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Combined Effects of H-7 and Cytochalasin B on Outflow Facility in Monkeys†

BAOHE TIAN[a], B'ANN T. GABELT[a], BENJAMIN GEIGER[b] AND PAUL L. KAUFMAN[a,*]

[a] *Department of Ophthalmology and Visual Sciences, University of Wisconsin, Madison, Wisconsin, U.S.A.;* [b] *Department of Molecular Cell Biology, Weizmann Institute of Science, Rehovot, Israel*

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The serine-threonine protein kinase inhibitor H-7 and the fungal metabolite cytochalasin B (CB) disrupt the actin microfilament network by different mechanisms, and increase outflow facility similarly in live monkeys. Their combined effect has therefore determined on total outflow facility in cynomolgus monkeys by 2-level constant pressure perfusion. (1) After unilateral anterior chamber (AC) bolus injection of H-7 [10 μM, 100 μM (subthreshold for increasing facility when given alone) or 500 μM (just-threshold)] followed by bilateral AC bolus injection of CB [2 μg (strong but submaximal for increasing facility when given alone)], no significant difference between eyes was observed. (2) After bilateral AC exchange with a subthreshold dose of H-7 (10 μM) followed by unilateral AC bolus injection of a subthreshold dose of CB (0·02, 0·05, 0·1 or 0·5 μg), 10 μM H-7 plus 0·1 or 0·5 μg CB increased facility by $\sim$40 or 80% compared to 10 μM H-7 alone. (3) After bilateral AC exchange with a maximal dose of H-7 (300 μM), followed by unilateral AC bolus injection of a subthreshold dose of CB (0·1 or 0·5 μg), 300 μM H-7 plus 0·5 μg CB increased outflow facility by 47% compared to 300 μM H-7 alone. (4) After unilateral AC exchange with a maximal dose of H-7 (300 μM) followed by bilateral AC bolus injection of a near-maximal dose of CB (2 μg), 300 μM H-7 plus 2 μg CB increased the facility by 67% compared to 2 μg CB alone. The significant effect of combined subthreshold doses of H-7 and CB on outflow facility, the potentiation of the facility-increasing effect of a maximal H-7 dose by both subthreshold and near-maximal CB doses, and the known cytoskeletal effects of both compounds, may suggest that both increase facility by disrupting actin filaments in the trabecular meshwork.

Key words: actin cytoskeleton; aqueous humor outflow facility; cynomolgus monkey; cytochalasin B; H-7; trabecular meshwork.

1. Introduction

Cytochalasin B (CB), a fungal metabolite, disrupts actin microfilaments by a complex mechanism which includes capping the filaments and preventing their elongation, thereby affecting a wide variety of actin-dependent cellular events including cell morphogenesis, motility and endocytosis (Davies and Allison, 1978; Godman and Miranda, 1978; Brenner and Korn, 1979; Brown and Spudich, 1981). Initially, however, CB can induce intense cellular contraction (Kolega, Janson and Taylor, 1991). In monolayer cultures of human trabecular meshwork (HTM) cells, CB caused the cells to separate (Polansky et al., 1984). In monolayer cultures of HTM cells grown on filters, CB induced a dramatic dose-related increase in hydraulic conductivity, accompanied by retraction of the cells and widening of the intercellular spaces (Perkins et al., 1988). In living monkeys, CB (Kaufman and Bárány, 1977; Kaufman, Bill and Bárány, 1977; Svedbergh et al., 1978; Kaufman and Erickson, 1982; Kaufman, 1987) dramatically increased outflow facility.

H-7 (1-(5-isoquinolinyl-sulfonyl)-2-methylpiperazine), a wide-range protein kinase inhibitor, blocks the phosphorylation activity of diverse serine-threonine kinases including protein kinase C, myosin light chain kinase (MLCK) and rho kinase (Hidaka et al., 1984; Chrzanowska-Wodnicka and Burridge, 1996). Addition of H-7 to cultured cells inhibits acto-myosin contractility, leading to deterioration of the actin microfilament system and perturbation of its membrane anchorage. This, in turn, weakens cell-extracellular matrix focal contacts (more so than cell–cell adherens junctions) in Swiss 3T3 cells, PTK2 cells, chick lens epithelial cells and fibroblasts, porcine or bovine aortic endothelial cells, and HTM cells (Birrell et al., 1989; Yu and Gotlieb, 1992; Volberg et al., 1994; Epstein, Skinner and Roberts, 1997; Liu et al., 1998; Tian et al., 1998). In living monkeys, H-7 increased outflow facility to a similar degree as CB (Tian et al., 1998).

In previous studies in cultured MDCK cells (Citi, 1992; Citi et al., 1994). H-7 prevented the fall in transepithelial electrical resistance induced by cyto-

* Address all correspondence to: P. L. Kaufman, Department of Ophthalmology and Visual Sciences, University of Wisconsin-Madison, F4/328 CSC, 600 Highland Ave., Madison, WI 53792–3220, U.S.A.

† The University of Wisconsin & the Weizmann Institute of Science have filed a related patent application related to H-7; accordingly, Drs Kaufman (UW) & Geiger (WIS) have a proprietary interest. Alcon Laboratories, Inc., Fort Worth, TX has paid a fee to UW and WIS for an option to license this technology, and has provided some financial support in this general area. Dr Kaufman has served as a paid consultant on unrelated issues for Alcon and several other pharmaceutical corporations.

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chalasin D (CD, a cytochalasin with effects on actin filaments similar to those of CB) (Brenner and Korn, 1979) or calcium chelators. Since the fall in electrical resistance caused by calcium removal is due to an increase in the permeability of the paracellular 'shunt' pathway (Martinez-Palomo et al., 1980), the inhibitory effect of H-7 is believed to result from inhibiting dissociation of cellular junctions by interfering with contractility of the adherens junction-associated microfilaments following treatment with calcium chelators or CD (Citi, 1992; Janson, Kolega and Taylor, 1991; Kolega, Janson and Taylor, 1991; Citi et al., 1994). Those data suggested that although both CB and H-7 could interfere with the same cytoskeletal components, the cellular consequences of their effect on the actin system are quite different. In cultured cells CB and CD induce rapid and often quite vigorous cell contractions by decreasing the gel structure (solation) in the cellular contractile system (Janson, Kolega and Taylor, 1991; Kolega, Janson and Taylor, 1991), which, in conjunction with weakening of the adherens junctions consequent to microfilament disruption, leads to cell–cell separation. H-7 induces relaxation by inhibiting acto-myosin contractility, which stabilizes adherens junctions against disruption by CD-induced contraction (Citi, 1994), but weakens focal contacts by disrupting actin stress fibers (Volberg et al., 1994).

We considered that the application of both drugs at subthreshold levels might increase outflow facility without causing adverse effects on other anterior segment tissues, and thereby provide insights into developing a novel effective and safe treatment strategy for glaucoma. We therefore studied the combined effects of H-7 and CB on outflow facility in living monkeys.

2. Materials and Methods

Animals and Anesthesia

Normal cynomolgus monkeys (*Macaca fascicularis*), weighing 2·5–5·2 kg, were studied. All investigations were in accordance with University of Wisconsin and NIH guidelines, and with the ARVO Statement on the Use of Animals in Ophthalmic and Vision Research. Anesthesia for anterior chamber (AC) perfusion was induced by intramuscular (i.m.) ketamine (10 mg kg^{-1}), followed by i.v. pentobarbital-Na (15 mg kg^{-1}) or i.m. pentobarbital-Na (35 mg kg^{-1}).

Drugs and Chemicals

H-7 and CB were obtained from Sigma Chemical Co. (St. Louis, MO, U.S.A.), and DMSO (dimethyl sulfoxide) from Sigma or Research Industries Corporation (Salt Lake City, UT, U.S.A.). H-7 (0·1–5 mM for bolus AC injection or 10 and 300 μM for AC exchange infusion) solutions were freshly prepared in Bárány's mock aqueous humor (Bárány, 1964). For CB, a 0·5% stock solution in DMSO was stored at $-20°C$, and diluted with Bárány's solution immediately before administration to provide 0·02, 0·05, 0·1, 0·5 or 2·0 μg of CB in 10 μl of 20% DMSO for bolus AC injection. CB bolus doses $\leqslant 0{\cdot}5$ μg are subthreshold, and the 2·0 μg CB dose is strong but submaximal, when given alone (Kaufman and Erickson, 1982). The 10 μM and 300 μM H-7 AC exchange doses are subthreshold and maximal respectively (Tian et al., 1998 + unpublished data). H-7 bolus AC injection doses (10 μl of 0·1, 1 and 5 mM), which produce 10, 100 and 500 μM initial AC concentration in the ~ 100 μl cynomolgus AC (Erickson et al., 1984), are sub-threshold (10 and 100 μM) or just-threshold (500 μM), respectively, for increasing facility (Tian et al., 1998; Tian and Kaufman, unpublished data, 1997).

Outflow Facility Measurement

Total outflow facility was determined by 2-level constant pressure perfusion of the AC with Bárány's solution (Bárány, 1964), correcting for internal apparatus resistance (Bárány, 1965). The eyes were cannulated with a branched needle, with one branch connected to the pressure transducer and the other branch to a reservoir inflow line. For AC exchange the eyes were cannulated with an additional single needle connected to an infusion pump. During the exchange, the tubing between the reservoir and the branched needle was disconnected temporarily and used as an outflow line while maintaining the intraocular pressure at ~ 10 mmHg. Most monkeys had undergone more than one prior perfusion but none within the preceding 5–6 weeks; all were free of anterior chamber cells and flare by biomicroscopy. Baseline facility was determined bilaterally for 35 min in all perfusion experiments.

Unilateral H-7 bolus + bilateral CB bolus Following baseline facility measurement, 10 μl of 0·1, 1 or 5 mM H-7 was injected via a t-piece into the inflow tubing of one eye [10, 100 or 500 μM in the ~ 100 μl anterior chamber (Erickson et al., 1984)], vehicle (Bárány's solution) to the opposite eye. Five minutes were allowed for the drug to wash into the AC, followed by 3 min of convection mixing of the AC contents by cold air blown on the cornea. The reservoirs were then closed for 30 min, after which 2·0 μg CB in 10 μl was administered into the inflow tubing (reservoirs open) of both eyes, 8 min allowed for wash-in and convection mixing, and the reservoirs were closed again for 15 min. The reservoirs were then opened and post-drug facility determined for 45 min.

Bilateral H-7 exchange + unilateral CB bolus Following baseline facility measurement, the ACs were exchanged bilaterally (2 ml for 10 min) with 10 or 300 μM H-7 solution, and the reservoirs filled with the corresponding exchange solutions. The reservoirs were closed for 30 min. Ten microliters of CB (0·02, 0·05, 0·1 or 0·5 μg) or vehicle (20% DMSO, which

TABLE I

H-7 and CB combined effects on outflow facility

	Treated eye (H-7 + CB)			Control eye (H-7 or CB alone)			Treated eye/Control eye		
	BL	Rx	Rx/BL	BL	Rx	Rx/BL	BL	Rx	Rx/BL
A. ($n = 6$): unilateral H-7 (10 μM) Bo; bilateral CB (2 μg) Bo	0·39 ± 0·07	0·81 ± 0·14	2·20 ± 0·24‡	0·36 ± 0·09	0·83 ± 0·12	3·00 ± 0·69†	1·19 ± 0·10	0·98 ± 0·13	0·84 ± 0·11
B. ($n = 4$): unilateral H-7 (100 μM) Bo; bilateral CB (2 μg) Bo	0·23 ± 0·03	0·59 ± 0·10	2·62 ± 0·27‡	0·24 ± 0·05	0·52 ± 0·10	2·26 ± 0·23†	1·04 ± 0·17	1·17 ± 0·09	1·19 ± 0·13
C. ($n = 4$): unilateral H-7 (500 μM) Bo; bilateral CB (2 μg) Bo	0·29 ± 0·04	0·69 ± 0·14	2·41 ± 0·46*	0·28 ± 0·02	0·70 ± 0·12	2·55 ± 0·49*	1·02 ± 0·10	1·01 ± 0·20	0·99 ± 0·14
D. ($n = 6$): Bilateral H-7 (10 μM) Ex; unilateral CB (0·02 μg) Bo	0·39 ± 0·04	0·41 ± 0·04	1·11 ± 0·16	0·36 ± 0·04	0·38 ± 0·07	1·06 ± 0·19	1·12 ± 0·17	1·30 ± 0·30	1·18 ± 0·25
E. ($n = 6$): Bilateral H-7 (10 μM) Ex; unilateral CB (0·05 μg) Bo	0·46 ± 0·06	0·61 ± 0·09	1·37 ± 0·15	0·50 ± 0·08	0·61 ± 0·11	1·23 ± 0·07†	0·97 ± 0·17	1·04 ± 0·12	1·15 ± 0·16
F. ($n = 6$): Bilateral H-7 (10 μM) Ex; unilateral CB (0·1 μg) Bo	0·39 ± 0·05	0·65 ± 0·13	1·66 ± 0·20†	0·38 ± 0·09	0·38 ± 0·11	1·00 ± 0·18	1·24 ± 0·23	2·24 ± 0·57	1·82 ± 0·24†
G. ($n = 8$): Bilateral H-7 (10 μM) Ex; unilateral CB (0·5 μg) Bo	0·40 ± 0·05	0·56 ± 0·06	1·43 ± 0·09‡	0·44 ± 0·05	0·43 ± 0·04	1·02 ± 0·08	0·94 ± 0·11	1·33 ± 0·15	1·43 ± 0·09‡
H. ($n = 7$): Bilateral H-7 (300 μM) Ex; unilateral CB (0·1 μg) Bo	0·36 ± 0·07	0·83 ± 0·15	2·52 ± 0·30‡	0·40 ± 0·11	0·77 ± 0·17	2·18 ± 0·26‡	0·97 ± 0·08	1·10 ± 0·06	1·20 ± 0·13
I. ($n = 6$): Bilateral H-7 (300 μM) Ex; unilateral CB (0·5 μg) Bo	0·26 ± 0·03	0·82 ± 0·16	3·06 ± 0·43‡	0·28 ± 0·02	0·58 ± 0·08	2·09 ± 0·17‡	0·96 ± 0·11	1·35 ± 0·11†	1·47 ± 0·16†
J. ($n = 7$): unilateral H-7 (300 μM) Ex; bilateral CB (2 μg) Bo	0·22 ± 0·02	0·85 ± 0·07	3·85 ± 0·60‡	0·26 ± 0·05	0·56 ± 0·05	2·36 ± 0·35‡	0·91 ± 0·10	1·67 ± 0·27†	1·67 ± 0·14‡

Cytochalasin B (CB) always administered 30 min after H-7. Bo, Bolus; Ex, Exchange; BL, Baseline; Rx, Post-drug facility. Post-drug data encompass 45 min, beginning 15–20 min after CB administration. Facility data are mean ± S.E.M. (μl min^{-1} mmHg^{-1}) for n animals, each contributing one treated eye receiving H-7 + CB and one control eye receiving CB or H-7 alone; ratios are unitless. *$P < 0·054$, †$P < 0·05$, ‡$P < 0·01$ for ratios different from 1·0 by the 2-tailed paired t test.

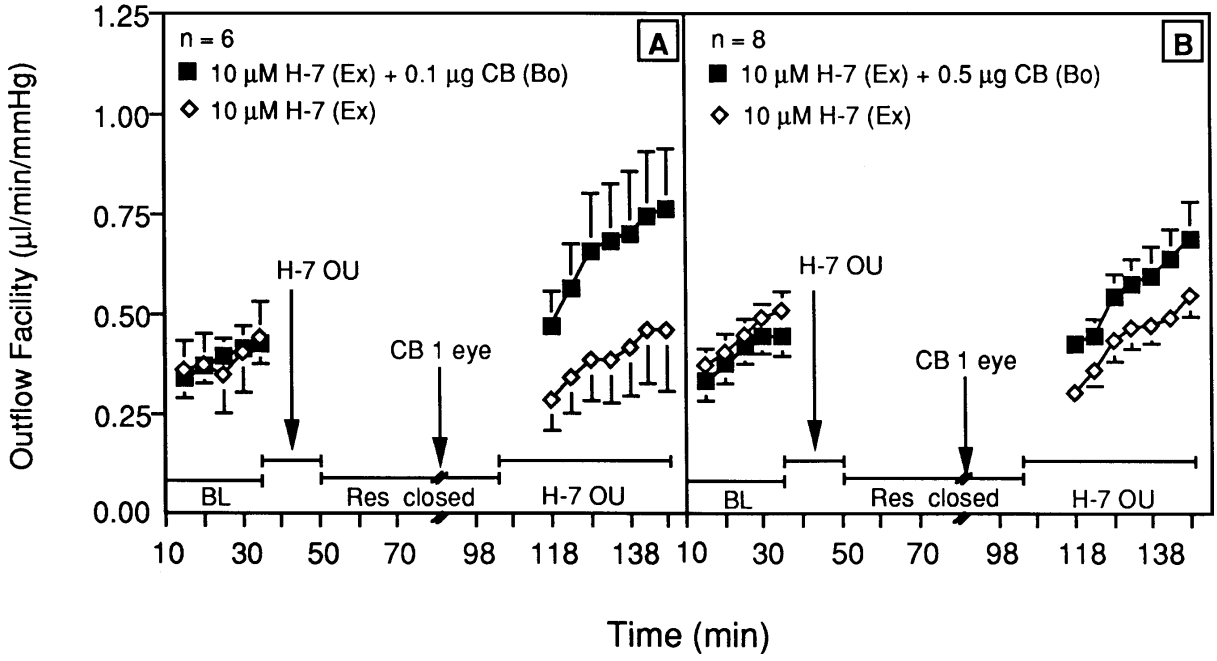

FIG. 1. Effect of bilateral intracameral exchange (Ex) of a subthreshold dose of H-7 (10 μM) followed by unilateral bolus (Bo) injection of subthreshold doses of CB (A: 0·1 μg; B: 0·5 μg) on outflow facility. Reservoir (Res) was filled with the corresponding H-7 solution after intracameral Ex. BL, baseline. Data are mean $\pm$ S.E.M. μl min^{-1} mmHg^{-1} for n monkeys, each contributing 1 eye receiving H-7 and 1 eye receiving H-7 and CB.

produces a 2% initial DMSO concentration in the 100 μl cynomolgus AC) was then injected into the inflow tubing of opposite eyes with the reservoirs open. Wash-in and convection mixing were as above. The reservoirs were then re-closed for 15 min after CB administration and reopened for 45 min of post-drug facility measurement.

Unilateral H-7 exchange + bilateral CB bolus Baseline facility measurement was followed by AC exchange with one eye receiving 300 μM H-7 and the other receiving vehicle (Bárány's solution). The reservoirs were then filled with the corresponding exchange solutions and closed for 30 min. The reservoirs were then opened and 2·0 μg CB was injected via t-pieces to both eyes, followed by wash in and convection mixing, as above. The reservoirs were re-closed for 15 min, then opened and facility was determined for 45 min.

Data Analysis

Data are presented as mean $\pm$ S.E.M. for n eyes or animals. Pre- or post-drug treated vs. contralateral control; post-drug or post-vehicle vs. ipsilateral baseline; and baseline corrected post-drug treated vs. control comparisons were made using the 2-tailed paired t test for ratios vs. 1·0. For example, a treated/control ratio of 1·25 $\pm$ 0·10 indicates that the treated value exceeds the control value by 25%, with an SEM of 10%. The ratio 1·25 $\pm$ 0·10 is tested against 1·00 $\pm$ 0·00 (a ratio of 1·0 representing no change) to determine if the apparent increase is statistically significant.

3. Results

Unilateral H-7 Bolus + Bilateral CB Bolus

In the present study, bolus intracameral injection of 2·0 μg CB more than doubled facility, far in excess of the 15% or 0·5% min^{-1} increase typically induced by perfusion with vehicle alone (perfusion induced resistance—washout; Kaufman and Bárány, 1977; Svedbergh et al., 1978; Erickson and Kaufman, 1981; Kaufman and Erickson, 1982; Kaufman, True-Gabelt and Erickson, 1988; [Table I(A)–(C)]. The facility increase was of similar magnitude in the contralateral eyes receiving bolus injection of 10–500 μM H-7 prior to 2·0 μg CB [Table I(A)–(C)].

Bilateral H-7 Exchange + Unilateral CB Bolus

Similar to previous data (Tian et al., 1998), AC exchange with 10 μM H-7 alone [Table I(F), (G)] had no effect on facility but subsequent bolus injection of 0·1 μg or 0·5 μg CB increased the facility by 82 $\pm$ 24% ($P < 0·02$) or 43 $\pm$ 9% ($P < 0·005$), relative to baseline and adjusted for washout occurring in the 10 μM H-7-only treated eyes [Fig. 1(A), (B), Table I(F), (G)]. 10 μM H-7 + 0·02 or 0·05 μg CB produced insignificant 18% and 15% facility increases respectively [$P > 0·05$, Table I(D), (E)]. AC exchange with 300 μM H-7 alone doubled facility relative to ipsilateral baseline [118% or 109%, Table I(H), (I)]. However, 300 μM H-7 AC exchange + 0·1 or 0·5 μg CB bolus increased facility by 152% or 206% compared to ipsilateral baseline. When compared to 300 μM H-7-only treated eyes, the CB induced facility increase

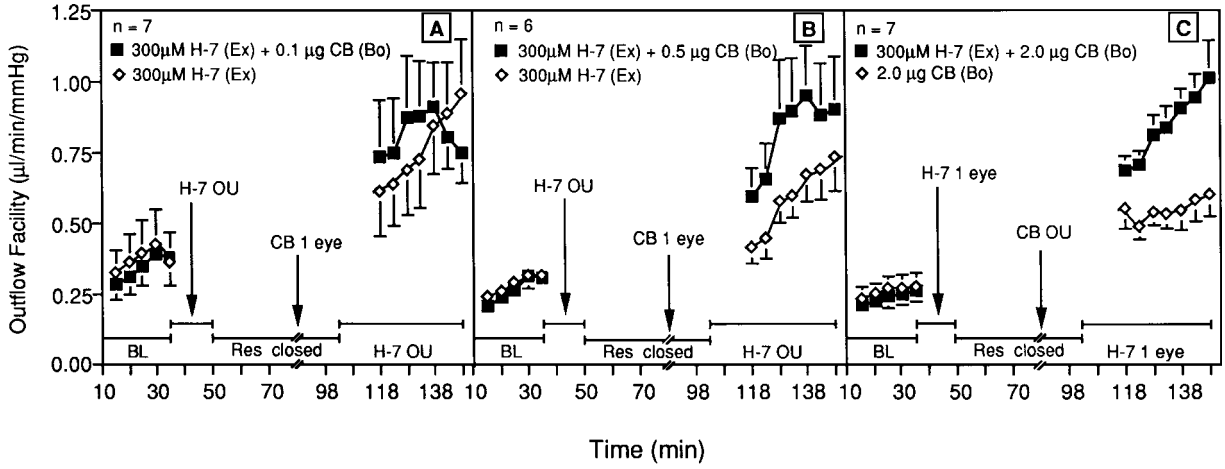

FIG. 2. Effect of bilateral intracameral exchange (Ex) with a maximal dose of H-7 (300 μM) followed by unilateral bolus (Bo) injection of subthreshold doses of CB [(A) 0·1 μg; (B) 0·5 μg], or unilateral Ex with a maximal dose of H-7 followed by bilateral Bo injection of a near-maximal dose of CB (C: 2·0 μg) on outflow facility. Reservoir (Res) was filled with corresponding H-7 or vehicle solution after intracameral Ex. BL, baseline. Data are mean $\pm$ S.E.M. μl min^{-1} mmHg^{-1} for n monkeys, each contributing 1 eye receiving one drug and 1 eye receiving both drugs.

proportional to baseline was another $20 \pm 13\%$ (NS) or $47 \pm 16\%$ ($P < 0{\cdot}05$) higher, respectively [Fig. 2(A), (B); Table I(H), (I)].

Unilateral H-7 Exchange + Bilateral CB Bolus

Bolus intracameral injection of 2·0 μg CB alone or AC exchange with 300 μM H-7 alone each increased facility $\sim$ 2-fold compared to ipsilateral baseline [136% for 2·0 μg CB, Table I(J); 118% or 109% for 300 μM H-7, Table I(H), (I)]. However, AC exchange with 300 μM H-7 + bolus intracameral injection of 2·0 μg CB increased facility up to $\sim$ 3-fold compared to ipsilateral baseline (285%, Table I(J)]. When compared to the 2·0 μg CB-only treated eyes, facility in the H-7 + CB-treated eyes was increased an additional $67 \pm 14\%$ ($P < 0{\cdot}005$), proportional to baseline [Fig. 2(C); Table I(J)]. When compared to the 300 μM H-7-only treated eyes [Table I(H) or (I)], facility in the H-7 + CB-treated eyes [Table I(J)] was increased an additional 77% ($P < 0{\cdot}05$) or 84% ($P < 0{\cdot}025$; 2-sample unpaired t test).

4. Discussion

Longer-term exposure to DMSO concentrations in the range we used might damage cells in culture (Layman, 1987; Crawford and Braunwald, 1991; Rinkes et al., 1992), and DMSO has been reported to induce formation of actin filament networks in cultured rat hepatocytes (Kojima et al., 1997) and the formation of microtubules in cultured arterial smooth muscle cells (Katsuda, Okada and Nakanishi, 1987). However, the situation may be quite different in the living eye under our relatively brief perfusion conditions. The actual concentration 'seen' by the TM cells in vivo may be less than that administered into the central AC, the cells may have 'coatings' or other 'defense' mechanisms not available in a cell culture environment, the duration of exposure may be too short to induce major functional or structural changes, etc. In the living perfused monkey eye, $[DMSO]_{AC} \geqslant 4\%$ decreased outflow facility, while lower concentrations had no effect on the facility (Kiland et al., 1997). In the present study, the initial $[DMSO]_{AC}$ following CB bolus injection was only 2%, and declined thereafter. Moreover, we used 2% DMSO in both eyes, so our comparisons were always to a contralateral control receiving the same vehicle.

In the present study, the initial intracameral concentrations of 10–500 μM H-7 presumably achieved after bolus injection did not alter the outflow facility response to 2·0 μg CB. Bolus injection to achieve initial intracameral concentrations of 10 or 100 μM H-7 alone is ineffective, but 500 μM H-7 alone is a threshold dose for increasing facility (Tian and Kaufman, unpublished data, 1997), as is AC exchange with 100 μM H-7 (Tian et al., 1998). The dose ratio of $\sim$ 5:1 between bolus and exchange infusion holds also for pilocarpine hydrochloride (Gabelt, Crawford and Kaufman, 1991; Gabelt and Kaufman, 1992), and may be consequent to incomplete mixing and rapidly decreasing AC drug concentration following bolus injection. Therefore, the lack of synergy in the H-7 + CB bolus combinations may be due to: (1) insufficient H-7 concentration and/or time in the AC; (2) a facility response near to the system's physiological maximum produced by 2·0 μg CB itself (see below); and (3) the difference between responses to 2·0 μg CB and 2·0 μg CB + 10, 100 or 500 μM H-7 being too small for resolution by the perfusion technique.

To clarify these uncertainties we then used protocols in which H-7 was administered by AC exchange while CB doses were given by bolus AC injection. We found that: (a) combining subthreshold doses of CB and H-7 produced significant facility increases beyond perfusion-induced resistance washout; (b) combining

subthreshold doses of CB and a maximal dose of H-7 produced facility increases significantly higher than those produced by H-7 alone; and (c) combining a near-maximal dose CB and a maximal dose of H-7 produced a facility increase of greater magnitude than either alone. Findings (a) and (b) could indicate that the pathways by which H-7 and CB increase outflow facility converge at some point presumably involving deterioration of actin microfilaments in TM/Schlemm's canal cells (Robinson and Kaufman, 1991; Robinson and Kaufman, 1994), with consequent alterations of cell–cell and/or cell-extracellular matrix adhesions. Finding (c) also could be consistent with this interpretation, but alternatively could suggest that H-7 and CB may increase facility at least in part by different mechanisms.

In a previous study, combined treatment with maximal facility-effective doses of CB and epinephrine did not increase facility by more than the $\sim 150\%$ induced by CB alone (Robinson and Kaufman, 1991). Therefore, it was hypothesized that once cell shape had changed to the extent produced by the large CB dose, further shape changes are not possible or have no additional consequences for facility. However, in the present study, the facility-increasing effect of a maximal dose of H-7 was potentiated dose-dependently by subthreshold and near-maximal CB doses. Furthermore, when compared to ipsilateral predrug facility, facility in the eyes receiving 300 μM H-7 plus either subthreshold (0·1 or 0·5 μg) or near-maximal (2·0 μg) doses of CB was increased by 152%, 206% or 285% [$\sim 130\%$, $\sim 180\%$ or 260%, adjusted for the 0·5%/min 'washout' seen in other comparably-timed perfusions without drugs (Erickson and Kaufman, 1981; Kaufman and Erickson, 1982)], equivalent or considerably more than the facility increase ($\sim 150\%$) induced by a maximally effective dose of either CB [5·0 μg (Kaufman and Bárány, 1977; Kaufman and Erickson, 1982)] or H-7 [300 μM (Tian et al., 1998)] alone. These findings could be due to CB-induced cellular contractions perturbing cell–cell adhesion (Citi et al., 1994), complimenting H-7's predominant effects on cell-extracellular matrix adhesion (Tian et al., 1998).

Preliminary ultrastructural studies indicate that administration of H-7 into monkey eyes has rather mild effects on cell–cell adhesion, but induces a generalized relaxation and apparent expansion of the TM and Schlemm's canal (Gabelt et al., 1999). Following AC exchange infusion of H-7 in living monkey eyes, the initial facility value was only slightly elevated, but continued drug-free perfusion produced substantial facility elevation even as the drug concentration declined (Tian et al., 1998). This suggests that TM architecture may be only slightly affected by H-7, but that at higher-pressure gradients/flow rates (e.g. in perfusion), the slightly destabilized TM architecture is further disrupted sufficiently to substantially reduce flow resistance. Following perfusion with CB, the TM in monkey eyes was greatly distended and the cells in the juxtacanalicular region and the inner wall of Schlemm's canal were pulled apart (Svedbergh et al., 1978; Johnstone et al., 1980). Therefore, both compounds increase facility during perfusion most likely by weakening cell adhesions, leading to loosening and distension of overall TM architecture. CB could contribute to the labilization of actin-associated structures, which are relatively insensitive to H-7 (i.e., cell–cell junctions), while the anti-contractile effects of H-7 might moderate the disruption of tissue architecture induced by CB. Combined administration of carefully chosen small doses might reduce the actions of each agent on ocular tissues not subjected to trans-tissue flow, while preserving flow-dependent facility effectiveness, which seems to require flow across the TM.

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