FOR DISCUSSION - Inactivation of Hepatitis Virus(es) by Detergents

Hitherto, attempts at inactivating HV have been non-specific, i.e. they do not make use of any intrinsic properties of the virus which might improve the ratio of inactivation (HV:FVIII:C). One exception to this is the proposal to use an immobilised antibody, e.g. polyclonal, monoclonal, mouse or human. Unfortunately, there is a theoretical limit, set by the affinity of the antibody and reflected in both the lower limit of RIA sensitivity and adsorbed/non-adsorbed equilibrium. Since experience shows that infectivity is several logs greater than the lower limit of antigen detection (approx. 1 ng/ml) it is unlikely that this approach will significantly remove infectivity when applied to contaminated final product.

One other approach, used by Kabi with FIX concentrates, makes use of the known hydrophobic coat protein of the HBV to adsorb it out onto alkyl C₈ solid phases. By appropriate choice of ligand length, the affinity can be raised to the point where it is capable (in theory) of reducing infectivity significantly in final product. Unfortunately, this approach is unlikely to work with FVIII concentrates as the alkyl C₈ group also binds strongly to FVIIIIC:Ag and irreversibly.

Nevertheless, it does point the way to designing inactivation systems which are targeted more specifically at the hepatitis viruses. As with other known viruses, the coat serves two purposes; (a) it protects the nucleic acids against degradation outwith the host cell and (b) permits specific affinity for and injection into the host cell type - in this case the liver. By removing the viral protein coat, e.g. by detergent we can reduce the infectivity by two mechanisms; (i) depolymerisation of DNA by endogenous contaminating DNAases in process material or after injection in the patients, or by adding exogenous DNAases (ii) reduce penetration of residual intact DNA into host liver by loss
of affinity and/or membrane transfer mechanisms.

Detergents exist or have been developed specifically for membrane protein extraction. In the case of Triton X-114, phase separation can be achieved by raising the temperature from 0°C to 20°C (the "cloud point") when hydrophobic proteins separate out in the sedimented oily phase. Soluble material remains in the detergent depleted supernatant, e.g. DNA and hydrophilic proteins. By choosing appropriate alkyl chain length detergents which are available in the Triton series, it may be possible to get (a) dissociation of viral coat protein, (b) phase separation of viral coat protein, (c) little or no loss of FVIII:C and (d) little or no systemic toxicity.

A semisynthetic detergent series (CHAPS, CHAPSO) has been developed from the natural bile salts sodium cholate and sodium deoxycholate specifically for membrane protein extraction without concomitant denaturation, and these may also be readily metabolised in vivo. Presumably bile salts themselves would be intrinsically non-toxic at low levels of use.

A third series of totally synthetic detergents (Betaines, Zwittergents) have been produced with +ve and -ve charges in the same molecule. They have had some success in protein solubilisation and also some topical human use (hair shampoo), so that some acute toxicity will have been done. A wide range of chain length, solubility and cloud points are also available.

A fourth series of non-ionic semisynthetic detergents have been made by condensing sugar residues with fats to produce the C6 to C12 alkyl - gluco or maltosides.

I would like to propose that we screen as many detergents as possible as soon as possible for two things; (i) ability to dissociate 125I-HBsAg particles and (ii) non-inactivation of FVIII:C. Additionally, we need to consider ways of testing for either endogenous or exogenous DNAase induced depolymerisation of 32P labelled HBV-DNA and to look for clues about whether the protein :DNA could reassemble to any extent in vivo and information on the toxicity of chosen detergents.
A great attraction of this approach is that at least \( \frac{3}{4} \) of the experiments can be performed in our existing laboratory facilities. Thus, if promising detergent(s) are chosen, infectivity assays will only be needed as a final confirmation of laboratory results.

Another feature of this approach is that it can easily be transferred to other products, e.g. immunoglobulins, ATIII, plasminogen etc. etc.

From an intellectual point of view, we might also look back on the mechanism of inactivation of HV by ethanol. It seems unlikely that pure precipitation/fractionation alone could account for loss of infectivity. Perhaps alcohol works by stripping the hydrophobic coat proteins and allowing access to endogenous nucleases. This latter step would be time, temperature and step dependent and might account for the peculiar variability of ethanol inactivation from product to product and occasional batch failures.

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