Acta Ophthalmologica

Comparison of the efficacy of topical insulin with autologous serum eye drops in persistent epithelial defects of the cornea

David Diaz-Valle,[1] Barbara Burgos-Blasco,[1] Daniela Rego-Lorca,[1] Virginia Puebla-Garcia,[2] Pilar Perez-Garcia,[1] Jose M. Benitez-del-Castillo,[1] Rocio Herrero-Vanrell,[3] Marta Vicario-de-la-Torre[3] and Jose A. Gegundez-Fernandez[1]

[1]Servicio de Oftalmología, Instituto de Investigación Sanitaria, Hospital Clínico San Carlos (IdISSC), Hospital Clinico San Carlos, Madrid, Spain
[2]Servicio de Farmacia. Hospital Clinico San Carlos, Madrid, Spain
[3]Departamento de Farmacia y Tecnología Farmacéutica, Facultad de Farmacia, Universidad Complutense de Madrid, Madrid, España

ABSTRACT.

Purpose: **To investigate the effect of topical insulin on epithelization in persistent epithelial defects (PED) refractory to usual treatment compared to autologous serum.**

Design: **Retrospective, consecutive case–control series.**

Methods: **The charts of 61 consecutive patients with PED treated with topical insulin (case group) and 23 treated with autologous serum (control group) were reviewed. Primary efficacy end points were the percentage of patients in which epithelization was achieved, as well as the rate and time until epithelization. Secondary efficacy point was need for amniotic membrane transplantation (AMT) or other surgeries.**

Results: **Mean time between PED diagnosis and start of topical insulin was 22.7 ± 18.5 days (range 13–115) and the mean area was 14.8 ± 16.2 mm^2 (range 1.1–70.6). In the control group, mean time was 27.9 ± 16.8 days, mean epithelial defect area being 18.6 ± 15.0 mm^2 (range 1.7–52.9). No differences in baseline characteristics were found between groups ($p > 0.05$). Epithelization was achieved in 51 patients (84%) on insulin and 11 patients (48%) on autologous serum ($p = 0.002$). In those patients, mean time until reepithelization was 32.6 ± 28.3 days (range 4–124) in the insulin group and 82.6 ± 82.4 days (range 13–231) in the autologous serum group ($p = 0.011$). The need for AMT was significantly lower in the insulin group ($p = 0.005$). PED recurrence was higher in patients treated on autologous serum (43%) compared with insulin (11%) ($p = 0.002$).**

Conclusions: **Topical insulin is an effective treatment and safely promotes healing of PED. In our series, topical insulin presented better epithelization outcomes than autologous serum and could thus be considered as a first-line treatment.**

Key words: autologous serum – corneal epithelial defect – insulin

DD-V and BB-B contributed equally.

Acta Ophthalmol.

doi: 10.1111/aos.14997

Introduction

Persistent epithelial defects (PED), defined as corneal defects that do not improve after two weeks of conventional treatment, can be challenging. Multiple causes may be responsible for the absence of epithelization of the corneal surface by the limbal stem cells, including altered epithelial adhesion, limbal stem cell deficiency, trauma, medications and infections (Vaidyanathan et al. 2019).

PED treatment starts with conservative management and progresses to medical or even surgical treatments if reepithelization is not achieved (Ziaei, Greene & Green 2018; Vaidyanathan et al. 2019). Non-invasive conventional treatment includes aggressive non-preserved lubrication, withdrawal of epitheliotoxic medication, prophylactic antibiotics, as well as occlusion and the use of bandage soft contact lenses or punctal plugs (Rosenthal, Cotter & Baum 2000; Sacchetti & Lambiase 2014). As a second line of treatment, the use of autologous serum or other haemoderivatives such as platelet-rich plasma has proven effective (Tsubota et al., 1999a, 1999b; López-Plandolit et al. 2010). Surgical treatments, such as debridement and amniotic membrane transplantation (AMT), are sometimes mandatory when the former options fail and there is risk of perforation (Sacchetti & Lambiase 2014; Ziaei, Greene & Green 2018).

Recently, new non-invasive medical treatments have shown utility for the treatment of PED, including recombinant nerve growth factor (NGF), epidermal growth factor (EGF) and topical insulin (Moon et al. 2020; Pflugfelder et al. 2020).

The effect of topical insulin was initially studied in rodent models, and Zagon et al. noted that diabetic rats treated with topical insulin presented smaller epithelial defects than those without insulin, although no promising results were obtained in non-diabetic rats (Zagon et al. 2007). In humans, only a limited number of case series (including from our group) and case reports have described the effect of topical insulin on corneal wound healing. (Aynsley 1945; Bastion & Ling 2013; Wang et al. 2017; Diaz-Valle et al. 2020).

The mechanism by which insulin may improve epithelization is not fully known. Insulin-like growth factors (IGF) play a central role in growth, differentiation and proliferation of corneal epithelial cells. Corneal keratocytes and epithelial cells express IGF-I, its receptors and insulin receptors (Trosan et al. 2016; Titone, Zhu & Robertson 2018). Interestingly, insulin, a potent anabolic hormone, is closely related to IGFs and has been found in tear film (Rocha et al. 2002) In studies in diabetic animals, insulin eye drops restored decreased DNA synthesis in basal epithelial cells to normal levels when measured 48 hours after the injury. Therefore, cell proliferation may be a plausible mechanism for normalization of the reepithelization process (Zagon et al. 2007). Other investigators have hypothesized that insulin may be involved in receptor homeostasis in corneal epithelial cells (Titone, Zhu & Robertson 2018).

Given the limited number of studies examining the effect of topical insulin in PEDs, the paucity of homogenous data and the lack of comparison with established treatment options, the main objective of the present study was to evaluate topical insulin for PEDs refractory to usual treatment in a large series of patients and to analyse how this therapy improves epithelization compared with autologous serum.

Methods

In this case–control study, patients with PED treated between 1st October 2019 and 31st March 2021 with off-label ophthalmic application of insulin eye drops at Hospital Clinico San Carlos in Madrid were included as case group. For the control group, patients with PED who had been treated with autologous serum were retrospectively selected. The study was conducted in accordance with the ethical guidelines of the Declaration of Helsinki and was approved by the hospital's Ethics Committee.

Since October 2019, patients with PED, defined as an epithelial defect unresponsive to conventional treatment for approximately 2 weeks (Vajpayee et al. 2003), with no improvement after standard treatment are offered treatment with topical insulin by our Corneal Unit. The risks, benefits and treatment alternatives are always discussed with all patients and verbal consent is obtained for the off-label use of insulin. This is a retrospective case series of those patients, whose charts were retrospectively reviewed. Only patients with incomplete follow-ups, because they had failed to attend the scheduled visits, and patients who had an absence of slit-lamp images were excluded.

The historic control group was made of patients with PED who had been treated with autologous serum between 1st October 2018 and 30th September 2019, which were consecutively recruited. The same exclusion criteria were applied.

From the included patients, the following variables were recorded: age, sex, diabetes mellitus history, previous ocular surgeries, ophthalmologic conditions, PED aetiology (determined by medical history and ophthalmological examination), time since diagnosis, concomitant treatment, best corrected visual acuity (BCVA) and PED area (measured in mm^2) before starting treatment with topical insulin or autologous serum. Patients in the insulin group that had been treated previously with autologous blood serum were not reflected in the autologous blood serum group in terms of a control in this study. In all patients, the same artificial tears were prescribed (hyaluronic acid 0.15%, no preservatives).

Patients were divided according to PED aetiology in the following groups: infectious, neurotrophic, chronic alterations of the ocular surface and immune-mediated. Infectious PED included those PED with active infections, while post-infectious PEDs were considered neurotrophic. The neurotrophic group also included herpetic keratitis, intracranial space-occupying lesions and neurosurgical procedures that damage the trigeminal ophthalmic branch.

To evaluate the PED area, anterior segment photography of the cornea was performed at each visit after administration of fluorescein dye at a magnification of 10× using a camera attached to a slit-lamp microscope. The images were analysed with an image analysis system (ImageJ software) by a single observer to determine the PED area.

The insulin and autologous serum drops were prepared in the hospital's Pharmacy Service using a sterile technique. Each patient was instructed by the pharmacist for the correct handling and administration of the drops, as well as the conditions of conservation. Based on the information available in the literature and our excellent results with this technique (Wang et al. 2017; Galvis et al. 2019; Diaz-Valle et al. 2020), the insulin eye drops were prepared at a concentration of 1 IU/ml, using fast-acting insulin (Actarapid; Novo Nordisk A/S, Søborg, Denmark) in solution for subcutaneous injection. The insulin solution (100 IU/ml) was diluted in a polyethylene glycol and polypropylene glycol base. A sterile filtration was performed, and the final solution was packaged in sterile amber glass eye drop bottles. The drops were refrigerated and used up to one month after preparation.

For autologous serum, all patients were screened for syphilis, hepatitis B and C viruses, and HIV serology before preparation of the eye drops. Twenty millilitres of blood were extracted from each patient and left in a vertical position for two hours at room temperature. Then, the serum was centrifuged for 10 min at 1370 g. The supernatant serum was removed in a sterile eye drop bottle, and 12 ml of patient serum were diluted with 48 ml of 0.9% sodium chloride for a 20% concentration.

All insulin patients were prescribed insulin eye drops every 6 hours (four times a day) on a compassionate basis for the treatment of refractory PED after standard treatment had failed. Patients on autologous serum were also

prescribed such treatment every 6 hours with the same criteria.

After initiating insulin or autologous serum treatment, patients were followed on a frequent basis in order to truly identify the rate of epithelialization. The variables recorded on each visit were BCVA, epithelial defect area (measured in the slit-lamp photographs), topical treatment, need for AMT or other surgeries and recurrence. Follow-up was also registered.

The primary efficacy end points were whether epithelization was achieved with the treatment, and in those cases, the rate (initial PED area divided by days till epithelization, in mm^2/day) and time until complete healing of the epithelial defect (defined as the absence of fluorescein staining of the cornea) were also included. When PED healed within two weeks, it was labelled as qualified success and when it healed within one month, it was labelled as partial success. Secondary efficacy point was need for AMT or other surgeries.

A descriptive statistical analysis was performed and the variables are presented as mean, standard deviation and range when quantitative and number and percentage when qualitative. To compare variables between both treatment groups, Fisher test and Mann–Whitney *U* test were performed accordingly. Kruskal–Wallis and Chi-squared tests were used to compare the variables depending on PED aetiology with each group. A Kaplan–Meier analysis evaluating time till epithelization was performed. $P < 0.05$ was considered statistically significant.

Results

The study population comprised 61 patients treated with insulin eye drops (35 females and 26 males), with a mean age of 71.5 ± 19.3 years (range 10–95). For the control group, 23 patients (15 females and 8 males) with PED treated with autologous serum were included, mean age being 72.3 ± 17.9 years (range 24–94). No differences in age or sex were noted between groups ($p > 0.05$).

Table 1 summarizes patient demographics, previous ophthalmologic conditions and ocular surgeries, along with baseline PED aetiology, characteristics and treatment. Occlusion included patching and tarsorrhaphy. Most common

Table 1. Baseline characteristics of patients included in each treatment group

Baseline characteristics	Insulin (n = 61)	Autologous serum (n = 23)	p
Age			
Mean ± SD	71.5 ± 19.3	72.3 ± 17.9	0.960†
Range	10–95	24–94	
Sex			
Male. N (%)	26 (43%)	8 (35%)	0.621*
Female. N (%)	35 (57%)	15 (65%)	
Diabetes mellitus. N (%)	8 (13%)	7 (30%)	0.107*
On insulin. N (%)	4 (7%)	2 (9%)	0.663*
Previous ocular surgery			
None. N (%)	21 (34%)	7 (30%)	0.800*
Cataract. N (%)	19 (31%)	8 (35%)	0.796*
Cornea. N (%)	16 (26%)	7 (30%)	0.785*
Glaucoma. N (%)	13 (21%)	1 (4%)	0.099*
Retina. N (%)	7 (11%)	2 (9%)	1.000*
Other. N (%)	1 (2%)	2 (9%)	0.181*
Previous ophthalmologic conditions			
None. N (%)	17 (28%)	10 (43%)	0.197*
Glaucoma. N (%)	17 (28%)	4 (17%)	0.405*
Retinal disorders. N (%)	9 (15%)	2 (9%)	0.719*
Corneal alterations. N (%)	22 (36%)	8 (35%)	1.000*
Uveitis. N (%)	8 (13%)	1 (4%)	0.433*
Other. N (%)	1 (2%)	1 (4%)	0.475*
PED aetiology			
Infectious. N (%)	20 (33%)	6 (26%)	0.608*
Bacterial. N (%)	11 (18%)	3 (13%)	
Mycotic. N (%)	2 (3%)	1 (4%)	
Acanthamoeba. N (%)	2 (3%)	0	
Unknown. N (%)	5 (8%)	2 (9%)	
Neurotrophic. N (%)	21 (34%)	9 (39%)	0.800*
Herpetic eye disease. N (%)	6 (10%)	3 (13%)	
Damage of the trigeminal nerve. N (%)	6 (10%)	1 (4%)	
Lagophthalmos. N (%)	3 (5%)	3 (13%)	
Post-infectious. N (%)	3 (5%)	1 (4%)	
Ophthalmologic surgery. N (%)	3 (5%)	1 (4%)	
Chronic alterations of the ocular surface. N (%)	18 (30%)	6 (26%)	1.000*
Bullous keratopathy. N (%)	6 (10%)	1 (4%)	
Calcium keratopathy. N (%)	5 (8%)	2 (9%)	
Drug toxicity. N (%)	4 (7%)	1 (4%)	
Severe dry eye disease. N (%)	2 (3%)	1 (4%)	
Other. N (%)	1 (2%)	1 (4%)	
Immune-mediated. N (%)	2 (3%)	2 (9%)	0.301*
Days with PED pre-treatment			
Mean ± SD	22.7 ± 18.5	27.9 ± 16.8	0.147†
Range	13–115	13–59	
VA pre-treatment			
Mean ± SD	0.07 ± 0.14	0.05 ± 0.18	0.881†
Range	0–0.5	0–0.3	
Area pre-treatment (mm^2)			
Mean ± SD	14.8 ± 16.2	18.6 ± 15.0	0.144†
Range	1.1–70.6	1.7–52.9	
Previous treatment			
Intensive lubrication. N (%)	61 (100%)	23 (100%)	1.000*
BCL. N (%)	37 (61%)	15 (65%)	0.804*
Occlusion. N (%)	15 (25%)	4 (17%)	0.570*
Antibiotics. N (%)	57 (93%)	20 (87%)	0.386*
Antivirals. N (%)	10 (16%)	5 (22%)	0.542*
Doxycycline. N (%)	22 (36%)	13 (57%)	0.136*
Corticosteroids. N (%)	15 (25%)	10 (43%)	0.112*
Autologous serum. N (%)	9 (15%)	-	-
Cyclosporine. N (%)	2 (3%)	2 (9%)	0.301*
AMT. N (%)	3 (5%)	3 (13%)	0.339*

AMT = amniotic membrane transplantation, BCL = bandage contact lens, PED = persistent epithelial defect, SD = standard deviation, VA = visual acuity.

* Fisher test.

† Mann–Whitney *U* Test.

aetiology of PED was neurotrophic in both groups. For the study subjects, the mean time between diagnosis and start of topical insulin was 22.7 ± 18.5 days (range 13–115) and the mean epithelial defect area at the beginning of treatment was 14.8 ± 16.2 mm^2 (range 1.1–70.6). In the control group, mean time of PED prior to autologous serum treatment was 27.9 ± 16.8 days (range 13–59), mean epithelial defect area being 18.6 ± 15.0 mm^2 (range 1.7–52.9). No differences in baseline characteristics were found between groups ($p > 0.05$).

Table 2 summarizes patients' epithelization results. Epithelization was achieved in 51 patients (84%) on insulin and 11 patients (48%) on autologous serum ($p = 0.002$) (Fig. 1). More patients achieved epithelization during the first 14 days in the insulin group (16 patients, 26%) compared with the autologous serum group (0 patients; $p = 0.004$). In patients where PED closure was achieved, the mean time until reepithelization was 32.6 ± 28.3 days (range 4–124) in the insulin group and 82.6 ± 82.4 days (range 13–231) in the autologous serum group ($p = 0.011$). No statistically significant differences in the daily epithelization rate were detected between groups (insulin: 0.51 ± 0.55 mm^2/day; autologous serum 0.33 ± 0.30 mm^2/day; $p = 0.407$). Also, no differences were found in the daily epithelization rate between diabetics and non-diabetics ($p > 0.05$). The need for AMT was significantly lower in the insulin group (10 patients; 16%) compared with the control group (11 patients; 48%) ($p = 0.005$). Mean time since start of treatment with topical insulin or autologous serum was 20.8 ± 15.9 and 32.1 ± 25.5 days, respectively ($p = 0.244$). Of the three patients with previous AMT treated with insulin, two of them healed and the remaining required another AMT. In the autologous serum group, the same results were observed.

In addition, patients were compared divided by aetiology among each treatment group and between groups (Table 3). Among autologous serum, no differences were detected. In insulin patients, pre-treatment PED area was higher in those of infectious aetiology, along with a reduction in epithelization achievement and an increased prevalence of AMT compared with PEDs of other aetiology. When insulin patients and those on autologous serum were compared, infectious PEDs in the former group presented increased epithelization rates. As for neurotrophic PEDs, pre-treatment areas were higher in the insulin group, but so was the proportion of patients where epithelization was achieved and there was less need of AMT. No differences were noted in patients with chronic alterations of the ocular surface.

Figures 2 and 3 depict the epithelization rate in both treatment groups depending on aetiology and PED pre-treatment area. Infectious PED present a statistically significant increased epithelization rate when treated with insulin compared with autologous serum ($p = 0.039$).

Topical insulin was well tolerated and no adverse events were reported with the treatment. PED recurrence was higher in patients treated on autologous serum (10 patients; 43%) compared with insulin (7 patients; 11%) ($p = 0.002$) and so was follow-up given that controls were historic ($p < 0.001$). The follow-up period for this study group will extend beyond that which is reported in the present study.

Table 2. Epithelization results in patients treated with insulin and autologous serum

Epithelization results	Insulin ($n = 61$)	Autologous serum ($n = 23$)	p
Epithelization achieved. N (%)	51 (84%)	11 (48%)	**0.002***
Qualified success. N (%)	16 (26%)	0	**0.004***
Partial success. N (%)	17 (28%)	3 (13%)	0.250*
Time till epithelization (days)			
Mean ± SD	32.6 ± 28.3	82.6 ± 82.4	**0.011**†
Range	4–124	13–231	
Epithelization rate (mm^2/day)			
Mean ± SD	0.51 ± 0.55	0.33 ± 0.30	0.407†
Range	0.04–2.33	0.03–0.99	
Epithelization failure			
AMT. N (%)	10 (16%)	11 (48%)	**0.005***
Other surgeries. N (%)	1 (2%)	3 (13%)	0.061*
Recurrence. N (%)	7 (11%)	10 (43%)	**0.002***
Follow-up (months)			
Mean ± SD	8.6 ± 5.4	23.0 ± 3.7	**<0.001**†
Range	2–17	18–30	

AMT = amniotic membrane transplantation, SD = standard deviation.
* Fisher test.
† Mann–Whitney *U* Test.

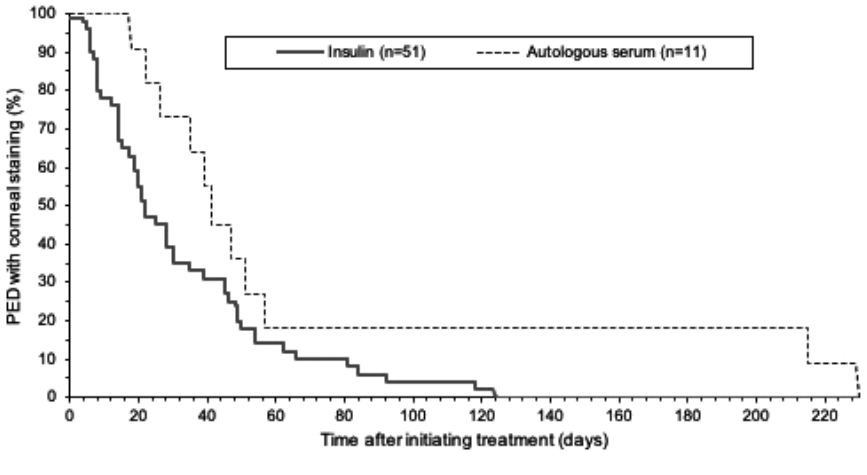

Fig. 1. Kaplan–Meier curves for days till epithelization in patients with persistent epithelial defects on topical insulin (solid line) and autologous serum (broken line). Only patients who achieved epithelization (defined as absence of corneal fluorescein staining) are included in this analysis.

Discussion

PED is a sight-threatening condition that can result in corneal melting, perforation and severe vision loss.

Table 3. Comparison between insulin and autologous serum patients according to aetiology

	Insulin group (n = 61)				
	Infectious (n = 20)	Neurotrophic (n = 21)	Chronic alterations of the ocular surface (n = 18)	Immune-mediated (n = 2)	p*
Time with PED					
Mean ± SD	23.2 ± 17.2	25.0 ± 23.8	20.4 ± 13.9	14.5 ± 0.7	0.885[†]
Range	13–73	13–115	13–74	14–15	
VA pre-treatment					
Mean ± SD	0.05 ± 0.12	0.14 ± 0.18	0.01 ± 0.02	0.20 ± 0.28	**0.048**[†]
Range	0.05–0.2	0–0.5	0–0.1	0.005–0.4	
Area pre-treatment					
Mean ± SD	24.2 ± 20.4	9.4 ± 7.6	12.2 ± 15.3	2.7 ± 1.2	**0.013**[†]
Range	1.6–70.6	1.1–27.2	1.3–52.9	1.8–3.5	
Epithelization results					
Epithelization achieved	13 (65%)	20 (95%)	16 (89%)	2 (100%)	**0.048**[&]
Qualified success	4 (20%)	5 (24%)	6 (33%)	1 (50%)	0.627[&]
Partial success	5 (25%)	9 (43%)	3 (17%)	0	0.178[&]
Time till epithelization (days)					
Mean ± SD	31.5 ± 26.2	29.8 ± 26.8	37.1 ± 33.6	32.0 ± 25.5	0.923[†]
Range	4–84	6–124	7–118	14–50	
Epithelization rate (mm^2/day)					
Mean ± SD	0.72 ± 0.68	0.41 ± 0.34	0.51 ± 0.64	0.14 ± 0.15	0.219[†]
Range	0.10–2.25	0.04–1.19	0.04–2.33	0.04–0.25	
Epithelization failure					
AMT	7 (35%)	1 (5%)	2 (11%)	0	**0.048**[&]
Other surgeries	1 (5%)	0	0	0	0.555[&]
Recurrence	0	4 (19%)	3 (17%)	0	0.211[&]

Autologous serum group (n = 23)					Aetiology comparison (p insulin versus autologous serum)		
Infectious (n = 6)	Neurotrophic (n = 9)	Chronic alterations of the ocular surface (n = 6)	Immune-mediated (n = 2)	p*	Infectious	Neurotrophic	Chronic alterations of the ocular surface
27.0 ± 16.8	20.8 ± 11.0	36.8 ± 19.6	35.5 ± 29.0	0.279[†]	0.689[¶]	0.857[¶]	0.057[¶]
13–58	13–47	13–59	15–56				
0.05 ± 0.08	0.02 ± 0.03	0.04 ± 0.08	0.16 ± 0.21	0.859[†]	0.976[¶]	0.250[¶]	0.787[¶]
0.001–0.2	0–0.1	0.001–0.2	0.01–0.3				
18.1 ± 14.4	19.2 ± 12.7	15.1 ± 15.0	28.0 ± 35.2	0.639[†]	0.646[¶]	**0.033**[¶]	0.976[¶]
4.7–39.4	5.9–38.8	1.7–37.8	3.1–52.9				
3 (50%)	4 (44%)	4 (67%)	0	0.434[&]	0.644[§]	**0.005**[§]	0.251[§]
0	0	0	0	1.000[&]	0.543[§]	0.286[§]	0.277[§]
2 (33%)	1 (11%)	0	0	0.333[&]	1.000[§]	0.204[§]	0.546[§]
92.7 ± 119.0	78.0 ± 86.6	85.5 ± 86.6	-	0.750[†]	0.346[¶]	0.066[¶]	0.237[¶]
22–230	18–231	35–215	-				
0.18 ± 0.09	0.53 ± 0.37	0.26 ± 0.36	-	0.317[†]	**0.039**[¶]	0.477[¶]	0.385[¶]
0.07–0.25	0.12–0.99	0.05–0.74	-				
3 (50%)	4 (44%)	2 (33%)	2 (100%)	0.434[&]	0.644[§]	**0.020**[§]	0.251[§]
2 (33%)	1 (11%)	0	0	0.333[&]	0.123[§]	0.300[§]	1.000[§]
3 (50%)	3 (33%)	3 (50%)	0	0.571[&]	**0.008**[§]	0.640[§]	0.139[§]

AMT = amniotic membrane transplantation, PED = persistent epithelial defect, SD = standard deviation, VA = visual acuity.
* Compares infectious, neurotrophic and chronic alterations of the ocular. Statistically significant differences are marked in bold.
[†] Kruskal–Wallis.
[&] Chi-squared test.
[¶] Mann–Whitney U test.
[§] Fisher test.

Treating PED is challenging for ophthalmologists and surgical intervention, such as AMT or tarsorrhaphy, or even new techniques such as corneal neurotization are occasionally indicated to manage refractory cases that are unresponsive to medical therapy (Katzman & Jeng 2014; Giannaccare et al. 2020). Recently, the use of topical insulin has reportedly yielded satisfactory results in several small case series of PED patients, posing as a possible effective alternative to autologous serum (Diaz-Valle et al. 2020).

Autologous serum eye drops have proved to be useful for the treatment of ocular surface conditions including severe dry eye, PED, recurrent erosion

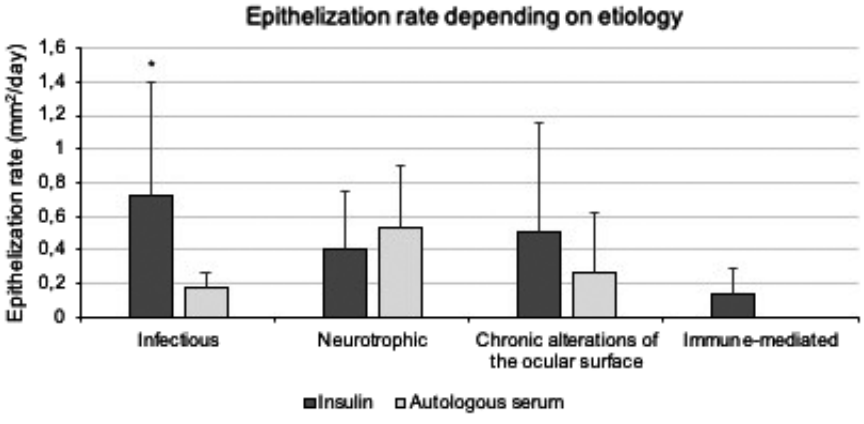

Fig. 2. Daily epithelization rate in both treatment groups depending on persistent epithelial defect (PED) aetiology. Infectious PED present a statistically significant increased epithelization rate when treated with insulin compared with autologous serum ($p = 0.039$). $*^{\text{indicates } p < 0.05.}$

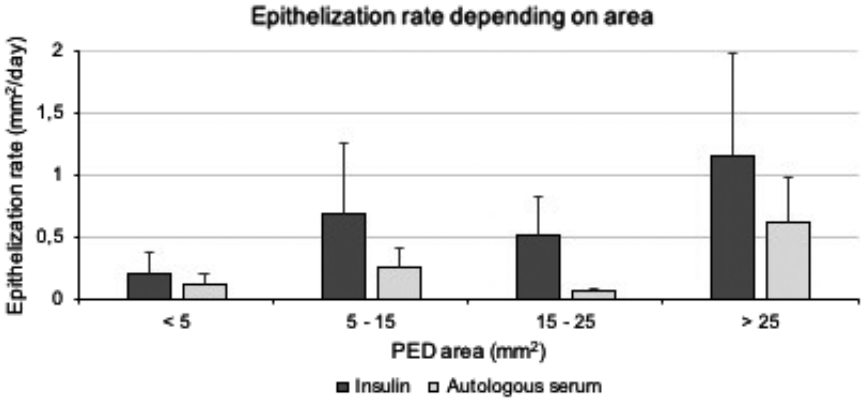

Fig. 3. Daily epithelization rate in both treatment groups depending on persistent epithelial defect (PED) pre-treatment area. No statistically significant differences were noted between groups.

syndrome and superior limbic keratoconjunctivitis (Jeng & Dupps 2009; Kim, Shin & Kim 2012; Cho et al. 2013; Lekhanont et al. 2013; Semeraro et al. 2014). Its effect relies on that human serum contains multiple biological factors such as EGF, transforming growth factor beta, platelet-derived growth factor, IGF, neurotrophic factors, fibronectin and vitamins; some of which are necessary for the proliferation, migration and maturation of the corneal epithelial cells (Tsubota et al., 1999a, 1999b). The American Academy of Ophthalmology has recently reviewed the effectiveness and safety of using autologous serum-based eye drops for the treatment of ocular surface disease. Of the four studies which were evaluated, all showed substantial improvement in the epithelial defects and noted a reduction of 71–100% in the size of the PED. However, they acknowledge that conclusions are limited owing to the absence of controlled trials. In addition, microbial contamination during preparation or storage was reported a considerable risk in patients who have a compromised ocular surface (Shtein et al. 2020).

For the past few years, topical insulin has been demonstrated to promote and accelerate corneal reepithelization, offering many benefits over other therapeutic options. However, since first reported as effective in five cases of epithelial defects unresponsive to treatment by Aynsley in 1945 (Aynsley 1945), studies in humans are scarce, being limited to case series and case reports. In healthy corneas of diabetic patients undergoing epithelial debridement for retinal surgery, Bastion and Ling noted an increase in the healing rate of the defects with topical insulin (Bastion & Ling 2013). Another series was reported by Wang et al. including six patients with neurotrophic corneal ulcers or epithelial defects refractory to standard treatment who were prescribed topical insulin. Complete epithelization was achieved within 7 to 25 days (Wang et al. 2017). Notwithstanding, our group has presented the largest series to date and includes the results of 21 patients with refractory PED (which are also included in the present study). Mean PED area before treatment was 17.6 ± 16.5 mm^2 (range 3.9–70.6), and by the end of the follow-up period, 17 patients (81%) had achieved reepithelization (mean time 34.8 ± 29.9 days; range 7–114) (Diaz-Valle et al. 2020).

Our results reveal that topical insulin achieved epithelization in a higher number of patients compared to autologous serum (84% versus 48%; $p = 0.002$), especially during the first 14 days of treatment. More importantly, not only the mean time until reepithelization was lower in the insulin group, but the need for AMT was also significantly lower (16% versus 48%; $p = 0.005$). AMT is an effective surgery for PED but is usually reserved for lesions unresponsive to medical treatment as a last resort or in those with severe corneal melting or at risk of perforation (Sacchetti & Lambiase 2014). Therefore, a decrease in the need for AMT with topical insulin should also be considered as an important step towards epithelization (Solomon et al. 2002; Mead, Tighe & Tseng 2020). For this reason, we have adopted insulin treatment as a first choice within second-line treatment options, that is, when epithelization is not achieved in two weeks with standard initial treatment. It would also seem to us an effective and valid first-line of treatment option, to avoid prolonging the re-epithelialization of PED and the appearance of possible associated complications.

As for insulin effectiveness compared with autologous serum depending on PED aetiology, significant differences were detected in the epithelization rate of infectious PEDs. In our series, infectious PEDs presented increased pre-treatment areas, worse epithelization achievement and higher rates of AMT, suggesting that infectious PEDs might be the real treatment challenge. This might be due to the release of multiple enzymes that contribute to stromal keratolysis by the injured corneal epithelium, the infiltrating neutrophils and some infectious organisms (Brown, Bloomfield & Tam 1974; Kim et al. 2001). Moreover,

toxicity due to topical fortified antibiotics and a possible neurotrophic component due to involvement of the corneal plexus could also hinder epithelization (Gilbert, Wilhelmus & Osato 1987). It is in these specific cases that insulin exhibited the best results compared with autologous serum (Fig. 4). Within the autologous serum group, no differences in epithelization variables were detected, probably because of the size sample.

The current study includes a large series with a long follow-up (in our former series, patients had a mean follow-up of 107 days after starting on insulin) and we have added a control group of patients with refractory PEDs who were prescribed autologous serum before we started using topical insulin at our centre. When recruiting patients to medical treatment who were prescribed autologous serum, we found that there were far fewer patients than treated with insulin in the same period of time. Before insulin was prescribed at our centre, fewer patients with refractory PEDs were treated with autologous serum because it takes about 10 days to prepare in our centre and many PEDs worsened or even perforated, requiring AMT or other surgeries beforehand. However, a considerably good number of controls was included.

Our study is the first comparative study evaluating the effectiveness of topical insulin and autologous serum in refractory PEDs and subdividing patients by aetiology. Large collections of patients with PEDs are rare, and comparative studies are scarce. No other studies analysing in such detail the effect of topical insulin are available to date. We observed that topical insulin is more effective than autologous serum in PEDs in many ways. In addition, need for multiple blood tests, accessibility and cost are substantial barriers to the use of autologous serum, while insulin presents faster dispensation to the patient, good availability, low cost, excellent tolerance and no adverse side effects.

However, a few limitations of the present study must be acknowledged, mainly its retrospective nature and the historic control group. Our case series presents a wider time range until healing in both groups compared with other series, probably because of the greater number and more complicated cases given that we are a specialized referral centre and that epithelization was considered when no cornea staining with fluorescein was noted, while other groups consider epithelization when PED area is below 0.5 mm^2. The latter explains the larger range of data. In the insulin group, few patients were prescribed autologous serum. Since we were under the clinical impression that it was effective and it takes at least 10 days to make the autologous serum in our centre, many patients were directly prescribed insulin. In addition, it is complicated to find a completely homogeneous sample so as to be comparable, although in our sample, no statistical differences in the baseline characteristics were found between both groups. It is therefore difficult to generalize to all PEDs. Finally, multiple p-values are presented and statistical differences could be a result of this.

While the rationale for using AMT is stated, it is a subjective call to make. Nevertheless, our clinical impression is that insulin is much more effective than autologous serum and that since we started to prescribe topical insulin less AMT are required for PEDs in our

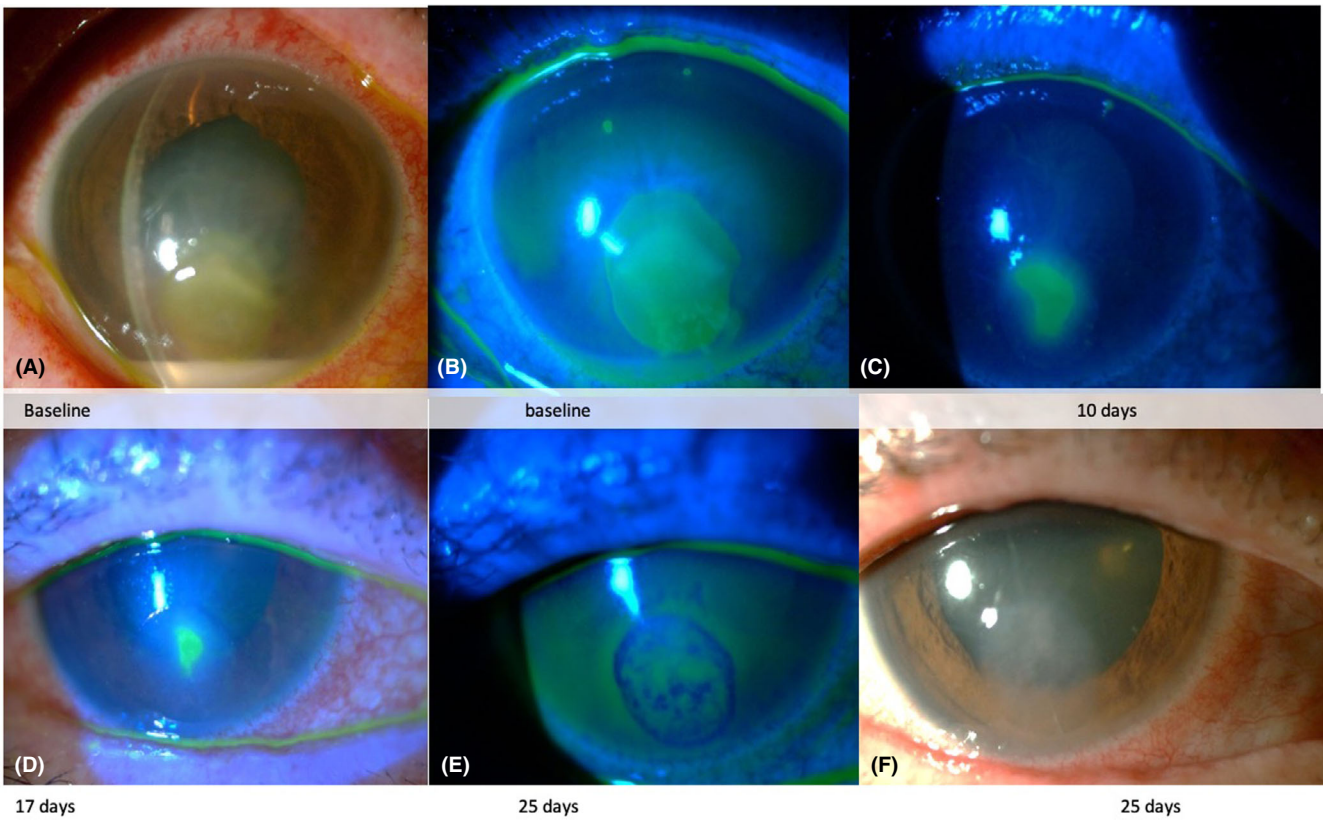

Fig. 4. (A–F). Serial slit-lamp images of an infectious persistent epithelial defect treated with topical insulin.

centre. Based on this, we have modified our treatment algorithm for PEDs refractory to routine treatment and included insulin as the first line of treatment in unresponsive cases, above autologous serum, PRGF and NGF. This is a pilot study to confirm our clinical impression that insulin was effective, but undoubtedly, now randomized prospective studies will be necessary to corroborate these preliminary results and provide a greater degree of evidence of the efficacy of topical insulin in the treatment of PEDs.

In conclusion, topical insulin is an effective treatment and safely promotes healing of PED in patients unresponsive to standard treatment. A larger clinical trial is appropriate to fully establish the differences between the two treatment methods.

References

Aynsley TR (1945): The use of insulin in the treatment of corneal ulcers. Br J Ophthalmol **29**: 361–363.

Bastion MLC & Ling KP (2013): Topical insulin for healing of diabetic epithelial defects?: A retrospective review of corneal debridement during vitreoretinal surgery in Malaysian patients. Med J Malaysia **68**: 208–216.

Brown SI, Bloomfield SE & Tam W (1974): The cornea-destroying enzyme of *Pseudomonas aeruginosa*. Invest Ophthalmol **13**: 174–180.

Cho YK, Huang W, Kim GY & Lim BS (2013): Comparison of autologous serum eye drops with different diluents. Curr Eye Res **38**: 9–17.

Diaz-Valle D, Burgos-Blasco B, Gegundez-Fernandez JA, Garcia-Caride S, Puebla-Garcia V, Peña-Urbina P & Benitez-del-Castillo JM (2020): Topical insulin for refractory persistent corneal epithelial defects. Eur J Ophthalmol 11206721209 5830.

Galvis V, Niño CA, Tello A, Grice JM & Gómez MA (2019): Topical insulin in neurotrophic keratopathy after resection of acoustic neuroma. Arch Soc Esp Oftalmol **94**: 100–104.

Giannaccare G, Bolognesi F, Biglioli F et al. (2020): In vivo and ex vivo comprehensive evaluation of corneal reinnervation in eyes neurotized with contralateral supratrochlear and supraorbital nerves. Cornea **39**: 210–214.

Gilbert ML, Wilhelmus KR & Osato MS (1987): Comparative bioavailability and efficacy of fortified topical tobramycin. Invest Ophthalmol Vis Sci **28**: 881–885.

Jeng BH & Dupps WJ (2009): Autologous serum 50% eyedrops in the treatment of persistent corneal epithelial defects. Cornea **28**: 1104–1108.

Katzman LR & Jeng BH (2014): Management strategies for persistent epithelial defects of the cornea. Saudi J Ophthalmol **28**: 168–172.

Kim J-S, Kim J-C, Hahn T-W & Park W-C (2001): Amniotic membrane transplantation in infectious corneal ulcer. Cornea **20**: 720–726.

Kim KM, Shin Y-T & Kim HK (2012): Effect of autologous platelet-rich plasma on persistent corneal epithelial defect after infectious keratitis. Jpn J Ophthalmol **56**: 544–550.

Lekhanont K, Jongkhajornpong P, Choubtum L & Chuckpaiwong V. (2013): Topical 100% serum eye drops for treating corneal epithelial defect after ocular surgery. BioMed Res Int **2013**: 1–7.

López-Plandolit S, Morales MC, Freire V, Etxebarría J & Durán JA (2010): Plasma rich in growth factors as a therapeutic agent for persistent corneal epithelial defects. Cornea **29**: 843–848.

Mead O, Tighe S & Tseng SG (2020): Amniotic membrane transplantation for managing dry eye and neurotrophic keratitis. Taiwan J Ophthalmol **10**: 13.

Moon HS, Li L, Yoon HJ, Ji YS & Yoon KC (2020): Effect of epidermal growth factor ointment on persistent epithelial defects of the cornea. BMC Ophthalmol **20**: 147.

Pflugfelder SC, Massaro-Giordano M, Perez VL et al. (2020): Topical recombinant human nerve growth factor (Cenegermin) for neurotrophic keratopathy: A multicenter randomized vehicle-controlled pivotal trial. Ophthalmology **127**: 14–26.

Rocha EM, Cunha DA, Carneiro EM, Boschero AC, Saad MJA & Velloso LA (2002): Identification of insulin in the tear film and insulin receptor and IGF-1 receptor on the human ocular surface. Invest Ophthalmol Vis Sci **43**: 963–967.

Rosenthal P, Cotter JM & Baum J (2000): Treatment of persistent corneal epithelial defect with extended wear of a fluid-ventilated gas-permeable scleral contact lens. Am J Ophthalmol **130**: 33–41.

Sacchetti M & Lambiase A (2014): Diagnosis and management of neurotrophic keratitis. Clin Ophthalmol **8**: 571–579.

Semeraro F, Forbice E, Braga O, Bova A, Di Salvatore A & Azzolini C (2014): Evaluation of the efficacy of 50% autologous serum eye drops in different ocular surface pathologies. Biomed Res Int **2014**: 1–11.

Shtein RM, Shen JF, Kuo AN, Hammersmith KM, Li JY & Weikert MP (2020): Autologous serum-based eye drops for treatment of ocular surface disease. Ophthalmology **127**: 128–133.

Solomon A, Meller D, Prabhasawat P, John T, Espana EM, Steuhl K-P & Tseng SC (2002): Amniotic membrane grafts for nontraumatic corneal perforations, descemetoceles, and deep ulcers. Ophthalmology **109**: 694–703.

Titone R, Zhu M & Robertson DM (2018): Insulin mediates de novo nuclear accumulation of the IGF-1/insulin Hybrid Receptor in corneal epithelial cells. Sci Rep **8**: 4378.

Trosan P, Javorkova E, Zajicova A, Hajkova M, Hermankova B, Kossl J, Krulova M & Holan V (2016): The supportive role of insulin-like growth factor-I in the differentiation of murine mesenchymal stem cells into corneal-like cells. Stem Cells Dev **25**: 874–881.

Tsubota K, Goto E, Fujita H, Ono M, Inoue H, Saito I & Shimmura S (1999): Treatment of dry eye by autologous serum application in Sjogren's syndrome. Br J Ophthalmol **83**: 390–395.

Tsubota K, Goto E, Shimmura S & Shimazaki J (1999): Treatment of persistent corneal epithelial defect by autologous serum application. Ophthalmology **106**: 1984–1989.

Vaidyanathan U, Hopping GC, Liu HY, Somani AN, Ronquillo YC, Hoopes PC & Moshirfar M (2019): Persistent corneal epithelial refects: A review article. Med Hypothesis Discov Innov Ophthalmol J **8**: 163–176.

Vajpayee RB, Mukerji N, Tandon R, Sharma N, Pandey RM, Biswas NR, Malhotra N & Melki SA (2003): Evaluation of umbilical cord serum therapy for persistent corneal epithelial defects. Br J Ophthalmol **87**: 1312–1316.

Wang AL, Weinlander E, Metcalf BM, Barney NP, Gamm DM, Nehls SM & Struck MC (2017): The use of topical insulin to treat refractory neurotrophic corneal ulcers. Cornea **36**: 1426–1428.

Zagon IS, Klocek MS, Sassani JW & McLaughlin PJ. (2007): Use of topical insulin to normalize corneal epithelial healing in diabetes mellitus. Arch Ophthalmol (Chicago, Ill 1960) **125**: 1082–1088.

Ziaei M, Greene C & Green CR (2018): Wound healing in the eye: Therapeutic prospects. Adv Drug Deliv Rev **126**: 162–176.

Received on June 1st, 2021.
Accepted on August 4th, 2021.

Correspondence:
Barbara Burgos-Blasco
Departmento de Oftalmología
Hospital Clinico San Carlos
Calle Prof Martin Lagos s/n 28040
Madrid
Spain
Email: bburgos171@hotmail.com

The authors declare no funding was received.

DD-V and BB-B contributed equally.