INFECTED BLOOD INQUIRY

BRENDON GRAY WITNESS STATEMENT

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CLOTTING FACTOR CONCENTRATES IN 1997

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Clotting factor concentrates are available today in great variety, safe from transmitting most viruses, at declining prices. Great advances have been made in less than two decades. We now have the luxury of choosing among many excellent products. The frequency of recent withdrawals of concentrates is not an indication of lesser safety but of increased vigilance, often excessive, over every aspect of plasma collection and testing and of concentrate manufacture. If any step deviates from perfection, if a donor later remembers a possible risk, a lot may be recalled. We are frustrated with recalls for trivial reasons, sometimes leading to destruction of large amounts of safe products, with shortages. In the USA, the FDA and fractionators are working together to avoid such wastage.

THE RAW MATERIAL -- DONORS AND PLASMA

Donors should be healthy persons at low risk for viral infections commonly transmitted in blood products. There is no easy formula for selecting good donors. In each part of the world, persons with insight into local risks and behavior must interview and examine donation candidates. Questions about risky behavior or exposure usually are refined after a great deal of experience with misinterpretation. At the moment, Creutzfeld-Jacob disease is receiving attention. There is no evidence that it can be transmitted by blood transfusion but, given its serious nature, long incubation period and sometimes familial occurence, donors are questioned about the presence of the disorder in family members as well as about personal exposure to human brain tissue (e.g. growth hormone injections) or dura mater transplants.

Passionate debates circle around the desirability of not compensating donors at all versus compensating them with money or with some other benefit such as a day off work. All systems have been used successfully in various places at various times when the donors are selected well.

At the present time, most plasma collected for commercial fractionnation is obtained by plasmapheresis of paid donors in the USA. In some European countries, volunteer plasmapheresis donors have provided a large proportion of domestic plasma needs. Plasma also is salvaged from whole blood donations, nearly all of which comes from unpaid donors, in North America and western Europe.

Which are better -- volunteer whole blood donors or paid plasmapheresis donors? The argument rages. The most popular view is that volunteers always are better. However, whole blood donors often donate only occasionally, and are less well-known to the blood bank than repeat plasmapheresis donors.
When plasma is salvaged from whole blood, the blood already may have been stored for days or weeks. Some degeneration of clotting factors make have taken place. According to one current hypothesis, degenerated factor VIII in plasma separated late from whole blood donations may have been the cause of an outbreak of inhibitors in previously-heavily-treated recipients of one brand of concentrate.
Plasma from plasmapheresis is centrifuged promptly, usually by skilled personnel dedicated to that task.

Serologic tests on each donor help reduce the possibility of viremia. Standard screens in the USA include tests for syphillis, hepatitis B antigen, hepatitis C antibody, HIV 1 and 2 antibody, the HIV marker p-24 antigen and the liver enzyme alanine aminotransterase (ALT). Recently, some fractionators in the USA and Europe have tested pooled plasma and/or final containers by nucleic acid techniques (PCR, polymerase chain reaction, and similar methods) for such viruses as hepatitis A, B and C, HIV, and parvovirus B-19. As this technology becomes more efficient, it will become widespread. Individual donors whose serologic (antigen and antibody) tests were negative may be identified as infected by nucleic acid techniques. All tests have lower limits of sensitivity, so a lightly-infected donor can escape detection by the most sensitive modern method. Some plasma banks quarantine donations of plasma until a donor returns and again tests negative, proving he was not in the early stages of infection at the time of the previous donation. Some banks will not use plasma from a donor who never returns, or from the final donation of a repeat donor.

Some persons question the size of pools, that is, the number of donations of plasma put together for fractionation. Modern fractionation methods require large volumes of plasma for efficiency and cost-effectiveness. Reducing the number of donations per pool from, for example, 20,000 to 5000, would have major deleterious effects on production efficiency but give little benefit to patients. It is assumed that most large pools contain donations from a few individuals with low-level, undetected infections. A single undetected infected donor is diluted more in a pool of 20,000 donations than in a pool of 5,000, but exposure to four lots of product each made from a pool of 5000 donors is equivalent to exposure to one lot made from a pool of 20,000 donors. Most patients with moderate to severe hemophilia

receive transfusions from multiple lots, so are exposed to a large number of different pools. Even if a patient could limit his exposure to one lot, he could only hope that he chose a lot free of undetected infection.

FRACTIONATION AND VIRAL INACTIVATION

Methods of separation of factor VIII from plasma include precipitation or separation with ethanol, cold (cryoprecipitation), glycine, glycine with heparin, polyethylene glycol, gel filtration, ion exchange chromatography or affinity chromatography which often is performed using monoclonal antibodies as attractants. Prothrombin complex or factor IX separation methods include polyethylene glycol precipitation, DEAE absorption (sometimes with heparin), tri-calcium phosphate absorption, and affinity chromatography which is sometimes performed using monoclonal antibodies as attractants. Recombinant DNA technology has been used to produce both factor VIII and factor IX in hamster cell cultures. Recombinant factor VIII is suspended in human plasma-derived albumin but recombinant factor IX does not use human proteins.

Which method is ideal? The goal for plasma-derived products is to separate the clotting factor efficiently, not activating or otherwise disturbing the factor molecule, leaving the residual plasma proteins in good condition for fractionation into other products. There may be an advantage to separating the targeted factor from those factors with which it associates, that is, to achieving a high level of purification, in order to avoid any complication from the unwanted factors (such as thrombosis from un-needed activated factors of prothrombin complex) or to separate the wanted factor from any contaminant microbes. Affinity chromatography is especially suitable for the latter, because, while the factor is attached to the attractant, the column can be washed to rid it of much of the unwanted proteins and contaminants. A high level of separation, however, may necessitate buffering of factor VIII with added human albumin.Heat-treated albumin has an excellent safety record. Highly purified factor VIII made with monoclonal-antibody affinity chromatography contains no von Willebrand factor, a lack which may be of importance in misdiagnosed patients, those with liver disease or those using very large amounts of the concentrate, as for surgery, instances in which the patient's own von Willebrand factor may not suffice to transport factor VIII in circulation.

Recombinant clotting factors made in cultures of cells into which normal human genes for factor VIII or IX have been inserted have been produced in recent years. The advantage is that a large supply can be made without reliance on human plasma as a raw material. To separate the human factor VIII or IX from the culture medium, a process such as monoclonal antibody affinity chromatography must be used. Do recombinant clotting factors have disadvantages? To date, their use has increased in popularity in developed countries. The common perception is that the danger of transmitting a viral infection is avoided. For some brands, however, it is alleged that many batches are discarded because of microbial growth in cell culture.

Viral inactivation has developed rapidly over the past two decades as methods became available to protect coagulation factors from deleterious effects and as methods became available to evaluate the efficacy of various treatments. Early viral-inactivated concentrates were produced in very small amounts by highly-inefficient methods. The most common methods of viral inactivation in use today are heat and solvent-detergent treatment. Heat can be applied during processing while concentrate is in solution (pasteurization), or after lyophilization while suspended in hot steam under pressure or while suspended in an organic solvent, or after bottling by baking ("dry") the final vial. To survive heating, clotting factors must be suspended in stabilizers. Pasteurization and vapor heating have been effective against HIV and hepatitis. Baking dry at lower temperatures (e.g. 68 C.) proved effective against HIV but higher temperatures, 80-100 C., were needed for adequate hepatitis virus kill. Solvent-detergent treatment of plasma or intermediate products causes little damage to clotting factors but kills lipid-enveloped virusus including HIV, HBV and HCV efficiency, but has little inherent effect against non-enveloped viruses such as hepatitis A. Sodium thiocyanate treatment is used for one factor IX concentrate. B-19 parvovirus can resist all known inactivation methods. Factor IX is a sufficiently small molecule to make use of ultrafiltration or the more advanced nanofiltration to allow factor IX to pass through the filter while holding back all the known pathogens including B-19 parvovirus.

Methods used to viral-inactivate whole plasma, including solvent-detergent treatment or addition of methylene blue with white-light irradiation, may provide a product for unusual clotting factor deficiencies such as factor V or factor X for which no concentrate exists, or may provide improved starting material for cryoprecipitate or other concentrate production.

In the USA, as well as in other countries, surveillance of patients with hemophilia for viral infections has been carried out for over a decade. No-one in the USA has acquired HIV from concentrate prepared from HIV-tested donors and viral-inactivated by any method. The last HIV seroconversion was in 1987. Some hepatitis B and C seroconversions were suspected but they were rare. Hepatitis A, which is transmitted infrequently by blood products, infected some patients through solvent-detergent treated concentrate, presumably due to the presence of asymptomatic viremic donors. The emergence of hepatitis A as a blood-borne pathogen has led to campaigns to vaccinate frequently-

transfused patients against hepatitis A as well as against hepatitis B, and to viral-inactivate concentrate by two methods, one of which, at least, kills or filters out hepatitis A. Some factor IX concentrates now are treated with solvent-detergent (or heat, or, in one instance, sodium thiocyanate) followed by filtration. Some factor VIII concentrates now are treated with both solvent-detergent and heat methods.

Two European concentrates were found to provoke inhibitors in previously-heavily-treated patients and were withdrawn. One concentrate was produced by a new separation technology and heated in solution.The other was treated with both solvent-detergent and heating in solution. For a period of time, heating in solution became suspect. However, other pasteurized or dual-inactivated concentrates have not provoked excess inhibitors. The relationship, if any, between provocation of inhibitors and the type of concentrate or its viral-inactivation is not yet clear and requires careful ongoing surveillance of recipients, incuding identification of their gene mutations, for certain mutations are much more associated with inhibitors than others. Careful management of plasma to avoid factor deterioration also may be important, as mentioned above.

CONSIDERATIONS IN CHOOSING A CONCENTRATE

Some preferences are based on well-documented medical evidence, some on poorly-documented medical evidence and some on hypothetical medical advantages. Preference also may be based on reliable availability and on price, a major consideration nearly everywhere in the world. Advertising to physicians and to families sometimes creates demand for certain products, which is not always based on proven advantages.

Highly-purified factor IX concentrate (that is, containing very little or no contamination with other fators of the prothrombin complex) is preferred strongly over prothrombin complex in situations in which thrombosis is more likely than usual: surgical operations, massive trauma with release of tissue thromboplastin, CNS trauma, intensive and lengthy use of concentrate, patients with a history of thrombosis, patients with severe liver disease who have diminished ability to clear activated factors, and newborn infants with immature livers. Children and adults without the above risk factors who need factor IX can be treated with prothrombin complex concentrate.

Highly-purified factor VIII concentrate has been advocated for use by HIV+ patients because some studies have shown a slower decline in CD4 counts in patients using such concentrates than in patients treated with intermediate purity products. In highly-developed countries, highly-purified concentrate usually is given to HIV+ patients in the hope that it may be of benefit and in fear of criticism if such concentrates are not used in those patients. There is no evidence that the use of highly-purified concentrate prolongs life, or prolongs the period of between seroconversion and the emergence of symptomatic AIDS. Since highly-purified concentrates do not contain von Willebrand factor (except for the brand called "Alphanate" from Alpha or "Fandhi" from Grifols), and since some patients with advanced HIV infection have serious liver dysfunction and respond less well to concentrates without vWF, a few patients are put back on intermediate-purity concentrates (or on "Alphanate" or "Fandhi"). HIV+ patients for whom only intermediate-purity concentrates are available should not feel deprived of a major advantage. The evidence that high-purity concentrate is beneficial is very weak.

Recombinant ooncentrates have been chosen or advocated in some countries because of the presumption that contamination with human viruses, known and unknown, will not be present. It is unlikely that viruses survive in the pasteurized albumin used to stabilize recombinant factor VIII concentrates. Temperatures used to cultivate cells may encourage growth of microbes during the manufacture of recombinant concentrates. No-one, as yet, is known to have been infected by a recombinant concentrate. These relatively new products need to be subjected to the same recipient surveillance as do plasma-derived concentrates.

Concentrates available today from western European and North American commercial sources have excellent safety records. Choosing among them often becomes a matter of price, propaganda and availability. With increasing use of recombinant concentrates in some wealthy countries, a surplus of plasma-derived concentrates may ensue with correspondingly lower prices. Negotiation is the key to a good concentrate price, but, in too many countries, the profit to the intermediate agent exceeds the wholesale price of the product. A modest profit to hemophilia centers, which have few other means of support, is justified. When choosing between a lower-price and a higher-price concentrate, both with excellent records of safety, when the total budget is fixed, it is better to allow patients a larger supply of lower-price concentrate than to buy a lesser amount of a higher-price product. In most of the world, patients are under-treated. Commercial companies often direct propaganda both to doctors and to patients to try to convince them to demand a particular brand of concentrate.

The availability of a given brand of concentrate may change overnight, for example, because of a factory breakdown. If is often useful to have on-going contracts with more than one fractionator. It also is useful to have a good national or regional blood bank service where well-screened plasma can be obtained. Some countries fractionate their

own plasma so that they have the mechanism and facilities for some of their own needs, and supplement their self-sufficiency , if necessary, with imports. Other countries promote partial self-sufficiency by means of contract fractionation, that is, sending domestic plasma to a large, efficient fractionator (usually in another country) to be processed into plasma products which are returned to the country of origin. If preferences in concentrate types change, the contract can be changed to a different fractionator. The least expensive alternative usually is importation of concentrate made from foreign plasma. Contract fractionation is nearly always more expensive. Building fractionation plants for domestic concentrate production is very expensive. Small, pre-fabricated fractionation units have been developed in Sweden (Pharmacia) but of the several in use around the world, only one produces factor VIII concentrate at the moment and none produce factor IX concentrate.

The availability of concentrates for rare clotting factor disorders also is important. At the moment, commercial concentrates of varying availability include activated prothrombin complex (Baxter, Immuno) and plasma-derived (LFB,France) or recombinant (NovoNordisk) activated factor VII for patients with factor VIII or IX inhibitors, porcine factor VIII concentrate for patients with factor VIII inhibitors (Speywood), factor XI concentrate (BioProducts and LFB) for congenitally-deficient patients, unactivated factor VII concentrate for factor VII deficient patients (Bioproducts, LFB and Immuno), and factor XIII (BioProducts and Immuno) for deficient patients. We lack a factor V or a factor X concentrate,. The latter, factor X, would not be difficult to produce. Concentrates for rare deficiencies cannot be produced at a profit to the company. Thanks are owed to those companies which produce them as a service to the community. Easier methods of licensing and importing such concentrates are needed around the world.

More new concentrates are expected over the next several years, in part to improve products and in part to allow companies to compete with each other. It will be important to track recipients, that is, to keep records of every lot infused in every patient, to know the safety level of concentrates. It is important not to be dazzled because a method is new, but to compare new products with older, known products. We need to encourage development and distribution of concentrates for rare disorders as well as choose among the wide array of concentrates for hemophilia A and B.

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