Effect of SC5b-9 on Rat Pulmonary Microvascular Endothelial Permeability in Hyperoxia-induced Acute Lung Injury

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Introduction

Pulmonary hyperpermeability is a typical pathophysiological change in animals exposed to hyperbaric oxygen at pressures ranging from more than 0.6-2.0 ATA. Previous studies had showed that complement complex SC5b-9 was involved in the endothelial barrier dysfunction. However, the role of SC5b-9 in pulmonary oxygen toxicity and the signaling pathways participate in SC5b-9-induced hyperpermeability has not been investigated. In the present study, we use both in vivo animal model and endothelial cells derived from rat pulmonary microcirculation to perform detailed analysis on the role of SC5b-9 in hyperoxia-induced lung injury.

Materials and Methods

To induce the lung injury elicited by hyperoxia exposure, rats were placed in a sealed Plexiglas chamber that was flushed with 100% oxygen at a flow rate of 3 liters/minute. The pressure in the chamber was monitored by a manometer and maintained at 2 ATA. Permeability of the rat lung to macromolecules after hyperoxia exposure was evaluated by Evans Blue dye assay. An in vitro endothelial barrier model with SC5b-9 stimulation was used to clarify the mechanisms of SC5b-9-induced hyperpermeability.

Results

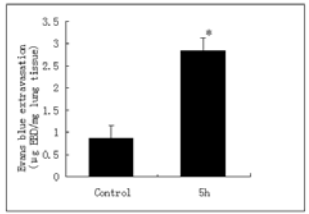

Fig. 1. Effect of hyperoxia on systemic complement activity in rats. Percent changes in 50% hemolytic unit of complement serum (CH50) of the rat sera after 5 h of 200 kPa hyperoxia exposure compared with air-treated animals is significantly different, *P < 0.05. Results are Mean±SE from 5 animals in each group.

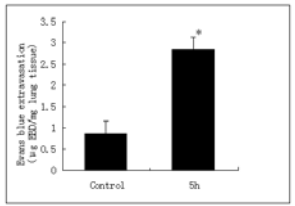

Fig. 2. Extravasation of Evans blue dye (EBD) into the rat lung tissue after 200 kPa hyperbaric oygen exposure. Results are expressed as total EBD/lung. There was a 3-fold increase in the lung/total dose ratio of EBD after hyperoxia exposure. *P <0.01. Results are Mean±SE from 5 animals in each group.

Results

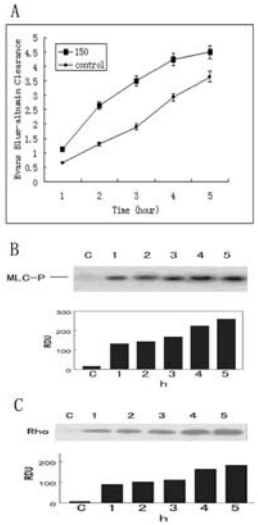

Fig. 3. Effect of SC5b-9 on endothelial permeability, MLC phosphorylation and Rho activation in rat pulmonary microvascular endothelial cells(RPMVECs). RPMVECs were treated with vehicle (M199) or SC5b-9(150 μg/ml) for the indicated time periods. A. Application of SC5b-9 to rat pulmonary microvascular endothelial cell monolayers resulted in increases in permeability. B. SC5b-9-induced MLC phosphorylation. C. SC5b-9 stimulation rapidly increased the levels of Rho-GTP indicating Rho activation. Shown are representative results of three independent experiments.

Conclusions

These data suggest that complement SC5b-9 contribute to pulmonary microvascular dysfunction elicited by hyperoxia exposure. This hyperpermeability was shown to be dependent on both activation of RhoA/ROCK pathway and phosphorylation of MLC.

Acknowledgement

We thank Dr. Kai-zhong Tao and Qian Ma for their kind help on the detection of complement acitivity. This work was funded by grants from the National Natural Science Foundation of China (NSFC) (30600216) and Second Military Medical University (05QN15).

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