Item I of 7.2.85

0065

FVIII STUDY GROUP - 7 FEBRUARY 1985

UPDATE PAPER ON VIRICIDAL ACTION SINCE LAST MEETING ONE YEAR AGO

SUMMARY

Considerable action has taken place on the heating of FVIII, both wet and dry. The latter is not entirely satisfactory for elimination of hepatitis B (probably ≈ 2 logs), whilst the effect on NANB is still uncertain in the absence of a titred infectious source. Dry heat appears to be satisfactory for the inactivation of AIDS virus (HTLV III) since this agent has been shown to be far more heat sensitive than HBV. Wet heating has the greatest potential for killing viruses. However, it is in abeyance at the moment for two reasons: firstly, the scale-up of the zinc high purity project has been shelved due to other developments in FVIII manufacture and secondly, because of pressure on PFC to complete dry-heat treatment of all existing batches of FVIII.

A number of notable developments in the last year include the isolation of HTLV III (?AIDS virus) and its culture, detection and antibody assay, as well as cloning and sequence of its genome. Last but not least was the publication of the structure of the FVIII:C gene and protein.

RECENT PUBLISHED WORK

Physical purification of FVIII complex from HBV has been claimed for polyelectrolyte PE5 by both ^{125}I-tracer and DNA-hybridisation; however the data do not support better than 2 logs decrease (Galpin et al, 1984).

The German group continue to publish on β-propiolactone and UV (together with Aerosil for serum) and 3% Tween 80 for FVIII concentrates, one other group has also taken up the β-PL and UV approach

(Yoshizawa et al) and report that it is capable of inactivating NANB hepatitis infectivity as judged by em of liver biopsies in chimpanzees. In order to improve the yield of FVIII from cold sterilised plasma, Stephan et al (1983) applied UV irradiation first, 3% Tween 80 second, then a reduced concentration of 0.05% (reduced from 0.25%) β-propiolactone. This was followed by PEG fractionation and Aerosil adsorption. A yield of 60% in the sterilisation steps is claimed. Prince et al (1983a) studied the chimpanzee infectivity of the above process with hepatitis B and non-A non-B. Unfortunately, the study was complicated by the presence of both viruses in one concentrate. They conclude that NANB can prevent or delay the onset of HBV. They conclude that the Tween/UV/β-PL/PEG process kills $10^{6.9}$ CID of HBV and an unknown quantity of NANB. Prince et al (1984a) also report on the inactivation of Hutchinson strain NANB by treatment with Tween 80 and ether.

Related topics of interest include the detection of virus particles or antibodies in vitro or in vivo. Of special interest was the report by Karayiannis et al (1983) on the experimental infection of tamarins (Saquinus labiatus) with NANB hepatitis. Duermeyer (1983a, 1983b) have confirmed by chimpanzee infectivity that their "DS-antigen" is not synonymous with infectivity of NANB since chimpanzees which showed an incubation time of 5-7 weeks for NANB hepatitis had not formed antibodies to DS-Ag even after 10 months. Nevertheless, the antigen does show a significant association with post transfusion hepatitis (65%) and the antibody is present in 48% of haemophilia A patients.

An interesting approach (Neurath and Strick, 1983) from the NY Blood Centre is to try and detect any foreign (non-human) DNA in plasma by a radioimmunoassay that can detect picogram quantities of DNA, however, interpretation of results is a problem. Lemonne et al (1983)

and Pontisso et al (1984) report the detection of HBV infection in cultured human lymphoblastoid cells and peripheral blood leukocytes respectively. Gordon et al (1983) report the occurrence of Burkitt-lymphoma in a patient with classical haemophilia receiving FVIII concentrates. Finally, we note (Froebel et al, 1983) that Scottish FVIII concentrates appear to produce depression of T cell helper : suppressor ratios as has been seen with other manufacturers, and that many of our haemophiliacs have anti-HTLV III antibodies.

HEAT / γ RADIATION

A patent (US 4,440,679) has been issued to Cutter in which saturated sucrose (80% w/v!) is used to pasteurise FVIII at 60°C for 10 hr, but the various claims for yield of FVIII:C range from 6% to 63%. More interesting is the report of Margolis and Eisen (1984) in which low concentrations of amino-acid mixtures (1-4 mg per unit of FVIII:C) are added to freeze dried concentrates prior to dry heating at 37-85°C or γ-irradiation (1-4 M rad) in which stability of FVIII:C and solubility were dramatically improved. In view of the likely identity of AIDS virus with HTLV III (Gallo et al, 1984) a cytopathic retro-virus, it is reasonable to extrapolate from the known heat sensitivity of other type 'C' retroviruses to expect that dry heat will be sufficient for their inactivation. The details on the genome are still awaited, but a report is in press describing the cloning and sequence of the genome. Information on the genome size of the single stranded RNA will also allow estimates of its susceptibility to γ-irradiation. The report on the improved solubility of γ-irradiation FVIII in the presence of amino acids is also important for two reasons. Firstly, it confirms our preliminary data that γ-radiation per se was not the only cause of lost FVIII:C bioactivity, but that insolubility of the sample was the major problem. Secondly, it suggests that we do not need high purity (≡ low fibrinogen) concentrates to study

γ-irradiation if amino acids are able to stabilise our concentrates. It seems worthwhile to plan a series of pilot experiments with PFC as Brenda Griffin is interested in doing the work for her thesis and Ethicon are still prepared to irradiate small batches of material for us at 2.5 M rad free of charge. Unfortunately, from Margolis' data, a loss of ca 25% FVIII:C can be expected at 2.5 M rad. It is interesting to recall the early work of Aronson (1962) when the critical target MW of FVIII:C was estimated to be 180,000 - this is not far from the subunit size following the first activation/cleavage by thrombin (≈ 150,000). Despite these reservations, γ-irradiation still remains the 2nd best option after heating - or in combination with dry heating. This is because it fits easily into the production process and adds no potentially toxic materials to the products. Capital investment would also be low since space can be rented in Ethicon's facility.

DETERGENTS / ORGANIC SOLVENTS

These both function in a similar fashion by disrupting the hydrophobic (?lipid) coat of viruses allowing the contents to be physically disrupted and/or receptors to be lost and/or nucleic acids to be metabolised. Obviously if a virus does not have a lipid coat, it is not a likely candidate for inactivation by this process. So far as we know, HBV, NANB and CMV etc are all likely candidates, although one form of NANB (Bradley, 1983) is reported to be chloroform resistant. The advantage of detergents over solvents is that they are more effective at disrupting hydrophobic coated viruses (including HBV) and there is a wide choice of materials available and they are almost totally innocuous as regards loss of FVIII:C activity. However, their disadvantage is the need to remove them afterwards by further fractionation and the almost total lack of data on their acute or long term toxicity.

The advantage of organic solvents is their ready removal, eg by flash evaporation, phase separation/dialysis or freeze drying, but they tend to be less efficacous than detergents at disruption of lipoproteins and more prone to denature soluble proteins. No data are available on the log kill of detergents used alone with HBV or NANB.

CHEMICAL METHODS

No further details are available on the two Immuno processes (probably ethoxyformicanhydride and bile salts). In general, chemical methods are unattractive since they inactivate a large proportion of the FVIII:C ($\geq$ 40%), can result in neo-antigen formation and are highly suspect as regards toxicity. No other chemical (covalent) methods of inactivation have been proposed other than β-propiolactone and this negative evidence can be taken as a general pessimistic view about the practicability of this approach. A range of viruses are now commercially available that have been "inactivated" by photo-activation of psoralen dyes intercalated within their DNA, thus the protein/lipid structures are maintained intact. It is unlikely that any agency would consider licensing these compounds for in-vivo products in view of their mode of action which can be potentially skin carcinogenic. Nevertheless, they represent a useful laboratory source of "nasty" virus for serological work without the need for microbiological contaminant facilities.

PROPOSALS FOR FUTURE WORK

1 Heated FVIII:C concentrates should be carefully monitored in vitro and in vivo for untoward reactions (see Appendix) and with 'spiked' viruses. Suitable systems for evaluation are currently being explored by PFC. Available information will be presented at the meeting.

2 γ-irradiation (2.5 M rad, 20°C) of high purity concentrates and intermediate purity concentrates with added amino acids (4 mg/unit) should be done.

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3 A "watching brief" should be kept on detergents and organic solvents.

4 DNA-probe hybridisation methodology should be made available within the SNBTS.

APPENDIX

Methods for evaluation of heat damage in heat treated FVIII.

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