Protein aggregates in plasma-derived factor IX (pdFIX) concentrates support platelet activation and microparticle formation in vitro

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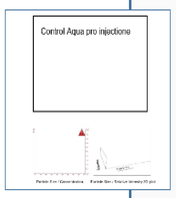
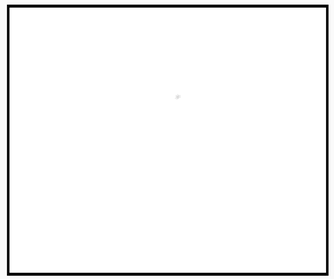
Bachground and objectives: Misfolded protein aggregation, forming subvisible particles, is a prevalent problem occurring in many biopharmaceutical manufacturing processes. Misfolded proteins have been shown to activate platelets (Herczenik et al., 2007). As protein aggregates were found in coagulation factor concentrates, we tested the effect of recombinant (r) and plasma-derived (pd) factor IX products on platelet function. To verify the role of protein aggregates we removed misfolded protein/protein aggregates by affinity binding to the chaperone GRP78 and to a ADAM15 peptide.

Methods: The influence of rFIX and pdFIX, used in clinically relevant concentrations, on platelet activation and microparticle formation of human platelets was studied by flow cytometry. Experiments were done in the presence of melagatran to inhibit thrombin formation dependent secondary platelet activation. Nanosight nanoparticle Tracking Analysis (NTA), a technique that combines laser light scattering microscopy with a charge-coupled device (CCD) camera enabled the visualization and recording of protein aggregates Subvisible particles < 1000nm were examined in 1 rFIX product (Benefix®) and 7 pdFIX products (Alphanine ®, Berinine®, Factor IX Biotest®, Immunine®, Haemonine®, Mononine®, Octanine®). Particle size distribution and concentration of protein particles was analysed using NanoSight NTA 2.3 Software. Misfolded proteins were removed by affinity ligation to GRP78 and to a ADAM15 peptide. The influence of rFIX and pdFIX on platelet activation and microparticle formation before and after removal of protein aggregates was compared.

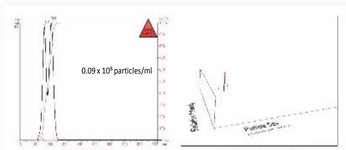
Results:



rFIX

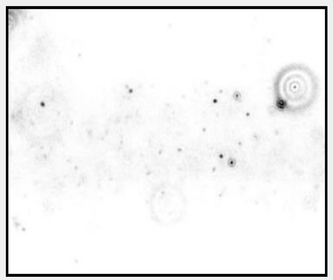


Recombinant FIX product: BeneFix

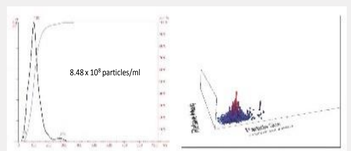


Particle Size / Concentration Particle Size / Relative Intensity 3D plot

pdFIX

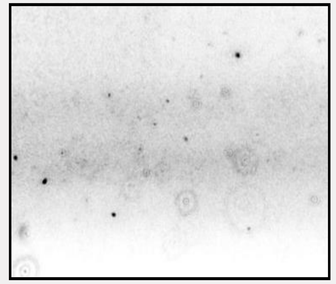


Alphanine

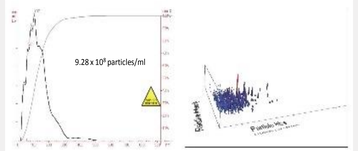


Particle Size / Concentration Particle Size / Relative Intensity 3D plot

pdFIX

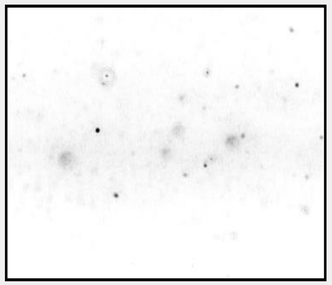


Berinine

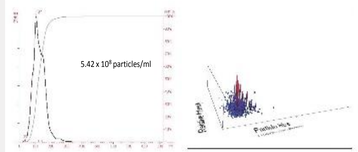


Particle Size / Concentration Particle Size / Relative Intensity 3D plot

pdFIX

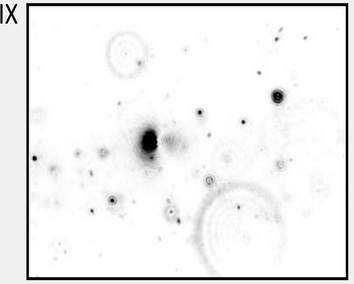


Factor IX Biotest

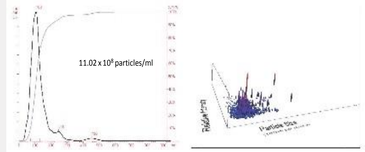


Particle Size / Concentration Particle Size / Relative Intensity 3D plot

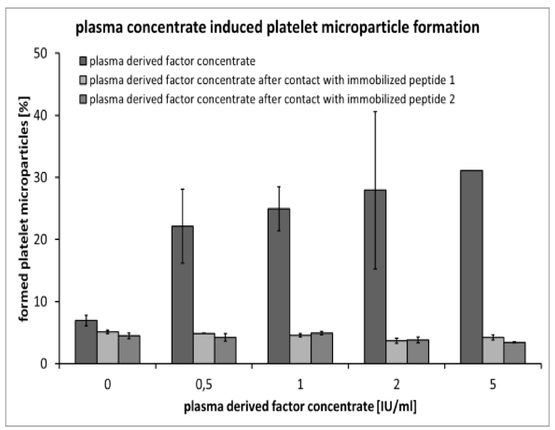
pdFIX



Haemonine

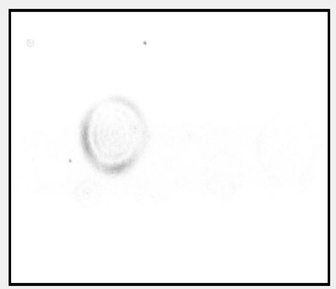


Particle Size / Concentration Particle Size / Relative Intensity 3D plot

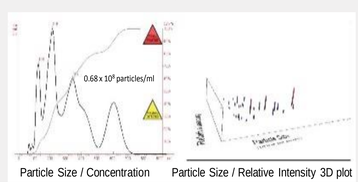


0-5IU/ml plasma derived factor concentrate was incubated with 10µg/ml immobilized peptide 1 or peptide 2, that binds misfolded proteins, for 1 hour. The supernatant was tested for induction of platelet microparticle formation.

pdFIX

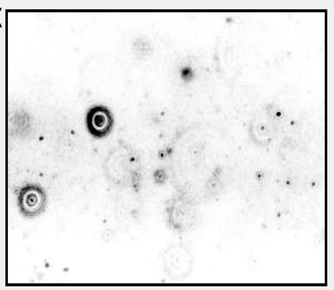


Immunine

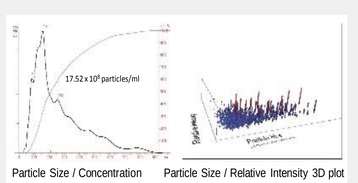


Particle Size / Concentration Particle Size / Relative Intensity 3D plot

pdFIX

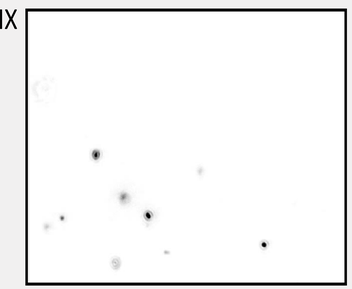


Mononine

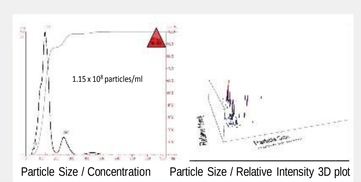


Particle Size / Concentration Particle Size / Relative Intensity 3D plot

pdFIX



Octanine



Particle Size / Concentration Particle Size / Relative Intensity 3D plot

Results: While we found in BeneFIX only sporadically small singular particles, pdFIX concentrates contained a lot more particles. Size distribution was different in different pdFIX concentrates. PdFIX supported platelet activation and microparticle formation induced by low concentrations of agonists, while rFIX did not. Removal of protein aggregates from pdFIX prevented the activation effect on platelets, without having and influence on FIX activity.

Conclusions: As platelets are not only involved in haemostasis but also in inflammation, the clinical relevance of protein aggregates in coagulation factors should be studied ex vivo.

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The poster was presented on the WFH 2016 in Orlando, USA