Radiol Oncol 1999; 33(4): 297-301.

Reorganization of microtubules in V-79 cells after treatment with cytohalasin B

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Background. *The aim of this work was to study the configuration of the microtubules in the cytochalasin B treated V-79 cells in connection to the cell shape and to see whether there are any similarities to the phenomena taking place in phospholipid vesicles.*

Subjects and methods. *An experiment was performed where cytochalasin B was added to the V-79 cells (lung fibroblasts of Chinese hamster).*

Results. *The cell shape changed from an elongated one into the shape with a profile resembling the Greek letter ϕ. The microtubules were found to be organized into a rod within the symmetry axis of the cell.*

Conclusion. *As similar shapes were previously observed also in the phospholipid vesicles with entrapped microtubule rods, our results support the hypothesis that similar physical mechanisms may pertain in simple systems as well as in living cells.*

Key words: cell culture - drug effects; cytochalasin B; microtubules -drug effects

Introduction

The cytoplasm of eucaryotic cells is spatially organized by a network of protein filaments - the cytoskeleton which contains three main types of filaments: microtubules, actin filaments, and intermediate filaments. Actin filaments are dynamic structures. They are organized into an actin cortex, a layer just beneath the plasma membrane, and thin stress fibers within the cells.[1] Actin-rich cortex as well as the cytoskeleton within the cytoplasm determine the mechanical properties of the cell and therefore control the shape of most animal cells. [2]

It was previously observed that the disintegration of the cytoskeleton affects the morphology of the fibroblasts grown in culture.[3] Cytochalasin B prevents actin polymerization to actin filaments. By disrupting the equilibrium of depolymerization-polymerization, the addition of cytochalasin B causes disaggregation of the actin filaments while it has no direct effect on microtubules.[4] However, upon disaggregation of the actin filaments, the microtubules may get reorganized as their interactions with the surrounding structures are changed. The reorganization of the cytoskeleton may affect the cell shape. In

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cytochalasin B treated cells, the effect of the actin filaments on the cell shape is diminished while the effect of the microtubule configuration on the cell shape becomes pronounced.

The distortion of the shape by entrapped microtubules was studied on the phospholipid vesicles.[5,6] The tubulin, encapsulated by the vesicles, polymerized and assembled into a rod within the vesicle. The rod grew and increased in length, causing initially a sphere-to-ellipsoid change in shape while, upon further growth of the rod, the vesicle developed regions of negative curvature and eventually transformed into a shape that had a profile resembling the Greek letter ϕ. The effect of the microtubule rod on the vesicle shape was also theoretically described by taking into account the elastic properties of the vesicle membrane.[5-8]

We observed that after addition of the cytochalasin B the shape of the body of the V-79 cells transformed from elongated to more globular while the cell took the ϕ shape. On the basis of the similarity with the observed morphology of the phospholipid vesicles with entrapped microtubule rods we assumed that the shape transformation of the V-79 cells is due to physical mechanisms similar to the ones taking place in phospholipid vesicles. Therefore, we wanted to determine whether the microtubules would get organized into a rod-like structure within the cell.

The aim of this work was to study the configuration of the microtubules in the cytochalasin B treated V-79 cells in connection to the cell shape and to see whether there are any similarities to the phenomena taking place in phospholipid vesicles.

Materials and methods

Cells

The V-79-379 A (diploid lung fibroblasts of Chinese hamster) were grown in Eagle MEM (minimal essential medium - GIBCO) supplemented with 10% fetal calf serum (FCS - FLOW), penicillin (100U/ml) and streptomycin (100 μg/ml) at 37°C in a CO_2 incubator.

Cytohalasin B treatment

The cells (2.10^5) were seeded in 50mm plastic Petri dishes. After 24 hours, the cells were treated with cytochalasin B (SIGMA) (final concentration of 2μg/ml) for one hour. At first, the cells were observed with a phase contrast microscope and then prepared for tubulin staining. The cells were simultaneously fixed and permeabilized with a mixture of 4% formaldehyde, microtubule stabilizing buffer[9] and 0.5% Triton at 37°C for 30 min. After washing in PBS and blocking an unspecific labelling with 1% BSA, the cells were immunolabelled with monoclonal anti β-tubulin (SIGMA) over night. The FITC-labelled secondary antibodies (SIGMA) were applied for 2 hours at 37°C. After washing the cells were mounted in vectashield with DAPI (VECTOR) and examined in fluorescent microscope (LEITZ Laborlux S).

Results and discussion

The morphological appearance of control V79 fibroblasts in cell culture was flat and mainly spread over the substrate while the microtubules were radially oriented within the cell body (Figure 1). After cytochalasin B treatment, the cell body became globular and the area of contact with the substrate diminished while the cell exhibited long cylindrical protrusions (Figure 2). With time, the cells more and more resembled the ϕ shape. The fluorescence microscope image showed that the microtubules were organized into rod-like structures emanating from the nuclear area (Figure 2).

While observing the ϕ shape of the cell in the phase contrast microscope, our assump-

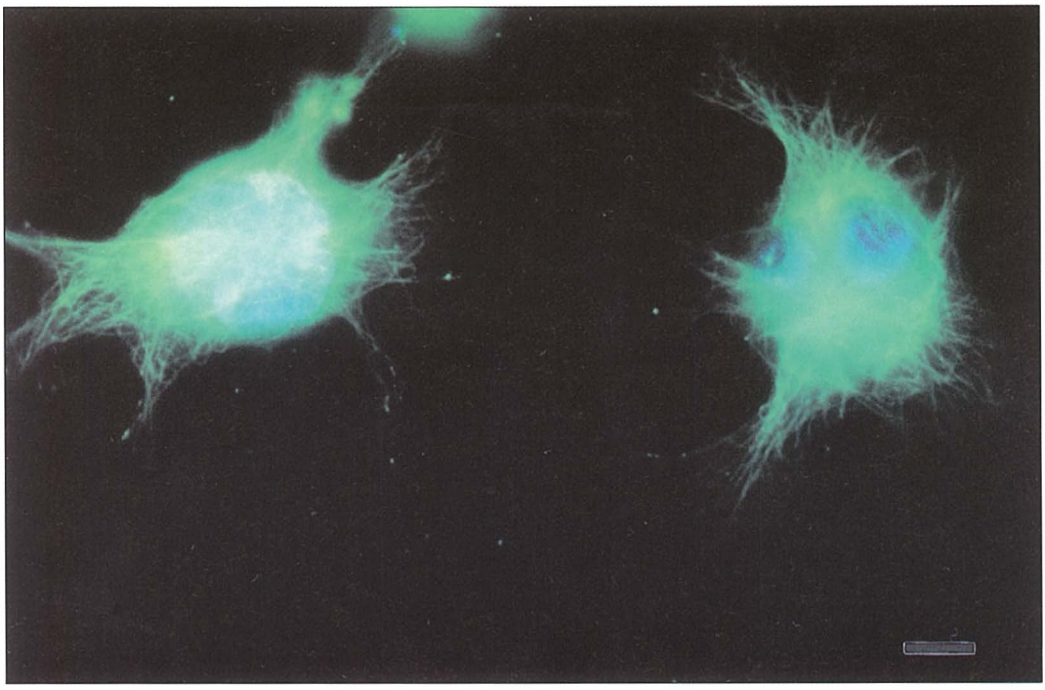

Figure 1. Control V-79 cells observed in fluorescence microscope showing the microtubules labelled with FITC (green) and nucleus with DAPI (blue). Bar - 10μm.

tion that there is a rod-like structure acting upon the membrane was therefore confirmed. A similarity can be drawn from the ϕ shape of V-79 cell with long tubular protrusions containing microtubule rod, and the shape of the phospholipid vesicle with a long entrapped microtubule rod. [6] There is however a major difference in interpreting the origin of the stability of the shape in phospholipid vesicles and in V-79 cells. In the determination of the shape of the phospholipid vesicles, the membrane bending energy is minimized at relevant geometrical constraints.[10] On the other hand, the fibroblasts are modelled as fluid drops bounded by actin cortex under persistent tension and possessing area elasticity.[11] The plasma membrane usually exhibits wrinkling, in contrast to the surface of the phospholipid vesicles where it is smooth.

There could be many possible reasons responsible for the microtubule reorganization after disaggregation of the actin filaments. In the experiments with phospholipid vesicles, the microtubules spontaneously associated into rodlike structure indicating that such configuration is energetically favourable. These processes could also be present in the cytochalasin B treated cells. However, in intact cells, the microtubules in the cell body have radial orientation that is maintained by the integrity of the whole cytoskeleton. After the disaggregation of the actin filaments, the radial orientation of the tubules may become unfavourable as the surface structure would impose a force on the microtubules leading to the bending of the microtubules. The microtubules may redistribute as to avoid energetically unfavourable bending.

In order to explain the observed shape of V-79 cells treated by cytochalasin B in more detail, additional experimental evidence

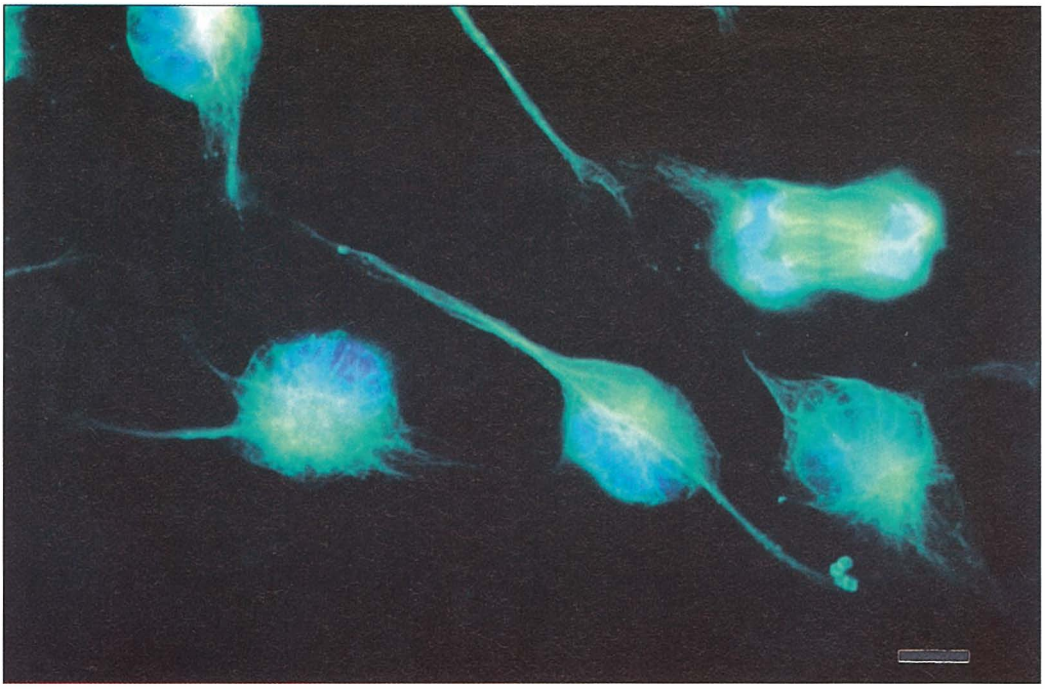

Figure 2. V-79 cells after treatment with cytochalasin B for 60 min in fluorescence microscope. Bar - 10 μm.

should first be collected. It should be established to what extent the membrane cortex has been preserved. Also, it would be of interest to see whether the wrinkling of the plasma membrane is increased with respect to untreated cells.

A related effect has been observed also in the erythrocytes of the patients with the sickle-cell disorder. These cells in the deoxygenated blood develop long protrusions of the membrane which apparently are caused by polymerized hemoglobin S in the cell interior.[12]

Conclusion

Based on the similarity of the shape of the cytochalasin B treated cells and phospholipid vesicles with entrapped rod-like structure, we suggest that the elastic properties of the surface structure determine the shape of the cytochalasin B treated cells as well as of the phospholipid vesicles subject to tension. Our results also support the hypothesis[13] that the shape of the intact cells is mainly determined by the configuration of the actin filaments.

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