Effect of Low-Dose Latrunculin B on Anterior Segment Physiologic Features in the Monkey Eye

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Objectives: To determine if low doses of topical latrunculin B (LAT-B) will increase outflow facility and decrease intraocular pressure without damaging the cornea and if they will inhibit miotic and accommodative responses to pilocarpine in monkeys.

Methods: We measured intraocular pressure (Goldmann tonometry) before and after 1 and 9 doses of 0.005% and 0.01% topical LAT-B and vehicle given twice daily on successive weeks; outflow facility (perfusion) following 15 doses; central corneal thickness (ultrasonic pachymetry) before and after 1 and 9 doses of 0.01% LAT-B and vehicle; pupillary diameter (calipers); and accommodation (refractometry) before and after 1 dose of 0.005% and 0.02% LAT-B.

Results: Latrunculin-B dose-dependently decreased intraocular pressure, multiple doses more than a single dose. Maximal mean ± SEM hypotension after 1 dose was 2.5 ± 0.3 mm Hg (0.005% LAT-B; n=8; $P<.001$) or 2.7 ± 0.6 mm Hg (0.01% LAT-B; n=8; $P<.005$); maximal mean ± SEM hypotension after 9 doses was 3.2 ± 0.5 mm Hg (0.005% LAT-B; n=8; $P<.001$) or 4.4 ± 0.6 mm Hg (0.01% LAT-B; n=8; $P<.001$). Outflow facility was increased by mean ± SEM 75% ± 13% (n=7; $P<.005$). Central corneal thickness was not changed after 1 or 9 doses of 0.01% LAT-B. Miotic and accommodative responses to intramuscular pilocarpine were dose-dependently inhibited. With 0.02% LAT-B, inhibition of miosis was substantial, whereas the inhibition of accommodation was only about 25%. With 0.005% LAT-B, the effects were trivial.

Conclusions: In ocular normotensive monkeys, 0.005% and 0.01% LAT-B administered topically increases outflow facility and/or decreases intraocular pressure without corneal effects. Multiple doses reduce intraocular pressure more than a single dose. Latrunculin-B dose-dependently relaxes the iris sphincter and ciliary muscle, with some separation of miotic and accommodative effects.

Clinical Relevance: Multiple treatments with low topical doses of LAT-B may substantially reduce outflow resistance in eyes with glaucoma without adversely affecting the cornea.

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LATRUNCULINS, MACROLIDES isolated from the marine sponge *Latrunculia magnifica*, are specific and potent actin-disrupting agents that sequester monomeric G-actin, leading to the disassembly of actin filaments.[1-3] Latrunculins A and B (LAT-A and LAT-B) are the 2 most common latrunculins, which cause reversible dose-dependent and time-dependent destruction of actin bundles and associated proteins in varieties of cultured cells including human trabecular meshwork (TM) cells.[1-7] In living monkeys, both LAT-A and LAT-B increase outflow facility and decrease intraocular pressure (IOP).[6,8,9] Latrunculin-B also increases outflow facility in the organ-cultured anterior segment of porcine eyes,[5] suggesting a direct effect on outflow resistance in the conventional drainage pathway. The latter has been confirmed by a recent morphological study of the TM cells in the live monkey eye.[10] Because LAT-B, compared with LAT-A, is more potent in increasing outflow facility[6,8] and produces fewer transient increases in aqueous humor formation, corneal endothelial permeability, and protein concentration in the anterior chamber (AC),[9] LAT-B may be a better candidate than LAT-A as a potential antiglaucoma medication. However, a single dose of 20 μL of 500-μM (approximately 0.02%) LAT-B administered topically, which decreases IOP in living monkeys,[9] still produces a transient increase in corneal thickness when applied to the central cornea as 4 drops of 5-μL volume.[9] Presumably, multiple treatments with the high

concentration of LAT-B might induce more apparent adverse effects in the cornea.

We hypothesized that repetitive lower concentrations and total doses in higher solution volumes, spread out over the entire corneal or conjunctival surface in the larger human eye, might minimize or avoid toxic effects on the cornea induced by high concentrations of cytoskeletal drugs without attenuating their effects on outflow resistance.[9,11] To test this hypothesis, we determined the effects of a single or multiple doses of 0.005% or 0.01% topical LAT-B on outflow facility, IOP, and/or central corneal thickness (CCT) in normotensive monkey eyes. To learn more about the drug-induced changes in the anterior segment physiologic features, the pupil diameter and accommodation following 0.005% and 0.02% topical LAT-B administration were also determined.

METHODS

ANIMALS AND ANESTHESIA

Twenty-seven adult normal cynomolgus monkeys (*Macaca fascicularis*) of both sexes, weighing 3 to 8 kg, were studied; 8 for the tonometry and perfusion protocols, 5 for the pachymetry protocol, and 14 for the pupil and accommodation protocols (8 in the 0.02% LAT-B group and 6 in the 0.005% LAT-B group). All monkeys contributed 1 eye treated with the drug and 1 contralateral eye treated with vehicle. All experiments were conducted in accordance with University of Wisconsin-Madison and National Institutes of Health, Bethesda, Md, guidelines and with the Association for Research in Vision and Ophthalmology Statement on the Use of Animals in Ophthalmic and Vision Research. All monkeys were free of AC cells and flare by slitlamp biomicroscopy when studied. Anesthesia for tonometry or pachymetry was induced with intramuscular ketamine hydrochloride (10 mg/kg) and maintained with supplemental intramuscular injections as required (usually 5 mg/kg every 30 to 45 minutes). Anesthesia for AC perfusion or refractometry was induced with intramuscular ketamine hydrochloride (10 mg/kg) followed by intravenous pentobarbital sodium (15 mg/kg).

DRUG PREPARATION AND ADMINISTRATION

Latrunculin-B was obtained from Sigma Chemical Co (St Louis, Mo) and stored as a 2mM stock solution in dimethyl sulfoxide (DMSO) (Sigma Chemical Co) at −20°C. Latrunculin-B solutions for topical administration were freshly prepared in the Bárány solution[12] with 25% DMSO. Twenty microliters of 0.005% (1 μg/20 μL), 0.01% (2 μg/20 μL), or 0.02% (4 μg/20 μL) LAT-B were composed of 1.26, 2.53, or 5.00 μL of 2mM LAT-B stock solution in DMSO and 15 μL of the Bárány solution, with an additional 3.74 or 2.47 μL of DMSO added into the 0.005% or 0.01% drug solution, so that each drug solution had 25% DMSO. Twenty-five percent DMSO served as a vehicle control. In IOP protocols, the drug or vehicle solution was administered to the central cornea of opposite eyes of either ketamine-anesthetized (day 1 and day 5; 4 × 5-μL drops at each treatment) or fully conscious and manually restrained monkeys (day 2 through day 4; 2 × 10-μL drops at each treatment) twice daily for 4.5 days at 8 AM and 4 PM. Eyedrops were administered at 30- to 60-second intervals with blinking prevented between drops. Following the 0.01% LAT-B IOP experiment, the monkeys were treated with 0.01% drug and vehicle solution at 4 PM on day 5 and then once (days 6 and 7) or twice (day 8) daily (2 × 10 μL at each treatment) for 3 additional days while fully conscious and manually restrained. On day 9, these monkeys were treated again with the same dose of the drug (4 × 5 μL at each treatment) after receiving ketamine anesthesia 2 hours before the AC perfusion. For pachymetry, different monkeys were treated with 0.01% LAT-B twice daily for 4.5 days after receiving ketamine anesthesia. For refractometry and pupil-diameter measurement, monkeys were treated with 0.005% and 0.02% LAT-B (4 × 5 μL at each treatment) 1 time after receiving ketamine and pentobarbital anesthesia. Administering the drug and vehicle solution to fully conscious and manually restrained monkeys in the IOP and outflow facility protocol was designed to reduce any potential cumulative effect of repeated ketamine administration on IOP or outflow facility during the multiple treatments.[13]

IOP MEASUREMENT

Intraocular pressure was determined on day 1 (before and after the first dose) and on day 5 (before and after the ninth dose) with a minified Goldmann applanation tonometer,[14] using half-and-half creamer solution (Borden Inc, Columbus, Ohio) as the tear film indicator, with the monkey lying prone in a head holder. For each eye, 3 IOP readings were averaged as a baseline or pretreatment IOP before administration of the first or ninth dose of 0.005% or 0.01% LAT-B or vehicle, and single IOP readings were taken after the drug and vehicle administration hourly for 6 hours. The 2-dose IOP protocols were conducted within a successive period of 2 weeks to observe the cumulative dose-response relationship during a short period. The same eyes of the same animals were treated with the drug in the 2 IOP protocols, with the 0.01% LAT-B experiment performed the week immediately following the 0.005% LAT-B experiment. Since there was only a 2-day washout period, some ocular hypotensive effect of the 0.005% dose may have been carried over to the 0.01% dose protocol, but this cumulative dose-response strategy, which is often used in pharmacological studies, does not affect our overall conclusions.

OUTFLOW FACILITY MEASUREMENT

Total outflow facility was determined by 2-level constant pressure perfusion of the AC with the Bárány mock aqueous humor,[12] using a 1-needle technique and correcting for internal apparatus resistance.[15] Outflow facility was measured for 90 minutes 2 hours after the fifteenth dose of 0.01% LAT-B or vehicle on day 9.

CCT MEASUREMENT

Central corneal thickness was determined by ultrasonic pachymetry (DGH-1000 ultrasonic pachymeter; DGH Technology, Inc, Solana Beach, Calif) on day 1 (before and after the first dose) and day 5 (before and after the ninth dose). For each eye, 3 readings were averaged as a baseline or pretreatment value before administration of the first or ninth dose of 0.01% LAT-B or vehicle, and single readings were taken after the drug and vehicle administration every 30 minutes for 4 hours and then hourly for 2 hours.

PUPIL AND ACCOMMODATION MEASUREMENT

Accommodation (difference between baseline and postdrug refraction) was determined with a Hartinger coincidence refractometer (Zeiss-Jena, Jena, Germany). Pupil diameter was measured with Vernier calipers under normal room light (350 lux). Baseline refraction and/or pupillary diameter were measured, followed by topical application of 2.5% phenylephrine (stimu-

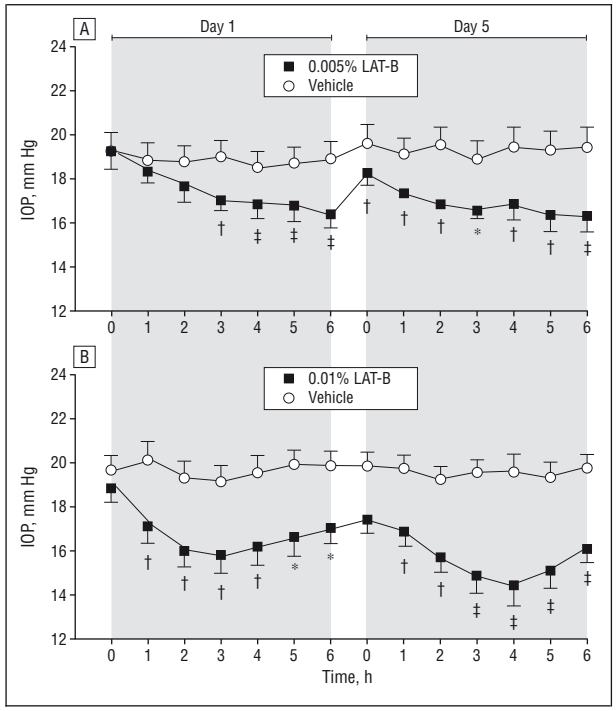

Figure 1. We administered 0.005% (A) or 0.01% (B) latrunculin B (LAT-B) and vehicle to the opposite eyes of monkeys topically twice daily for 4.5 days. Intraocular pressure (IOP) was measured before and after the first (on day 1) and ninth (on day 5) treatment. The same eyes of the same monkeys were treated with the drug in the 2-dose studies, and the higher-dose experiment was conducted the week immediately following the lower-dose experiment, with only 2 days' drug-free interval between studies. The IOP before the first treatment in each study was used as a baseline. Data are expressed as mean±SEM for 8 animals. The IOP difference between eyes corrected for baseline was tested for differences vs 0.0 or by the 2-tailed paired *t* test. * indicates $P<.01$; †, $P<.005$; ‡, $P<.001$.

lates the iris dilator muscle without influencing the iris sphincter and ciliary muscle,[16,17] facilitating measurement of miosis and accommodation[18]). Refraction and/or pupillary diameter were measured again approximately 30 minutes later, after which 20 μL (4×5 μL for each treatment) of 0.005% or 0.02% LAT-B were administered topically to one eye and vehicle to the other. Refraction and pupillary diameter were determined 85 minutes after LAT-B administration. Five minutes later, approximately 3 mL of pilocarpine solution were infused intramuscularly in the thigh (1.5 mg/kg) across 10 minutes. Refraction was determined every 5 minutes after pilocarpine infusion until stable, and final pupillary diameter was then measured. The intramuscular infusion of pilocarpine as described earlier allowed us to measure dose-dependent accommodation during the drug administration, where, in effect, time becomes the dose. This method allowed us to look for differences in the absolute amplitude of the accommodative response and for any leftward or, more likely, rightward shift in the "dose-response" curve of the eye treated with LAT-B relative to the contralateral control eye.[19,20] Furthermore, systemic administration assures that both eyes receive the same pilocarpine dose at all times, making the comparison between the eyes at each point still more valid and precise.

SLITLAMP EXAMINATION

Slitlamp biomicroscopy was performed before drug administration, during IOP measurement (1, 3, and 6 hours after drug administration), and before pachymetry and AC perfusion. The integrity of the corneal epithelium and endothelium, the presence of flare or cells in the AC, and the clarity of the lens, were noted. All animals were free of preexisting ocular abnormalities when studied.

DATA ANALYSIS

Data are given as mean ± SEM for number of eyes or animals. Predrug or postdrug treated vs contralateral control; postdrug or postvehicle vs ipsilateral baseline; and baseline-corrected postdrug treated vs control comparisons were made using the 2-tailed paired *t* test for differences vs 0.0 or ratios vs 1.0. The baseline IOP used for the data analysis in the 0.005% LAT-B or 0.01% LAT-B protocol was the IOP measured immediately before the first treatment of the corresponding dose of the drug or vehicle.

RESULTS

INTRAOCULAR PRESSURE

A single dose of 0.005% LAT-B lowered IOP from mean ± SEM 19.3 ± 0.8 to 16.4 ± 0.7 mm Hg within 6 hours. After adjustment for baseline and contralateral IOP,[21] the maximal mean ± SEM hypotension of 2.5 ± 0.3 mm Hg (n=8; $P<.001$) occurred at hour 6. Multiple doses (9 doses) of 0.005% LAT-B reduced IOP similar to a single dose but the significant IOP reduction occurred earlier (hour 1 vs hour 3) and the maximal ocular hypotension was slightly greater (mean ± SEM, 3.2 ± 0.5 mm Hg; $P<.001$). Intraocular pressure at 16 hours after the eighth treatment (IOP at 0 hours on day 5) in the eye treated with LAT-B was significantly lower than that in the contralateral control eye (mean ± SEM, −1.4 ± 0.3 mm Hg; $P<.005$) (**Figure 1**A). A single dose of 0.01% LAT-B lowered IOP from mean ± SEM 18.8 ± 0.7 to 15.7 ± 0.8 mm Hg within 6 hours. After adjustment for baseline and contralateral IOP, the maximal mean ± SEM hypotension of 2.7 ± 0.6 mm Hg (n=8; $P<.005$) occurred at hour 3. Multiple doses (9 doses) of 0.01% LAT-B induced a greater IOP reduction than a single dose, with the maximal mean ± SEM hypotension of 4.4 ± 0.6 mm Hg ($P<.001$) at hour 4. The IOP measured before the ninth treatment (IOP at 0 hours on day 5) in the eye treated with LAT-B tended to be lower than that in the contralateral control eye (mean ± SEM, −1.7 ± 0.7 mm Hg; $P=.056$). Although the monkeys had not received any treatment for 3 days after the ninth treatment with 0.005% LAT-B, the baseline IOP (IOP at 0 hours on day 1) in the eye treated with LAT-B in the 0.01% LAT-B protocol (Figure 1B) did not return to the level before the first treatment with 0.005% LAT-B (Figure 1A).

OUTFLOW FACILITY

Latrunculin B significantly increased outflow facility by mean ± SEM 75% ± 13% (n=7; $P<.005$) during the overall 90-minute postdrug perfusion beginning 2 hours after the 15th treatment of 0.01% LAT-B. The total number of monkeys was 7 rather than 8 because 1 monkey died on day 6 of a disease unrelated to the experiment. In analysis of three 30-minute perfusion periods, the drug increased outflow facility by mean ± SEM 35% ± 14%, 69% ± 14%, and 100% ± 14% in the first, second, and third 30-minute durations, respectively (**Table 1**). **Figure 2**

Effect of Latrunculin B (LAT-B) on Outflow Facility in Monkeys*

	Outflow Facility (µL/min per mm Hg)		
	LAT-B	Vehicle	LAT-B-Vehicle Ratio
90 min	0.93 ± 0.19	0.51 ± 0.08	1.75 ± 0.13†
First 30 min	0.58 ± 0.10	0.43 ± 0.05	1.35 ± 0.14‡
Second 30 min	0.89 ± 0.19	0.51 ± 0.08	1.69 ± 0.14†
Third 30 min	1.19 ± 0.28	0.51 ± 0.11	2.00 ± 0.14§

*Following 15 doses of 0.01% LAT-B or vehicle (Figure 2), outflow facility was measured by 2-level constant pressure perfusion for 90 minutes. No baseline outflow facility was determined, but all monkeys were selected from those that had similar baseline facilities in both eyes per previous studies. Data are expressed as mean ± SEM for 7 animals. Ratios are unitless. The difference between eyes was tested for ratios unequal to 1.0 by the 2-tailed paired *t* test.
†$P<.005$.
‡$P<.05$.
§$P<.001$.

shows that the increase in outflow facility was both time dependent and pressure dependent. There was no facility increase initially when the perfusion was started at the spontaneous IOP of the monkey eye 2 hours following administration of LAT-B or vehicle (spontaneous IOP of pentobarbital-anesthetized normal monkeys is typically <10 mm Hg[22]), but a progressive increase occurred across time during perfusion at an elevated IOP (15 or 25 mm Hg) even though the drug concentration in the AC must have been decreasing because of the infusion of drug-free fluid and secretion of drug-free aqueous humor into the eye.

CENTRAL CORNEAL THICKNESS

On day 1, mean ± SEM baseline CCT was 456.3 ± 17.0 µm in the eye treated with LAT-B and 457.7 ± 18.2 µm in the contralateral control eye. Mean ± SEM CCT after the first treatment varied between 454.6 ± 17.2 and 462.4 ± 17.0 µm in the eye treated with LAT-B and between 453.4 ± 15.4 and 458.6 ± 18.7 µm in the contralateral control eye during 6-hour pachymetry. On day 5, the mean ± SEM CCT measured before the ninth treatment was 448.4 ± 17.9 µm in the eye treated with LAT-B and 455.9 ± 18.6 µm in the contralateral control eye. The mean ± SEM CCT after the ninth treatment varied between 454.4 ± 16.4 and 462.2 ± 18.2 µm in the eye treated with LAT-B and between 452.2 ± 18.8 and 457.2 ± 19.6 µm in the contralateral control eye during 6-hour pachymetry. Collectively, the mean ± SEM CCT in the eye treated with LAT-B was only 0.9 to 8.1 µm ($P=.08$-$.82$) or 3.1 to 7.1 µm ($P=.07$-$.37$) thicker than that in the eye treated with vehicle after 1 or 9 doses of 0.01% LAT-B, after adjustment for ipsilateral baseline (**Figure 3**).

PUPIL AND ACCOMMODATION MEASUREMENT

Pupillary Diameter

Baseline pupil diameters of both eyes in all monkeys were similar (**Figure 4**A and C). Twenty-five minutes after

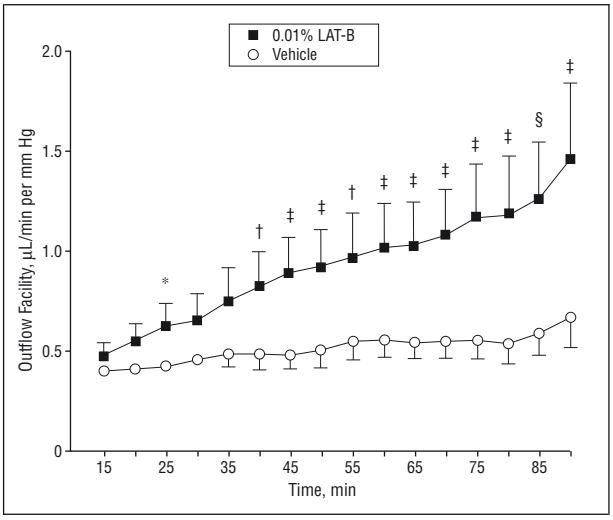

Figure 2. Following the 0.01% latrunculin B (LAT-B) intraocular pressure protocol (Figure 1B), treatment with 0.01% LAT-B or vehicle to opposite eyes of monkeys topically once or twice daily was continued without interruption for 3 additional days. Outflow facility was measured by 2-level constant pressure perfusion for 90 minutes on day 9 (2 hours after the 15th treatment). No baseline outflow facility was determined, but all monkeys were selected from those that had similar baseline facilities in both eyes per previous studies. Data are expressed as mean±SEM for 7 animals (n=7 rather than 8 because 1 monkey died in its cage of a disease unrelated to the experiment before perfusion). The difference between eyes was tested for differences vs 0.0 or by the 2-tailed paired *t* test. * indicates $P<.05$; †, $P<.03$; ‡, $P<.02$; §, $P<.01$.

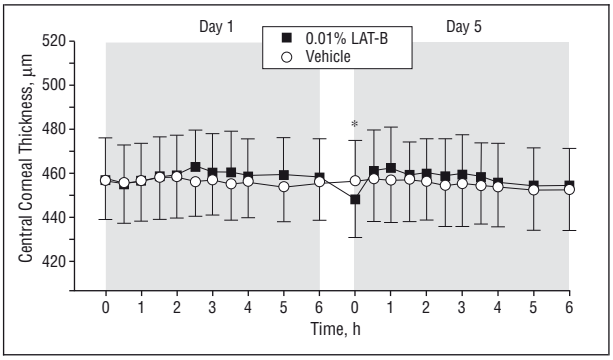

Figure 3. We administered 0.01% latrunculin B (LAT-B) and vehicle to the opposite eyes of monkeys topically twice daily for 4.5 days. Central corneal thickness (CCT) was measured before and after the first (on day 1) and ninth (on day 5) treatment. The CCT before the first treatment was used as a baseline. Data are expressed as mean±SEM for 5 animals. The CCT difference between eyes corrected for baseline was tested for differences vs 0.0 or by the 2-tailed paired *t* test. * indicates $P<.05$.

phenylephrine administration, both pupils dilated equally (in the 0.02% LAT-B protocol, mean ± SEM, 7.2 ± 0.3 mm vs 7.2 ± 0.3 mm; n=8; $P<.20$) (Figure 4A) (in the 0.005% LAT-B protocol, mean ± SEM, 7.0 ± 0.3 mm vs 7.0 ± 0.3 mm; n=6; $P<.40$) (Figure 4C). Eighty-five minutes after topical administration of 20 µL of 0.02% LAT-B, the pupils in the eyes treated with LAT-B dilated further relative to the contralateral controls (to mean ± SEM 8.0 ± 0.3 mm vs 7.0 ± 0.4 mm; $P<.005$) (Figure 4A). However, 85 minutes after 20 µL of 0.005% LAT-B, the pupils in the eyes treated with LAT-B were only slightly larger than those in the eyes treated with vehicle. When pilocarpine was infused intramuscularly in the thigh, the control pupils constricted but the pupils treated with 0.02%

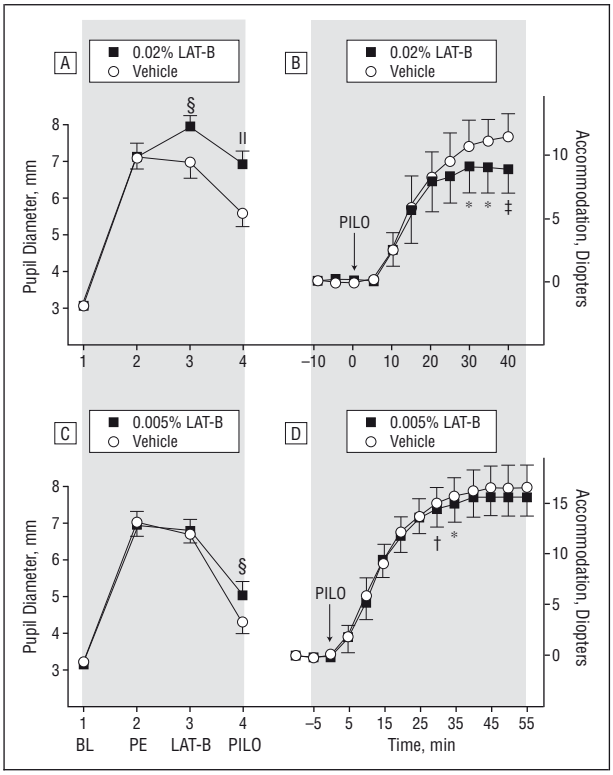

Figure 4. Pupillary and accommodative responses to topical phenylephrine (PE), topical latrunculin B (LAT-B) (0.02% [A and B] or 0.005% [C and D]) and intramuscular pilocarpine (PILO) (1.5 mg/kg) in monkeys. Data are expressed as mean ± SEM for 8 animals (A), 5 animals (B), and 6 animals (C and D) (accommodation data for 3 animals in the 0.02% LAT-B protocol are not available). The difference between eyes was tested for differences vs 0.0 or by the 2-tailed paired *t* test. * indicates $P<.05$; †, $P<.02$; ‡, $P<.01$; §, $P<.005$; ‖, $P<.001$; BL, baseline.

LAT-B did not (mean ± SEM, 5.6 ± 0.3 mm in controls vs 7.0 ± 0.4 mm in eyes treated with LAT-B; $P<.001$) (Figure 4A). The inhibition of miosis was substantial when compared with the pre–LAT-B value (mean ± SEM, 7.0 ± 0.4 mm vs 7.2 ± 0.3 mm). The miosis was only slightly inhibited by 0.005% LAT-B (mean ± SEM, 5.0 ± 0.4 mm in eyes treated with LAT-B vs 4.3 ± 0.3 mm in control eyes).

Accommodation

No significant differences between pilocarpine-induced accommodation in eyes treated with LAT-B vs control eyes were observed initially after 20 μL of 0.02% LAT-B (Figure 4B). However, the accommodation plateau in the eyes treated with LAT-B occurred earlier than that in the control eyes (30 vs 40 minutes after the intramuscular pilocarpine). A statistically significant difference between eyes was observed during the period of 30 to 40 minutes after intramuscular pilocarpine, with the eyes treated with LAT-B accommodating approximately mean ± SEM 2.5 ± 0.5 diopters (D) (approximately 25% ± 8%) less than the controls (8.9 vs 11.4 D; n = 5; $P<.01$) (Figure 4B). The accommodation was only slightly inhibited by 0.005% LAT-B (Figure 4D). The accommodative amplitude appears greater in the eyes treated with vehicle in the 0.005% LAT-B group compared with the eyes treated with vehicle in the 0.02% LAT-B group, which may be owing to the different durations of the measurement in the 2 groups. Other factors might also be involved, such as animal age (not available for some monkeys in the 0.02% LAT-B group), different accommodative amplitudes in different animals, differences in lag time for systemic bioavailability or distribution of the drug, and/or body weight of the animals, that might affect muscle mass and therefore distribution of the drug. In any case, the difference was not statistically significant by the 2-tailed unpaired *t* test (mean ± SEM, 11.4 ± 1.7 D vs 16.5 ± 2.2 D; $P>.60$), and the "different" accommodative amplitudes did not affect the major conclusions from the data obtained from comparison between contralateral eyes of the same monkey.

SLITLAMP EXAMINATION

During IOP measurement, most monkeys had mild punctate corneal epithelial defects at 3 to 6 hours after the drug administration, but the defects in eyes treated with LAT-B were similar to those in control eyes. Additionally, the punctate corneal epithelial defects seen during tonometry after the first treatment on day 1 had disappeared in both eyes of almost all monkeys at approximately 16 hours after the eighth dose (before tomometry on day 5). No other abnormality was observed in any monkey in any protocol during slitlamp examination. The heavily pigmented monkey conjunctivas precluded the evaluation of conjunctival hyperemia.

COMMENT

This study has shown that LAT-B administered topically decreases IOP in normotensive monkeys in a dose-dependent manner, with multiple doses producing greater IOP reduction than a single dose. This is consistent with many current clinical and experimental antiglaucoma drugs that have greater effects following multiple treatments in both normotensive[21,23] and glaucomatous[24,25] monkeys. Some ocular hypotensive effect of multiple administrations of LAT-B appears to last more than 16 hours, evidenced by the lower IOP in the eyes treated with LAT-B than in the eyes treated with vehicle at 16 hours after the eighth treatment in both the 0.005% and 0.01% LAT-B protocols (Figure 1A and B) and by the tendency toward slightly lower baseline IOP in the eyes treated with a drug than in the control eyes 3 days after the ninth treatment of 0.005% LAT-B (Figure 1B). The latter indicates that the cumulative effect of 0.005% LAT-B may affect the apparent IOP response to 0.01% LAT-B given subsequently. However, the IOP measured on day 5 in the 0.01% LAT-B protocol tended to increase 4 hours after the ninth treatment, which did not occur in the 0.005% LAT-B protocol. This seems to imply that it is more difficult for the drug to maintain a larger IOP reduction than a smaller one, although a higher dose is used. A more rapid rate of decrease in AC drug concentration due to greater resistance washout and greater reduction of the pressure gradient between the AC and the Schlemm canal following the higher dose than the lower dose may account for this phenomenon. Additionally, the same monkeys may have different IOPs or different responses to the drug on different occasions for a variety of rea-

sons, including anesthetic considerations. Nevertheless, the IOP at 6 hours after the higher dose was still lower than that at 6 hours after the lower dose, which is consistent with the statement made earlier. In a previous study,[9] a single dose of 20 μL of 500-μM (approximately 0.02%) LAT-B maximally decreased IOP by 3.1 mm Hg, which is slightly greater than the maximal IOP reduction (–2.7 mm Hg) induced by a single dose of 0.01% LAT-B and apparently smaller than the IOP reduction (–4.4 mm Hg) induced by multiple doses of 0.01% LAT-B, in the current experiments. This further indicates that LAT-B dose dependently decreases IOP and that multiple doses of LAT-B are more effective than a single dose. In the present study, 15 treatments with 0.01% LAT-B time dependently and pressure dependently increased outflow facility in the monkey eye, which, in conjunction with our previous findings,[5,8] suggests that LAT-B decreases IOP by reducing outflow resistance in the TM.

In the previous study,[9] a single dose of 0.02% topical LAT-B also transiently increased the CCT of the monkey eye by up to 47 μm within 3 hours. Unlike the higher dose studied previously, a single and multiple doses of 0.01% LAT-B administered topically in the present study do not change the CCT. This indicates that the 0.01% concentration of the drug does not significantly affect the corneal endothelium. By slitlamp biomicroscopy, 0.01% LAT-B is also less toxic to the corneal epithelium than the higher dose studied before.[9] The LAT-B doses used in this study did not produce any additional punctate corneal epithelial defects in the eyes treated with LAT-B compared with the eyes treated with vehicle. The mild punctate corneal epithelial defects in both eyes, occurring 3 to 6 hours after the drug administration, are a common phenomenon during tonometry in ketamine-anesthetized animals, presumably owing to reduced blinking under ketamine anesthesia and frequent IOP measurements. All these seem to support our hypothesis from previous studies[9,11] that repetitive lower concentrations and total doses in higher solution volumes, spread out over the entire corneal or conjunctival surface, may minimize or avoid toxic effects on the cornea.

A recent morphological study[10] revealed that LAT-B induces formation of numerous cytoplasmic projections of the subcanalicular cells and massive "ballooning" of the juxtacanalicular region, leading to a substantial expansion of the space between the subcanalicular cell layer and the trabecular collagen beams. Additionally, LAT-B also significantly increases the junction-to-junction distance of the inner wall cells of the Schlemm canal,[10] although the increase is not as great as that after the serine-threonine kinase inhibitor H-7.[26,27] All these structural changes in the TM may be consequent to the drug-induced cellular relaxation and account for the drug-induced decrease of outflow resistance in the TM. The current physiologic data indicate that LAT-B dose-dependently relaxes intraocular smooth muscles. This further supports that cellular relaxation could be an important mechanism by which LAT-B decreases outflow resistance in the TM, since H-7, which decreases outflow resistance primarily by relaxing the TM,[26,27] also relaxes the iris sphincter in vivo and ciliary muscle strips in vitro.[19] More interestingly, although 0.02% LAT-B appears to substantially, if not completely, inhibit the miotic response of the monkey eye to pilocarpine, it only inhibits the accommodative response to the muscarinic agonist by up to 25%. Phenylephrine-induced bilateral mydriasis may allow LAT-B's inhibition of the pilocarpine-induced miosis to be observed more easily but does not affect the conclusion since phenylephrine was administered bilaterally. The reason for the separation is not clear, but a pharmacokinetic explanation seems plausible.[19] Pilocarpine is a classical antiglaucoma medication that indirectly increases outflow facility by contracting the ciliary muscle. However, the induced miosis, which reduces vision especially in elderly patients with incipient cataract,[28] restricts its use. Although higher doses of pilocarpine may be more resistant to inhibition by LAT-B, the relative dissociation of miotic and accommodative responses to pilocarpine (as used in this study) after LAT-B administration provides a possibility that the combination of a low but still facility-effective topical dose of pilocarpine with a facility-effective and cornea-safe topical dose of LAT-B may induce a facility increase greater than that induced by either drug alone, without damaging the cornea or constricting the pupil. Further studies are needed to prove this hypothesis.

Collectively, the fact that 0.005% and 0.01% topical LAT-B increase outflow facility and/or decrease IOP without adversely affecting the cornea suggests that a low dose of topical LAT-B may have potential as a safe and TM-selective antiglaucoma medication.

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Drs Okka and Tian contributed to this study equally.

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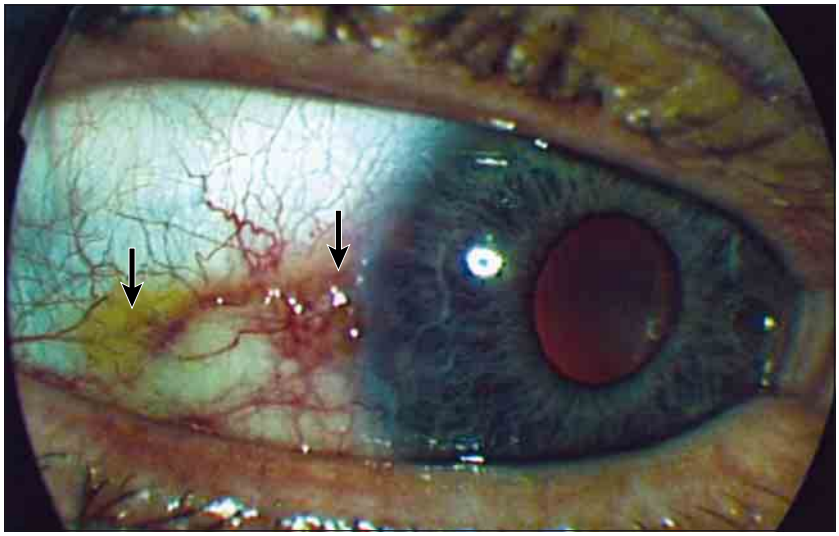
ARCHIVES Web Quiz Winner

We stumped you this month! The correct answer to our June challenge was ocular ochronosis. For a complete discussion of this case, see the Clinicopathologic Reports, Case Reports, and Small Case Series section in the July ARCHIVES (Barrios PC, Font RL. Pigmented conjunctival lesions as initial manifestation of ochronosis. *Arch Ophthalmol.* 2004;122:1060-1063).



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