Omega-3 polyunsaturated fatty acids attenuate thrombin formation in human vascular smooth muscle cells

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Background: Atherosclerosis is a chronic inflammatory disease where phenotypic and functional changes in vascular smooth muscle cells (VSMCs) contribute to some features of this disease, such as thrombosis and vascular calcification¹. VSMCs provide a significant surface for local thrombin formation in the vascular wall. Thrombin is a potent protease, which in addition to its procoagulant activity exerts proinflammatory effects and participates in the proteolytic metabolism of matrix proteins², including osteopontin (OPN). Although, uncleaved OPN is a strong inhibitor of vascular calcification³, the thrombin-cleaved form of OPN (OPN-N half) is associated with carotid-plaque inflammation⁴ and stimulates cardiovascular calcification⁵. Importantly, thrombin generation is increased in VSMCs from spontaneously hypertensive rats⁶ and by cyclic stress⁷ through binding of prothrombin to activated $\alpha_v\beta_3$ integrin. At the same time, omega-3 polyunsaturated fatty acids (n-3 PUFAs) contribute to decreased inflammation in atherosclerosis⁸ and the thrombotic potential in healthy subjects⁹. Of interest, the recent REDUCE-IT trial reported a 25% relative risk reduction for cardiovascular events of a high dose of the n-3 PUFA eicosapentaenoic acid (EPA) compared with placebo¹⁰, potentially mediated by the action of an antithrombotic mechanism, which remains to be elucidated.

Hypothesis: The aim of the present study was to investigate the effects of n-3 PUFAs on VSMCs' thrombin generation and hence to assess their potential role on the thrombin-mediated OPN cleavage.

Figure 1. n-3 PUFAs reduce thrombin generation at the surface of human vascular smooth muscle cells (VSMCs) (a) Representative thrombin generation curves and (b) % decrease (from vehicle) in endogenous thrombin potential (ETP) of VSMCs incubated with different concentrations of n-3 PUFA for 48h in pooled platelet poor plasma. (c) ETP fold change of VSMCs preincubated with 100nM n-3 PUFA for 15 min, 6 h, 24 h or 48 h before initiation of thrombin generation. Results are presented as mean $\pm$ SEM from at least 3 experiments in triplicates.

Figure 2. n-3 PUFA-mediated reduction in VSMC thrombin formation is dependent on exogenous prothrombin. (a) Prothrombin mRNA (left) and protein (right) levels from VSMCs incubated with 100nM n-3 PUFA and vehicle for 48h. (b) Thrombin generation by VSMCs pretreated with vehicle or 100nM n-3 PUFA for 48h in the presence of prothrombin-deficient plasma. Results are mean $\pm$ SEM (a) or representative curve (b) from at least 3 experiments.

Figure 3. n-3 PUFA-mediated reduction in VSMC thrombin formation is dependent on $\alpha_v\beta_3$ integrin. Fold change in endogenous thrombin potential (ETP) from vehicle treated VSMCs pre-incubated with either scrambled GRT or RGT peptides at 0,25 mM for 30 min prior to thrombin generation. Results are mean $\pm$ SEM from at least 3 experiments in triplicates.

Figure 4. n-3 PUFA reduce thrombin-cleaved Osteopontin N-half. (a) Osteopontin (OPN) mRNA (left) and full-length protein levels (right) from VSMCs incubated with 100nM n-3 PUFA for 48h. (b) Concentration levels of thrombin-cleaved OPN N-half in the supernatants of VSMCs incubated with 100nM n-3 PUFA for 48h. Results are presented as mean $\pm$ SEM from at least 3 experiments in triplicates.

Summary – Conclusions

- ❖ n-3 PUFAs reduce thrombin generation in human VSMC
- ❖ The n-3 PUFA-mediated reduction of thrombin in human VSMC is dependent on prothrombin and $\alpha_v\beta_3$ integrin activation
- ❖ n-3 PUFAs reduce the pro-calcifying thrombin-cleaved OPN-N half in human VSMC
- ❖ These effects may contribute to the vascular protection of n-3 PUFAs

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