Efficacy of a Recombinant ADAMTS13 in a Mouse Model of TTP

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Objective

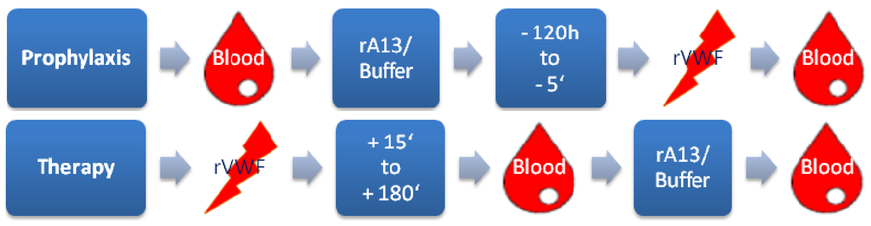
Baxter is developing a recombinant ADAMTS13 (rADAMTS13, a disintegrin and metalloproteinase with a thrombospondin type 1 motif, member 13) product for the potential prophylaxis and treatment of thrombotic thrombocytopenic purpura (TTP).

We established a disease model in ADAMTS13 ko mice (B6.129-ADAMTS13^{tm1Dgi}) in which the animals simultaneously and consistently develop TTP-like symptoms by challenge with a high dose of human recombinant von Willebrand factor (rVWF) containing ultra-large VWF multimers.[1] A dose-dependent efficacy of rADAMTS13 in this model was shown in previous studies.

Methods

All animals were challenged with a high i.v. dose of rVWF to induce TTP-like symptoms. Groups of 10 ADAMTS13 ko mice (5m/5f) received prophylactic or therapeutic treatment with 200 FRETS-U/kg rADAMTS13 or 5 mL/kg buffer.

Efficacy was defined as the degree of prevention of platelet drop, schistocytosis and increase in LDH.



Prophylactic efficacy of Baxter's rADAMTS13 was tested by administration of rADAMTS13 either 120h, 72h, 48h, 24h, 3h or 5min before challenge with rVWF. Buffer served as the negative control item and was administered 5min before challenge. On day 0, a pre-treatment platelet count was assessed and set to 100% for each individual animal. On day 1, the animals were humanely killed and necropsied. Blood was sampled to assess platelet count, hematocrit, schistocytosis and LDH. Individual results and means are presented.

Therapeutic efficacy of rADAMTS13 was tested by administration of 200 FRETS-U/kg 15min, 30 min or 180min after challenge with rVWF. Buffer served as the negative control item and was administerd 15min after challenge. Since thrombocytopenia progresses rapidly after challenge with rVWF, pre-treatment platelet count was assessed immediately before the application of the test or control item. See above for study procedures on day 1.

All animal experiments accorded with Austrian laws governing animal experimentation and were additionally approved by the Institutional Animal Care and Use Comittee (IACUC).

Results

Morbidity in buffer-treated control animals was 100%: All animals receiving rVWF containing ultra-large VWF multimers were severely thrombocytopenic and showed increased LDH levels, schistocytosis and organ damage.

Efficacy of rADAMTS13 was treatment interval-dependent in both studies. Platelet count at termination of all rADAMTS13-treated groups was statistically superior to buffer-treated controls (p≤0.0001). However, animals that received prophylactic treatment 120h before administration of rVWF showed severe thrombocytopenia and clinically relevant protection was only seen for treatment intervals ≤72h.

Therapeutic treatment with rADAMTS13 stabilized the platelet count and prevented further development of thrombocytopenia. Other endpoints, including LDH, schistocytosis and organ damage, confirmed the treatment interval-dependent efficacy observed for platelet count.

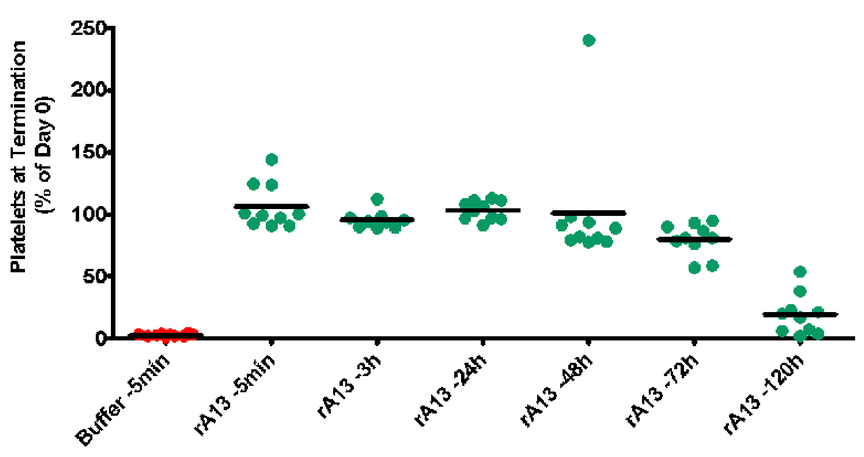


Fig. 1: Platelet count after prophylactic treatment with rADAMTS13

All buffer-treated control animals (red) had a severe thrombocytopenia (2.8% of their baseline level). Prophylactic treatment with 200 FRETS-U/kg rADAMTS13 (green) 5min-24h hours prior to challenge protected the animals from developing thrombocytopenia. Animals treated 48-72h prior to administration of rVWF showed a trend towards developing a mild thrombocytopenia. Animals that received prophylactic treatment 120h prior to challenge with rVWF were thrombocytopenic with a mean platelet count of 19.2% of their baseline level.

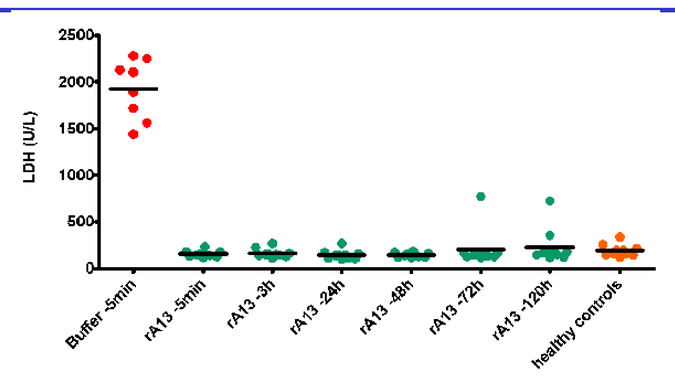


Fig. 2: LDH after prophylactic treatment

Mean serum LDH in untreated ADAMTS13 ko mice (orange) was 193 U/L (118-337 U/L). Mean serum LDH in buffer-treated controls (red) was 1922 U/L. Prophylactic treatment with 200 FRETS-U/kg rADAMTS13 (green) 5min-120h prior to application of rVWF prevented LDH increase (144-227 U/L; p≤0.0001). However, 1/10 animals treated 72h and 1/10 animals treated 120h before challenge showed a 4-fold increase in serum LDH level to 772 and 724 U/L, respectively.

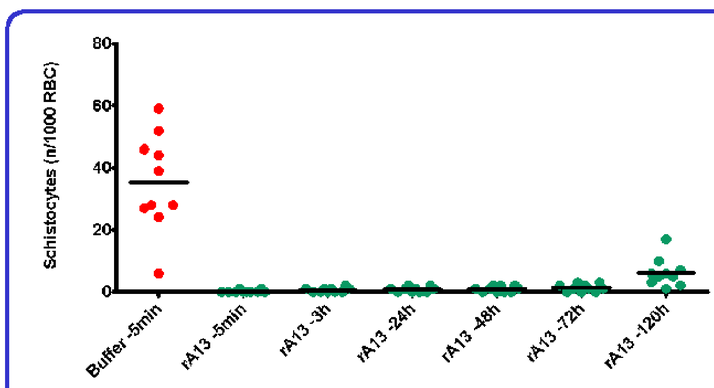


Fig. 3: Schistocytosis after prophylactic treatment

Schistocytes (sc) were present in all buffer-treated animals (red; 35.3 sc/1000 RBC), indicating hemolytic anemia. Prophylactic treatment with rADAMTS13 (greeb) ≤72h prior to challenge with rVWF decreased the incidence to 2-6/10 animals and the severity to a maximum of 3 sc/1000 RBC. Schistocytes were found in all animals treated 120h prior to challenge with r, however, the severity of schistocytosis was decreased to a maximum of 17 sc/1000 RBC.

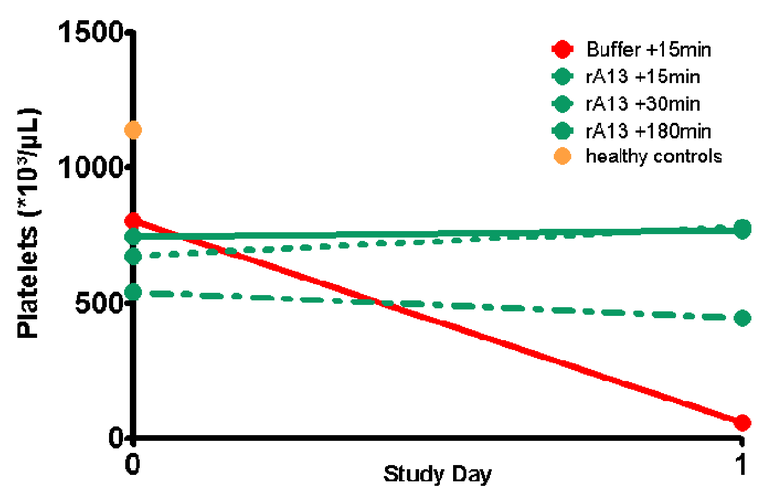


Fig. 4: Platelet count after therapeutic treatment with rADAMTS13

At the time of administration of rADAMTS13 (green) animals already showed a mild to moderate thrombocytopenia compared with healthy controls (orange), depending on the treatment interval. Therapeutic administration of rADAMTS13 prevented further development of thrombocytopenia in all groups, whereas buffer-treated animals (red) suffered a progression of thrombocytopenia. Platelet count of all rADAMTS13-treated groups was statistically superior to buffer-treated controls (p≤0.0001).

Conclusions

- Baxter's rADAMTS13 was effective in an rVWF-induced animal model closely mimicking the situation in patients with hereditary TTP.
- The efficacy of Baxter's rADAMTS13 was treatment-interval dependent in both a prophylactic and therapeutic setting.

References

1. Schiviz et al (2012) Blood

Acknowledgements

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