

Improved treatment for Haemophilia A: platelet transport of coagulation Factor VIII

Introduction: Haemophilia A is a congenital X-linked bleeding disorder. Because of the absence of coagulation Factor VIII (FVIII) in the plasma of Haemophilia patients, their capacity to stop bleeding (hemostasis) can be severely compromised and bleeding episodes may occur. Besides the obvious direct risks involved, recurrent bleeding in the joints can lead to bone destruction and reduces the mobility of these patients. At present,



Figure 1. Joint bleeding in a child with Haemophilia A.

patients with Haemophilia are treated by supplementation of their blood with exogenous FVIII, either purified from donor plasma or recombinantly produced.

The circulating half-time of FVIII products is between 10-20 hours and the use of them is associated with the risk of developing inhibiting antibodies against FVIII. These antibodies render further therapy useless and form a severe obstacle in the management of Haemophilia. Therefore, there is a need for improved Haemophilia therapy that functions longer and has a reduced immunogenicity compared to the current therapeutics.

A new strategy: We have recently developed a strategy to address this challenge. This strategy is based on the existing homology between FVIII and coagulation Factor V (FV). Unlike

FVIII, FV is taken up by cells in the bone marrow. As a result, up to 25% of all produced FV is present in circulating platelets, rather than freely available in plasma. Platelets are small anucleate cells that home in on wound areas and stick together and release their content. The platelet-pool of FV is sufficient to support its role in hemostasis, even when the plasma levels of FV are 0% (Duckers et al. *Blood* 2010). At present, the mechanism by which FV is incorporated into platelets is largely elucidated (Fig.1A). More importantly, the domains of the FV protein which mediate its uptake are known. Very recent un-published results from our group indicate that therapeutic FVIII can be taken up by blood platelets *in vitro*, although not extremely efficient (Fig.1B,C). This suggests that that by increasing the efficacy of incorporation of FVIII into platelets, a platelet-type FVIII may be artificially generated. This may provide significant advantages for the treatment of haemophilia by improving the half life of FVIII, while reducing immunogenicity (since the protein does not need to circulate freely in order to fulfill its role).

We would like to develop a novel form of recombinant FVIII, which is more efficiently transported by platelets, compared to normal FVIII. Hereto, we will replace the domains of FVIII with the corresponding domains of FV that are responsible for FV incorporation into platelets.

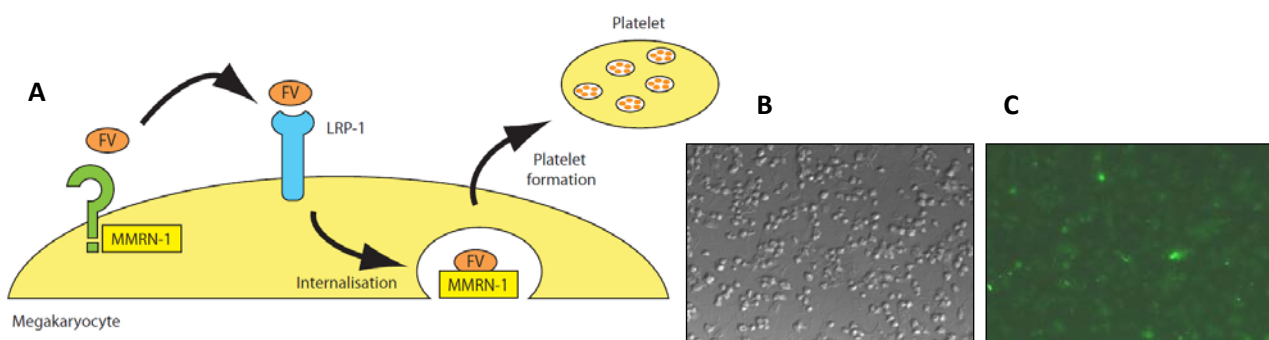
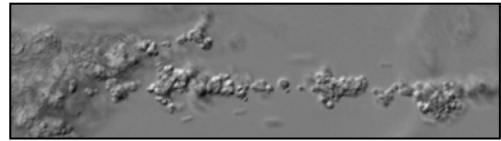


Figure 1. Incorporation of FV into platelets is a multi-step process, involving multimerin (MMRN-1) and LRP-1 (A). Bright-field (B) of platelets that have been incubated with therapeutic FVIII and fluorescence immunohistochemistry against FVIII in the same platelets (C).

Aims:

1) To generate FVIII-FV chimeras. At first, complete domains shall be swapped (Figure 2). Additionally, a region of interest will be determined by replacement of smaller sequences from FV.

2) To characterize these mutants in the context of Clinical Chemistry *in vitro*. Blood coagulation assays will be performed and the build-up of blood clots under flow will be studied by real-time microscopy.



3) To characterize these chimeras *in vivo*. A FVIII-deficient mouse strain is available. The chimeras will be intravenously administered to the mice and their effects on improving hemostasis will be evaluated by tail-bleeding assays.

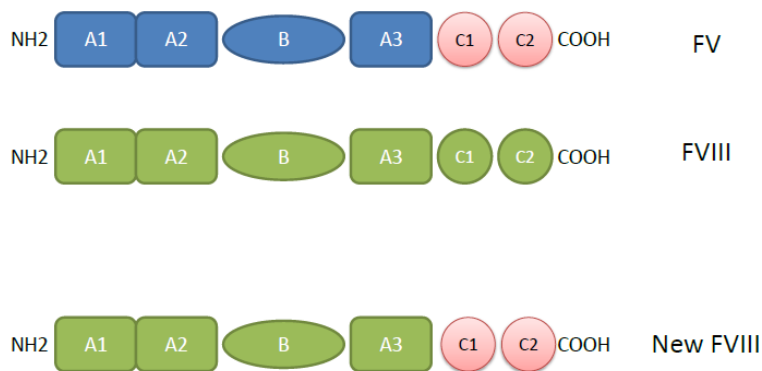


Figure 2. Primary strategy for the generation of an improved FVIII product. The domains that mediate FV incorporation into platelets are indicated in red.

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