SNBTS FVIII STUDY GROUP

FIRST REPORT OF THE SAFETY ACTION GROUP

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PRELIMINARY MEETINGS WERE HELD AT H.Q. LABORATORY:

Duncan Pepper and Bruce Cuthbertson on Tuesday, 9th February 1982
Duncan Pepper and Bobby Sommerville on Wednesday, 10th February 1982.

SUMMARY

Any attempts to inactivate or remove infectious agents presuppose a satisfactory infectivity assay. This should be assured or developed by the group. Any attempts to heat or irradiate the concentrates of FVIII:C presuppose a more purified/more stable concentrate than those presently available. At the present time the efficacy appears to be heat > irradiation > adsorption.

INTRODUCTION

Before considering what has to be achieved and what we proposed to do, I would like to raise several questions for discussion:

(i) What will be the effect of doing nothing at this time? It may well be that this is an appropriate and valid course of action.

(ii) What is the nature and quantity of the risks in Scotland at this time? Are we worrying about a problem which exists elsewhere (England? W. Germany? USA?).

(iii) Are current developments in other associated areas moving at such a pace that any realistic time scale for our projected work may well end in shelving the whole project, e.g. genetic engineering of FVIII and/or synthetic antigen vaccines or production of neo-classical vaccines?

I personally believe that political and economic considerations force us to act as we are in effect competing with commercial companies, thus (i) above is not appropriate. Published data, although several years out of date, indicates that although hepatitis-B is decreasing to levels lower than non-A, non-B hepatitis, there are significant amounts of the latter in England, and Scotland may also have a significant problem but more data is urgently required. Obviously, we will have to rank our action in terms of priority as regards cost, feasibility and time scale. Likewise, we have (however difficult) to estimate the time scale and impact of R & D in areas outwith our own.

Finally, it is clearly unwise to embark upon a course of inactivation if the benefit (reduced infectivity) cannot be proven.

1. SUMMARY OF WHAT IS KNOWN:

We have restricted ourselves to viral risks, which include in addition to hepatitis B, a probable two (or more) hepatitis non-A, non-B, CMV, herpes, human polyomas and Kreutzfeld-Jakob agent. Considerable progress has been made in the last three years in evaluating the protein and DNA structures in hepatitis B. Significant advances include the production of a vaccine (MSD-inactivated 22 nm particles), the purification

of a 30,000 surface polypeptide (prototye pure vaccine), the incorporation of HBsAg genome into E. Coli (actually done in Edinburgh in 1979!) and the total chemical synthesis of surface exposed peptide sequences (as potential synthetic vaccines). Relatively less hard scientific data is available on non-A, non-B hepatitis. However, incidence data by manufacturer, incubation times and liver biopsy all indicate that two or more agents are responsible. There are rumours of an RIA from the N.Y.B.C. Symposium last year, and immuno-electron microscopy has shown a positive result in some cases. It seems likely that developments in non-A, non-B will follow the same route as hepatitis B, but over a considerably shorter time span, (e.g. 5 years vs. 10 years) due to technological gains, notably in genetic manipulation.

As much of the most relevant information upon which we must base our decisions is unpublished (i.e. < 2 years old), we must make strenuous efforts to confirm data, preferably by direct personal contact, e.g. letter/telephone/visit. In this latter category we can anticipate some foreign travel inevitably.

Presumably, further improvements in the efficiency of hepatitis-B by e.g. RIA, will only happen slowly and at greater cost than previous improvements. On the other hand, we can anticipate rapid progress in screening for non-A, non-B.

No discussion of possible inactivation strategies can take place without concurrent evaluation of the impact (cost and effectiveness) of immunisation, screening, etc. Obviously, to be attractive, inactivation must be cheap, reliable and capable of killing more than one virus. I would also add that in my opinion it should be developed within two years. Any longer than this is too unpredictable as regards other developments which may make inactivation obsolete.

Turning first to γ-irradiation as a method of inactivation, it is possible to calculate the dose required for a given degree of inactivation and is largely a function of the degree of double strandedness of the

nucleic acid, to a lesser extent, MW also has an influence. In practice, other physical and chemical factors such as temperature and reducing agents also have a modifying effect. Unfortunately, we do not have any data on the DNA/RNA of non-A, non-B viruses, and the situation is complex with hepatitis-B due to variable double strandedness, repair kinase and incomplete genome. All these factors lead us to conclude that some sort of trial inactivation is desirable with a model virus (e.g. SV-40 or polyoma) which is readily available and can be easily bioassayed for infectivity following irradiation. Hopefully, more model viruses can be included to generalise the findings in respect of MW, type of nucleic acid and degree of double strandedness. As with all inactivation tests, we have to ask ourselves how much inactivation is worthwhile? Calculations suggest that γ-irradiation will achieve at most 10^2 - 10^3-fold inactivation of hepatitis-B. However, bearing in mind that screening has now reduced infectivity to such titres, it could be attractive.

An alternative to γ-irradiation is heating (pasteurisation). This has been attempted by Behringewerke who now market "Faktor VIII HS" in which HS implies "safe from hepatitis". Unfortunately only one paper has been published (in German) and no details are given of solution compositions or yields. However, estimates by P.F.C. indicate 8% yield which is rather low. Examination of the process shows that by RIA of HBsAg and chimpanzee infectivity a total reduction of 10^7 - 10^8-fold occurred, but much of this must be due to removal of antigen by physical means (RIA for HBsAg $<$ 1 ng/ml in final product). In fact the process clearly only works because large amounts of protein (fibrinogen) are removed prior to the heating step and these preliminary steps may well be responsible both for the removal of hepatitis and the low yields. Thus we conclude that heating of itself may not be an inherently low-yield step, nevertheless it is clear that low fibrinogen (≡ high purity) is a desirable product for both heat inactivation or γ-irradiation processes.

A third approach which has been used for FIX concentrates (but not for FVIII concentrates) is a combination of β-propiolactone and UV irradiation. This has been used by Biotest Serum Institute (Frankfurt) but very little practical information is to hand. Published information is contradictory; one paper states that a total of 10^4-fold reduction is achieved (10^3-fold inactivation and 10^1-fold purification) whereas a more recent abstract claims 10^7-fold total reduction or 10^8-fold if a third treatment step (aerosil adsorption) was used. This latter step was only applied to normal serum pools but it implies that (a) adsorption is feasible to reduce infectivity at low concentrations and (b) that Biotest are not totally satisfied with β PL-UV on their own. Furthermore, since about 40% of FII is inactivated, we may predict that FVIII:C will be greatly inactivated, though no published data is to hand.

Finally, although little data is available, some attention has been given to non-specific filtration/adsorption of hepatitis virus. Kabi have published a method in which dextran sulphate was used to purify HBsAg and more recently caprylhydrazide agarose was used to adsorb out HBsAg from FIX concentrates. Obviously, further work and information are needed in this area, but it has one great attraction, namely that removal of infectivity can reasonably be equated with removal of HBsAg assayed by RIA and thus obviating the need for infectivity assays during development work. However, such assays are not yet available for non-A, non-B virus and the adsorbents may well have to be redeveloped and tested for each virus type.

Assay of infectivity is the major problem to be faced in this work. At present only one assay is established, that in chimpanzees. This service is available commercially and without a serious waiting list (at present). We are informed by Dr. Kellner of the N.Y. Blood Bank that acquisition of suitable chimpanzees cost £8,000 per animal and a further £1,400 per month for laboratory testing and care. Thus each chimpanzee will cost about £10,000 per 6 month trial, and a straightforward experiment (3 samples, 3

controls) would cost £60,000 minimum.

More recently, reports from Panama indicate that Owl monkeys can simulate closely the human hepatitis-A virus infection. If they can also be infected with other human hepatitis viruses, then the cost of acquisition and availability of animals would improve. However, we should not anticipate that such a colony would be any cheaper to run and it would still be (geographically) very inconvenient. It also has to be developed to the same state as the chimpanzees which may take some time.

Finally, the most attractive possibility would be a tissue culture assay for hepatitis virus. No such assay exists, but we should actively pursue those working in the area to see if such an assay is likely to appear in the near future. Also, we might consider undertaking such work ourselves. A cell line ('Alexander') of human hepatoma has been established which appears to have incorporated the genome corresponding to the HBsAg but not to the core antigen, i.e. it does not produce infective material but this does not constitute a tissue culture assay of infectivity.

2. PROPOSALS FOR ACTION:

Bruce Cuthbertson (P.F.C.) - will investigate charged diatomite filters (AMF Cuno) which have performed well in removing pyrogens from FVIII concentrates. If suitable laboratory facilities can be found, this work will be extended to include HBsAg by RIA. Other filters might be included, especially capryl hydrazide as the techniques are similar. We also suggest that the heat inactivation work initiated by Alex McLeod be continued, specifically to look at the behaviour of FVIII:C/C:Ag/R:Ag in higher purity concentrates and in the presence of stabilising salts.

Duncan Pepper (H.Q.) - will investigate irradiation, as a function of temperature, additives and fibrinogen content. Marker viruses will also be added to "dope" intermediate FVIII concentrates. Most of this work will be

done at fixed doses in the range 1-3 Mrad. Water content may also be an investigated variable if feasible. Literature searches will also be carried out on other irradiation techniques (e.g. UV).

Bobby Sommerville (Belvidere Hospital) - will carry out a literature search and personal contacts on the biological problems of infectivity models, tissue culture, antibody localisation assays etc. Dummy viruses as candidates for inactivation will also be provided.

3. RESOURCES REQUIRED:

It is difficult to quote actual figures at this stage, but general areas where expenditure could be incurred are listed with typical costs.

Staff: Presently not needed, but may need one research assistant in future, say £7,000/year.

Laboratory Space: Not a problem except for category 'B' virus work, which will have to be borrowed.

Raw Materials: Commercial FVIII:C concentrates, < £1,000 in total.

Animals: 10 Chimpanzee infectivity assays, approx. £100,000.

Specialised Assays: e.g. infectivity of viruses (other than hepatitis) hopefully free of charge at Belvidere Hospital, but gifts in kind might help with reagents.

Equipment: At present only freeze drying may be a problem, as infectious agents are involved. D.S.P. is prepared to do this with existing H.Q. Lab freeze drier, but this may not be 'legal' for hepatitis-B. If a new freeze drier is needed, approx. £3,000.

Irradiation Facilities: Local firms (Arbrook Products, Livingston and Ethicon, Sighthill) are both able to offer 2.5 Mrad fixed dose free of charge for small items. For temperature controlled work or other complex manoeuvres, we will have to use Harwell or Medical Physics (Belvidere). It would be wise to allow £1,000 for this item.

Travel: Could be a big item (e.g. B. Sommerville to Panama or Liberia!), also within the U.K. and Europe (esp. W. Germany and Sweden). Minimum £500 - £5,000 maximum.

Literature Surveys: Including computer searching, telephone calls etc., may be a significant item so budget a minimum of £1,000.

Several of these items will, of course, be spread over 2-3 years so costs per year are ÷ 2 or ÷ 3.