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ORIGINAL RESEARCH

A 1-Year Randomized Study of the Clinical and Confocal Effects of Tafluprost and Latanoprost in Newly Diagnosed Glaucoma Patients

Paolo Fogagnolo · Angelica Dipinto · Elisa Vanzulli · Emanuele Maggiolo · Stefano De Cilla' · Alessandro Autelitano · Luca Rossetti

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ABSTRACT

Introduction: The aim of the present study was to compare the confocal and clinical features of newly diagnosed glaucoma patients receiving unpreserved prostaglandins (tafluprost) versus preserved prostaglandins (latanoprost).

Materials and Methods: 40 patients were randomized to tafluprost 0.0015% (20 patients; 32 eyes) or latanoprost 0.005% + benzalkonium chloride 0.02% (20 patients; 35 eyes) once daily for 1 year. Inclusion criteria were new glaucoma diagnosis, and no ocular treatments for 6 months before the study. Patients were evaluated at baseline and every 3 months with a complete ophthalmologic evaluation, Schirmer's test, break-up time test, confocal microscopy of the central cornea, and measurement of intraocular pressure (IOP). Investigators were masked to treatment. Both eyes were analyzed if they fulfilled inclusion criteria. Treatments and changes between follow-up and baseline were compared by analysis of variance (ANOVA), *t* test and Chi-square test.

Results: At baseline, the two groups had similar age, ocular surface and confocal findings; keratocyte activation was present in 40%, branching pattern in 85%, and beading in 75%, with no inter-group differences. At follow-up, no significant clinical changes were detected, apart from a drop of IOP by 3.6–4.2 mmHg in the two groups ($p < 0.001$, with no difference between treatments). Despite inter-treatment ANOVA for confocal microscopy being negative, subtle changes were present. During follow-up, all eyes without nerve branching pattern at baseline progressively developed it when treated with latanoprost, whereas no change occurred using tafluprost treatment ($p = 0.05$). None of the eyes without beading at baseline developed it at the

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P. Fogagnolo (✉) · A. Dipinto · E. Vanzulli · E. Maggiolo · S. De Cilla' · A. Autelitano · L. Rossetti
Eye Clinic, Dipartimento Testa-Collo, Ospedale San Paolo, University of Milan, Milan, Italy
e-mail: fogagnolopaolo@googlemail.com

S. De Cilla'
Unit of Ophthalmology, Ospedale Maggiore della Carità, Novara, Italy

end of the study in the tafluprost group, whereas beading did occur in 75% of patients treated with latanoprost ($p = 0.05$). Both treatments were associated with increased keratocyte activation at follow-up; the change from baseline was statistically significant after month 3 with latanoprost ($p = 0.02$) and after month 6 with tafluprost ($p = 0.04$).

Conclusions: The two study treatments had similar clinical effects, but tafluprost had a more favorable profile for some confocal parameters of the cornea.

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Keywords: Confocal microscopy; Cornea; Glaucoma; Intraocular pressure (IOP); Latanoprost; Tafluprost

INTRODUCTION

The beneficial effect of intraocular pressure (IOP) lowering treatments to reduce glaucoma progression has been demonstrated by a number of multicenter, randomized studies [1–4]. On the other hand, more recent studies have also shown the detrimental effects of medical treatments for glaucoma on the ocular surface [5–11]. It has been shown that prostaglandin analogs have inflammatory effects [5–9, 11], yet the vast majority of side effects are due to preservatives, in particular benzalkonium chloride (BAK), which is the most toxic and most used of ophthalmic preservatives [5–11]. BAK effects are dose dependent [7–12], and this is relevant considering that most glaucoma patients receive more than one IOP-lowering treatment [4]. Chronic BAK exposure is also associated with reduced efficacy of glaucoma surgery [13]. As a consequence, preservative-free treatments are preferable for glaucoma, as for all chronic eye diseases [14].

Confocal microscopy is a recent technique which enables ophthalmologists to detect subtle inflammatory and toxic changes of the ocular surface [15]. By means of confocal microscopy, BAK has been shown to reduce the density of conjunctival goblet cells [16, 17], of conjunctival and corneal epithelial cells [17], and to deteriorate the normal characteristics of corneal nerves [18–20].

Still, timing of occurrence of ocular surface changes when starting IOP-lowering treatments is an unexplored issue. Tafluprost is the most recent unpreserved prostaglandin analog introduced in clinical practice and it is characterized by the absence of BAK.

To the best of the author's knowledge, this is the first study to investigate and compare, from both clinical and confocal viewpoints, the effects of preserved and unpreserved prostaglandin analogs in newly diagnosed glaucoma patients with normal ocular surface.

MATERIALS AND METHODS

A randomized, masked, prospective study was carried out to test the primary hypothesis that treatment with preserved prostaglandins induces confocal changes of the cornea (both stromal inflammation and toxic damage to the sub-basal nerves) and that these anatomical changes would induce clinical changes, as detected during a general ophthalmic examination.

Inclusion Criteria

Inclusion criteria for the present study were: diagnosis of ocular hypertension (OH), primary open-angle glaucoma (POAG), pseudoexfoliative glaucoma or normal tension glaucoma, according to the definitions of the

European Glaucoma Society Guidelines [21]; no previous treatments to reduce IOP and no treatment with any BAK-preserved eye drop for at least 6 months before the study; no fluorescein staining at baseline and no observable signs of ocular surface disease.

Exclusion Criteria

Exclusion criteria for the present study were: unwillingness to sign informed consent; aged <18 years; any ocular condition that was of safety concern or interfering with the study results; any ocular condition requiring the use of eye drops during follow-up (i.e., dry eye); closed/barely open anterior chamber angles or history of acute angle closure; ocular surgery or argon laser trabeculoplasty within the last year; ocular inflammation/infection occurring within 3 months prior to pre-trial visit; presence of the following ocular conditions: dry eye, moderate–severe blepharitis, Rosacea, Sjogren syndrome, pterygium or use of contact lens(es); hypersensitivity to BAK or to any other component of the trial drug solutions; any corneal pathology; diabetes at any stage; other abnormal condition or symptom preventing the patient from entering the trial (need for more than 1 IOP-lowering treatment), according to the investigator's judgment; refractive surgery patients; women who were pregnant, of childbearing potential and not using adequate contraception or nursing; inability to adhere to treatment/visit plan.

Clinical Plan

The study protocol comprised 5 visits (performed at Eye Clinic of San Paolo Hospital, Milan, Italy): Baseline, Month 3, Month 6, Month 9 and Month 12.

At baseline, a clinical evaluator performed a complete ophthalmologic evaluation to confirm diagnosis. The following examinations were done in the following sequence: anterior segment examination, Schirmer's test and break-up time test. Thereafter, a confocal evaluator performed confocal microscopy of the central cornea. Finally, contact measurements were carried out in the following order: IOP, pachymetry and gonioscopy. A 15-min interval between two consecutive tests was observed.

A study coordinator recorded medical history and then randomized patients into two groups: one group to receive unpreserved (tafluprost 0.0015%, Saflutan®, Santen Pharmaceutical, Osaka, Japan) and one group to receive preserved prostaglandins (latanoprost 0.005% + BAK 0.02%, Xalatan®, Pfizer S.r.L., Latina, Italy) once daily to both eyes (randomization of 1:1, by means of a list of random numbers). Being patients treated to both eyes, a control group was not available. During the study, patients were instructed not to use any other topical treatment other than the study medication. The confocal and the clinical investigators were masked to treatment.

Confocal and clinical examinations, as described above, were repeated at months 3, 6, 9 and 12.

Adherence to treatment, medical history, and side effects were checked by study coordinator at follow-up visits. Adverse effects were recorded. Symptoms were evaluated by means of comparison of ophthalmic medications for tolerability (COMTOL) questionnaire [22].

Corneal Confocal Biomicroscopy

The second version of Heidelberg Retina Tomograph (Heidelberg Engineering,

Heidelberg, Germany) is endowed with a lens system called the [Rostock Cornea Module (RCM)], and allows an in vivo confocal study of the ocular surface. The laser source used in the RCM is a diode laser with a wavelength of 670 nm. The acquired two-dimensional images have a definition of 384 × 384 pixels over an area of 400 μm × 400 μm with lateral digital resolution of 1 μm/pixel and a depth resolution of 2 μm/pixel.

After administration of one drop of 0.4% oxybuprocaine and one drop of a lubricant gel (0.2% carbomer), the patient was asked to fixate on a small, bright, red light as the examination was performed in the contralateral eye. Correct alignment and contact with the cornea were monitored using the images captured by a camera tangential to the eye. The distance from the cornea to the microscope was kept stable using a single-use contact element in sterile packaging, (TomoCap, Heidelberg Engineering, Heidelberg, Germany). The examination took about 7 min per eye; 5 images of each cornea layer and of the sub-basal layer were collected, both in central area. The highest resolution images taken of the different layers were considered for the analysis.

Test–retest variability of confocal microscopy of the central cornea was tested at the beginning of the study using the following method. 5 eyes of 5 volunteers were tested 3 times each: twice during the same day (at 9 a.m. and at 11 a.m.) and once the day after (at 9 a.m.). The confocal operator evaluated these images and found an agreement of 80% or more for all parameters.

Sample Size Calculation

Given the paucity of information available on the effects of treatments with BAK-free prostaglandin on the ocular surface studied by confocal imaging, sample size calculation for this pilot study may be imprecise. The outcome of the study was corneal inflammation at confocal microscopy (defined as activation of anterior stroma, changes of nerve morphology, increase of dendritic cells). If a worth-detecting difference of 40% between the two groups is assumed, the presence of subclinical inflammation in 30% of normal cases, a one-tailed distribution in favor of the BAK-free arm of the study, $\alpha = 0.05$, $\beta = 0.2$, a sample of 20 eyes would be necessary [20, 23, 24]. It was decided to overpower the study including all treated eyes (a control group was absent in any case, being patients treated to both eyes), and this gave a study power of nearly 90%.

Statistical Analysis

All available data were analyzed (i.e., all eyes receiving study product were analyzed). The dataset was analyzed by means of linear and generalized, mixed-effect models of analysis of variance (ANOVA), with a post hoc test. In case of multiple comparisons, *t* test and Chi-square tests with Bonferroni–Holm correction were used. R open-access software was used (version 3.1.3, R foundation for statistical computing, Vienna, Austria).

Compliance with Ethics

This present study was performed at the Eye Clinic, Department of Medicine, Surgery and Odontoiatry, San Paolo Hospital, University of Milan, Italy.

All procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation (University of Milan, Italy) and with the Helsinki Declaration of 1964, as revised in

2013. Informed consent was obtained from all patients for being included in the study.

RESULTS

Forty consecutive patients with new diagnosis of glaucoma or ocular hypertension were enrolled between January and July 2013. The study included 32 and 35 eyes in the tafluprost and latanoprost groups, respectively. Demographic characteristics of the study population and main study results are given in Tables 1, 2 and 3. The two groups had similar age and ocular surface and confocal findings at baseline (Figs. 1, 2). At the beginning of the study, activation of anterior stromal keratocytes was present in 40% of total patients (28% and 50% of subjects in latanoprost and tafluprost groups, respectively, $p = 0.08$); branching pattern was present in about 85% of patients, and beading in 75% of cases.

During a 1-year interval from treatment beginning, no significant clinical changes were detected, apart from a drop of IOP of 3.6–4.2 mmHg in the two groups ($p < 0.001$, with no statistically significant difference between treatments; ANOVA).

Confocal microscopy was similar between groups and between time points when analyzed by ANOVA. Yet, subtle changes occurring on the morphology of the cornea were shown at follow-up. All patients without branching pattern of sub-basal nerves at baseline progressively (from 9 to 12 months) developed this pattern when treated with latanoprost, whereas no change occurred at follow-up in subjects treated with tafluprost ($p = 0.04$, month 12). None of the patients without beading at baseline developed beading at the end of the study in tafluprost group, whereas this occurred in 6/8 (75%) patients treated with latanoprost ($p = 0.05$).

Both treatments were associated with an increase of activation of anterior stromal keratocytes at follow-up; the change from baseline was statistically significant 3 months after starting treatment with latanoprost ($p = 0.02$) and 6 months after tafluprost ($p = 0.04$).

A small and not significant increase of dendritic cells density occurred over time, with no difference between treatments.

No significant side effects were detected with any treatment during the study. No significant changes of symptoms were found, as evaluated by COMTOL scale, at follow-up in the two groups. Adherence to treatment was high (96%), and no study discontinuation occurred.

Table 1 Demographic and main ocular features of the study population

	Tafluprost	Latanoprost	Total
Number of patients	20	20	40
Number of eyes	32	35	67
Age years (SD)	68.5 ± 12.3	63.4 ± 14.4	65.9 ± 13.5
Sex f/m	7/10	8/10	15/20
Refraction	0.98 ± 0.28	1.1 ± 0.28	1.03 ± 0.28
IOP mmHg (SD)	18.5 ± 4.0	18.5 ± 5.5	18.5 ± 5.0

IOP intraocular pressure, *f/m* female/male, *SD* standard deviation

DISCUSSION

This paper explored the effects of tafluprost and latanoprost on a population of newly diagnosed POAG and OH with normal ocular surface, and the two treatments were found to have the same IOP-lowering effect and clinical tolerability over

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