ENZYMATIC VITRECTOMY BY INTRAVITREAL AUTOLOGOUS PLASMIN INJECTION AS INITIAL TREATMENT FOR MACULAR EPIRETINAL MEMBRANES AND VITREOMACULAR TRACTION SYNDROME

VITRECTOMÍA ENZIMÁTICA POR INYECCIÓN INTRAVÍTREA DE PLASMINA AUTÓLOGA COMO TRATAMIENTO INICIAL DE LAS MEMBRANAS EPIRRETINIANAS MACULARES Y EL SÍNDROME DE TRACCIÓN VITREOMACULAR

DÍAZ-LLOPIS M1, UDAONDO P2, CERVERA E3, GARCÍA-DELPECH S4, SALOM D2, QUIJADA A5, ROMERO FJ6

ABSTRACT

Purpose: To determine the effectiveness of intravitreal plasmin injection in the treatment of macular epiretinal membranes (MEM) and vitreomacular traction syndrome (VMTS) without associated pars plana vitrectomy.

Methods:
Design: Interventional, prospective, case series pilot study.
Patiens: Seven patients were enrolled in the study, 4 with MEM and 3 with VMTS.
Treatment: 0.2 ml of autologous plasmin intravitreally injected under topical anesthesia was administered.

RESUMEN

Objetivo: Determinar si la inyección intravítrea de plasmina es efectiva en el tratamiento de las membranas epirretinianas maculares (MEM) y el síndrome de tracción vitreomacular (STVM), sin necesidad de asociar vitrectomía quirúrgica.
Material y método
Diseño: Estudio piloto prospectivo, de intervención, serie de casos.
Pacientes: Siete pacientes, cuatro con membrana epirretiniana macular y tres con síndrome de tracción vitreomacular.

Received: 19/5/08. Accepted: 12/1/09.
1 Ophthalmology Dept.
2 Ophthalmology Dept. La Fe Hospital. Valencia. Spain.
3 Ophthalmology Dept. General University Hospital of Valencia. Spain.

Correspondence:
Manuel Díaz Llopis
C/. Ruzafa, 19
46004 Valencia
Spain
E-mail: manuel.diaz@uv.es
to all patients. The plasmin was obtained by a simplified method with urokinase.

**Main outcome measures:** Degree of detachment of the MEM and the VMTS measured by optical coherence tomography (OCT), and the best corrected visual acuity (Snellen scale) before and one month after the plasmin injection.

**Results:** The follow-up period was completed by all the patients. The MEM remained attached to the retina in all cases, as measured either by biomicroscopy or OCT. The VMTS was completely detached from the foveal area in all cases, with the disappearance of secondary tractional retinal folds and recovery of the normal macular anatomic architecture as measured by OCT. Visual acuity was not modified in any of the MEM patients, and improved in all VMTS patients. No adverse effects were observed.

**Conclusion:** In our case series, intravitreally injected autologous plasmin was not effective in the treatment of MEM, but resolved VMTS successfully, improving the visual acuity and releasing the retinal traction without the need for associated pars plana vitrectomy. Larger studies to confirm the efficacy of this technique and the possibility of success after repeated injections are warranted (Arch Soc Esp Oftalmol 2009; 84: 91-100).

**Key words:** Autologous plasmin, pharmacologic vitreolysis, epiretinal membrane, macular pucker, vitreomacular traction syndrome, enzymatic vitrectomy, enzymatic vitreolysis.

**INTRODUCTION**

The formation of vitreoretinal and epiretinal membranes, and a vitreomacular pathological adhesion, are characteristic signs both of proliferative vitreoretinopathy and macular preretinal fibrosis («macular pucker»), the vitreomacular traction syndrome and macular holes (1-3). The extracellular matrix of the vitreoretinal and epiretinal membranes contain abundant fibronectin and laminine (4,5), and are in permanent formation and biological degradation. The proteolitic cascade measured by plasminogen activation, by means of the formation of plasmin, is an important proteolitic mechanism of the physiological degradation in the permanent dynamics of tissue remodeling of the extracellular matrix of said membranes (6).

Cases of spontaneous occasional separation of the epiretinal macular membrane have been described in the context of posterior vitreous detach-
ment (7,8), and up to 11% of vitreomacular traction syndrome cases, with immediate improvement of visual acuity and disappearance of metamorphopsia (9-14). Also, the presence of a posterior vitreous detachment facilitates surgical peeling during the vitrectomy of the membranes (15,16).

Pharmacologic vitreolysis has been proposed by several authors (16-20). Plasmin is a protease with enzymatic action on laminin and fibronectin (21-25), which is located between the posterior vitreous cortex and the internal limiting membrane of the retina, and the main molecules involved are considered responsible for the firm adhesion between both surfaces (26,27). Several studies have described the efficacy of the injection of plasmin, immediately before vitrectomy in cases of proliferative diabetic retinopathy and/or refractory macular edema, macular holes and premature retinopathy (28-34), simplifying surgery to facilitate the surgical detachment of the posterior hyaloids and the vitreoretinal membranes (15).

The intravenous injection of plasmin—without associated surgical vitrectomy— as a single treatment, which has been termed «pharmacologic and enzymatic vitrectomy» (16,18), has shown its efficacy in the treatment of diffuse diabetic macular edema both as initial treatment and in cases refractory to other treatments, by inducing a posterior detachment of the vitreous (DPV) enzymatically (35) (Díaz-Llopis M, Udaondo P, García-Delpech S, Salom D, Cervera E, Quijada A. Intravitreal autologous plasmin without associated vitrectomy for refractory diffuse diabetic macular edema. Eye 2008; in press).

The objective of the present pilot study, on a series of patients with macular epiretinal membrane (MEM) and vitreomacular traction syndrome (VMTS), is to establish the efficacy of the autologous injection of plasmin—without associating vitrectomy— as the initial and single therapeutic option.

**SUBJECTS, MATERIAL AND METHOD**

This study is a prospective pilot study comprising therapeutic intervention. The patients were recruited between September 2006 and December 2007 in the Macular Units of the Ophthalmology Services with the approval of the Research Committees. Seven eyes of seven consecutive different patients were included in the study for treatment with intravitreal plasmin. All the patients signed an informed consent, clearly explaining to them the objective of the study and the potential risks of the intravitreal injection (retinal detachment, endophthalmitis, vitreous hemorrhage, lens traumatism, etc) and the possibility of requiring later additional treatment (surgical vitrectomy, mechanical tinction and peeling of membrane).

**Patients: Inclusion and exclusion criteria**

**Inclusion criteria**

Only those patients that had confirmation of their macular epiretinal membrane or vitreomacular traction syndrome by optical coherence tomography (Stratus OCT-3, Zeiss). Only VMTS and/or MEM primary and idiopathic cases were included, unrelated to any other previous ocular pathology (diabetic retinopathy, uveitis, detachment of retina, etc.) anatomically and visually established three months before the plasmin injection.

**Exclusion criteria**

1. Any concomitant ocular pathology (glaucoma, diabetic retinopathy, etc).
2. Previous ocular surgery in the last six months.
3. Visual acuity above 0.4 (Snellen scale).

**Preoperative examination**

All the patients received a complete ophthalmologic examination before the injection of plasmin, including exploration by slit lamp, applanation tonometry, indirect ophthalmoscope, macular scanner by optical coherence tomography scanner (OCT), photography of the background of the macular area and fluorescence angiography. The OCT and the biomicroscope confirmed the adherence of the membrane in the macular area in all the cases, as well as allowing to clearly differentiate the epiretinal membranes of the vitreomacular traction syndrome.
Preparation of the autologous plasmin

The plasmin was prepared in the operating theatre immediately before the injection, as described above (15,35), with the modification of substituting streptokinase as plasminogen activator with urokinase (36-40). A sample of the patient’s blood was extracted from a peripheral vein. Once the blood had been centrifuged at 4,000 rpm for 15 minutes, the plasma was transferred to a vial with urokinase, which had been incubated previously for 15 minutes at 37ºC. The urokinase was mixed with plasma agitating the vial vigorously for five minutes. The solution was incubated 10 additional minutes at 37ºC. Finally, the solution was sterilized through a Millipore filter of 0.22 mm, and was then ready to be injected.

Injection techniques

Before the injection, topical anesthesia with 1% tetracain eye drops was carried out at least 3 times, with conjunctiva washing with povidona solution as well as paracentesis of anterior chamber with a 27 G needle, to avoid the posterior reflux on carrying out the intravitreal injection given the volume to inject. Subsequently an intravitreal injection of 0.2 ml of the autologous plasmin solution was carried out with a 30 G needle, at 3.5 or 4 mm of the limbus depending on whether the patient was pseudophakic or phakic respectively. An absorbing sponge was applied at the injection point to avoid reflux. Ciprofloxine and dexametason eyedrops 4 times a day were administered five days post-op. A sole injection of plasmin in the eye was carried out independently of the initial response.

Main measurements

The main measurements of the degree of efficacy and response to the treatment were changes in visual acuity and the separation of the retina membrane. To avoid bias and interobserver differences, all the visual acuities (Snellen optotypes) and the measurements in the Optical Coherence Tomography (OCT-3, Zeiss) were measured by a single explorer that did not know the clinical data of the patients (SGD). The measurements were carried out the previous day, at 7 days –data not shown– and 4 weeks after the injection. The case series did not allow the statistical study.

RESULTS

Seven eyes were included in the study from a total of seven different patients. The average age of the patients was 66 years (range 42-81). Four were women and three men. Three were pseudophakic and four phakic. Plasmin was injected in three right eyes and four left eyes. All the patients had an epiretinal macular membrane or a vitreomacular traction syndrome as sole ocular pathology (table I).

Visual acuity

No changes occurred in the visual acuity of any of the patients with MEM (0.05, 0.1, 0.2, 0.3). In the three patients with VMTS the visual acuity improved from 0.1 to 0.6, 0.4 to 0.9 and from 0.2 to 0.8 respectively.

Separation of the retina membrane

There were no changes either in the ophthalmoscope or in the OCT of the four patients with MEM (figs. 1 and 2), and if in the three with VMTS where a clear trend to normalization of the macular structure was observed (figs. 3 and 4).

Secondary effects

No secondary effects of vitritis, endophthalmitis, vitreous hemorrhage, retinal tearing or retinal detachment were identified.

DISCUSSION

The results of this pilot and prospective study show the efficacy of the intravitreal injection of plasmin in improving the visual acuity and reducing the thickening of the retinal macular in patients with vitreomacular traction syndrome (VMTS), and open the possibility of beginning to evaluate and consider a simple and safe alternative before trying surgical maneuvers, such as peeling of the membra-
Table I. Clinical characteristics of the patients with vitreomacular traction syndrome and epiretinal membrane, visual acuity before and 1 month after the intravitreal injection of autologous plasmin, without associated vitrectomy

<table>
<thead>
<tr>
<th>Patient #</th>
<th>Age</th>
<th>Diagnosis</th>
<th>AV Preinjection*</th>
<th>#AV Postinjection**</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>72</td>
<td>MEM</td>
<td>0.1</td>
<td>0.1</td>
</tr>
<tr>
<td>2</td>
<td>67</td>
<td>MEM</td>
<td>0.05</td>
<td>0.05</td>
</tr>
<tr>
<td>3</td>
<td>52</td>
<td>MEM</td>
<td>0.3</td>
<td>0.3</td>
</tr>
<tr>
<td>4</td>
<td>76</td>
<td>MEM</td>
<td>0.2</td>
<td>0.2</td>
</tr>
<tr>
<td>5</td>
<td>81</td>
<td>VMTS</td>
<td>0.1</td>
<td>0.6</td>
</tr>
<tr>
<td>6</td>
<td>42</td>
<td>VMTS</td>
<td>0.4</td>
<td>0.9</td>
</tr>
<tr>
<td>7</td>
<td>70</td>
<td>VMTS</td>
<td>0.2</td>
<td>0.8</td>
</tr>
</tbody>
</table>

VA: visual acuity; **: Pre and post intravitreal injection of autologous plasmin; VMTS: vitreomacular traction syndrome; MEM: epiretinal membrane.

Fig. 1: Epiretinal membrane (MEM) before (A and B) and after (C and D) the intravitreal injection of autologous plasmin (patient 3). Without neither anatomic nor visual changes.

Fig. 2: Optical Coherence Tomography of the epiretinal membrane (MEM) before (A) and after (B) the intravitreal injection of autologous plasmin (patient 1). Without any anatomical or visual changes.
Fig. 3: Vitreomacular traction syndrome (VMTS) before (A and B) and after (C and D) the injection of intravitreal plasmin. Observe the detachment of the traction of the macular area and the normalization of the macular anatomical structure in the OCT (patient 5). The visual acuity improved from 0.2 to 0.8.

Fig. 4: Vitreomacular traction syndrome (VMTS) before (A) and after (B) the injection of intravitreal plasmin. Observe the normalization of the retinal anatomical structure in the OCT (patient 6). The visual acuity improved from 0.4 to 0.9.
Intravitreal plasmin in the vitreomacular syndrome

nes. This efficacy was negative in the initial series of cases studied with epiretinal membrane (MEM). Until now the intravitreal injection of plasmin was only used immediately before the vitrectomy to facilitate intraoperative handling (16,27-34). Recently, the intravitreal injection of autologous plasmin as a unique treatment – without associated surgical vitrectomy - has shown to be partially efficient in diffuse diabetic macular edema (35). The efficacy of the isolated injection of plasmin in achieving pharmacological separation of the posterior vitreous cortex of the retina, along with its vitreolitic effect, known as enzymatic vitrectomy and pharmacological vitreolysis (16,17,41), open the therapeutic possibility not only of diabetic macular edema and of VMTS, but also of other macular edemas (venal occlusions, uveitis, post surgical, etc.) and other pathologies where the vitreoretinal tractions are clearly involved in their pathology (myopic macular traction syndrome, macular holes, tractional cystoid macular edema syndrome, etc.) (42), without the iatrogenic risks associated with vitreous surgery (cataracts > 80%, tears –20%–, retina detachments, vitreous hemorrhage, etc.) (43-45).

Although there could be no existing direct correlation between the improvement of visual acuity and the membrane detachment, this has occurred in our cases. The patients where the plasmin caused the detachment of the retinal membrane in the macular area –always VMTS cases– were those that increased their vision with the disappearance of the metamorphopsia and normalization of the visual qualitative parameters (clarity, luminosity, contrast and colorimeter).

We should remember that the plasmin injection was used as a first therapeutic option before trying treatment by means of surgical peeling of the macular epiretinal membrane (MEM) and of the VMTS membrane. The visual improvement occurred exclusively in VMTS patients and not in the MEM cases, as perceived by the patient very early (2-3 days), maintaining stability during the complete follow up. This is a pilot study and for this reason its results cannot be generalized. Studies with a greater number of cases and follow ups on the long term are necessary to establish its efficacy as monotherapy, and their possible efficiency of reinjections to consolidate its efficacy in failed MEM cases. It is not theoretically impossible that plasmin reinjections would be capable of achieving the MEM detachment in the cases where it was not achieved in the first injection, but it depends on future studies to establish the number and frequency with which the injections should be carried out before considering the technique failed and proceed to operate.

The big difference in efficacy of the intravitreal injection of autologous plasmin among MEM and VMTS patients is an important point of reflection. In the first place, the Optical Coherence Tomography (OCT) allows a clearer differentiation of both syndromes which could easily be confused by biomicroscope, increasing the identification of these pathologies against the biomicroscope in 10% of the MEM cases and up to 300% in VMTS cases (46-48). In figures 1, 2, 3 and 4 the tomographic difference between both pathologies can be appreciated. In the second place, the absence of efficacy of plasmin in the MEM cases can be explained because over 95% have associated a previous posterior vitreous detachment (49-52). There are MEM cases with adhered vitreous exclusively in young patients (53) –none of them included in our series–, where the efficacy of the surgical vitrectomy was greater to that carried out on patients with DPV (53). Perhaps this subgroup of MEM –with hyaloids adhered on young subjects– is the only one which can be treated with intravitreal plasmin. In the third place, we should remember that the new generations of high definition 3D OCT makes both syndromes (MEM versus VMTS) progressively more complex and difficult to differentiate. In this way, Koizumi and cols (55) observed the coexistence in 10 out of 12 VMTS cases an underlying MEM. This tomographic observation of clinical superposition of both syndromes has been histological corroborated (56-60), where the membranes of both pathologies turn out to be similar with the only difference of associating fragments of the internal limiting membrane in the MEM. Finally, in the fourth place, in the VMTS cases where the intravitreal plasmin has been efficient, this option emerges as an initial minimally invasive therapeutic option, knowing that its evolution is unforeseeable without treatment, with some cases spontaneously evolving towards the complete detachment and resolution between 1 and 6 months from the beginning of the symptoms (11-14), and in others towards the progression to a complete macular hole (61).

The efficacy and toxicity of autologous plasmin is dose- and time-dependent. Previous studies have shown that the intravitreal injection of 0.4 UI of
plasmin is enough to separate the posterior vitreous cortex of the internal limiting membrane, without known toxicity in concentrations of 3-4 UI (62-64). The quantity of autologous plasmin obtained and injected by the method employed in this study is on average 0.26 UI (15.38), but has shown to be enough to be efficient in all the cases treated for vitreomacular traction syndrome (VMTS), and it is hypothetical but not impossible that greater concentrations would have achieved efficiency in epiretinal membranes (MEM). Gandorfer and cols. (26,63,65) found a direct correlation between exposure to plasmin time and the degree of vitreoretinal separation. In vitro studies have shown different exposure to plasmin times for the different main molecules in vitreoretinal adhesion: the lamina is completely degraded in 15 minutes, while the fibronectin continues to degrade under the plasmin action for 60 minutes, with no effects being detectable after 24 hours (65-67). Starting from the principle that residual autologous plasmin in previous studies has always been evacuated from the vitreous in the surgical maneuver immediately after the injection, we believe that from our results intravitreal plasmin should be used as an alternative previous to surgical vitrectomy in VMTS cases rather than surgical coadjuvant. The exposure time of the vitreoretinal surface to autologous plasmin has shown in the present study to be sufficient and not toxic. Also, the change from streptokinase to urokinase as an activator from plasminogen to plasmin (29,30,37,38), has the added advantage of a lower potential for intraocular toxicity as well as avoiding the initial clouding of the vitreous that occurred in the majority of cases when the plasmin was prepared with streptokinase (16,35,36). None of the cases exhibited any complications such as endophthalmitis, vitreous hemorrhaging, uveitis, retina detachment, increase of intraocular pressure or cataract progression.

One of the main problems that limited the use of autologous plasmin has always been its sophisticated system of long, costly and complex preparation, requiring specialized equipment only accessible in determined hematological units. The advantage of the simplified technique of preparation of autologous plasmin is the possibility of preparing it in the Ophthalmology operating room minutes before use in a quick, easy and relatively inexpensive procedure (16,37). The future commercialization of microplasmin (Trombogenics, Ireland) will allow a more universal use of this technique (69) (Stalman P. MIVI-2T Trial. A randomized, double-masked clinical trial of microplasmin intravitreal injection for nonsurgical treatment of vitreomacular traction. Indian Wells, California: American Society of Retina Specialist; 2007) (Brown D. MIVI III Trial. Boston: Retina Society Annual Meeting; 2007).

In conclusion, the enzymatic and pharmacologic vitrectomy carried out only with intravitreal autologous plasmin injection has shown to be efficient and safe in reducing and eliminating macular traction and improving visual acuity in vitreomacular traction syndrome cases (VMTS), avoiding vitrectomy surgery and in none of the macular epiretinal membrane (MEM) cases of the series studied. Future studies should qualify the most adequate and efficient intravitreal dosage of plasmin in the vitreomacular syndrome, the possibility of increasing its efficacy in the initially resistant MEM cases with successive reinjections and/or higher doses, as well as its role facilitating detachment during posterior surgical vitrectomy.

REFERENCES

on surgical decision making for epiretinal membranes and vitreomacular traction. Retina 2007; 27: 552-556.


