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Tear cytokine profile of glaucoma patients treated with preservative-free or preserved latanoprost

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ABSTRACT

Purpose: To determine variations in cytokine levels of glaucoma patients treated either with preservative-free latanoprost or preserved latanoprost, relative to healthy individuals.

Methods: Tear samples were collected from 39 healthy subjects, 20 glaucoma patients treated with preserved latanoprost, and 20 patients treated with preservative-free latanoprost. A set of 27 inflammatory cytokines was analyzed in each group, including interleukin (IL)-1 β , IL-1ra, IL-2, IL-4, IL-5, IL-6, IL-7, IL-8, IL9, IL-10, IL-12 (p70), IL-13, IL-15, IL-17, eotaxin, fibroblast growth factor (FGF) basic, granulocyte colony stimulating factor (G-CSF), granulocyte monocyte colony stimulating factor (GM-CSF), interferon (IFN)- γ , interferon gamma-induced protein (IP)-10, monocyte chemo attractant protein (MCP)-1MCAF, macrophage inflammatory protein (MIP)-1 α , MIP-1 β , platelet-derived growth factor (PDGF)-BB, regulated on activation, normal T cell expressed and secreted (RANTES), tumor necrosis factor (TNF)- α and vascular endothelial growth factor (VEGF). Cytokine concentrations were obtained by the Bio-Plex Human Cytokine Immunoassay. Non-invasive tear breakup time (NI-TBUT), tear meniscus height, corneal fluorescein staining, conjunctival hyperemia and ocular surface disease index (OSDI) were assessed in patients treated with preservative-free and preserved latanoprost.

Results: The levels of IL-2, IL-5, IL-10, IL-12 (p70), IL-13, IL-15, IL-17, FGF basic, PDGF-BB, and TNF- α were significantly higher in patients receiving preserved latanoprost, compared to normal controls ($p < 0.05$). The expression of all the cytokines studied remained statistically invariable in patients receiving preservative-free latanoprost, compared to healthy subjects ($p > 0.05$). Ocular surface parameters were not significantly different in both glaucoma groups, and no correlation between these clinical parameters and cytokine levels was observed.

Conclusions: Treatment with preserved latanoprost has a direct impact on tear cytokine levels, whereas this effect is not observed upon preservative-free latanoprost instillation.

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Abbreviations: IL, interleukin; FGF, fibroblast growth factor; G-CSF, granulocyte colony stimulating factor; GM-CSF, granulocyte monocyte colony stimulating factor; IFN, interferon; IP, interferon gamma-induced protein; MCP, monocyte chemo attractant protein; MIP, macrophage inflammatory protein; PDGF, platelet-derived growth factor; RANTES, regulated on activation, normal T cell expressed and secreted; TNF, tumor necrosis factor; VEGF, vascular endothelial growth factor; IOP, intraocular pressure; PGA, prostaglandin analogue; BAK, benzalkonium chloride; OSD, ocular surface disease; ELISA, enzyme-linked immunosorbent assay; SD, standard deviation; NI-TBUT, non-invasive tear breakup time; OSDI, Ocular Surface Disease Index.

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1. Introduction

Glaucoma affects over 60 million people worldwide and is the second leading cause of blindness [1]. Elevated intraocular pressure (IOP) is the main risk factor for development of glaucoma. Thus, the goal of the current standard of care is to lower IOP levels. Following this rationale, different topical hypotensive compounds have emerged, including prostaglandin analogues (PGAs), carbonic anhydrase inhibitors, β -adrenergic receptor antagonists, α -2 selective adrenergic agonists, and non-selective adrenergic agonists. Remarkably, their high IOP-reducing efficacy places PGAs as one of the first-line treatments for glaucoma [2].

In PGAs-based formulations, supplementation with preservatives to protect from microbial contamination entails several ocular side effects [3,4]. The presence of benzalkonium chloride (BAK) in latanoprost, the most frequently prescribed PGA, is associated with conjunctival hyperemia, superficial punctate keratopathy, or squamous metaplasia [5–7]. Furthermore, long-term exposure to BAK-preserved medications may cause or aggravate pre-existing ocular surface diseases (OSDs), such as dry eye, blepharitis, meibomian gland dysfunction, chronic conjunctival inflammation, or corneal surface impairment [8,9]. These ocular disorders result in eye irritation, blurred vision or tearing, and in a general deterioration of patients' quality of life [10]. To circumvent these detrimental effects, efforts have been made to develop preservative-free formulations for the management of glaucoma. Following this approach, preservative-free latanoprost was developed as a topical glaucoma treatment. Compared with its preserved counterpart, preservative-free latanoprost showed equivalent efficacy in reducing IOP, was better tolerated, and reduced the number of associated ocular adverse events, such as conjunctival hyperemia [11–13]. However, the mechanisms behind these favorable effects are not completely understood.

Inflammation is the key underlying mechanism fueling preservative-associated OSDs. Hypotensive eye drops induce the expression of several cytokines, including interleukins, interferon (IFN)- γ , and tumor necrosis factor (TNF)- α [8,14]. Therefore, cytokines have become reliable biomarkers for the diagnosis, monitoring, and classification of OSDs. Their increasing importance is evidenced by the number of tools applied to detect cytokines in tears, including proteomic techniques, enzyme-linked immunosorbent assay (ELISA), membrane microarrays, and multiplex bead assays [15].

In this study, we compared the cytokine profile of patients treated either with preservative-free latanoprost (Monoprost[®]; Thea, Clermont-Ferrand, France) or preserved latanoprost (Xalatan[®]; Pfizer, New York, USA), by using multiplex bead technology. We evaluated changes in the concentration of 27 inflammatory cytokines in glaucoma patients, treated with or without preservatives, relative to healthy patients. To the best of our knowledge, this is the first study comparing the impact in cytokine concentration of long-term instillation with either preserved or preservative-free eye drops.

2. Material and methods

2.1. Participants

This prospective, observational study was conducted at the Hospital Clínico San Carlos (Madrid, Spain). A total of 79 subjects were prospectively recruited between December 2015 and April 2016. Glaucoma patients were receiving either Monoprost or Xalatan for periods ranging from 6 to 120 months. The study also enrolled 39 healthy age- and sex-matched controls.

The study adhered to the tenets of the Declaration of Helsinki

and the Spanish legislation, and was approved by the local Institutional Clinical Research Ethics Committee. Written legally-binding informed consent was obtained from all participants before recruitment.

Exclusion criteria included glaucoma surgery within the last six months, secondary glaucoma (including congenital, traumatic, steroid, uveitic, or angle closure), treatment with concomitant topical medications (such as lubricant eye-drops), any ocular surface disease that could have influenced treatment election, other serious eye disease or syndrome, or any physical or mental problem hindering the realization of tests or examinations required for the study.

Each patient was examined and medical history was recorded in a single visit. The information collected included demographic data, treatment duration, and the type (Monoprost or Xalatan) of eye drops received.

2.2. Tear collection

Tear samples were collected using a sterile microcapillary tube from a single eye of each individual. Samples were frozen at -80°C and stored until analysis. To avoid evaporation or degradation, the elapsed time between collection and storage was kept below 15 min. Since tear volumes ranged from 2 to 15 μL , saline buffer (Braun Mini-Plasco[®]) was added to each sample before storage to reach 50 μL (the minimum volume for analysis).

2.3. Cytokine quantification

Cytokine concentrations were determined in tear samples using the Bio-Plex Pro Human Cytokine 27-Plex Immunoassay (Bio-Rad Laboratories). This platform is based on fluorescent magnetic beads and allows the simultaneous detection of samples in 96-microwell plates, requiring low sample volumes [16–18]. With this technology, up to 27 relevant inflammatory cytokines could be detected in a single run. The set of cytokines detected were: interleukin (IL)-1 β , IL-1 α , IL-2, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, IL-10, IL-12 (p70), IL-13, IL-15, IL-17, eotaxin, fibroblast growth factor (FGF) basic, granulocyte colony stimulating factor (G-CSF), granulocyte monocyte colony stimulating factor (GM-CSF), interferon (IFN)- γ , interferon gamma-induced protein (IP)-10, monocyte chemo attractant protein (MCP)-1MCAF, macrophage inflammatory protein (MIP)-1 α , MIP-1 β , platelet-derived growth factor (PDGF)-BB, regulated on activation, normal T cell expressed and secreted (RANTES), tumor necrosis factor (TNF)- α and vascular endothelial growth factor (VEGF).

Briefly, 50 μL of tear samples were dispensed into defined wells of the 96-microwell plate. Samples were simultaneously read on the Luminex MAGPIX reader (Luminex Corporation, Austin TX USA). Cytokine concentrations were derived by interpolating the measured fluorescence intensities to standard curves, and correcting by the corresponding dilution factor employed to achieve the minimum volume for analysis. Bio-Plex Manager[™] software was employed to calculate cytokine concentrations.

2.4. Ocular surface measurements

The ocular surface parameters evaluated included corneal fluorescein staining, non-invasive tear breakup time (NI-TBUT), tear meniscus height, conjunctival hyperemia and ocular surface disease index (OSDI). These parameters were assessed at least 48 h before or after tear sample acquisition.

NI-TBUT and conjunctival hyperemia were measured by the Keratograph 5 (Oculus, Wetzlar, Germany), and the lower tear meniscus height was evaluated by the Spectralis Fourier-Domain OCT (Heidelberg Engineering GmbH, Germany) [19].

All eyes were examined using a standard slit lamp at $\times 16$ magnification. The cornea was stained using fluorescein staining (Minims-fluorescein sodium 2.0%; Chauvin Pharmaceuticals Ltd.). Corneal staining was graded using the 6-point Oxford scale (0 = no staining; 5 = severe staining). Since all the patients evaluated exhibited a maximum corneal staining score of 1, this variable was subsequently graded as negative or positive (score 0 or 1, respectively).

Global OSDI score was calculated for each participant from the following equation: $12.5 \times [(\text{sum of individual question scores}) / (\text{number of questions answered})]$, which yields a global score ranging from 0 to 100.

2.5. Statistical methods

Statistical analyses were performed using SAS software for Windows, version 9.2. To evaluate differences between study groups, quantitative variables were analyzed using the Student's *t*-test for independent samples, whereas categorical variables were compared using the Chi-square test. Spearman rank correlation was used to analyze the correlations between tear cytokine levels and ocular surface parameters. The adjusted level was established by Bonferroni test. $p < 0.05$ was the threshold to determine statistically significant differences.

3. Results

3.1. Demographic characteristics of study groups

Demographic characteristics of the participants are listed in Table 1. A total of 79 subjects (median age, 70 years; range, 29–88 years) participated in this study; 34 were males (43.0%) and 45 were females (57.0%). The study enrolled 40 glaucoma patients, and 39 individuals with no history of ocular disease were enrolled as untreated controls. Among glaucoma patients, 20 were receiving Monoprost and the other 20 Xalatan as topical anti-glaucoma medication. Mean time of treatment did not statistically differ between the two groups (29.6 ± 27.2 months for Monoprost and 33.8 ± 26.5 months for Xalatan; $p = 0.619$). The demographic variables assessed were not significantly different between groups ($p > 0.05$).

3.2. Effects of Xalatan on tear cytokine levels

A set of 27 relevant inflammatory cytokines were analyzed in Xalatan-treated and control tears. The absolute concentration of each cytokine is presented in Table 2. Cytokine levels were systematically greater in patients receiving Xalatan, compared to healthy participants (Fig. 1). Among all the cytokines evaluated, there was a significant increase ($p < 0.05$) in the levels of IL-2, IL-5, IL-10, IL-12 (p70), IL-13, IL-15, IL-17, FGF basic, PDGF-BB, and TNF- α in Xalatan-treated versus control subjects. Remarkably,

Table 2
Cytokine concentrations in Xalatan-treated and control subjects.

Cytokine	Control	Xalatan	<i>p</i> -value
IL-1 β	51.99 \pm 44.38	73.74 \pm 60.01	0.120
IL-1ra	18,120.10 \pm 17,296.27	14,150.58 \pm 10,067.61	0.359
IL-2	79.48 \pm 67.76	130.22 \pm 84.72	0.028*
IL-4	58.93 \pm 40.28	75.10 \pm 53.41	0.198
IL-5	150.56 \pm 133.52	258.91 \pm 221.34	0.024*
IL-6	161.79 \pm 150.37	185.92 \pm 131.44	0.546
IL-7	442.35 \pm 343.28	631.08 \pm 475.92	0.086
IL-8	573.88 \pm 465.33	475.80 \pm 314.45	0.402
IL-9	230.65 \pm 168.14	339.96 \pm 244.85	0.050
IL-10	243.01 \pm 182.77	392.86 \pm 295.55	0.019*
IL-12 (p70)	334.28 \pm 222.28	505.92 \pm 328.43	0.021*
IL-13	120.99 \pm 95.26	193.62 \pm 129.48	0.017*
IL-15	149.26 \pm 107.10	228.37 \pm 130.37	0.047*
IL-17	343.83 \pm 332.85	634.36 \pm 472.46	0.009*
Eotaxin	396.77 \pm 251.72	512.45 \pm 327.51	0.140
FGF basic	303.67 \pm 215.90	547.02 \pm 355.85	0.002*
G-CSF	621.68 \pm 483.07	700.72 \pm 489.66	0.560
GM-CSF	168.91 \pm 124.44	227.03 \pm 136.41	0.123
IFN- γ	2034.27 \pm 1331.88	2677.13 \pm 1857.30	0.134
IP-10	25,632.69 \pm 28,754.16	14,130.95 \pm 15,142.54	0.102
MCP-1 (MCAF)	489.70 \pm 304.50	633.31 \pm 388.62	0.124
MIP-1 α	54.92 \pm 38.41	70.14 \pm 44.04	0.176
PDGF-BB	126.17 \pm 113.44	234.96 \pm 201.35	0.010*
MIP-1 β	174.03 \pm 176.80	124.14 \pm 77.96	0.249
RANTES	320.03 \pm 218.74	394.26 \pm 228.71	0.231
TNF- α	317.12 \pm 263.14	602.44 \pm 502.04	0.006
VEGF	476.41 \pm 286.34	495.52 \pm 282.81	0.808

Data are expressed as mean (pg/mL) \pm SD.

Statistical significance between groups was determined using Student's *t*-test.

*Indicates a statistically significant difference ($p < 0.05$).

Abbreviations: IL = interleukin; FGF = fibroblast growth factor; G-CSF = granulocyte colony stimulating factor; GM-CSF = granulocyte monocyte colony stimulating factor; IFN = interferon; IP = interferon gamma-induced protein; MCP1(MCAF) = monocyte chemo attractant protein; MIP = macrophage inflammatory protein; PDGF = platelet-derived growth factor; RANTES = regulated on activation, normal T cell expressed and secreted; TNF = tumor necrosis factor; VEGF = vascular endothelial growth factor; SD = standard deviation.

PDGF-BB and TNF- α showed the highest increase in concentration (1.9-fold) in patients treated with Xalatan, relative to normal subjects.

3.3. Effects of Monoprost on tear cytokine levels

Cytokine concentrations in Monoprost-treated and control tears are given in Table 3. Levels of cytokines were highly coincident for Monoprost and control groups (Fig. 1). Likewise, changes in cytokine concentrations between patients receiving Monoprost and healthy subjects failed to reach statistical significance for all the 27 cytokines studied ($p > 0.05$). Remarkably, those cytokines with significantly increased levels in the previous comparison (Xalatan versus control), remained statistically invariable when Monoprost-treated patients were compared with healthy patients.

Table 1
Demographic characteristics of study participants.

	Control	Monoprost	Xalatan	Total	<i>p</i> -value
Gender, N (%)					
Male	16 (41%)	10 (50%)	8 (40%)	34 (43%)	0.765
Female	23 (59%)	10 (50%)	12 (60%)	45 (57%)	
Total	39 (100%)	20 (100%)	20 (100%)	79 (100%)	
Age (years), Mean \pm SD	72.72 \pm 8.67	70.35 \pm 13.92	71.65 \pm 9.93	71.85 \pm 10.43	0.713

Data are expressed as number (percentage), or mean \pm SD.

Abbreviations: N = number of patients; SD = standard deviation.

Statistical significance between groups was determined using either the Student's *t*-test or the Chi-square test for continuous or categorical variables, respectively.

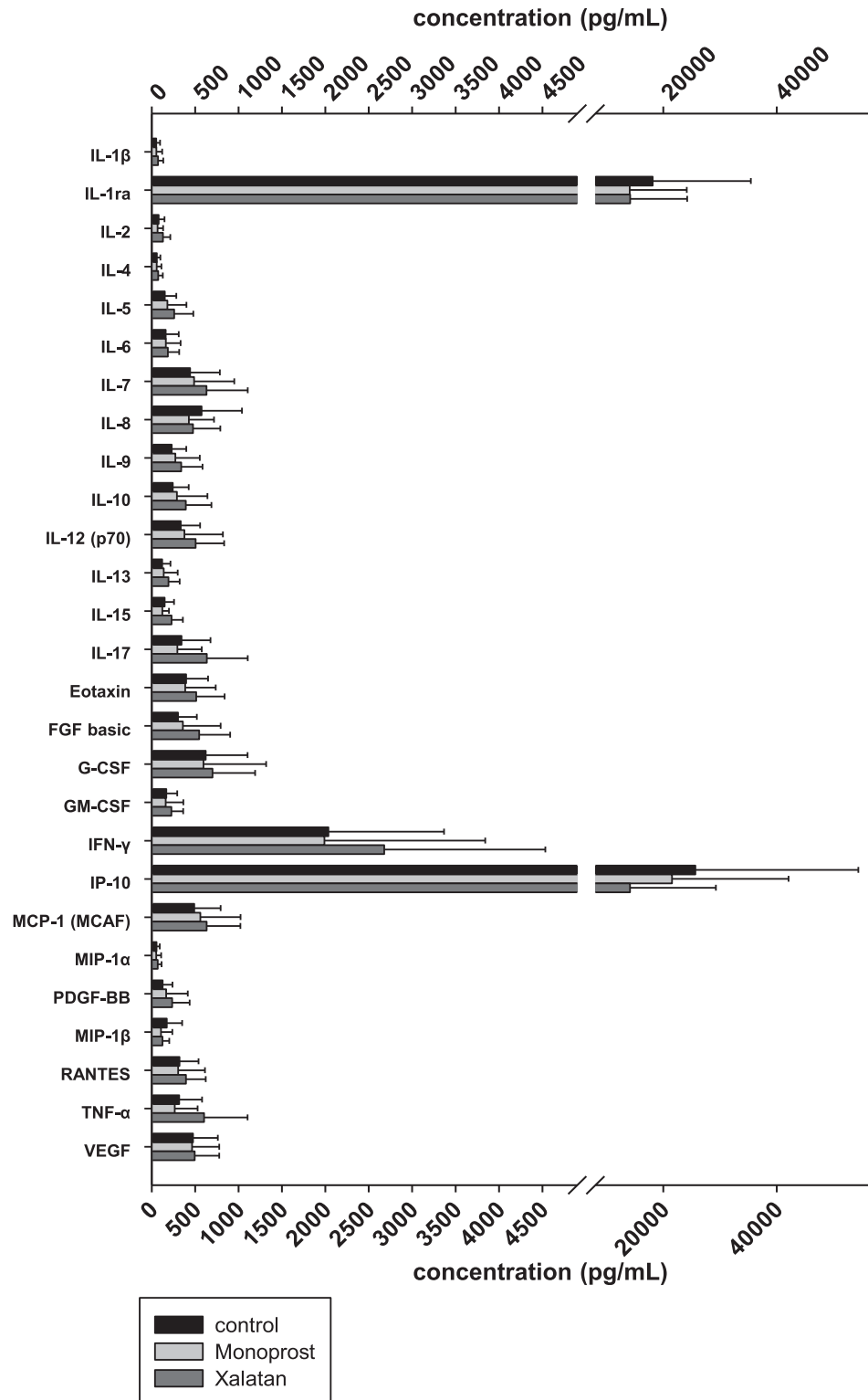


Fig. 1. Bar graph showing cytokine concentrations in patients treated with Xalatan or Monoprost and in control subjects. The bar graph shows the concentration of each of the 27 cytokines determined by the Bio-Plex Pro Human Cytokine 27-Plex Immunoassay: interleukin (IL); fibroblast growth factor (FGF); granulocyte colony stimulating factor (G-CSF); granulocyte monocyte colony stimulating factor (GM-CSF); interferon (IFN); interferon gamma-induced protein (IP); monocyte chemo attractant protein (MCP1(MCAF)); macrophage inflammatory protein (MIP); platelet-derived growth factor (PDGF); regulated on activation, normal T cell expressed and secreted (RANTES); tumor necrosis factor (TNF); vascular endothelial growth factor (VEGF). Values are expressed as mean (pg/mL) \pm standard deviation (SD).

3.4. Cytokine profiles and clinical parameters in Monoprost and Xalatan groups

The comparison of Xalatan- and Monoprost-treated tears

revealed that, except for IP-10, all the cytokines evaluated were higher in the Xalatan group, with the levels of IL-2, IL-15, IL-17 and TNF- α reaching statistical significance ($p < 0.05$) (Table 4, Fig. 1).

Clinical parameters of ocular surface showed similar levels in

Table 3
Cytokine concentrations in Monoprost-treated and control subjects.

Cytokine	Control	Monoprost	p-value
IL-1 β	53.10 \pm 67.05	51.99 \pm 44.38	0.939
IL-1ra	14,069.58 \pm 10,054.80	18,120.10 \pm 17,296.27	0.349
IL-2	68.69 \pm 63.21	79.48 \pm 67.76	0.592
IL-4	57.48 \pm 54.07	58.93 \pm 40.28	0.908
IL-5	181.62 \pm 217.64	150.56 \pm 133.52	0.503
IL-6	162.51 \pm 171.08	161.79 \pm 150.37	0.987
IL-7	488.20 \pm 461.86	442.35 \pm 343.28	0.668
IL-8	428.52 \pm 289.48	573.88 \pm 465.33	0.219
IL-9	272.26 \pm 281.40	230.65 \pm 168.14	0.492
IL-10	290.68 \pm 351.42	243.01 \pm 182.77	0.494
IL-12 (p70)	377.24 \pm 443.19	334.28 \pm 222.28	0.620
IL-13	138.74 \pm 160.53	120.99 \pm 95.26	0.596
IL-15	122.86 \pm 76.12	149.26 \pm 107.10	0.442
IL-17	295.49 \pm 280.22	343.83 \pm 332.85	0.604
Eotaxin	385.54 \pm 352.04	396.77 \pm 251.72	0.889
FGF basic	358.46 \pm 435.10	303.67 \pm 215.90	0.519
G-CSF	596.67 \pm 722.30	621.68 \pm 483.07	0.879
GM-CSF	162.25 \pm 201.80	168.91 \pm 124.44	0.882
IFN- γ	1989.44 \pm 1852.67	2034.27 \pm 1331.88	0.916
IP-10	21,529.78 \pm 20,541.95	25,632.69 \pm 28,754.16	0.584
MCP-1 (MCAF)	560.90 \pm 463.19	489.70 \pm 304.50	0.481
MIP-1 α	52.88 \pm 55.96	54.92 \pm 38.41	0.870
PDGF-BB	166.56 \pm 248.59	126.17 \pm 113.44	0.393
MIP-1 β	107.33 \pm 129.72	174.03 \pm 176.80	0.173
RANTES	305.15 \pm 308.74	320.03 \pm 218.74	0.832
TNF- α	265.71 \pm 263.83	317.12 \pm 263.14	0.488
VEGF	464.41 \pm 312.76	476.41 \pm 286.34	0.883

Data are expressed as mean (pg/mL) \pm SD.

Statistical significance between groups was determined using the Student's t-test. Abbreviations: IL = interleukin; FGF = fibroblast growth factor; G-CSF = granulocyte colony stimulating factor; GM-CSF = granulocyte monocyte colony stimulating factor; IFN = interferon; IP = interferon gamma-induced protein; MCP1(MCAF) = monocyte chemo attractant protein; MIP = macrophage inflammatory protein; PDGF = platelet-derived growth factor; RANTES = regulated on activation, normal T cell expressed and secreted; TNF = tumor necrosis factor; VEGF = vascular endothelial growth factor; SD = standard deviation.

both Monoprost and Xalatan groups. Concretely, tear meniscus height, OSDI, NI-TBUT, and conjunctival hyperemia were statistically equivalent in Monoprost- and Xalatan-treated patients ($p > 0.05$) (Table 5). Corneal fluorescein staining and the presence of epitheliopathy were not significantly different between Monoprost and Xalatan groups ($p = 0.491$).

No significant correlation was observed between cytokine levels and clinical parameters of ocular surface disease (OSDI, NI-TBUT, conjunctival hyperemia, and tear meniscus height), when analyzing those cytokines with significantly different levels in Monoprost and Xalatan groups (IL-2, IL-15, IL-17 and TNF- α).

4. Discussion

Due to the chronic nature of glaucoma, many glaucomatous patients require lifetime treatment. The current goal of anti-glaucoma therapy is to lower IOP without compromising patients' quality of life [10,20]. The use of PGAs as topical medication entails several adverse events, which often derive from the presence of preservatives in these ophthalmic preparations [21,22]. Adverse events include inflammation or corneal impairment, among other symptoms of discomfort, and can result in treatment discontinuation [23–25]. In this regard, there is increasing concern about the risks associated with the use of preservatives in anti-glaucoma medications.

For this reason, developing new anti-glaucoma formulations without preservatives appears to be a successful therapeutic avenue for the management of glaucoma. Different preservative-free PGAs are currently available that are no less effective in

Table 4
Cytokine concentrations in Monoprost- and Xalatan-treated subjects.

Cytokine	Monoprost	Xalatan	p-value
IL-1 β	53.10 \pm 67.05	73.74 \pm 60.01	0.312
IL-1ra	14,069.58 \pm 10,054.80	14,150.58 \pm 10,067.61	0.980
IL-2	68.69 \pm 63.21	130.22 \pm 84.72	0.022*
IL-4	57.48 \pm 54.07	75.10 \pm 53.41	0.307
IL-5	181.62 \pm 217.64	258.91 \pm 221.34	0.273
IL-6	162.51 \pm 171.08	185.92 \pm 131.44	0.637
IL-7	488.20 \pm 461.86	631.08 \pm 475.92	0.341
IL-8	428.52 \pm 289.48	475.80 \pm 314.45	0.629
IL-9	272.26 \pm 281.40	339.96 \pm 244.85	0.433
IL-10	290.68 \pm 351.42	392.86 \pm 295.55	0.326
IL-12 (p70)	377.24 \pm 443.19	505.92 \pm 328.43	0.303
IL-13	138.74 \pm 160.53	193.62 \pm 129.48	0.241
IL-15	122.86 \pm 76.12	228.37 \pm 130.37	0.016*
IL-17	295.49 \pm 280.22	634.36 \pm 472.46	0.014*
Eotaxin	385.54 \pm 352.04	512.45 \pm 327.51	0.245
FGF basic	358.46 \pm 435.10	547.02 \pm 355.85	0.142
G-CSF	596.67 \pm 722.30	700.72 \pm 489.66	0.603
GM-CSF	162.25 \pm 201.80	227.03 \pm 136.41	0.267
IFN- γ	1989.44 \pm 1852.67	2677.13 \pm 1857.30	0.248
IP-10	21,529.78 \pm 20,541.95	14,130.95 \pm 15,142.54	0.207
MCP-1 (MCAF)	560.0 \pm 463.19	633.31 \pm 388.62	0.595
MIP-1 α	52.88 \pm 55.96	70.14 \pm 44.04	0.285
PDGF-BB	166.56 \pm 248.59	234.96 \pm 201.35	0.345
MIP-1 β	107.33 \pm 129.72	124.14 \pm 77.96	0.636
RANTES	305.15 \pm 308.74	394.26 \pm 228.71	0.306
TNF- α	265.71 \pm 263.83	602.44 \pm 502.04	0.013*
VEGF	464.41 \pm 312.76	495.52 \pm 282.81	0.743

Data are expressed as mean (pg/mL) \pm SD.

Statistical significance between groups was determined using the Student's t-test.

*Indicates a statistically significant difference ($p < 0.05$).

Abbreviations: IL = interleukin; FGF = fibroblast growth factor; G-CSF = granulocyte colony stimulating factor; GM-CSF = granulocyte monocyte colony stimulating factor; IFN = interferon; IP = interferon gamma-induced protein; MCP1(MCAF) = monocyte chemo attractant protein; MIP = macrophage inflammatory protein; PDGF = platelet-derived growth factor; RANTES = regulated on activation, normal T cell expressed and secreted; TNF = tumor necrosis factor; VEGF = vascular endothelial growth factor; SD = standard deviation.

Table 5
Ocular surface parameters in Monoprost- and Xalatan-treated patients.

	Monoprost	Xalatan	p-value
OSDI	11.220 \pm 9.799	6.439 \pm 7.052	0.121
Conjunctival Hyperemia	1.706 \pm 0.522	1.400 \pm 0.473	0.088
Tear Meniscus Height	324.000 \pm 108.312	333.059 \pm 104.711	0.809
NI-TBUT	8.836 \pm 4.73,136	11.365 \pm 6.045	0.189

Statistical significance between groups was determined using either the Student's t-test or the Chi-square test for continuous or categorical variables, respectively.

Abbreviations: OSDI = ocular surface disease index; NI-TBUT = non-invasive tear breakup time.

reducing IOP than their preserved counterparts [26–28]. Furthermore, switching studies revealed that changing from preserved to preservative-free medications significantly improved signs and symptoms related to OSDs [29,30].

Among PGAs, we focused on latanoprost, the most frequently prescribed formulation, because of its high tolerability and the lesser number of side effects associated with its use [7]. Despite its potency, various adverse events have been associated with the presence of BAK in latanoprost formulation [5–7]. Preservative-free latanoprost (Monoprost) was therefore conceived to maintain the drug potency of latanoprost while reducing the associated adverse events.

A variety of studies support the advantages of Monoprost as topical anti-glaucoma medication. In an *in vivo* study, Pauly and colleagues revealed that epithelial cell viability was higher after treatment with preservative-free latanoprost compared to

treatment with preserved latanoprost [11]. A randomized, multinational trial demonstrated equivalent efficacy between preserved and preservative-free latanoprost in reducing IOP levels [12]. A meta-analysis of randomized clinical trials confirmed that preservative-free latanoprost presented equivalent safety and reduced adverse events, such as conjunctival hyperemia, compared to preserved latanoprost [31]. A phase 2 study reported no differences between latanoprost with or without preservative, in terms of safety and patient tolerance [32]. Additionally, commercialization in single-dose format ensures Monoprost sterility.

Several studies have revealed important differences between preserved and preservative-free latanoprost, but none has directly compared their impact on tear cytokine levels. Inflammation is a key cause and effect of the onset and progression of OSDs, which can be reliably monitored by measuring cytokine levels. In this study, we compared differences in cytokine concentrations promoted either by Monoprost or Xalatan in glaucoma patients. We observed that treatment with Xalatan resulted in great variations of cytokine levels in tears. However, and interestingly, upon treatment with Monoprost, we only observed mild changes that did not reach statistical significance.

We simultaneously determined the concentration of 27 cytokines employing multiplex technology, thus eliminating inter-assay variability. The cytokine profile of patients treated with Xalatan revealed a significant increase in IL-2, IL-5, IL-10, IL-12 (p70), IL-13, IL-15, IL-17, FGF basic, PDGF-BB, and TNF- α . Among them, PDGF-BB and TNF- α showed the highest increase in concentration (1.9-fold) in patients treated with Xalatan, whereas their expression was statistically unchanged in patients treated with Monoprost. TNF- α is a pleiotropic protein eliciting different cellular responses, such as apoptotic cell death or inflammation [33]. Consistently, both apoptosis and inflammation participate in ocular adverse events associated with the use of preservatives in ophthalmic preparations. Indeed, BAK is able to interact with cell death receptors, inducing apoptosis and inflammation [34,35]. This fact, together with the elevated levels of TNF- α in patients treated with Xalatan, suggests that apoptosis could also play a role in these associated side effects. In addition to playing this role, TNF- α is a recognized marker of dry eye disease [18,36]. In line with this, growth factors such as PDGF-BB and FGF basic play a critical role in the maintenance of corneal function and imbalances in their expression are found in different ocular pathologies [37,38]. In addition, IL-17 (the IL presenting the highest increase) is also associated with the severity of different OSDs, such as uveitis, scleritis and dry eye disease [39–41]. Overall, patients treated with Xalatan presented an increased expression of different OSD markers, which could indicate that they are more prone to suffer or exacerbate these ophthalmic pathologies.

Nevertheless, other relevant markers of OSDs, such as IL- β 1 or IL-6, are not differentially expressed in patients treated with Xalatan [42]. This apparent discrepancy could be explained considering that early and late inflammatory responses greatly differ in their cytokine profile. In this regard, this study was conducted in individuals already under treatment for long-term periods (ranging from 6 to 120 months). This fact represents an important strength of our study, since it reflects the actual context of patients chronically exposed to anti-glaucoma treatments. In contrast, clinical trials are usually performed according to short-term schedules, thus underestimating potential long-term effects. Unexpectedly, no relevant differences in ocular surface measurements (corneal staining, conjunctival hyperemia, NI-TBUT, and tear meniscus height) were observed between Xalatan- and Monoprost-treated patients. In addition, cytokine levels and these ocular surface parameters were not statistically correlated in either the Xalatan or the Monoprost groups. Thus, although the presence of

BAK is not apparently influencing these ocular surface markers, it could be impacting other clinical outcomes, such as the success of posterior glaucoma surgery. The lack of correlation indicates that the inflammatory status achieved after long-term treatment with either Monoprost or Xalatan is not directly translated in detectable differences by exploratory procedures. This fact could be explained considering the wide variation of treatment durations that could compensate potential differences in clinical measurements between both groups. This idea is in accordance with previous studies in which transition from latanoprost to BAK-free tavoprost showed that hyperemia was not significantly altered at 1 month, but it was significantly decreased after 3–12 months of treatment [43]. Hence, it would be interesting to unveil the inflammatory profile of patients as a function of treatment duration, to distinguish between acute and chronic adverse events derived from the use of preservatives, and to establish its potential correlation with ocular surface markers.

A limitation of this study is that basal levels of cytokines were not determined before treatment initiation. However, since patient selection was not based on clinical characteristics or previous treatment intolerances, we can assume that cytokine levels were balanced between both groups. Further studies should be conducted using a crossover design switching from preserved to preservative-free latanoprost, and vice versa. By quantifying cytokine levels before and after each treatment course, we could rule out any baseline effect.

Taken together, the results of this study support previous reports of the beneficial effects of Monoprost in patients, and highlights the importance of assessing the cytokine profile to characterize inflammatory effects of topical medications.

5. Conclusions

This study compared the cytokine profile of patients treated with preservative-free or preserved latanoprost. The lack of inflammation associated with preservative-free latanoprost treatment is likely responsible for its significant reduction of adverse events. These findings may have special relevance for patients receiving long-term treatment, and with OSDs.

Disclosure

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