세포골격의 해체에 의한 혈관 내피세포의 형태학적,생리학적 변화에 관한 연구

Morphological and Physiological Changes of Endothelial Cell induced by Disruption of Cytoskeleton

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Introduction

The cytoskeleton(CSK) of eukaryotic cells is composed of three types of filamentous protein elements such as microfilament(MF), microtubule (MT), and intermediate filament(IF). The MF of endothelial cells is mainly composed of actin, tropomyosin, and myosin and are thought to mediate various responses of the endothelial cells (EC) such as contraction, motility, migration, attachment, and shape against external stimuli. The function of the endothelial IF is considered as the positioning of organelles to specific domains within the cytoplasm. The endothelial MTs are dispersed throughout the cytoplasm and composed of tubulin. They appear to radiate from a single focal point located in the cell center[1].

The anti-cytoskeletal drugs such as cytochalasin D, nocodazol, and acrylamide disrupt these filamentous elements and then induce the morphological changes of the ECs [2].
In vitro cultured human endothelial cells secrete a basal amount of PGI_2, which depends on cell density and incubation time[3]. Resting cells do not synthesize or store PGI_2 *in vivo*.

In this study, we tried to find the relationship between the cytoskeletal changes by those drugs and the physiological response of the ECs in order to investigate the effect of the morphological change on the endothelial cellular physiology.

Materials and Methods

Cell Culture

ECs were isolated from human umbilical veins and grown in Medium 199 containing 20% fetal bovine serum, 100 IU/ml penicillin, 2 mM L-glutamin, 25 mM Hepes buffer. ECs were seeded on fibronectin(FN)-coated glass at the concentration 10,000 cells per square centimeter and grown for 2 days before the experiment. FN coating is achieved by incubation for 2 hrs at room temperature with phosphate buffered saline(PBS).

Anti-cytoskeletal drug treatment

Cytochalasin D is known to inhibit actin polymerization. Nocodazol depolymerize the MT. Acrylamide leads to the IF disruption. Three drugs were added to the EC culture well alone or combinatory for changing the morphology of the ECs. The methods of the treatment and incubation time were summarized in Table 1 and the concentrations of cytochalasin D, nocodazol, and acrylamide were 0.1 μg/ml, 10 μg/ml, and 4 mM, respectively. After drug treatment, the ECs were incubated at 37 °C.

Immunofluorescence Staining

FITC conjugated phalloidin was used for immunofluorescence staining. All drug was removed and the cells rinsed twice with PBS. For immunofluorescence, the cells were fixed in formaldehyde and were washed twice with PBS. The sample soaked in 0.1 % Hepes-Triton X-100 buffer for 3 - 5 minutes and were washed twice with PBS and were incubated with 0.01 mg/ml FITC-conjugated phalloidin in PBS containing 1 % dimethyl sulfoxide for 30 min at room temperature

Table 1. Incubation time of human umbilical vein endothelial cells with anti-cytoskeletal drug

Treatment	Control	Cytochalasin D (Cyto D)	Nocodazol (Noco)	Acrylamide (acryl)	Cyto D+ Noco*	Cyto D+ Acryl**	Noco+ Acryl***
Incubation time	-	1 hr	2 hrs	4 hrs	3 hrs (1/2 hrs)	5 hrs (1/4 hrs)	6 hrs (2/4 hrs)

* : Cyto D+ Noco : treated cytochalasin D for 1 hr on endothelical cells and washed out the drug and incubated for 2 hrs upon treatment of nocodazole .
** : Cyto D+ Acryl : cytochalasin for 1 hr and acrylamide for 4 hrs.
*** : Noco+Acryl : cytochalasin D for 2 hr and acrylamide for 4 hrs.

in humid atmosphere. The morphology of cytoskeleton was observed with fluorescence microscopy.

Destabilization of CSK with Anti-Cytoskeleton Drugs

Before 4 days the ECs were seeded in polystyrene 24 well, which were coated with 5 $\mu g/cm^2$ of FN. The cell density were $1x10^4$ cells/cm^2. The cytochalasin D, nocodazol, and acrylamide were added into 4 wells. The combination of cytochalsin D with nocodazol, cytochalsin D with acrylamide were poured into 4 wells. After removal of drugs, the samples were washed twice with 10mM Hepes buffer containing 0.14 M NaCl, 0.004 M KCl, 0.01 M glucose and then incubated in 1 ml of Hepes buffer supplemented with 0.3 U thrombin to simulate prostacyclin formation. After 30 min incubation at 37 °C, supernatant was removed, centrifuged at 1000 g for 2 min and stored frozen at - 80 °C[3]. Cell monolayers were treated with trypsin for counting with a hemocytometer. PGI_2 formation is determined by measuring excretion of 6-Keto-prostaglandin F1α, the stable hydrolysis product of PGI_2.

Results and Discussion

Figure 1a shows cytoskeletal changes of ECs treated with cytochalasin D. Figure 1b shows changes of cytoskeletal morphology induced by nocodazol which depolymerize the MT. Acrylamide was added for intermediate filament disruption(Figure 1c).

The morphology of ECs was converted to a spider's thread form when cytochalasin D was added. Nocodazol -treated with ECs show the localization of actin microfilament at the cell periphery and acrylamide-treated ECs have multilateral forms in their actin cytoskeleton. Figure 2 summarizes the changes of PGI_2 production by drug treatment on 5 $\mu g/cm^2$ concentration. FN coated glass PGI_2 production was significantly decreased in the acrylamide treated cells compared to the control cells.

The production of PGI_2, measured by radioimmunoassay of the stable metabolite 6-Keto-PGF1α, decreased after treating anti-cytoskeletal drugs. Prostacyclin production of cytoskeleton destabilized endothelial cell was shown in Fig 2. Compared to the control cells, cytochalasin D-treated cells, nocodazol-treated cells, and acrylamide-treated cells show 56.6, 70, and 92.3 % decreased productions of PGI_2, respectively. The detail mechanism of decreased PGI_2 production induced by morphological changes of ECs is not clear until now.

The relationship between degree of morphological changes and PGI_2 production must be investigated in the future study.

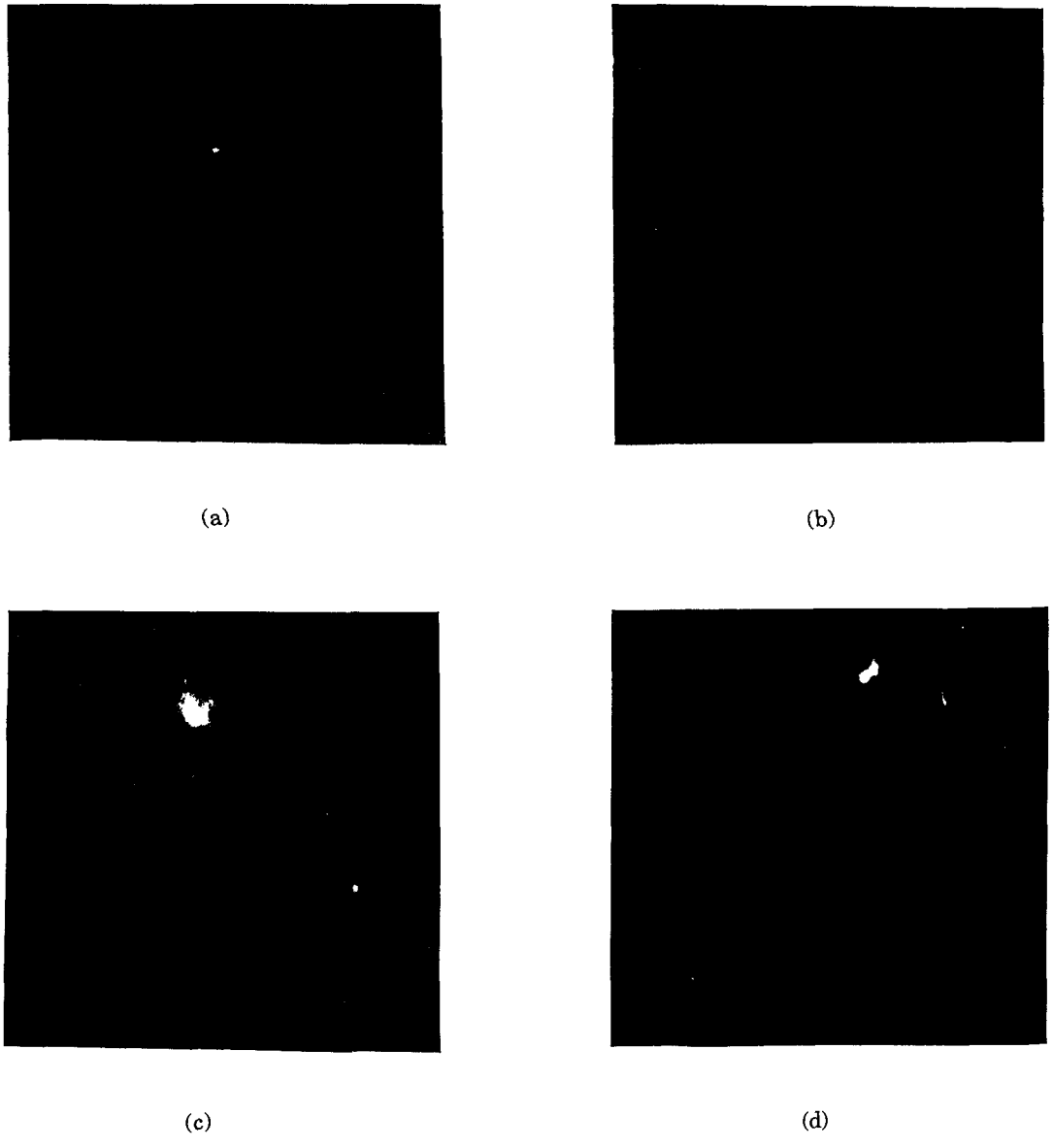

Figure 1. ECs were incubated for 1 hr in 0.1 μg/ml cytochalsin D (a), for 2 hrs in 10 μg/ml nocodazol (b), for 4 hrs in 4 mM acrylamide (c), control(d).

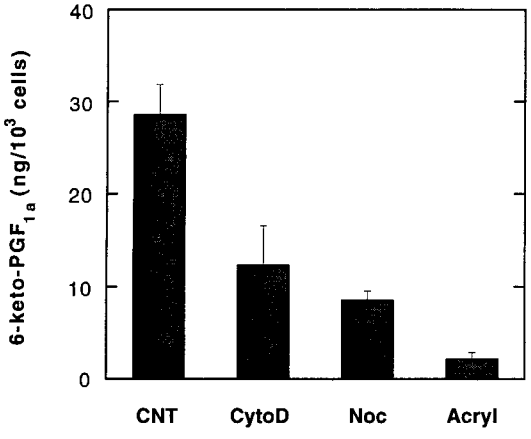

Figure 2. PGI_2 production of cytoskeleton disrupted EC

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