noteworthy that the convention of reporting the success of immunisation as a percentage of those who seroconvert (ie, a 4-fold rise over pre-vaccination antibody levels) is of little relevance when maternal antibody is high, as was the case in many of the children immunised with the EZ vaccine. What matters is the level of antibody at 9 months—by then maternal antibody is no longer present and so this measurement reflects the childs true response to vaccination and his state of immunity.

The EZ vaccine offered clinical protection against measles which was as good after vaccination as that offered by the Schwarz vaccine: measles developed in 2 of 28 children in the EZ group, known to be exposed to measles after vaccination, and in 2 of 14 children in the SW group. In addition, a further 5 children in the SW group had measles before vaccination. The results for short-term and long-term morbidity after the large dose of EZ vaccine were encouraging and exposure to measles in the EZ children was not associated with any untoward reactions. Thus this study showed that the EZ vaccine, given in a dose of 40 000 pfu to children aged 18 weeks, offered safe and effective protection against measles up to at least 18 months of age.

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EFFECT OF DRY-HEATING OF COAGULATION FACTOR CONCENTRATES AT 80°C FOR 72 HOURS ON TRANSMISSION OF NON-A, NON-B HEPATITIS

STUDY GROUP OF THE UK HAEMOPHILIA CENTRE DIRECTORS ON SURVEILLANCE OF VIRUS TRANSMISSION BY CONCENTRATES*

Summary 32 patients with coagulation factor deficiencies and likely to be susceptible to non-A, non-B hepatitis (NANBH) virus infection were treated with a total of 20 batches of a factor VIII concentrate and 10 batches of a factor IX concentrate, both heated at 80°C for 72 h in the freeze-dried state. Serial measurements of serum aminotransferase levels for 4 months revealed no patterns of rises attributable to NANBH. Severe dry heating appears to have reduced the risk of NANBH transmission from about 90% in untreated concentrates to a statistically determined rate of 0–9%. No evidence was found in recipients of infection with hepatitis B or human immunodeficiency virus.

Introduction

UNHEATED coagulation factor concentrates, even those made from plasma of volunteer donors in Britain, transmit non-A, non-B hepatitis (NANBH) to almost all patients receiving treatment for the first time.[1,2] In 1985, the Blood Products Laboratory 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°C for 72 h. This treatment is much more severe than heating at 60°C or 68°C, which almost completely eliminates human immunodeficiency virus (HIV) transmission by coagulation factor concentrates,[3-5] but because of the effect on heat-stable viruses such as vaccinia in the laboratory, it was thought that this high temperature treatment might inactivate the virus or viruses causing NANBH, which withstand heating to 60°C.[6] Since chimpanzees seem not to be a satisfactory model for studying infectivity in man,[6,7] and since acute NANBH is often symptomless, the only convincing way to examine infectivity of a concentrate is to measure aminotransferase concentrations in serial plasma samples 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 Committee on Thrombosis and Haemostasis (ICTH) on such trials became firmly established. This analysis is restricted to those cases approximating to compliance with ICTH 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.

Patients and Methods

Concentrates

Factor VIII concentrate (8Y) and factor IX concentrate (9A were prepared by standard methods from the plasma of volunteer donors of the National Blood Transfusion Service at the Blood Products Laboratory, Elstree, or at its pilot plant, the Plasma Fractionation Laboratory, Oxford. All donations were negative for HBsAg. They were not screened for aminotransferase levels or anti-HBc. Both fractionation processes ended with heating the freeze-dried product in its final container at 80°C for 72 h. Routine batches were taken at random and nominated for individual patients, to ensure that at least some batches were injected into more than 1 patient.

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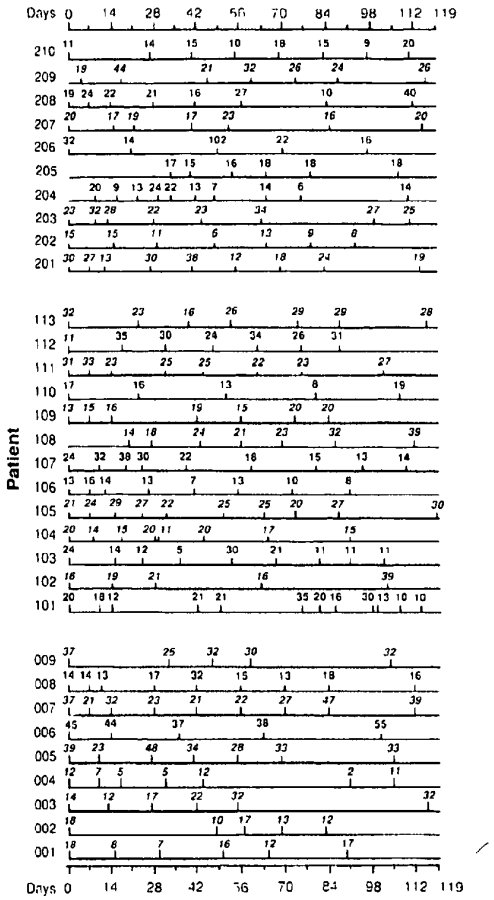

Serial serum aminotransferase levels in 32 patients after first infusion with factor VIII or factor IX concentrate.

Day 0 = day of first injection.
ALT concentrations (in normal type) and AST concentrations (in italics) given as units/l.

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, whole blood, or fresh-frozen plasma.

Testing Regimen

Participating physicians were asked to take blood samples for serum aminotransferase measurements before infusion with concentrate, every fortnight to 12 weeks, monthly to 6 months, and at 9 and 12 months. This schedule was seldom rigidly adhered to for reasons such as missed or delayed appointments or reluctance to take blood from infants. A patient was included in the analysis 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 the first injection with concentrate. Serum aminotransferase concentrations were determined in the participants' local laboratories and interpreted according to local normal ranges. Approximately every 3 months, samples were analysed for markers of hepatitis B (HB) and HIV infection; since the results were uniformly negative, they are not discussed here.

Endpoint

The definition of transmission of NANBH was a rise in alanine aminotransferase (ALT) or aspartate aminotransferase (AST) to $>2{\cdot}5\times$ the upper limit of normal (ULN), which was confirmed at a further test, preferably within 2 weeks of the first, and which could not be accounted for by hepatitis A, hepatitis B, Epstein-Barr virus, or cytomegalovirus infections.

Second Treatments

24 patients received only one batch of concentrate. A patient who received further batches of concentrate within 14 days of the first was considered to have been exposed to all batches. Batches given later during the study period were not considered to be under trial. Second treatments after completion of the first were excluded from this analysis, but there were no suspicious events when study patients were followed up for a second time.

Results

Three categories of patient were considered: series 001–009 consisted of 9 patients receiving 8Y without previous exposure to any blood product; series 101–113 of 13 patients receiving 8Y after previous exposure to only single-donor products; and series 201–210 of 10 patients receiving 9A with no previous exposure to any blood product. These 32 patients received a total of 20 batches of 8Y and 10 batches of 9A, representing approximately 200 000 donations of plasma. Production of these concentrates started before the screening of blood donations for anti-HIV became mandatory in the UK; 23 of these patients received at least one batch made from unscreened plasma. The patients received between 370 IU and 27 800 IU (mean 3260 IU) of factor VIII or factor IX concentrate within the first 14 days of treatment. 16 patients were vaccinated against hepatitis B, 5 before and 11 during the follow-up period.

Even though a 14-week sample was not requested in this study as it is in ICTH guidelines, a mean of 6·5 tests was performed on these patients between days 7–119 after injection with concentrate. Only 7 patients had an interval of >35 days between consecutive tests in the 4-month study period, attributable in most cases to missing the sample at about 14 weeks.

In the 4 months of follow up none of the 32 eligible patients had an AST or ALT level $>2{\cdot}5\times$ ULN, as confirmed by a prompt repeat test. Two events deserve comment. Patient 206 showed a rise of ALT to 102 u/l ($<2{\cdot}5\times$ ULN) on day 49, but was normal on day 21 and day 70, the nearest dates on which he could be tested. The batch of 9A given to this patient was also given uneventfully to patients 207 and 208. Patient 101 had a rise of ALT to 107 u/l ($>2{\cdot}5\times$ ULN) on day 133; this rise was not confirmed 5 days later, and AST and other liver function tests were normal throughout. He 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-infusion aminotransferase level, and that rise was marginal. In the 3 cases where a pre-injection sample could not be obtained, the earliest post-infusion aminotransferase levels were normal.

Discussion

In none of 32 patients exposed to factor VIII or factor IX concentrate, dry-heated at 80°C for 72 h, did NANBH develop as defined by ICTH criteria. This may be interpreted statistically as indicating a true incidence (95%

confidence limits) of NANBH transmission in a range of between 0 and 9%[8]—a range similar to that for pasteurisation in solution[9] and better than that for factor VIII heated in non-aqueous immiscible fluid.[10] However, the quality of the data requires careful assessment.

ICTH recommend that patients entering such trials should have normal liver function and no prior exposure to blood products. Only 1 of the patients described in this study had pre-infusion ALT or AST outside the normal range, and 13 had been exposed to between 1 and 100 units of cryoprecipitate or plasma. A study on the incidence of post-transfusion hepatitis in the UK in 1983[11] suggested that approximately 0·3% of blood donations may transmit NANBH. In two recent studies[2,12] a total of 11 haemophiliacs treated with only cryoprecipitate (mean 71 bags) underwent serial aminotransferase measurements; in none did NANBH develop. The small but significant risk that some of these 13 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 13 patients from this analysis. Moreover, there is a lack of published evidence to support one assumption behind the ICTH exposure criteria—namely, that earlier NANBH infection obscures the detection of re-infection by a later treatment. In one study,[11] 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; 6 of 9 previously untreated patients were infected and 4 of 6 infrequently treated patients were infected.

The only four published studies reporting a substantial incidence of NANBH transmission by clotting factor concentrate[1,2,6,12] offer little support for a rigid insistence on fortnightly measurement of serum aminotransferases. Of the 44 published histories showing significant rises in aminotransferases to $>2{\cdot}5 \times$ ULN, 42 would have shown at least one significant rise if tested only at 4, 8, 12, and 16 weeks; the remaining 2 would have been observed only by continuing tests beyond 20 weeks. Indeed, testing every 4 weeks for 16 weeks would almost certainly have picked up raised aminotransferases on at least two occasions in 38 of these 42 cases, and possibly in another 3 (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 aminotransferase rises seen in this and other studies. The patterns of testing in the present study would have revealed virtually all aminotransferase rises published in all four relevant studies.

The sensitive and specific methods of screening used indicated that the donations from which these batches were derived were unlikely to have contained hepatitis B virus. Before the days when plasma was screened for HIV antibody about 1 in 50 000 donations used for making concentrate probably contained HIV antibody.[13] By 1986, when donor screening became effective the proportion was probably 1 in 10^6. Two-thirds of our patients received a batch made from plasma unscreened for HIV antibody. In contrast, all production batches were likely to be made from pools containing at least one donation capable of transmitting NANBH, even when diluted with thousands of non-infective donations. Because at least one previous study[12] had shown that failure to inactivate NANBH might be confined only to a small proportion of batches treated, we thought it preferable to use a large number of batches in the present trial.

In addition to these 32 patients, many other patients not meeting entry or compliance criteria for this study have been followed up less formally. Among 25 patients who have had some aminotransferase measurements, 2 have shown single rises not attributable to NANBH infection. 1 other patient had a sustained ALT and AST rise consistent with NANBH; his incompletely documented treatment with single-donation products before and subsequent to his first injection of 8Y makes it difficult to draw conclusions on an association between treatment and NANBH. No other patient who complied with the trial received the implicated batch.

After this demonstration that dry heating at 80°C is highly effective in inactivating NANBH virus in coagulation factor concentrates, a second trial of 8Y and 9A, rigorously in line with ICTH criteria, has been started to quantify more precisely any residual risk. In the absence of objective and specific tests for the virus(es) transmitting NANBH, clinical trials cannot prove that transmission has been eliminated by an inactivation method. When 0·3% of the apparently normal healthy population may transmit NANBH, and many more may have sporadic rises in aminotransferases, occasional misattributions can be expected to occur. 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 should continue to prescribe as if all blood products still carry a diminishing but finite risk of transmitting blood-borne viruses, particularly the agents for NANBH.

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