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Photodynamically induced endothelial cell injury and neutrophil-like HL-60 adhesion

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Photodynamic therapy (PDT) is a treatment modality based on photochemical reaction and the resultant cytotoxic reactive oxygen species. The platelet thrombus formation leading to stasis observed in vivo during PDT is called vascular shut down (VSD) effect. To investigate the mechanism of the VSD effect, we observed Human Umblical Vein Endothelial Cell (HUVEC) injury induced by photochemical reaction. We observed cell retraction and blebbing after PDT. It seems that the injury was not fetal and only morphological change. Then, the cytoplasm was stained by Calcein-AM and subendothelial area was evaluated from fluorescence microscopy. The rate of subendothelial area after PDT increased significantly. Second, we investigated interaction between neutrophils and HUVEC. Human promyelocytic leukemia cells (HL-60) were differentiated into neutrophil by incubation with all-trans retinoic acid. Calcein-AM labeled neutrophil adhesion to HUVEC was evaluated from fluorescence microscopy. PDT-induced neutrophil adhesion to HUVEC depended more on the exposure of subendothlial area than on neutrophil activation. This result suggests that there is a certain interaction between neutrophil and HUVEC during PDT.

Key words : photodynamic therapy, vascular shut down, thrombus, neutrophil, endothelial cell

INTRODUCTION

Photodynamic therapy (PDT) is a treatment modality based on photochemical reaction and the resultant cytotoxic reactive oxygen species. The platelet thrombus formation leading to stasis observed in vivo during PDT is called vascular shut down (VSD) effect. To investigate the mechanism of the VSD effect, we observed Human Umblical Vein Endothelial Cell (HUVEC) injury and neutrophil adhesion to HUVEC induced by photochemical reaction.

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MATERIALS AND METHODS

Cell culture. HUVEC was purchased from Clonetics (USA) and cultured in MCDB131 medium supplemented with 10% fetal bovine serum in 75 cm^2 culture flasks precoated overnight with collagen. When grown to confluence, the cells were trypsinized and subcultured in collagen-coated 35mm dishes. For experiments, only confluent monolayers at least 2days after subculture were used. Human promyelocytic leukemia cell (HL-60) were purchased from RIKEN cell bank and cultured in RPMI 1641 medium supplemented with 10% fetal bovine serum, 10^5 units/l penicillin, 100mg/l streptomycin.

Preparation of neutrophils. HL-60 cells were incubated with 1μM all-trans retinoic acid for 4 days. For experiments, we confirmed about 90% differentiation into neutropils with nitroblue tetrazolium.

PDT protocol. The photosensitizer zinc coproporphyrin III tetrasodium salt (Zn CP-III) was purchased from Porphyrin Products, Inc., USA. HUVEC and HL-60 were separately incubated with 100 μg/ml Zn CP-III for 1 hour at 37°C, 5% CO_2 and 100% humidity. The cells were irradiated with LED array (5.0 mW/cm^2 at 530 nm) for 10 minutes. After irradiation, the cells were washed two times with pre-warmed medium.

Determination of subendothelial area. After washing, HUVEC were incubated with Calcein-AM for 20 minutes at 37°C and washed with pre-warmed medium and observed with fluorescence microscope.

Determination of neutrophil adherence. After washing, HL-60 were incubated with Calcein-AM for 20 minutes at 37°C and washed with medium. After co-incubation of HUVEC and HL-60 for 10 minutes, HUVEC were washed with medium to remove the non-adherent cells.

RESULTS AND DISCUSSION

We observed PDT-induced cell injury with confocal laser scanning microscope (CLSM). As shown in Fig.1, cell retraction and blebbing was observed immediately after PDT. Then, we confirmed that PDT-treated cell viability was at least 80% from trypan blue exclusion assay (Fig. 2). It seems that PDT-induced endothelial cell injury was not fetal and only morphological change. To investigate the morphological change in detail, the cytoplasm of HUVEC was stained by Calcein-AM and subendothelial area was evaluated from fluorescence microscopy (Fig. 3). The observed fluorescence images were divided into two value (gray level) parts. Non-fluorescent part in which cells did not exist was defined as a subendothelial area and estimated as the rate of all the parts.

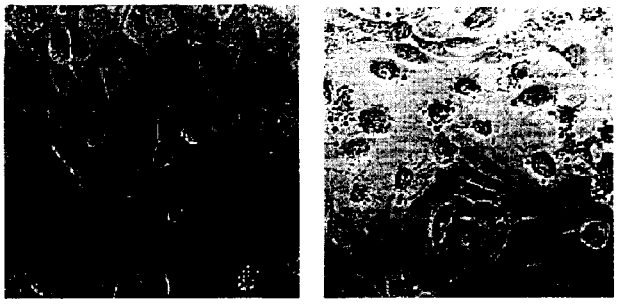

Figure 1. DIC images acquired by CLSM. Bar=100μm. (left) non-treated HUVEC. (right) HUVEC after PDT.

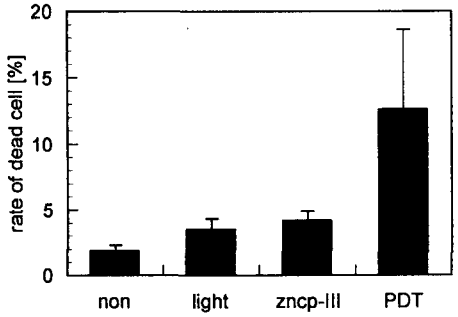

Figure 2. HUVEC viability after PDT.

The rate of subendothelial area after PDT increased significantly (Fig.4), and it depended on photosensitiser concentration and irradiation time. Then, we investigated interaction between neutrophil and HUVEC. Calcein-AM labeled neutrophil adhesion to HUVEC was evaluated from fluorescence microscopy (Fig.5). The number of HL-60 cells adherent to HUVEC after PDT was increased significantly (Fig. 6).

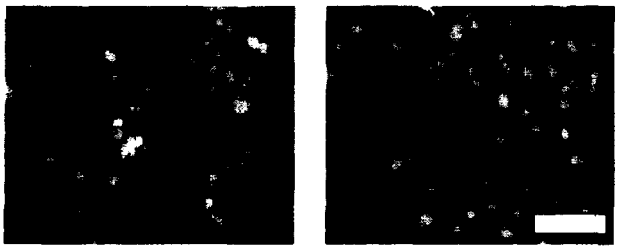

Figure 3. Fluorescence images of HUVEC. (left) Non-treated. (right) After PDT. Bar=100μm.

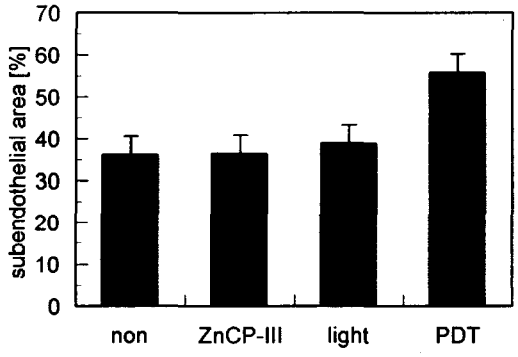

Figure 4 Evaluation of subendothelial area from fluorescence images.

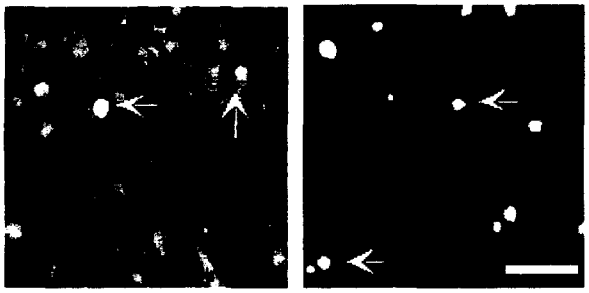

Figure 5. Fluorescence images of HL-60 adhesion to HUVEC. (left) Non-treated. (right) After PDT. Bar=100μm.

PDT-induced HL-60 adhesion to HUVEC depended more on the exposure of subendothlial area than on neutrophil activation. In addition, neutrophil did not adhere to only collagen, which we used to coat the endothelial culture dishes. This result suggests that there is a certain interaction between neutrophil and HUVEC during PDT.

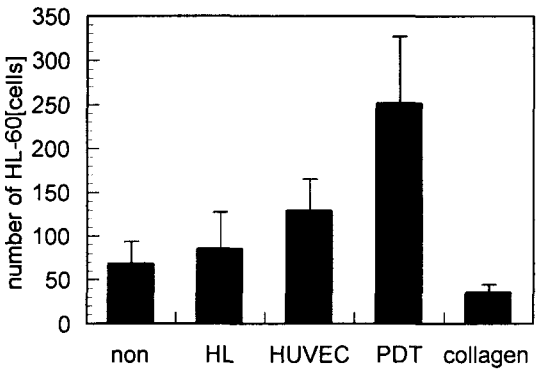

Figure 6. Effect of PDT on HL-60 adhesion to HUVEC.

CONCLUSION

The major finding of this study was that PDT-induced HUVEC injury was not fetal [1] and only morphological change. This morphological change led to the increase of subendothelial area. This led to an increased adherence of HL-60 to the subendothelial area. This is an evidence that leukocyte-endothelium interaction is one of the cause of the VSD effect during PDT.

REFERENCES

1.Roosje M. A. V. Gorp et al. (1999) Peroxide-induced membrane blebbing in endothelial cells associated with glutathione oxidation but not apoptosis. *Am J Physiol.* 1999 Jul; 277(1 Pt 1):C20-8.