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9th March, 1988.

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Dear Brian,

Surveillance for evidence of NANBH transmission by BPL concentrates 8Y and 9A

I have at last drawn together the first draft of a manuscript for publishing our results on 8Y and 9A from 1985-7, i.e. before the more rigorous trial to be co-ordinated by Dr. Rizza and Dr. Kernoff. It is not very elegant since I will certainly receive many suggestions for wide-ranging alterations. A few data are still coming in (hence the discrepancy between 29 in the text and 30 in the abstract, etc.) but their inclusion will not greatly affect the conclusions. The Figure will be presented as printed ALT (bracketed AST where appropriate) values along an axis of days since injection as in the Schimpf NEJM paper.

Taking together all the advice I have had on the preferences and time constants of various journals, I propose to offer it successively to Lancet, BMJ and Clin.Lab.Haemat., taking the view that reasonably rapid publication of important results is preferable to polishing imperfect data.

Given the number of views to be reconciled without making the manuscript too apologetic or bland, I would be grateful if you would provide specific text in making counter-suggestions, and indicate the strength of your feeling on a scale from "I'd rather" to "over my dead body". I anticipate difficulty with editors over such particulars as authorship and will probably have to ask you to sign a covering letter for the final manuscript, possibly on several occasions. In replying, please ensure that your letter carries sufficient detail of your present post, title, address, etc. to satisfy all editors. You will have 10 days from receipt of any draft to propose changes, after which you will be taken to have acquiesced.

As you know, a poster will be exposed at ISBT in July, and I will carry the basic data to WFH in May in case there are questions in some symposia. I would also like to give a similar version of these results, with attribution, at an International Workshop on "The Present and Future of Haemophilia Care" on 19th April.

Thank you for your painstaking original contributions. I hope you will agree that this last heave is well worthwhile.

With my best wishes.

Yours sincerely,

J.K. Smith.
Chief Project Scientist.

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SURVEILLANCE OF NON-A NON-B HEPATITIS TRANSMISSION BY COAGULATION FACTOR CONCENTRATES, DRY-HEATED AT 80°.

(Alternative title:

DRY-HEATING COAGULATION FACTOR CONCENTRATES AT 80° GREATLY REDUCES THE RISK OF TRANSMITTING NON-A NON-B HEPATITIS.)

bythe Study Group of the UK Haemophilia Centre Directors on Surveillance of Virus Transmission by Concentrates.

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Abstract

30 patients likely to be susceptible to infection with the virus(es) transmitting non-A non-B hepatitis (NANBH) were treated with 18 batches of a factor VIII concentrate and 10 batches of a factor IX concentrate, both heated at 80° for 72h in the freeze-dried state. Serial measurements of serum transaminase levels for four months revealed no patterns of elevations attributed to NANBH. Severe dry heating appears to have reduced the risk of NANBH transmission from about 90% in untreated concentrates to a rate between 0 and 10%, at least as low as has been demonstrated for any other virus inactivation method.

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Introduction

It has been shown that unheated coagulation factor concentrates, even when made from plasma of unremunerated NBTS donors, transmit non-A non-B hepatitis (NANBH) to almost all patients receiving treatment for the first time (1, 2).

In 1985, Blood Products Laboratory, Elstree, introduced new concentrates of human factor VIII (type 8Y) and of factors IX, II and X (type 9A), both heated in the freeze-dried state in the final container at 80° for 72 hours. This dry-heat treatment was much more severe than heating at 60° or 68° which has been almost completely successful in eliminating HIV transmission by coagulation factor concentrates (3, 4, 5). By inference from laboratory experiments on heat-stable viruses such as vaccinia, it was thought that the higher temperature might be successful in inactivating the virus or viruses causing NANBH, where 60° heating has failed (6). Since experiments in chimpanzees appear to provide an unsafe model for infectivity in human patients (6, 7) and since acute NANBH is often symptomless, the only convincing way to demonstrate infectivity of a concentrate was to carry out serial plasma transaminase determinations for 3-4 months after the first injection into susceptible patients.

The Study Group began collecting data on NANBH transmission before the recommendations of the International Society on Thrombosis and Haemostasis (ISTH) on such trials became firmly established. This analysis is restricted to those cases approximating to compliance with ISTH criteria in terms of previous exposure to blood products and in frequency of testing. Evidence from further categories of patients not meeting entry or testing criteria is discussed briefly and some limitations on the interpretation of this and earlier studies are addressed.

Methods

Concentrates

Factor VIII concentrate (8Y) and factor IX concentrate (9A) were prepared from the plasma of unremunerated donors of the National Blood Transfusion Service at the Blood Products Laboratory, Elstree or at its pilot plant, the Plasma Fractionation Laboratory, Oxford by standard methods. Every donation had been screened and found negative for HBsAg but no screening was carried out for transaminase levels or anti-HB_c. Both fractionation processes ended with heating the freeze-dried product in its final container at 80° for 72h. Routine batches were taken at random and nominated for individual patients trying only to ensure that most some batches were injected into several patients.

Entry criteria

All patients described in the study needed treatment with concentrate and gave informed consent. They had previously been exposed to no large-pool concentrates and no more than 100 units of single-donor products such as cryoprecipitate.

Testing regime

Participants were asked to take blood samples for serum transaminase determinations; before infusion with concentrate; two-weekly to 12 weeks; monthly to six months; and at nine and 12 months. The latter samples were analysed for markers of HB and HIV infection and, being uniformly negative, are not discussed here. Because of missed or delayed appointments, reluctance to take blood from infants, etc., this regime was seldom rigidly adhered to. Data on any patient are analysed here only if he had been successfully sampled at least three times between days 7-91, and at least four times between days 7-119 after first injection with concentrate. Serum transaminase concentrations were determined in the participants' local laboratories and interpreted according to local normal ranges.

Endpoint

The definition of transmission of NANBH was a rise to >2.5 x the

upper limit of normal (ULN), confirmed at a further test, preferably within two weeks of the first, and after the exclusion of hepatitis A, hepatitis B, EBV and CMV as probable alternative explanations. No duplicate samples were stored, e.g. for repetition of these determinations in a central laboratory.

Second treatments

When a patient received a second batch of concentrate within 14 days of the first, he was considered to have been exposed to both batches. Batches given later during the study period were not considered to be under trial. Although there were no suspicious events when such patients were followed up for a second time, second treatments were excluded from this analysis.

Results

In Figure 1 serial serum transaminase levels are plotted against the interval since first infusion of concentrate. Three categories of patient were considered:

Series 001: 7 patients receiving 8Y without previous exposure to any blood product.

Series 101: 12 patients receiving 8Y after previous exposure only to single-donor products.

Series 201: 10 patients receiving 9A with no previous exposure to any blood product.

Even though a 14-week sample was not requested in this study as it is in ISTH guidelines, a mean of 6.8 tests was performed on these patients between days 7-119 after injection with concentrate. Only five patients had a gap in testing >35 days in the four-month study period.

In these 29 eligible patients adequately followed for four months, none had an AST or ALT level >2.5 x the upper limit of locally defined normal, confirmed by a prompt repeat test. Two events deserve comment. Patient 206 showed a rise of ALT to 102 u/L (<2.5 x ULN) on day 34, but was normal on day 6 and day 55, the nearest dates on which he could be tested. Patient 101 had a rise of ALT to 107 u/L (>2.5 x ULN) on day 133; this rise was not confirmed five days later and AST and other liver function tests were normal throughout. The batch of 9A given to patient 206 was also given uneventfully to patients 207 and 208. Patient 101 received three batches of 8Y, two of which had not been given to any other patient in this study and one which was given uneventfully to patient 104.

Only patient 006 had an abnormal pre-injection transaminase level, and that elevation was marginal. In the ___ cases where a pre-injection sample could not be obtained, the earliest post-infusion transaminase levels were normal.

These 29 patients received a total of 18 batches of 8Y and 10 batches of 9A, containing approximately 180,000 donations of plasma.

Discussion

A superficial conclusion from this study is that none of 29 patients exposed to factor VIII or factor IX concentrate, dry-heated at 80° for 72h, developed NANBH according to ISTH criteria. This may be interpreted statistically as indicating a true incidence (95% confidence limits) of NANBH transmission in a range between 0 and 10% (8). However, the quality of the data requires careful assessment.

ISTH recommend that patients entering such trials should have normal liver function and no prior exposure to blood products. Only one of the patients described in this study had pre-injection ALT or AST outside the normal range, and 12 had been exposed to between 1 and 100 units of cryoprecipitate or plasma. The incidence of post-transfusion hepatitis in the UK in 1983 (9) suggested that approximately 0.3% of blood donations may transmit NANBH. The small but significant risk that some of these 12 patients were previously exposed to NANBH from single-donor products might reasonably be held to weaken statistical conclusions by the equivalent of one or two cases, but should not exclude all 12 patients from this analysis. Moreover, there is a lack of published evidence to support one assumption behind the ISTH exposure criteria, namely that earlier NANBH infection obscures the detection of re-infection by a later treatment. In one study (10) which included both untreated patients and some who had been treated only infrequently with large-pool concentrates, infective batches of unheated concentrate affected both groups equally; six of nine previously untreated patients were infected and four of six infrequently treated patients were infected.

The only four published studies reporting a substantial incidence of NANBH transmission (1, 2, 6/7, 11) offer little support for a rigid insistence on two-weekly determination of serum transaminases. Of a total of 44 published histories showing significant elevations of transaminases to >2.5 x ULN, 42 would have shown at least one significant

elevation if tested only at 4, 8, 12 and 16 weeks; the remaining two would have been observed only by continuing tests beyond 20 weeks. Indeed, testing at four-weekly intervals to 16 weeks would almost certainly have found elevated transaminases on at least two occasions in 38 of these 42 cases, and possibly in another three (the uncertainty in interpretation being due to the different graphical methods and probable approximations used by authors to illustrate the time course of infection). This further diminishes the significance of isolated transaminase elevations seen in this and other studies. There is an unquantifiable statistical risk that even two-weekly testing for 16 weeks may miss an atypically brief or late rise in transaminase levels. Gaps in testing or reduced frequency obviously increase the likelihood of missing an atypical response to NANBH, but failure to comply with an arbitrary testing regime does not justify the exclusion of cases approximating to at least four-weekly testing. The patterns of testing achieved in the present study would have revealed virtually all transaminase elevations published in all four relevant studies.

The donations from which these batches were derived were unlikely to have contained HB after sensitive and specific screening. Early batches made from plasma unscreened against HIV antibody may have been exposed to an incidence of 0.002% HIV antibody-positive donations (12), reducing to perhaps 1 in 10^6 in batches released during 1986, when donor screening became effective. Although evidence of HB and HIV transmission continues to be sought in these and other groups of patients, the apparent absence of transmission so far provides little evidence of inactivation of these viruses by dry heating. In contrast, all production batches were likely to carry at least one donation capable of transmitting NANBH, even when diluted with thousands of non-infective donations. Because at least one previous study (11) had shown that failure to inactivate NANBH might be confined only to a small proportion of batches treated, it was considered preferable to use a large number of batches in the present trial. This becomes a disadvantage only when a

batch falling under suspicion is found to have been used by only one or a few patients in full compliance.

In addition to these 29 patients, many other patients not meeting entry or compliance criteria for this study have been followed less formally. Among 25 patients who have had some transaminase determinations, two have shown single elevations not attributed to NANBH infection, and one had a sustained ALT and AST elevation consistent with NANBH; his treatment prior to and subsequent to his first injection of 8Y makes it difficult to confirm an association between treatment and NANBH, and no other patient in trial compliance received the implicated batch.

Earlier surveillance for NANBH transmission by a pasteurised factor VIII concentrate gave equivocal results (13, 14) and led to the most satisfactory study to date, complying totally with ISTH recommendations; there were no significant elevations of serum transaminase levels in 26 patients with no prior treatment with blood products, tested at two-weekly intervals (15). Similarly, having demonstrated here that dry heating at 80° is highly effective in inactivating NANBH virus in coagulation factor concentrates, a second trial of 8Y and 9A, rigorously in line with ISTH criteria, has been started to quantitate more precisely any residual risk. In the absence of objective and specific tests for the virus(es) transmitting NANBH, clinical trial is incapable of proving that transmission has been eliminated by any inactivation method. When 0.3% of the apparently normal healthy population may transmit NANBH, and many more may have sporadically elevated transaminases, occasional misattributions can be expected to occur, and there can be no perfect safeguard against trial patients receiving other products than those intended. Thirty patients would be required to show that any single batch has a 0-10% risk of transmitting NANBH, and any failures will probably continue to be limited to occasional individual batches until more generous margins of inactivation can be achieved, perhaps by the application of more than one virucidal process during manufacture.

Physicians who care for haemophiliacs should resist the comfortable assumption that clinical trial alone can demonstrate the complete success of any process designed to inactivate NANBH, and should continue to prescribe as if all blood products still carry a diminishing but finite risk of transmitting blood-borne viruses, particularly NANBH.

This is the first study showing that sufficiently severe dry heating in the final container may eliminate or greatly diminish the former virtually inevitable transmission of NANBH by coagulation factor concentrates made from large pools of plasma. The range of statistically probable incidence is the same as that shown for pasteurisation in solution (15), and better than that shown for factor VIII heated in a non-aqueous immiscible fluid (11). Although clinical proof of inactivation of HIV is unattainable, laboratory (16, 17) and clinical evidence (3, 6) strongly justify the inference that HIV is much more heat-sensitive than NANBH.

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