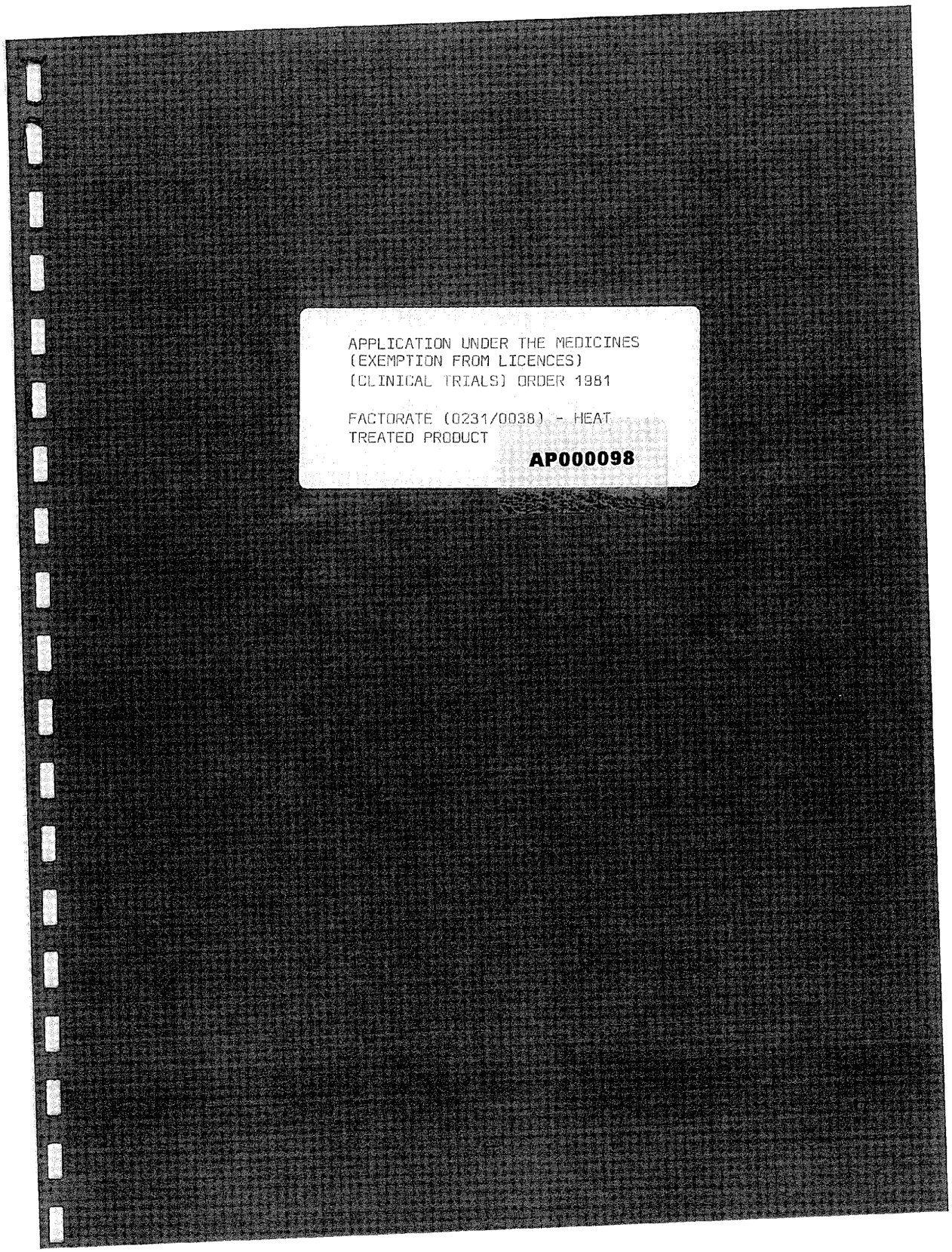


ARMOUR001096

APPLICATION UNDER THE MEDICINES (EXEMPTION FROM LICENCES)
(CLINICAL TRIALS) ORDER 1981

FACTORATE (0231/0038) - HEAT TREATED PRODUCT

CONTENTS




WJT/JJ
AUGUST 1983

ARMOUR PHARMACEUTICAL COMPANY LTD.,
ST. LEONARDS HOUSE,
ST. LEONARDS ROAD,
EASTBOURNE,
EAST SUSSEX,
BN21 3YG

APPENDIX 1

MEDICINES ACT 1968
NOTICE UNDER THE EXEMPTION FROM LICENCES

(CLINICAL TRIALS) ORDER 1981

PART I

Form: MLA 164
Page 1

| | |
|---|---|
| Name of Product: | FACTORATE |
| Full name and address of supplier for the purposes of clinical trial: | Armour Pharmaceutical Company Ltd.,<br>St. Leonards House,<br>St.Leonards Road, Eastbourne,<br>East Sussex, BN21 3YG |
| Any other name under which the supplier carries on business: | Division of<br>Revlon Health Care (UK) Ltd.,<br>Address as at 2. |
| Supplier's reference number: | PL 0231/0038 |
| Details of earlier notices or applications: | None |
| Scientific Evidence: | (i) Chemistry and Pharmacy 2 pages<br>(ii) Experimental and Biological Studies 6 pages<br>(iii) Other studies 1 pages |

I/We hereby give notice of my/our intention to sell or supply, or procure the sale, supply or manufacture or assembly of, medicinal products of the description set out in the following pages and summaries for the purposes of a clinical trial. I/We enclose the particulars and summaries required by Article 4(a)(i) of the Medicines (Exemption from Licences) (Clinical Trials) Order 1981.

I/We undertake to inform the Licensing Authority of:-

(a) any adverse reaction or effects associated with the administration of the medicinal product,

(b) any other matter coming to my/our attention which might reasonably cause the licensing authority to think that the medicinal product could no longer be regarded as a product which could safely be administered for the purposes of the clinical trial or as a product which was of satisfactory quality for those purposes,

(c) any changes in respect of any of the matters specified in Schedule 2 to the Medicines (Exemption from Licences) (Clinical Trials) Order 1981,

(d) any refusal to approve the clinical trial by a Committee established or recognised by a health authority constituted under the National Health Service Act, 1977 or, as the case may be, by a health board constituted under either the National Health Service (Scotland) Act 1978 or the Health and Personal Social Services (Northern Ireland) Order 1972 or by the Medical Research Council, to advise on the ethics of research investigations on human beings.

9. Signatures:

Date 12/8/83 Signature of the supplier .... GRO-C ....

In this notice, the expression "supplier" means a person selling or supplying, or procuring the sale, supply, manufacture or assembly of, a medicinal product for the purposes of a clinical trial.

9.1 I have satisfied myself that the attached summaries are an accurate account of the data obtained by the proposed supplier and having regard to the content of those summaries, I am of the opinion that it is reasonable for the proposed clinical trial to be undertaken.

Date 12/8/83 Signature GRO-C

Dr. H. L. Shaw, MB BS MRCGP,
Medical and Technical Director,
Revlon Health Care (UK) Ltd.,
St. Leonards House,
St. Leonards Road,
Eastbourne,
East Sussex.

*Medical Adviser in the employment of

(insert name of proposed supplier)
working at:

(insert full address and country in which employed)

Medical and scientific qualifications:

*consultant to:

(insert name of proposed supplier)
working at:

(address and country in which the consultant is working)

Medical and Scientific qualifications:

*delete whichever is inapplicable

AP000101

ARMOUR001099

ARMO0000121_0004

PART II

PARTICULARS OF MEDICINAL PRODUCT
AND TRIAL



1. Name and address of supplier, if different from Part I:

   As Part I.

2. Name and address of any person taking part, in the course of a business carried on by him, in the manufacture or assembly of the product: (for an imported product, give the name and address of the manufacturer or assembler of the product in the form in which it is to be imported.)

   Manufactured by: Armour Pharmaceutical Company Limited, Kankakee, Illinois, U.S.A.

   Heat treatment carried out by: Armour Pharma, Eschwege, West Germany.

3. Name of the product, or the designation by which the supplier identifies it:

   Factorate (Heat-Treated)

4. Active constituents:

   Human Anti-haemophilic Factor.

5. Pharmaceutical form in which the product is to be administered:

   Lyophilised powder for intravenous injection after reconstitution with sterile Water for Injections.

6. Indications:

   Therapy of classical haemophilia (Haemophilia A).

7. Proposed dosage, duration and the methods and routes of administration of the product:

   As licensed under PL 0231/0038.

8. Full details of the proposed trial, together with:

8.1 names and qualifications of each investigator:

Dr. C. R. Rizza, MD MB ChB FRCP,
Consultant Physician,
Oxford Haemophilia Centre,
Churchill Hospital,
Headington,
Oxford, OX3 7LT

8.2 duration of the trial:

One year.

8.3 number of patients involved:

Approximately 25 depending upon availability of patients fulfilling inclusion criteria.

8.4 criteria used in the selection, exclusion or withdrawal of patients from the trial:

As detailed on pages 3 and 4 of the attached protocol.

8.5 description of the safety monitoring procedures:

As detailed on pages 6 and 7 of the attached protocol.

## PART III

## FURTHER INFORMATION REQUIRED BY SCHEDULE I OF THE ORDER

rovided in Schedule 1 of the Order, the following information should be rnished in addition to that given in Parts I and II above.

A statement of the chemical structural formula for each active Constituent, the specification of the product, including qualitative and quantitative composition; active and inactive constituents; colouring matter, flavouring agents and perfumes. The approved name should be stated in respect of each constituent; otherwise a designation apart from a laboratory code by which it can be readily identified should be provided. Where any constituent is the subject of a monograph, the monograph name may be given instead.

A description of the containers used for the product and any special directions given by the manufacturer for storage and transport.

Summaries of pharmaceutical data in respect of:

11.1 the method of synthesis of each active constituent and the results of any physico-chemical tests to substantiate the structure of the compound. The monograph name may be given instead;

11.2 the specification of each constituent unless one has not been established, in which case provide a batch characterisation for each batch to be used in the trial. The monograph name may be given instead.

11.3 the quality control procedures and methods to be applied to ensure compliance with the specification for each Constituent;

11.4 the methods of manufacture or assembly of the product;

11.5 the procedures and methods employed, and specifications used, in the process of manufacture and assembly to ensure uniformity;

11.6 evidence of the stability of the product and of its bioavailability for the use intended;

11.7 methods to be employed during manufacture for determining the identity, purity and potency of the product, and the address of the premises where these procedures will be carried out.

Summaries of reports and evaluations of any experimental and biological dies and of other pre-clinical, clinical or laboratory studies carried with each product or its constituents which, in the view of the supplier, are vant to the assessment of the safety, quality or efficacy of the product, ther with references to relevant publications or other clinical trials.

SCHEDULE 1 Article 4(1)(a)(i)

Particulars and summaries which are to accompany a notice given or sent under Article 4(1)(a).

1. The name and address of the supplier and any other name under which he carries on business.

2.-(a) The name and address of any person taking part, in the course of a business carried on by him, in the manufacture or assembly of the medicinal product, and

(b) in the case of an imported product, the name and address of the manufacturer or assembler of the medicinal product in the form in which it is to be imported.

3. The name or proposed name of the medicinal product or where the medicinal product has not been given a name, the designation by which the supplier identifies the product.

4. The chemical structural formula for each active constituent. Where an active constituent is the subject of a monograph, the monograph name may be given instead of the formula.

5. A description of the pharmaceutical form in which the medicinal product is to be administered.

6. The specification of the medicinal product including a statement of its qualitative and quantitative composition giving the constituents whether active or not, including all colouring matter, flavouring agents and perfumes.

7. In respect of each constituent, whether active or not -

(a) the approved name or the monograph name, or

(b) where there is no approved name or monograph name, a designation other than a laboratory code by which it can be readily identified.

8. A description of the containers used for the medicinal product and a statement of special directions given by the manufacturer for storage and transport.

9. The clinical use to be investigated.

10. A description of the proposed clinical trial including the names and qualifications of each investigator, the duration of the trial, the number of patients involved, a statement of the criteria to be used in the selection for, or exclusion or withdrawal of patients from, the trial and a description of how safety will be monitored during the trial.

11. The proposed dosage and its duration, and the methods and routes of administration of the medicinal product.

12. A summary of pharmaceutical data in respect of:-

(a) the method of synthesis of each active constituent and where appropriate, the result of physico-chemical tests to substantiate the structure of the compound. Where the active constituent is the subject of a monograph, the monograph name may be given instead of those data;

(b) the specification of each constituent whether active or not unless a specification has not been established for a constituent, in which case a batch characterisation for each batch of that constituent to be used in the clinical trial. Where a constituent is the subject of a monograph, the monograph name may be given instead of the specification;

(c) in the case of each constituent, whether active or not, the quality control procedures and methods to be applied to ensure compliance with the specification;

(d) the method of manufacture or assembly of the medicinal product;

(e) the procedures and methods employed and specifications used in the process of manufacture or assembly to ensure the uniformity of each medicinal product. Evidence of the stability of the medicinal product and of its bioavailability for the use intended;

(f) the methods to be employed during manufacture for determining the identity, purity and potency of the medicinal product and the address of the premises where such procedures are to be carried out.

13. Summaries of reports and evaluations of any experimental and biological stu e and of other preclinical, clinical or laboratory studies carried out with ach medicinal product or its constituents, which in the view of the supplier are elevant to the assessment of the safety, quality or efficacy of the medicinal product, together with references to relevant publications or other clinical trials.

SCHEDULE 2 Article 4(1)(c)(iii)

Matters in respect of which the licensing authority shall forthwith be informed changes.

1. The name or proposed name of the medicinal product or of the designation by which the medicinal product is identified.

2.-(a) The name and address of any person in the United Kingdom taking part, in the course of a business carried on by him, in the manufacture or assembly of the medicinal product; or

(b) in the case of an imported medicinal product, the name and address of the manufacturer or assembler of the medicinal product in the form in which it is imported.

3. The dosage or its duration or the methods or routes of administration of the medicinal product.

4. The active or inactive constituents, or the method of manufacture or assembly of the medicinal product where such change will affect the bioavailability and/or the shelf life of that medicinal product.

The method of synthesis of any active constituent where such change will affect the range or level of impurities produced.

The clinical use to be investigated.

The criteria used in connection with the clinical trial in respect of the selection for, or exclusion or withdrawal of patients from, the trial.

The investigator.

The nature and purpose of the trial.

AP000108

# PROTOCOL FOR THE STUDY OF

# HEAT-TREATED FACTORATE (FACTORATE H.T.)

# IN LONG TERM TREATMENT OF HAEMOPHILIA

# PROTOCOL VIII - 201

Revlon Health Care (U.K.) Limited
St. Leonard's House
St. Leonard's Road
Eastbourne
East Sussex

May, 1983

HAT/EB

## INTRODUCTION

One of the primary concerns in the use of coagulation factors in the haemophiliac patient who has mild to moderate disease which requires infrequent treatment or in the newly diagnosed patient, is the knowledge that each exposure presents a risk of causing hepatitis.

An intense effort to reduce the hepatitis infectivity of coagulation factors has been ongoing. All donors are now screened to eliminate identifiable hepatitis carriers. Currently, process modifications are being implemented to reduce infectivity of coagulation proteins. Our current approach is to treat with heat the Factor VIII Concentrate and although animal studies have shown reduced infectivity the ultimate test is the use of the new product in patients requiring coagulant therapy to maintain hemostasis or to end a bleeding episode.

## OBJECTIVE

It is the purpose of this study to use our specially prepared Factorate product exclusively for an extended period of time in a number of previously untreated patients or in those who have received minimal treatment to determine if infectivity of the product has been eliminated.

Minimal treatment is defined as having received no Concentrate or cryo-precipitate during the preceeding six (6) months and not having undergone major surgery requiring large amounts of blood and blood products at one time during the preceeding three (3) years and having no history of hepatitis, yellow jaundice, sub-clinical hepatitis or any abnormal liver function tests.

## DEFINITION OF HEPATITIS

A patient will be considered to be suffering from acute hepatitis if he develops clinical symptoms and signs or shows an increase of at least two and a half times the upper limit of normal serum aminotransferase levels, having had normal values previously.

Hepatitis will be classified as acute icteric (raised serum bilirubin)

... anicteric

... symptomless

This may be of two varieties - hepatitis B or non-A, non-B. Hepatitis A, cytomegalovirus infection, glandular fever and toxoplasmosis will be excluded by appropriate laboratory tests.

STUDY DESIGN

Selected study sites will be haemophilia centres run by recognized experts in haemophilia care, who have an adequate number of patients to assure the recruitment of at least five (5) untreated subjects each over a one (1) year study period. In addition, these centres will be asked to recruit an equal number of patients who have had infrequent treatment and are free of hepatitis markers. These markers include hepatitis-B surface antigen, antibody to surface antigen, and antibody to core. They will also have normal liver function studies and have no history of hepatitis.

It will be essential for the centres recruited to have close control over their patients to ensure that those entered into the study have access to and use only the trial Factorate. Any break in this rule will end the study for that subject at the time the non-study product is used. Patients will be entered into the study as they require Factorate H.T.

The end point of this study will be the presence or absence of hepatitis as measured by hepatitis markers and liver chemistries taken serially over the one year period of study.

The final study design will be consistent with the study centre medical and administrative management procedures. Every attempt will be made to have the study design fit smoothly into already-established study centre practices. Any deviation from the protocol will be reported to the study monitor by telephone. A written report should be sent promptly following the call. Should it be necessary to drop a patient from the study a determination of the length of follow-up will be made based on the dose and duration on protocol study and the reason for termination.

All patients or their guardians will have the purpose of the study carefully explained and will sign an Informed Consent. They will understand and agree to the use of study Factorate exclusively for the one year period of the study. However, in the best interests of their patients, the physicians may prescribe any treatment considered necessary. If this includes Factor VIII other than the study material the patients will continue to be followed but not included in the analysis.

PLAN OF STUDY

1. Entry Criteria - Group A

   a) Diagnosis of Haemophilia A established by Factor VIII C levels < 20%, with evidence of bleeding.

   b) No history of use of any blood products.

   c) Normal liver function studies.

   d) Negative hepatitis-B markers.

   e) No history of hepatitis - clinical or sub-clinical.

   f) An attempt will be made to screen immediate family contacts before entry into the study in order to include only those where there is no history or laboratory evidence of hepatitis. The tests will include serology for hepatitis A & B and liver function tests.

2. Entry Criteria - Group B

   a) Diagnosis of Haemophilia A established by Factor VIII C levels < 20% of normal with evidence of prolonged bleeding.

   b) History of infrequent use of cryo-precipitate or concentrate (no use in past six (6) months). No history of use of blood products other than Factor VIII for surgery or trauma in past three (3) years.

   c) No evidence of hepatitis markers.

   d) Normal liver function studies.

3. Exclusion Criteria

a) Any hepatic abnormality.

b) Other disabling diseases which would interfere with objectives of the study. This could include those with known defects which may require packed red cells or whole blood or any serious illness.

c) A member of a transient family group.

d) Parents, guardians, or adult patients with limited intelligence and difficulty understanding or accepting the restrictions of this study should be excluded.

4. Study Design

prior to entering the study, a history, including details of previous transfusions, will be recorded, a complete physical examination performed, and blood collected for baseline laboratory studies. These will include a full blood count, liver function tests and hepatitis A & B antibody.

If the patient is seen as an emergency, then as many tests will be performed as is compatible with the situation.

Each patient entered into the study will agree to use only Factorate H.T. for treatment or prophylaxis of any bleeds from whatever cause. A careful explanation will be given to the parent, guardian, or responsible patient of all risks and benefits they are taking in agreeing to this one (1) year period of study. Informed Consent will be signed.

The patients will be mild haemophiliacs and therefore not on home treatment. Any intercurrent illness will be recorded with date and time of such illness. Instructions on maintaining telephone contact with the investigator will be given with reasons for making contact.

The patient records or duplicates will be segregated at the treating institution to ensure that the investigator or staff will be notified at any time the patient enters the institution either as an out-patient or in-patient.

In the absence of transfusion hepatitis patients will be followed for 1 year following treatment with heat treated Factorate. Liver function tests and tests for hepatitis A & B markers, CMV & EBV will be carried out at appropriate intervals. Blood will be collected pre-treatment and at weeks 1, 2, 4, 8, 12, 16, 20, 24, 28, 32, 40 and 52 post-transfusion. In the event of the patient developing evidence of acute hepatitis, his liver function tests and hepatitis B serology will be followed fortnightly until his condition resolves or for 3 months after the onset and if his condition has not resolved then monthly for 6 months. Follow-up after this will be 3 monthly for the next 3 years.

This study will continue for twelve months. A complete physical examination will be repeated at the twelve month visit.

Those patients whose liver function tests remain elevated for one year after the attack of non-A, non-B hepatitis or become carriers of hepatitis B virus will be referred to the local liver clinic for investigation of chronic liver disease. Liver biopsy will only be carried out if clinically indicated.

LABORATORY TESTS

The basic tests will be conducted at the chosen local Haemophilia Centres. In addition, it is proposed that sera from patients who have received heat-treated Factorate should be made available to the Hepatitis Working Party for use when tests for non-A, non-B hepatitis become available. A 2.0 ml aliquot serum obtained in the follow-up period will be sent to Dr. Craske at the Public Health Laboratory, Withington Hospital, Manchester, M20 8LR for this purpose.

(a) Tests performed prior to study and at 1, 2, 4, 8, 12, 16, 20, 24, 28, 32, 40 and 52 weeks post-transfusion to include :-

1) Haematocrit, Haemoglobin, white blood count and differential count.
2) Absolute lymphocyte count, percent T cells and B cells) if locally
3) Factor VIII-C and Factor VIII ag, IgG, IgM, IgA ) available
4) Urine analysis including microscopic examination of the sediment.
5) Blood creatinine.
6) Hepatitis screen to include Hepatitis-B surface antigen, hepatitis-B surface antibody, hepatitis-B core antibody.
7) CMV and EBV.

8) Liver enzymes to include SGOT, SGPT, and alkaline phosphatase. Abnormal values will be repeated to confirm the tests.

(b) Liver biopsy only if indicated.

DRUG SUPPLIES

The principal investigator will be provided with adequate supplies of Factorate H.T. for each patient enrolled. These supplies should be refrigerated and specifically assigned to the enrolled subjects. Provisions must be made to prevent the inadvertent substitution of other products, whether from Armour or other manufacturers, to replace any of the study material. As each vial is assigned, a record of the lot number and quantity will be recorded on the subject's record and case report form.

Those vials assigned to home care will likewise be recorded on the subject's report form, as well as on the patient's self-kept record of product use. Patients should confirm the presence of adequate refrigerated storage at home.

The dose of Factorate will be determined by the principal investigator based on the severity of the bleed and experience in treating patients. As a general rule, 1 unit of AHF activity per Kg of body weight, will increase plasma circulating AHF levels by 2%.

Infusion rates of the reconstituted product should be adjusted to a rate comfortable to the patient about 2 ml per minute.

ADVERSE EFFECTS

Any untoward reaction to the infusion of Factorate H.T. should be reported to the investigator who in turn will maintain a record in the case report form.

Abnormal laboratory values should be reported to the sponsor by telephone as soon as practical. Severe reactions should be reported immediately to the sponsor. Written reports will follow the telephone reports.

## ANALYSIS OF DATA

The end point to be analysed is the presence or absence of hepatitis as manifested by serial liver enzymes and hepatitis markers.

All reported side effects will be listed and tabulated.

Laboratory data will be summarized.

Clinical control of bleeding will be evaluated.

## DOCUMENTATION

Signed Informed Consent which conforms to the guidelines of the Food and Drug Administration will be obtained by the investigator.

Institutional Review Board approval will be submitted to the sponsor prior to the study.

Case Report Forms and Patient Report Cards, provided by the sponsor, will be completed and submitted at the end of the study.

## WITHDRAWAL FROM FURTHER TREATMENT WITH HEAT-TREATED FACTORATE

1. Will occur on request of patient or guardian.

2. Patients unable to follow the protocol.

3. Patients whose liver function studies become abnormal if the abnormality is due to Factorate. Repeat testing will be done to exclude an aberrant value and every effort will be made to exclude any other causative agent.

4. Patients whose hepatitis markers become positive. Family contacts will be screened to exclude them as a source of infection.

5. Patients receiving whole blood or blood products other than study drug.

**AP000116**

Follow-up of Subjects:

Every effort will be made to follow every subject who has received at least one treatment with heat-treated Factorate. This will be for a full year as set out above or more frequently or in greater depth if clinically indicated.

Only those patients who, in the course of the one year study period have received whole blood, blood products other than the study material or, who have family contacts proven to have developed hepatitis and/or a source of infection will be excluded from the analysis.

Those patients requiring a second dose of Factor VIII will, as far as is clinically reasonable, receive heat treated Factorate.

APPLICATION UNDER THE MEDICINES (EXEMPTION FROM LICENCES) CLINICAL TRIALS ORDER 1981

FACTORATE (0231/0038) - HEAT TREATED PRODUCT

BACKGROUND

The US research divisions of Armour Pharmaceutical Company and Revlon Health Care Group have devoted time in the past two years, to the development of presentations of Factorate and High Potency Factorate (PL 0231/0044) with reduced risk of transmission of hepatitis. Initial studies revealed that heat-treatment, a commonly accepted concept in the destruction of heat-labile viruses, was insufficient alone to prevent transmission of Hepatitis B although there was evidence to suggest non-A non-B virus(es) might be removed by this process. Hence subsequent studies were devoted to the development of a product containing Hepatitis B immune serum globulin, as well as being heat-treated, in order to provide reduced risk of transmission of both types of hepatitis.

It is apparent that, even though hepatitis B transmission could not be erradicated by heat treatment alone, reduced risk of transmission of non-A non-B hepatitis virus could be of considerable benefit to patients. There are in existence screening methods for the detection of hepatitis B in plasma which although not totally accurate, are used to assess the material used in production of blood products with consequent reduction of risk. Unfortunately no such tests are available for detection of non-A non-B virus and this type of hepatitis is the most commonly transmitted form in haemophiliacs. Thus potential reduction of this risk by heat-treatment would be of considerable advantage.

In addition, the recent upsurge in incidence of Acquired Immune Deficiency Syndrome (AIDS) has highlighted the problem of possible transmission of illness through blood products. The nature of the AIDS syndrome and lack of knowledge of the background aetiology are such that it is impossible to determine whether procedures such as heat-treatment might afford protection. Nevertheless there is a growing body of opinion that heat-treatment of material could maximise safety without detriment to the product or its clinical efficacy.

The present study is proposed to assess the value of heat-treatment on transmission of non-A non-B hepatitis. The discovery of common interests led to an approach to the UK Haemophilia Centre Directors' Working Party on Hepatitis with resultant collaboration in design of the study.

The Haemophilia Centre Directors expressed a wish that the study be conducted with Factorate, rather than High Potency Factorate as, for economic reasons this is the product most commonly used in the UK. The development work on heat-treatment was conducted on the High Potency Factorate product because the intermediate product Factorate, is not sold in all markets.

This situation has been discussed informally with DHSS professional staff and it was agreed that this present application could be supported with development data on the High Potency Factorate product provided that stability data on Factorate were supplied.

We believe that this study, performed under the auspices of the country's leading haemophilia specialists, studying in depth a group of highly investigated patients is the definitive course to follow at this time in order to prove the efficacy of heat-treated Factorate in the prevention of transmission of non-A non-B hepatitis.

## PART III - FURTHER INFORMATION REQUIRED BY SCHEDULE 1

1. The chemical structure, specification etc. of Factorate products used in these studies will be as licensed under PL 0231/0038.

2. Factorate used in these studies will be supplied in Type I glass vials with grey butyl rubber stoppers as with the existing marketed products.

3. Pharmaceutical Data

   3.1. Factorate products contain Dried Human Factor VIII Fraction BP. Processing of the material will be as currently licenced.

   3.2. No changes to the existing specification are envisaged as a results of the modified processing of the finished product (see 3.4.)

   3.3. Quality Control procedures will be as for the licensed products.

   3.4. The method of manufacture will be as for the existing licensed product with the exception that the finished lyophilised product will be subjected to heat-treatment in a water-bath at an attained temperature of 60 ± 1°C for a period of thirty hours.

   3.5. In-process specifications are identical to those already in use for the existing non-heated products and no deviations are envisaged due to heat-treatment.

   3.6. A stability study has been conducted with Factorate material subjected to heat treatment. The material has been stored at refrigerated temperature for six months to date and the following results of potency determination and reconstitution time obtained.

   Batch W12011

| Duration of Storage | Treatment | Potency | % Original | Reconstitution |
|---|---|---|---|---|
| 0 | Control, unheated | 201 u | - | 4' 15" |
| 6 | Control unheated | 192 u | 95.5 | 3' 45" |
| 0 | Heated | 197 u | - | 3' 45" |
| 6 | Heated | 185 u | 93.4 | 3' 30" |

In addition to this study it is intended that samples of all three batches of Factorate currently available for use in clinical trials will be entered in an accelerated stability study.

Additional stability data on other Factorate products has confirmed the stability of these following heat-treatment.

A batch of Factorate with additional Hepatitis B immune globulin (equivalent to approximately 3 mg of material per vial) has been heat-treated and placed on accelerated stability study. The following potency results have been obtained to date:

| | Potency | | |
|---|---|---|---|
| Duration | 2 - 8°C | 15 - 30°C | 37°C |
| 0 | 285 | - | - |
| 3 months | 250 (88%) | 310 (109%) | 305 (107%) |

Stability studies have been carried out with two batches of High Potency Factorate (PL 0231/0044) which have been subjected to heat treatment as described above. Sample vials were tested for potency, appearance, reconstitution time and potency three hours after reconstitution at varying periods after storage at refrigerated (2 - 8°C), room (15 -30°C) and elevated (37°C) temperatures. The results indicated the possibility of small loss of potency between non-heated material but it was not possible to differentiate between loss due to heat treatment and the normal variation inherent in biological assays. Results of further testing over a period of one year at refrigerated and elevated temperatures, indicated that the potency of the heated material did not decrease significantly and it was concluded that heat-treatment had no detrimental effect on the stability of the product either at reconstitution or when tested three hours later. The appearance of the product was satisfactory at all stages and reconstitution time was within twenty minutes in all cases except the control (unheated) initial sample for one batch.

3.7. The licence particulars for the product indicate that Quality Control will be carried out at Armour Pharmaceutical Company, Kankakee and at Armour Pharmaceutical Company Limited, Hampden Park, Eastbourne (now Revlon Health Care (UK) Limited). In addition batches of these products are subject to release by NIBS & C. Materials used in this study have already passed through quality control procedures and protocols have been submitted to NIBS & C for approval. Sufficient material for the study has been dispatched to our affiliate company, Armour Pharma of Eschwege, Germany where heat-treatment was carried out. Samples of each batch were then sent to Armour Kankakee for repeat potency assay and results of these are as follows:

| Batch | Potency iu/vial | Potency of Heated Material iu (% Original) | Potency of Unheated Material Tested Comcomitantly iu/Vial |
|---|---|---|---|
| X23102 | 200 | 190 (95%) | 210 |
| X24302 | 200 | 220 (110%) | 210 |
| X25203 | 200 | 205 (103%) | 200 |

4. Summary of Experimental and Biological Studies

The purpose of this clinical study is the evaluation of heat-treated Factorate on the premise that heat-treatment may reduce the risk of transmission of Non-A, Non-B hepatitis. Consequently the experimental studies carried out with the High Potency Factorate products and summarised in this section, have been conducted primarily to determine whether heat-treatment has produced any modification in the nature of the product or in its effect. The studies presented are in two parts ie 4.1. Biochemical Studies and 4.2. Animal Studies.

4.1. Biochemical Studies

4.1.1. Effect of Heat-Treatment on Potency of Factorate

Potency assays have been carried out on material from seven batches of Factorate (High Potency product) before and after heat-treatment. Six of the batches were heated at 60°C for thirty hours and one batch (Batch TM 223) at 60°C for seventy-two hours.

The following results were obtained:

| Batch No. | Potency before Heating | Potency after Heating | % of Unheated |
|---|---|---|---|
| W 77405 | 1170 | 1150 | 98% |
| W 78105 | 1005 | 1020 | 101% |
| W 103306 | 1150 | 1095 | 95% |
| A 1607-020 (V 28602) | 728 | 674 | 93% |
| A 1607-022 (U 26712) | 782 | 689 | 88% |
| A 1607-024 (V 44106) | 826 | 783 | 95% |
| TM 223 | 891 | 884 | 99% |

All potency values were within the permitted limits of 80 - 125% for the assay. There was no significant loss of potency ascribable to heat-treatment.

4.1.2. Acute Stability of Reconstituted Factorate Before and After Heat-Treatment

Three vials of High Potency Factorate (Batch TM 223) were heated at 60°C for 72 hours. After heating, two of the vials together with a vial of unheated control product were reconstituted with 30 ml Water for Injections and assayed over a period of 25 days. In addition a portion of each vial was diluted to a concentration of 1 unit/ml aseptically, using sterile citrate-saline buffer and assayed at the same times as the undiluted product.

The study was terminated at 25 days because AHF activity in all three diluted samples had fallen to levels almost undetectable in the assay. Comparison of assays on heated and control unheated material showed no significant difference in potency of either concentrate or diluted material at any of the reference points. It was concluded that the heating procedure had not affected the stability of the product.

4.1.3. Effect of Heat-Treatment on Thrombin Activation

Thrombin has been shown previously to produce a pronounced increase in Anti-Haemophilic Factor activity, measured in a one-stage assay. This phenomenon may be of importance as an amplification mechanism in the intrinsic clotting cascade and consequently experiments have been carried out to determine whether heat-treated AHF retains this characteristic. Heat-treated material used in this experiment was prepared by heating at 70°C for 25 hours. Studies on the time-course of thrombin activation showed a peak of activation after 2 - 5 minutes incubation at which time the AHF activity increased twelve-fold over starting values. The effect gradually diminished with further incubation until at 15 - 20 minutes values were at or below initial results. Comparative tests with heated and non-heated material, using an incubation period of 2 minutes and measuring activity by a one-stage partial thromboplastin time test, showed no significant difference between the two materials.

4.2. Animal Studies

4.2.1. Immunochemical Testing in Rabbit

Immunological testing was carried out in order to determine whether heating of the complex protein of anti-haemophilic factor might lead to structural modification.

Antibodies to heated and non-heated Factorate were developed in New Zealand white rabbits (3 per group) by monthly subcutaneous injections of protein emulsions. The antibodies produced were then used in Ouchterlony gel diffusion and two-dimensional immunoelectrophoresis experiments in an attempt to detect the presence of new antigens. Results of both series indicated no difference in the patterns obtained for heated and non-heated material attributable to the heat-treatment. Similar tests with antisera specific for various plasma proteins, eg immunoglobulin, fibrinogen, albumin and transferren also failed to show any differences between the two products.

4.2.2. Half-Life and Recovery of Unheated and Heated Factorate in Dogs

Studies on the half-life, recovery and various haematological parameters of heated and non-heated AHF were carried out in male dogs (weight 27.3 - 36.4 kg) with haemophilia A. One dog received unheated and two dogs heated material, infused at 8 ml/min over a period of 3.5 to 4 minutes. The total dose administered was 770 units/dog. Blood samples were obtained by venepuncture at 15, 60, 90 minutes and 3, 5, 8 and 24 hours after infusion. Blood was collected into 3.8% citrate and divided, one part being used to prepare platelet-free plasma for measurement of F VIII C and F VIII RA levels. Blood samples were tested, in addition, for White Blood Cell Count, Haematocrit, Total Protein and Platelet Count.

Respiration rate, pulse rate and rectal temperature of the dogs were recorded at each test point.

Measurements of F VIII C indicated a rise in the level of this activity in all dogs at 15 minutes after dosing, the increases being 76% in the dog treated with unheated material and 258 and 134% in the two dogs receiving heated material. The F VIII activity of the dog treated with unheated material fell to a level below the initial value at 24 hours, whereas the levels in dogs treated with heated material were 43% and 34% above initial values at this time. Calculations of half-life from this data gave values of 7.8 hours for unheated material and 18.2 and 22.5 hours respectively for dogs receiving heated material. Recovery of exogenous AHF was 65% in the control dog (unheated material) and 97% and 81% respectively in the two dogs receiving heated AHF.

Plasma VIII RA activity increased in all animals at 15 minutes by 26% (control) and 46 and 51% (heated AHF) respectively. At 24 hours values in the control dog were 26% below baseline whereas those in dogs receiving heated AHF were elevated by 24% and at baseline value respectively.

Both AHF preparations produced decreases in haematocrit values but all values remained within the normal range for dogs of the age used (ie 35 - 53%).

The levels of blood proteins fluctuated in all dogs during the study with indications that AHF treatment might reduce these levels; however values were within normal range for proteins in dogs, ie 4.9 - 9.6 gm %, at all times.

AHF treatment produced fluctuations in levels of white cell count and platelets during the experiment. There were indications that heated material may have induced leukopenia although it was felt by the investigator that these reductions were not signficant as fluctuations in white cells are not uncommon in haemophilic dogs. The fluctuations in platelet-count during the experiment were attributed to the stress of the experiment rather to the specific treatment administered.

Assessment of physiological parameters showed increase in respiration rate, apparently unrelated to treatment and possibly due to stress in all animals. Pulse pressure was reduced dramatically in one dog receiving heated material but only slightly in the other. The results for this latter dog and those of the control animal were all within the normal range of 105 - 150 mm Hg for dogs.

There were no dramatic changes in rectal temperature during the experiment. The temperature of the control dog fell slightly at 5 - 8 hours post dose but had returned to initial value by 24 hours. Rectal temperatures increased in both dogs receiving heated material during the period up to 3 hours post dose, by 1 - 1.2°C, followed by a fall in temperature at 8 hours.

It was concluded that heat-treatment of AHF does not adversely effect the recovery or half-life of F VIII C nor F VIII RA in haemophilic dogs compared with standard material. Neither material altered haematocrit or total blood protein and although there were marked fluctuations in WBC and platelet counts following treatment, these responses did not appear to be drug related. Respiration rate, pulse rate and rectal temperature appeared to be little affected by either treatment.

4.2.3. Effect of Heat-Treated Factorate on Heart-Rate and Arterial Blood Pressure in Anaesthetised Dogs

Eight male Beagle dogs weighing 9 - 11.3 kg, fasted overnight, were used in the study. Anaesthesia was induced with sodium pentathol (15 mg/kg) and maintained with -chloralose (60 mg/kg). Four dogs were treated with control, unheated Factorate (High Potency) 100 u/kg and four with the same dose of material which had been heat-treated at 60°C for 24 hours, heart rate and arterial blood pressure were monitored throughout the study. All dogs received Water for Injections at an injection rate of 4.4 ml/ml for 4.5 minutes prior to testing with AHF.

Infusion of Water for Injections had little effect on heart-rate or arterial pressure, the maximum changes observed being a decrease of 15 beats per minute in one dog and an increase of 20 beats per minute in another for heart rate and an increase of 13 mm Hg blood pressure in one dog.

Administration of unheated AHF caused maximal increases of 14, 30 and 40 bpm in three dogs and a decrease of 5 bpm in the fourth. Arterial pressure changed slightly, the range of effect being a reduction of 7 mm Hg to an increase of 18 mm Hg. Heated AHF caused reduction of heart-rate in two dogs by 16 and 20 bpm respectively and an increase of 6 and 20 bpm in the other two dogs. Mean arterial pressure was elevated in all dogs, increases ranging from 4 - 22 mm Hg.

It was concluded that infusion of AHF at 100 u/kg to anaesthetised dogs produced minor changes in heart-rate and mean arterial pressure. These effects were usually immediate and transient in nature. Heat treatment of the material did not increase the incidence of these effects.

4.2.4. Studies in Chimpanzees

4.2.4.1. AHF Infected with HBV Strain (ay) (BOB-NIAID)

A preliminary study was conducted with AHF, to which had been added $10^{3.5}$ chimpanzees infective dose 50 ($CID_{50}$) Hepatitis B Virus, strain (ay), per 30 ml vial. Some vials were heated by immersion in a water bath at 60°C for 30 hours and the contents of two such vials were administered to two chimpanzees. A further chimpanzee was given HBV infected, unheated material as a control and the animals were observed for development of Hepatitis B viral infection. Parameters assessed were liver enzymes, Hepatitis B markers and liver histology.

All chimpanzees in the study developed Hepatitis B infection shown by antigenaemia, enzyme elevation and antibody formation and it was concluded that heat-treatment did not eliminate transmission of infection due to Heptitis B strain (ay). The control animal receiving unheated material exhibited elevation of enzyme levels (ALT) early in the study - an effect which did not appear in the test animals. Evaluation of the results of the study, together with blind reading of histological specimens suggested that the material used in the study may have been fortuitously infected with Non-A, Non-B Virus resulting in the development of infection in the animal receiving unheated material. The fact that infection did not develop in animals receiving heated material was taken to mean that heat treatment had inactivated the Non-A, Non-B Virus.

4.2.4.2. AHF Infected with Hutchinson (NIH) Non-A, Non-B Hepatitis

Vials of commercial High Potency Factorate were infected with approximately 300 chimpanzee hepatitis doses (CHD) of Non-A, Non-B hepatitis virus, Hutchinson strain. Some vials were then heated at 60°C for thirty hours before injection of the contents of single vials into each of two chimpanzees. A further chimpanzee received one vial of control, unheated material which had been infected with Non-A, Non-B hepatitis virus. Following this treatment the animals were assessed weekly for liver enzyme activity. Liver punch biopsies and observation of Hepatitis Markers (AUSRIA, AUSAB, HAVAB and CORAB) were carried out at four week intervals.

Following injection of the animals no changes in enzyme activity (SGOT, SGPT, GGT) were observed within a period of 22 weeks. Consequently two reserve animals were treated, one with heated, infected material and one with unheated infected material. Enzyme level studies during the 12 weeks following injection of these two animals showed the onset of acute infection in the positive control animal with SGPT levels greater than 10 x baseline and elevation of of GGT and SGOT. No significant changes were observed in the animal receiving heated material.

The three original animals were challenged with a dose of 300 CHD of Hutchinson strain Non-A, Non-B Virus to assess their susceptibility to infection with this virus. Enzyme levels increased in the two animals which had previously received heated, infected AHF but no such changes were observed in the animal which had received unheated, infected material. It was hypothesised that this animal might be resistant or immune to infection with this strain of virus or may not demonstrate such infection on serological testing. In the latter case infection may be demonstrated by histological examination at the end of the experiment, which had not terminated at the time these reports were written.

Full evaluation of the experiment will not be possible until the experiment is completed and histological examination is made. However it is concluded on an interim basis, that the control animal used in the intial part of the experiment was resistant to infection with the Non-A, Non-B Virus used. The animals receiving heated, infected material showed no signs of developing infection although they subsequently developed signs of infection when challenged with the same dose of hepatitis virus alone. These results, together with those of the second part of the experiment where the animal receiving heated infected material failed to show signs of infection, suggest that heat treatment substantially decreases or totally removes the risk of infection with Non-A, Non-B heaptitis virus at the dose examined.

5. Clinical Studies

A clinical half-life study has been carried out in the USA with Factorate, containing hepatitis immune globulin, which has been heat-treated as specified above (3.4).

Six male patients, four with severe and two with moderate Haemophilia A were given the heat-treated material in a cross-over study against unheated material. Blood sampling was carried out at 0, 15, 30 and 60 minutes and 2, 4, 6, 8, 24 and 48 hours after injection and patients were subjected to general clinical assessment at 0 and 15 minutes and 1, 2 and 4 hours. Circulating levels of Factor VIII activity were calculated from the biological response in tests of coagulation function. The results indicated absence of adverse reactions or local intolerance to infusions. No clinically significant effects of measured parameters were detected as a result of the treatments. Evaluation of the half-life characteristics of the two preparations showed that these were practically identical. The biological half-life values (ie elimination half-lives) of the two preparations were calculated to be 10.88 ± 4.11 hours for heat-treated Factorate product and 10.88 ± 3.57 hours for the unheated Factorate product. Recovery values calculated using the formula:

$$\text{Recovery (K)} = \frac{\text{kg body weight x Factor VIII rise (u/dl)}}{\text{dose of Factor VIII administered (u)}}$$

were 1.8 ± 0.2 for heated material and 1.9 ± 0.2 for unheated material.