Thermal Stability of Bimatoprost, Latanoprost, and Travoprost Under Simulated Daily Use

Thomas V. Johnson,^{1,2} Preeya K. Gupta,³ Daljit K. Vudathala,^{4,5} Ian A. Blair,⁴ and Angelo P. Tanna⁶

Abstract

Purpose: To determine the stability of bimatoprost, latanoprost, and travoprost under conditions of simulated daily use and varying degrees of thermal stress.

Methods: Commercially available bimatoprost, latanoprost, and travoprost were obtained in their original bottles as distributed by the manufacturers. Bottles were stored in calibrated, nonhumidified, light-free incubators maintained at 27°C, 37°C, or 50°C for 3, 9, 15, or 30 days. Capped bottles were inverted and left uncapped for 1 min daily to simulate patient use; no drops were expelled. Bimatoprost concentration was analyzed using liquid chromatography with ultraviolet detection at 210 nm. Latanoprost and travoprost concentrations were analyzed by liquid chromatography/tandem mass spectrometry (MS/MS) using selected reaction monitoring.

Results: Off-the-shelf control bottles of bimatoprost contained 102% of the labeled concentration. In all combinations of stress temperature and duration, mean bimatoprost concentration ranged from 100% to 116% of the labeled concentration with no measurable degradation. Off-the-shelf control bottles of latanoprost contained 115% of the labeled concentration. Mean latanoprost concentration ranged from 97% to 120% of the labeled concentration. Latanoprost was stable at 27°C. When stressed at 37°C or 50°C, latanoprost degraded at a rate of 0.15 or 0.29 µg/mL/day, respectively. Off-the-shelf control bottles of travoprost contained 120% of the labeled concentration. Mean travoprost concentration ranged from 83% to 142% of the labeled concentration. Travoprost was stable at 27°C and 37°C, although concentration measurements at 37°C exhibited high variability. When stressed at 50°C, travoprost degraded at a rate of 0.46 µg/mL/day.

Conclusions: Higher than expected concentrations for stressed drug samples are likely a result of evaporation. Under the conditions of thermal stress tested in this study, bimatoprost remained stable for all conditions tested. Latanoprost degradation was measurable only in samples stressed at 37°C and 50°C, whereas travoprost degradation was statistically significant only in samples stressed at 50°C.

Introduction

GLAUCOMA IS A PROGRESSIVE neurodegenerative optic neuropathy and the leading cause of irreversible blindness in the world.^{1,2} The only clinically proven intervention to prevent the onset or slow the progression of glaucoma is intraocular pressure reduction.^{3–8} Topical prostaglandin analogs (PGAs) have become the first-line ocular hypotensive therapy for the treatment of glaucoma.^{9–13} The most commonly prescribed PGAs include bimatoprost (an

amide pro-drug of 17-phenyl-PGF_{2α}, sometimes classified as a prostamide), latanoprost, and travoprost (both ester pro-drugs of PGF_{2α}). Each of these drugs reduces intraocular pressure by increasing aqueous humor drainage primarily through the uveoscleral pathway and possibly also by increasing trabecular outflow facility.^{14–20}

A fundamental determinant of pharmacological efficacy over time is compound stability, which may be affected by storage conditions, including ambient temperature.²¹ The package inserts for all three PGAs indicate that they should

¹Centre for Brain Repair and Department of Ophthalmology, University of Cambridge, Cambridge, United Kingdom.

²Molecular Mechanisms of Glaucoma Section, Laboratory of Molecular and Developmental Biology, National Eye Institute, National Institutes of Health, Bethesda, Maryland.

³Department of Ophthalmology, Duke University Eye Center, Durham, North Carolina.

⁴Centers of Excellence in Environmental Toxicology and Cancer Pharmacology, Department of Pharmacology, University of Pennsylvania, Philadelphia, Pennsylvania.

⁵Department of Pathobiology, Toxicology, University of Pennsylvania, School of Veterinary Medicine, Kennett Square, Pennsylvania.

⁶Department of Ophthalmology, Northwestern University Feinberg School of Medicine, Chicago, Illinois.

be stored at or below 25°C after opening for up to 6 weeks in the case of latanoprost²² or for an unspecified period of time for bimatoprost²³ and travoprost.²⁴ However, unlike the other two drugs, latanoprost is stated to require refrigeration before opening,²² possibly owing to a lower thermal stability.

Patient compliance has been highlighted as an issue affecting topical ocular hypotensive treatment efficacy with regard to self-administration,^{25–27} but the importance of compliance with recommended drug storage conditions should also be considered. In certain climates where it is not uncommon for temperatures to reach above 40°C, and where indoor temperature regulation is not available or consistently utilized, it is possible that prescribed medications are exposed to destabilizing thermal conditions contraindicated by the package inserts. Moreover, as PGAs become available for generic production and consequently more widely distributed throughout the world, including in developing nations where refrigerated transport and storage are not always practical, thermal stability may be an important determinant of overall efficacy.

Several published studies have assessed the stability^{28,29} or efficacy^{30,31} of PGAs under conditions of thermal stress or patient storage, and one has compared the stability of patient-stored samples of latanoprost and travoprost.³² However, to our knowledge, no published study exists comparing the thermal stability of all three PGAs when stored under identical controlled experimental conditions while also simulating normal use by patients. The current study was conducted to assess and compare the stability of three clinically approved, commercially available PGA formulations over periods of up to 1 month when stored at 27°C, 37°C, or 50°C.

Methods

Drugs and reagents

For calibration of the analysis system, standard reference samples of bimatoprost and latanoprost with purity of 99% or greater were purchased from Cayman Chemicals (Ann Arbor, MI), and the travoprost reference sample (250 ppm in 35% acetonitrile in water) was donated by Alcon Laboratories (Fort Worth, TX). For stability experiments, unopened bottles ($N=42$ per drug) containing commercial formulations of bimatoprost 0.03% (Lumigan®; Allergan Inc., Irvine, CA), latanoprost 0.005% (Xalatan®; Pfizer Inc., New York, NY), or travoprost 0.004% (Travatan®; Alcon Labs.) were used. Acetonitrile, water, formic acid, and ammonium acetate of high performance liquid chromatography (LC) grade were purchased from Fisher Scientific (Fair Lawn, NJ).

Drug storage conditions

Bimatoprost, latanoprost, and travoprost were obtained as commercially supplied in individual 4 mL polypropylene (travoprost) or 5 mL polyethylene (bimatoprost and latanoprost) bottles containing 2.5 mL drug fill volume per bottle. All bottles within each drug group were from the same lot. All external packaging was removed and bottles were opened for 1 min before recapping. Bottles were then stored in calibrated, nonhumidified, light-free incubators maintained at 27°C, 37°C, or 50°C for 3, 9, 15, or 30 days ($N=3$ bottles for each drug at each combination of temperature and duration of stress). Each day, bottles were inverted while capped, and then left uncapped for 1 min to simulate patient

use without overtly reducing drug volume in the container. After thermal stressing for the specified temperatures and durations, the samples of each drug were frozen and stored at –80°C in their original bottles until analyzed. Additional six bottles per drug were frozen before opening, immediately after removal from their external packaging, and used as “off-the-shelf” controls.

Analysis of latanoprost and travoprost

Latanoprost and travoprost samples were analyzed by LC performed on Waters 2690 equipped with an autosampler and a vacuum degasser. This was coupled to a Thermo-electron triple quadrupole TSQ 7000 mass spectrometer fitted with an electrospray ionization source. The analytical LC column was YMC ODS-AQ S 3 micron 120 Å, 2.0×150 mm used in isocratic run with 0.2 mL/min flow rate. The mobile phase was 45% by volume consisting of 5 mM ammonium acetate with 0.02% formic acid and 55% by volume of 5 mM ammonium acetate in acetonitrile/water (95/5; v/v) with 0.02% formic acid. The samples were maintained in autosampler set at 4°C. The mass spectrometer operating conditions were as follows: heated capillary at 175°C, nitrogen was used as the sheath gas (60 psi) and the auxiliary gas (5 U). LC/tandem mass spectrometry (MS/MS) was conducted by using 518→321 transition for travoprost at collision offset energy of –12 eV and 450→337 transition for latanoprost at collision energy of –22 eV.

Analysis of bimatoprost

The samples for bimatoprost were assayed by using a Hitachi LC system with an ultraviolet (UV) detector L-2400 set at 210 nm, autosampler L-2200 maintained at 10°C, and pump L-2130 and data processed by EZ-Chrome Elite. The analytical column and the mobile solvent used were as specified above for latanoprost and travoprost analysis.

Preparation of drug samples

Baseline drug samples were analyzed in sextuplet and samples for each time/temperature combination were stressed and analyzed in triplicate. Bimatoprost samples were thawed and vortexed for 30 s, diluted 1:30 with 50% acetonitrile in water, spiked with 10 µg/mL latanoprost, and injected at 50 µL into LC/UV system. Latanoprost samples were thawed and vortexed for 30 s, diluted 1:10 with 50% acetonitrile in water, spiked with 10 µg/mL travoprost, and injected at 50 µL into LC/MS/MS system. Travoprost samples were thawed and vortexed for 30 s, diluted 1:10 with 50% acetonitrile in water, spiked with 10 µg/mL latanoprost, and injected at 50 µL into LC/MS/MS system.

Validation of methods

Using a stock solution of 50 µg/mL travoprost in 35% acetonitrile in water, calibration solutions of 1.0, 2.0, 4.0, 6.0, and 8.0 µg/mL were prepared daily and spiked with 10.0 µg/mL latanoprost as internal standard. Using a stock solution of 100 µg/mL latanoprost in 50% acetonitrile in water, calibration solutions of 1.25, 2.5, 5.0, 7.5, and 10.0 µg/mL were prepared daily and spiked with 5.0 µg/mL travoprost as internal standard. Using a stock solution of 100 µg/mL bimatoprost in 50% acetonitrile in water, calibration

solutions of 2.0, 5.0, 8.0, 10.0, 12.0, and 15.0 µg/mL and were spiked with 10.0 µg/mL of latanoprost as internal standard. Three samples of each concentration were prepared fresh daily and measured to check within-day accuracy and precision. Between-day accuracy and precision were assessed on three different days. Accuracy was evaluated by calculating the percent relative error (% RE) of the concentration measurements relative to the known concentrations (% RE = true concentration – mean measured concentration / true concentration × 100) and the precision was measured as coefficient of variation.

Statistical analysis

Multivariate linear regression analyses were employed with storage time, storage temperature, and the time-by-temperature interaction as independent predictors of drug concentration. An interaction occurs when the effect of one variable is significantly modified by another variable; the time-by-temperature interaction term describes how cumulative temperature exposure (expressed as °C days) affects drug concentration. Univariate linear regression models were employed individually for each stress temperature using time as an independent predictor of drug concentration. Linear regression plots are depicted in Figs. 1–3, where the solid line represents the regression and dotted lines indicate the 95% confidence interval. Statistical analyses were performed using SPSS Software (SPSS Inc., Chicago, IL).

Results

Validation

The within-day and between-day validation data for concentration measurements of bimatoprost, latanoprost, and travoprost are presented in Table 1. The coefficients of variation for concentration measurements of travoprost tended to be the highest of the three drugs (range 2.9%–6.6%), whereas those for bimatoprost tended to be the lowest (range 0.1%–0.8%). A similar trend was observed for % RE of concentration measurements.

Bimatoprost

The bimatoprost concentration in off-the-shelf control bottles was 306.7 ± 18.8 µg/mL (mean ± standard deviation) or 102% of the labeled concentration (300 µg/mL). In all combinations of stress duration and temperature, mean bimatoprost concentration ranged from 300.5 ± 20.8 to 347.0 ± 6.5 µg/mL, or from 100% to 116% of the labeled concentration (Fig. 1). A multivariate linear regression model using storage time, storage temperature, and the time-by-temperature interaction as independent predictors of concentration yielded an overall

FIG. 1. Stability of bimatoprost under conditions of thermal stress. Commercially available bottles of bimatoprost were stored in nonhumidified, light-free incubators at 27°C (A), 37°C (B), or 50°C (C) for 3, 9, 15, or 30 days before high performance liquid chromatography/ultraviolet detection analysis of remaining drug concentration. Solid lines represent linear regressions of the data, and dotted lines represent the 95% confidence interval of the regression. “P” represents the likelihood of the null hypothesis that the slope of the regression line is not different from zero.

ANOVA P value of 0.180 and no significant associations for temperature (coefficient = $0.455 \mu\text{g/mL}/^\circ\text{C}$, $P = 0.069$), time (coefficient = $-0.517 \mu\text{g/mL/day}$, $P = 0.559$), or the interaction variable (coefficient = $0.002 \mu\text{g/mL}/^\circ\text{C day}$, $P = 0.932$). Subsequent univariate linear regression analyses conducted after grouping data based on storage temperature and using time as an independent predictor of concentration revealed no significant change in bimatoprost concentration when stored at any of the three temperatures assessed (Fig. 1).

Latanoprost

The mean latanoprost concentration in off-the-shelf control bottles was $57.7 \pm 3.2 \mu\text{g/mL}$ or 115% of the labeled concentration ($50 \mu\text{g/mL}$). In all combinations of stress duration and temperature, mean latanoprost concentration ranged from 48.3 ± 1.0 to $60.1 \pm 4.0 \mu\text{g/mL}$, or from 97% to 120% of the labeled concentration (Fig. 2). The multivariate linear regression model yielded an overall ANOVA P value of <0.001 and a significant correlation between latanoprost concentration and the time-by-temperature interaction (coefficient = $-0.011 \mu\text{g/mL}/^\circ\text{C day}$, $P = 0.004$) and but not with time (coefficient = $0.264 \mu\text{g/mL/day}$, $P = 0.060$) or temperature (coefficient = $-0.036 \mu\text{g/mL}/^\circ\text{C}$, $P = 0.351$), suggesting that latanoprost concentrations tended to be lowest in the bottles stressed at the highest temperatures over the longest periods of time—those with the greatest cumulative temperature exposure. Subsequent linear regression analyses conducted after grouping data based on storage temperature and using time as an independent predictor of concentration revealed no significant change in concentration over time for bottles stored at 27°C , but a significant loss of concentration over time for bottles stored at 37°C (rate = $-0.15 \mu\text{g/mL/day}$, $P = 0.05$) and 50°C (rate = $-0.29 \mu\text{g/mL/day}$, $P < 0.001$, Fig. 2). Extrapolation of these data, based on the assumption that drug degradation continues linearly after the range of observation, suggests that the T90 (time required for the formulation to lose 10% of the labeled drug concentration) is 33.3 days for latanoprost stored at 37°C and 17.2 days when stored at 50°C , assuming a starting concentration equal to the labeled concentration ($50 \mu\text{g/mL}$). The corresponding T90s calculated using actual baseline drug concentration from this study are 84.7 and 43.8 days, respectively. At the end of 6 weeks of storage at 37°C or 50°C , the remaining latanoprost concentration is expected to be $43.7 \mu\text{g/mL}$ (87% of the labeled concentration) or $37.8 \mu\text{g/mL}$ (76% of the labeled concentration), respectively, assuming a starting concentration equal to the labeled concentration; the corresponding values using actual measured baseline concentration are $51.4 \mu\text{g/mL}$ (103% of the labeled concentration) and $45.5 \mu\text{g/mL}$ (91% of the labeled concentration), respectively.

FIG. 2. Stability of latanoprost under conditions of thermal stress. Commercially available bottles of latanoprost were stored in nonhumidified, light-free incubators at 27°C (A), 37°C (B), or 50°C (C) for 3, 9, 15, or 30 days before liquid chromatography/mass spectrometry analysis of remaining drug concentration. Solid lines represent linear regressions of the data, and dotted lines represent the 95% confidence interval of the regression. “ P ” represents the likelihood of the null hypothesis that the slope of the regression line is not different from zero.

Travoprost

The mean travoprost concentration in off-the-shelf control bottles was $48.1 \pm 2.4 \mu\text{g/mL}$ or 120% of the labeled concentration ($40 \mu\text{g/mL}$). In all combinations of stress duration and temperature, mean travoprost concentration ranged from 33.1 ± 5.1 to $56.7 \pm 0.6 \mu\text{g/mL}$, or from 83% to 142% of the labeled concentration (Fig. 3). The multivariate linear regression model yielded an overall ANOVA P value of 0.057 and a significant correlation between travoprost concentration and the time-by-temperature interaction (coefficient = $-0.024 \mu\text{g/mL}/^\circ\text{C day}$, $P = 0.020$) and but not with time (coefficient = $0.679 \mu\text{g/mL/day}$, $P = 0.069$) or temperature (coefficient = $0.071 \mu\text{g/mL}/^\circ\text{C}$, $P = 0.481$), suggesting that the travoprost concentration tended to be lowest in the bottles with the greatest cumulative temperature exposure. Subsequent linear regression analyses conducted after grouping data based on storage temperature and using time as an independent predictor of concentration revealed no significant change in concentration over time for bottles stored at 27°C or 37°C , but a significant loss of concentration over time for bottles stored at 50°C (rate = $-0.46 \mu\text{g/mL/day}$, $P = 0.001$, Fig. 3). Extrapolation of these data, based on the assumption that drug degradation continues linearly after the range of observation, suggests that the T90 for travoprost stored at 50°C is 8.7 days, assuming a starting concentration equal to the labeled concentration ($40 \mu\text{g/mL}$). The corresponding T90 calculated using actual baseline drug concentration from this study is 26.3 days. At the end of 6 weeks of storage at 50°C , the remaining travoprost concentration is expected to be $20.7 \mu\text{g/mL}$ (52% of the labeled concentration) assuming a starting concentration equal to the labeled concentration; the corresponding value using actual measured baseline concentration is $28.8 \mu\text{g/mL}$ (72% of the labeled concentration).

Discussion

The results of this study indicate that all three commercially available PGA formulations that were assessed are stable for at least 30 days when stored at room temperature, as suggested by the package inserts and in agreement with the published literature. In a prior study, Reis et al.³⁰ reported no loss of ocular hypotensive efficacy for travoprost after opening and storage at room temperature for 6 weeks compared with freshly opened bottles, though thermally stressed travoprost was not assessed. Similarly, Mochizuki et al.³¹ observed no difference in the intraocular pressure (IOP)-lowering capability of latanoprost stored for 4 weeks at 30°C compared with 4°C . However, storage conditions imposed by patients are unlikely to be ideal and it is possible that these drugs may be maintained at temperatures well

FIG. 3. Stability of travoprost under conditions of thermal stress. Commercially available bottles of travoprost were stored in nonhumidified, light-free incubators at 27°C (A), 37°C (B), or 50°C (C) for 3, 9, 15, or 30 days before liquid chromatography/mass spectrometry analysis of remaining drug concentration. Solid lines represent linear regressions of the data, and dotted lines represent the 95% confidence interval of the regression. " P " represents the likelihood of the null hypothesis that the slope of the regression line is not different from zero.

TABLE 1. VALIDATION DATA FOR PROSTAGLANDIN ANALOG CONCENTRATION MEASUREMENTS

Actual drug concentration ($\mu\text{g/mL}$)	Measured drug concentration ($\mu\text{g/mL}$), N = 3 per measurement	Coefficient of variation	Percent relative error
<i>Bimatoprost</i>			
<i>Within-day measurements</i>			
2.00	2.01	0.3	−0.7
5.00	5.01	0.2	−0.3
8.00	8.04	0.2	−0.5
10.00	9.84	0.4	1.5
12.00	12.07	0.3	−0.6
15.00	15.02	0.1	−0.2
<i>Between-day measurements</i>			
2.00	2.01	0.7	−0.4
5.00	5.01	0.7	−0.6
8.00	8.04	0.3	−0.2
10.00	9.84	0.6	1.2
12.00	12.06	0.2	−0.5
15.00	15.04	0.8	−0.2
<i>Latanoprost</i>			
<i>Within-day measurements</i>			
1.25	1.28	2.6	−3.0
2.50	2.47	2.3	1.3
5.00	4.99	2.8	0.2
7.50	7.49	1.5	0.1
10.00	10.12	2.2	−0.2
<i>Between-day measurements</i>			
1.25	1.34	3.2	−7.6
2.50	2.46	3.6	1.4
5.00	4.88	3.2	2.3
7.50	7.49	2.5	0.0
10.00	10.05	2.6	−0.5
<i>Travoprost</i>			
<i>Within-day measurements</i>			
1.00	1.05	5.7	−5.3
2.00	2.04	4.5	−1.9
4.00	3.95	3.8	1.2
6.00	5.79	5.5	3.4
8.00	8.16	4.9	−2.0
<i>Between-day measurements</i>			
1.00	1.05	6.6	−5.2
2.00	1.97	4.2	1.5
4.00	3.99	3.1	0.1
6.00	5.91	5.1	1.4
8.00	8.07	2.9	−0.8

above what is recommended. Our data indicate that latanoprost exhibited statistically significant loss of concentration over time when stored at temperatures $\geq 37^\circ\text{C}$ as did travoprost when stored at 50°C . Bimatoprost, in contrast, appeared to be relatively stable at all storage temperatures assessed.

Although the current data appear to fit a linear model of drug degradation within the observation period of this study, drug degradation at time points beyond 30 days may no longer be linear. Therefore, estimations of drug concentrations after 6 weeks and estimations of T90s that were longer than 30 days may contain some level of error. Nonetheless, as the current study was limited to 30 days, extrapolations based on models that fit the data within that observational time frame are the best predictors available of

drug concentration at later time points, and were employed here. Extrapolations of our data suggested that at the end of 6 weeks, the concentration of latanoprost stored continuously at 50°C is expected to remain $>35\text{ }\mu\text{g/mL}$ and that of travoprost is expected to remain $>20\text{ }\mu\text{g/mL}$. Although this does represent a significant loss of drug compared with the labeled concentration, previous dosing studies for latanoprost have demonstrated potent ocular hypotensive effects at even lower concentrations.^{33,34} Thus, it is possible that some level of efficacy could be retained after exposure to elevated temperatures, even after 6 or more weeks in these highly stressed samples. However, the bioactivity of pure drug at lower concentrations may be different from partially degraded drug with respect to both intraocular

pressure reduction and potential side effects, such as hyperemia. Therefore, the actual therapeutic efficacy of thermally stressed and/or degraded PGAs remains to be determined.

The current data correlate reasonably well with thermal degradation rates for latanoprost previously published by Morgan et al.,²⁹ who reported that latanoprost was stable at 4°C and 25°C but measured T90s of 8.3 and 1.3 days when stored at 50°C and 70°C, respectively. Other studies have measured the remaining concentration of PGAs collected directly from glaucoma patients after patient-directed storage with varying results. Varma et al.²⁸ reported that 94% of patients' bottles collected 4–6 weeks after opening contained within 10% of the labeled concentration of latanoprost. Paolera et al.³² found that 100% of patients' bimatoprost bottles assessed contained over 100% of the labeled drug concentration when returned ~6 weeks after opening. In contrast, more than 17% of latanoprost bottles in that study contained <80% of the labeled concentration and over 50% contained <90% of the labeled concentration. The results of the latter publication corroborate our observations that suggest bimatoprost might be more thermally stable than latanoprost.

Off-the-shelf control bottles of all three PGAs analyzed in the present study were found to contain, on average, more than 100% of the labeled drug concentration. Although the excess concentrations were relatively minor, they did lead to reasonably large variability in the calculated T90s as well as expected concentrations of drug remaining at the end of 6 weeks under conditions of thermal stress. For this reason, these calculations were presented using both the measured and labeled drug concentrations as a baseline.

At baseline, the mean concentrations for all drugs were measured to be within 102% to 120% of the labeled concentration. At higher storage temperatures, we observed a non-significant trend for the concentration of bimatoprost to increase over time. We attribute this temperature-dependent effect to evaporation of water from the formulation. Indeed, measured concentration of all three drugs is affected by two simultaneous processes—drug degradation and water evaporation. Because evaporation occurs concomitantly with drug degradation, and because evaporation reduces formulation volume and increases drug concentration, measured drug concentration reduction rates were likely lower than actual drug degradation rates. Importantly, evaporation rates might be different for each of the drugs, owing to differences in container design and/or overall formulation. If bottle design strongly influences evaporation rates, then different results might be obtained by placing the drugs in different containers. However, the ultimate impact of evaporation on the clinical applicability of the current data is likely to be minimal, because drug concentration is ultimately determines efficacy. Any differences in evaporation rates among drugs that may have affected measured drug concentration in this study would be similarly reflected during daily use by clinical patients. It is for this reason that our primary outcome measure was drug concentration when stored in commercially supplied containers (as used by patients), and evaporation rates were not directly measured.

The analyses of latanoprost and travoprost were carried out using LC/MS/MS; however, mass spectrometry was found to yield inconsistent results during preliminary analyses of bimatoprost standard samples. This inconsistency is postulated to have resulted from variable vaporization of the

compound under the extreme conditions contained within the source of the mass spectrometer. Therefore, bimatoprost analyses were conducted using high performance LC with UV detection. Importantly, all measurements were calibrated using precise standard curves for each drug, and so differences in measurement technique are unlikely to account for any differences noted in drug concentration at any point. Validation experiments demonstrated very good accuracy and precision for measurements of bimatoprost and latanoprost concentration in standardized samples, with lower precision being observed for travoprost (Table 1). Indeed, the measurements for experimental samples of travoprost were also more variable within stress condition groups compared with the other two drugs (Figs. 1–3), making the data for this particular drug somewhat less conclusive. While temperature dependence for the stability of travoprost and a clear degradation of travoprost when stored at 50°C were observed, variability in our measurements may account for the high (borderline significant) multivariate ANOVA *P* value observed and may have obscured drug instability at lower temperatures leading to a type II error. Therefore, the stability of travoprost at mid-range temperatures (near 37°C) should be interpreted with caution.

Studies aimed at assessing patient adherence with self-administration of topical ocular hypotensive medications suggest that over 40% of patients take their medications on 75% or less of the days that they are supposed to.^{35,36} It seems possible that compliance with drug administration might take precedence over drug storage and so one could expect that an even lower percentage of patients carefully store their drug bottles as directed. Our results suggest that this could seriously affect drug stability if the default storage conditions are at high temperature. In some regions throughout the United States, seasonal outdoor temperatures exceed 37°C. Indoor temperatures can be even higher when air conditioning is not utilized, for instance, if no one is home during the day. Temperatures in enclosed spaces such as automobiles can easily rise to exceed 50°C. In addition to storage temperature, exposure to UV radiation also has a significant effect on PGA stability. Indeed, Morgan et al.²⁹ showed that exposure to UV radiation at levels similar to summer sunlight can degrade latanoprost at a rate corresponding to a T90 of just a few hours. Therefore, handling and storage of PGAs are likely to impact their efficacy. As both of these factors are time dependent, the impact of improper storage may be even further exacerbated by failure to discard unused medication after the recommended amount of time (6 weeks after opening for latanoprost,²² not indicated in the package inserts for bimatoprost²³ or travoprost²⁴). In light of this, proper storage of PGAs should be stressed by prescribing ophthalmologists.

Another consideration involving the stability of PGAs centers on the use of these compounds in geographic areas with limited resources. Glaucoma is a major cause of visual impairment throughout the world,^{1,2} including in developing countries where potent ocular hypotensive medications are not as readily available as in the US or Europe. The patent for latanoprost will be the first for the three PGAs to expire in 2011 (US Patent #5,296,504), making it available for generic production, whereas travoprost is protected by patent until at least 2013 (US Patent #5,510,383) and bimatoprost until at least 2014 (US Patent #5,688,819). In considering whether generic forms of topical prostaglandins might be

useful and cost effective for the treatment of glaucoma in developing nations with extremely warm climates, the ability to transport and store these compounds (especially latanoprost and travoprost) at temperatures below 25°C should be taken into account. If loss of efficacy resulting from reductions in drug concentration after extended periods of thermal stress proves to be problematic, it may be beneficial to package the drugs in smaller volumes that can be consumed more quickly after opening.

On the basis of the current results, we conclude that storage of topical PGAs at or below room temperature for a period of 1 month results in no measureable loss of drug concentration. However, patients should be cautioned not to store these compounds, especially latanoprost and travoprost, in conditions that exceed room temperature, as significant drug degradation may occur and to discard unused drug after the recommended period of time. The efficacy of these compounds in parts of the world in which refrigerated transport and storage are not available may be compromised by continued exposure to thermal stress. The greater stability of bimatoprost compared with latanoprost and travoprost might indicate greater efficacy in situations where long-term storage at lower temperatures is not possible.

Acknowledgments

T.V.J. was supported by a National Institutes of Health OxCam Scholarship, the Gates-Cambridge Trust. I.A.B. was supported by NIH Grants R01CA091016 and P30ES013508. D.K.V. was supported by Pennsylvania Animal Diagnostic Laboratory System. A.P.T. was supported by an unrestricted grant from Research to Prevent Blindness to the Northwestern University Feinberg School of Medicine Department of Ophthalmology. The authors are grateful to S. R. Grossman for helpful advice with the statistical analyses.

Author Disclosure Statement

Angelo P. Tanna: Alcon, Allergan, and Apotex (consultant); Alcon, Allergan, and Pfizer (educational grant support). The other authors report no conflicts of interest with any material presented in this article.

References

- Quigley, H.A., and Broman, A.T. The number of people with glaucoma worldwide in 2010 and 2020. *Br. J. Ophthalmol.* 90:262–267, 2006.
- Resnikoff, S., Pascolini, D., Etya'ale, D., et al. Global data on visual impairment in the year 2002. *Bull. World Health Organ.* 82:844–851, 2004.
- Maier, P.C., Funk, J., Schwarzer, G., Antes, G., and Falck-Ytter, Y.T. Treatment of ocular hypertension and open angle glaucoma: meta-analysis of randomised controlled trials. *BMJ* 331:134, 2005.
- Kass, M.A., Heuer, D.K., Higginbotham, E.J., et al. The Ocular Hypertension Treatment Study: a randomized trial determines that topical ocular hypotensive medication delays or prevents the onset of primary open-angle glaucoma. *Arch. Ophthalmol.* 120:701–713; discussion 829–830, 2002.
- Heijl, A., Leske, M.C., Bengtsson, B., Hyman, L., Bengtsson, B., and Hussein, M. Reduction of intraocular pressure and glaucoma progression: results from the Early Manifest Glaucoma Trial. *Arch. Ophthalmol.* 120:1268–1279, 2002.
- The Advanced Glaucoma Intervention Study (AGIS): 7. The relationship between control of intraocular pressure and visual field deterioration. The AGIS Investigators. *Am. J. Ophthalmol.* 130:429–440, 2000.
- The effectiveness of intraocular pressure reduction in the treatment of normal-tension glaucoma. Collaborative Normal-Tension Glaucoma Study Group. *Am. J. Ophthalmol.* 126:498–505, 1998.
- Comparison of glaucomatous progression between untreated patients with normal-tension glaucoma and patients with therapeutically reduced intraocular pressures. Collaborative Normal-Tension Glaucoma Study Group. *Am. J. Ophthalmol.* 126:487–497, 1998.
- Bean, G.W., and Camras, C.B. Commercially available prostaglandin analogs for the reduction of intraocular pressure: similarities and differences. *Surv. Ophthalmol.* 53 Suppl 1:S69–S84, 2008.
- Holmstrom, S., Buchholz, P., Walt, J., Wickstrom, J., and Aagren, M. Analytic review of bimatoprost, latanoprost and travoprost in primary open angle glaucoma. *Curr. Med. Res. Opin.* 21:1875–1883, 2005.
- Marquis, R.E., and Whitson, J.T. Management of glaucoma: focus on pharmacological therapy. *Drugs Aging* 22:1–21, 2005.
- Schwartz, K., and Budenz, D. Current management of glaucoma. *Curr. Opin. Ophthalmol.* 15:119–126, 2004.
- Hylton, C., and Robin, A.L. Update on prostaglandin analogs. *Curr. Opin. Ophthalmol.* 14:65–69, 2003.
- Johnson, T.V., Fan, S., Zhan, G., Camras, C.B., and Toris, C.B. Efficacy and mechanisms of intraocular pressure reduction with latanoprost and timolol in participants with ocular hypertension: a comparison of 1 and 6 weeks of treatment. *J. Glaucoma* 19:356–364, 2010.
- Lim, K.S., Nau, C.B., O'Byrne, M.M., et al. Mechanism of action of bimatoprost, latanoprost, and travoprost in healthy subjects. A crossover study. *Ophthalmology* 115:790–795.e4, 2008.
- Toris, C.B., Gabelt, B.T., and Kaufman, P.L. Update on the mechanism of action of topical prostaglandins for intraocular pressure reduction. *Surv. Ophthalmol.* 53 Suppl 1:S107–S120, 2008.
- Toris, C.B., Zhan, G., Fan, S., et al. Effects of travoprost on aqueous humor dynamics in patients with elevated intraocular pressure. *J. Glaucoma* 16:189–195, 2007.
- Christiansen, G.A., Nau, C.B., McLaren, J.W., and Johnson, D.H. Mechanism of ocular hypotensive action of bimatoprost (Lumigan) in patients with ocular hypertension or glaucoma. *Ophthalmology* 111:1658–1662, 2004.
- Toris, C.B., Camras, C.B., and Yablonski, M.E. Effects of PhXA41, a new prostaglandin F2 alpha analog, on aqueous humor dynamics in human eyes. *Ophthalmology* 100:1297–1304, 1993.
- Ziai, N., Dolan, J.W., Kacere, R.D., and Brubaker, R.F. The effects on aqueous dynamics of PhXA41, a new prostaglandin F2 alpha analogue, after topical application in normal and ocular hypertensive human eyes. *Arch. Ophthalmol.* 111:1351–1358, 1993.
- Novack, G.D., and Evans, R. Commercially available ocular hypotensive products: preservative concentration, stability, storage, and in-life utilization. *J. Glaucoma* 10:483–486, 2001.
- Xalatan [Package Insert]. Pharmacia & Upjohn Company, Division of Pfizer Inc.; New York, NY: January 2009. www.pfizer.com/files/products/uspi_xalatan.pdf. Accessed July 27, 2010.
- Lumigan [Package Insert]. Allergan Inc.; Irvine, CA: September 2006. www.allergan.com/assets/pdf/lumigan_pi.pdf. Accessed July 27, 2010.

24. Travatan [Package Insert]. Alcon Laboratories, Inc.; Fort Worth, TX: 2004. http://ecatalog.alcon.com/pi/travatan_us_en.pdf. Accessed July 27, 2010.
25. Hahn, S.R. Patient-centered communication to assess and enhance patient adherence to glaucoma medication. *Ophthalmology* 116:S37–S42, 2009.
26. Tsai, J.C. A comprehensive perspective on patient adherence to topical glaucoma therapy. *Ophthalmology* 116:S30–S36, 2009.
27. Budenz, D.L. A clinician's guide to the assessment and management of nonadherence in glaucoma. *Ophthalmology* 116:S43–S47, 2009.
28. Varma, R., Winarko, J., Kiat-Winarko, T., and Winarko, B. Concentration of latanoprost ophthalmic solution after 4 to 6 weeks' use in an eye clinic setting. *Invest. Ophthalmol. Vis. Sci.* 47:222–225, 2006.
29. Morgan, P.V., Proniuk, S., Blanchard, J., and Noecker, R.J. Effect of temperature and light on the stability of latanoprost and its clinical relevance. *J. Glaucoma* 10:401–405, 2001.
30. Reis, R., dos Santos, L.C., Vila, M.P., and Magacho, L. Effects of travoprost 0.004% ophthalmic solution, six weeks after its laminated packaging had been removed, in primary open-angle glaucoma: a randomized, controlled, investigator-blinded study. *Clin. Ther.* 26:2121–2127, 2004.
31. Mochizuki, H., Itakura, H., Takamatsu, M., and Kiuchi, Y. Efficacy of latanoprost when stored at room temperature. *Hiroshima J. Med. Sci.* 57:69–72, 2008.
32. Paolera, M.D., Kasahara, N., Umbelino, C.C., and Walt, J.G. Comparative study of the stability of bimatoprost 0.03% and latanoprost 0.005%: a patient-use study. *BMC Ophthalmol.* 8:11, 2008.
33. Fristrom, B., and Nilsson, S.E. A double masked comparison of the intraocular pressure reducing effect of latanoprost 0.005% and 0.001% administered once daily in open angle glaucoma and ocular hypertension. *Br. J. Ophthalmol.* 81:867–870, 1997.
34. Diestelhorst, M., Roters, S., and Krieglstein, G.K. The effect of latanoprost 0.005% once daily versus 0.0015% twice daily on intraocular pressure and aqueous humour protein concentration in glaucoma patients. A randomized, double-masked comparison with timolol 0.5%. *Graefes Arch. Clin. Exp. Ophthalmol.* 235:20–26, 1997.
35. Friedman, D.S., Okeke, C.O., Jampel, H.D., et al. Risk factors for poor adherence to eyedrops in electronically monitored patients with glaucoma. *Ophthalmology* 116:1097–1105, 2009.
36. Okeke, C.O., Quigley, H.A., Jampel, H.D., et al. Interventions improve poor adherence with once daily glaucoma medications in electronically monitored patients. *Ophthalmology* 116:2286–2293, 2009.

Received: July 18, 2010

Accepted: September 13, 2010

Address correspondence to:

Dr. Angelo P. Tanna

Department of Ophthalmology

Feinberg School of Medicine

Northwestern University

645 North Michigan Avenue, Suite 440

Chicago, IL 60611

E-mail: atanna@northwestern.edu