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TAP-SSL5 FUNCTIONS AS AN ANTICOAGULANT AND ANTI-INFLAMMATORY PROTEIN TO INHIBIT THROMBOSIS IN RAT AND ATTENUATE ATHEROSCLEROSIS IN APOE-KNOCKOUT MOUSE

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Objectives This study aims to inhibit the inflammatory response and thrombosis in atherosclerotic lesions, by using the

staphylococcal superantigen-like protein-5 (SSL5) to inhibit neutrophil activation, and tick anticoagulant peptide (TAP) to inhibit factor Xa (FXa) activation. To achieve this, we made a novel fusion protein TAP-SSL5 and tesed its dual anticoagulant and anti-inflammatory properties.t

Methods We put six flexible amino acids sequence between TAP and SSL5 genes as a linker. The polycistronic fragment was constructed and then subcloned into a pET22b(+) expression vector. The binding ability of TAP-SSL5 to P-selectin glycoprotein ligand-1 (PSGL-1) on leukocytes was verified by flow cytometry. The binding of TAP-SSL5 to PSGL-1 inhibited the adhesion of leukocytes to P-selectin-coated surface. FXa activity was determined by chromogenic substrate assay.

Results We have observed that TAP-SSL5 inhibited FXa activity in a concentration-dependent manner and TAP-SSL5 reduced ferric chloride-induced thrombosis in the inferior vena cava of rat. In a high-fat diet induced atherosclerotic model by using ApoE-knockout ($ApoE^{-/-}$) mouse, male $ApoE^{-/-}$ mice were grouped to receive intraperitoneal treatment with either TAP-SSL5 (3 mg/kg/day), SSL5 (2 mg/kg/day) or vehicle separately. After 12 weeks of the respective treatment, we have observed that TAP-SSL5 reduced the atherosclerotic plague formation by 48% compared to controls. We also found TAP-SSL5 could down-regulate several kinds of inflammatory cytokine expressions in vascular wall which were detected by mouse inflammatory antibody array.

Conclusions TAP-SSL5 fusion protein has promising anti-inflammatory and anti-thrombosis properties, and it acquires the potential for the prevention of atherosclerotic lesion and thrombus formation.