## 22. Inhibitory effects of Deep Sea Water on collagen induced human platelet aggregation by PI3K/Akt signaling

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### 1. Introduction

Heart disease leads to sudden symptoms and death. Acute myocardial infarction due to thrombosis is a very fatal symptom among various causes of these heart diseases. Platelet aggregation is one of the major causes of thrombosis. Therefore, inhibiting platelet aggregation is an effective way to prevent cardiovascular disease. Platelets are stimulated by agonists, and activated platelets secrete substances that promote platelet aggregation, such as thromboxane $A_2$($TXA_2$) and serotonin. It is well known that various second mediators such as Phosphatidylinositol 3,4,5-trisphosphate kinase(PI3K), Protein kinase B(Akt, PKB) and mitogen-activated protein kinase(MAPK) play important roles in secretion of $TXA_2$ and serotonin. In this study, we investigated the effect of deep sea water (DSW) on the $TXA_2$, serotonin secretion, phosphorylation of PI3K, Akt and MAPK in human platelets.

### 2. Methods

Human platelet-rich plasma (PRP) anticoagulated with acid-citrate-dextrose solution (0.8% cirtric acid, 2.2% sodium citrate, and 2.45% glucose) was purchased from Korean Red Cross Blood Center (Changwon, Korea). PRP was washed twice with washing buffer (138 mM NaCl, 2.7 mM KCl, 12 mM $NaHCO_3$, 0.36 mM $NaH_2PO_4$, 5.5 mM glucose, and 1 mM $Na_2EDTA$, pH 6.5). The washed platelets were suspended in suspention buffer (138 mM NaCl, 2.7 mM KCl, 12 mM $NaHCO_3$, 0.36 mM $NaH_2PO_4$, 5.5 mM glucose, 0.49 mM $MgCl_2$, and 0.25% gelatin, pH 6.9) to a final concentration of $5 \times 10^8$/ml. DSW pretreated human washed platelet ($10^8$/ml) were stimulated with collagen (3μg/ml) for 5 min. Aggregation was monitored using an aggregometer (Chrono-Log, Corporation) at a constant stirring speed of 1,000 rpm. Phosphorylation of PI3K, Akt and MAPK were analyzed by western blotting and release of $TXA_2$ and serotonin were analyzed by ELISA kit.

### 3. Conclusion

DSW inhibited platelet aggregation induced by collagen (3μg/ml) in a hardness-dependent manner. Based on these results, it was found that the DSW inhibited phosphorylation of PI3K, Akt, and MAPK. In addition, DSW decreases the release of serotonin, which is a granular substance in platelets, and $TXA_2$, a product of platelet membrane. As a result, it is considered to inhibit platelet aggregation. Therefore, the results of study suggested that DSW could prevent the cardiovascular disease associated with activation of platelets.

### 4. References

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