The Effects of a Long-Acting Factor IX Product (N9-GP) on Wound Healing

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Background

Recent efforts in hemophilia B therapy have focused on extending the half-life of FIX. To this end, glycopegylated factor IX (nonacog beta pegol, N9-GP) is under development and has demonstrated efficacy in a clinical trial (1). N9-GP bears a 40-kDa polyethylene glycol (PEG) moiety on the activation peptide of FIX and has a half-life of 93 hours (2).

Hemophilia B (HB, FIX knockout) mice have a defect in cutaneous wound healing compared to wild-type (WT), with delayed time to wound closure, reduced macrophage influx, increased iron deposition and excessive angiogenesis (3). In this model, hemostatic therapy for 7 days is required to normalize healing (4). The purpose of the present study was to use the wound healing model to evaluate the ability of a single dose of N9-GP to provide long-term hemostasis and support wound healing in HB mice .

We and others have proposed that extra-vascular FIX plays an important role in hemostasis and wound healing. We hypothesized that N9-GP might not enter the extravascular space in sufficient amounts to improve wound healing in HB mice.

Methods

HB mice were untreated or received 285 or 470 IU/kg N9-GP intravenously 30 min before wounding. A 3mm punch biopsy skin wound was made down to (but not into) the muscle (3). Wound dimensions were measured daily. At days 2, 4, 6, 8 and 10, some mice were sacrificed and skin samples were collected, fixed in 10% formalin for 24 hrs. Staining for macrophage influx, iron deposition and vessels was as described (3).

Bleeding was assessed in the Whinna bleeding model (5), which has been shown to be responsive to clinically relevant levels of FVIII. In this model the saphenous vein is transected with a 23G needle, then a longitudinal incision made in the distal portion of the vessel. Blood is wicked away with a Kimwipe until haemostasis is achieved; the clot is then disrupted and blood wicked away until bleeding stopps again. Clot disruption is repeated after every incidence of haemostasis until 30 minutes after the initial injury, and all clot disruption and haemostasis time points are recorded .

Results

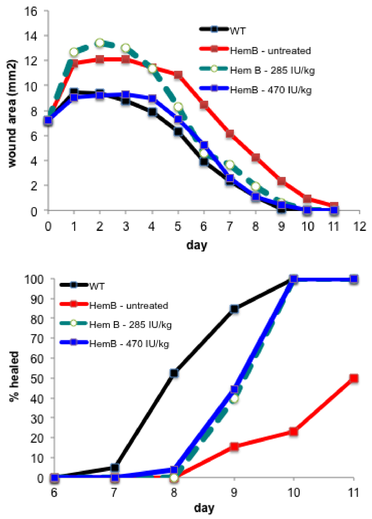

Figure 1. N9-GP Improves wound healing in HB mice. Mice were injected with the indicated treatment on day 0, immediately before placement of a punch biopsy wound on each animal.

Top panel – Wound areas (shown are mean area ± SEM) on untreated HB mice are significantly different from WT on all days; mice treated with 470 IU/kg N9-GP are similar to WT. The 285 IU/kg N9-GP and WT are significantly different on days 1-5 and 9 ($p<0.05$).

Bottom Panel - % of mice healed on each day. Both doses of N9-GP improved the time to healing, with all wounds closed by 10 days. Each group began with at least 26 mice, with some being sacrificed on even-numbered days for histology; at least 10 mice remained in each group at complete wound closure

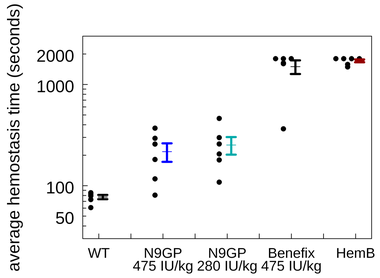

Figure 2. Hemostasis remains enhanced in HB mice 7 days after iv injection of N9-GP. Average time to hemostasis in a saphenous vein bleeding model is shown for WT and HB mice treated 7 days earlier with N9-GP or BeneFIX. Each dot represents an animal (6 mice/group), with lines indicating mean ± SEM. The N9-GP-treated HB mice were significantly different from untreated ($p<0.05$), but Benefix-treated were not different from untreated HB mice at 7 days after infusion .

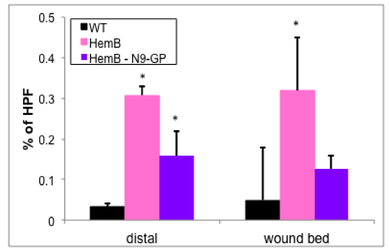

Figure 3. N9-GP improves, but does not completely normalize, tissue iron deposition after wounding. Data are mean percentage (± SEM) of a high power field (HPF) occupied by iron staining within the wound bed and at sites distal to the wound bed on day 6 after wounding. In all of the histological analyses, there were no significant differences between the two N9-GP doses. Therefore, we combined the data into a single N9-GP-treated group.
* indicates $p<0.05$ compared to WT.

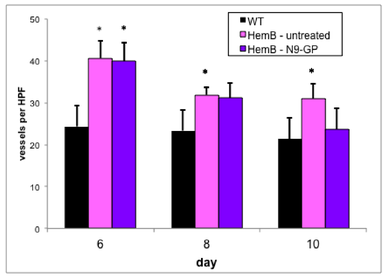

Figure 4. N9-GP improves, but does not normalize wound angiogenesis.
Data shown are the number of vessels per 40x field (mean ± SEM) on days 6 and 8 after wounding. HB and N9-GP-treated groups are both are significantly different from WT ($p<0.05$) at day 6. Only the untreated HB group is significantly different from WT on days 8 and 10.

Summary & Conclusions

- As expected, hemostatic activity persisted 7 days after iv injection of 470 or 285 IU/kg N9-GP
- Unlike wild-type FIX, a single iv injection of 285 or 470 IU/kg N9-GP normalized the time to closure of cutaneous punch biopsy wounds
- N9-GP did not normalize wound site iron deposition or angiogenesis following wounding
- **We conclude that N9-GP, that remains primarily in the intravascular space, can significantly improve wound healing in a mouse model of HB**

References

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Conflict of Interest

This work was supported by a grant from Novo Nordisk and ME is an employee of Novo Nordisk