Hemarthrosis results in an increase in synovial fibrinolytic activity in hemophilic mice.

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Introduction

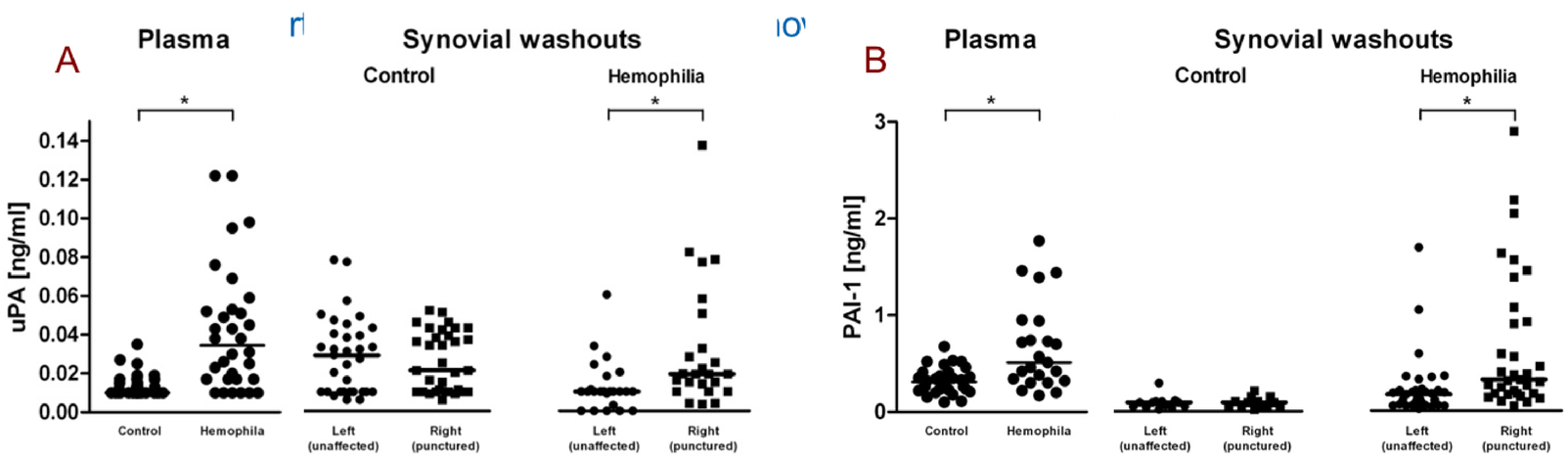
Recurrent joint bleeding is the most common manifestation of hemophilia resulting in hemophilic arthropathy (HA). One in three patients with severe hemophilia develop a so-called target joint, defined as a joint in which recurrent bleedings have occurred on four or more occasions during the previous 6 months or 20 times during the patient's life. Considering that target joints are all synovial joints and that synovial tissue is potentially able to contribute to local fibrinolytic activity, we hypothesize that synovial fibrinolytic activity is increased following hemarthrosis.

Materials & methods

Three to four months old FVIII deficient (n=150) and control (n=138) mice were anesthetized, the hair over both knee joints was removed, the knee joints were examined, visual bleeding score (VBS) was assessed, and the diameter of the joint was measured. Subsequently, hemarthrosis was induced in the right knee using a 30 Gauge needle. The left knee served as an unaffected control joint. After 24 hours blood was collected via a cheek puncture, mice were sacrificed, knee joints were examined, and VBS and joint diameter was determined again. Patellae and surrounding synovium were isolated and shortly pre-washed in tris buffered saline (TBS) to remove blood adherence. Subsequently, a patella-synovial washout was performed for 1 hour at room temperature in a solution consisting of one part trisodiumcitrate-dihydrate and nine parts of 3% Bovine Serum Albumin – TBS. Levels of functionally active murine urokinase-type plasminogen activator (uPA), plasminogen activator inhibitor 1 (PAI-1), plasmin, and alpha-2-antiplasmin (A2AP) were measured in blood plasma and in the synovial washouts.
In a subset of mice (n=6), knee joints were isolated, fixed in 4% paraformaldehyde, cut and used for immunohistochemistry to examine synovial uPA expression.

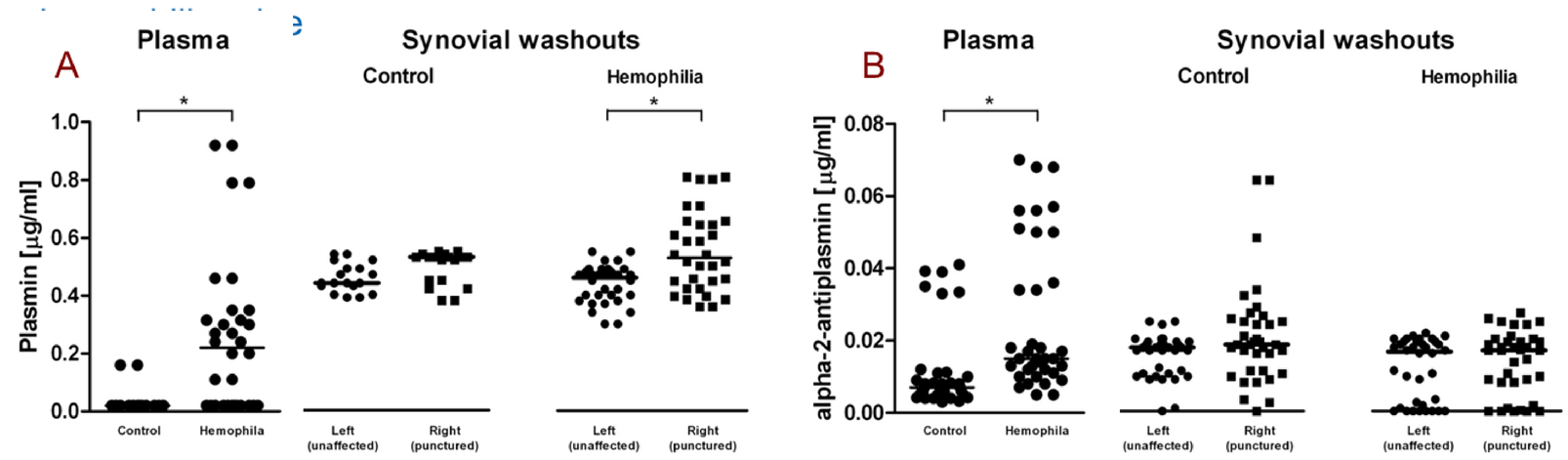
Results

Puncture of the right knee in hemophilic mice resulted in hemarthrosis. The left unaffected knee joint showed no signs of bleeding. Puncture of the right knee in control mice did not resulted in induction of hemarthrosis. Also, no sign of bleeding was seen in the left unaffected knee joint. In hemophilic mice, the mean VBS of the right knee increased 24h after puncture of the right knee as compared to baseline 0.03 (± 0.02) vs. 1.88 (± 0.12) ($p<0.001$). Also, the mean knee joint diameter increased from 4.20mm (± 0.04) at baseline to 5.51mm (± 0.18) 24h after induction of the joint bleeding ($p<0.001$). No alterations in VBS and joint diameter were seen in the left knee of hemophilic mice and in both knee joints of control mice. In control mice the median (range) percentage uPA positive synovial lining cells was similar for the left 41% (range 10) and right knee 40% (range 9) ($p=0.586$). In hemophilic mice, an increase in the median percentage uPA positive synovial lining cells in the right punctured knee following hemarthrosis was observed: 43% (range 21) for the left knee versus 47% (range 15) for the right knee ($p=0.028$).
Plasma levels of uPA were elevated in hemophilic mice 0.035ng/ml (range 0.011) as compared to control mice 0.01ng/ml (range 0.025) ($p<0.001$) (figure 1A). Following hemarthrosis, synovial uPA levels increased in the right punctured knee joint 0.016ng/ml (range 0.133) in comparison with the left unaffected knee joint 0.01ng/ml (range 0.077) in hemophilic mice ($p=0.03$). Plasma levels of PAI-1 were statistically significant higher in hemophilic mice 0.51ng/ml (range 1.6) as compared to control mice 0.31ng/ml (range 0.58) ($p=0.001$). In hemophilic mice a significant higher synovial PAI-1 level 0.32ng/ml (range 2.84) in the right punctured knee was measured in comparison to the left unaffected knee joint 0.17ng/ml (range 1.67) ($p<0.001$) (figure 1B).



Plasma levels of plasmin were statistically significant higher in hemophilic mice 0.22µg/ml (range 0.9) ($p=0.027$). (figure 2A) In the right punctured knee joint 0.53µg/ml (range 0.45) of hemophilic mice an increase in synovial plasmin levels was noticed 24h after the induction of hemarthrosis as compared to the left control knee joint 0.46µg/ml (range 0.25) ($p=0.01$). A2AP levels were statistically significant higher in hemophilic mice 0.015µg/ml (range 0.07) as compared to control mice 0.007µg/ml (range 0.04) ($p=0.006$). (figure 2B) No difference in synovial A2AP levels of the left unaffected knee joint 0.021µg/ml (range 0.001) and the right punctured knee joint 0.021µg/ml (range 0.001) in hemophilic mice were found ($p=0.146$).

Figure 2: Following hemarthrosis synovial plasmin, but not A2AP, levels are increased in



Conclusion

In this study, using an in vivo murine hemophilia model, we provide evidence that hemarthrosis stimulates the local synovial fibrinolytic system resulting in increased synovial uPA expression and increased synovial uPA, PAI-1, and plasmin levels. The increased synovial fibrinolytic activity makes the joint more vulnerable for prolonged and subsequent bleedings and may attribute to the onset of hemophilic arthropathy.

Acknowledgements

This study was financially supported by an unrestricted grant of Novo Nordisk

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DOI: 10.3252/pso.eu.WFH2012.2012