The Cytoskeleton of the Cynomolgus Monkey Trabecular Cell

II. Influence of Cytoskeleton-Active Drugs

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The effects of cytochalasin B (10⁻⁶ M and 10⁻⁵ M), taxol (10⁻⁵ M), nocodazole (10⁻⁵ M), and colchicine (10⁻⁵ M) on the cytoskeleton of cynomolgus monkey trabecular cells were examined with Nomarski observations, fluorescent labeling as well as extraction, S-1 labeling, and critical-point drying. Changes in actin, microtubules, and intermediate filaments, the three major cytoskeletal systems, were correlated with changes in the overall shape and organization of the monkey trabecular cell. Incubation with cytochalasin B caused a marked alteration on actin filament structure, as well as cell shape and cytoskeletal organization. Effects on microtubule structure were noted with taxol, nocodazole, or colchicine; however, no marked changes in overall cell shape or other cytoskeletal structures were observed. These studies demonstrate the importance of actin filaments in regulating the shape and cytoskeletal organization of cynomolgus monkey trabecular cells. Invest Ophthalmol Vis Sci 27:1312-1317, 1986

Substantial evidence has accumulated during the past several years that the cytoskeleton is an essential component of many cell processes.¹⁻³ In a prior study,⁴ transmission electron microscopy was employed to obtain stereo pairs of whole cynomolgus monkey trabecular cells that had been extracted, S-1 labeled, and critical-point dried in order to simultaneously identify actin, microtubules, and intermediate filaments, the three major cytoskeletal systems, and visualize their three-dimensional nature. A double fluorescence technique for actin and microtubules was also used to provide a broad view of cytoskeletal relationships within the cell. In the current investigation, we evaluated the effects of cytoskeleton-active drugs on these systems. Furthermore, we correlated changes in these cytoskeletal systems with changes in the overall shape and organization of the monkey trabecular cell.

Materials and Methods

Monkey trabecular cells were grown to confluence (7-10 days of culture) on 1 cm² glass coverslips (with or without attached grids), using previously described methods.⁵ The cells were then incubated for 1 hr at 37°C with either: (1) 0.1 M phosphate buffered saline (PBS) (control); (2) 0.1 M PBS with 0.5% DMSO (control); (3) 10⁻⁵ M cytochalasin B (Sigma; St. Louis) in 0.1 M PBS with 0.5% DMSO; (4) 10⁻⁶ M cytochalasin B in 0.1 M PBS with 0.5% DMSO; (5) 10⁻⁵ M taxol in 0.1 M PBS with 0.5% DMSO; (6) 10⁻⁵ M nocodazole (Sigma; St. Louis) in 0.1 M PBS with 0.5% DMSO; or (7) 10⁻⁵ M colchicine (Sigma; St. Louis) in 0.1 M PBS.

The cells, attached to the coverslips, were then processed for either (1) Nomarski observations, (2) fluorescent labeling, or (3) extraction, S-1 labeling, and critical-point drying using the methods described previously.⁴,⁶

Results

No difference in cell shape (Fig. 1A) or cytoskeletal organization (Figs. 2A, B) was noted between cells incubated in PBS alone or with PBS with 0.5% DMSO (controls).

Light Microscopic Observations

Incubation with the antiactin drug, cytochalasin B, caused marked alterations in cell shape and cytoskeletal organization. At 10⁻⁵ M cytochalasin B the cell body rounded up (Fig. 1B) leaving fine branching projections (or “microarborizations”). These morphologic changes were not noted at 10⁻⁶ M cytochalasin B (Fig. 1C). At both concentrations, a slight increase in blebbing at the free surface was noted. At 10⁻⁵ M cytochalasin B,
Fig. 1. Nomarski micrographs of cynomolgus monkey trabecular cells after 1 hr of incubation with selected cytoskeletal drugs. A, After incubation with phosphate buffered saline (control), the cells are flat and polygonal shaped. B, After treatment with $10^{-5}$ M cytochalasin B, the cell body has rounded up, leaving fine branching peripheral cell processes ("microarborizations"). Some blebbing (arrows) is noted on the cell surface. C, Although the cell shape is similar to the control after treatment with $10^{-6}$ M cytochalasin B, there is some cell surface blebbing (arrow). D, Although cell shape has not significantly changed after treatment with $10^{-5}$ M taxol, blebbing is present (arrows). E, After treatment with $10^{-5}$ M nocodazole, cell shape has not markedly changed and there is pronounced blebbing (arrows). F, After treatment with $10^{-5}$ M colchicine, there is marked blebbing without change in cell shape (as with nocodazole) (all micrographs X2,600).

double labeled fluorescence views of the organization of actin and microtubules revealed marked changes in the cytoskeleton. There was a near total alteration of the actin filament structure into globular condensations with a few residual stress fibers (Fig. 3A). At $10^{-6}$ M cytochalasin B, these globular condensations were also noted within intact stress fibers (Fig. 4A). A peripheral reduction and condensation of microtubules was also noted at $10^{-6}$ M (Fig. 4B).

No marked changes in cell shape were noted with taxol, nocodazole, or colchicine, drugs which are known to effect microtubules (Figs. 1D–F). However, a marked increase in free surface blebbing was noted, especially with nocodazole and colchicine (Figs. 1E, F). The overall actin stress fiber organization remained intact with these three drugs (Figs. 5A, 6A, 7A). However, each drug had a marked effect on microtubule organization. With $10^{-5}$ M taxol there was a reduction in microtubule staining at the periphery, with a concentration at the cell nucleus (Fig. 5B). With $10^{-5}$ M colchicine or nocodazole, there was a marked reduction in all microtubule staining (Figs. 6B, 7B). Rather, microtubule staining was seen in globular and granular condensations as well as within short lengths of microtubules radiating outward from residual centrioles.

Electron Microscopic Observations

Low power transmission electron microscopic (TEM) observations of extracted, critical-point dried
cells at $10^{-3}$ M also revealed the near complete alteration of stress fiber organization (Fig. 8). In addition, the distribution of granules and vesicles was homogeneous throughout the cytoplasm, as opposed to concentrated around the nucleus as seen in controls. TEM observations of cells treated with $10^{-3}$ M taxol revealed no marked changes in the structure of microtubules, intermediate filaments, and actin filaments. With $10^{-5}$ M nocodazole or colchicine (Fig. 9), the overall stress fiber structure remained intact.

High power TEM observations of cells incubated with cytochalasin B demonstrated that actin condensed primarily into globular condensations (Fig. 10). However, both microtubules and intermediate filaments remained intact. With $10^{-5}$ M colchicine or nocodazole (Fig. 11), there was an absence of microtubules among the intact stress fibers, actin filaments, and intermediate filaments.

**Discussion**

Cytochalasins B and D, actin disrupting drugs, have been reported previously by Kaufman to increase outflow facility after intracameral infusion in cynomolgus monkeys. Morphologic evaluation of the trabecular meshwork of cynomolgus and rhesus monkeys under...
these conditions revealed separation of trabecular cells, washout of extracellular material, and disruptions of the inner wall of Schlemm's canal. These effects were fully reversible and there were no detectable alterations in the trabecular meshwork 6 days after treatment. Based on this physiologic and morphologic data, Kaufman has likened the effects of cytochalasin on the trabecular meshwork to a “pharmacologic trabeculocanalotomy” and suggested that they may be useful for normalizing outflow facility and intraocular pressure in glaucoma.

Analogous morphologic effects occurred in vitro. Using scanning electron microscopy, we have previously reported that $10^{-5}$ M cytochalasin B or D distorted the morphology of cultured human trabecular cells within 10 min and that a reversal of this effect was observed beginning 1 hr after the drug was removed. Similar morphologic and cytoskeletal effects were observed in the current study when cultured cynomolgus monkey trabecular cells were exposed to $10^{-5}$ M cytochalasin B. Through the use of double fluorescence and critical-point drying techniques, we were also
able to observe the effects of cytochalasin B on the intracellular organization of microtubules and intermediate filaments as well as intracellular granules and vesicles. At a lower dose of cytochalasin B ($10^{-6}$ M) there was no marked change in cell shape. However, a partial dissolution of the stress fiber organization into globular condensations was observed. These observations suggest that changes in cytoskeleton may precede actual changes in overall cell shape. Observations of critical point-dried cells treated with $10^{-3}$ M or $10^{-6}$ M cytochalasin B indicated intact microtubules and intermediate filaments. However, fluorescence studies demonstrated a condensation of the microtubule staining pattern at the periphery, while TEM studies revealed a redistribution of granules from the perinuclear region in controls to a diffuse distribution throughout the cytoplasm. Thus, we have demonstrated the critical role of actin in regulating trabecular cell shape, as well as the organization of other cytoskeletal elements and intracellular organelles.

We also investigated the effects on cell shape and cytoskeletal organization of colchicine and nocodazole, microtubule depolymerizing drugs, and taxol, a microtubule stabilizing drug. Colchicine has been reported to reduce intraocular pressure, although the basis for this effect is not known. The cytoskeletal effects of these three drugs were similar to their effects in vitro in other cells. Taxol induced a perinuclear condensation of mi-
crotubules, while colchicine and nocodozole obliterated the peripheral microtubule structure, leaving a residual centriole region. Besides changing the overall cell shape minimally, these drugs had little effect on the organization of actin filaments in stress fibers, intermediate filament structure, and intracellular granule and vesicle organization. A marked "blebbing" of the upper surface of cells exposed to colchicine and nocodozole was observed, however. Such blebbing was also observed to a lesser extent with taxol and cytochalasin B. This may be the result of subtle changes in the underlying cytoskeletal organization, or may represent a release of granules and vesicles due to the elimination of microtubule or actin structure. This latter hypothesis is supported by pharmacological studies which demonstrate the intracellular redistribution or release of pigment granules, lysosomal granules, and fibroblast secretory vesicles after treatment with antimicrotubule or antiactin drugs. Thus, although microtubules may not affect the shape of the trabecular cell, they may directly influence its phagocytic and secretory functions. Such a hypothesis may be confirmed by correlating the comprehensive morphologic techniques of this study with functional and biochemical studies.

Key words: trabecular cells, cytochalasin B, taxol, nocodozole, colchicine

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