Induction of Immune Tolerance in Patients With Hemophilia A and Inhibitors Treated With Porcine VIIIC by Home Therapy

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Home therapy with porcine factor VIIIC was safe and effective when administered to five hemophilic patients over periods of 8 1/2, 6, 4, 3 1/2, and 2 years. No significant transfusion reactions occurred. Before treatment with porcine factor VIIIC, all five had high-level, high-responding anti-human VIIIC inhibitors initially lacking anti-porcine factor VIIIC activity. Although specific anti-porcine VIIIC inhibitors arose in all patients, these were generally transient, and only one patient became refractory to treatment. We believe that porcine factor VIIIC is the treatment of choice in patients whose inhibitors do not cross-react. All five patients lost their original anti-human VIIIC inhibitors after starting treatment with porcine VIIIC, permitting the reintroduction of human VIIIC in three of them. There has been no recurrence of anti-human VIIIC inhibitor activity during 2 to 3 years of regular treatment with human VIIIC in these patients. This suggests that tolerance to human VIIIC has arisen as a result of treatment with porcine VIIIC. Porcine VIIIC may have a role in the desensitization of some factor VIIIC inhibitor patients.

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PATIENTS AND METHODS

Anti-human factor VIIIC inhibitor activity was measured using the Bethesda Method4 and pooled normal plasma. Anti-porcine VIIIC inhibitor activity was measured using the Bethesda Method and a porcine concentrate (Hyate:C, Speywood Laboratories) reconstituted in hemophiliac plasma.

Factor VIII clotting activity was measured using a one-stage method in three centers17 and a two-stage method in two,18 with a pooled normal human plasma standard calibrated against national standards. Human immunodeficiency virus (HIV) antibody was detected in 1985 and 1986 by radioimmunossay, but later by enzyme-linked immunosorbent assay (ELISA) and confirmed by Western blotting. T-cell subsets were estimated by flow cytometry.

Five patients with severe hemophilia-A (VIIIC less than 0.01 U/mL) from centers in England and France were regularly treated with porcine VIIIC (Hyate:C, Speywood Laboratories) for between 2 and 8 1/2 years. These patients were selected for regular treatment with porcine VIIIC because they all had high-responding VIIIC inhibitors precluding satisfactory replacement therapy with human VIIIC, and because their factor VIII inhibitors were found to lack measurable cross-reactivity to porcine VIIIC.

Porcine VIIIC was administered under close hospital supervision for the first few weeks or months. When no significant treatment-related problems were encountered, the treatment was given on an outpatient basis, and then as self-administered home therapy. The treatment regimes used varied according to the frequency of hemarthroses and the personal preference of the clinician. The three patients with most frequent hemarthroses were treated with 20 to 60 U/kg every second day, and the two with less frequent hemarthroses with 20 U/kg on demand.

Patient 1. This 35-year-old patient developed a factor VIII inhibitor at the age of 4 years that reached a peak of 28 Bethesda Units (BU) during adolescence. He was subsequently treated with factor VIII concentrate combined with plasmapheresis, prothrombin...
complex concentrates, factor VIII in combination with anti-
fibrinolytic drugs, and immunosuppression with cyclophosphamide,
all with little success. He became disabled with severe painful
arthropathy of both knees, and arthropathy of his elbows compi-
lcated by right ulnar nerve palsy.

Treatment with porcine VIIIC began in October 1980 when his
inhibitor was found to lack cross-reactivity to porcine VIIIC. He has
been treated almost exclusively with this product since that time, and
has used no blood products of human origin since 1984. He uses 20
U/kg every second day for prophylaxis, and 40 U/kg for hemar-
throses, and his clinical response has always been excellent. Conse-
quently, he has been able to have remedial surgery, resulting in a
considerable reduction in pain, and improvement in mobility and
work pattern. No significant reactions or thrombocytopenia have
been observed. He is anti-HIV seronegative on repeated testing.

Patient 2. This 32-year-old patient has been confined to a
wheelchair by hemophilic arthropathy from the age of 8. An
anti-human VIIIC inhibitor of 18 BU was detected when he was 15
years old. He was treated irregularly with human factor VIIIC until
1977 when regular treatment with FEIBA prothrombin complex
concentrate began. This treatment was ineffective.

In October 1980 his anti-human inhibitor increased to 10 BU after
the administration of 500 U of human VIIIC for an ankle bleed.
Because no cross-reactivity with porcine VIIIC could be demon-
strated at this time, treatment on demand with porcine VIIIC began.
His clinical response to 20 U/kg has been consistently excellent and
he continued on home therapy, treating his hemarthroses exclusively
with porcine VIIIC approximately twice a month over the next 6
years. His inhibitor having disappeared, regular treatment with
human VIIIC resumed in 1986. He remains anti-HIV seronegative
on repeated testing.

Patient 3. This 22-year-old patient developed an inhibitor at age
7. Treatment with human factor VIII continued intermittently, but
was not very effective. He became disabled with severe painful
arthropathy affecting his left hip, both knees, and both ankles, and
he began to abuse dihydrocodein. He became infected with HIV
during 1984.

In March 1985 he suffered a fractured femur requiring internal
fixation, and was treated for 10 days with human VIIIC. This was
associated with a rapid anamnestic increase of his anti-human VIIIC
inhibitor to 56 BU, the patient became refractory to treatment. Porcine VIIIC was
discontinued, and regular treatment with human VIIIC restarted.
The patient became HIV-seropositive in 1983, shortly before start-
ing porcine VIIIC.

RESULTS

No significant reactions were encountered with porcine VIIIC. Mild pyrexial
reactions and a transient modest decrease in platelet count of between 30 and
40 × 10^9/L were experienced with occasional earlier batches of concen-
trate by patients 1 and 2. Thrombocytopenia was not observed.

The administration of porcine factor VIIIC did not cause an
anamnestic increase in anti-human VIIIC inhibitor activity
in any of our patients (Figs 1 through 5).

A rapid initial decline in anti-human VIIIC inhibitor activity
was observed in all patients during the first few months of therapy. A slower decrease in anti-human VIIIC

![PORCINE VIIIC](https://www.bloodjournal.org/)

Fig 1. Patient 1: inhibitor levels 1980 through 1989.
inhibitor followed, and the inhibitor was undetectable 1 to 4 years after the last treatment with human VIIIIC. Patient one showed occasional anamnestic increases in inhibitor level associated with human VIIIIC therapy until 1984. He has been treated exclusively with porcine VIIIIC since this time, and his inhibitor has been undetectable since 1987.

Anti-porcine VIIIIC inhibitor activity arose in all patients (Figs 1 through 5). This reached a maximum level of 0.5, 2, 0.6, and 1.3 BU in patients 1 through 4, respectively, and did not compromise their clinical responses. These inhibitors were transient in patients 1 and 3 but disappeared more slowly, over 2 to 3 years, in patients 3 and 4 despite continued therapy with porcine VIIIIC. Remarkably, patient 5 developed an anti-porcine VIIIIC inhibitor lacking cross-reactivity to human VIIIIC soon after starting porcine VIIIIC. This reached a maximum level of 6 BU after 2 years, causing him to become refractory to porcine VIIIIC. Because he had lost his original anti-human VIIIIC inhibitor it was possible to resume regular treatment with human VIIIIC. This did not provoke an anamnestic response, and his anti-porcine VIIIIC inhibitor gradually disappeared over the next 18 months.

Porcine factor VIIIIC recovery and half-life, estimated in four patients (1, 2, 4, and 5), reflected inhibitor activity against porcine VIIIIC. A half-life of 8 to 9 hours and
recovered of between 1.5% and 2%/U/kg was observed in all patients at the start of treatment. Recovery and half-life assessed serially in patients 4 and 5 declined to a minimum of 1%/U/kg and 4 hours in patient 4, and 0.3%/U/kg and 1 hour in patient 5. Recovery and half-life returned to normal in patient 4 when his anti-porcine VIIIC inhibitor could no longer be detected.

Regular home therapy with human VIIIIC was re-established in three patients (2, 4, and 5), 1 to 3 years after their anti-human VIIIIC inhibitor had last been detected. Regular treatment with human VIIIIC has continued for between 2 and 3 years in these patients, without any recurrence of anti-human VIIIIC inhibitor activity (Figs 2, 4, 5). Patients 4 and 5 are HIV-seropositive. Two patients (1 and 3) have not been rechallenged with human VIIIIC because they refuse treatment with human factor VIIIIC.

Two patients (1 and 2) have remained anti-HIV seronegative on repeated testing. Patients 3, 4, and 5 were infected with HIV before starting porcine VIIIIC. Their T4-helper cell counts at the beginning of therapy with porcine VIIIIC were 0.8, 1.04, and 0.85 × 10^9/L respectively, and 0.15 and 0.65, in patients 4 and 5 when treatment with human VIIIIC was resumed. Although patients 3 and 4 have now mild acquired immunodeficiency syndrome (AIDS)-related complex (ARC), this did not develop until 1988, and patient 5 is asymptomatic. All three maintain protective levels of hepatitis-B surface antibody (HBsAB).

**DISCUSSION**

Porcine factor VIIIIC was well-tolerated by five patients with anti-human factor VIIIIC inhibitors lacking measurable cross-reactivity. No significant reactions or thrombocytopenia were observed. This concurs with the results of a recent multi-center trial that reported minor reactions only with 4% of infusions of porcine VIIIIC.7 The low incidence of reactions reported in more recent series may reflect increasing product purity, specific activity having increased from less than 25 U/mg in 1981 to greater than 140 U/mg in 1989.19 We suspect that the risk of reactions with current preparations of porcine VIIIIC has been overstated.

Although our patients all developed anti-porcine VIIIIC inhibitors at some stage, these were transient in four, despite continued treatment with porcine VIIIIC. This suggests that they had become tolerant of porcine VIIIIC. The induction of immune tolerance in patients with anti-porcine VIIIIC inhibitors probably follows the same principles as the induction of immune tolerance in patients with anti-human VIIIIC inhibitors by repeated exposure to human factor VIIIIC.3,10-12

No measurable anamnestic increase in anti-human VIIIIC inhibitor activity followed the use of porcine VIIIIC during 2 to 8½ years of regular treatment, and the inhibitor disappeared in all patients. An anamnestic increase in anti-human VIIIIC inhibitor is reported to follow between 20% and 27% of infusions of porcine VIIIIC,6,8,14,11 but may be less likely to occur in the 10% to 20% of patients reported to have inhibitors with little or no affinity for porcine VIIIIC.8,9,15

The failure of three of these patients to mount an anamnestic response when rechallenged with human VIIIIC suggests that they have become tolerant of human VIIIIC. This state of immune tolerance, or specific immune unresponsiveness, has probably arisen as a result of treatment with porcine VIIIIC, since these patients all had high-responding inhibitors before starting porcine VIIIIC. Tolerance develops for specific antigenic determinants and not particular antigens. Therefore, tolerance to one antigen may be induced by administration of immunogenic doses of a second antigen sharing the epitope to which the subject had originally been sensitized. Porcine VIIIIC may have induced tolerance to human VIIIIC in this way, and may be a better tolerogen than human VIIIIC in some patients because it is less immunogenic.9,14,15 Indeed, immune tolerance may have been difficult to achieve conventionally using human VIIIIC in our patients, since tolerance is only achieved in between 30% and 50% of patients with high-responding inhibitors often after many months of treatment.3,10-13 Porcine VIIIIC may have a limited role in the induction of tolerance in patients with high-responding factor VIIIIC inhibitors lacking cross-reactivity.

Although the unit cost of porcine VIIIIC is twice that of intermediate-purity human VIIIIC and similar to that of immunoaffinity-purified VIIIIC, it is a cost-effective alternative to the high doses of human factor VIIIIC often used to treat inhibitor patients.

Inhibitor loss has been reported in occasional patients with marked abnormalities of B-cell function and AIDS. However, this is not a general observation. It appears to be a feature of full-blow AIDS or severe ARC and has not been reported in asymptomatic patients.20,21 Two of our patients have remained HIV-seronegative, and none of the three HIV-positive patients had AIDS or ARC when treatment with porcine VIIIIC began or when human factor VIII was reintroduced. Continued B-cell competence in these three patients is suggested by the maintenance of protective levels of hepatitis-B surface antibody and the development of specific anti-porcine VIIIIC inhibitors. Therefore, HIV infection is unlikely to account for the loss of inhibitor or development of immune tolerance in these patients.

A group of inhibitor patients can be identified by their lack of cross-reactivity to porcine VIIIIC in whom prolonged home therapy with this product is safe and effective and may be associated with inhibitor loss and the development of immune tolerance. Although close monitoring of the early phases of treatment is clearly desirable, we would consider porcine VIIIIC to be the treatment of choice for inhibitor patients with little or no cross-reactivity.

**REFERENCES**

5. Sjamoedien LJM, Heijnen L, Mauser-Bunschoten EP, van...


19. Manufacturer’s information: Porton Products, Wrexham, Clwyd, UK


Induction of immune tolerance in patients with hemophilia A and inhibitors treated with porcine VIIIIC by home therapy

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