0.5) (table 1).

Discussion

This study has demonstrated that intravitreal injections of ranibizumab 0.5 mg in patients with AMD does not give rise to toxic effects at the corneal endothelium level. No statistically significant differences were found between the pre- and post-injection values in the intervals of the study (7 days and 6 months), in the corneal thickness or in any of the analyzed endothelial morphometric parameters such as corneal endothelial cell density, cell size variation coefficient and percentage of cell hexagonality.

To the best of our knowledge, the toxic effect of ranibizumab on corneal endothelium for treating AMD has not been studied by mirror microscopy. In this regard, our study exhibits some limitations related to the small size of the sample (N=26) and the broad range of pre-op endothelial cell density. Accordingly, for this pilot study to reach statistical significance for a study potency of 80% and assuming an alpha error of 5%, 50-60 patients would be required, provided that the differences encountered between the pre- and post-injection figures for corneal endothelial cell density maintained the same tendency.

To conclude, it can be said that repeated intravitreal injections of ranibizumab 0.5 mg for treating choroidal neovascularization secondary to AMD do not have significant toxic effects on the corneal endothelium in this study at six months of follow-up.

Conflict of interest

The authors declare that they have no conflict of interest.

References


Table 1 – Corneal endothelial morphometric analysis before and after intravitreal injection of 0.5mg ranibizumab (Lucentis®) for treating age-related macular degeneration

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<thead>
<tr>
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<th>Media and SD (range)</th>
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<tbody>
<tr>
<td></td>
<td>N=26</td>
</tr>
<tr>
<td></td>
<td>Preinjection</td>
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<tr>
<td>Endothelial cell density (cells/mm²)</td>
<td>2.339.73 SD 367.08</td>
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<tr>
<td></td>
<td>(1.020 to 2.862)</td>
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<tr>
<td>Cell size variation coefficient</td>
<td>35.19 SD 6.35</td>
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<td>(27 to 49)</td>
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<tr>
<td>% Hexagonality</td>
<td>51.15 SD 7.30</td>
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<td>(32 to 60)</td>
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<tr>
<td>Corneal thickness (µm)</td>
<td>0.501 SD 0.038</td>
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<td>(0.411 to 0.577)</td>
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AMD: age-related macular degeneration.


