SUMMARY OF Ph.D. DISSERTATION

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Title

Screening of bioactive secondary metabolites that regulate endothelial functions.

Abstract

Vascular endothelial cells are intimately involved in various physiological processes. It is also involved in various pathological processes including cancer, atherosclerosis, and inflammation. In the present study, I searched among microbial secondary metabolites for compounds that could inhibit the growth of endothelial cells or the cellular adhesion to the endothelial cells.

In the course of our screening for the selective growth inhibitors of human umbilical vein endothelial cells (HUVEC), sangivamycin was isolated from the culture filtrate of *Streptomyces*. It inhibited the growth of HUVEC at about a 30 times lower concentration than that needed to inhibit the growth of WI-38 human fibroblasts. Structurally-related nucleosides, such as toyocamycin, tubercidin, and formycins A and B, did not show the differential inhibition. Then, sangivamycin effectively inhibited S-phase induction in HUVEC. Next, sangivamycin was found to inhibit DNA synthesis selectively in HUVEC. Thus, sangivamycin was shown to be a new selective growth inhibitor of HUVEC acting on DNA synthesis. Therefore, sangivamycin may be a suitable core structure to be modified to develop new anti-angiogenesis agents.

Lipopolysaccharide (LPS) is considered to cause various inflammatory reactions. I searched among microbial secondary metabolites for compounds that could inhibit LPS-stimulated adhesion between HUVEC and human myelocytic leukemic cell line HL-60 cells. In the course of our screening, I isolated a novel cyclic depsipeptide, which I named heptadepsin, from the whole culture broth of *Paenibacillus* sp. The addition of heptadepsin prior to LPS stimulation decreased HL-60 cell-HUVEC adhesion without showing any cytotoxicity. It also inhibited the cellular adhesion induced by lipid A, the active component of LPS, but it did not inhibit TNF-α or IL-1β-induced cell adhesion. The result of surface plasmon resonance (SPR) analysis revealed that heptadepsin interacted with lipid A directly. Thus, heptadepsin, a novel naturally-occurring cyclic heptadepsipeptide, was shown to inactivate LPS by direct interaction with LPS.

Thus, I found two bioactive low-molecular-weight compounds from microorganisms. Sangivamycin was shown to inhibit the cell growth of endothelial cells selectively. Newly discovered heptadepsin was found to be a selective inhibitor of LPS signal transduction, interacting with LPS itself. They may have therapeutic potentials for vascular diseases such as cancer, Gram-negative bacterial sepsis, and atherosclerosis.