（第３号様式）

学 位 論 文 要 旨

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論 文 名　スタチンによるメバロン酸経路阻害はマスト細胞の脱顆粒反応を抑制する

学位論文要旨

Introduction

Statins are well known inhibitors of 3-hydroxy-3-methylglutaryl-CoA reductase, which inhibit the mevalonate pathway, and their activity not only decreases cholesterol level, but also ameliorates inflammation and modulates the immune system. The mevalonate pathway is important in producing lipid molecules that are used in post-translational modification of proteins related to exocytosis with farnesyl pyrophosphate and geranylgeranyl pyrophosphate.

The exocytotic process is complex and orchestrated by many proteins, including small G proteins such as RhoA, Cdc42, Rab, that contribute to actin rearrangement and intracellular vesicle/granule trafficking in the early exocytosis stage. SNARE proteins (SNAP, syntaxin, VAMP) and Ca^{2+}-binding proteins (Doc2a, synaptotagmin, munc13) are important in the final docking process between granules and plasma membrane. In mast cells, the exocytotic process is important in mediating allergic inflammation pathogenesis. In the present study, the effects of simvastatin on the exocytotic process of mast cells were investigated using rat basophilic leukemia (RBL-2H3) cells, a tumor analogue of mucosal mast cells.

Methods

(1)Histamine release measurement: RBL-2H3 cells were sensitized with monoclonal IgE against dinitrophenylated bovine serum albumin (DNP-BSA), and stimulated with 20 ng/mL DNP-BSA

as an antigen, 1 μM ionomycin (Ca^{2+} ionophore) or 0.5 μM thapsigargin (Ca-ATPase inhibitor of endoplasmic reticulum) after incubation with various concentrations of simvastatin for 24h. Released histamine was determined by HPLC-fluorometry. The recovery from inhibitory effect of simvastatin on histamine release was performed with co-incubation of simvastatin with geranylgeraniol, farnesol, or mevalonolactone.

(2)Ca^{2+} influx measurement: The Ca^{2+} signals after DNP-BSA, ionomycin, and thapsigargin treatment were detected using fluorescent Ca probe, fura-2, at 340/380 nm excitation and 510 nm emission wavelengths.

(3)Detection of mRNA expression of Ca^{2+}-binding protein: mRNA expressions of Ca^{2+}-binding proteins (Doc2a, synaptotagmin2 and munc13-4) were determined by RT-PCR method.

(4)Detection of exocytosis proteins using immunofluorescence staining: The expression of VAMP7, SNAP23, Doc2a and Rab27a proteins were observed by immunofluorescence staining.

(5)Visualization of protein interaction using in situ proximity ligation assay: The interaction between Doc2a and Rab27a in the exocytosis complex was detected using PLA methods. The interaction signal was detected as red spots using fluoromicroscopy.

Results and Discussion

(1)Simvastatin inhibited histamine release in RBL-2H3 induced by DNP-BSA, ionomycin, and thapsigargin in a dose dependent manner. This inhibition is unrelated with the Ca^{2+} influx triggered by all three secretagogues.

(2)The inhibition of histamine release induced by simvastatin was counteracted by co-administration of mevalonolactone or geranylgeraniol, but not farnesol.

(3)Ca^{2+}-dependent exocytosis proteins (Doc2a, synaptotagmin2 and munc13-4), Rab protein (Rab27a) and SNARE proteins (SNAP23 and VAMP7) were detected in RBL-2H3 cells using RT-PCR method and immunofluorescent staining.

(4)PLA method clearly exhibited the interaction between Rab27a and Doc2a after antigen stimulation, which was disturbed by treatment of simvastatin.

These data suggest that simvastatin interfered the mevalonate pathways and disturbed the interaction of proteins involved in exocytosis process, resulting in decrease of degranulation in mast cells.

Conclusion

Inhibition of mevalonate pathway by simvastatin resulted in the depletion of geranylgeranyl pyrophosphates, that disturbed the interaction between Rab27a and Doc2a of the exocytosis complex and suppressed degranulation in mast cells. Finally statins may plays roles in improvement of allergic status derived from mast cell activation.

キーワード（３～５）	ラット好塩基球性白血病細胞(RBL-2H3)，ヒスタミン遊離， 低分子GTPタンパク質(Rab27)，Ca結合タンパク質(Doc2)