Introduction

- Highly purified Factor VIII:C (FVIII:C) concentrates, extracted from plasma or recombinant and B-domainless.
- Chromogenic assay BIOPHEN Factor VIII:C* (Xa generation).
- FVIII:C deficient plasma, immunodepleted (FVIII:C < 0.1%).
- Assay buffer: Tris-Saline buffer supplemented with 1% BSA and PEG.
- Concentrates: tested pre-diluted in FVIII:C deficient plasma or in the assay buffer (1 unit/ml), then diluted 1:40 (standard assay dilution) with the assay diluent.
- Method calibrated with the NIBSC secondary plasma standard, lot 3.
- Isolation of plasma fractions: normal plasma or FVIII:C deficient plasma
- Methods: fractionation. Fractions were then added to the FVIII:C diluent in order to analyse which ones support the FVIII:C activity.
- The most reactive fraction was then further purified using ion exchange chromatography and gel filtration.

* Patent pending

Results

- Assay calibration curves yielded A405 values from about 2.00 (200% FVIII:C) to 0.020 (0%).
- When highly purified extracted or recombinant FVIII:C were tested diluted in protein plasma fractions. The first one contained ceruloplasmin and the second one α-1-Acid-Glycoprotein or AGP. We confirmed the role of these two proteins by testing purified ceruloplasmin or AGP from Sigma in the diluent. The combination of the dialysable fraction with AGP was the most active for FVIII:C activity recovery.
- By testing the different divalent ions (Ca++, Li++, Ni++, Cu++, Mg++, Mn++., Zn++) we showed that adding Cu++, or AGP in the diluent was able to restore most of the FVIII:C reactivity. Using Cu++ and AGP, was still more effective and slightly enhanced by trace amounts of Zn++.
- This observation allowed us to develop a diluent containing Cu++, Zn++, and AGP.
- Recovery of both concentrates was then restored to about 100% and 85%. The supplemented diluent did not change the FVIII:C concentrations measured in plasma.

Conclusions

- Presence of Cu**, and of Cu**, or Zn**-binding proteins is required for allowing a full expression of FVIII:C activity in chromogenic assays.
- An optimized diluent containing Cu++ (the most relevant), potentiated by Zn++ (trace amounts) and AGP (which some isoforms can bind Cu++), was developed for testing FVIII:C concentrates.
- Using this diluent, highly purified FVIII:C concentrates or recombinant Factor VIII:C preparations (B-domainless) yield the same reactivity than when diluted in FVIII:C deficient plasma. FVIII:C measurements in plasma remain unchanged.

Material and Methods

- FVIII:C concentrates extracted from plasma or recombinant and B-domainless.
- Chromogenic assay BIOPHEN Factor VIII:C* (Xa generation).
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References