In-vivo efficacy of human recombinant Factor IX produced by the Hepatoma cell line HuH-7

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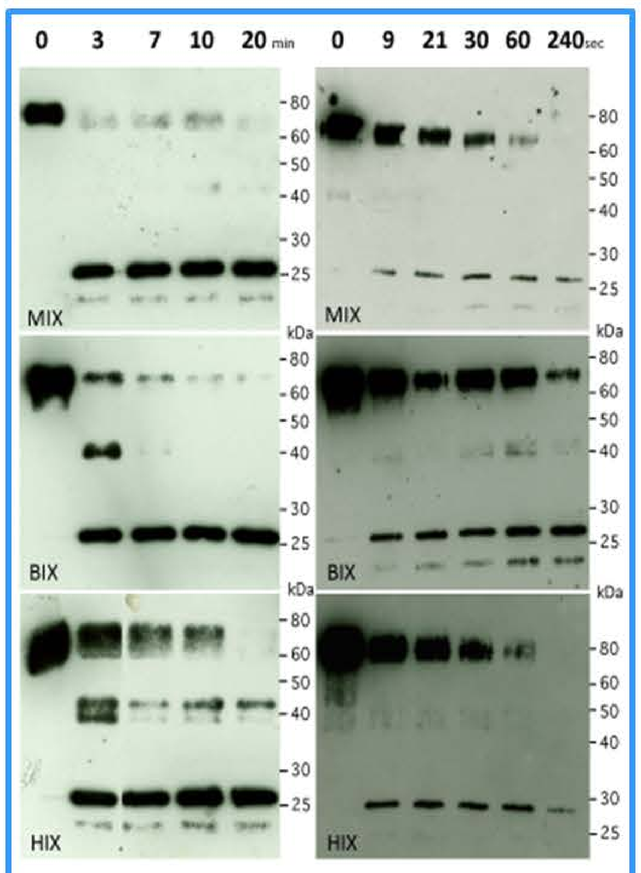
OBJECTIVES

For hemophilia B treatment, a strong need exists for a new recombinant FIX (rFIX) preparation which could be administered in lower dosage than conventional rFIX from CHO cells for obtaining the same in-vivo recovery than observed with plasma-derived FIX. This is accompanied by the desire to improve cell lines in order to achieve higher titers and a better product quality. Our previous studies described a methodology for obtaining a highly efficient cellular clone from a human hepatoma-cell line Huh-7 secreting FIX. This rFIX (HIX) has been described having better post-translational modification (PTM) profile than rFIX produced by CHO cells (*Enjolras et al., 2012*).

METHODS

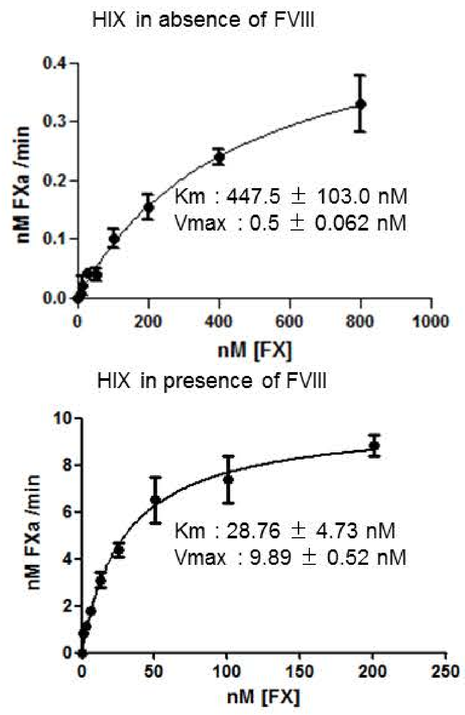
HIX has been produced in a bioreactor (Wave® 2/10L, GE Healthcare) in Ultraculture medium (Lonza®) and then purified from supernatant via anion-exchange and heparin-affinity chromatographies (HiTrap FF-Sepharose and HiTrap Heparin, GE Healthcare).
To study the biochemistry and clotting function of HIX, Benefix® (BIX) and Mononine® (MIX), activation courses of molecules have been studied using FXIa or FVIIa-TF. Aliquots were removed at timed intervals and the reaction was visualized with SDS-PAGE.
Activation of FX by HIXa/Factor VIIIa (0.4nM)/phospholipid (Intrinsic Tenase Complex) by studied as described by (Larson et al., 1996).
The three molecules were then administrated (i.v.) to FIX-knockout mice at a dosage of 10µg/20g body weight (17 to 20 animals per molecule). Two minutes after injection, blood samples were collected in presence of 10% citrate and subjected to human FIX-specific-ELISA and thrombin generation tests (TGT).

RESULTS



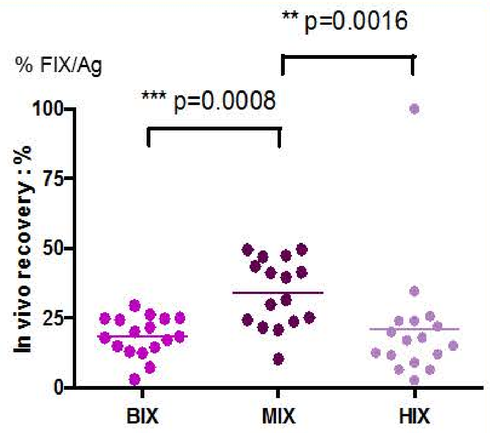
Activation by FVIIa/TF **Activation by FXIa**

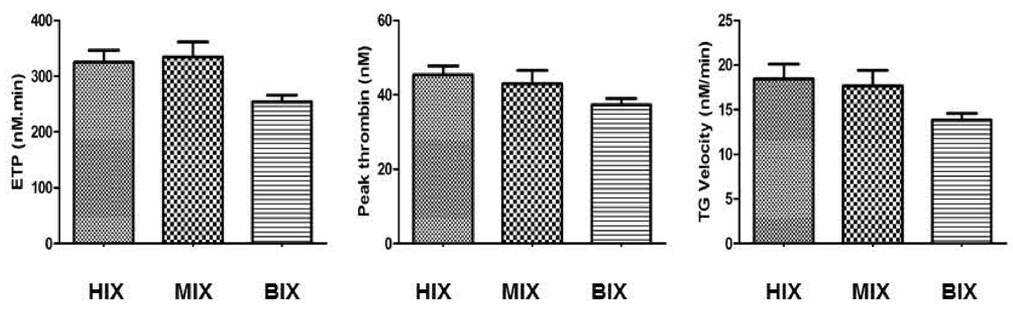
To study the biochemistry and clotting function of HIX, activation studies revealed that HIX was totally activated after 20 min in presence of FVIIa/TF and after 4 min in presence of FXIa as did activation of BIX and MIX.



In the absence or presence of FVIIIa, kinetic parameters of the HIXa were evaluated and appeared normal. For example, in presence of FVIIIa the Km for HIXa molecule was similar to those observed for recombinant FIXawt already described by *Lin et al., 2010* (Km 23.10 ± 4.87 ; Vmax : 24.33 ± 1.53) and *Kao et al., 2013* (Km : 30.12 ± 9.98 ; Vmax : 34.10 ± 1.55).

Two minutes after injection of three molecules to FIX-knockout mice, blood samples were collected and subjected to human FIX-ELISA and thrombin generation tests (TGT). Circulating HIX did not present any significant difference in term of antigen value with Benefix® (BIX).





Mann Whitney	HIX vs BIX	MIX vs BIX	HIX vs MIX
ETP	p=0.014	p=0.0036	p=0.41
Peak IIa	p=0.02	p=0.04	p=0.89
TG Velocity	p=0.023	p=0.05	p=0.65

Though, intriguingly, TGT values were clearly exhibiting a better velocity and a higher thrombin peak height for HIX than for BIX and MIX. These data suggested that HIX may improve *in-vivo* coagulant efficacy in comparison with the two commercial FIX injected at the same dose.

CONCLUSIONS

In conclusion, HuH-7 cells could represent an effective cellular system for production of rFIX. If enhancement of in-vivo recovery was not demonstrated for HIX versus Benefix®,TGT studies demonstrated that this rFIX from human hepatoma cell clone could be used *in-vivo* at the same concentration than Benefix® to achieve Mononine® efficacy.

References

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WFH2014 35-P-W
Clotting Factor Concentrates
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Poster presented at: WFH 2014 World Congress
PosterSessionOnline.com