ENZYMATIC VITRECTOMY BY INTRAVITREAL AUTOLOGOUS PLASMIN INJECTION, AS INITIAL TREATMENT FOR DIFFUSE DIABETIC MACULAR EDEMA

VITRECTOMÍA ENZIMÁTICA POR INYECCIÓN INTRAVÍTREA DE PLASMINA AUTÓLOGA COMO TRATAMIENTO INICIAL DEL EDEMA MACULAR DIABÉTICO DIFUSO

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ABSTRACT

Purpose: To determine whether intravitreal injection of plasmin is effective in treating diffuse diabetic macular edema (DDME).

Design: A prospective, comparative, interventional case study.

Patients: Eighteen patients with bilateral DDME received, as their primary therapeutic treatment, an intravitreal injection of plasmin in one eye, with their contralateral untreated eye serving as a control.

Intervention: Intravitreal 0.2 ml of autologous plasmin injected under topical anesthesia. The plasmin was obtained by a simplified method.

Main Outcome Measures: Central macular thickness (CMT), determined by optical coherence tomography (OCT), and Best Corrected Visual Acuity (LogMAR), assessed at one and three months of follow-up.

Results: All patients completed the 3-month follow-up assessments. Before the injection, the CMT was 525.22 SD 80.12 µm [mean ± standard deviation (SD)] in the eyes to be injected, compared to 525.44 SD 78.13 µm in the control eyes. One month after the injection, the CMT was 323.72 SD 44.87 µm in the treated eyes, which is statistically significant compared to the control eyes.

RESUMEN

Objetivo: Determinar si la inyección intravítrea de plasmina es efectiva en el tratamiento del edema macular diabético difuso (EMDD).

Diseño: Estudio piloto prospectivo, comparativo, de intervención, serie de casos.

Pacientes: Dieciocho pacientes con edema macular diabético bilateral, recibieron como primera actuación terapéutica una inyección intravítrea de plasmina en un ojo, sirviendo el ojo contralateral como control.

Tratamiento: Inyección intravítrea de 0,2 ml de plasmina autóloga bajo anestesia tópica. La plasmina fue obtenida por un método simplificado.

Principales medidas: Engrosamiento macular central (EMC) medido por tomografía de coherencia óptica (OCT) y agudeza visual (escala LogMAR), al mes y los 3 meses.

Resultados: Todos los pacientes completaron el seguimiento de 3 meses. Antes de la inyección el EMC 525,22 DE 80,12 µm [media ± desviación estandard (DE)] en los ojos inyectados, versus 515,44 DE 78,13 µm en los ojos control. Un mes tras la inyección el EMC era 323,72 DE 44,87 µm.
INTRODUCTION

Diffuse diabetic macular edema (DDME) is the main cause of visual impairment in diabetic patients (1). The efficacy of grid laser photocoagulation has proven to be limited (2). Several options to laser treatment have been suggested, with varying results as far as efficacy is concerned: intravitreal triamcinolone (3,4), intravitreal bevacizumab (5), protein kinase inhibitors (ruboxistaurin) and somatostatin analogues (octeotride) (6,7).

Incidence of diabetic macular edema in eyes suffering from spontaneous posterior vitreous detachment (PVD) is lower when compared to those suffering from a thickened and adhered hyaloid membrane, where the macular traction of the vitreous cortex plays a significant role in the increase and chronification of macular edema (8,9). This fact explains why vitrectomy, with or without peeling of the internal limiting membrane, relaxes macular traction and aids in edema resolution (10,11). However, one must keep in mind that this surgical procedure may entail serious secondary complications (12).

Several authors have advocated the use of pharmacologic vitreolysis by means of autologous plasmin injections (13,14). Plasmin is a protease involved in fibrinolysis. Its enzymatic action affects laminin and fibronectin, both located between the posterior vitreous cortex and the retinal internal limiting membrane and are thought to be the molecules mainly responsible for firmly adhering both surfaces (15,16). Different studies reported on the efficacy of plasmin injections immediately prior to vitrectomy in patients suffering from diabetic retinopathy and macular holes. Plasmin injections simplified surgery by inducing surgical detachment of the vitreoretinal membranes (17-19).

The goal of the present controlled, prospective pilot study on a series of diabetic patients with bilateral diffuse macular edema is to determine the efficacy and safety of plasmin autologous injections—not associated to vitrectomy—as the only initial therapeutic option in the treatment of DDME. Its efficacy is assessed based on the changes obtained in both visual acuity and optical coherence tomography.

SUBJECTS, MATERIAL AND METHOD

The present study is a prospective pilot study aimed at providing therapeutic intervention.
Patients were recruited between September 2006 and February 2007 in the Ophthalmologic Services’ Diabetes Unit and was approved by the hospital’s Research Committee. Thirty-six eyes belonging to eighteen consecutive patients were included in the study, using one eye for control purposes and the contralateral eye for treatment with intravitreal plasmin.

**Patients: Inclusive and Exclusive Criteria**

**Inclusive Criteria**

The study included only diabetic patients suffering from bilateral diffuse macular edema. The diffuse macular edema had to fulfill the following criteria for both eyes: a) Macular thickening confirmed by means of biomicroscopy and 90D lens; b) widespread leaking around the macular area under fluorescein angiography; c) absence of previous retinal laser photocoagulation (either macular or peripheral); d) macular thickening greater than 360 µm (normal =/≤ 200 µm) confirmed by optical coherence tomography (Stratus OCT-3, Zeiss).

**Exclusive Criteria**

1. Uncontrolled, systolic and diastolic blood pressure greater than 150 and 90 mmHg, respectively.
2. Unstable glycemia levels. Glycosylated hemoglobin (Hb A1c) greater than 9.5 percent.
4. Ocular history of ocular hypertension, glaucoma.
5. Signs of ischemia in the macular area or around the retinal periphery under fluorescein angiography.
6. Eye surgery during the previous six months.
7. Previous posterior vitreous detachment, diagnosed by means of biomicroscopy and +90D lens and/or optical coherence tomography.

All patients signed an informed consent and clearly understood the purpose of the study and its potential risks (retinal detachment, endophthalmitis, vitreous hemorrhage, lens trauma, etc.). and the possibility of requiring subsequent additional treatments to address their diabetic retinopathy (laser, intravitreal injections of anti-VEGF drugs or triamcinolone).

**Pre-surgical Examination**

All patients underwent full ophthalmologic exploration before the plasmin injection, including slit lamp exploration, applanation tonometry, indirect ophthalmoscopy, macular scan by means of optical coherence tomography, fundus photograph of the macular area and fluorescein angiography. In all cases, the optic coherence tomography and biomicroscopy discarded a vitreous detachment and confirmed adherence of the vitreous cortex in the macular area.

**Autologous Plasmin Preparation**

As already described (20), plasmin was prepared in the surgery room immediately prior to applying the injection. A blood sample from patients was taken from a peripheral vein. Once the blood had been spin-dried at 4,000 rpm for 15 minutes, the plasma was transferred to a vial containing streptokinase (Streptase®, ZLB BEHRIG Laboratories), previously incubated for 15 minutes at 37ºC. The diluted streptokinase was then combined with plasma by vigorously shaking the vial for 5 minutes. The resulting solution was again incubated for 15 additional minutes at 37ºC. Finally, the solution was sterilized using a 0.22 mm Millipore filter, and ready to be injected.

**Eye Selection and Injection Technique**

The eyes were randomly assigned to either the plasmin or the control group. Before the injection, topical anesthesia with 1% tetracaine ophthalmic drops was administered at least three times, followed by conjunctival wash with povidone solution. Anterior chamber paracentesis was performed with a 25 G needle. Subsequently, intravitreal injection of the autologous plasmin solution was applied with a 30 G needle, 3.5 or 4 mm from the limbus depending on whether the patient was pseudophakic or phakic, respectively. An eye sponge was placed over the injection spot in order to avoid reflux. Ciprofloxacin and dexamethasone eye drops were prescribed four times a day during five days after surgery.
Key Measurements

Main measurements focused on changes in visual acuity and central macular thickening (CMT). In order to avoid biases and intra-observer differences, every visual acuity (ETDRS optotypes) and macular thickening measurement obtained through an optic coherence tomography (OCT-3, Zeiss-Humphrey, Dublin, CA, USA) performed by single explorer who ignored the patients’ clinical data (P.U). For statistical purposes, the best-corrected visual acuity measurement (BCVA) was converted into the equivalent logMAR of the minimum angle of resolution. Measurements were taken on the day prior to the injection as well as 4 and 12 weeks after. The mean value for a normal central retinal thickness was set at 200 µm.

Statistical Study

Only one eye per patient underwent treatment. All data were processed using SPSS 13.0 software for Windows (SPSS for Windows, SPSS Inc, Chicago, USA). In order to perform a statistical analysis of paired data, every eye treated was paired to the non-treated one for the same patient. The statistical analysis was performed using Wilcoxon’s signed-ranks test for matched pairs, considering P < 0.05 as statistically significant.

RESULTS

Thirty-six eyes belonging to a total of eighteen patients were included in the study, always using one of the eyes for control purposes —for observation only— and the contralateral eye for plasmin intraocular injection. The patients’ average age was 65 (range between 56 and 83). Twelve were female and six were male. Thirteen were pseudophakic and five were phakic. Plasmin was injected in nine right eyes and nine left eyes. All patients suffered from non-proliferating diabetic retinopathy.

Visual Acuity (Table I)

No statistically significant differences were found between the mean pre-surgical visual acuity in the control group (1.04, data not shown) and that of the group treated with intravitreal autologous plasmin (IAP) (1.03) (P > 0.05). No significant changes took place in the control group’s BCVA after the three-month follow-up (mean BCVA 1.03), whereas the mean BCVA for the group treated with plasmin improved to 0.53. In other words, the mean BCVA improvement was 0.50 log units of the minimum angle of resolution in the eye treated with plasmin and barely 0.01 in the control group. Such difference is clinically and statistically significant (P < 0.001).

VA improved in sixteen out of the eighteen eyes treated: in seven cases, it improved by one visual line, two lines in four eyes, three lines in two eyes and four lines in three eyes.

Central Macular Thickness (Table II)

The mean central macular thickness (CMT) of the eye to undergo plasmin injection treatment (525.22 SD 80.12 µm) was slightly greater than that of the control eye (515.44 SD 78.13 µm), although statistically not significant (p > 0.05). One month after administering the plasmin injection, mean central macular thickness in the eye treated and the control eye stood at 322.72 SD 44.87 µm and

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Table I. Best-Corrected Visual Acuity (BCVA) for eyes treated with intravitreal autologous plasmin injection (IAP). Values before treatment and one month and three months after treatment (converted to the logMar scale)

<table>
<thead>
<tr>
<th>Patient No</th>
<th>Visual acuity eyes treated with autologous plasmin</th>
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<tbody>
<tr>
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<td>Before treatment</td>
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<tr>
<td>1</td>
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<tr>
<td>2</td>
<td>0.48</td>
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<tr>
<td>3</td>
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<tr>
<td>4</td>
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<tr>
<td>5</td>
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<tr>
<td>6</td>
<td>0.48</td>
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<tr>
<td>7</td>
<td>0.6</td>
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<tr>
<td>8</td>
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<tr>
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<td>10</td>
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<td>0.9</td>
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518.44 SD 78.13 µm, respectively. This statistically
significant difference (P < .001, Wilcoxon’s signed-
ranks test for matched pairs) between both groups
persisted three months later (310.55 SD 15.58 µm
—plasmin— versus 517.66 SD 80 µm —control—).
While no significant changes were found during
follow-up in the control group, central macular
thickness decreased in all cases in the group treated:
bymore than 70 percent in four eyes; from 60 to 70
percent in eleven eyes; and more than 50 percent in
the remaining three eyes, taking into account that nor-
mal central macular thickness was set at ≤ 200 µm.

<table>
<thead>
<tr>
<th>Patient No.</th>
<th>Eyes treated IAP 1 month</th>
<th>3 months</th>
<th>Control Eyes 1 month</th>
<th>3 months</th>
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<tr>
<td>Media±SD</td>
<td>525.22±80.12</td>
<td>322.72±44.87</td>
<td>310.55±35.58</td>
<td>518.72±78.54</td>
</tr>
</tbody>
</table>

SD: standard deviation; PAI: autologous plasmine injection; Note : OCT values are expressed in µ.

Side Effects

No side effects were observed during follow-up.

DISCUSSION

The results of the present prospective, controlled
study seem to prove the efficacy of intravitreal plas-
min injections in improving visual acuity and reduc-
ing retinal thickening in patients suffering from
diffuse diabetic macular edema (DDME). Until
now, intravitreal plasmin injections had always
been used immediately after vitrectomy in order to
facilitate intra-surgical handling (17,19,21), although
the present study showed the efficacy of isolated
plasmin injections for treatment purposes. The
pharmacologic detachment of the retinal posterior
vitreous cortex by means of intravitreal plasmin
injections only, not associated with vitrectomy,
should be termed «enzymatic vitrectomy», opening
the doors to a potential therapeutic treatment not
only of diabetic macular edema, but also of other
macular edemas (venous occlusions, etc). and diffe-
rent other pathologies where vitreous-retinal trac-
tion is clearly involved in their pathogenesis (myo-
opic macular traction syndrome, etc), with the asso-
ciated iatrogenic risks of vitreal surgery (retinal tea-
rings and detachments, vitreous hemorrhage, etc).

Although there could be no direct correlation bet-
ween improved visual acuity and the decrease in
diabetic macular edema after the plasmin injection,
the edema decreased by more than 50 percent in all
eyes treated (100%), while visual acuity improved
by two or more lines in 50 percent of cases. One
should bear in mind that plasmin injections were
used as the first therapeutic option for diffuse dia-
betic macular edema before attempting treatment
with laser, triamcinolone, pegaptanib, ranibizumab
or intravitreal bevacizumab. This visual improve-
ment is noticed by patients very early on (2-3 days),
remaining stable from the first until the third month
during follow-up. This is a pilot study, and thus its results cannot be generalized. Studies involving a larger number of cases and long-term follow-up are needed in order to determine its efficacy as monotherapy or whether it may be associated with the remaining treatments described above as to guarantee its efficacy.

On the other hand, the present study revealed a full posterior vitreous detachment (PVD) in the macular area in all the patients targeted (fig. 1), clinically documented by means of biomicroscopy or optical coherence tomography, with a significant decrease in retinal macular thickening—anatomical macular edema—in all cases (100%). Resolution

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Fig. 1: A,B: Eye fundus imaging and angiography corresponding to a diabetic patient with a large diffuse macular edema – patient no. 2-. C, D, E, F, G: Series of optical coherence tomography (OCT) images corresponding to the same patient before (C, D), one week (E) and one month after (F, G) treatment with autologous plasmin; the arrows show in detail the posterior hyaloid membrane’s traction before injection and its detachment after the plasmin injection, improving the macular edema significantly and simultaneously and bringing macular thickening from 624 down to 305 microns.
of the macular edema remained stable during the three-month follow-up, but long-term studies are a must if one wishes to determine the occurrence of relapses and/or the need to associate other therapeutic options (laser, anti-VEGF or intravitreal triamcinolone).

The efficacy and toxicity of autologous plasmin is dose-dependent. In previous studies, a single intravitreal injection containing 0.4 UI of plasmin proved to be enough to detach the posterior vitreous cortex from the internal limiting membrane, and no toxicity has been reported in concentrations of up to 3-4 UI (4,17,22,24,25,27).

The amount of autologous plasmin obtained and injected with the method used in the present study was 0.26 UI (20) on average, and yet it proved to be sufficient in terms of efficacy. On the other hand, Gandorfer et al (13) found a direct correlation between plasmin exposure time and the degree of vitreous-retinal detachment based on the principle that, in previous studies, residual autologous plasmin was always removed from the vitreous with a surgical maneuver right after the injection. In the present study, the time of exposure of the vitreoretinal surface to autologous plasmin proved to be sufficient and non-toxic. After reviewing PubMed, we concluded that this was the longest exposure time ever reported in the literature (25,26). No complications such as endophthalmitis, vitreous hemorrhage, uveitis, retinal detachment, increased intraocular pressure or cataract progression have been reported either.

Traditionally, one of the main issues limiting the use of autologous plasmin was its sophisticated, long, costly and complex preparation, which required special equipment only available in certain hematology units. The benefit of this simplified autologous plasmin preparation technique is that it may be used in the ophthalmology surgery room a few minutes before use in a way that is quick, easy and relatively cheap (20).

In other words, enzymatic vitrectomies performed only in combination with autologous plasmin injections have proven to be efficient and safe when attempting to reduce diffuse diabetic macular edemas and improving visual acuity, at least in the short term. It also seems to be a good, first and initial therapeutic option for these patients before trying other procedures (laser, intravitreal triamcinolone, etc.). In the future, studies should specify the ideal plasmin dose, the possibility of relapses taking place in the long term and the convenience of complementary associated treatments that may be necessary.

REFERENCES


