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NOT FOR PUBLICATION

COMMERCIAL - IN CONFIDENCE

COMMITTEE ON SAFETY OF MEDICINES

Sub-Committee on Biological Substances

Product licence Number	PL/0215/0003
Report by	Dr. Thomas
Meeting	January 1973
Therapeutic Class	Blood product

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MEDICINES ACT 1968 - APPLICATION FOR A PRODUCT LICENCE

Summary and Report

1. PRODUCT SUMMARY

1.1 Name of Product:

Kryobulin - Human antihaemophilic fraction.

1.2 Period of Validity:

5 years.

1.3 Licence to be held by:

Serological Products Limited,
Regina House,
5 Queen Street,
London EC4.

1.4 Description:

A sterile lyophilised preparation of purified Factor VIII (antihaemophilic factor).

1.5 Manufacturer:

Österreichisches Institut Für Haemoderivate Ges M.B.H. (Immuno Ag),
Vienna,
Austria.

1.6 Proposed Method of Sale:

Prescription only (Hospitals and Haemophilia Treatment Centres)

1.7 Consideration of this application by other Sub-Committees

a Sub-Committee on Chemistry, Pharmacy and Standards - not referred.

b Sub-Committee on Toxicity and Clinical Trials - February 1973.

2. PHARMACEUTICAL FORM

The product is a sterile, lyophilized substance. After reconstitution it consists of:-

(a) A solution for intravenous injection, containing 100, 200 or 500 units of Factor VIII, and

(b) A solution for intravenous transfusion, containing 500 or 1000 units of Factor VIII.

3. COMPOSITION

When dissolved in the volume of Water for Injection B.P., stated on the label, the solution contains not less than 5 units (± 10%) per ml; the solution also contains not more than 2.5% w/v fibrinogen and not more than 3% w/v of total protein.

4. PHYSICAL CHARACTERISTICS

A white to yellowish amorphous powder or friable solid without any characteristic odour.

5. CLINICAL USE

5.1 Recommended Clinical Use

Treatment of bleeding caused by Factor VIII deficiency in patients with:

Haemophilia A
von Willebrand's Disease
Haemophilia caused by Factor VIII inhibitors
Thrombocytopenia with decreased Factor VIII activity
Combined coagulation disorders, also including reduced Factor VIII activity (consumption coagulopathy, autoimmune diseases, neoplasms, etc).

5.2 Route of Administration

(i) intravenous injection

100 units of Factor VIII (10 ml. injection)
250 units of Factor VIII (20 ml. injection)
500 units of Factor VIII (20 ml. or 40 ml. injection)

(ii) intravenous transfusion

500 units of Factor VIII (100 ml. transfusion)
1000 units of Factor VIII (100 ml. transfusion)

5.3 The Recommended dosage for Adults and Children

The amount required may vary considerably according to the response of the individual. As a simple rule, to achieve an increase in the Factor VIII concentration of 1%, it is necessary to administer one unit of Factor VIII per kilogramme of bodyweight.

Initial treatment requires doses at shorter intervals than maintenance therapy, because of excessive Factor VIII consumption. Replenishment of the treatment should be controlled by a specific Factor VIII assay, as partial thromboplastin times result in a less accurate value when large quantities of Kryobulin must be used. If large quantities are used volume overloading may arise and partial removal of patient plasma by plasmapheresis should be considered.

Bleeding from skin, nose and oral mucous membrane

The initial dose should be 10 units of Factor VIII per kg of bodyweight followed by a maintenance dose of 5 to 10 units of Factor VIII per kg of bodyweight at 6 to 12-hourly intervals.

Haemarthrosis

Approximately 10 units of Factor VIII per kg of bodyweight should be given as an initial dose. The maintenance dose should be 5 to 10 units of Factor VIII per kg of bodyweight at 6 to 12-hourly intervals.

Heavy bleeding into muscles

The initial dose ranges from 15 to 20 units of Factor VIII per kg of bodyweight followed by 10 units of Factor VIII per kg of bodyweight at 6-hourly intervals from the first to second day and at 12-hourly intervals from the third to the fifth day.

Haematuria

An initial dose of 15 to 20 units of Factor VIII per kg of bodyweight will be sufficient. For maintenance, 10 units of Factor VIII per kg of bodyweight should be given at 12-hourly intervals.

Major surgery on haemophilic patients

For initial treatment, the administration of at least 25 to 50 units of Factor VIII per kg of bodyweight is recommended. The maintenance dose should be 20 to 40 units per kg of bodyweight starting at 4-hourly intervals from the first to fourth day, then at 8-hourly intervals from the fifth to eighth day and later, at 12-hourly intervals until all wounds are healed.

The effect of treatment must be checked daily. Factor VIII activity should not fall below 50% of the normal average value of 100%. Besides the repeated control of Factor VIII, tests for occasionally developing Factor VIII inhibitors should also be carried out.

Dental extractions

The amount of Factor VIII to be infused depends on the number and type of teeth to be extracted and on the severity of the haemophilia.

6. STANDARD PROVISIONS

No exemptions required.

7. MANUFACTURE AND ASSEMBLY

The product will be manufactured, filled under sterile conditions into final containers, freeze-dried for stability in storage, labelled and packed by Österreichisches Institut Für Haemoderivate Ges. M.B.H., Industriestrasse 72, 1220 Vienna, Austria, and imported into the U.K. by Serological Products Limited. The product will be imported in labelled final containers. (See 9 below).

8. QUALITY CONTROL

(a) Quality control will be exercised for identity and Factor VIII activity.

(b) Person responsible for deciding whether a batch of the product is of acceptable quality for marketing: Prof. Dr. W. Zischka-Konorsa M. Dr., a.o. Professor of Pathology, the Faculty of Medicine, Vienna University.

Requirements set out in the Therapeutic Substances Act relating to preparations of human blood, and Regulations 4 g, h, e, i, of the Therapeutic Substances (Manufacture and Importation) General Regulations 1963 Statutory Instrument 1963 number 1450, will be complied with.

9. CONTAINERS

(a) KRYOBULIN - 100 units Factor VIII (injection)
- 250 units Factor VIII (injection)
- 500 units Factor VIII (injection)

is filled into glass puncture bottles, labelled and packed into labelled cartons together with a labelled glass puncture bottle containing Water for Injections B.P., three disposable needles and one disposable syringe.

(b) KRYOBULIN - 500 units Factor VIII (transfusion)
-1000 units Factor VIII (transfusion)

is filled into glass transfusion flasks, labelled and packed into labelled cartons together with a labelled glass puncture bottle containing Water for Injections B.P., one transfer tube and one transfusion set with filter.

The product will be stored at a temperature between 2° and 6°C, protected from light.

10. LABELLING

Cartons and final containers will be labelled in accordance with the regulations provided for under T.S.A., and the B.P. The text of the proposed labels are included in the submission as enclosures Nos. 1 to 4.

11. METHOD OF SALE AND SUPPLY

The product will be made available to Hospitals and Haemophilia Treatment Centres.

11.1 Labelling

A copy of the draft package insert is included in the Submission. It contains, inter alia, the following information:

Manufacture and Composition

Kryobulin is prepared from the pooled plasma of healthy donors and freeze-dried for stabilization. All donors, whose plasma is used for the production of Kryobulin, are tested at each donation for their GPT level and the absence of Hepatitis Associated Antigen (HAA). Any donor, who has a history of a pathological transaminase level or positive HAA test is permanently excluded from the donor programme. Despite these precautions, the risk of transmission of serum hepatitis can only be diminished and not completely eliminated.

Precautions

Though the danger of volume overloading is small with the use of Kryobulin, in cases of major surgery, the control of the patient's central venous pressure, blood pressure and chest-X-ray should be carried out repeatedly as required. If symptoms of volume overloading become apparent, therapeutic plasmapheresis is recommended.

In patients suffering from consumption coagulopathy with a significantly low Factor VIII level, intravascular coagulation must be interrupted by the administration of Heparin before the therapy with Kryobulin is started.

Side Effects

Side effects are rarely observed during treatment with Kryobulin though the following reactions may occur:

(a) Allergic Reactions:

All forms of allergic reactions from mild and temporary urticaria to severe anaphylactic shock are possible when human plasma derivates are administered. If these occur, treatment with Kryobulin must be interrupted at once. Allergic reactions should be controlled with antihistamines and gluco-corticoids and routine shock-treatment given for anaphylactic shock.

(b) Despite the precautions taken in the selection of donors, the risk of transmission of homologous serum hepatitis cannot be entirely excluded when administering human coagulation factors.

(c) During every type of therapy involving blood or Factor VIII concentrates, the appearance of a circulating Factor VIII inhibitor is possible. The time at which such an inhibitor is produced cannot be predicted and neither depends on the amount of Factor VIII administered nor on the frequency of administration.

Shelf Life and Storage

One and a half years when stored between 2° and 6°C, protected from light.

Packs

(a) Kryobulin (injection)

- 1 puncture bottle containing lyophilized Kryobulin equivalent to 100, 250 or 500 units of Factor VIII,
- 1 puncture bottle containing Water for Injection, B.P. (10, 20 or 40 ml.)
- 1 disposable syringe; 1 filter; 3 disposable needles.

(b) Kryobulin (transfusion)

- 1 transfusion bottle containing lyophilized Kryobulin equivalent to 500 or 1000 units of Factor VIII,
- 1 puncture bottle containing Water for Injection, B.P. (100 ml.)
- 1 transfer tube
- 1 transfusion set with filter

12. CHEMISTRY AND PHARMACY

12.1 Names

Approved Name

Human Antihaemophilic Fraction

Monograph Name

Human Antihaemophilic Fraction (British Pharmacopoeia, Addendum 1971, Page 12).

U.S. Adopted Name

Antihaemophilic Globulin (Human)

International Non-Proprietary Name

Antihaemophilic Globulin (Human); Antihaemophilic Factor (Human); Factor VIII Concentrate.

Proprietary or Trade Name

Currently marketed in Europe (mainly Austria, West Germany and Italy) and overseas by the manufacturers, under the trade name of Kryobulin.

12.2 Description

Kryobulin - Human Antihaemophilic Fraction is a preparation of human blood as defined in Regulation 2 (1) of S.I. 1963, No. 1456. It meets the requirements set forth in the B.P. 1968, page 116, items (a) to (d) concerning whole human blood and also complies with the monograph for Human Antihaemophilic Fraction included in the Addendum 1971 to the B.P.

13. METHOD OF MANUFACTURE

Factor VIII concentrate is prepared by large scale fractionation of fresh human plasma, obtained from a plasma pool of 1000 HAA negative donors. Plasma is obtained from human donors aged between 18 and 65 years at 6 plasmapheresis stations in Austria and Germany.

13.1 Criteria for accepting donors prior to donation

Good conditions of health, normal temperature, no increased transaminase values (above 15 I.U. per litre), HAA - negative, cardiolipin negative, and haemoglobin not less than 12.5% w/v (female donors), or 13.3% w/v (male donors) (B.P.), protein at least 6%.

13.2 Equipment used for Plasmapheresis

Sterile, pyrogen-free disposable plastic bags containing 75 ml Anticoagulant Citrate Dextrose Solution U.S.P. XVIII, Formula A: contains 16.8 m.eq of Sodium.

Each ml contains: 0.8 g Citric Acid (hydrous) U.S.P.
2.2 g Sodium Citrate U.S.P.
2.45 g Dextrose (hydrous) U.S.P.

13.3 Plasmapheresis

About 500 ml blood are collected. After completion of the bleeding the taking needle remains in the vein; a new transfusion set is adjusted and Ringer solution transfused during the time the blood is centrifuged and the plasma separated from the erythrocytes. The erythrocytes are suspended in Ringer's solution and re-transfused to the donor. A precise system is employed, including exact labelling of the bottles and determination of the donor's blood group as well as his erythrocytes suspension before re-transfusion. The process is then repeated.

After separation of plasma and cells, the plasma is centrifuged again for 30 min/2500 rpm and the supernatant if taken off, frozen and stored.

The entire operation is supervised and controlled by a registered medical practitioner.

13.4 Precautions taken during Plasmapheresis

(a) Only sterile, pyrogen-free, plastic equipment is used and an aseptic procedure is employed in all phases of plasmapheresis.

(b) The plastic bag into which the donor's blood is collected shows the donor's name and number. Direct ABO blood grouping is carried out while the blood donation is taken and immediately before infusion of autologous red cells.

(c) Approximately 500 ml of plasma are taken per visit.

(d) Withdrawn plasma is fresh-frozen and kept in frozen state. During this time several tests, such as Australia Antigen detection, transminase etc., are carried out. Only Australia antigen-free plasma is used for further processing.

13.5 Method of Processing

The preparation follows the method of Pool (J. G. Pool and A. E. Shannon, New Engl.J.Med., 273:1443, 1965). The plasma of the donor is frozen and thawed in the cold. The insoluble cryoprecipitate containing mainly fibrinogen and Factor VIII is centrifuged, the supernatant plasma taken off and the precipitate results. In order to achieve a further specific concentration of Factor VIII, the cryoprecipitate is submitted to further purification. The major part of the non-coagulation active fibrinogen is removed by selective elution of Factor VIII. For the purpose of concentration, the eluate is freeze-dried. A Factor VIII activity assay is carried out on the intermediate product. Depending on the results of the same, the intermediate product is reconstituted, the stabilizer added, and the resulting preparation filtered and filled into final containers under sterile conditions. Immediately after filling into the final containers, the product is freeze-dried for stability in storage and again assayed for Factor VIII activity.

The final composition of Kryobulin is as follows:

When dissolved in the volume of Water for Injection, B.P., stated on the label, the solution contains not less than 5 units ($\pm$ 10%) per ml: the solution also contains not more than 2.5% w/v of fibrinogen, not more than 3.0% w/v of total protein, not more than 200 milliequivalents of sodium ions per litre and not more than 165 milliequivalents of citrate ions per litre, with the exception of Kryobulin 500 units reconstituted with only 20 ml of Water for Injections, which contain up to 3% w/v of fibrinogen and up to 6% w/v of total protein.

13.6 Method and Time of Filtration of the Solution

Filtration is carried out with the use of a membrane filter, approximately 30 ml of solution per square centimetre per hour. The pressure is kept to 0.2 kg. per square centimetre.

13.7 Filling

Semi-automatically under strictly aseptic conditions.

14. QUALITY CONTROL

14.1 Tests performed on the donor prior to donation:

Haemoglobin value:

according to the B.P., 1968.

14.2 Tests performed on the plasma of each donation:

(a) Evidence of syphilitic infection:

Cardiolipin test.

(b) Presence of Hepatitis-Associated Antigen:

laboratory's own method (crossover electrophoresis).

(c) SGPT-activity:

U.V. Test.

(d) Protein content:

by colorimetric assay (BIURET).

14.3 Tests performed during manufacture of the product:

(a) Assay of Factor VIII activity of freeze-dried "intermediate product"

Method employed: see Assay of Factor VIII activity on the final product.

(b) Determination of pH value:

according to the B.P., 1968.

(c) Sterility

5 ml in 60 ml Fluid Thioglycollate
5 ml x 1 ml in 10 ml Fluid Thioglycollate
Incubation temperature: 32°C
Incubation time: 14 days

14.4 Tests performed on the final product:

(a) Solubility in water:

according to the B.P., 1968, Addendum 1971.

(b) Stability:

No formation of Fibrin for at least 30 minutes after reconstitution.

(c) Identification:

(i) By Precipitation Test:

according to the B.P., 1968, Addendum 1971, the test shall be made on the contents of a final labelled container which has been selected at random from the fillings of each lot or portion of a lot. The test includes a positive test for human serum protein, and a negative test for any other animal serum protein.

(ii) By Factor VIII activity assay as described in the B.P., 1968, Addendum 1971.

(d) Loss on drying:

according to the B.P., 1968, Addendum 1971.

(e) Test for Freedom from Pyrogenic Substances:

according to the B.P., 1968, using 10 units per kg of the rabbit's weight.

(f) Test for sterility:

under aerobic and anaerobic conditions.
Number of final containers tested: 20
Quantity per final container tested: 2 ml
Number of culture tubes:

20 x 1 ml on 10 ml fluid Thioglycollate
20 x 1 ml on 10 ml Soya Bean Casein Digest Medium

Number of days incubation: 14 days
Temperature of incubation: Thioglycollate at 32°C; Soya Bean Casein Digest Medium at 20 - 25°C.

(g) Test for Innocuity:

Subcutaneous injection of 0.5 ml into two mice not exceeding 20 grammes of weight.

Subcutaneous injection of 5.0 ml into at least two guinea pigs not exceeding 400 grammes of weight.

Observation period: 7 days.

The product passes the test if all animals:

1. survive the test period

2. do not exhibit any abnormal signs during the test period

3. weigh at seven days not less than at the time of injections.

(h) Assay for total protein: Kjehldahl

The following tests will be carried out according to the B.P., 1968, Addendum 1971:

(i) Assay for total fibrinogen

(j) Assay for Sodium ions

(k) Assay for Citrate ions

(l) Assay for potency

15. ASSAY FOR POTENCY

Methods employed:

15.1 Laboratory own method (one-stage Factor VIII assay)

Reagents:

(a) Factor VIII deficient plasma: Citrated plasma from a patient with severe haemophilia A (Factor VIII below 1%), stored deep-frozen.

(b) Phospholipid-kaolin suspension: Phospholipid concentrate (Tachostyptan, Hormon Chemie München) is diluted 1:2000 in Owren's buffer (11.75 g sodium diethylbarbiturate and 14.67 g sodium chloride in 1570 ml distilled water plus 430 ml 0.1 N hydrochloric acid; pH 7.35). Kaolin is added to a concentration of 0.5% w/v.

(c) Dialysed aged EDTA plasma as diluent for samples: Nine parts blood from a healthy donor are mixed with one part 40 mM disodium ethylendiaminetetraaceticacid (Na_2EDTA) containing 0.7% NaCl. The resulting plasma is incubated at 37°C for 48 hours and then dialysed for 48 hours. Storage: deep frozen in portions.

(d) M/20 calcium chloride

Procedure:

The reagents (during testing stored in an icebath) are pipetted into glass tubes in the following way:

0.1 ml Factor VIII deficient plasma
0.1 ml phospholipid-kaolin suspension
0.1 ml sample (serial dilutions of testing sample: Factor VIII concentrate or normal plasma pool respectively).
five minutes incubation at 37°C
0.1 ml M/20 calcium chloride

The time from the addition of calcium chloride until clot formation is measured with a stopwatch.

Calculation of Factor VIII concentration:

A standard plasma is prepared by pooling plasma samples from at least 15 healthy donors. A calibration curve is prepared by plotting the clotting times of serial plasma dilutions of this plasma pool (conc., 1:2, 1:4.....) against the respective concentrations on a double logarithmic paper.

The Factor VIII concentrations of the testing sample dilutions are expressed as a percentage of Factor VIII in normal plasma by using the calibration curve. The amount of Factor VIII in one bottle of concentrate is calculated by the following formula:

$$\text{Units Factor VIII} = \frac{(\%\ \text{Factor VIII}) \times (\text{Volume})\ (\text{ml})}{100}$$

1 unit Factor VIII is equivalent to the Factor VIII activity of 1 ml average fresh citrated plasma.

15.2 Biological Assay of Human Antihaemophilic Fraction, Addendum 1971, page 120, to the B.P. 1968.

16. STABILITY

A batch (No. 0938770/2) of Kryobulin was tested for Factor VIII activity after two years of storage below 6°C. The material was stored in siliconized, sterilized glass bottles. Factor VIII activity was assayed by a Thrombo-plastin Generation Test and a 1-stage method. It is claimed that during a period of two years no change of Factor VIII activity occurred, but no data are given. The physical characteristics of the product remained unchanged during storage, and no evidence of degradation products was found employing microzone electrophoresis.

16.1 In vitro tests

After reconstitution of samples of Kryobulin 500 units in 100 ml of solvent, the solutions were assayed for Factor VIII activity and fibrinogen concentration. The values of Factor VIII activity given below correspond to the average values of six determinations and of fibrinogen to the average value of two assays.

Factor VIII (% of the standard)	Fibrinogen mg %
490	1432
515	1387
478	1408
462	1455
534	1286
512	1421
467	1272
488	1471
521	1395
494,9	1388,5 ± 99

17. CLINICAL STUDIES

17.1 Results of a Clinical Study using Kryobulin 500 and Kryobulin 250

(Dr. Helmut Vinazzer, Linz, Austria).

Between January 1st, 1969 and June 30th, 1972, 20 patients with haemophilia A received a total of 415 packs of Kryobulin 500 and 63 packs of Kryobulin 250. The indications for treatment were mainly haemarthroses, haematuria and dental extractions. In vivo recovery of Factor VIII was calculated in three patients, who were all severe cases of haemophilia with a Factor VIII activity below 1%. The ratio of Factor VIII activity found to Factor VIII activity expected was, respectively, 67.5%, 66.6% and 63%. In none of the patients treated did an inhibitor develop against Factor VIII. 14 patients were observed at 2-4 week intervals over a period of 6 months following treatment. Attention was paid to SGPT, SGOT, LBH, and bilirubin levels in the serum and urobilinogen in the urine. In no case was evidence of an overt or latent hepatitis found. Dosage was ascertained for each patient and was calculated according to the Factor VIII desired. Ordinarily, a PTT assay was carried out before the treatment and at 12 to 24 hour intervals after treatment.

17.2 Billroth's Operation (B.11) in Cases of Haemophilia A (Factor VIII Deficiency) (M. Fischer et al., Vienna, Austria)

Prior to the operation the patient received 3 units AHG "500" and 1 unit AHG "250" which corresponds to a Factor VIII content of 1750 ml fresh plasma. A Factor VIII level of 107% in the plasma of the patient was achieved. Thus increased tendency to bleeding was eliminated and the operation could be started. During the operation an additional 4 units AHG "250" equivalent to 1000 ml fresh plasma were administered, the Factor VIII level increased to 160%.

Post-operatively a total of 14 units AHG "500", 24 AHG "250" and 75 units Plasma Fraction 1 (Cohn) were given. This corresponds to an equivalent of approximately 25 l fresh plasma. The single doses were administered until the 9th post-operative day at intervals of 4 hours, until the 12th post-operative day at intervals of 6 hours. During and after the operation no bleeding occurred, and the wound healed per primam.

18. MEDICAL COMMENT

This submission is deficent in certain respects. For example, the information given on the manufacturing process is very sparse, no details of the stability data on the product are given, and little clinical evidence of effectiveness is provided. However, the donors are well screened and a real attempt is made to meet B.P. requirements for blood. The factory has not been visited recently, and the Sub-Committee may consider that the Austrian Authorities should be requested to carry out an inspection.

Recommendation - That the granting of a Product Licence be deferred pending further information about the product.

D. P. Thomas