NOT FOR PUBLICATION DR M E DUNCAN

COMMERCIAL IN CONFIDENCE

COMMITTEE ON SAFETY OF MEDICINES

THE SAFETY OF IMMUNOGLOBULIN PREPARATIONS

BACKGROUND

In July 1985 CSM(B) reviewed the Safety of Intravenous Immunoglobulins licensed for use in the UK and advised that licence holders be asked to provide additional information as follows:-

a. further data concerning the ability of the manufacturing process to inactivate viruses

b. ongoing evidence of the safety in clinical use of the product

c. information regarding the company's plans for screening of donors.

There are 5 licence holders - Miles, Biotest, Sandoz, Kabi-Vitrum and PFC Edinburgh. All have been asked to supply additional information and all have indicated their willingness to do so, but so far no new data have been received from any of them.

In addition to the 5 licensed materials, intravenous immunoglobulin prepared by BPL Elstree is distributed under Crown Immunity. We understand that the present method of manufacture is the same as that used by PFC Edinburgh.

Following consideration of the intravenous immunoglobulins, it was considered appropriate that the sub-committee be asked to review the safety of the intramuscular preparations and to endorse a recommendation that additional safety data be required for these preparations as well as for the intravenous ones.

There are 2 licensed products, prepared by Kabi and by Immuno, and 2 unlicensed products, distributed under Crown Privilege by BPL, Elstree and PFC, Edinburgh. Although the Edinburgh material is unlicensed, samples from each batch prepared are submitted to NIBSC.

It was felt that it was now no longer the case to ask manufacturers what plans they had to screen donors for antibodies to HTLV-III but to require that, for all immunoglobulin preparations, individual donations of plasma should be screened using an ELISA test - the details of the test to be made available to the licensing authority and to NIBSC. Indeed, in November 1985, CSM(B) remarked that:-

"The Committee are anxious that individual donations for all blood products should be screened for HTLV-III from the earliest possible date. Manufacturers should be requested to confirm that donations are being screened and to provide information about the nature of the screening tests used."

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The Main Committee endorsed this remark when it met at the end of November 1985.

RECENT REPORTS

a. Intramuscular Immunoglobulins

Since early November 1985, NIBSC has been screening samples of immunoglobulin received by them under the batch release procedure. In late November they reported to the Licensing Authority that intramuscular immunoglobulin from both licence holders (Kabi and Immuno) had given a positive test for antibodies to HTLV-III.

Three batches of material are involved, two from Kabi and one from Immuno. All were positive in 2 different ELISA tests, and were again positive when the tests were repeated. These results have been confirmed by immunoblotting. The two companies have been asked to state the source of the plasma used, and to give details of any screening carried out by them for antibodies to HTLV-III (nature of test, stage of manufacture when it was carried out).

Material from PFC, Edinburgh, screened at the same time by the same ELISA tests has given negative results. Thus the present position is that of 4 possible sources of supply of intramuscular immunoglobulin 2 are producing end product which appear to have antibodies to HTLV-III, and have therefore not been relased by NIBSC, one is producing a 'clean' final product, and in the 4th case the final product has not been available for examination.

b. Intravenous Immunoglobulins

Exactly one week after NIBSC had first communicated their findings with intramuscular immunoglobulins Sandoz reported to us that retrovirus, morphologically indistinguishable from HTLV-III, had been isolated from 2 patients at Northwick Park. Both patients had been treated with intravenous immunoglobuli prepared by Sandoz.

The background to this report is complex, and the details need not be entered into here. Briefly both patients are diagnosed as having common variable hypogammaglobulinaemia, and at Northwick Park such patients are being screened for retrovirus as part of a research programme. Isolation of the virus resul from routine screening in the course of this investigation.

In one case the link with Sandoglobulin is tenuous. The patient has received a variety of treatments over many years. These have included intramuscular gammaglobulin and plasma as well as intravenous immunoglobulins from Elstree and Miles (Gamimune) in addition to Sandoglobulin. Only 1 viral isolate has been obtained, and subsequent attempts to isolate the virus have been negative. It has been suggested that the first isolate may have been a laboratory contaminant.

In the second case the picture is different. Virus isolated on more than one occasion is morphologically identical to HTLV-III although there are some differences in immunoblotting patterns. The patient is an 18 year old girl who has no history of contact with high risk groups and who has received no blood products other than Sandoglobulin. Clinically she is suffering from AIDS, and the possibility that this might have been transmitted by Sandoglobulin cannot be excluded.

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Batches of material received by the 2 patients have been traced and quarantined. They will be tested for antibody to HTLV-III, and attempts will be made to isolate and culture virus. Other recipients of these batches will be screened for HTLV-III.

A 20 month old infant who received material from one of the suspect batches is reported to be suffering from AIDS. In this case, however, the mother is a known heroin addict whose husband is a drug pusher, and the disease may have been transmitted from her to the infant. Further information is awaited.

CURRENT POSITION

Whatever else has emerged from the events of the last few weeks it is clear that, certainly under present conditions of manufacture, there cannot be any confidence in the safety of immunoglobulin preparations which have not been derived from individually screened plasma donations. However an insistence that only 'donation tested' products be used could lead to a temporary difficulty in maintaining adequate supplies of material.

a. Intramuscular Immunoglobulin

- PFC Edinburgh will have material from individually screened donations by March/April 1986. The material currently available is from unscreened donations but screened plasma pools. Final material is also screened.

- Immuno intend to issue only "donation tested" batches after 1st January 1986.

- Kabi have been screening individual plasma donations since early this year, but we have no information on how long it will be before material prepared exclusively from individually tested donations will be available.

- BPL Elstree, on the basis of their current stocks of plasma, will not have material from individually tested donations for 18 months to 2 years. Current material is from screened plasma pools. Final material is also screened.

b. Intravenous Immunoglobulin

- Miles material is not, at present, being released by NIBSC because the strucuture and functional integrity of the molecule is in doubt. It is now known when material prepared exclusively from individually tested donations will be available, but in any case the company has obviously got more than one problem to sort out.

- Biotest have been screening individual donations since July 1985 and expect to have material prepared exclusively from these sources by January 1986. They also screen plasma pools for antibody to HTLV-III.

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- Kabi material has not yet been launched on the UK market. It is not known how long it will be before they can supply material exclusively from individually tested plasma donations, although they have been screening individually plasma donations, since early 1985.

- Sandoz will have material from individually screened plasma donations by March/April 1986. Current material has not been subjected to any screening.

- PFC Edinburgh will have material from individually screened donations by March/April 1986. Current material is from screened plasma pools. Final material is also screened.

- BPL Elstree on the basis of their current stocks of plasma, will not have material from individually tested donations for 18 months to 2 years. Current material is from screened plasma pools. Final material is also screened.

SUMMARY

In the light of recent events it may be appropriate that only those immunoglobulin preparations made from individually screened plasma donations should be used. In the short term this could cause a supply problem.

BY January 1986 we expect to have at least one source of intramuscular immunoglobulin and one source of intravenous immunoglobulin prepared from individually screened plasma donations. By March/April 1986 at least one additional source of intramuscular immunoglobulin and two additional sources of intravenous immunoglobulin should be available.

In the event of a shortfall of material in the first few months one option would be to allow the use of material where the plasma pool has been screened. Another would be to restrict the use of material to certain very serious clinical situtations. It is possible that a combination of these options will have to be considered, and expect guidance is required.

SPECIFIC IMMUNOGLOBULINS

With the exception of Humotet, an antitetanus immunoglobulin prepared by Immuno amd marketed by Wellcome, the national requirement for the various specific immunoglobulins is met by PFC, Edinburgh and BPL, Estree who manufacture and distribute these preparations under Crown Privilege. Obviously the same requirements must apply to these products, ie individually screened plasma donations and provision or protocols and samples to NIBSC, and ideally all manufacturers should also be licence holders.

RECOMMENDATIONS

1. No immunoglobulin preparations should be used other than those made from plasma where individual donations have been screened for antibodies to HTLV-III (and for HBsAg).

2. Details of the nature and sensitivity of the screening test used should be provided.

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3. In the event of a shortfall of material prepared from individually screened plasma donations, consideration shoud be given to

a. restricting the use of the material to certain serious clinical situations

b. allowing some use of material prepared from screened plasma pools

4. Data should be provided on the ability of the manufacturing process to inactivate viruses.

5. Evidence should be provided of the safety of the product in clinical use.

APPENDICIES

1. Intramuscular immunoglobulins (NIBSC paper).

2. Intravenous immunoglobulins (Secretariat paper).

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NIBSC

Appendix 1

Comment on the safety of human immunoglobulin products intended for intramuscular use

Introduction

Although there has recently been considerable concern over the transmission of viral infection by intravenous administration of human immunoglobulin preparations which are prepared specifically for intravenous use, 'intramuscular' products have an outstanding safety record. The principal problem associated with intravenous immunoglobulin preparations has been incidences of raised liver enzymes which are directly attributable to use of intravenous IgG products (Ochs et al. 1985 Lancet I, 404; Leon et al. 1984 Lancet II, 1062). Some of these patients were subsequently diagnosed as having suffered from non A - non B hepatitis. To date there are no reports of transmission of HTLV-III/LAV injection by either intramuscular or intravenous immunoglobulin preparations.

This paper briefly reviews the safety aspects of human immunoglobulin prepared for intramuscular use.

Human immunoglobulin products intended for intramuscular use

At present four intramuscular immunoglobulin products are used routinely in the UK. Two of these, which are made by KabiVitrum (Kabiglobulin) and Immuno (Gammabulin) are licensed (PLR and PL respectively) and subject to the batch release procedure involving evaluation of samples and protocols at NIBSC.

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The other two products, which are produced by the Scottish BPL and BPL Elstree are issued under Crown Privilege and are therefore not subject to the Batch Release process. However samples and protocols of the Scottish BPL immunoglobulin are routinely sent to NIBSC, and are subjected to the same testing as the licensed products. Although samples and protocols of the BPL Elstree material have in the past been sent to NIBSC, none have been submitted since April 1982.

All human immunoglobulin products intended for intramuscular or intravenous use are prepared using the Cohn (cold ethanol) fractionation technique or modifications of this procedure (Hein et al. 1985 Lancet i, 405). Intravenous products are normally further purified or treated to reduce immunoglobulin aggregates, prekallikrein activator and/or other impurities. Intramuscular IgG preparations are not usually subjected to further processing after cold ethanol fractionation; they are all essentially Cohn II fractions.

Clinical indications for use of intramuscular immunoglobulins includes the treatment of primary immunodeficiency (hypogammaglobulinaemia), severe bacterial infections and burns. The preparations are also used for prophylaxis of hepatitis A, measles and rubella.

Batch release of immunoglobulin products intended for intramuscular use

Samples submitted to NIBSC are tested to establish purity, identity, sterility, antibody content against viruses and bacteria, antibody dependent complement fixation, and

pyrogenicity. The following specific tests are applied:

(1) Assay of human IgG by single radial immunodiffusion using monoclonal antibodies.

(2) Immunoelectrophoresis.

(3) SDS polyacrylamide gel electrophoresis run under reducing and non-reducing conditions and also immuno-blotting using monoclonal antibodies.

(4) Sterility.

(5) Pyrogenicity (using limulus amoebocyte lysate).

(6) Testing for the presence of hepatitis B surface antigen (IRMA).

(7) Assay of prekallikrein activator.

(8) Estimation of antibodies against:

(i) Polio virus (viral neutralization)

(ii) Rubella (single radial haemolysis)

(iii) Measles (" " ")

(iv) Hepatitis B virus (IRMA)

(v) Tetanus toxin (ELISA)

(vi) Diptheria toxin (ELISA)

(vii) Tests for the presence of antibodies to LAV/HTLV-III (ELISA, Immunoblotting).

All batches submitted for evaluation at NIBSC have been found to comply with license specifications in terms of purity, potency and biological function as claimed in the specification by the manufacturer. This contrasts with some intravenous immunoglobulin preparations and is probably due to the relatively simple manufacturing procedure, which produces little alteration of the 'native' immunoglobulin structure.

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Safety of intramuscular immunoglobulin

Problems associated with the use of human immunoglobulin preparations can be divided into two principal groups, ie the occurrence of adverse reactions and the transmission of viral agents.

a) Adverse reactions

It is well documented that adverse reactions do occur following injection of human immunoglobulin and such reactions, which can be severe, are of the anaphylactic or anaphylactoid type. These adverse reactions have been attributed tentatively to immunoglobulin aggregates present in the preparations, allergic reactions to preservatives, production of antibodies by the recipient to IgA or subtypes of other immunoglobulins present in the preparations, or possibly to PKA, which is present in high levels in some but not all preparations (Shemin 1968, JAMA 203, 113; Kamme et al. 1966, Acta. Med. Scand. 179, 679; Kleinman & Weksler 1973, J. Paediatrics 83, 827; Lederman & Winkelstein 1985 Medicine 64, 145).

Such reactions often occur if immunoglobulin preparations intended for intramuscular use are injected intravenously and this has been interpreted to suggest that procedures used to further purify or modify Cohn fraction II as used for production of intravenous immunoglobulin help to eliminate or reduce such adverse side effects. Adverse reactions occur mainly in patients being treated for hypogammaglobulinaemia and this may reflect the relatively large doses used for this indication. Pain associated with

administration of such large volumes can also be problematical in such patients, (Soothill, 1971 in Hypogammaglobulinaemia in the UK, MRC special report series No 310 p106), and intravenous immunoglobulin is usually preferred for this reason.

Transmission of viral diseases

Human immunoglobulin preparations intended for intramuscular use have an outstanding safety record regarding transmission of viral agents (Iwarson et al. 1985, Transfusion 25, 15, Gerety & Aronson 1982 Tranfusion 22, 347). For example Kabiglobulin has been licensed in the UK since 1973 and there has been no reported incidence of transmission of viral disease. However it has been shown using chimpanzees that it is possible in rare instances to transmit hepatitis B using human immunoglobulin (Tabor & Gerrety 1979, Lancet ii, 1293). There is also a single report of transmission of non A - non B hepatitis by Anti-D (Rho) immunoglobulin prepared from the sera of donors who had been immunized with erythrocytes from an individual affected with the disease (Renger et al. 1981, Z. Aerztl Fortbild 75, 894). There is no description of the procedure(s) used to purify this immunoglobulin. This report has probably little relevance to immunoglobulin products used in the UK.

In general it can be assumed that the intramuscular products licensed in the UK are unlikely to cause viral diseases. This safety record contrasts with that of intravenous immunoglobulin where transmission of non A - non B hepatitis is now a known potential hazard. As production of both intra-

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muscular and intravenous immunoglobulins initially involves fractionation using cold ethanol (as Cohn fraction II), this conflicting record of transmission of non A - non B hepatitis is difficult to explain. It is possible that the intravenous route is advantageous for viral infection or that viral contamination of immunoglobulin preparations occurred during the post Cohn fractionation procedures used in the preparation of intravenous immunoglobulin, or that the manufacturer did not follow the Cohn type procedure. Intramuscular immunoglobulin preparations are known to contain anti- hepatitis B antibodies and this fact together with the nature of the fractionation procedure used would suggest that risk of hepatitis B infection is very low with such materials.

To date there have been no reports of cases of AIDS transmission attributable to human immunoglobulin preparations. However the potential risk of such infection has resulted in discussion in the medical literature (Kane & Geiko 1984, JAMA 252, 1057). Also antibodies to LAV/HTLV-III have been found in a German intramuscular immunoglobulin preparation high in antibodies against hepatitis B virus ('gamma protect hepatitis' - Biotest Pharma), although an intravenous immunoglobulin preparation (Intraglobin) produced by the same manufacturer did not contain such antibodies (Tedder et al. 1985, Lancet i, 815). It has been reported that the cold ethanol fractionation process used to prepare intramuscular immunoglobulin causes inactivation of LAV/HTLV-III (Spire et al. 1984, Lancet ii, 899). Investigation concerning the inactivation of LAV/HTLV-III by such procedures are in

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progress at NIBSC.

Conclusions

The preparations of human immunoglobulin intended for intramuscular use which are licensed in the UK are safe products when used as intended. Serious adverse reactions may occur with patients suffering from hypogammaglobulinaemia, and in these cases the use of intravenous immunoglobulins may be preferred.

The risk of transmission of viral disease is minimal. In this context it is essential that manufacturers screen all individual plasma donations and product batches for the absence of hepatitis B surface antigen and evidence of infection with LAV/HTLV-III virus.

It is recommended that manufacturers provide detailed information in licence applications and manufacturing protocols for individual production batches, confirming that screening of individual donors for LAV/HTLV-III is undertaken and detailed information on the nature and sensitivity of the screening tests used.

Tests for antibody to LAV/HTLV-III virus are routinely carried out at NIBSC on all batches of immunoglobulins submitted for batch release purposes.

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APPENDIX 2
CSM/BSC/1985/3rd Meeting
CSM/1986/7th Meeting

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COMMITTEE ON SAFETY OF MEDICINES

SAFETY OF INTRAVENOUS IMMUNOGLOBULINS

Human Immunoglobulin preparations for intramuscular administration have a long history of safety in clinical use and when, more recently, preparations for intravenous use became available, there seemed no reason to suppose that they would be any less safe.

Since January 1984, four intravenous preparations have been licensed in the UK, and PL applications for a further two products are pending. In addition, human i.v. immunoglobulin is manufactured and distributed under Crown Immunity by B.P.L. Elstree.

Manufacturer	Product	Licensing Position
Miles Laboratories	Gamimune PL 0055/0104	Granted 16/1/84
Biotest Pharm.	Intraglobin PL 4500/0002	Granted 22/6/84
Sandoz	Sandoglobulin PL 0101/0181-2	Granted 13/11/84
Kabi-Vitrum	Gammonativ PL 0022/0056	Granted 18/2/85
BPL Elstree	i.v. Immunoglobulin type GGV	Distributed under Crown Immunity
PFC Edinburgh	Human Immunoglobulin PL 3473/0011	Pending
Immuno	Endobulin PL 0215/0023	Pending

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ntravenous immunoglobulin like intramuscular immunoglobulin is prepared by cold ethanol fractionation of pooled plasma. The Cohn fraction II is then treated to make it suitable for i.v. administration. It was widely believed that Cohn fractionation of plasma was capable of inactivating virus. The first indication that this might not always be the case came with a report from BPL[1] that 12 patients treated with their i.v. preparation had all developed non A non B (NANB) hepatitis. Correspondence in the Lancet[2] suggested that there might now be a case for incorporating into the process a step capable of inactivating hepatitis viruses and HTLV-III, while a leading article in the same journal[3] noted the finding of American workers that blood donors with a raised serum ALT are more likely to transmit non A non B hepatitis than are those with normal transaminases. They estimated that elimination of donations with an ALT greater than 60 units would prevent 29% of transfusion NANB hepatitis with loss of only 1.6% of donations.

There has now been another published report[4] of NANB hepatitis occurring in 7/16 patients treated with the Hyland (Travenol) material. There is a further (unpublished) report of NANB hepatitis in some patients treated with the Kabi preparation, 'Gammonativ', but no details are available.

In view of these reports it seems timely to stop and take a critical look at the products already available in the UK and at those for which licence applications are pending; to consider, particularly with regard to HBV, HTLV-III and NANB virus,

1. the acceptability of the starting material

2. evidence of virus inactivation during the preparation of the products

3. evidence of clinical safety with respect to transmission of viral infection.

Acceptability of the starting material essentially means screening of individual donors. At the moment all are screened for HBsAg, and it is intended that all should be screened for presence of HTLV-III antibodies as soon as a reliable test is available. Licence holders and applicants should be asked to confirm that this will be the case.

There is no screening test for NANB, but in the light of the American findings it seems desirable to eliminate donations from subjects with a raised serum ALT. This might not be easy to achieve since not all laboratories have the facilities for doing ALT estimations, but it is worth suggesting.

Evidence of virus inactivation during manufacture

A variety of methods is used to render Cohn fraction II immunoglobulin suitable for intravenous use. Each manufacturer should provide evidence that his procedure is also capable of inactivating viruses.

Evidence of clinical safety

Ultimately the evidence of safety must be provided by adequate follow-up of patients. For monitoring of liver function by ALT, timing of samples is important, and BPL regard a pre-sample, 1 week post, and subsequently fortnightly up to 16 weeks as the minimum acceptable for NANB follow-up. For Hepatitis B and HTLV-III longer follow-up is required (a minimum of 6-12 months).

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UK PRODUCTS: EVIDENCE OF SAFETY

Product	Procedure	Evidence of viral inactivation by procedure used	Evidence of clinical safety
Gamimune (Miles)	- Reduction by dithiothreitol - Alkylation with Iodocetamide	None provided	66 patients followed up for 2-5 years. No details given
Intraglobin (Biotest)	- Acetylation with B-propiolactone	None provided but is known to be effective	- 28 volunteers followed up at 3-week intervals for 35 weeks. - Data on large numbers of children, in some cases up to 6 years
Sandoglobulin (Sandoz)	- Limited pepsin digestion at pH4	None provided but is known to be effective	- Several hundred patients treated for 2 years
Gammonativ (KabiVitrum)	- Adsorption on DEAE Sephadex	None provided	- 43 patients treated for up to 9 months
Endobulin (Immuno)	PEG precipitation	None provided	16 patients followed up for average of 14 months. Monitoring included estimates of liver enzyme levels before and after each infusion

(Contd.)

Product	Procedure	Evidence of viral inactivation	Evidence of clinical safety
Human Immuno-globulin (PFC, Edinburgh)	Limited pepsin digestion at pH4	None provided but is known to be effec-tive	No volunteer(5) or patient (26) develope clinical or labora-tory features of NANB hepatitis and nc changes in Hepatitis B serology occurred during follow-up of 2-9 months
i.v. Immuno-globulin type GGV (BPL Elstree) - present method	Same method used as at PFC Edinburgh		
i.v. Immuno-globulin type IV (BPL Elstree) - former method	Gel filtra-tion (Sephadex G25)		NANB reported in 12 patients

From the table, it is clear that none of the manufacturers has provided evidence of the ability of the procedure to inactivate viruses. However pepsin digestion and treatment with B propiolactone are both known to do so.

There is adequate supporting evidence for only 3 licensed or licence pending products

- Human Immunoglobulin (PFC, Edinburgh)
- Sandoglobin (Sandoz)
- Intraglobin (Biotest)

Manufacturers of the other 3 products have not provided evidence of viral inactivation by the process used nor of safety in clinical use, and they should be required to do so

- Gamimune (Miles
- Gammonativ (Kabi-Vitrum)
- Endobulin (Immuno)

Endobulin is not licensed but the other 2 products are, and it is suggested that, in the first instance, an informal approach be made to Miles and to Kabi-Vitrum to ask them to supply the necessary information.

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P.F.C. Edinburgh has provided an interesting and useful pre-publication document, and BPL has provided copies of the protocols used for follow-up of haemophiliacs, and which could be modified for follow-up of patients receiving i.v. immunoglobulin. Unfortunately neither of these documents was available in time to be abstracted in the paper, and they are attached as Appendices 1 and 2 respectively. A copy of a 'pre-draft draft' of FDA requirements for Immunoglobulins is also attached.

Index of Appendices

Appendix 1 : Paper from PFC, Edinburgh

Appendix 2 : Protocols for clinical follow-up studies, from BPL, Elstree

Appendix 3 : Draft of FDA requirements for immunoglobulins

References

1. LANE, R. S., 1983, Lancet ii 974 - 975

2. WELCH, A. G. et al, 1983, Lancet ii 1198 - 1199

3. Leading Article, Lancet, 1983, ii 1077 - 1078

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THE RISK OF INFECTIVITY ASSOCIATED WITH INTRAVENOUS IMMUNOGLOBULIN

- A GENERAL OVERVIEW

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WITN5281065_0018

THE RISK OF INFECTIVITY ASSOCIATED WITH INTRAVENOUS IMMUNOGLOBULIN

- A GENERAL OVERVIEW

1. INTRODUCTION

All plasma pools used for the manufacture of therapeutic blood products are liable to be contaminated with a range of viruses. From experience, the most significant of these are Hepatitis B virus, Hepatitis Non-A Non-B virus(es) (NANB), HTLVIII(LAV).

Certain Human blood products have a high probability of viral transmission and, for example, 100% of haemophiliacs exposed to unheated FVIII for the first time are liable to develop NANB hepatitis[1]. In contrast, intramuscular immunoglobulins manufactured by cold-ethanol (Cohn) fractionation[2] have an excellent safety record with, to date, no recorded cases of AIDS transmission (WHO Expert Committee - reported by A. Zuckerman at Symposium on AIDS In Blood Transfusion, 3rd April 1985) and very few reported cases of Hepatitis transmission (3-5).

The impressive safety record of intramuscular immunoglobulin led to the belief that IgG manufactured by cold-ethanol fractionation is inherently safe. This conclusion has required modification in the light of recent reports of Non-A, Non-B hepatitis transmission by intravenous immunoglobulin products prepared by three different manufacturers, these being BPL (Elstree)[6,7], Nyland[8] and Kabi (Hanson, L.A. and Björkander, J. personal communication). There is, however, no evidence that administration of immunoglobulin products by the intravenous route is inherently infective. In this brief paper, an overview will be given of factors which are likely to contribute to the manufacture and validation of a safe intravenous product.

2. FACTORS CONTRIBUTING TO PRODUCT SAFETY

As stated above, all plasma pools used in the manufacture of blood products are likely to contain viral contamination. The infectivity of intravenous immunoglobulin derived from contaminated plasma is likely to depend on the interaction of several different factors. These factors are:-

2.1 Plasma Quality

Viral inactivation procedures rarely give absolute assurance that no infective virus will survive. For example, it has been shown that pasteurisation of human albumin by heating at 60°C for 10 hours will inactivate no more than 10^4 chimpanzee infective doses of Hepatitis B virus[9]. Therefore, the plasma pools used in the manufacture of blood products should contain the lowest possible levels of viral contamination, and so minimise the demands on any inactivation processes, including neutralisation by specific antibodies in the plasma pool. The level of viral contamination is likely to depend on a number of factors:-

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2.1.1 Exclusion Of Potentially Infective Donations

The most efficient means of excluding infective donations is by the use of assays for specific markers of potential infectivity. We believe that all input plasma must be tested for HBsAg by the most sensitive "third generation" assay methods available. Exclusion of HTLVIII antibody positive donations would also be desirable once the technology has been validated.

The exclusion of some infective donations can also be achieved by persuading donors in "high-risk" categories, particularly homosexuals and intravenous drug abusers, that they should not donate. This exclusion process should be mandatory.

2.1.2 Donor Type

It has been demonstrated[10] that plasma pools from unpaid volunteer donors are generally of lower infectivity than pools derived from paid donors.

2.2 Fractionation Technology

The use of cold-ethanol fractionation could, potentially, contribute to product safety in a number of ways.

2.2.1 Fractional Separation of Viral Contaminants

Viral contaminants may be preferentially fractionated into other protein fractions. For example, there is evidence[11-13] that HBsAg is not fractionated into Cohn Fraction II to any significant extent.

2.2.2 Direct Inactivation By Contact With Ethanol

Alcohol inactivates viruses with a lipid envelope. A recent example of this phenomenon was reported by Spire et al[14] who found that 19% ethanol rapidly inactivated HTLVIII on exposure at room temperature. Ethanol concentrations above this value are routinely used in the manufacture of immunoglobulin preparations and, despite the use of lower temperatures in the fractionation process, there is still reason to believe that some inactivation of lipid-enveloped viruses will occur.

In view of these comments, it seems reasonable to expect that protein separation procedures should follow established cold-ethanol methodologies for the isolation of the IgG fraction, until further information on the consequences of alternative methods is available.

2.3/

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2.3 Finishing Options

After fractionation, an IgG preparation is generated which still contains significant levels of ethanol. This must be removed and other finishing steps may also be necessary to generate a product in its final formulation. The potential virucidal impact of typical finishing technologies can be assessed as follows:-

2.3.1 Intramuscular Immunoglobulin

In the manufacture of intramuscular immunoglobulin, a freeze-drying step is normally employed for the removal of ethanol from the IgG solutions. Serum or albumin solutions of similar protein concentration are known to stabilise viruses during freeze-drying operations[15]. However, the initial presence of ethanol may result in a significant degree of viral inactivation during the freeze-drying procedure.

2.3.2 Intravenous Immunoglobulin

2.3.2.1 Ethanol Removal

In intravenous immunoglobulin manufacture, ethanol is removed under conditions designed to prevent the formation of aggregated immunoglobulin.

Typically, ethanol removal may be by

i. Freeze-drying in the presence of sugar stabilisers.

ii. Diafiltration.

iii. Gel filtration chromatography.

It seems improbable that any of these techniques will lead to any significant degree of viral inactivation.

2.3.2.2 Further Treatment

The manufacture of intravenous immunoglobulin usually involves further processing to reduce the level of spontaneous anti-complementary activity and of vasoactive enzymes. Several of the techniques used at this stage have recognised or potential virucidal activity, and these are listed below. References are given where the stage is known to be virucidal.

a. Reduction and Alkylation.

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b. Reduction and Sulphonation.

c. Beta-propiolactone and ultraviolet irradiation [16].

d. Acid treatment (pH4.0)[17].

e. Complete proteolysis using plasmin.

f. Limited or complete proteolysis using pepsin at low Ph [18,19].

It is believed that one of the three products known to have transmitted NANB Hepatitis received no further treatment following ethanol removal (by gel filtration chromatography) and that ion-exchange chromatography was used in the further treatment of the other two products. Thus, none of these products were subjected to a recognised virucidal finishing procedure.

2.4 GMP failure

Good Manufacturing Practice is of vital importance in all manufacturing processes. GMP failure could contribute to infectivity in two ways.

2.4.1 Failure to properly carry out the effective virucidal stage of a manufacturing process.

2.4.2 Cross contamination due to failure to correctly sanitise equipment used in the manufacture of other batches of blood product. Of particular concern are equipment and materials which are difficult to sterilise (eg chromatography gels) and items such as freeze-driers which are used in the manufacture of other blood products, such as FVIII, which carry a high risk of viral contamination.

3. NON-CLINICAL ASSESSMENTS OF PRODUCT SAFETY

Having chosen a technology which is believed to be safe, it is necessary for a manufacturer to demonstrate that the expected degree of safety is achieved. The best measure of safety is by infusion into patients with careful clinical follow-up (see section 4). Two other methods are available for product evaluation, as follows:-

3.1 Chimpanzee Studies

Since NANB and Hepatitis B viruses cannot be cultured in vitro, the chimpanzee infectivity model has been adopted for a range of products in order to demonstrate that products are safe or processes effective. While such studies have been of value throughout/

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throughout the development of the fractionation industry, a number of disadvantages have come to be associated with this approach.

3.1.1 The value of information from chimpanzee studies is uncertain as at least one report is available of a blood product (FVIII, Hyland) which was found to be non-infective in chimpanzee studies[20] but which has caused NANB hepatitis on clinical evaluation (Mannucci, personal communication).

3.1.2 The chimpanzee is an endangered species. This severely limits the availability of suitable animals for proper scientific study and experiments are inevitably compromised by lack of data.

3.1.3 Results take a long time to accumulate. A typical experiment must run for 18 months before a procedure can be declared "non-infective" on the basis of champanzee studies.

3.2 In-Vitro Viral Inactivation Models

In vitro models have the advantage of being rapid and relatively cheap. They are, however, of restricted value as they are limited to the investigation of viruses which can be cultured. Nevertheless, they do have some value in evaluating manufacturing procedures for the following reasons:-

3.2.1 They can confirm the general virucidal activity of a process step or an entire manufacturing procedure.

3.2.2 They can be used to evaluate the relative performance of alternative manufacturing procedures.

3.2.3 They can be used to evaluate the likely effect of minor or major changes in a manufacturing procedure.

There is therefore a role for this type of study and there is a continuing programme of work on this topic at the PFC.

4. CLINICAL EVALUATION OF PRODUCT INFECTIVITY

There is no simple formula for defining suitable clinical studies which unequivocally demonstrate product safety. The interpretation of clinical trial results may be hampered by low patient numbers and previous exposure to other blood products. Such constraints emphasise the need for detailed prospective patient follow-up and interpretation of clinical data.

Thus, whilst it is not appropriate to propose a universal clinical trial design at the present time, guidelines may be constructed which take account of potential risk of product infectivity and defined areas of product application. It is suggested that for hypogammaglobulinaemia, a minimum of 20 patients should be followed up over a period of six months each/

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each.. It would be expected that a minimum of five different product lots would be included in the evaluation. Trial patients should have no pre-existing markers of infection determined over a suitable period before/
before commencement of the trial. If risk of product infectivity exists then it seems sensible and good ethical practice, in trialling a new product, to minimise patient exposure, consistent with acquisition of sufficient interpretable scientific data.

Criteria for assessment of infection in patients requires definition.

4.1 Hepatitis B

Following the first infusion, patients should be monitored for at least six months for appropriate markers of Hepatitis B infection (HBsAg, HBsAb, HBcAb). The latter two markers are unlikely to appear in hypogammaglobulinaemic patients.

Samples should be taken at approximately monthly intervals.

4.2 NANB Hepatitis

Liver function tests (either ALT or AST) are the accepted method for the detection of NANB hepatitis. Infectivity is indicated by elevated ALT or AST levels (2.0 times upper normal limit) over two consecutive monthly samples, in the absence of other known causes of elevated liver enzymes.

Follow-up should be for a duration of six months post infusion. Pre-infusion patterns of ALT or AST levels are clearly of significance in such a study since transient episodes of hepatitis may occur which are not product related and may modify interpretation of post infusion follow-up measurements.

The reported incidents of NANB Hepatitis transmission have been unequivocal, involving sustained elevations of ALT or AST levels.

4.3 HTLVIII

In hypogammaglobulinaemic patients, seroconversion is improbable and clinical symptoms are the only available indicator of HTLVIII infection. In non-immunodeficient individuals, e.g. ITP patients, the possibility of HTLVIII infection should be excluded by testing for seroconversion to HTLVIII at a suitable period (eg six months) after the initial exposure to the immunoglobulin preparation.

5. SUMMARY

It is apparent that intravenous immunoglobulin is capable of transmitting NANB Hepatitis. It is our belief that maximal safety will be achieved if the following manufacturing procedures are adopted.

5.1 Plasma is collected from healthy unpaid volunteer donors.

5.2/

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5.2 Where possible, plasma donations are screened to eliminate contaminated donations.

5.3 Cold-ethanol fractionation is employed to isolate the IgG fraction.

5.4 Finishing of the immunoglobulin must include a step recognised as being virucidal.

5.5 The highest standards of Good Manufacturing Practice are adopted.

In addition, the need for detailed clinical follow-up studies is emphasised.

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