Extended Factor IX Activity Improves Joint Healing In Hemophilic Mice

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OBJECTIVE

Healing in skin wounds of hemophilia B mice is delayed when compared to hemostatically normal wild type (WT) mice, and is characterized by persistence of iron deposition, inflammation, and neovascularity. Following a dermal wound, restoring normal hemostasis with a single hemostatic dose of recombinant factor IX (FIX) did not normalize cutaneous wound healing. Instead, hemostasis was required throughout one week of healing.[1-3]

In this study we observed **healing following hemarthrosis** in WT and factor IX knockout ($FIX^{-/-}$) mice, examining the parameters previously studied in the dermal wound model.

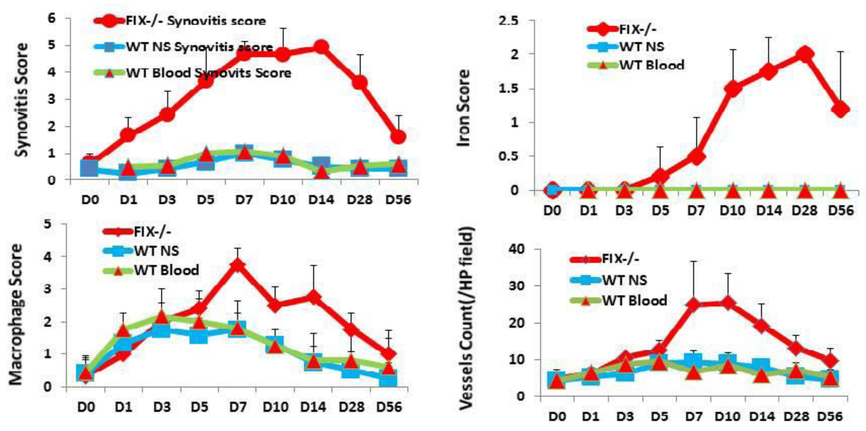
We additionally explored the **hypothesis that the use of a FIX protein with extended FIX** activity (N9-GP, Novo Nordisk) **could achieve improved synovial and osteochondral wound healing** in hemophilic mice, when compared to treatment with unmodified recombinant FIX (rFIX) in an established joint bleeding model.[4,5]

CONCLUSION

- Hemostatically normal mice tolerate this joint bleeding challenge, clear blood from the joint, and heal with minimal pathology.
- Hemophilia B mice have impaired wound healing following induced joint hemorrhage. Impaired features of wound healing, previously observed in a cutaneous wound model, have close parallels in hemophilia B mice following hemarthrosis.
- Significantly **improved synovial wound healing and preservation of normal osteochondral architecture** are achieved by extending FIX activity after hemarthrosis using N9-GP when compared to an equivalent dose of rFIX.
- These results suggest that treating joint bleeding only until hemostasis is achieved may not result in optimal joint healing, which is improved by extending factor activity.

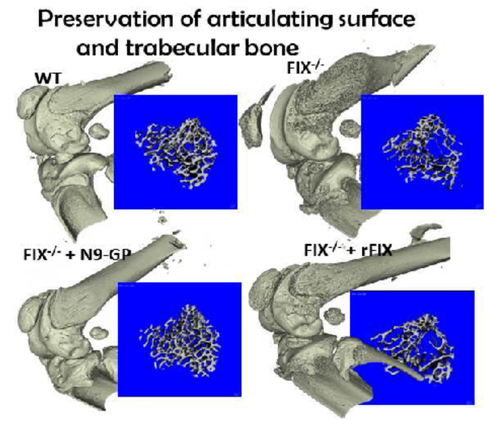
RESULTS

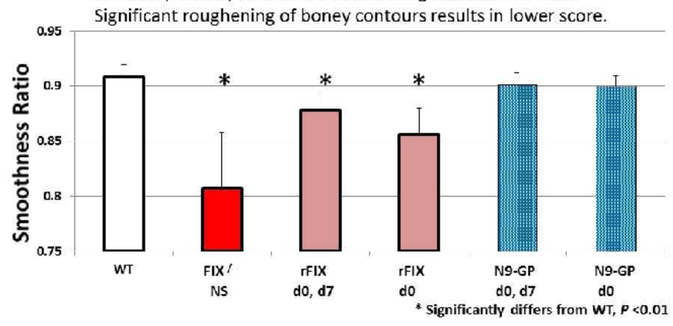
$FIX^{-/-}$ mice: Abnormal wound healing following haemarthrosis



$FIX^{-/-}$: Hemophilia B mice received needle puncture of the knee joint capsule to induce intra-articular (I.A.) hemorrhage
WT NS: WT mice received I.A. injection of 5 µl of normal saline (NS) at the time of joint injury
WT Blood: WT mice received I.A. injection of 5 µl of autologous blood at the time of joint injury

Knee joint tissues were harvested from separate groups of mice at the indicated time points (day 0 to 8 weeks after wounding). Synovitis was scored using the Valentino Score (scale of 0-10)[4,5] Stains were: H&E for synovitis, Prussian blue for iron, CD68+ for macrophages, VWF for vessels.

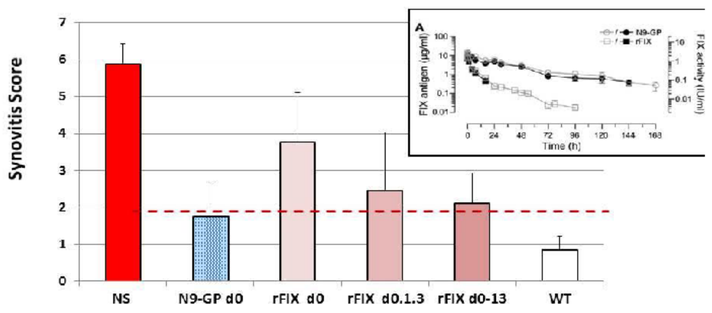




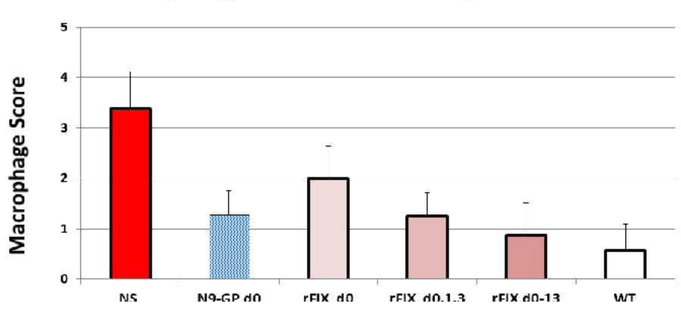
$FIX^{-/-}$ or WT mice experienced induced hemarthrosis.
$FIX^{-/-}$ mice were treated within 20 minutes of wounding with either I.V. NS(**$FIX^{-/-}$ NS**), OR rFIX 250 IU/kg at day 0 (**rFIX d0**), OR 2 doses of rFIX 250 IU/kg at day 0 and day 7 ("**rFIX d0,d7**") .
Two additional groups of $FIX^{-/-}$ mice were treated with N9-GP 250 IU/kg at day 0 ("**N9-GP d0**") OR 2 doses of N9-GP at day 0 and day 7 ("**N9-GP d0,d7**").

TOP: MicroCT examination of the left knee joint of mice taken at 2 weeks after joint wounding shows relative roughening of the contours of the articulating surface of bones and (inset) the relative density of trabecular bone. BOTTOM: Mathematical modeling of deviation from perfectly smooth surface (assigned a value of 1) allows quantitation of relative protection from roughening of the articulating surfaces of the joint.

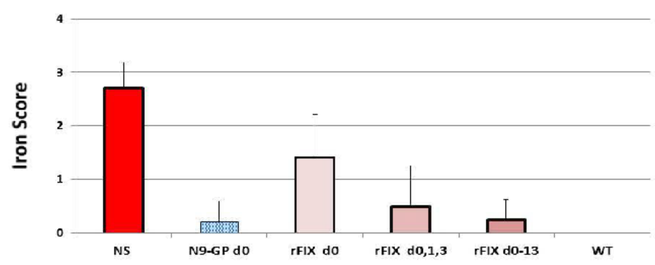
Synovitis: N9-GP vs. Repeated rFIX



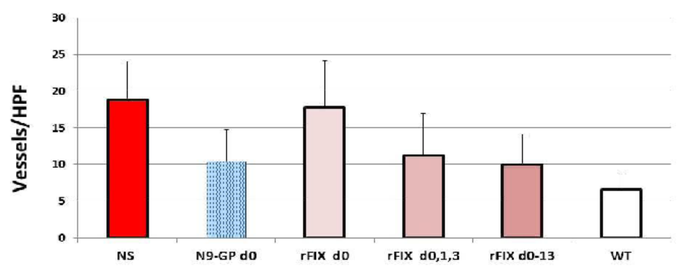
Macrophage: N9-GP vs. Repeated rFIX



Iron: N9-GP vs. Repeated rFIX



Angiogenesis: N9-GP vs. Repeated rFIX



$FIX^{-/-}$ or WT mice underwent induced hemarthrosis. $FIX^{-/-}$ mice were treated with either I.V. normal saline ("**NS**"), OR single dose N9-GP 250 IU/kg ("**N9-GP d0**") OR rFIX 250 IU/kg ("**rFIX d0**"), OR 3 doses rFIX at day 0, 1 and 3 ("**rFIXd0,1,3**") OR 8 doses of rFIX on an alternate day schedule over two weeks("**rFIX d0-13**"). Inset (top left) shows the reported PK of N9-GP in mice.[6]

METHODS

Comparison of wound healing in the absence of therapy $FIX^{-/-}$ or WT mice received unilateral needle puncture of the knee joint capsule to induce intraarticular (I.A.) hemorrhage. An additional group of WT mice received I.A. injection of 5 µl of autologous blood at the time of needle puncture.
Evaluation of replacement clotting factor **N9-GP is a recombinant FIX that has been modified with a 40 kDa polyethylene glycol (PEG) molecule to prolong the FIX half-life.** $FIX^{-/-}$ mice were treated following wounding with either I.V. normal saline (NS), with single dose N9-GP 250 U/kg, or with rFIX 250 U/kg as a single dose at the time of hemorrhage or one of two multiple-dose schedules. One group received 250 IU/kg of rFIX at the time of injury, on the next day, and again on the day 3. Another group received 8 replacement doses of rFIX given on an alternate days throughout the entire two weeks following hemarthrosis. Joints were examined two weeks after the injury using histology and microcomputed tomography (mCT) at 10 micron resolution.

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