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Effects of *Vibrio vulnificus* cytolysin on platelet aggregation and lysis

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Vibrio vulnificus is an estuarine bacterium that has been associated with septicemia and serious wound infection in person. Cytolysin has been incriminated as one of the important virulence determinants. Little is known about the target cell of *Vibrio vulnificus* cytolysin in the body. Recently, we observed cytolysin-induced blood coagulation in rat. Platelets are pivotal for haemostasis and thrombosis. In this study, we examined the effects of *Vibrio vulnificus* cytolysin on rat platelet. *Vibrio vulnificus* cytolysin induced a concentration-dependent and irreversible platelet aggregation and lysis. When LDH activity was measured, cytolysin was shown to induce a concentration-dependent lysis of platelets in rat washed platelets.

Staphylococcus α -toxin, which has a similar action to *Vibrio* cytolysin is known to form small pores on erythrocyte membrane. Using patch clamp technic, we observed that *Vibrio vulnificus* cytolysin also formed pores in calcium-free buffer. The slope conductance of pore was 46.1pS in symmetrical 118 mM KCl. These results show ECF calcium is not essential for pore formation.

ECF calcium is essential for platelet lysis. When we changed buffer for calcium-free to investigate relationship with ECF calcium and cytolysin, LDH release is decreased from $64.3 \pm 3.4\%$ to $1.3 \pm 0.4\%$. (3HU cytolysin). The ability of cytolysin to alter intracellular calcium was examined using the fluorescent calcium indicator fura-2. Cytolysin caused a elevation of the cytosolic calcium concentration in the presence of extracellular calcium. Verapamil, organic calcium channel blocker, and mefenamic acid, a non-selective calcium channel blocker had no effect on calcium elevation. Since Co^{+2} , Cd^{+2} , Mn^{+2} are known to interfere with calcium influx through calcium channels at plasma membrane, we further investigated the effects of these divalent cations. All of tested divalent cations (Co^{+2} , Cd^{+2} , Mn^{+2} , Zn^{+2}) did not inhibit intracellular calcium increase. Raffinose and sucrose were known to prevent the hemolysis as an osmotic protectant. Incubation with raffinose (50 mM) did not inhibit the lytic activity of *Vibrio vulnificus* cytolysin. From the above results, we concluded that *Vibrio vulnificus* cytolysin induced platelet aggregation and lysis as a result of intracellular calcium elevation. And, this was related to pore formation of *Vibrio vulnificus* cytolysin.