Treatment of haemophilia A in Tunisia: efficacy and inhibitor study

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Haemophilia A, factor VIII, recovery, inhibitors

Summary
Cryoprecipitate is the principal type of factor VIII (FVIII) concentrate used for treating haemophilia A in Tunisia. Allergic reactions, viral transmission, and inhibitor formation remain the most serious complications of FVIII therapy. The aims of the study presented here were to evaluate the efficacy of FVIII therapy, to investigate the inhibitor prevalence, and the factors which may affect inhibitor formation in our haemophilia A patients. Plasma samples were screened for FVIII inhibitors by the Bethesda method. 30 minutes FVIII recovery was also determined for each patient. In this prospective study, 18 previously treated haemophilia A patients, four with severe (FVIII concentration <2%) and 14 with moderate haemophilia, were closely followed up during administration of 223 FVIII concentrates (cryoprecipitate and/or fresh frozen plasma). The median age of the patients involved in the study was 13.5 years (range 5 to 53). Clinical response to FVIII was consistently good to excellent. In the majority of cases, actual and predicted FVIII recovery correlated well. Adverse reactions were not observed. Five patients, aged less than 18 years and minimally treated (<36 FVIII exposure days), were found to have low titre FVIII inhibitors (<10 Bethesda units) at the end of the study. Inhibitor activity was detected in one patient with severe and in four patients with moderate haemophilia. In conclusion, FVIII therapy was effective, well tolerated, and low titre inhibitors identified did not preclude continued on demand FVIII therapy. Our study has also demonstrated that patients’ age and treatment regimen do not affect inhibitor formation. Further studies are necessary to confirm these findings.

Schlüsselwörter
Hämophilie A, Faktor VIII, Inhibitorbildung

Zusammenfassung

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Hae mouldia A is a recessive, X-linked, hereditary disease caused by a great variety of mutations within the factor VIII (FVIII) gene (1). It is the most common severe bleeding disorder, characterized by low or undetectable levels of functional blood clotting FVIII (2). The conventional preferred treatment for bleeding episodes and preventive treatment is the substitution with FVIII concentrates. Cryoprecipitate (cryo) made from locally supplied blood is the principal blood component used for treating haemophilia A in Tunisia. Allergic reactions, transmission of blood borne viruses and development of antibodies (inhibitors) against the FVIII protein remain the most serious complications of haemophilia A treatment. These inhibitors render FVIII therapy ineffective. Although the mechanisms of inhibitor formation are not fully understood, several patient-related factors may influence inhibitor development including age and genetics, severity of the haemophilia, type and frequency of bleeding episodes, type and duration of FVIII therapy (3).

The aims of the study presented here were to evaluate the efficacy of FVIII therapy, to investigate the inhibitor prevalence and the factors which may affect inhibitor formation in our haemophilia A patients.

Patients and methods

All patients with haemophilia A, admitted to the Haematology Department between June 2000 and May 2001, were included in this prospective study. The age, body mass (kg), and the patients’ baseline FVIII levels were documented from the patients’ hospital records. However, the inhibitor history
of these patients was unknown. The patients with FVIII coagulant activity (FVIII : C) assay values <2% were considered to have severe haemophilia, those with values of ≥2% up to ≤5% were considered to have moderate haemophilia, and those with values >5% were considered to have mild haemophilia (4).

The kind of FVIII replacement therapy used, the previous exposure days (number of days a given patient was exposed to any source or amount of FVIII) as well as the frequency (number of exposure days) and the consumption of FVIII (UI/kg) in the previous year prior to the study were noted.

Patients were closely followed up during the treatment or the prevention of a bleeding episode. The location and the type of haemorrhage were recorded. Transfusions of FVIII concentrates were monitored for significant changes in vital signs, other adverse reactions and clinical response to treatment. The dose of FVIII administration was noted. A blood sample was drawn from each patient before transfusion with FVIII for the measurement of pre-transfusion FVIII level and inhibitor study. Every patient was investigated for the presence of FVIII inhibitors at least twice during the study period. Blood samples were also drawn 30 minutes after transfusion in order to assess FVIII recovery determined at least once for each patient during this study.

4.5 ml of venous blood was drawn from patients or healthy blood donors directly into 0.5 ml of trisodium citrate (0.109 mol/l). Immediately after collection, plasma samples were prepared by two cycles of centrifugation at 2000g for 15 min and stored in small aliquots at -70°C until assayed.

**FVIII : C level** was measured by a one stage clotting assay based on the kaoline activated partial thromboplastin time and expressed as a percentage of FVIII : C present in pooled normal human plasma (5). Reference curves were established using a pool of normal plasma. Plasma samples were diluted with buffer (OWREN-KOLLER®). Kaoline partial thromboplastin (cephalin kaolin) was commercially available from Biomaghréb® (Tunisia). FVIII : C plasma deficient was provided from Diagnostica stago® (Asnieres, France). FVIII : C assays were performed manually in a water bath.

**FVIII recovery:** Plasmatic FVIII : C level was measured in the pre-transfusion and the 30 minutes post transfusion samples as previously described. FVIII recovery was assessed by calculating the ratio of the peak level of FVIII measured post infusion to the predicted achievable peak level of FVIII using the specific dose infused (6). The predicted FVIII level was calculated on the basis of the expectation that 1 IU of FVIII/kg body mass would raise plasma FVIII activity by 2%. A ratio of actual to predicted FVIII recovery ≥0.8 was considered to be within normal limits.

**Inhibitor study:** Inhibitor measurements were performed using the Bethesda method. The Bethesda Unit (BU) is the amount of inhibitor neutralizing 50% of FVIII activity in a pool of normal plasma (7). All determinations were assayed after 2 h incubation at 37°C of plasma or plasma dilutions with pooled normal plasma. Patients were classified as inhibitor positive if their inhibitor levels were ≥1 BU/ml on two separate occasions. Inhibitor levels were considered to be low if ≤10 BU/ml or high if >10 BU/ml (3, 8).

**Statistical analysis:** The computer program SPSS (Windows 10.0 ) was used to test the results. The χ² test, the unpaired t-test and the logistic regression test were used for analysing the parameters, p values <0.05 were considered statistically significant.

### Results

**Patients, FVIII utilization, clinical efficacy**

In May 2001, 18 patients (median age 13.5 years; range: 5-53 years) with haemophilia A were enrolled in this study. None of them had an additional disease. Nine patients have family members with haemophilia, and three patients’ brothers were also enrolled. The degree of haemophilia A was severe in four patients and moderate in 14. All patients were previously treated with cryo, one patient received both cryo and fresh frozen plasma (FFP). FFP is generally used when cryo is not available. Nine patients received less than 50 exposure days (ED) to FVIII, six patients between 50 and 100 and three patients more than 100 ED. In the year preceding the study, the median number of FVIII ED for the 18 patients was 7 (range 1 to 16) and the mean consumption of administered FVIII was 156.81 ± 105.42 IU/kg per year (range 20 to 400). One patient received packed red blood cells on at least one occasion. Patients’ selected characteristics are presented in Table 1 (inhibitor patients) and in Table 2 (non inhibitor patients).

A total of 223 FVIII concentrates (cryo and/or FFP ) were administered to treat (n = 30) or to prevent (n = 2) bleeding events in the 18 patients involved in the study. The bleeding episodes largely consisted of haemarthroses and extra-articular soft tissue haemorrhages. Two

### Tab. 1 Selected characteristics of haemophilia A patients with FVIII inhibitor formation (FVIII : C: Factor VIII coagulant activity, cryo: cryoprecipitate, FFP: fresh frozen plasma)

<table>
<thead>
<tr>
<th>case (patient initials)</th>
<th>age (years)</th>
<th>baseline FVIII : C levels (%)</th>
<th>kind of FVIII therapy</th>
<th>in the previous year prior to the study</th>
<th>most recent inhibitor titre (BU/ml)</th>
<th>most recent recovery (ratio)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>frequency of FVIII therapy (days)</td>
<td>annual consumption of FVIII (IU/kg)</td>
<td></td>
</tr>
<tr>
<td>1 (BA)</td>
<td>8</td>
<td>4.5</td>
<td>cryo</td>
<td>1</td>
<td>20</td>
<td>2</td>
</tr>
<tr>
<td>2 (BS)</td>
<td>14</td>
<td>4.5</td>
<td>cryo</td>
<td>16</td>
<td>400</td>
<td>3</td>
</tr>
<tr>
<td>3 (FM)</td>
<td>13</td>
<td>2.55</td>
<td>cryo</td>
<td>3</td>
<td>45.71</td>
<td>4</td>
</tr>
<tr>
<td>4 (FA)</td>
<td>17</td>
<td>2.55</td>
<td>cryo/FFP</td>
<td>8</td>
<td>100</td>
<td>6</td>
</tr>
<tr>
<td>5 (AJ)</td>
<td>10</td>
<td>1.25</td>
<td>cryo/FFP</td>
<td>8</td>
<td>180</td>
<td>7</td>
</tr>
</tbody>
</table>
patients received prophylactic therapy before undergoing an elective circumcision for one patient, and dental care for the second one. Follow-up observation revealed no adverse reactions during the monitored FVIII transfusions. Clinical response to FVIII was consistently good to excellent. Among the 30 bleeding episodes, 28 (93%) responded as expected to FVIII transfusions at conventional doses. The two remaining bleeding episodes (6.66%) required increased doses of FVIII to manage the bleeding which occurred in patients with low levels of inhibitors. FVIII therapy was effective in the two patients who received preventive treatment.

**FVIII recovery, inhibitors**

A total of 32 FVIII levels were measured 30 min post transfusion. 29 were performed coincident with the treatment of an acute bleeding event. One recovery (patient 5, Tab. 1) was performed coincident with a follow-up transfusion that was given as continuing therapy for a pre-existing haemorrhage. For this patient, the FVIII recovery ratio was corrected for the pre-transfusion plasma FVIII level. The two last recoveries were performed when patients (patient 5, and 11, Tab. 2) were in the non bleeding state. The ratio of FVIII recovery was less than 0.8 in four patients. Low recoveries occurred in three patients with inhibitors (patients 1, 3, 5, Tab. 1). No inhibitor was detected for the last patient with abnormally low recovery (patient 2, Tab. 2). There was no relationship between inhibitor titre and FVIII recovery ratio. For example, patient 1 (Tab. 1) had a low recovery ratio of 0.57 at an inhibitor titre of 2 BU/ml, whereas patient 4 (Tab. 1) had a normal recovery ratio of 0.84 when his inhibitor titre was 6 BU/ml.

Five (28%) of the 18 studied patients, aged less than 18 years, formed FVIII inhibitors at the end of the study. One patient was among the four patients with severe haemophilia (25%) and four were among the 14 with moderate haemophilia (31%). Among the five inhibitor patients (Tab. 1), patients 1 and 2 are brothers, as well as patients 3 and 4. The mean age was 12.4 ± 3.51 years in patients with inhibitors, and 19.15 ± 13.55 years in patients without inhibitors. The difference was statistically not significant (p = 0.295). The patients with inhibitors were in the initial period of FVIII therapy (i.e. <36 ED). The mean frequency of FVIII therapy (i.e. FVIII ED) in the year prior to the study was 7.2 ± 5.81 ED in patients with inhibitors, and 7.46 ± 3.78 ED in patients without inhibitors. The risk of inhibitor development was similar in both groups (p = 0.911). Furthermore, there was no statistically significant difference in patients with or without inhibitors, when they were compared to each other according to FVIII consumption (IU/kg per year) in the same period: 149.14 ± 153.03 versus 159.76 ± 88.90 IU/kg per year (p = 0.855).

In patients with inhibitor formation, the inhibitor titres were low (i.e. <10 BU/ml) (Tab. 1). The three inhibitor patients with inhibitor titres <5 BU/ml (patient 1, 2, 3, Tab. 1) continued to respond well to FVIII therapy at conventional doses. The remaining two patients with inhibitor titres >5 BU/ml (patient 4 and 5, Tab. 1), required increased doses of FVIII to control the bleeding episodes. Patients with inhibitors continue to receive treatment with FVIII “on demand” (as necessary). During the study period, patients received the same FVIII concentrates (cryo and/or FFP) as before. New inhibitors did not arise during this period.

**Discussion**

In this prospective study, FVIII therapy was effective in both the treatment of acute haemorrhage and for surgical prophylaxis. Indeed, the response of bleeding was appropriate in the majority of patients, and recovery studies showed plasma levels of FVIII that approached the predicted (calculated) levels. Among the abnormally low recoveries, one occurred in a non inhibitor patient. We cannot rule out the possibility of a low responder inhibitor in him, especially as he was in the initial phase of therapy, and therefore at higher risk. Recent studies have shown that the Bethesda assay lacked specificity for the low range of activity (<0.8 BU/ml) (9). Alternatively, it is possible that the peak of the plasmatic FVIII level was reached at a time later than 30 min post transfusion. Both

<table>
<thead>
<tr>
<th>case (patient initials)</th>
<th>age (years)</th>
<th>baseline FVIII : C levels (%)</th>
<th>frequency of FVIII Therapy (days)</th>
<th>prior to the study</th>
<th>most recent recovery (ratio)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 (JAB)</td>
<td>8</td>
<td>2</td>
<td>9</td>
<td>260</td>
<td>1.12</td>
</tr>
<tr>
<td>2 (JAL)</td>
<td>5</td>
<td>2</td>
<td>1</td>
<td>20</td>
<td>0.57</td>
</tr>
<tr>
<td>3 (LA)</td>
<td>8</td>
<td>1.75</td>
<td>6</td>
<td>175</td>
<td>2.25</td>
</tr>
<tr>
<td>4 (RN)</td>
<td>9</td>
<td>1.25</td>
<td>10</td>
<td>327.27</td>
<td>1.32</td>
</tr>
<tr>
<td>5 (BH)</td>
<td>9</td>
<td>4.45</td>
<td>6</td>
<td>190</td>
<td>0.89</td>
</tr>
<tr>
<td>6 (KF)</td>
<td>30</td>
<td>3.8</td>
<td>12</td>
<td>200</td>
<td>1.86</td>
</tr>
<tr>
<td>7 (AH)</td>
<td>53</td>
<td>1.75</td>
<td>5</td>
<td>89.28</td>
<td>1.26</td>
</tr>
<tr>
<td>8 (BT)</td>
<td>20</td>
<td>2.25</td>
<td>16</td>
<td>258</td>
<td>2.8</td>
</tr>
<tr>
<td>9 (MM)</td>
<td>32</td>
<td>3.8</td>
<td>6</td>
<td>79.41</td>
<td>1.16</td>
</tr>
<tr>
<td>10 (AT)</td>
<td>19</td>
<td>2.25</td>
<td>6</td>
<td>92</td>
<td>2.81</td>
</tr>
<tr>
<td>11 (SA)</td>
<td>17</td>
<td>4.5</td>
<td>8</td>
<td>160</td>
<td>1.6</td>
</tr>
<tr>
<td>12 (TA)</td>
<td>27</td>
<td>4.5</td>
<td>4</td>
<td>66.03</td>
<td>1.27</td>
</tr>
<tr>
<td>13 (LH)</td>
<td>12</td>
<td>3.7</td>
<td>8</td>
<td>160</td>
<td>0.92</td>
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</tbody>
</table>
the volume of distribution and the timing of the peak plasma level are variables that exert significant impact on in vivo recovery (10). Among inhibitor patients, there was no correlation between titre and in vivo recovery. This was in accordance with the findings reported by others (11). These authors suggest that the half life of FVIII : C may be more closely related to inhibitor titre.

FVIII therapy was well tolerated in our patients and not associated with short term adverse effects. However, two patients had a history of urticarial rashes. Allergic reactions are a well known complication of FVIII concentrates of low purity (12, 13). This kind of reaction was also described with high purity and recombinant FVIII concentrates (14, 15).

In our study, the prevalence of inhibitor development (28%) was higher than what we expected since we included all haemophiliacs. Indeed, severe and moderate clinical forms, minimally treated as well as multitransfused patients of all ages were included. The prevalence of inhibitor reported in all patients with haemophilia A ranged between 5.4 and 19.5% (16-19). Some reports demonstrated that when patients with moderate or mild haemophilia were included in the study group, there was a decrease in inhibitor prevalence (17).

The increased prevalence in our study may be associated with the small number of patients enrolled. A cooperative study between the different Tunisian centres or departments responsible for haemophilia care will allow to establish the more realistic inhibitor prevalence. Another reason for the increased prevalence in our study is probably the fact that nine patients out of the 18 received less than 50 ED to FVIII. It is well known that the majority of inhibitors develop before the first 50 ED (20). Indeed, five out of the nine patients with few ED to FVIII developed inhibitors. However, the four remaining patients still at high risk should be closely followed up. Furthermore, four patients among the five inhibitor patients have haemophilic brothers’ with inhibitor. Haemophiliacs from families with a history of inhibitor development exhibited a higher risk than patients from families without such history (21). We examined the patients’ inhibitor levels at least twice during the study period. None of the patients developed new inhibitors during follow up, perhaps because we used the same FVIII concentrate (mostly cryo).

Most investigators exclude patients with mild or moderate disease from their analysis to get a more homogeneous group, but patients with mild or moderate haemophilia with high-titre inhibitors were described (11, 22, 23). In our study, inhibitor development was detected in four out of the 14 patients with moderate haemophilia. Thus, the number of inhibitor patients with moderate haemophilia is not small.

The comparison of inhibitor frequency among previously untreated patients with haemophilia A has demonstrated that there is no differences in the incidence of high responder inhibitors (>10 BU/ml) between patients treated with recombinant FVIII and those treated with plasma derived FVIII of varying purity, whereas a lower incidence of low titre inhibitors was observed in patients treated with plasma derived FVIII (24). These data suggest that the low incidence observed may be related to less frequent inhibitor testing. Only prospective studies can detect these low titre inhibitors. An important outcome of the present study is a greater appreciation of low titre inhibitors frequency in haemophilia A patients previously treated (minimally treated <36 FVIII ED) with less purified FVIII concentrates. Indeed, all the studied patients were prospectively screened by means of inhibitor assays, regardless of clinical indication.

In our study, all the inhibitor patients were rather young (<18 years). No differences in age between patients with and without inhibitors were found. According to the literature inhibitors mostly develop before the age of 20 years, and the cumulative risk of developing an inhibitor at age of 25 years is 24% (11, 25). Likewise, analysis of our patients’ clinical data showed no differences in therapy regimen (i.e. frequency and consumption of FVIII therapy) between patients with and without inhibitors in agreement with the findings reported by others (11).

In the past, the treatment with FVIII would have been stopped in most of the cases after inhibitor detection, even in patients with low titres. At present, FVIII replacement therapy will not be stopped in many cases, because it may act as an immune tolerance regimen which may reduce the incidence of high-responders (26-27). Thus, our patients continue to receive FVIII therapy as necessary.

Conclusion

FVIII therapy was effective and well tolerated in our haemophilia A patients. We observed a relatively high prevalence of low titre inhibitors among patients previously treated with less purified FVIII concentrates (mostly cryo). Although only clinical data of a small number of patients were available, our study demonstrated that neither patients’ age nor treatment regimen affect inhibitor formation. Further studies are required for confirmation.

References


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