INHIBITORS
IN HEMOPHILIA A OR B
2012

Carol K. Kasper, M.D.
Hemophilia Treatment Center
Los Angeles Orthopaedic Hospital
2400 S. Flower Street
Los Angeles, California 90007
Inhibitors are antibodies that neutralize the activity of a clotting factor. Inhibitor antibodies to factor VIII or IX may arise as allo-antibodies in patients with hemophilia A or B who have been transfused with exogenous factor VIII or IX, respectively. Inhibitor antibodies to factor VIII may arise as auto-antibodies in non-hemophilic persons. Non-neutralizing antibodies also may arise and may be detected with suitable ELISA tests.

**Incidence and Prevalence**

The number of new inhibitors in a population in a given period of time (incidence) reflects transient inhibitors as well as enduring ones. As the frequency of testing increases, more transient inhibitors are identified and the apparent incidence of inhibitors increases. The number of inhibitors present in a population at any given time (prevalence) primarily reflects long-standing inhibitors. The incidence of inhibitors to factor VIII in patients with severe hemophilia A within the first few years of life is typically reported as about 20-30%. The prevalence is lower. The incidence of inhibitors in severe hemophilia B is about 4%. Clinically-apparent factor VIII auto-antibodies are uncommon; the incidence is difficult to assess because patients are not centralized. My personal estimate is one or two per million population per year. Factor IX auto-antibodies are very rare.

**Issues affecting incidence in hemophilia**

**Genetic factors** strongly influence the probability of inhibitor formation. Inhibitors arise much more commonly in severe hemophilia than in mild or moderate hemophilia. If the fetus is exposed to a version of the relevant clotting factor, even if it is non-functional, the factor is likely to be recognized as “self” and the fetus to become tolerant.

Inhibitors are especially frequent in patients with mutations that prevent formation of the clotting factor, such as deletions of large portions of the gene and nonsense mutations causing premature stop codons. With other, less drastic mutations, a version of the relevant molecule may be made. A few missense mutations causing mild hemophilia are associated with a high frequency of inhibitors. One such mutation in factor VIII prevents binding of T cells to factor VIII, thus interfering with tolerance development in fetal life.

The strong tendency of inhibitors to run in families is due in part to the mutual causative mutation but in part to other, as yet ill-defined, circumstances. Some variants of immune-competent genes and HLA phenotypes are slightly more common than others in patients with inhibitors.

A striking example of a genetic influence other than the mutation was seen in dogs in Canada. A male with hemophilia A was mated to a related female and also to an unrelated female of another breed, in order to establish a colony of hemophilic dogs. Hemorrhages were treated with canine cryoprecipitate. After such treatment, inhibitors developed in two-thirds of the hemophilic descendants of the unrelated female but not in the hemophilic descendants of the related female. Susceptibility to inhibitor development may have been inherited through the immune system of the female ancestor.

In the USA, the inhibitor incidence in hemophilia A patients of black African descent or of Latin-American descent is twice as high as in white patients. The probable cause is racial variation in FVIII gene and protein structure. All white persons and a majority of black persons have one of two FVIII genes differing only in a single-nucleotide polymorphism (SNP) coding for the B-domain, not a notably immunogenic region. A quarter of black persons, however, have FVIII genes with SNPs coding for the A2 or C2 domains, which contain immunogenic epitopes. The greatly increased vulnerability of these patients to inhibitors probably accounts for the overall racial difference in inhibitor incidence. These patients are exposed to FVIII...
concentrates that are mis-matched to their fundamental FVIII structure. FVIII genes in patients or Latino descent have not yet been characterized.

Genetic predisposition also is suggested by the observation that strong inhibitor antibodies typically arise early in life, after treatment with the exogenous clotting factor on only a few occasions. In prospective studies of previously-untreated patients (mostly babies) followed closely, inhibitors arose after a median of 8 to 9 exposure-days. A few inhibitors, predominantly weak ones, may arise later in life, notably after periods of intensive treatment with the clotting factor, or with inflammatory disorders.

**The role of concentrate type** has been scrutinized. The medical community was wary of viral inactivation with heat when it was introduced as pasteurization (heating in solution) at the end of the 1970’s in Germany and as dry heat (baking) in 1983 in the USA, fearing that the FVIII might be slightly denatured by heat and thus more antigenic. These fears were unfounded: the early viral -inactivated concentrates did not provoke excess inhibitors.

Two FVIII concentrates made in Europe in the early 1990’s, however, did provoke new inhibitor development in several patients who were thought to be at low risk of forming new inhibitors because previously they had been heavily treated with FVIII concentrates. One of these concentrates, made in The Netherlands by the controlled-pore-silica fractionation method, had not elicited an excess number of inhibitors when viral-inactivated with dry heat but did so when viral inactivated with pasteurization. The other concentrate, made in Austria, became more antigenic when pasteurization was added to a solvent-detergent viral inactivation process. An additional problem in the latter concentrate may have been partial degeneration of FVIII, possibly due to delay in separating plasma from donated blood.

When recombinant concentrates were introduced in the 1990’s, previously-untreated patients (PUPs) were studied with frequent inhibitor tests. In these early, limited studies, the incidence of inhibitors, other than transient ones, was not higher than that reported for PUPs on plasma-derived concentrates. A recent meta-analysis of inhibitor incidence in 2094 PUPs, from 24 studies, found an overall incidence of 14.3% in patients using plasma-derived FVIII and 27.5% in patients using recombinant FVIII. A prospective, randomized trial is underway.

**Non-hemophilic persons**

Inhibitors occasionally arise as auto-antibodies in post-partum women, patients with auto-immune disorders or other illnesses, or in older adults without underlying diseases.

Clinically-apparent auto-antibodies may be only the tip of an iceberg. The incidence of subclinical auto-antibodies may be much greater but most are controlled quickly by the immune system. As many as 17% of normal blood donors have been reported to have low-level inhibitor antibodies to factor VIII, together with anti-idiotypic antibodies that suppress the inhibitory action. Such antibodies are more common in multiparous women and older donors, the groups subject to clinically-apparent auto-antibodies. Patients with hemophilia whose inhibitors have vanished (either spontaneously or after induction of tolerance) often have demonstrable anti-idiotypic antibodies.

**Characteristics of Inhibitor antibodies**

Inhibitors are IgG antibodies. Anti-factor VIII antibodies react with active sites of the factor VIII molecule, primarily with epitopes in the A2, A3, C1 and C2 domains. It has been proposed that less-immunogenic recombinant molecules be designed with alternate amino acid sequences at these sites.

Antibodies to factor VIII are unlikely to fix complement or to precipitate. The union of factor VIII with its inhibitor is not associated with allergic reactions. The union of factor IX with its inhibitor, however, may cause serious allergic reactions, including anaphylaxis. Such reactions can occur with the first infusion of exogenous factor IX given after the inhibitor develops, that is,
before the inhibitor has been diagnosed. Factor IX-inhibitor complexes can precipitate and the nephrotic syndrome can result.

The reaction of factor VIII with its inhibitor is time-dependent both in vitro and in vivo, an observation relevant to measurement and to clinical treatment. The higher the level of the inhibitor, the more rapidly it inactivates FVIII. If a patient’s inhibitor level is low to moderate, a high therapeutic dose of factor VIII may be able to interact in coagulation before being neutralized.

Two patterns of reaction kinetics are seen. Inhibitors with “Type 1” or “simple” kinetics completely neutralize factor VIII and are neutralized themselves in the reaction. Most inhibitors in patients with hemophilia are Type 1. Inhibitors with “Type 2” or “complex” kinetics do not completely neutralize factor VIII and, after reaction, retain some ability to neutralize additional factor VIII. Although some factor VIII may remain measurable in the presence of type 2 inhibitors, the patient may bleed as profusely as if he had no factor VIII at all. The reason for this confusing presentation is unknown. Type 2 reaction kinetics are more common in auto-antibodies than in alloantibodies.

**Clinical presentation**

In hemophilia, an inhibitor is suspected when a patient’s hemorrhage is not promptly controlled with his usual dose of clotting factor concentrate. The presence of an inhibitor does not change the typical site, frequency or severity of bleeding. The inhibitor makes control of hemorrhages more difficult.

Patients with auto-antibodies to factor VIII may have severe bleeding in multiple sites, usually in muscles and soft-tissue, with extensive bruising. “Elder abuse” may be suspected. The startling pattern of bleeding in these patients is unexplained.

The immune response to exogenous factor VIII (or IX) may be weak or robust. Most inhibitors are “high responders”, that is, within a few days of exposure to exogenous clotting factor, the inhibitor level rises briskly, peaking within the first month. Without further exposure, the inhibitor level gradually falls in most patients and, after years, may become undetectable.

A few low-level inhibitors in hemophilia patients are “low responders”, that is, their levels do not rise notably after exposure to the exogenous clotting factor. Thus, such patients can continue treatment with the factor in a sufficiently-high dose. Auto-antibodies usually do not rise after exposure to exogenous clotting factor.

**Simple reaction kinetics:**

\[ \text{FVIII Ag + inhibitor Ab} = \text{AgAb}; \text{ (no factor VIII activity, no inhibitor activity)} \]

**Complex reaction kinetics:**

\[ \text{FVIII Ag + inhibitor Ab} = \text{Ag (low factor VIII activity) + Ab (diminished inhibitor activity)} \]
When repeated doses of factor VIII are given to a low responder, as depicted by the solid line, anamnesis is not seen. The first dose of factor VIII, given to the high responder (dashed line) when the inhibitor level is low, does provoke anamnesis but subsequent doses, given while the inhibitor level still is high (at its own ceiling) provoke no further rise.
Laboratory diagnosis

The most common screening test for inhibitors is an APTT on a mixture of patient and normal plasma incubated to-
gether for one to two hours at 37°C. In the presence of an inhibitor, the APTT after incubation is prolonged compared to controls without inhibitor, as in the following example:

<table>
<thead>
<tr>
<th>INCUBATION MIXTURE:</th>
<th>APTT at outset of incubation, seconds:</th>
<th>APTT after two hours, seconds:</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal plasma alone</td>
<td>32</td>
<td>40</td>
</tr>
<tr>
<td>Factor VIII deficient plasma alone</td>
<td>90</td>
<td>95</td>
</tr>
<tr>
<td>Normal plasma plus factor VIII deficient plasma with</td>
<td></td>
<td></td>
</tr>
<tr>
<td>No inhibitor</td>
<td>37</td>
<td>45</td>
</tr>
<tr>
<td>1 Bethesda unit</td>
<td>37</td>
<td>53</td>
</tr>
<tr>
<td>5 Bethesda units</td>
<td>43</td>
<td>64</td>
</tr>
<tr>
<td>20 Bethesda units</td>
<td>54</td>
<td>92</td>
</tr>
</tbody>
</table>

In a person with hemophilia, a prolonged APTT of a mixture of patient or normal plasma strongly suggests the presence of an inhibitor to the relevant clotting factor. In a non-hemophilic person, however, such a result indicates the presence of some inhibitor, but not the identity of the antigen. Similar results might be seen with a “lupus” (anti-phospholipid) inhibitor. Specific tests for lupus inhibitor can be performed to rule out that condition. Clotting factor assays are performed to determine which factor is the antigen.

Assays of the target antigen are low whether the patient plasma is tested at a small or a great dilution. Assays of other clotting factors also may be affected by the inhibitor and may be low if the patient plasma is not much diluted.

For example, if a patient plasma is low in factor VIII and also contains excess antibody to factor VIII, the effects on the factor VIII and the factor IX assays might be as follows:

<table>
<thead>
<tr>
<th>Factor VIII  APPT-based assay system, Reagents:</th>
<th>Factor IX  APPT based assay system, Reagents:</th>
</tr>
</thead>
<tbody>
<tr>
<td>Known factor VIII deficient plasma “substrate” (supplies all factors except FVIII) used full-strength</td>
<td>(1) Known factor IX deficient plasma “substrate”, (supplies all factors except factor IX, supplies FVIII) used full-strength</td>
</tr>
<tr>
<td>(2) Patient plasma (low level FVIII, plus excess inhibitor to FVIII) tested at various dilutions</td>
<td>(2) Patient plasma (low level of FVIII, normal factor IX plus excess inhibitor to FVIII) tested at various dilutions</td>
</tr>
<tr>
<td>(3) Phospholipid, surface activator, calcium</td>
<td>(3) Phospholipid, surface activator, calcium</td>
</tr>
<tr>
<td>Result: factor VIII is the only deficient factor; factor VIII level is low as measured at all dilutions of patient plasma.</td>
<td>Result: excess inhibitor neutralizes some or all of the factor VIII in substrate, the only source of factor VIII in the system. Clotting times are prolonged, thus, the factor IX level appears to be low. As patient plasma is diluted, less substrate factor VIII is destroyed.</td>
</tr>
</tbody>
</table>
In the following example, the levels of four clotting factors were assayed in plasma containing an inhibitor to factor VIII. Each clotting factor was measured in a one-stage APTT based assay. The level of factor VIII is similarly low whether the patient plasma is slightly or greatly diluted in buffer. The apparent levels of factors IX, XI and XII increase as patient plasma is diluted. As the inhibitor in the patient plasma is “diluted out”, it has a decreasing detrimental effect on the FVIII in the reagent substrate plasmas deficient in factor IX, in factor XI or in factor XII.

If plasma from the patient below is assayed at only two dilutions, for example, 1:5 and 1:10, the laboratory might report that the patient has a severe deficiency of FVIII and a mild deficiency of other clotting factors. Laboratories commonly assay plasma at only one or two dilutions unless otherwise instructed.

<table>
<thead>
<tr>
<th>Patient plasma, diluted:</th>
<th>Factor VIII</th>
<th>Factor IX</th>
<th>Factor XI</th>
<th>Factor XII</th>
</tr>
</thead>
<tbody>
<tr>
<td>1:5</td>
<td>&lt;1</td>
<td>28</td>
<td>20</td>
<td>38</td>
</tr>
<tr>
<td>1:10</td>
<td>&lt;1</td>
<td>36</td>
<td>29</td>
<td>47</td>
</tr>
<tr>
<td>1:20</td>
<td>&lt;1</td>
<td>42</td>
<td>38</td>
<td>54</td>
</tr>
<tr>
<td>1:50</td>
<td>&lt;1</td>
<td>60</td>
<td>51</td>
<td>72</td>
</tr>
<tr>
<td>1:100</td>
<td>&lt;1</td>
<td>74</td>
<td>63</td>
<td>84</td>
</tr>
</tbody>
</table>

Inhibitors with simple reaction kinetics are quantified easily by the Bethesda test but those with complex kinetics can be quantified only roughly.

In the “Nijmegen” modification of the Bethesda test (introduced in 1995 and now in widespread use), the control consists of normal plasma incubated with immuno-depleted FVIII-deficient plasma instead of buffer.

Measurement of the in vivo recovery and, especially, the half-life of a bolus of FVIII infused into the patient is more sensitive to traces of inhibitor than the above screening tests.

The figure illustrates the reference graph for the Bethesda test. If the residual factor VIII, after incubation, is 100% of the level in the control incubation mixture, then the inhibitor level is zero. If the residual factor VIII is 50% of that of the control, then the inhibitor level is one Bethesda unit. The graph is used between 25 and 75% (solid line). Results of more than 75% are within the error of the assay. If the result is less than 25%, then the patient plasma is tested at various dilutions until the result can be read off the graph. The result then is multiplied by the dilution to assign Bethesda units. For example, if a plasma sample is diluted 1:5 before incubation and the residual factor VIII then is 50%, or one unit, $1 \times 5 = 5$ Bethesda units.
STRATEGIES FOR MANAGEMENT OF BLEEDING

Control of acute bleeding is sometimes a challenge. If one approach fails, another must be undertaken. Patients are best managed in experienced centers with laboratories that are able to follow factor levels and inhibitor levels closely.

(1) Assay inhibitor ahead of need

The level of inhibitor should be quantified when the diagnosis of an inhibitor is made, whether or not the patient is bleeding. Re-quantification at intervals after that, e.g. monthly, is a good idea so that the most recent results can be used to make a quick choice of the most appropriate treatment if a hemorrhage occurs. In patients with factor VIII inhibitors, the level of inhibition of porcine FVIII also should be measured in advance of need.

(2) Use conservative aids

Minor bleeding may subside with restriction of motion of the affected area, to prevent weak clots in the damaged vessels from being dislodged. Cold packs, to constrict blood vessels, are useful for fresh hemorrhages.

(3) Consider raising plasma factor levels

If the plasma factor level can be raised to around 30 to 50 U/dL, the chance of securing hemostasis at that time is excellent (close to 100%) whereas the chance of securing hemostasis with the first dose of a bypassing agent is about 50% (see table of controlled experiments). In patients with low-responding inhibitors, the obvious choice is to give exogenous clotting factor to raise the plasma level. In patients with high-responding inhibitors, and very dangerous hemorrhages, an attempt may be made to raise the plasma factor level if the baseline inhibitor level is not too high. In such an instance, one trades an immediate benefit, a good chance of securing hemostasis, for an evil, the inevitable subsequent rise in the inhibitor level.

(4) Consider using a bypassing agent

If a patient has a high-responding inhibitor and a minor or routine hemorrhage (including most joint hemorrhages), then a "bypassing agent" which does not contain the relevant clotting factor is preferred in order to avoid stimulating a rise in the inhibitor level (anamnesis). One hopes that the inhibitor level will fall again to a low level before any dangerous hemorrhage occurs.

If the inhibitor level is high at the time of a hemorrhage, a bypassing agent may be the only available treatment.

When the inhibitor level is unknown and the hemorrhage is serious, one may give a large dose of the deficient factor and also a dose of a bypassing agent.

(5) Beware of inhibitors in hemophilia B

Patients with hemophilia B and inhibitors to factor IX which cause allergic reactions are best treated with recombinant activated factor VII, the only bypassing agent which does not contain factor IX.
Methods of management of hemorrhages

Lowering inhibitor levels

If time and circumstances permit, the level of an inhibitor may be lowered in order to make it feasible to give sufficient exogenous clotting factor to neutralize the remaining circulating inhibitor and raise the plasma factor level. Exchange plasmapheresis can be performed efficiently with continuous flow cell-separation centrifuges which can replace a liter of plasma in ½ to one hour in a typical adult. Replacement of three to four liters of plasma in typical adults temporarily reduces plasma inhibitor levels by 40% or more. If time permits, e.g. before an urgent but not emergency surgical operation, exchanges may be performed on two or three consecutive days, if needed. Anamnesis is likely to be provoked within a few days of exposure to normal plasma, thus, in exchanges over several days, the patient's plasma should be replaced with saline. Factor VIII (or IX) concentrate should be given promptly after the final plasmapheresis to achieve a maximal peak of the factor in the patient's plasma. Any surgery should be timed to coincide with that peak.

In well-equipped centers, the efficacy of plasmapheresis can be improved by pouring the plasma, while outside the body, through columns containing protein A sepharose, which binds most IgG. Over a period of 6 or more hours, some 5 to 9 liters of patient plasma can be processed with reduction of inhibitor levels by two-thirds or more.

Intravenous infusion of normal human gamma globulin, in high doses, by itself or after plas-
mapheresis, may lower inhibitor levels immediately, perhaps by the action of anti-idiotypic antibodies. Some degree of long-term immune suppression also may be seen. IVIgG has been especially useful in treatment of autoantibodies.

Raising plasma factor levels

**DDAVP (desmopressin, Stimate®)**

DDAVP mediate the release of factor VIII and von Willebrand factor from cellular storage sites into the plasma. Its use may elevate plasma FVIII levels temporarily in those inhibitor patients who have the fundamental capacity to make FVIII, that is, non-hemophilic patients or those with mild hemophilia A, in the presence of a low-level inhibitor (e.g. two Bethesda units). In these special circumstances, DDAVP may release enough FVIII to neutralize the circulating inhibitor and raise the plasma level of FVIII slightly, sufficient to stop minor bleeding or allow minor surgical procedures.

**Exogenous human factors**

A large bolus of human factor VIII (for hemophilia A) or purified human factor IX (for hemophilia B) may be infused directly, if the inhibitor level is not too high (under 5 BU, sometimes higher) to try to achieve a plasma level of 30 U/dl or more. The amount of concentrate needed is only roughly correlated with the inhibitor level. A suggested initial factor VIII doses is 20-40 U/kg plus 20 U/kg per BU. The factor level should be measured immediately after infusion so that further concentrate can be given if needed. Once an inhibitor is neutralized with boluses of concentrate, the dose needed for subsequent infusions that day and the next few days will be lower, until anamnesis supervenes.

Some clinicians infuse factor VIII, in the presence of an inhibitor, without attempting to attain measurable plasma factor VIII levels, with some success. If the inhibitor level is not very high, factor VIII may have time to act in coagulation before it is neutralized. In one such protocol, patients with factor VIII inhibitors of less than 30 BU receive a bolus of 70-140 factor VIII U/kg followed by a continuous infusion of 4-14 U/kg/hour. Hemostasis often is achieved although measurable plasma factor VIII levels are not always reached.

Some clinicians infuse factor VIII, in the presence of an inhibitor, without attempting to attain measurable plasma factor VIII levels, with some success. If the inhibitor level is not very high, factor VIII may have time to act in coagulation before it is neutralized. In one such protocol, patients with factor VIII inhibitors of less than 30 BU receive a bolus of 70-140 factor VIII U/kg followed by a continuous infusion of 4-14 U/kg/hour. Hemostasis often is achieved although measurable plasma factor VIII levels are not always reached.

**Porcine factor VIII**

Porcine factor VIII interacts well in the human coagulation sequence but attaches anti-human-factor VIII antibodies poorly. Inhibitors arising in persons with hemophilia A neutralize, on average, only 20-25% as much porcine factor VIII as human factor VIII. (The range is wide, from zero to 75%). Cross-reactivity of auto-antibody inhibitors tends to be lower that that of hemophilic allo-antibodies. For many years, porcine FVIII made from the plasma of pigs was available as “Hyate-C®”. It was discontinued in early 2004. A recombinant B-domain-deleted porcine FVIII is now in clinical trials.

Porcine factor VIII was given to patients with low anti-porcine-factor VIII inhibitor levels, in doses sufficient to neutralize that inhibitor activity and achieve a therapeutic circulating factor VIII level. Some patients developed antibodies to porcine factor VIII after several days of treatment, or after several episodes of treatment.

**Bypassing agents**

Activated clotting factors such as Xa or VIIa may trigger some degree of coagulation in the absence of factor VIII or IX or in the presence of an inhibitor to one of them, thus they may “bypass” the need for factor VIII or IX. Prothrombin complex concentrate (PCC) contains clotting factors II (prothrombin), VII, IX and X, which travel together in plasma fractionation. A portion of each of these factors activates spontaneously during processing. Activation can be enhanced deliberately to create “activated” prothrombin complex (APCC), also known as anti-inhibitor coagulant complex (AICC). PCC and APCC have been used to treat bleeding in patients with inhibitors since the early 1970's. Nowadays,
The above graph depicts anti-human-factor VIII-inhibition (solid bars) and anti-porcine-factor VIII-inhibition in the first 29 patients with anti-factor VIII-inhibitors studied in my laboratory. Patients with lower levels of antibody to human factor VIII are shown in the upper graph and those with higher levels on the lower graph. Inhibition of porcine factor VIII was lower than that of human factor VIII in all
PCCs are rarely used for this purpose but APCC (FEIBA®) is popular.

A single factor can be separated and activated. A concentrate of plasma-derived factor Xa, with phospholipid, was developed and used successfully in animal trials in the 1980’s, but human trials were not attempted. A concentrate of plasma-derived factor VIIa was developed in the early 1980’s and used successfully in patients with inhibitors. A recombinant factor VIIa (rVIIa) concentrate was then prepared (NovoSeven®). FVIIa needs tissue thromboplastin to initiate the activation of factor X. Tissue thromboplastin is present at sites of injury but is not abundant elsewhere, thus, the coagulant effect of exogenous FVIIa may be focused on sites of injury, a probable safety advantage. The potency of PCC is described in factor IX units. The potency of the two APCCs (Autoplex®, which was discontinued in 20005, and FEIBA®) is described in terms of factor VIII correctional or bypassing units, which are unique to each brand. The potency of rVIIa is expressed in weight in micrograms or in KIU (thousands of international units of factor VII).

Some clinicians give one dose of an APCC or of rVIIa daily or every other day as prophylaxis in hemophilic patients with inhibitors who hemorrhage frequently. Some clinicians alternate doses of FEIBA® and NovoSeven®.

In the above simplified coagulation scheme, factor X may be converted to activated factor X either by the contact-activation (“intrinsic”) sequence on the left, or the factor VII-tissue thromboplastin (“extrinsic”) sequence on the right. (These sequences are depicted as separate for clarity; in reality, they interact.) Activated factor X is one of the essential catalysts of the conversion of prothrombin to thrombin.

**Efficacy**

It is difficult to know when internal bleeding (for example, in a joint) stops. Diminution of pain is the first, albeit subjective, indicator. Range of motion improves more slowly. By the late 1970’s doubts had arisen that PCC was effective at all, so comparison to albumin placebo was justified. The first controlled trial of PCCs for joint hemorrhages in persons with hemophilia and inhibitors confirmed that a single dose of PCC was associated with symptomatic improvement within a few hours in about half of instances. Trials of “Autoplex” (an APCC) versus “Proplex” (a PCC) in the USA, and of “FEIBA” (an APCC) versus “Prothromblex” (a PCC) in Europe gave similar results. Uncontrolled reports of the efficacy of repeated doses of APCC, with evaluation performed after two or three days, showed a higher success rate. Clinical trials of NovoSeven (rVIIa) all featured repeated dosing at intervals of 2.5 to 3 hours. In rough summary, two doses or rVIIa successfully controlled about half of joint hemorrhages. A trial of NovoSeven versus an APCC is underway. Summaries of the most comparable completed trials are appended.
<table>
<thead>
<tr>
<th>Study Details</th>
<th>“Effective” (subjective)</th>
<th>ROM improved 10° or more</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Lusher et alia, 1980, observed at six hours after a single infusion</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hemophilia A, no inhibitor, open-label</td>
<td>100%</td>
<td>65%</td>
</tr>
<tr>
<td>Usual dose of factor VIII</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hemophilia A, inhibitor, double-blind</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Konyne (PCC) 75 U/kg</td>
<td>48%</td>
<td>34%</td>
</tr>
<tr>
<td>Proplex (PCC) 75 U/kg</td>
<td>53%</td>
<td>33%</td>
</tr>
<tr>
<td>Albumin placebo</td>
<td>29%</td>
<td>18%</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th><strong>Lusher et alia, 1983, observed at six hours after a single infusion</strong></th>
<th>“Effective” (subjective)</th>
<th>ROM improved 10° or more</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hemophilia A, inhibitor, double-blind</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Proplex (PCC) 75 U/kg</td>
<td>50%</td>
<td>43%</td>
</tr>
<tr>
<td>Autoplex (APCC) 50 U/kg</td>
<td>56%</td>
<td>48%</td>
</tr>
<tr>
<td>Autoplex (APCC) 75 U/kg</td>
<td>52%</td>
<td>52%</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th><strong>Sjamsoedin et alia, 1981, observed at 24 hours after a single infusion</strong></th>
<th>“Effective” (subjective)</th>
<th>ROM improved 30° or more</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hemophilia A, inhibitor, double-blind</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Prothromblex (PCC) 48 U/kg</td>
<td>46%</td>
<td>7%</td>
</tr>
<tr>
<td>FEIBA (APCC) 88 U/kg</td>
<td>66%</td>
<td>25%</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th><strong>Key et alia, 1998, USA</strong></th>
<th>Subjective efficacy</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hemophilia A, inhibitor, open label, home treatment</td>
<td></td>
</tr>
<tr>
<td>NovoSeven, 90 ug/kg, every 3 hours until effective</td>
<td>Median 2.2 doses needed</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th><strong>Santagostino et alia, 1999, Italy</strong></th>
<th>Subjective efficacy</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hemophilia A, inhibitor, open label, home treatment</td>
<td></td>
</tr>
<tr>
<td>NovoSeven, 90 ug/kg, every 3 hours until effective</td>
<td>Median 2 doses needed</td>
</tr>
</tbody>
</table>
A GENERAL ALGORITHM FOR MANAGEMENT OF AUTO-ANTIBODY INHIBITORS:

Anti-human-factor VIII Inhibitor level:

- **1-2 Bethesda units**
  - Try dose of DDAVP to raise plasma factor VIII level

- **< 5 Bethesda units**
  - Human FVIII, enough to raise plasma FVIII level to 30% or more

- **> 5 Bethesda units**
  - Measure anti-porcine inhibitor level, if porcine FVIII is available
    - **< 5 BU anti-porcine**
    - Porcine FVIII, enough to raise plasma FVIII level to 30% or more
    - **5-50 BU anti-porcine**
    - Plasmapheresis to reduce inhibitor level, then give porcine or human FVIII
    - **> 50 BU anti-porcine**
    - Bypassing agents

A GENERAL ALGORITHM FOR MANAGEMENT OF ALLO-ANTIBODY (HEMOPHILIC) INHIBITORS:

- **“Low-responder”**
  - Inhibitor < 5 BU
    - Human factor VIII, enough to raise plasma factor VIII level to 30% or more
    - Non-critical hemorrhage
      - Bypassing agent (to avoid anamnesis)
    - Critical hemorrhage
      - Anti-human-factor VIII < 5 BU
      - Anti-porcine Factor VIII 5 or more BU
      - Bypassing agent

- **“High-responder”**
  - Human factor VIII, enough to raise plasma factor VIII level to 30% or more
  - Anti-human-factor VIII 5 or more BU
  - Anti-porcine Factor VIII 5 or more BU
NovoSeven has been used effectively, in frequently-repeated doses, for a few urgent and elective major surgical operations. FEIBA® also has been used effectively, in less-frequent doses, for a few urgent surgical operations.

An occasional side-effect of PCC or APCC, seen only in a few sensitive patients with hemophilia A, is an anamnestic rise in the factor VIII inhibitor. These plasma-derived products may contain residual denatured factor VIII.

Other adverse side-effects of bypassing agents, seen predominantly after intensive use, may include DIC, deep vein thrombosis (DVT) and myocardial infarction. As of 2000, there were published reports of myocardial infarction in 16 patients, most of them young, who had received PCC or APCC. On autopsy, when performed, hemorrhagic myocardial infarction without coronary thrombosis was seen. Because of this mysterious but catastrophic complication, closely-spaced repeated doses of PCC or APCC are avoided. Early reports suggested that rVIIa may be associated with fewer instances of DIC and DVT with intensive use than are other bypassing agents. The frequency of adverse events with these products is the subject of ongoing controversy. Continued vigilance is essential because adverse events often are not even recognized, much less reported.

INDUCTION OF IMMUNE TOLERANCE

Bonn protocol and similar regimens

Tolerance to the deficient clotting factor can be achieved in a large majority of patients with alloantibodies to factor VIII by giving frequent (usually daily) doses of factor VIII over a prolonged period of time. During the first month of treatment, anamnesis usually occurs, but in the second month, inhibitor levels typically fall rapidly. Thereafter, they may fall more slowly. Complete tolerance consists of a sustained zero inhibitor level and normal T1/2 of infused factor VIII. The duration of treatment until complete hemostasis is achieved varies from a few weeks to more than a year. In Germany, tolerance then is maintained with the low-level prophylaxis customary there for all patients with severe hemophilia. Without prophylaxis, inhibitors may recur at low levels. Some patients achieve and maintain partial tolerance, that is, their inhibitor does not disappear completely but remains at a low level, behaving as a low-responding

<table>
<thead>
<tr>
<th>Historic highest inhibitor level, Bethesda units:</th>
<th>Proportion of patients achieving tolerance on daily factor VIII dose of:</th>
</tr>
</thead>
<tbody>
<tr>
<td>Under 50</td>
<td>Under 50 units / kg</td>
</tr>
<tr>
<td>Under 50</td>
<td>38.7 %</td>
</tr>
<tr>
<td>50-500</td>
<td>25%</td>
</tr>
<tr>
<td>Over 500</td>
<td>Nil</td>
</tr>
</tbody>
</table>

The ideal dosage is highly debated. Doses used range from 300 FVIII U/kg every day in Bonn, Germany, where tolerance was pioneered, to 25 FVIII U/Kg every other day in The Netherlands. In North America, doses of 50-100 U/kg daily have been used. Some centers add short courses of low-dose prednisone or another corticosteroid if the fall in the inhibitor level is sluggish.

Reports from large registries of patients undergoing induction of immune tolerance agree that the lower the inhibitor level at the outset of tolerance induction, and the lower the inhibitor level in the patient’s past, the more likely he is to achieve tolerance. Dosage, on the other hand, is disputed. The registry maintained by Prof. Guglielmo Mariani of Italy (below) indicates that higher doses are correlated with a greater chance of success (and also with shorter duration of needed treatment, not depicted.). The North American registry, maintained by Dr. Donna DiMichele of New York, does not show a correlation of dose with chance of success. An international clinical trial is underway, comparing the lowest-dose (Dutch) protocol with the highest-dose (German) one.

Malmo protocol (intensive, inpatient regimen)

In Malmo, Sweden, an intensive two- to four-week inpatient tolerance protocol was used for many years. With this protocol, tolerance was achieved in

Prof. Guglielmo’s Registry as of 1993:

<table>
<thead>
<tr>
<th>Historic highest inhibitor level, Bethesda units:</th>
<th>Proportion of patients achieving tolerance on daily factor VIII dose of:</th>
</tr>
</thead>
<tbody>
<tr>
<td>Under 50</td>
<td>38.7 %</td>
</tr>
<tr>
<td>50-500</td>
<td>25%</td>
</tr>
<tr>
<td>Over 500</td>
<td>Nil</td>
</tr>
</tbody>
</table>
more than half of patients with the first round of treatment, and in 70% with one or more additional rounds of treatment. Success is achieved using the Malmo protocol with a lower total dose of factor VIII and with lower cost than with Bonn-style outpatient protocols. Suppression of factor IX inhibitors in patients with hemophilia B was achieved in 86% of instances, whereas in Bonn-style outpatient protocols of daily factor IX the rate of success has been low, about 10%, with a high frequency of nephrotic syndrome. A critical difference may be the initial removal of much of the factor IX inhibitor in the Malmo system.

After immune tolerance was induced, some patients developed new circulating antibodies that either complexed protectively with infused clotting factors or acted as anti-idiotypic antibodies to the inhibitors.

The Malmo protocol is no longer used in Malmo. Inhibitors are treated as soon as they are discovered (usually in babies) with the Bonn regimen.

(1) If the inhibitor is over 10 BU, lower it by means of plasmapheresis and extracorporeal adsorption of antibodies;
(2) Give sufficient factor VIII to neutralize remaining circulating inhibitor and to maintain plasma FVIII (or IX) levels at 30-80 U/dL;
(3) Give cyclophosphamide 12-15 mg/kg intravenously for two days then 2-3 mg/kg orally for 8-10 days;
(4) Give intravenous gamma globulin in a dose of 2.5-5 grams with the first dose of factor VIII and, at each of days 4-9, in a dose of 0.4 grams/kg.
REFERENCES

Incidence and Prevalence


Raw data from 8 studies on 451 patients with severe hemophilia A followed from birth with regular outpatient visits are combined. At age 3 years, the cumulative incidence of high-responding inhibitors was 10%; by age 18 years, it was 20%.


In Frankfurt, Germany, from 1976 to date of the article, inhibitors developed in 14/27 (52%) of boys with severe haemophilia A followed prospectively. (This incidence is the highest ever reported and is often quoted. In a larger study of a more extensive sample in Germany, the proportion was lower.)


German patients from several centers were evaluated prospectively over a 23-year period. An inhibitor developed in 22/72 (32%) of patients with hemophilia A after a median of 15 exposure-days. Amongst 46 patients with severe hemophilia A, 43% developed inhibitors. No difference was seen between those treated with plasma-derived concentrates and those treated with recombinant concentrates.


All issues influencing incidence, including patient characteristics and treatment history, are reviewed.


Development of inhibitors is reported in 26 patients with mild to moderate hemophilia A. Inhibitors usually appeared in young adults and usually after intensive therapy. Analysis of mutations causing hemophilia showed that certain missense mutations predispose to inhibitor formation.


Various series of patients are reviewed. Studies of patients using a single plasma-derived FVIII concentrate reported lower inhibitor incidence than those using multiple plasma-derived FVIII concentrates or a single recombinant concentrate.


The cumulative risk of inhibitor development was 16% at age 5 years and 36% at age 75 years in severe hemophilia A. The cumulative risk of inhibitor development was 6% at age 5 years and 8% at age 75 years in severe hemophilia B. In patients with severe hemophilia, without HIV infection, inhibitor development doubled mortality in the years 1977-92 but did not affect mortality rates in 1993-99.


Missense mutations in the carboxy-terminal part of the C1 domain predispose to the development of inhibitor antibodies in patients with mild to moderate hemophilia A. At least three such mutations alter T cell epitopes and may prevent T cells from becoming tolerant toward the corresponding epitope in normal factor VIII.


A purebred miniature Schnauzer with severe hemophilia A was bred to produce hemophilic descendants. Hemorrhages were treated with canine cryoprecipitate. The hemophilic dog was mated to a normal Brittany spaniel; six of her nine hemophilic descendants developed significant inhibitors after treatment with canine cryoprecipitate. The dog also was mated to his sister and only one of their 16 treated hemophilic descendants had an inhibitor, at a trace level, present only transiently. A genetic influence other than the hemophilia mutation is suggested.


The author reviews familial incidence of inhibitors and association with mutation type.


The incidence of inhibitors is related to mutation type. The highest frequency is with inversions of the factor VIII gene (40% have inhibitors), deletions of large parts of the gene (32% inhibitors) and in-frame stop codons (37% inhibitors) especially if that mutation is in one of six particular exons (60% inhibitors) versus other exons (8% inhibitors). RNA may skip an exon containing a stop codon or a splice site mutation, thus still producing some factor VIII, the presence of which permits tolerance development in fetal life.

At the time of a survey, inhibitors were present in 28/735 patients with severe haemophilia B, 1/644 with moderate haemophilia B and none of 588 with mild haemophilia B. The overall prevalence was 1.5%, or 3.8% in severe haemophilia B.


Among over 30 such patients, inhibitors and anaphylaxis developed early (median 11 exposure-days). Total gene deletions and major gene derangements were found in the majority. Hemorrhages are best managed with recombinant activated factor VII. Attempts at immune tolerance in such patients were rarely successful and sometimes complicated by the nephritic syndrome.

**Factor VIII concentrates with heightened antigenicity**


The Dutch Red Cross produced factor VIII concentrate by a controlled pore silica fractionation method. In 1990, the viral inactivation process was changed from dry heat to pasteurization. Dutch patient surveillance showed that in the previous 27 months on dry-heated concentrate, only 4 inhibitors developed, all in newly-exposed patients, that is, the most vulnerable. During 18 months of use of pasteurized concentrate, inhibitors were reported in 11 patients, all but two of whom were previously-heavily-exposed patients, that is, those believed to have low vulnerability.


The above pasteurized Dutch concentrate also was used in Belgium starting in 1990. Five of 50 patients with severe haemophilia A, all heavily transfused in the past, developed new high level inhibitors. Excellent patient surveillance allowed rapid identification of the problem. Belgium then switched totally to solvent-detergent-treated (SD) concentrate.


In 1995, Belgium replaced the SD concentrate mentioned above with a concentrate doubly viral inactivated with both SD and pasteurization. Eight of 140 previously-heavily-transfused patients with severe haemophilia A developed inhibitors shortly after changing products.


Investigating the above inhibitor outbreak, the authors found that the combination of SD plus pasteurization induced changes in factor VIII not found after other single or double viral inactivation processes.


Investigating the above inhibitor outbreak, these authors found that plasma obtained from certain blood banks already contained factor VIII breakdown products. (The plasma may not have been separated from red cells promptly.) Concentrate produced from these sources was associated with inhibitor formation.

**Inhibitors in normal persons**


Inhibitor activity against factor VIII, found in 85/500 (17%) of plasma samples from healthy blood donors, ranged from 0.4 to 2 BU. Mean levels of factor VIII activity were not different in persons with and without these antibodies.


A higher frequency of anti-idiotypic antibodies to factor VIII inhibitors was found in donors in older age groups and in multiparous women (reflecting the population who tend to acquire auto-antibodies to factor VIII). These donor groups may be good sources of such antibodies for therapeutic IV gamma globulin.


Traces of antibody to factor VIII were found in the serum of all ten normal subjects studied. The presence of anti-idiotypic antibodies also was strongly suggested.

**Characteristics of inhibitor antibodies**


The authors review studies of involved epitopes and also review type 1 and 2 kinetics.


The authors review the genesis of inhibitors and describe the involved epitopes on light and heavy chains of factor VIII and the association of inhibitors with anti-idiotypic antibodies.
Type 1 antibodies completely destroy factor VIII when antibody is present in high concentration, and may react with antigenic determinants near sites for procoagulant activity. Type II antibodies do not completely inactivate factor VIII, per haps due to steric inhibition by VWF. These antibodies react with more distant sites.

In this classic article, the authors defined “high-responding” patients as those in whom “the antibody titers increased after each antigenic stimulation or persisted for years in the absence of transfusion” whereas “low-responding” patients were those in whom antibody titers remained low, and in whom there was no significant difference in individual titers before and 8-10 days following transfusion.

A comprehensive immunological and biochemical review.

In 149 studies on 95 different adults with severe hemophilia A, who had not been transfused in the previous few days, the early-phase T1/2 of infused factor VIII, as concentrate, was defined as about 3.25 hours. The early T ½ was used as a sensitive, routine pre-operative screen for inhibitor.

Plasmapheresis
Cobcroft R,Tamagnini G, Dormandy KM. Serial plasmapheresis in a hemophiliac with antibodies to FVIII.
The authors describe the lowering of inhibitor levels with plasmapheresis alone using older equipment.

The authors describe the lowering of inhibitor levels in 10 patients on 19 occasions with plasmapheresis coupled with extracorporeal adsorption of antibodies given over a treatment period of 1-6 days. In all instances, it was possible either to eliminate the inhibitor totally or to reduce it to low levels at which remaining inhibitor was neutralized easily with concentrate infusions.

An excellent description is provided of the use of plasmapheresis with extracorporeal adsorption of antibodies in 13 patients with inhibitors to factor VIII measuring 18 to 540 Bethesda units, treated on a total of 89 occasions. At each session, lasting about 3.8 hours, about seven liters of plasma were processed with a mean reduction in inhibitor level of about 72% per session.

DDAVP
The patient had a factor VIII auto-antibody of 1.8 Bethesda units was given DDAVP on two occasions and had a seven-to nine-fold rise in plasma factor VIII (to over 50 FVIII U/dL). Plasma factor VIII levels remained in the hemostatic range for three hours after each infusion, allowing dental procedures to be carried out.

The authors present an excellent summary of the use of DDAVP in 22 patients with auto-antibodies to factor VIII. Successful hemostasis was correlated with low inhibitor levels.
Human factor VIII


Stimulated by in vivo observations, the authors incubated seven brands of factor VIII concentrate in vitro with seven different inhibitors to factor VIII. Several inhibitors caused notably less neutralization of factor VIII when it was present together with VWF (in concentrates “Humate-P” and “Koate HP”) than when it was presented as factor VIII alone, in plasma-derived or recombinant concentrates. The authors suggest that some inhibitor patients may respond better to concentrates containing factor VIII with VWF.

Porcine factor VIII


A polyethylene-glycol fractionated porcine factor VIII concentrate was first used in London in 1980. The chance of hemostasis after infusion was related to the ability to raise the plasma factor VIII to a measurable level, and that response was related to a low anti-porcine-factor VIII inhibitor level. One patient had thrombo-cytopenia. Some patients had no immune reaction (no anamnesis) after infusions and could use the concentrate repeatedly.


The median cross-reactivity in these patients was 32%. Anamnesis occurred after 9/22 (41%) of treatments. Two instances of severe thrombocytopenia were seen.


Five patients received a total of 60 infusions. Excellent graphs demonstrate that, a few days after infusion, there was zero to low increase of anti-porcine factor VIII inhibitor but some increase in anti-human factor VIII inhibitor.


In 65 patients with auto-antibodies to factor VIII, who were treated with porcine factor VIII for a total of 74 acute hemorrhages, there was an overall good to excellent response in 78%. These authors did not find a relationship between the probability of hemostasis and the initial antibody level or plasma factor VIII level attained after infusion.


A retrospective international survey showed that the median cross-reactivity in 137 patients was 15%. No rise in antibodies to porcine factor VIII after infusion was seen in 29% of recipients, an intermediate response was seen in 40% and a brisk response in 31%. Seven patients, “non-responders”, were treated on-demand at home for 1.5 to 13 years (median 6.2 years) and another 23 patients were treated regularly with porcine FVIII in the hospital for 2-7 years (median 3). These non-responder patients used porcine FVIII for 2000 bleeding episodes. The risk of transfusion reaction was dose-related. Such reactions were rare in the home setting, where dosage was low. A post-infusion fall in platelet count was common but usually transient and clinically insignificant. Occasional marked falls in platelet counts were seen, usually with intensive replacement therapy.


Porcine sequences were substituted for highly antigenic epitopes of human FVIII, to make potential therapeutic agents, i.e. recombinant hybrid FVIII molecules

Bypassing agents


This first controlled study of bypassing agents is the “gold standard” of such studies. All patients had severe hemophilia A and were treated for acute joint hemorrhages. As a non-blind control, 20 patients who did not have inhibitors were treated with a single dose of factor VIII and observed the same way as inhibitor patients; at six hours, all had symptomatic improvement and 65% had improved joint range of motion (ROM) of at least 10 degrees. None had any change in joint circumference. In the double-blind controlled group, 51 patients with inhibitors were treated for 157 joint hemorrhages with a single dose of either Konyne (a PCC) 75 factor IX U/kg, Proplex (a PCC) 75 factor IX U/kg, or an intravenous albumin placebo. Six hours after the infusion, patients judged the treatment efficacious in 29% of hemorrhages treated with albumin, 48% with Konyne, and 53% with Proplex. Range of motion was improved in 18% on placebo, 34% on Konyne and 33% on Proplex. Results with the two PCCs were significantly better than with albumin.


In a trial of similar design to that above, acute joint hemorrhages were treated with single doses either of Proplex (75
factor IX units/kg) or Autoplex (either 50 or 75 “factor eight correctional units” per kg.). Six hours after the infusion, patients judged the treatment efficacious in 50% of hemorrhages treated with Proplex, 56% with lower-dose Autoplex, 52% with higher-dose Autoplex. Differences were not significant.


A large majority of hemorrhages were into joints. Hemorrhages were treated with single doses either of Prothromblex (a PCC, 48 factor IX units/kg) or FEIBA (88 factor eight inhibitor bypassing units/kg). Subjective evaluation at 24 hours showed Prothromblex effective or partly effective in 46% and FEIBA in 66% of instances. A 30-80% improvement in range of motion was seen in 7% of hemorrhages treated with Prothromblex and in 25% of those treated with FEIBA.


Various doses of infused activated factor X and phospholipids were given to hemophilic and to normal dogs to determine the therapeutic dose (shortening of the cuticle bleeding time) and toxic dose (DIC).


Purified factor VIIa from human plasma, activated spontaneously during the purification process, was given in doses of 9-20 ug/kg (equivalent to 700-1000 U/kg). Plasma levels of factor VII were 330% to 610% 15 minutes after infusion. With the lowest dose, a second dose clearly was needed for hemostasis. As dose levels went up, a second dose was not always clearly needed.


Doses of 35 or 70 ug of rVIIa/kg were given at intervals of 2.5 hours, up to a maximum of 6 doses, for treatment of 119 joint hemorrhages in patients with hemophilia A (of whom 27/33 had an inhibitor) or B (of whom half had an inhibitor). Response was judged by assessing subjective pain and measuring decrease in size of hemorrhage at 8-14 hours after initiating treatment. In 144 hemarthroses (20 in non-inhibitor patients), treated was judged effective or excellent in 71% of those given 35 ug/kg and 71% of those given 70 ug/kg. The median number of doses given for those having effective or excellent responses was two.


NovoSeven (rVIIa), 90 ug/kg, was given every three hours for a maximum of four doses. Once the patient judged it effective, one more dose was given for “maintenance” and then treatment was stopped. In about 47-48% of the courses of treatment judged effective, a median of 2.2 doses had been given prior to the maintenance dose.


NovoSeven, 90 ug/kg, was given every 3 hours for a maximum of four doses for hemorrhages treated within an hour of onset. The authors state, “two infusions were sufficient to achieve a successful outcome in more than half of the bleeding episodes.”


Various doses of infused activated factor X and phospholipids were given to hemophilic and to normal dogs to determine the therapeutic dose (shortening of the cuticle bleeding time) and toxic dose (DIC).


Purified factor VIIa from human plasma, activated spontaneously during the purification process, was given in doses of 9-20 ug/kg (equivalent to 700-1000 U/kg). Plasma levels of factor VII were 330% to 610% 15 minutes after infusion. With the lowest dose, a second dose clearly was needed for hemostasis. As dose levels went up, a second dose was not always clearly needed.


Doses of 35 or 70 ug of rVIIa/kg were given at intervals of 2.5 hours, up to a maximum of 6 doses, for treatment of 119 joint hemorrhages in patients with hemophilia A (of whom 27/33 had an inhibitor) or B (of whom half had an inhibitor). Response was judged by assessing subjective pain and measuring decrease in size of hemorrhage at 8-14 hours after initiating treatment. In 144 hemarthroses (20 in non-inhibitor patients), treated was judged effective or excellent in 71% of those given 35 ug/kg and 71% of those given 70 ug/kg. The median number of doses given for those having effective or excellent responses was two.


NovoSeven (rVIIa), 90 ug/kg, was given every three hours for a maximum of four doses. Once the patient judged it effective, one more dose was given for “maintenance” and then treatment was stopped. In about 47-48% of the courses of treatment judged effective, a median of 2.2 doses had been given prior to the maintenance dose.


NovoSeven, 90 ug/kg, was given every 3 hours for a maximum of four doses for hemorrhages treated within an hour of onset. The authors state, “two infusions were sufficient to achieve a successful outcome in more than half of the bleeding episodes.”


NovoSeven was used in doses of 35 ug/kg or 70 ug/kg (double-blind) and given pre-operatively, intra-operatively and for 48 hours post-operatively, every 2 hours as needed. If hemostasis was inadequate, as judged by individual investigators, an open-label “escape” dose of up to 180 ug/kg could be given. After the first 48 post operative hours, NovoSeven was given at the same dose at intervals of two to six hours, as determined by the investigator, for another three days. After post-operative day 5, a dose of 90 ug/kg, open-label, was given as often as the investigator determined. The higher (70ug/kg) dose proved adequate for 8 minor and 6 major surgeries through post-operative day 4. The 35 ug/kg dose was less satisfactory. Six patients left the study, five as treatment failures who were subsequently managed on factor VIII or FEIBA, and the sixth because of thrombosis of the internal jugular during central venous catheter placement.


In an unblinded study, the authors compared the efficacy of three regimens of NovoSeven. In a study of 58 bleeding episodes, a bolus of 90 ug/kg was given, followed with continuous infusion of 15 ug/kg/hour. Satisfactory hemostasis was achieved in 70% of patients. In another study of 72 bleeding episodes, a bolus of 180 ug/kg was given, followed by continuous infusion of 30 ug/kg/hour. Hemostasis was satisfactory in 72% of instances. In a third study, of 84 bleeding episodes, a single dose of 300 ug/kg was given without subsequent continuous infusion. The dose was repeated in 23% of episodes. The large dose (given once or twice) was efficacious in 77% of instances. The authors prefer to use the large dose, without continuous infusion.
Complications of bypassing agents


Dr. Green reviews reports of anamnesis, DIC and thrombosis after bypassing agents and of thrombocytopenia and severe allergic reactions after porcine factor VIII.


In a USA national study, factor VIII inhibitor levels were measured before and after PCC treatment of 261 bleeding episodes in 75 patients with inhibitors to factor VIII. A rise in inhibitor level to twice baseline or more was seen in 13.5% of episodes, in 27 patients.


Six of 20 patients with high-responder inhibitors to factor VIII occasionally had anamnestic inhibitor responses after treatment with PCC or APCC. Factor VIII antigen, measured in vials of one PCC and the two APCCs, was 11.7, 14.1 and 117 units/vial, respectively. Factor VIII was present predominantly as the light chain. Only small amounts of von Willebrand factor were found, and only in one APCC. Thus, the factor VIII present in these concentrates is modified, degraded and dissociated from vWF.


The authors review the first nine such patients reported, of whom six were under age 30 years, and four under age 20. Pathology findings were available for five patients. Myocardial hemorrhages were seen, but no evidence of atherosclerosis or thrombosis. Some patients also had old infarcts. Myocardial infarction tended to be associated with use of high doses of concentrate over several days.


A review of 16 published instances of myocardial infarct after use of PCC or APCC is included.


A 72 year old patient was given a bolus of 102 ug/kg and then a total of 1 mg by continuous infusion, as well as tranexamic acid, prior to a dental extraction. Immediately after the extraction, he had a myocardial infarct. The patient survived so no pathologic report is available. The authors wonder whether tissue factor, exposed on atherosclerotic plaques, attracts activated factor VII and thus leads to myocardial infarct.


Typical doses of rFVlla or of FEIBA were infused into rabbits with isolated vein segments. Both drugs were associated with minor thrombus formation after 10 minutes and definite thrombus formation at 30 minutes. FEIBA was associated with a significant decrease of platelet counts and fibrinogen levels at 3 hours whereas rFVlla was not.


For the most recent 10 year period of post-marketing surveillance, 16 thrombotic adverse events were documented, an incidence of 4.05 per 105 infusions. Included were 7 instances of DIC and 5 of myocardial infarction. Known risk factors were present in 13/16 instances, including overdose in half of instances.

Induction of Immune Tolerance

Bonn Protocol and similar outpatient programs


The first experience with induction of tolerance at Bonn is described.
factor VIII in intermediate or low dose. Br J Haematol 1986; 64:291-65-68


In this excellent study, performed because of doubts expressed about the success of immune tolerance, the authors evaluated 18 patients, mostly from the Bonn center, who had completed induction of immune tolerance. Twelve patients no longer had detectable inhibitor and six had questionable or very low inhibitors (maximum 2.4 Bethesda units) behaving as low-responders. All now could be treated with factor VIII.


In early years of immune tolerance, the Bonn protocol consisted of two phases. Initially, in phase one, 100 FVIII U/kg and 50 FEIBA U/kg were given twice daily until the inhibitor level fell to less than one BU and factor VIII could be measured in the plasma after infusion. In the second phase, 150 FVIII U/kg was given daily without FEIBA until the inhibitor disappeared completely and the T½ of infused factor VIII was normal. In recent years, Bonn patients are put on the second phase dosage immediately and do not receive FEIBA.

The authors review the response of 60 patients (36 high responders and 24 low responders) in whom induction of tolerance was either completed (# 52) or stopped (# 8). Tolerance was achieved in 52 patients (86.7%). In 41 patients whose treatment was continuous, the median time until the inhibitor was less than one BU was 3 months and the median time to a normal T½ was 11.6 months. In 11 patients whose tolerance programs were interrupted, the median time until the inhibitor was less than one BU was 18.8 months and the median time to a normal T½ was 39.9 months. They did achieve complete tolerance in five patients whose inhibitor at the outset was over 100 BU. Central lines were used in five patients and all lines became infected.

Ewing NP, Sanders NL, Dietrich SL, Kasper CK. Induction of immune tolerance to factor VIII in hemophiliacs with inhibitors. JAMA 1988; 259: 65-68

In this first North American series, nine of 12 patients achieved complete tolerance in less than 11 months on a dose of 50 factor VIII units/kg/day. The three unsuccessful patients included one with a large gene deletion (diagnosed at a later date) who did not respond at all, and two who had had intensive factor VIII treatment ending two months before the tolerance regimen was started. Those two patients did respond, but slowly, over years.


These Dutch investigators gave 25 factor VIII units/kg every other day to 18 patients with hemophilia A and inhibitors under 9 BU. They stopped induction of tolerance if the inhibitor level peaked at more than 80 BU. At the time of publication, 12 patients had achieved tolerance.


The authors updated their results over 13 years of low dose induction of immune tolerance, with 25 factor VIII U/kg every other day. Tolerance was achieved in 87% of 24 patients with severe hemophilia A and inhibitors. Excellent tables and graphs are presented.


This international registry included 204 patients who underwent immune tolerance. Of these, 82.3% were high responders with historic inhibitor peaks of more than 10 BU. Higher rates of success were seen in patients with lower inhibitor levels at the outset and in patients in whom higher doses of factor VIII, over 100 U/kg/day, were used.


Successful induction of immune tolerance correlated with lower inhibitor levels at the outset. No correlation was found in the dosage range studied, which was <50 to 199 factor VIII U/kg/day (but mostly 50-100 U/kg/day).


“A longitudinal study of ITI at our center showed a significantly decreased success rate since the introduction of high purity plasma derived and recombinant FVIII products using the Bonn protocol. In inhibitor patients who showed an unsatisfactory response to treatment with FVIII concentrates with very little or no VWF the change to concentrates containing high amounts of VWF increased success rates up to 90%. These observations raise the question of whether VWF plays an important role in the induction of immune tolerance.”

Malmo Protocol, intensive inpatient treatment


In 9 of 11 patients with hemophilia A and inhibitor, the inhibitor disappeared after 2-3 weeks of an intensive inpatient regimen of treatment and tolerance appeared to be stable on follow up. One such course of treatment sufficed in seven cases and two such courses were required in two patients.
Two had inadequate responses. The protocol was defined as follows:

1. Inhibitors of more than 10 BU were reduced by plasmapheresis with extracorporeal adsorption of antibodies;
2. Sufficient factor VIII was given to neutralize remaining circulating inhibitor and raise the plasma factor VIII level to 40-100 U/dL and maintain it on subsequent days at 30-80 U/dL;
3. Cyclophosphamide was given just prior to the first dose of factor VIII, at a dose of 12-15 mg/kg intravenously on each of two days, followed by 2-3 mg/kg orally for 8-10 days.
4. Intravenous gamma globulin, 2.5 to 5 grams, immediately after the first dose of factor VIII and then, starting day 4, at doses of 0.4 grams/kg for 5 days.

After the intense course of treatment, factor VIII was continued prophylactically to maintain suppression. The authors point out that the total amount of factor VIII used was equivalent to that used in a month or less on the Bonn protocol.


In excellent detail, the authors review results of induction of tolerance with the Malmo protocol. Tolerance was achieved in ten of 16 patients with hemophilia A and inhibitors to factor VIII and in six of seven patients with hemophilia B and inhibitors to factor IX. (One of the patients with hemophilia B relapsed at six months and another course of treatment did not re-induce tolerance.) Overall, twelve patients achieved tolerance after one course of the protocol and four more achieved tolerance after two or more courses. The chance of success was best in those with low inhibitor levels at outset, histories of low inhibitor levels and a long interval since previous replacement therapy. Actual duration of treatment in successful courses ranged from 13 to 39 days. The average factor VIII use over a mean of 20 days was 162,000 units. The average factor IX use over a mean of 23 days was 219,000 units. (Note that the rate of success in inducing tolerance in hemophilia B was good, in stark contrast to outpatient regimens.)


At 6-19 days into tolerance induction in four patients with hemophilia B and inhibitors, a new non-neutralizing IgG4 antibody appeared, which complexed with infused factor IX.


The authors demonstrated the presence of a new non-neutralizing IgG4 antibody in six patients with hemophilia A who had undergone induction of tolerance. The antibody fuses with infused factor VIII but does not neutralize it or reduce its T 1/2.


The authors followed two patients with severe hemophilia A and inhibitors during induction of immune tolerance. Anti-idiotypic antibodies were elicited which neutralize the inhibiting capacity of anti-factor VIII antibodies. The authors contend that anti-factor VIII inhibitors have not disappeared after induction of tolerance, but are disarmed by anti-idiotypic antibodies.

Auto-Antibodies to Factor VIII


In this international survey, incidence was about equal in males and females. More than half of patients were over 50 years of age. Inhibitors were post-partum in 7.3%, associated with recognized auto-immune disease in 18%, and not associated with any underlying disorder in 46.1%. Others had a variety of possibly-associated conditions. Major hemorrhages occurred in 87% of patients and 22% died.


In the major referral center in London, 24 patients with auto-antibodies to factor VIII were seen over a 28 year period. Two-thirds were female. The median age was 69 years.


Post-partum inhibitors occur most common after the first pregnancy, within three months of delivery. They may disappear spontaneously, over months or years. Retrospectively, the authors compared no treatment to treatment with corticosteroids and to use of other immunosuppressive drugs with or without corticosteroids. Use of immunosuppressive drugs with or without corticosteroids resulted in remission sooner than use of corticosteroids alone. No difference was seen in the rate of remission in patients treated with corticosteroids alone versus those not given medication.


A prospective, randomized multicenter trial was conducted with 31 patients who had auto-antibodies to factor VIII. All patients were treated with prednisone, 1 mg/kg for 3 weeks, and, if inhibitors still persisted, then were randomized either to continue prednisone, or to taper off it and begin cyclophosphamide 2 mg/kg, or to continue prednisone and add cyclophosphamide. Inhibitors disappeared in 10 patients during initial prednisone therapy, in 3 of 4 randomized to continue on prednisone, in 3 of 6 continued on cyclophosphamide alone, and in 5 of 10 continued on both agents.

Nine consecutive patients, ages 50-79 years, with autoantibodies to factor VIII were given daily cyclophosphamide, 100-200 mg orally, and prednisone 50-60 mg, until inhibitors disappeared. Remission was achieved in all patients, with a median treatment time of 12 weeks, and a maximum time of 270 days. This paper contains excellent graphs.


Auto-antibodies were suppressed successfully in three patients given rituximab together with other immunosuppressive agents and in a patient with mild hemophilia who had both an auto-antibody to his own factor VIII and an allo-antibody to exogenous factor VIII. The allo-antibody persisted longer than the auto-antibody. The authors believe that addition of rituximab, a monoclonal antibody against CD 20 T cells, to their other immunosuppressive drugs either improved a sluggish response or resulted in a quicker response than would have been expected.


Two patients with auto-antibodies treated with intravenous IgG had a rapid and prolonged but not total suppression of their inhibitors. Intravenous IgG was ineffective in two hemophilia A patients with inhibitors.


A 25% or more reduction of the levels of autoimmune factor VIII inhibitors was achieved in 6 of 16 patients given high-dose intravenous IgG.

Porcine factor VIII


A polyethylene-glycol fractionated porcine factor VIII concentrate was first used in London in 1980. The chance of hemostasis after infusion was related to the ability to raise the plasma factor VIII to a measurable level, and that response was related to a low anti-porcine-factor VIII inhibitor level. One patient had thrombo-cytopenia. Some patients had no immune reaction (no anamnesis) after infusions Mannucci PM. Use of porcine factor VIII in the management of seventeen patients with factor VIII antibodies. Thromb Haemost 1984; 51:379-384.

The median cross-reactivity in these patients was 32%. Anamnesis occurred after 9/22 (41%) of treatments. Two instances of severe thrombocytopenia were seen.
Porcine factor VIII


A polyethylene-glycol fractionated porcine factor VIII concentrate was first used in London in 1980. The chance of hemo- stasis after infusion was related to the ability to raise the plasma factor VIII to a measurable level, and that response was related to a low anti-porcine-factor VIII inhibitor level. One patient had thrombo-cytopenia. Some patients had no immune reaction (no anamnesis) after infusions Mannucci PM. Use of porcine factor VIII in the management of seventeen patients with factor VIII antibodies. Thromb Haemost 1984; 51:379-384.

The median cross-reactivity in these patients was 32%. Anamnesis occurred after 9/22 (41%) of treatments. Two instances of severe thrombocytopenia were seen.