Human donor plasma fractionation to produce therapeutic proteins

Part 1 of a two-part article

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Summary

Plasma fractionation, despite having had its 60th birthday, has not lost its relevance for clinical practice. The Pandora's box which is the world of the different plasma proteins of possible interest for patient care is too large. With the gain in predictive power of the biochemical markers measured in the clinical laboratory to delineate inflammation, immune dysfunction, impaired tissue oxygenation and endocrine alterations, more patients arrive at a disease stage where prescription of proteins extracted from healthy human donor plasmas may prolong their lives and improve their quality of life.

Beginnings of plasma fractionation

Human blood plasma is the liquid in which blood cells are suspended. The term plasma also encompasses the gas in which atoms have been ionised – that is to say, stripped of electrons – used in plasma-screen TV sets. The present two-part article is designed to draw medical laboratories' attention to determination of proteins usable as drugs. In pipette 01/2006 we stressed the importance of C-reactive protein (CRP) and other inflammatory markers, but these proteins are neither removed, nor downregulated, nor replaced, nor are they available as therapeutic agents. The position is completely different with a series of stable plasma derivatives. The clinical laboratory fulfills an important task when stable plasma derivatives such as intravenous (i.v.) or intramuscular (i.m.) immunoglobulins, clotting factors, antithrombin III, C1 inhibitor and albumin are prescribed for patients.

In most instances administration of these products is preceded by laboratory results pointing to reduced levels of IgG, factor VIII, antithrombin III or C1 inhibitor. In rare instances even normal levels may be associated with malfunction, e.g. when inhibitors and autoantibodies hamper functional integrity or when molecular defects of an otherwise normally concentrated protein, as is the case in dysfibrinogenaemia or von Willebrand disease, do not allow the protein to deploy its function. Inflammation, infections, sepsis and haemostasis problems are typical of the major disorders to be alleviated by blood donor plasma or its derivatives. When genetic engineering was developed to produce factors VII and VIII and erythropoietin, and when monoclonal antibodies became available, the pharmaceutical industry believed that proteins fractionated from plasma would disappear from the market. Quite impressively, neither of these techniques made plasma donation superfluous and, more than ever, the plasma-derived products are needed worldwide; shortages of i.v. immunoglobulins (IVIG) sometimes occur.

The initial driving force was human serum albumin (HSA) obtained by ethanol fractionation of plasma in sufficient quantity to serve as a plasma expander on the battlefields of World War II. Edwin Cohn asked Charles Janeway to test plasma fractions in clinical settings because he was already running the immunology laboratories at the Brigham Hospital in Boston. The Armour Company in Chicago was gearing up for production of bovine serum albumin (BSA) purified from bovine plasma in anticipation of a cheap and inexhaustible animal source of albumin. However, patients receiving BSA developed severe serum sickness (see: www.immune-complex.ch → serum sickness) which brought further clinical testing of BSA to an abrupt end. In the meantime, Janeway, in July 1941, a few months after failure of clinical trials with BSA, infused HSA into 8 patients in shock and 3 medical students who had been intentionally bled to the point of shock. The September issue of the New England Journal of Medicine published the excellent results with HSA in a report which further predicted a big future for the Cohn fractionation procedure for war victims. Ethanol fractionation of plasma soon became an internationally acknowledged procedure for production of HSA and aroused interest around the globe.

Progress in plasma fractionation in Switzerland in the 1960s

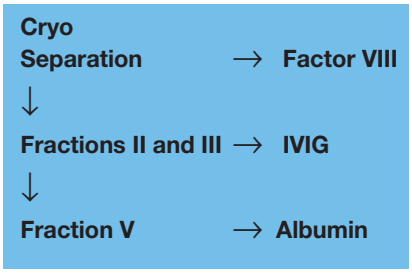
An important focus was Switzerland: the pharmacy of the Swiss Army during WWII served as background to the foundation of the Central Laboratory under the auspices of the Swiss Red Cross, and soon the modification of the Cohn process by *Kistler* and *Nitschmann* became standard. The reaction conditions for this method are given in Figure 1. Before ethanol is added to plasma, the cryoprecipitable proteins are separated in cold conditions: frozen plasma is thawed slowly in cold conditions to produce cryoprecipitate rich in fibrinogen, factor VIII, von Willebrand factor, factor XIII, and fibronectin. Several other coagulation factors, such as factor IX and protease inhibitors (e.g. C1 esterase inhibitor) may be separated from cryosupernatant plasma by chromatography before it is processed by alcohol fractionation to exploit the plasma donation to the greatest extent possible.

Five variables are used to obtain differential solubility of proteins in ethanol-water mixtures.
They include:
– Concentration variation of ethanol ranging from 8% to 40%
– PH levels ranging from 4.5 to 7.4
– Temperatures ranging from –5° to –7° C
– Ionic strength differentials from 0.14 to 0.01
– And protein concentrations from 5.1% at the outset to 0.8% at later stages of the process.

Processing is usually performed using batchwise treatment of the fractions in stirred tanks; continuous flow fractionation under automatic control has been abandoned in most producer plants. At the end, ethanol is removed by freeze-drying or by ultrafiltration and is reused.
Kistler was a biochemist in the pilot plant of the Central Laboratory of the Swiss Red Cross who investigated optimal conditions with which to obtain the best yields, and *Nitschmann* held the chair of biochemistry at the University of Bern.
Why has such an old procedure as ethanol fractionation survived so long that it is still the predominant procedure for preparation of stable plasma derivatives? Today, on the laboratory scale, biochemists use more precise protein purification methods such as gel filtration, ion exchange procedures, affinity absorption, immunosorption and the like. The answer is that neither of these laboratory-scale procedures meets the requirements for reproducible bulk processing in industrial quantities. After all, the current need for IVIG worldwide is estimated to be 50 tons. In addition, virus-inactivation steps that complement each other in their mode of action, one of which safely removes enveloped viruses, cannot be done on elution columns however large. This is why large scale ethanol fractionation is still in use and has been brought to perfection in downsized experimental laboratories, referred to as pilot plants.

Safety features of therapeutic plasma proteins

As with any drug, the therapeutic administration of plasma proteins transfused into recipients may be overshadowed by side effects. The specific side effects of each protein will be briefly discussed in the second part of this paper (pipette 03/2006). The reader should realize here that overdosage of i.v.immunoglobulins results in hyperviscosity, overdosage of C1 inhibitor suppresses complement activation beyond reach, and overdosage of antithrombin III results in dangerous bleeding.
Chief attention regarding safety features focuses on transmission of infectious agents, mainly viruses and prions. Selection of plasma from voluntary and non-remunerated donors is mandatory, to reduce the risk of infected subjects offering their blood. This might be especially important when plasma from countries with restricted safety features is involved. While blood donation in China and South America does not yet match up to standards in developed countries, excellent fractionation centres are being built up: thus, Shanghai RAAS Blood Products was established in 1988 and has progressed to where their products match USFDA, WHO and European Pharmacopeia Guidelines. According to their information, the plant obtains plasma from healthy Chinese donors vaccinated against hepatitis A and hepatitis B viruses in a rigorous programme (www.raascorp.com). Nearby fractionation centres such as www.zlbbehring.com or www.octapharma.com pursue the policy of excluding voluntary at-risk donors from donation and screening of donations for a range of relevant blood-borne viruses, as recently discussed by *Niederhaeuser* in "pipette". With plasma protein manufacture, the companies must maintain a tedious track-back system for each contributing donation, and many a production batch from plasma fractionation has had to be discarded and/or its products withdrawn from the market because a donor contributing to the pool later developed an infectious disease likely to be transmitted. Such window donors, i.e donations from infected people not yet detected by screening tests because seroconversion is not in effect, cause substantial financial losses for fractionators which are then reflected in the price of their products. PCR testing procedures give grounds for hope that this situation may change for the better.



Plasma fractionation with differential pH and temperatures. Simplified scheme – the interested reader is referred to www.niaid.nih.gov.

Once the hurdle of negative, non-reactive testing of the raw material has been cleared, it is adequate viral removal by effective inactivation or removal steps during or after the plasma fractionation which has become mandatory. Their advantage is that thus far unidentified viruses will most probably be removed as well. While life cellular products and cryoprecipitates have transmitted West Nile virus or HIV, fractionated HSA or IVIG never did despite contaminated starting plasma pools in the 1980s.

Progress in virology science helps plasma fractionators make products safer

Hepatitis A, B, C viruses, West Nile viruses, H5N1 viruses – we know they are dangerous and must be absent from plasma products; but how can we prove their absence? Fractionators must be able to spike fractionation cuts, then treat the product and demonstrate the extent of virus removal from the spiked sample. The decisive advance came with model viruses, e.g. Semliki Forest virus, a model for hepatitis B and others. Precipitation of Cohn fraction III typically results in a 3-4 $\log_{10}$ reduction for the lipid enveloped (LE) and non-lipid enveloped viruses (NLE), whereas a pH 4.4 treatment step typically shows variable inactivation of LE viruses and virtually no inactivation of NLE viruses. For immunoglobulin products a contributing virus-reduction effect of neutralising antibodies directed against parvovirus B19 and hepatitis A virus is described. Although immuno-

globulin products do have an excellent safety record, European regulators encourage implementation of additional effective virus-reducing steps into the manufacturing process to further ensure their safety, with nanofiltration currently in the forefront. It is hoped that such extensive safety requirements will not push up product prices, or that, when in effect, they will not involve regrettable loss of active compounds in the final product.

In addition to the current prion removal plans, some companies are developing their own state-of-the-art laboratories for the R&D virus and prion validation, such as that in the 2005-launched Frankfurter Innovations Zentrum (FIZ). In a recent scrapie model, sheep brain homogenate with infectivity titres measured in hamsters showed that substantial removal of the infectious PrP fibrils, as analysed by Western blot, was achieved by ethanol fractionation on its own.

On the viral side, representative LE and NLE model viruses are now used for studies such as bovine viral diarrhoea virus, canine parvovirus, encephalomyocarditis virus, pseudorabies virus and others. Such viruses may be chosen on the basis of European guidelines for virus-validation studies of plasma derivatives. Their LE and NLE status makes it possible to differentiate the virus inactivation capacity of such measures as pH 4.4 treatment, nanofiltration, solvent/detergent virolysis and others. Due to lack of space the author cannot dwell on this subject further, but it is recalled that WNV has recently been found in 0.03 percent of Mexican blood donors and measures are under way to reduce the risk of transfusion transmission.

One issue often neglected is reduction of product efficacy by some virus-removal procedures. Good studies on efficient virus removal must therefore end with clinical efficiency studies on the new products, usually focusing on platelet concentration correction in patients suffering from idiopathic thrombocytopenic purpura (ITP) or, alternatively, focusing on infection reduction in immunodeficient children.

Thanks to unceasing vigilance on the disease transmission issue, patients receiving plasma products may pass quiet nights recovering under administration of efficient product. We at Octapharma maintain a postmarketing surveillance programme which includes information on viral safety.

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References

1 Sanchez-Guerrer SA, Romeri-Estrella S, Rodriguez-Ruiz A et al: Detection of WNV in the Mexican blood supply. Transfusion 2006;46:111–117.
2 Gregori L, Maring JA, Mac Auley C et al: Partitioning of TSE infectivity during ethanol fractionation of human plasma. Biologicals 2004;32:1–10.
3 Niederhaeuser C. pipette 03/2005, 6–12.
4 Schetz MR, van den Berge G: Do we have reliable biochemical markers to predict the outcome of critical illness? Int J Artif Org 2005;28:1197–1210.