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Virus elimination during the recycling of chromatographic columns used during the manufacture of coagulation factors

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ABSTRACT

Various chromatographic procedures are used during the purification and manufacture of plasma products such as coagulation factors. These steps contribute to the overall safety of such products by removing potential virus contamination. Virus removal by two affinity chromatography procedures, i.e. monoclonal antibody chromatography and metal chelate chromatography (immobilised metal ion affinity chromatography), used during the manufacture of the high purity factor VIII (Replenate®) and factor IX (Replenine®-VF), respectively, has been investigated. In addition, as these columns are recycled after use, the effectiveness of the sanitisation procedures for preventing possible cross-contamination, has also been investigated.

Both chromatographic steps proved effective for eliminating a range of model enveloped and non-enveloped viruses by 4 to >6 and 5 to >8 log for the monoclonal and metal chelate columns, respectively. The effectiveness of the relatively mild column sanitisation conditions used, i.e. ethanol for factor IX and acetic acid for factor VIII, was confirmed using non-spiked column runs. The chemicals used contributed to virus elimination by inactivation and/or by physical removal of the virus. In summary, these studies demonstrate that potential virus contamination between chromatographic runs can be prevented when an effective column recycling and sanitisation procedure is included.

1. Introduction

The virus safety of plasma products such as coagulation factors is ensured by a combination of screening tests and the incorporation of dedicated virus inactivation/removal steps in the manufacturing processes [1]. Chromatographic steps are a major component of manufacturing processes that are used to produce high purity products. Such procedures can make a significant contribution to virus safety [2] when combined with dedicated and robust virus reduction steps. Different chromatographic steps are used during the manufacturing process for two coagulation factor concentrates manufactured at BPL, i.e. the factor VIII Replenate and the factor IX Replenine-VF. The factor VIII process includes a monoclonal antibody affinity (immunoaffinity) step [3] whilst the factor IX process includes a metal chelate affinity step (immobilised metal ion affinity chromatography) [4].

In the current study the effectiveness of these steps for eliminating viruses during the manufacturing process has been tested and the mechanism involved has been investigated. Furthermore, as the chromatography columns are recycled between each run, the effectiveness of the sanitisation procedure for removing any potential viral contamination, that may theoretically occur, has also been tested.

2. Materials and methods

2.1. Intermediates

In the case of factor IX, process intermediate at the stage immediately before the metal chelate affinity step, was obtained from BPL production. At this stage the intermediate contained solvent/detergent reagents 1% (v/v) polysorbate-80 and 0.3% (v/v) tri-n-butyl phosphate (TnBP). Intermediate without added solvent-detergent reagents was used as a control in some experiments. In the case of factor VIII, process intermediate at the stage immediately before the immunoaffinity chromatography step, was used.

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This contained solvent/detergent (1% v/v Triton X-100/0.3% v/v TnBP). Again, where indicated, the same intermediate without solvent/detergent was used.

2.2. Virus

Sindbis virus (strain Lister) and vaccinia virus (strain Lister) were propagated in BHK-21 cells grown in minimal essential medium (MEM) with 5% newborn-calf serum. Polio virus type 1 (Sabin type 1) was propagated in Vero cells grown in medium 199 with 5% foetal-calf serum. Bovine parvovirus (BPV; strain Haden) was propagated in MDBK cells grown in MEM with 5% newborn-calf serum. Hepatitis A (HAV; Strain HM175A2) was propagated in BSC-1 cells grown in MEM supplemented with 5% foetal-calf serum. Virus stocks were filtered to 0.2 or 0.45 μm before use to remove any virus aggregates that might potentially be present.

2.3. Metal chelate chromatography

This step is part of the factor IX process and uses a copper-charged Chelating Sepharose Fast-Flow column [4]. A small-scale model, which used the same column bed height, the same load per unit column volume and the same linear flow-rate as the full-scale process, was used. The intermediate, containing 500 mM NaCl, was applied to the column which was then sequentially washed with citrate–phosphate buffers containing NaCl i.e. 1) 500 mM pH 6.5, 2) 100 mM pH 7.0, 3) 100 mM pH 4.4, 4) 100 mM pH 7.0. The factor IX intermediate was eluted with buffer, 5) 100 mM pH 7.0 containing 20 mM glycine. The column was then washed with buffer, 6) 0.05 M EDTA to remove the copper ligand. Between runs the gel was sanitised by treatment with 85% (v/v) ethanol for 2 h and 70% (v/v) ethanol for 0.5 h at room temperature. For long term storage, 85% ethanol at 4 °C was used.

2.4. Immunoaffinity chromatography

This step is part of the factor VIII process and uses an immunoaffinity column with a monoclonal antibody to factor VIII:C, to purify factor VIII [3]. A small-scale model, which used the same column bed height, the same load per unit column volume and the same linear flow-rate as the full-scale process, was used. The column was equilibrated by washing with 0.8 M NaCl, 50 mM CaCl, 50 mM imidazole, solvent/detergent (1% v/v Triton X-100, 0.3% v/v TnBP) at pH 7.4. The factor VIII intermediate, i.e. redissolved cryoprecipitate after clarification at low temperature and followed by solvent/detergent treatment, was then loaded. The column was then washed with 40 mM calcium chloride, 50 mM Imidazole and 50% ethanediol pH 6.4. The factor VIII was eluted with 40 mM calcium chloride, 50 mM Imidazole, 40% ethanediol and 0.1% albumin pH 6.4. The column was then sequentially washed with 0.5 M NaCl, 0.1 M acetic acid (pH 2.9) for 2 h and 0.01 M acetic acid (pH 3.4) for 1 h to remove any remaining bound protein before storage in water.

2.5. Column spiking studies

The intermediate was spiked with virus at a dilution of 1/20 and applied to the column. The chromatographic process was carried out and each fraction retained for virus assay. After the virus spiked run, the standard column sanitisation procedure was carried out. The column run was then repeated but without spiking the column load with virus.

In all cases the samples were diluted in medium and adjusted to neutral pH where necessary. The conditions used had been determined to be non-toxic to the cells or interfere with any of the virus assay in preliminary experiments. Samples that contained solvent/detergent i.e. load and flow-through were diluted with medium 1/100, subsequent wash fractions 1/10 and the eluted product 1/2 to 1/10. Fractions containing EDTA were very toxic and it was thus necessary to dilute these by 1/1000.

2.6. Virus inactivation studies

The virus inactivation capacity of some of the chemicals used during the chromatographic processes was tested. For this, virus was spiked at a dilution of 1/20 into the test material or cell culture medium as a control. Samples were removed after various time periods, diluted in cell culture medium and adjusted to neutral pH where necessary. The conditions used had been previously determined to be non-toxic to the cells, or not to interfere with any of the virus assay.

2.7. Virus assay

Virus infectivity was determined by plaque assay using monolayers of Vero (SFV, Vaccinia), BHK-21 (Sindbis), MDBK (BPV), or BSC-1 (HAV) cells.

Serial log dilutions of virus were prepared in phosphate buffered saline and a volume of 0.5 ml of each dilution added to the cells. The minimum concentration assayed was that previously determined to have no toxic effect on the cells or to cause any interference to the virus assay. In order to increase the sensitivity of virus detection, larger volumes e.g. 7 ml of the least diluted samples were also assayed where appropriate. After a 45 min incubation period, an overlay of 0.4% high viscosity carboxymethylcellulose in complete cell culture medium was added.

Virus titres, in plaque forming units (pfu)/ml, were calculated from sample dilution, assay volume and plaque number. Where virus was undetectable, the titre was calculated assuming, as a worst case, that only one infectious virus unit had been present in the total volume assayed and the final titre expressed as a 'less than' value. The titre/ml was multiplied by the volume of each column fraction to calculate the total virus present. Virus reduction values were calculated by subtracting log total virus in the product eluate fraction from that determined in the product load.

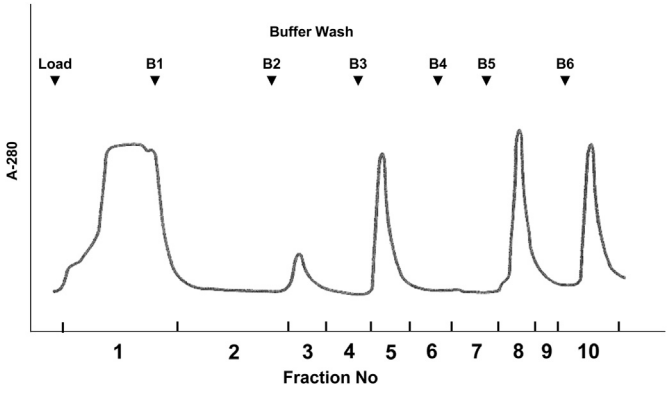

Fig. 1. Elution profile for the factor IX metal chelate chromatography process. After loading, the column was washed sequentially with buffers B1–B6 and fractions 1–10, including the product eluate (fraction 8), collected. The sensitivity of the UV absorbance monitor was increased ten-fold to enhance peak fractions 3 and 8/9.