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Role of Cholesterol in Toxic Mechanism of *Vibrio vulnificus* Cytolysin

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Abstract

The halophilic bacterium *Vibrio vulnificus* (*V. vulnificus*) is known to be a life-threatening pathogen that causes septicemia and serious wound infection in human. *V. vulnificus* infection is characterized by the high fatality rates of ca. 60% and the primary attack against a person who are immunocompromised or have underying diseases such as liver cirrhosis or hemochromatosis (1-2). *V. vulnificus* cytolysin (VVC) has been implicated as powerful virulence determinant for *V. vulnificus* infection. VVC showed hemolytic activity and acted as vascular permeability factor (3-6). The lysis of erythrocytes caused by VVC is colloid-osmotic in nature and that VVC, after binding to the erythrocyte membrane, oligomerize to form small pores in the membrane resulting in cell lysis (7), indicating that VVC lyses erythrocytes due to the formation of small pores on erythrocyte membrane by cholesterol-mediated oligomerization of the cytolysin. Exogenous cholesterol could inactivate VVC by converting active monomer cytolysin into inactive oligomer at cell free system (7-8). In addition, oligomerization of VVC is completely dependent on three-dimensional structure of cholesterol (9), suggesting that cholesterol is importantly involved in the hemolytic mechanism of VVC on erythrocytes. However, it has not been known whether cell associated cholesterol is a real target for VVC. In addition, role of cholesterol in the cytotoxicity of VVC on nucleated cells is not known yet. Here, we show that membrane-embedded cholesterol is a receptor for VVC. We depleted cholesterol on neutrophil by methyl-β-cyclodextrin (MbCD) extraction and analysed the effect of cholesterol depletion on cellular toxicity of VVC. VVC rapidly bound to normal neutrophils, but didn't bind to cholesterol depleted-neutrophils. Normal neutrophil showed cytotoxicity induced by VVC in a dose-dependent manner whereas cholesterol depleted neutrophil showed the significant inhibition of VVC-induced neutrophil cytotoxicity. In addition, cholesterol depletion inhibited VVC-induced decrease of cellular ATP, potassium efflux, elevation of the cytosolic free Ca^{2+}, and generation of reactive oxygen species (ROS), which are causing mechanisms of cell death. Moreover, cholesterol depletion suppressed VVC-induced pore formation, as evidenced by efflux of 2-deoxy-D-[^{3}H]glucose. These findings suggest

that plasma membrane cholesterol is an essential for cytotoxicity of VVC on human neutrophils.

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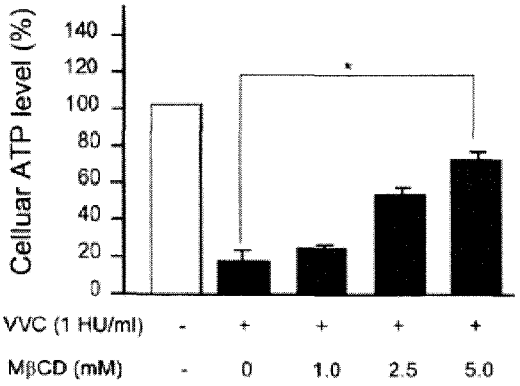

Figure 1. Effect of MβCD on ATP contents of neutrophils treated with VVC

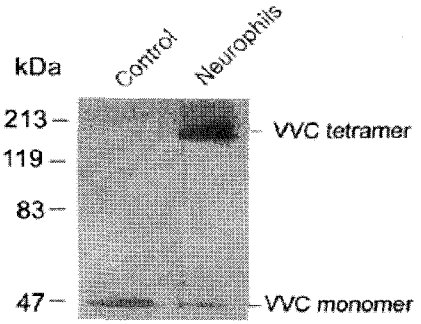

Figure 2. Oligomerization of VVC by neutrophil membranes

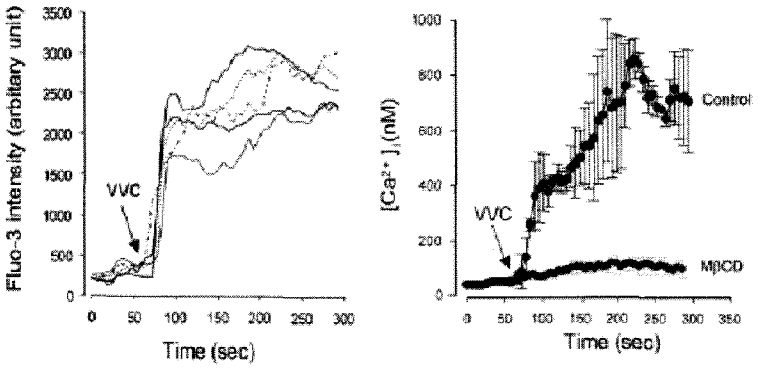

Figure 3. Effect of MβCD on VVC-mediated increase in $[Ca^{2+}]i$ of neutrophils

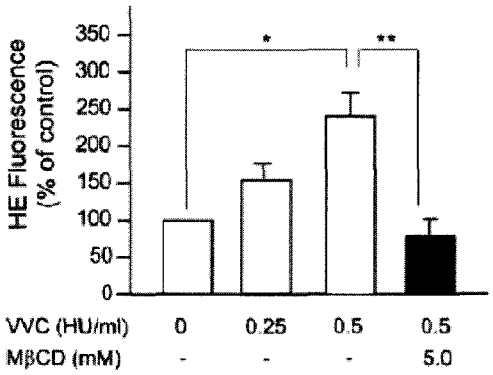

Figure 4. Effect of MβCD on VVC-mediated ROS generation in neutrophils.

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