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COMMEMORATIVE ARTICLE

Porcine factor VIII

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> "All animals are equal. But some animals are more equal than others." The creatures outside looked from pig to man, and from man to pig, and from pig to man again: but already it was impossible to say which was which.
>
> George Orwell: Animal Farm (1945)

Early use of animal plasma to treat haemophilia

A potential treatment for haemophilia was first identified in 1937 when a component of human plasma called 'antihaemophilic globulin' was described [1]. Although blood transfusion was routine at that time, the separation of plasma from donated whole blood on the large scale required to permit commercial fractionation only became a reality in the 1970s. Biggs, Macfarlane and Bidwell in Oxford estimated that every haemophilic patient would need the plasma from 1000 donors each year for the production of enough material for maintenance treatment. In recognition of the fact that this was 'wildly impractical', the group pursued the development of antihaemophilic globulin derived from animals as a potential treatment [2]. The first material purified was bovine factor VIII, extracted from plasma collected from animals sent to an abattoir for slaughter [3,4]. Approximately 3–4 L were obtained from each animal and the globulin was purified by fractionation in the presence of potassium phosphate and sodium citrate. Early reports were encouraging, but it soon became apparent that repeated treatment with this product was frequently complicated by severe allergic reactions and thrombocytopenia. Furthermore, patients also soon became refractory to the product, a phenomenon which was attributed to the development of alloantibodies against the bovine factor VIII protein. Porcine plasma was first used to treat a patient in 1954. The first recipient was a 22-year-old man from Norwich, who worked in a gun shop and who got shot in the loin by mistake by a customer who was trying out a rifle [2,5a]. He required surgery and was initially treated with bovine material. This was initially successful in controlling the bleeding, but soon the patient developed severe allergic reactions and the bleeding was no longer controlled owing to the appearance of inhibitory antibodies against the bovine material. In some desperation, porcine plasma was obtained from an abattoir in the neighbouring Norfolk village on a Sunday. By Wednesday of the following week, the Oxford group had prepared an extract of porcine antihaemophilic globulin, which saved this young man's life. Porcine antihaemophilic globulin turned out to be generally better tolerated than bovine material, although allergic reactions and resistance caused by antibody formation were still a problem after repeated infusions. A lyophilized concentrate of porcine antihaemophilic globulin was subsequently manufactured by S. Maw and Sons Ltd of north London [Fig. 1] and remained in use in the late 1950s and early 1960s. It must be emphasized that porcine and bovine preparations were initially used to treat all patients with haemophilia A, and not just those who had developed inhibitors to factor VIII. The development of cryoprecipitate in 1965 and the subsequent development of concentrates of fractionated human plasma transformed clinical practice, and the use of basic animal blood products was then abandoned.

The development of Hyate:C

The development of inhibitory antibodies to human factor VIII in a significant minority of patients with haemophilia A treated with concentrates derived from human plasma was already well recognized by the early 1970s. The treatment options at the time were limited to either infusions of high doses of human factor VIII or primitive prothrombin complex concentrates (PCCs) like Autoplex and Proplex. Neither of these options could guarantee control of haemostasis and the use of PCCs was also known to be associated with a risk of venous and arterial thromboembolism. A highly purified preparation of porcine factor VIII was developed in the early 1980s using polyelectrolyte chromatographic fractionation. This product was specifically developed to provide another treatment option for patients who had developed inhibitory antibodies to human factor VIII. The rationale was that porcine factor VIII was sufficiently similar to human factor VIII to work just like the natural product, but it was also sufficiently different in structure to render it less susceptible to inactivation by circulating inhibitory antibodies. The very early work was undertaken by Speywood Laboratories in Nottingham (which later became part of the Ipsen

Fig. 1. Polyelectrolyte resin ion-exchange fractionation of porcine plasma cryoprecipitate. The Polyelectrolyte (PE-E5) was a key purification step.

group) in conjunction with researchers in Oxford. An attractive offer from the Welsh Development Agency persuaded Speywood to set up its production facility for Hyate:C in Wrexham, where a fractionation plant was built to handle porcine plasma obtained from abattoirs in England [Figs 2–4].

Clinical experience with Hyate:C

The first published report of the clinical use of Hyate:C appeared in 1984 and described the successful use of the product in eight patients over an 18-month period [6]. A total of 297 infusions were given for the treatment of 45 distinct bleeding episodes. A clear advantage over other products was that measureable levels of factor VIII were obtained after infusion, which could be used to monitor treatment. In most cases, the inhibitory antibodies against human factor VIII showed little or no cross-reactivity with porcine factor VIII. Where no baseline antibody against porcine factor VIII was detectable, the mean postinfusion rise in plasma factor VIII was 1.29 U dL^{-1} per unit infused kg^{-1}. Furthermore, there was usually little or no anamnestic rise in antibody titre after treatment with Hyate:C, by contrast with the steep rise frequently reported after treatment with human factor VIII or activated prothrombin complex concentrates. Multiple and prolonged courses of therapy were used in this series without evidence of loss of clinical or laboratory efficacy. Apparently allergic reactions, including fever, were observed after approximately 10% of the infusions.

Hyate:C was licenced for clinical use in the UK in 1984 and approved in the United States of America 2 years later. This British product soon came to be used all over the world. It is interesting to note that this product was also used in countries where consumption

Fig. 2. Polyelectrolyte resin ion-exchange fractionation of porcine plasma cryoprecipitate. The Polyelectrolyte (PE-E5) was a key purification step (*Speywood archive material kindly supplied by Dr Phil Robinson*).

Fig. 3. Sterile suite at Speywood's Wrexham manufacturing facility showing Hyate:C filling and freeze-drying in process (circa 1985) (*Speywood archive material kindly supplied by Dr Phil Robinson*).

Fig. 4. Vials and packages of Hyate:C (circa 1985) (*Speywood archive material kindly supplied by Dr Phil Robinson*).

of pork products would not normally be permitted for cultural reasons, as religious authorities were prepared to make an exception for a product of medicinal value. The clinical experience with the new material was generally very positive, with a reported efficacy of 90% [7]. One important area where Hyate: C made a real impact on treatment was in the setting of surgery. Although, we now take it for granted that elective surgery may be carried out safely in haemophilic men

with inhibitors, this was by no means the case even a couple of decades ago. The accumulated experience with Hyate:C in 45 haemophilic patients undergoing surgery in seven countries over a 13-year period was published in 1993 [8]. The authors specifically commented that their intention was to encourage clinicians to consider surgical options in this type of patient, as they recognized that many physicians had hitherto been reluctant to recommend elective surgery in these challenging cases. Porcine factor VIII was advocated by many physicians as the first-line treatment option for patients with acquired haemophilia [8]. Such patients, typically elderly and frail, are at particular risk of thrombosis which is a recognized complication with activated PCCs. At the same time, the development of resistance is less of a concern, as most patients only require a few infusions whilst waiting for the effect of immunosuppression to kick in. The first large survey published included data from 47 centres in Europe and North America on the use of Hyate:C in the management of 74 acute bleeding episodes in 65 patients with acquired haemophilia [8]. Most of these patients showed a very clear difference in antibody titre between human and porcine factor VIII: the median initial anti-human factor VIII auto-antibody inhibitor level was 38 Bethesda unit (BU) per mL (range 1.2–1024), whereas that against porcine was 1 BU mL^{-1} (range 0–15). After therapy, no increase in the median level of anti-human FVIII or anti-porcine antibody was noted in the group as a whole, although 13 patients showed individual increases in either anti-human or anti-porcine antibody levels or both of more than 10 BU mL^{-1}. The treatment of acquired haemophilia is often exceedingly expensive and this can pose a problem even in more affluent countries. The conclusion of an independent cost-benefit analysis that initial treatment of acquired haemophilia with porcine FVIII was more cost-effective than activated PCCs or human factor VIII also helped to stimulate use in this particular indication [9]. Case reports also documented the successful use of porcine factor VIII over long periods of time for induction of immune tolerance and as secondary prophylaxis [10,11]. The reason for the selection of a porcine product in such cases was often concern about the possibility of transmission of human blood-borne infections at a time of heightened insecurity over the safety of plasma-derived human concentrates, when recombinant products were not yet available. The half-life of porcine factor VIII in patients with no detectable inhibitor was reported to be very similar to that of human factor VIII and in the range 8–12 h [6,10,12].

Thrombocytopenia and allergic reactions in association with porcine factor VIII therapy were a concern ever since the first crude preparations were used in the 1950s. These primitive products were acknowledged to contain an unspecified 'Platelet Aggregating Factor'. Clinicians reported at the time that patients often complained of flashes of light in their eyes after infusions of animal plasma, which was attributed to clumps of platelets passing through retinal blood vessels [5b]. Although, much purer than previous formulations, factor VIII only accounted for around 1% of the total protein in Hyate:C [13]. Adverse effects were still observed following Hyate:C infusions, but the incidence and severity were much lower in association with the use of this purer product [14,15]. However, the possibility of such reactions led some physicians to express reservations about the use of this particular product for home treatment. In one detailed review of adverse events, the observations related to 283 infusions of porcine FVIII given to 30 subjects over a decade were reported [16]. There was a median percentage fall in the baseline platelet count of 54% (range 8–86%). In the case of 10 courses, the subsequent drop was more severe with nadirs ranging 10–99 $\times 10^9$/L (median 67). Allergic reactions were seen in 15 of 30 patients (50%), in 20 of 63 courses (32%). The symptoms were generally mild and included fever, flushing, urticaria and shivering, but five courses were accompanied by more severe anaphylactoid reactions. The observed thrombocytopenia was shown to be caused by residual traces of porcine von Willebrand factor (VWF) in the concentrate [17]. Porcine VWF binds to the glycoprotein Ib receptor on platelets, leading to activation of the glycoprotein IIb/IIIa receptor, which in turn causes to platelet aggregation associated with binding of fibrinogen [18]. Flow cytometry also demonstrated activation of platelets following treatment with Hyate:C, reflected by an increase in the number of circulating platelets expressing CD62 and CD63 and annexin V [19]. The authors of this study speculated that the platelet activation caused by infusion of porcine factor VIII enhanced haemostasis through a quite separate, but complementary pathway to that simply because of increased circulating factor VIII.

Hyate:C was not subjected to any specific viral inactivation steps, such as pasteurization or solvent/detergent treatment during manufacture, unlike conventional plasma-derived products derived from human plasma. In late 1996, low levels of porcine parvovirus (PPV) were identified in some batches of product through routine screening. This contamination was initially identified during manufacture of Hyate:C by observation of cytopathic effects in cultured mammalian cells and PPV DNA was subsequently identified using polymerase chain reaction technology. Infection with human parvovirus B19 is very common in the general population and does not cause significant illness. Furthermore, it is well-documented that human parvovirus can be transmitted by modern plasma-derived concentrates subjected to virucidal treatment, such as heat-treatment and solvent/detergent treatment. The interruption of manufacture of Hyate:C may therefore seem in retrospect to have been a somewhat drastic step to take. However, this came at a time of heightened concern about zoonoses, as it was in this same year that vCJD was first reported in humans, and

acknowledged to be the result of transmission of prions from cattle infected with bovine spongiform encephalopathy (BSE). A subsequent retrospective study of 81 patients, who had received Hyate:C showed no serological evidence of infection with porcine parvovirus (PPV), encephalomyocarditis virus (EMCV) or porcine respiratory and reproductive syndrome virus (PRRSV) [20]. There was also no evidence of infection with these pathogens in 125 control subjects, who included workers in the pig abattoir and personnel involved in the manufacture of Hyate: C at the Wrexham plant, as well as recipients of porcine heparin or insulin. A separate study in the United States of America identified PPV DNA in 21 of 22 different batches of Hyate:C using nested PCR testing, although none of 98 Hyate:C recipients tested positive for PPV IgG antibodies [21]. Another study reported the presence of porcine endogenous retrovirus (PERV) particles in all of six batches of Hyate:C screened, although infectious virus was not detected [22]. PERV particles were shown to be a common contaminant of Hyate:C products, but the risk of actual transmission of PERV infection was deemed to be very low [23]. A change in the manufacturing process to incorporate a virucidal step, such as heat-treatment would have necessitated a formal clinical trial to satisfy the requirements of the regulatory agencies, which would have been a huge undertaking. The company decided instead to introduce serological screening of all porcine plasma for antibodies to PPV and only select seronegative plasma for fractionation. As PPV infection is very common amongst swine, this resulted in a significant reduction in plasma cleared for fractionation at the plant and thus the total number of vials produced. Manufacture of Hyate:C eventually ceased at the Wrexham plant in 2004, and the last vial was supplied for clinical use the following year. It is probably fair to say that the challenge posed by the introduction at around the same time of recombinant activated factor VII (NovoSeven, NovoNordisk), also played a significant part in the demise of Hyate:C. NovoSeven is a recombinant product free of the risk of transmission of blood-borne viruses. It is also not associated with adverse reactions, such as thrombocytopenia, allergic reactions or the development of resistance through antibody formation and thus it is suitable for home treatment.

Recombinant porcine factor VIII

Although, Hyate:C is no longer available, a recombinant factor VIII has been developed as a collaboration between Ipsen, Inspiration and Emory University. This is produced at a facility near Boston in the United States of America. There is a high degree of sequence and functional homology between human and porcine factor VIII. Both human and porcine factor VIII share a common sequence of A1–A2–B–A3–C1–C2 domains and both molecules are cleaved by thrombin to form a dimer composed of a light and heavy chain [24]. OBI-1 is a recombinant B-domain deleted form of porcine factor VIII synthesized in baby hamster kidneys cells grown in a serum-free medium. It does not contain any porcine von Willebrand factor. Phase III clinical trials in patients with acquired haemophilia and congenital haemophilia A complicated by alloantibodies are underway [25].

A goal of future research will be to attempt to produce a porcine factor VIII molecule, which is even less antigenic than the natural wild type. This could be done by substitution of specific amino acids through genetic engineering, eliminating those that seem to be particularly immunogenic, or creating human/porcine hybrid constructs [26,27]. Such modified molecules could then conceivably be used to treat noninhibitor patients too, or at least as treatment in the early period soon after diagnosis which is known to be the peak risk period for inhibitor development. There is already quite a wealth of information available on which parts of the porcine and human factor VIII molecules are particularly immunogenic. The antibody response to the individual FVIII domains in haemophilic mice immunized with human or porcine FVIII has provided important information [28].The overall immunogenicity of human and porcine FVIII is similar, but significant differences in domain recognition have been identified. Anti-A2 and anti-C2 antibodies constitute the majority of inhibitors in both the human and porcine FVIII groups, similar to inhibitors that develop in humans. The proportions of anti-A2 or anti-C2 antibodies were not significantly different between the two groups in one study. However, the proportion of anti-C1 antibodies was significantly higher in the human FVIII group, whereas anti-A3 antibodies were more common in the porcine FVIII group. The differential immune response to human and porcine FVIII supports the view that it may be possible to reduce the immunogenicity of porcine (and human) FVIII by mutagenesis at specific sites, within the A2, A3 and C1 domains.

Transgenic products

In a final twist to this tale, it appears that pigs may be able to provide us with human coagulation proteins like factor VIII and IX [29,30]. The development of transgenic animals offers the potential for the production of recombinant products which can be extracted from their milk. To express a recombinant protein in the milk of an animal, expression vectors containing a gene encoding the protein of interest are fused to milk-specific regulatory elements (such as casein, lactalbumin or lactoglobulin) and introduced by microinjection of a one-cell embryo, or alternatively transfected into a cell line suitable for somatic cell nuclear transfer. The mammary-gland specific transgene is transmitted in a Mendelian fashion, following integration into the germline. If expressed, it becomes a dominant genetic characteristic that will be predictably inherited by offspring of the animal, and the yield of transgenic protein in the milk is often high in the range of grammes

per litre. Transgenic expression delivers the advantages of mammalian cells (such as sophisticated molecular refolding machinery and glycosylation), as well as the potential for flexibility of scale in production and relatively low costs. Pigs have proved to be the best animals to produce human coagulation proteins: the γ-carboxylation profile of factor IX from pigs is much better than that observed in sheep. There is no evidence of transmission of porcine pathogens to humans and, in particular, pigs are not susceptible to BSE/vCJD. They also have a high reproductive rate and each sow can produce 200–300 L of milk per year, which is unusually rich in protein. Early experiments using transgenic factor IX in haemophilic dogs have already begun (WH Velander, personal communication).

In conclusion, blood products made from porcine plasma had an important role in the treatment of haemophilia for half a century from 1954 to 2004. A recombinant porcine factor VIII product is now at an advanced stage of clinical development. In the more distant future, the distinction between human and porcine products may become blurred through the production of hybrid human/porcine recombinant factor VIII, and even human factor VIII produced in transgenic pigs.

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