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Pharmacokinetics, efficacy and safety profiles of preserved and preservative-free tafluprost in healthy volunteers

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ABSTRACT.

Purpose: **Prostanoid $F_{2\alpha}$ ($PF_{2\alpha}$) analogues are commonly used as first-line treatment of glaucoma. Tafluprost is a newly synthesized $PF_{2\alpha}$ derivative and represents the first $PF_{2\alpha}$ analogue with a fully preservative-free formulation.**

Methods: **A randomized, investigator-masked, single-centre, crossover phase I study evaluated the pharmacokinetics, efficacy and safety profiles of preserved and preservative-free tafluprost 0.0015% eyedrops in healthy volunteers. Both formulations were administered once/day for 8 days each. Plasma concentrations and, consequently, area under the curve (AUC_{0-last}), maximum concentration (C_{max}) and time to maximum concentration (t_{max}) were determined for tafluprost acid, the biologically active metabolite. Intraocular pressure, adverse events, and ocular and systemic safety parameters were analysed.**

Results: **There were no statistically significant differences in pharmacokinetic parameters between preserved and preservative-free formulations after either single (day 1) or repeated (day 8) dosing. The mean (± standard deviation) results for preserved and preservative-free formulations on day 8 were, respectively: AUC_{0-last} 581.1 ± 529.9 pg/min/ml versus 431.9 ± 457.8 pg/min/ml ($p = 0.462$); C_{max} 31.4 ± 19.5 pg/ml versus 26.6 ± 18.0 pg/ml ($p = 0.294$), and median (range) t_{max} 10 (5–15) for both. Generally, plasma concentrations of tafluprost acid were low at all time-points and were cleared rapidly from the circulatory system. There were no unexpected safety findings. The incidence of ocular hyperaemia was similar in both formulations and was of predominantly moderate severity with preserved tafluprost and mild severity with preservative-free tafluprost.**

Conclusions: **Preservative-free tafluprost appeared to have similar pharmacokinetic properties to the preserved formulation and was generally well tolerated.**

Key words: benzalkonium chloride – glaucoma – pharmacokinetics – preservative-free – tafluprost

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Introduction

Glaucoma is the term used to describe a family of ocular diseases characterized by typical structural and functional alterations in the optic nerve head. If left untreated, glaucoma can lead to visual impairment and loss of sight. In general, glaucoma is the second leading cause of visual impairment in elderly people, particularly in developed countries. Elevated intraocular pressure (IOP) is the main risk factor for glaucoma. Reduction of IOP using topical ocular hypotensive agents can prevent the development of open-angle glaucoma (Kass et al. 2002) and slow the progression of glaucoma (AGIS Investigators 2000; Leske et al. 2003). The prostaglandin analogues latanoprost, bimatoprost and travoprost are currently available as topical anti-glaucoma agents and are recommended by the European Glaucoma Society Guidelines (2008) as first-line therapy for lowering IOP in glaucoma.) Tafluprost is a newly synthesized prostaglandin (PG) $F_{2\alpha}$ derivative that is rapidly hydrolyzed by corneal esterases to the biologically active metabolite tafluprost acid, which has a high affinity for and potent agonistic effects on prostanoid fluoroprostaglandin (FP) receptors (Nakajima et al. 2003; Takagi et al. 2004). In animal studies, and trials with healthy volunteers and patients

with glaucoma, tafluprost effectively lowered IOP and demonstrated a good safety profile (Takagi et al. 2004; Sutton et al. 2007; Hamacher et al. 2008).

Preservatives such as benzalkonium chloride (BAC) are commonly used in eyedrop formulations. In the case of some water-soluble molecules, BAC is also perceived to improve penetration through corneal epithelium (Burstein 1984; Okabe et al. 2005). However, the use of preservatives in glaucoma medications is associated with an increased incidence of adverse effects on the ocular surfaces. The chronic use of preserved topical eyedrops is associated with goblet cell loss (Liesegang 1998), conjunctival foreshortening and shrinkage leading to scarring conjunctivitis (Schwab et al. 1992) and infiltration of the substantia propria by inflammatory cells (Broadway et al. 1993, 1994a; Baudouin et al. 1999). Furthermore, the use of preserved therapies also increases patient reports of ocular signs and symptoms of glaucoma, including dry eye and irritation (Furrer et al. 2002; Pisella et al. 2002; Mundorf et al. 2004; Jaenen et al. 2007). Therefore, new formulations for ophthalmic indications, without BAC or other preservatives, are being developed.

This study was carried out to compare the pharmacokinetics and safety profiles of preserved and preservative-free tafluprost 0.0015% eyedrops in healthy volunteers. The primary aim of the study was to measure and compare the plasma concentrations of tafluprost acid after single and repeated dosing of the preserved and preservative-free formulations of tafluprost.

Materials and Methods

The study was carried out in accordance with current good clinical practice and the ethical standards outlined in the Declaration of Helsinki. The study protocol was evaluated by the Ethical Committee of Kuopio University Hospital. All volunteers gave written informed consent before participating.

Study design

The study was a randomized, investigator-masked, single-centre, crossover phase I trial of the pharmacokinetics and safety of preserved and preservative-free tafluprost 0.0015% eyedrops (Taflotan®; Santen Oy, Helsinki, Finland) in healthy volunteers.

A total of 16 healthy volunteers (eight per treatment sequence) received either preserved or preservative-free tafluprost 0.0015% eyedrops in two consecutive treatment sequences. The order in which subjects received either formulation was randomized. Eyedrops were administered in each eye once/day at 20.00 hours for 8 days per treatment sequence. After a washout period of ≥ 4 weeks, volunteers were switched to the alternative formulation for a further 8 days of treatment (Fig. 1). The total duration of the crossover treatment was 16 days.

Blood samples for pharmacokinetic analysis were taken from volunteers on days 1 and 8 before administration of tafluprost (preserved or preservative-free) and at 5, 10, 15, 30 and 45 mins, and 1, 2 and 4 hours after administration of tafluprost formulations.

Volunteers

A total of 16 healthy volunteers were enrolled into the study. Inclusion criteria required subjects to be aged 18–45 years, in good general health, willing and able to follow instructions and attend all study visits, have blood and urinary laboratory values within normal limits at baseline 1 (visit 2), and have a best corrected Early Treatment Diabetic Retinopathy Study (ETDRS) visual acuity (VA) score of ≥ 0.1 logMAR (logarithm of the minimal angle of resolution) in each eye. Women of childbearing age were allowed to participate in the study if they had a negative pregnancy test at screening and used a reliable method of contraception throughout the study.

Volunteers were excluded from the study if they had a history of any chronic, severe or other major disorder, a history of eye surgery (including refractive surgery), amblyopia in one or both eyes, chronic ocular disease (e.g. glaucoma) or any clinically significant abnormality in either eye during screening. Volunteers who had a known allergy or hypersensitivity to tafluprost or BAC, used systemic or ocular medications within a week of the screening visit or needed contact lenses within 1 week of screening or during the study were also excluded.

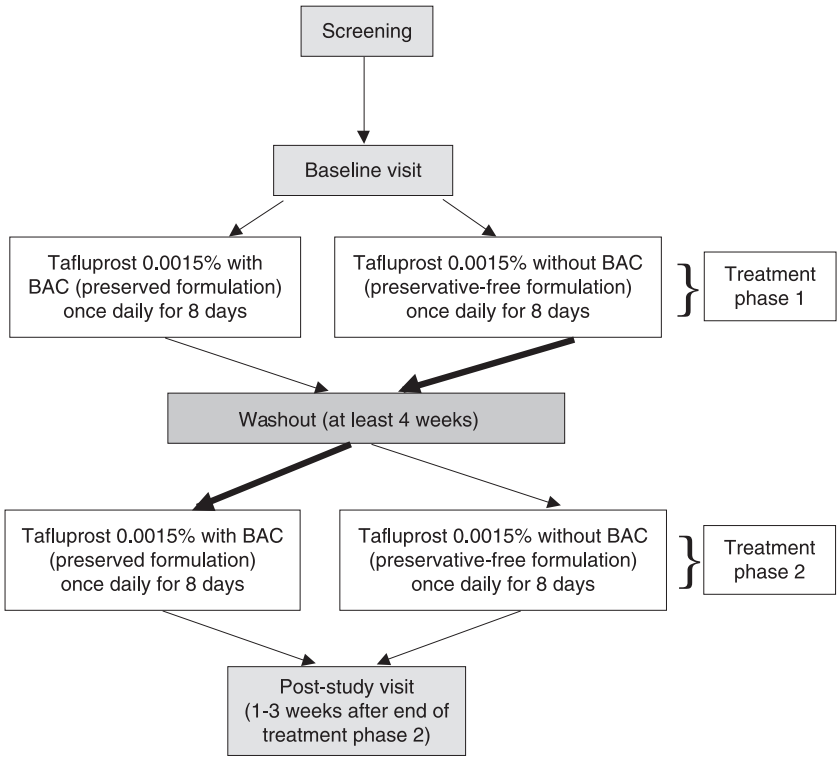

Fig. 1. Study design: a randomized, single-centre, crossover study. Blood samples for pharmacokinetic analysis were taken from volunteers on days 1 and 8 before and at 5, 10, 15, 30 and 45 mins, and 1, 2 and 4 hours after administration of tafluprost (preserved or preservative-free formulations). BAC = benzalkonium chloride.

Other exclusion criteria were: any corneal abnormality or other condition interfering with or preventing reliable use of applanation tonometry; IOP < 10 mmHg or > 21 mmHg (or a difference in IOP between the eyes of ≥ 4 mmHg) at screening, and current or recent participation (< 30 days) in another clinical trial involving investigational drugs or devices. Women of childbearing age were excluded from the study if they were pregnant, breastfeeding or planning a pregnancy.

Procedures

All subjects who met the enrolment criteria at the screening visit were randomized to treatment with tafluprost 0.0015% eyedrops at the first baseline visit according to one of the two treatment sequences (preserved followed by preservative-free or vice versa). The randomization procedure was carried out using randomly permuted blocks; eight volunteers were assigned to each treatment sequence.

After randomization, volunteers were instructed to administer one drop of the study medication in each eye once/day at 20.00 hours for 8 days for treatment period 1. During study visits, medication was administered by an independent third party. After a washout period of ≥ 4 weeks, volunteers switched medication and entered treatment period 2 for a further 8 days. Thus, each person received both preserved and preservative-free tafluprost eyedrops in a crossover manner.

Eyedrops were administered by the volunteers except during the study visits. Accurate timing of medication administration was strongly encouraged but a variation of up to 1 hour was considered acceptable. The use of concomitant ocular topical medications was permitted (e.g. for treatment of dry eye, antibiotics) during the study, except for those medications in the exclusion criteria. No concomitant ocular or systemic medications that might induce a change in IOP were to be initiated during the study period.

Analytical procedures

Tafluprost acid concentrations in plasma samples were determined by using high-performance liquid chromatography with tandem mass spectrometric (MS/MS) detection. The ratio of intensities of chromatographic responses for tafluprost acid and the added internal standard were used to calculate tafluprost acid concentrations in unknown plasma samples using a calibration curve. The calibration curve range is 10.0–5000 pg/ml for tafluprost acid, using a plasma sample volume of 0.500 ml. This analytical method was previously validated according to industry standards by Covance Bioanalytical Services (Indianapolis, IN, USA). Pharmacokinetic samples were analysed by Covance Bioanalytical Services and pharmacokinetic calculations performed in the University of Kuopio.

End-points

Following single and repeated administration of preserved and preservative-free tafluprost, plasma concentrations, area under the curve (AUC_{0-last}), maximum concentration (C_{max}) and time to maximum concentration (t_{max}) were determined for tafluprost acid.

The C_{max} and t_{max} values for tafluprost acid were read from concentration versus time data plots. The AUC_{0-last} was calculated using the trapezoidal rule from time 0 (just prior to application of tafluprost) to the last time-point at which a quantifiable concentration of tafluprost acid was measured (quantifiable limit 10.0 pg/ml). Tafluprost acid was only detectable in plasma for up to 1 hour after topical application, hence AUC_{0-4hr} could not be calculated and determination of the elimination half-life ($t_{½}$) was not possible.

Adverse events and other safety parameters were analysed. All adverse events were counted once for each volunteer per treatment period. This included assessment of the type of adverse event, severity, onset and duration, frequency, probability of relationship to study treatment, location (left or right eye, both, neither), action taken and outcome. An adverse event was counted once for each volunteer, and recorded by maximum severity and strongest causality. Ocular and non-ocular events were presented separately.

Ocular safety measurements recorded were: best corrected VA; IOP; biomicroscopic assessment of the structures of the eyelids, conjunctiva, cornea, anterior chamber and lens, and ophthalmoscopic assessment of the vitreous, retina and optic nerve head. Systemic safety measurements recorded at screening and post-study visits only were: blood pressure, heart rate, a 12-lead electrocardiogram (ECG), and laboratory assessments (biochemistry, haematology, urinalysis).

Statistical methods

Descriptive statistics were used to summarize the plasma concentrations of tafluprost acid, resulting pharmacokinetic parameters, adverse events and ocular/systemic safety variables. Statistical analyses were performed using two-sided tests. A p-value of < 0.05 was considered statistically significant.

In the analysis of the pharmacokinetic parameters of the preserved and preservative-free formulations of tafluprost, mean concentration–time curves on day 1 (single dose) and day 8 (after several doses) were compared using a repeated measures (RM) analysis of variance (ANOVA) model appropriate for a two-period crossover study. A non-parametric ANOVA model appropriate for a two-period crossover study design was used to compare AUC_{0-last} and C_{max} on days 1 and 8 between the two tafluprost formulations. Non-parametric 90% confidence intervals (90% CIs) (preserved or preservative-free) were used to quantify the relative bioavailability of the tafluprost formulations.

Results

A total of 16 volunteers (nine women, seven men) were randomized into the study; all 16 completed the study with 100% treatment compliance. All volunteers were White. Their mean age was 29.2 years. All protocol deviations that occurred were considered minor and the data from all volunteers was evaluable and included in the pharmacokinetic and safety analyses.

Pharmacokinetics

The systemic bioavailability of the preserved and preservative-free tafluprost formulations was comparable after both single and repeated dosing. Concentration–time curves for preserved and preservative-free tafluprost after a single topical dose (i.e. day 1) and repeated topical dosing (i.e. day 8) are shown in Fig. 2. Pre-dose concentrations of tafluprost acid were

below the lower limit of quantification for both formulations on both days. After single and repeated dosing of tafluprost 0.0015% eyedrops (preserved and preservative-free), plasma concentrations of tafluprost acid were low at all time-points, peaked after 10 mins and fell to unquantifiable levels within an hour after dosing.

The shapes of the plasma concentration–time curves of tafluprost acid were comparable between the preserved and preservative-free formulations of tafluprost ($p = 0.269$ RM ANOVA analysis of formulation by time interaction after a single dose; $p = 0.373$ RM ANOVA analysis of formulation by time interaction after repeated dosing).

Overall, there were no statistically significant differences between the preserved and preservative-free formulations of tafluprost in terms of the AUC_{0-last}, C_{max} and t_{max} parameters after either single or repeated dosing (Table 1). Mean concentrations, C_{max} values (31.4 pg/ml versus 24.4 pg/ml) and AUC_{0-last} values (581.1 pg/min/ml versus 405.9 pg/min/ml) of the preserved formulation were slightly higher on day 8 than on day 1. Mean concentrations, C_{max} values and AUC_{0-last} values were similar for the preservative-free formulation on days 1 and 8 (26.2 pg/ml and 26.6 pg/ml for C_{max}; 394.3 pg/min/ml and 431.9 pg/min/ml for AUC_{0-last}, respectively).

For both formulations of tafluprost, mean concentrations peaked at 10 mins and decreased rapidly after this point. At day 1, mean concentrations for the formulations at 10 mins were 21.95 pg/ml (with preservative) and 25.56 pg/ml (preservative-free). At day 8, mean concentrations at 10 mins were 30.43 pg/ml (with preservative) and 25.34 pg/ml (preservative-free). Thus, the differences in mean concentrations between the preserved and preservative-free formulations were small and statistically insignificant at both time-points.

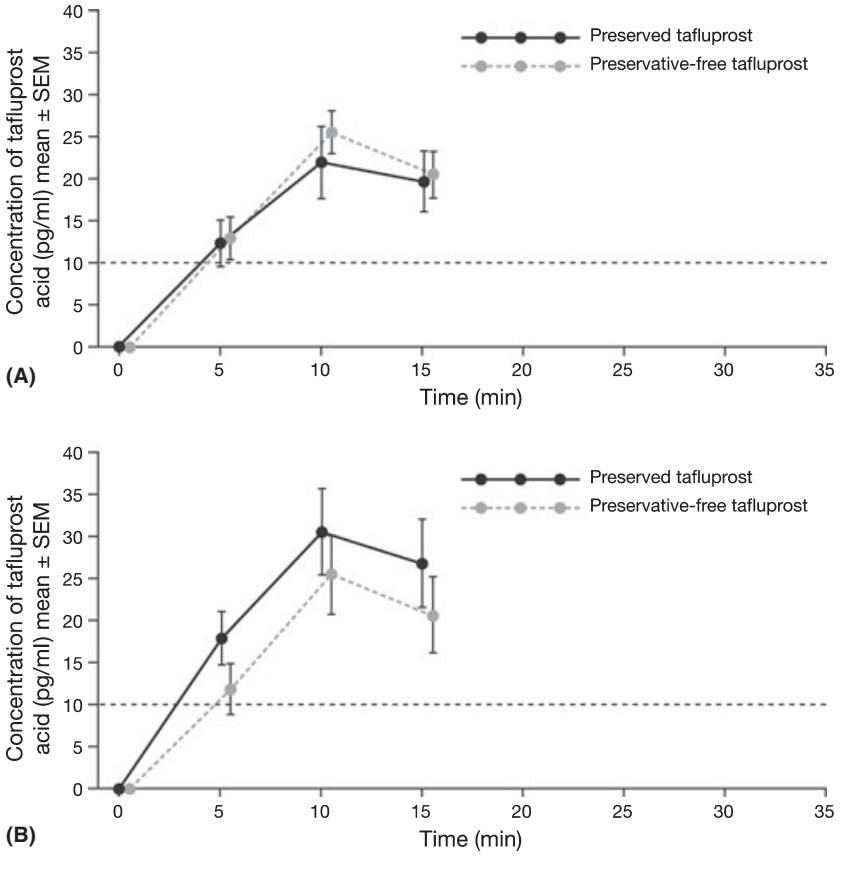

Fig. 2. Plasma concentration curves (mean ± SEM) of tafluprost acid following administration of preserved and preservative-free tafluprost 0.0015% eyedrops, showing results for (A) single-dose administration on day 1, and (B) repeated-dose administration on day 8. SEM = standard error of the mean.

IOP reductions

The IOP-lowering effects of the two tafluprost formulations were similar over the duration of the study (Fig. 3).

Safety

In general, both formulations were well tolerated. A total of 36 adverse events (29 ocular, seven non-ocular) were reported by 16 volunteers for the preserved formulation and 27 adverse events (24 ocular, three non-ocular) were reported by 16 volunteers for the preservative-free formulation. No serious adverse events, or withdrawals caused by adverse events, occurred during the study. None of the non-ocular adverse events were related to tafluprost treatment and all were mild or moderate in severity. There were no unexpected systemic safety findings. No clinically relevant changes in blood pressure, heart rate, volunteer ECGs or laboratory safety measurements were recorded.

The ocular adverse events reported for each formulation of tafluprost are summarized in Table 2. All ocular events were considered to be related to the study medications. All the ocular adverse events were of mild or moderate severity except for one case of severe ocular hyperaemia that occurred with the preserved formulation. The most prevalent ocular adverse event was ocular hyperaemia, which was reported by a similar number of volunteers for both formulations (all 16 volunteers for the preserved formulation and 15 for the preservative-free formulation). The ocular hyperaemia was mostly of moderate severity with the preserved formulation and mild severity with the preservative-free formulation.

Best corrected VA was evaluated at screening, on days 1 and 8 and post-study. The volunteers' VA (logMAR scores) remained stable throughout the study and no clinically relevant changes (> 0.2 logMAR) were observed. Biomicroscopy findings were reported for lids, conjunctiva, cornea, iris, anterior chamber and lens. No unexpected safety observations were made; conjunctival redness was reported more commonly for the preserved than the preservative-free formulation.

Table 1. Pharmacokinetic parameters after single (day 1) and repeated (day 8) dosing with either preserved or preservative-free formulations of tafluprost 0.0015% eyedrops.

	Day 1			Day 8		
Parameter	Preserved tafluprost solution	Preservative-free tafluprost	Comparison*	Preserved tafluprost solution	Preservative-free tafluprost	Comparison*
AUC_{0-last} (pg/ml/min) Mean ± SD	405.9 ± 395.2	394.3 ± 286.4	p = 0.600	581.1 ± 529.9	431.9 ± 457.8	p = 0.462
C_{max} (pg/ml) Mean ± SD	24.4 ± 15.8	26.2 ± 10.4	p = 0.529	31.4 ± 19.5	26.6 ± 18.0	p = 0.294
t_{max} (mins) Mean (range)	10 (10–15)	10 (5–15)		10 (5–15)	10 (5–15)	

* Non-parametric analysis of variance.
AUC_{0-last} = area under curve (time 0 to last measurable value); C_{max} = maximum concentration; SD = standard deviation; t_{max} = time to maximum concentration.

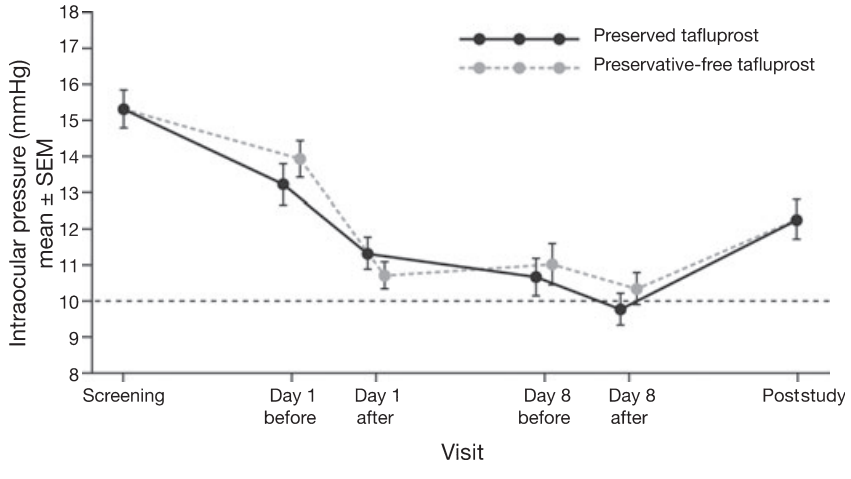

Fig. 3. Intraocular pressure measurements (mean ± SEM) in the two treatment groups before and after administration of preserved and preservative-free tafluprost 0.0015% eyedrops. SEM = standard error of the mean.

Table 2. Treatment-related ocular adverse events reported for preserved and preservative-free tafluprost 0.0015% eyedrops.

	Preserved formulation of tafluprost (n = 16)				Preservative-free formulation of tafluprost (n = 16)			
Adverse event	Mild	Moderate	Severe	Total	Mild	Moderate	Severe	Total
Conjunctival hyperaemia	6	9	1	16	9	6	0	15
Eye pain	3	1	0	4	1	0	0	1
Eye pruritis	2	0	0	2	2	0	0	2
Eye irritation	0	1	0	1	0	1	0	1
Foreign-body sensation	0	1	0	1	1	0	0	1
Erythema of eyelid	1	0	0	1	0	0	0	0
Eyelid sensory disorder	1	0	0	1	0	0	0	0
Eyelid oedema	0	0	0	0	0	1	0	1
Increased lacrimation	0	0	0	0	1	0	0	1
Photophobia	0	0	0	0	0	1	0	1

Discussion

This study showed that preserved and preservative-free formulations of tafluprost 0.0015% eyedrops have similar systemic pharmacokinetic profiles in healthy volunteers. Their systemic bioavailability was similar and there were no statistically significant differences in AUC_{0-last}, C_{max} and t_{max} measurements. Plasma concentrations of tafluprost acid, the biologically active metabolite of tafluprost, peaked at 10 mins, were low at all other time-points and cleared rapidly from the circulation, after both single and repeated topical dosing.

The comparability between the pharmacokinetic profiles of preserved and preservative-free tafluprost observed in this study is in accordance with findings of a previous *in vivo* study in rabbits, which demonstrated equal concentrations of tafluprost acid in aqueous humour after single instillations of preserved and preservative-free tafluprost 0.0015% eyedrops (Pellinen & Lokkila 2008).

Our findings demonstrated clear reductions in IOP within 1 week with both preserved and preservative-free formulations of tafluprost 0.0015%. Both formulations resulted in similar reductions in IOP from baseline. These findings are in agreement with those of a phase III randomized cross-over trial of 43 patients with either ocular hypertension or glaucoma, where similar reductions in IOP achieved by preserved and preservative-free tafluprost were sustained for 4 weeks (Hamacher et al. 2008).

In this study, we observed that preserved and preservative-free tafluprost eyedrops were both safe and generally well tolerated. Adverse effects were mainly ocular and mild or moderate in severity. As is common with topical ocular medications with prostaglandin analogues, ocular hyperaemia was the most prevalent adverse event. Notably, the ocular hyperaemia reported was predominantly moderate with the preserved formulation and mild with the preservative-free formulation. Together with the findings from a similar study in patients with glaucoma (Hamacher et al. 2008), these data suggest that both preserved and preservative-free tafluprost formulations are well tolerated.

There is accumulating evidence that preservative-free formulations may be useful in the future longterm management of patients with chronic eye diseases, such as glaucoma. Preservative-free formulations may be particularly relevant in patients suffering from concurrent dry eye syndrome and glaucoma, patients who discontinue therapy as a result of adverse events and those who may eventually need surgery. It should be remembered that almost 35% of glaucoma patients aged over 65 years also suffer from dry eyes (Smith et al. 2007). The impaired tear film characteristic of patients with dry eye syndrome may enhance the penetration of topical therapies applied to the eye and reduce conjunctival or corneal resistance to potential irritants. These patients would therefore be most susceptible to the effects of preservatives. In fact, preservative-free artificial tears for the treatment of dry eye syndrome when eyedrops are required 4–6 times/day are recommended by the practice guidelines of the American Academy of Ophthalmology Cornea/External Disease Panel (2003). These recommendations are echoed by the consensus views from a roundtable discussion, which support a trend towards preservative-free artificial tears as first-line therapy whenever possible (Asbell 2006). Various preservative-free formulations of artificial tears are available. The preservative-free formulations do not contribute to ocular surface damage or aggravate the symptoms of dry eye syndrome.

Studies also indicate that up to 80% of patients do not take antiglaucoma medication as prescribed (Olthoff et al. 2005; Schwartz 2005). Patients who identified adverse events as a 'significant problem' were most likely to have poor adherence to prostaglandins (Zimmerman et al. 2007). Therefore, it seems that preservative-free formulations would be beneficial in patients with sensitivity to preservatives, such as patients with dry eyes, and in those patients who discontinue medication early because of adverse events. Furthermore, it has been shown that long-term topical therapy may contribute to the failure of glaucoma surgery: in particular, patients who received two or more medications were found to experience significantly lower surgical success rates (Broadway et al. 1994b). Preservatives within medications can result in inflammatory responses (Broadway et al. 1994a; Baudouin et al. 2004). Conjunctival inflammation is an established predictor of surgical failure (Broadway & Chang 2001). Furthermore, chronic inflammation can lead to fibrosis and scarring, which can result in excessive postoperative wound healing time (Broadway et al. 1994b). Therefore, as glaucoma is a progressive disease and many patients with glaucoma require long-term antiglaucoma treatment, combination therapy, and may eventually require surgery, preservative-free formulations are likely to improve outcomes in all patients with glaucoma.

In conclusion, the pharmacokinetic profiles of tafluprost acid were similar following administration of preserved and preservative-free tafluprost 0.0015% eyedrops. Both formulations were equally efficacious and generally well tolerated.

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Conflict of interest

HU has received funding for research carried out in this work; KK has acted in an academic position without commercial conflicts; AR is an employee of Santen Oy.

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