Universiteit Utrecht

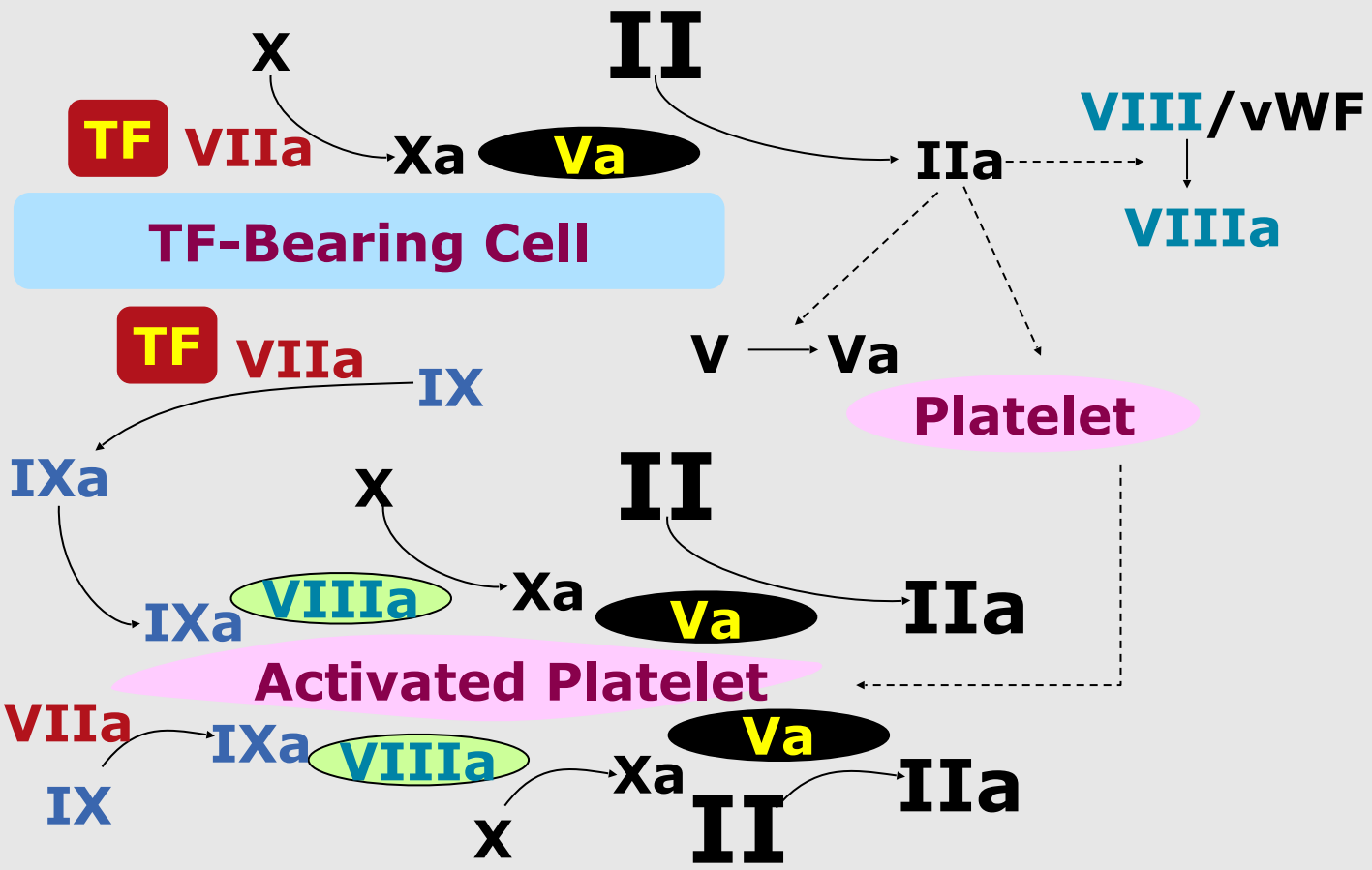
elevate

[Faculty of Science
Pharmaceutical Sciences]

Quality and Safety

Example: Factor VIII

Hemophilia A



Hoffman et al. Blood Coagul Fibrinolysis 1998;9(suppl 1):S61

History of Clotting Factor Concentrates

- Prior to 1950: whole blood
- 1952: Hemophilia A distinguished from B
- 1950-1960: Fresh Frozen Plasma and Cryoprecipitate
- Early 1970s: Commercial plasma-derived factor concentrates
- Mid-late 1970's: Home infusion practices
- 1981: First AIDS death in the Hemophilia community

Alessandro Gringeri - Blood Transfus. 9 (2011): 366–370

History of Clotting Factor Concentrates Continued

- Mid-1983: Factor concentrates heat treated for hepatitis
- 1985: All products heat treated for viral inactivation
- 1987: Recombinant factor concentrates
- 1992: Recombinant factor VIII (formulated with Albumin)

Factor VIII and factor IX concentrates licensed in Italy (listed in alphabetical order).

Product	Manufacturer	Production characteristics	
		Purification	Viral inactivation
Plasma-derived FVIII concentrates			
Alphanate®[1]	Grifols	Heparin ligand chromatography	S/D, dry heat
Beriate®	CSL Behring	Ion exchange chromatography	Pasteurisation
Emoclot D.I.®	Kedrion	Ion exchange chromatography	S/D, dry heat
Fanhdi®[1]	Grifols	Heparin ligand chromatography	S/D, dry heat
Haemate P®[1]	CSL Behring	Multiple precipitation	Pasteurisation
Haemoctin®	Biotest	Ion exchange chromatography	S/D, dry heat
Talate®[1]	Baxter	Ion exchange chromatography	Detergent, vapour
Recombinant FVIII concentrates			
Advate®[2]	Baxter	Immunoaffinity chromatography	S/D
Helixate NexGen®[3]	CSL Behring	Immunoaffinity chromatography	S/D, ultrafiltration
Kogenate Bayer®[3]	Bayer Healthcare	Immunoaffinity chromatography	S/D, ultrafiltration
Recombinate®[4]	Baxter	Immunoaffinity chromatography	-
Refacto AF®[5]	Pfizer	Immunoaffinity chromatography	S/D, nanofiltration

Morfini et al. Blood Transfus. 2013 11(Suppl 4): s55–s63.

History of Clotting Factor Concentrates Continued

- Early 1990s: factor VIII-inhibitor outbreaks
- 2001: 2nd generation recombinant factor VIII (sucrose)
- 2008: 3rd generation recombinant factor VIII ('protein'-free)

Alessandro Gringeri - Blood Transfus. 9 (2011): 366–370

Quality & Safety – Clinical study

- Guideline on the clinical investigation of recombinant and human plasma-derived factor VIII products (EMA: 28 jan 2016)

4. Efficacy: General aspects

Efficacy needs to be demonstrated in clinical trials to be conducted before marketing authorisation combined with the commitment to perform (a) post-authorisation investigation(s) to collect additional clinical data and to bridge in the long-term between the outcome from clinical trials and from routine use. When clinically evaluating human plasma-derived or recombinant coagulation factors for the treatment of haemophilia A, the initial trial typically examines the pharmacokinetics of the principal active factor. Appropriate pharmacokinetic data (incremental recovery, half-life, area under the curve (AUC), and clearance) are the most important surrogate endpoints for efficacy of a new factor VIII product. Furthermore, clinical efficacy of factor VIII treatment (e.g. prophylaxis, on demand) should be assessed during a period of a minimum of 50 exposure days by the patients themselves and treating physicians.

Quality & Safety – Clinical study

- Guideline on the clinical investigation of recombinant and human plasma-derived factor VIII products (EMA: 28 jan 2016)

5. Safety: General aspects

Safety aspects of factor VIII products include viral safety, immunogenicity and other adverse events. For recombinant products, the use of non-human cell-lines raises the possibility of different contaminants and altered immunogenic potential.

Quality & Safety – Clinical study

- Guideline on the clinical investigation of recombinant and human plasma-derived factor VIII products (EMA: 28 jan 2016)

6.1.1. Potency measurements

The potency assignments for factor VIII products covered by European Pharmacopoeia (Ph. Eur.) monographs have to be performed with the Ph. Eur chromogenic assay. However, 'with the agreement of the competent authority, alternative methods of analysis may be used for control purposes, provided that the methods used enable an unequivocal decision to be made as to whether compliance with the standards of the monographs would be achieved if the official methods were used.'[2].

Quality & Safety – Raw materials

FR Document en Français | PDF | Knowledge Database | General Monographs | General Notices

01/2008:1643

HUMAN COAGULATION FACTOR VIII (rDNA)

Factor VIII coagulationis humanus (ADNr)

DEFINITION

Human coagulation factor VIII (rDNA) is a freeze-dried preparation of glycoproteins having the same activity as coagulation factor VIII in human plasma. It acts as a cofactor of the activation of factor X in the presence of factor IXa, phospholipids and calcium ions.

Human coagulation factor VIII circulates in plasma mainly as a two-chain glycosylated protein with 1 heavy (relative molecular mass of about 200 000) and 1 light (relative molecular mass 80 000) chain held together by divalent metal ions. Human coagulation factor VIII (rDNA) is prepared as full-length factor VIII (octocog alfa), or as a shortened two-chain structure (relative molecular mass 90 000 and 80 000), in which the B-domain has been deleted from the heavy chain (moroctocog alfa).

Full-length human rDNA coagulation factor VIII contains 25 potential *N*-glycosylation sites, 19 in the B domain of the heavy chain, 3 in the remaining part of the heavy chain (relative molecular mass 90 000) and 3 in the light chain (relative molecular mass 80 000). The different products are characterised by their molecular size and post-translational modification and/or other modifications.

PRODUCTION

Human coagulation factor VIII (rDNA) is produced by recombinant DNA technology in mammalian cell culture. It is produced under conditions designed to minimise microbial contamination.

Purified bulk factor VIII (rDNA) may contain added human albumin and/or other stabilising agents, as well as other auxiliary substances to provide, for example, correct pH and osmolality.

The specific activity is not less than 2000 IU of factor VIII:C per milligram of total protein before the addition of any protein stabiliser, and varies depending on purity and the type of modification of molecular structure of factor VIII.

The quality of the bulk preparation is controlled using one or more manufacturer's reference preparations as reference.

Quality & Safety – Raw materials

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MANUFACTURER'S REFERENCE PREPARATIONS

During development, reference preparations are established for subsequent verification of batch consistency during production, and for control of bulk and final preparation. They are derived from representative batches of purified bulk factor VIII (rDNA) that are extensively characterised by tests including those described below and whose procoagulant and other relevant functional properties have been ascertained and compared, wherever possible, with the International Standard for factor VIII concentrate. The reference preparations are suitably characterised for their intended purpose and are stored in suitably sized aliquots under conditions ensuring their stability.

PURIFIED BULK FACTOR VIII (rDNA)

The purified bulk complies with a suitable combination of the following tests for characterisation of integrity of the factor VIII (rDNA). Where any substance added during preparation of the purified bulk interferes with a test, the test is carried out before addition of that substance. Where applicable, the characterisation tests may alternatively be carried out on the finished product.

Specific biological activity or ratio of factor VIII activity to factor VIII antigen. Carry out the assay of human coagulation factor VIII (2.7.4). The protein content, or where a protein stabiliser is present, the factor VIII antigen content, is determined by a suitable method and the specific biological activity or the ratio of factor VIII activity to factor VIII antigen is calculated.

Protein composition. The protein composition is determined by a selection of appropriate characterisation techniques which may include peptide mapping, Western blots, HPLC, gel electrophoresis, capillary electrophoresis, mass spectrometry or other techniques to monitor integrity and purity. The protein composition is comparable to that of the manufacturer's reference preparation.

Molecular size distribution. Using size-exclusion chromatography (2.2.30), the molecular size distribution is comparable to that of the manufacturer's reference preparation.

Peptide mapping (2.2.55). There is no significant difference between the test protein and the manufacturer's reference preparation.

Carbohydrates/sialic acid. To monitor batch-to-batch consistency, the monosaccharide content and the degree of sialylation or the oligosaccharide profile are monitored and correspond to those of the manufacturer's reference preparation.

FINAL LOT

It complies with the requirements under Identification, Tests and Assay.

Excipients: 80 per cent to 120 per cent of the stated content, determined by a suitable method, where applicable.

Quality & Safety – Raw materials

CHARACTERS

Appearance: white or slightly yellow powder or friable mass.

IDENTIFICATION

A. It complies with the limits of the assay.

B. The distribution of characteristic peptide bands corresponds with that of the manufacturer's reference preparation (SDS-PAGE or Western blot).

TESTS

Reconstitute the preparation as stated on the label immediately before carrying out the tests (except those for solubility and water) and assay.

Solubility. It dissolves within 5 min at 20-25 °C, giving a clear or slightly opalescent solution.

pH (2.2.3): 6.5 to 7.5.

Osmolality (2.2.35): minimum 240 mosmol/kg.

Water. Determined by a suitable method, such as the semi-micro determination of water (2.5.12), loss on drying (2.2.32) or near-infrared spectroscopy (2.2.40), the water content is within the limits approved by the competent authority.

Sterility (2.6.1). It complies with the test for sterility.

Bacterial endotoxins (2.6.14): less than 3 IU in the volume that contains 100 IU of factor VIII activity.

ASSAY

Carry out the assay of human coagulation factor VIII (2.7.4).

The estimated potency is not less than 80 per cent and not more than 125 per cent of the stated potency. The confidence limits ($P = 0.95$) are not less than 80 per cent and not more than 120 per cent of the estimated potency.

How to check your formulated protein?

- What is important for your formulated protein?
  - Primary structure?
  - Folding?
  - Glycosylation?
  - Monomers vs dimers/multimers/aggregates?
- How to investigate?
  - Multiple techniques available (e.g. chapter 2 Pharmaceutical Biotechnology)
  - What information and how reliable?
  - Good enough?
- Post-marketing surveillance!

Lessons

- Proteins aren’t small molecules
- Production, formulation and characterization
- Post-marketing surveillance!