Review Article

Inhibitors in mild/moderate haemophilia A: An update

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Summary

The development of inhibitors in patients with mild/moderate hemophilia A is an increasingly recognized occurrence and is manifested by the patients' bleeding pattern becoming more severe. Inherited (hemophilia genetic mutations) and acquired (type and delivery of factor VIII replacement therapy) factors have been associated with an increased likelihood of developing factor VIII inhibitors. Although the use of bypassing agents (i.e. activated prothrombin complex concentrates and recombinant factor VII activated) has been demonstrated to be effective in controlling bleeding episodes in patients who develop factor VIII inhibitors, the limited data available in the literature are insufficient to determine the optimal approach to the eradication of inhibitors (i.e. immune tolerance induction, immunosuppression or both) for this group. Particular attention should be directed to the prevention of this complication in those patients with mild/moderate hemophilia recognized to be at increased risk of developing a factor VIII inhibitor. In conclusion, large prospective trials are warranted in order to elucidate the many still unclear pathogenic and therapeutic aspects of the development of inhibitors in patients with mild/moderate hemophilia A.

Keywords

Inhibitors, bleeding, mild hemophilia A

Thromb Haemost 2006, 96: 113–8

Introduction

Inhibitors against factor VIII are a major problem in the treatment of patients with hemophilia. Although such inhibitors are most frequently seen in patients with severe hemophilia (1), the development of antibodies against factor VIII in hemophiliacs with a mild/moderate defect is often associated with considerable clinical problems as the bleeding phenotype in the majority of these patients changes from mild/moderate to severe, and thus they experience spontaneous severe bleeding (2). The epidemiologic, pathogenic, clinical and therapeutic features of inhibitors in mild/moderate hemophilia A patients are distinct from those arising in patients with severe hemophilia. All these various aspects will be discussed concisely in this review.

What is the real incidence of inhibitors in hemophiliacs with a mild/moderate factor VIII defect?

Until the late 1990s, the development of inhibitors in patients with mild or moderate hemophilia A was considered uncommon. In fact, the prevalence of inhibitors in this group was estimated to be between three and 13 percent, based on the little available literature data (3–6). In a prospective study of inhibitor incidence among 1,306 US hemophilia A patients, only 6% of the patients with inhibitors had factor VIII levels higher than 0.03 IU/ml (7). In contrast, a study published in 1998 by the UK Haemophilia Centre Doctors' Organization on the incidence of new inhibitors in UK hemophiliacs over a period of 7 years (1990–1997) found that 15 (28%) of the 57 cases of new inhibitor development occurred in patients with a mild or moderate defect (8). These data suggested an annual incidence of inhibitors in the UK of 0.84 per 1,000 patients registered with mild or moderately severe hemophilia compared to 3.5 per 1,000 patients with severe hemophilia. In a more recent study conducted by Sharathkumar et al. (9), four out of 54 boys (7.4%) with mild hemophilia A developed inhibitors. It should, however, be noted that only a few large, prospective studies have been conducted so far to estimate the prevalence of factor VIII inhibitors in such patients so that the real entity of this phenomenon is still unknown. Moreover, it is possible that improvements in diagnosis and increasing availability of treatment will make this complication more frequent than previously thought.

Why do some hemophiliacs with a mild/moderate factor VIII defect develop inhibitors?

As for the development of inhibitors in severe hemophilia A, several risk factors for the development of inhibitors in mild/moder-

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Received February 9, 2006
Accepted after resubmission June 26, 2006

Prepublished online July 19, 2006 doi:10.1160/TH06–02–0078

ate hemophilia A patients have been proposed; genetic factors may play a central role, although environmental influences can also be involved (Table 1) (10).

Genetic predisposition to the development of inhibitors in patients with mild/moderate hemophilia A

Hay et al. found that hemophilic relatives of patients with mild or moderate hemophilia A and inhibitors tend to develop inhibitors more frequently than patients with the same degree of hemophilia from kindreds lacking an inhibitor history (8), suggesting that some kindreds may have had a constitutional predisposition to develop inhibitors as occurs in severe hemophilia. Similarly, other authors described affected members of hemophilia A families who developed inhibitors, thus suggesting a familial predisposition (11, 12).

The existence of a genetic predisposition to the development of inhibitors in patients with mild to moderate hemophilia A is closely linked to the type of the mutation responsible for the hemophilia (13). Although mutations causing mild/moderate hemophilia A are evenly distributed throughout the factor VIII gene, those described in association with the development of factor VIII inhibitors tend to be clustered in or around residues 482–501 in the A2 domain and 2248–2312 in the C2 domain (14), these also being common inhibitor epitopes in patients with severe hemophilia (15). As these domains are crucial for the function of factor VIII (i.e. interaction with activated factor IX or von Willebrand factor), it is clear that antibodies reacting against these regions can severely reduce factor VIII activity (13).

It has been suggested that such mutations may give rise to conformational changes in the patient's factor VIII molecule, which would become antigenically distinct from the "wild-type" molecule. Thus, infused factor VIII would be recognized as non-self and result in the production of an inhibitor that, in turn, in most cases neutralizes normal infused factor VIII and also cross-reacts with the patient's own mutant factor VIII (13, 16). Certain missense mutations seem particularly likely to predispose to inhibitor formation in patients with mild/moderate hemophilia A (17–26). In the series reported by Hay et al. (8), seven out of nine mutations described were clustered in a restricted region within 100 bases of the junction between the C1 and C2 domain. In most other reported cases of mild/moderate hemophilia, clustering in these regions was confirmed, and some particular mutations seemed to be over-represented, such as Arg2150His (8, 11, 21, 26) and Arg593Cys (8, 18 ,19, 26). Among the 26 patients with mild/moderate hemophilia A described by Hay et al. (8), nine patients carried missense mutations introducing a new Cys residue (Tyr2105Cys, Trp2229Cys and Arg593Cys), which may affect the formation of disulphide bridges leading to stable abnormal conformations. However, Knobe et al. (12) proposed an alternative mechanism of action based on the evidence that the conformation of the A2 domain of the recombinant Arg593Cys mutant behaves in a way similar to that of the "wild-type" factor VIII molecule thus making it unlikely that the Arg to Cys mutation induces abnormal disulphide bonds or impairs 3-dimensional folding. The authors suggested that the local conformational changes induced by the mutation impede appropriate intracellular contacts during the processing of factor VIII, which, however, once secreted in the bloodstream should function essentially as the "wild-type" factor VIII. These data were supported by the observations of Roelse et al. (27) who revealed intracellular accumulation of the Arg593Cys mutant in transfected cells. Van der Brink et al. (28) conducted a longitudinal analysis of factor VIII inhibitors in a previously untreated patient with mild hemophilia A due to an Arg593Cys substitution. The authors suggested that after treatment of patients with the Arg593Cys mutation using "wild-type" factor VIII, the subsequent processing of the normal factor VIII antigen peptides containing amino acid Arg593 may evoke T-helper cell activation. This may result in loss of tolerance to the patient's Arg593Cys factor VIII, which coincides with the formation of B-cell clones expressing antibodies directed towards the major inhibitor epitopes on factor VIII. Finally, the authors hypothesized a prominent role for major histocompatibility complex (MHC) class II molecules in this setting, as these molecules would continuously present Arg593-containing peptides thus supporting the proliferation of B-cell clones specific for the Arg593Cys mutation.

The molecular background of another mutation involved in inhibitor formation was recently addressed by Jacquemin et al. (16). The authors analyzed T-cell response to factor VIII in a patient with mild hemophilia A caused by the Arg2150His substitution in the C1 domain and who presented with a high-titer inhibitor toward normal but not self factor VIII. The factor VIII-specific T-cell clone recognized a peptide encompassing residue Arg2150 but did not recognize recombinant factor VIII carrying the Arg2150His substitution. Thus, while these observations demonstrate that the C1 domain of wild-type factor VIII contains T-cell epitopes that are absent in mutated factor VIII, they also support the hypothesis that Arg2150His factor VIII and normal factor VIII can be distinguished by the immune system not only at the B-cell level but also at the T-cell level.

This pathogenic mechanism has been confirmed by further studies on similar and other mutations. In fact, the analysis of factor VIII produced by patients with mild/moderate hemophilia A demonstrated that mutations at residues Arg2150, Arg2159 or Ala2201 eliminate factor VIII epitopes recognized by monoclonal inhibitors antibodies (20, 25, 29). Interestingly, in the study by Jacquemin et al. (16) the synthetic peptides encompassing Arg2150 could interact with multiple HLA class II molecules thus suggesting that a restricted number of T-cell epitopes, which promiscuously interact with multiple HLA class II molecules,

Table 1: Genetic and non-genetic factors influencing inhibitor development in mild/moderate hemophilia A patients.

Genetic risk factors
1) Missense mutations in the A2 and C2 domains of the FVIII gene
2) HLA class II polymorphisms
Non-genetic risk factors
1) Immunological factors (T-cell response)
2) Surgery and trauma (increase of pro-inflammatory cytokines)
3) Treatment-related factors
– Modality of FVIII administration (continuous infusion)
– Intensive treatment with FVIII products
– Changes of FVIII product type

are involved in the initiation of immune responses in patients with an Arg2150His mutation. This phenomenon, which could explain why mild/moderate hemophilia A patients carrying the Arg2150His mutation have an increased propensity to develop factor VIII inhibitors, may similarly occur in patients carrying other mutations and thus explain the lack of association of HLA class II alleles with inhibitor formation recently found by Bril et al. (30) in patients with mild hemophilia A with an Arg593Cys mutation.

We have recently treated (unpublished data) a patient with mild hemophilia A, due to a Tyr2105Cys mutation in exon 22 of the C1 domain, who developed a high titer factor VIII inhibitor with a clinical picture of severe hemorrhages. This mutation was first described by Naylor et al. in 1993 (31). However, based on the paucity of subsequent reports, we can assume that this mutation occurs only rarely in mild/moderate hemophilia A (8, 12, 24, 31, 32). In fact, a web-based search in the international database for hemophilia A mutations, the Hemophilia A Mutation Structure, Test and Resource Site (HAMSTeRS, http://europium.csc.mrc.ac.uk), updated in November 2005, revealed only seven cases. Interestingly, four out of the seven cases reported developed inhibitors. Through a careful literature search, we identified six further cases occurring in the same family, described by Knobe et al. (12), three of whom developed high titer inhibitors. Although the incidence of inhibitors in such patients (7/13 cases = 54%) may be over-estimated, as it is plausible that those cases with inhibitor are preferentially reported, it is nevertheless evident that the Tyr2105Cys mutation in the C1 domain of the factor VIII molecule strongly predisposes to inhibitor development. As proposed by Knobe et al. (12), this substitution could induce local conformational changes (i.e. a destabilizing cavity in the factor VIII structure) thus generating a population of wrongly folded factor VIII molecules that would be degraded inside the cells. Moreover, the secreted mutant factor VIII molecule may be less stable due to the presence of the cavity or because of an impaired interaction with von Willebrand factor.

However, the fact that not all individuals carrying such mutations develop inhibitors makes it likely that other risk factors play a role.

Other risk factors involved in the development of inhibitors in patients with mild/moderate hemophilia A

Non-genetic risk factors for developing inhibitors in mild/moderate hemophilia A include the type of clotting factor concentrate used for treatment and the way in which the concentrate is delivered (33). Inhibitor formation has been reported in patients receiving a large variety of low, intermediate, high purity or recombinant products, but none of them appeared clearly associated with a higher rate of inhibitor development (8). Baglin and Beacham (34) reported the cases of two adult patients with mild hemophilia A who developed inhibitors following a change from intermediate to high purity factor VIII concentrate. However, as the concentrate was given to both patients by continuous infusion, it was questioned whether the inhibitor development was related to the change of product or to the modality of administration. To respond to this question, Yee and Lee (35) reviewed patients in their center who had been treated with continuous infusion. From 1995 until 1999 high purity monoclonal purified plasma-derived or recombinant factor VIII concentrates were used in continuous infusion for the treatment of 50 bleeding episodes in 19 patients with non-severe hemophilia and in 26 patients with severe hemophilia. Three of the 26 severe hemophiliacs with a past history of inhibitor formation received continuous infusion, but inhibitors remained undetectable. Two of the 19 patients with non-severe hemophilia developed inhibitors: however, they not only received concentrate by continuous infusion but also experienced changes of clotting factor over a short period of time. Thus, although the authors concluded that on the basis of these results it was not possible to establish causation between continuous infusion and inhibitor development, they advised that particular caution be taken on both the type and mode of delivery of concentrate in patients with non-severe hemophilia and pointed out that desmopressin (DDAVP) should be the treatment of choice for these patients.

White et al. (36) reported the cases of two patients with mild hemophilia who were treated with recombinant factor VIII by continuous infusion and who developed inhibitors. Based on the observation that over a period of 20 years 120 mild/moderate hemophilia A patients had been treated with factor replacement and none had developed an inhibitor, the authors concluded that the increase in inhibitor development reported by the UK Haemophilia Centre Directors' Organization (8) could reflect improved data collection or recent changes in clinical practice (i.e. a switch to higher purity or recombinant products or the use of continuous infusion). Other authors have reported inhibitor development after continuous infusion of concentrate in mild/moderate hemophiliacs (9, 37, 38). In a publication from Canada (9), the overall incidence of inhibitors in 54 patients with mild hemophilia A was 7.4 percent, and when the analysis was restricted to the patients exposed to factor VIII concentrates, the incidence was 14 percent (4/29) and four out of the seven (57%) patients who received factor VIII concentrates as a continuous infusion developed inhibitors. The most recent study on this topic was published by von Auer et al. (38). The authors conducted a retrospective study to investigate the development of inhibitors after continuous infusion of factor VIII concentrates in 13 hemophilia centers in Germany and, interestingly, found that five out of ten patients who developed inhibitors had mild to moderate hemophilia. They hypothesized modification of the factor VIII molecule into a more antigenic form during ex-vivo storage by dilution or by prolonged contact with plastic infusion material or with inflamed veins as a possible mechanism by which continuous infusion of factor VIII might result in a higher incidence of inhibitor formation. However, the authors mitigated their conclusions derived from this retrospectively conducted study in which a lack of documentation could also have been possible and advised that prospective multicenter studies be carried out to investigate inhibitor development after continuous infusion in such patients.

The intensity of exposure to factor VIII concentrates as a risk factor for inhibitor development in mild/moderate hemophilia also emerged in the series reported by Hay et al. (33), in which 16 out of the 26 inhibitors described were detected after intensive replacement therapy, although no particular concentrate was implicated. Our unpublished case described above developed inhibitors after intensive treatment with an intermediate purity

product. Thus, trauma and surgery appear to be conditions causing a particularly high risk of developing inhibitors as they usually require intensive treatment with FVIII concentrates and generate immunogenic pro-inflammatory cytokines (39).

Diagnostic and clinical aspects of inhibitors in patients with mild/moderate hemophilia A

Both type I and II inhibitor reaction-kinetics have been described for inhibitors in patients with mild/moderate hemophilia (11, 40, 41), although type II kinetics seem to be more frequent. This is in contrast with findings in patients with severe hemophilia and inhibitors, in whom type I kinetics are more frequently reported (2). In the series reported by Hay and colleagues, the median inhibitor titer at presentation was 11.6 Bethesda units (BU)/ml rising to a maximum median titer of 22.5 BU/ml following further treatment (8). The development of high-titer FVIII inhibitors (i.e. > 5 BU/ml) in most cases has been confirmed by other reports (10, 36).

Inhibitors in mild/moderate hemophilia occur more commonly later in life than in severely affected hemophiliacs, and the majority of reported cases occur in the second or third decade of life or in even older patients (33). The development of inhibitors at an older age may be explained by the fact that mildly/moderately affected hemophiliacs rarely require treatment with factor VIII concentrates during their youth as they do not bleed spontaneously. Replacement therapy is usually required for those patients who do not respond to desmopressin (or in whom desmopressin is not sufficient to provide an adequate hemostatic cover) on occasions of trauma or surgery. However, rare cases of inhibitors developing in children have been reported. One child described by Puetz et al. (42) had an anamnestic response following desmopressin and thus they suggested that he developed an autoantibody that cross-reacted with "wild-type" factor VIII.

The presence of inhibitors in patients with mild or moderate hemophilia A must always be suspected if hemorrhagic events resembling those normally seen in patients with severe hemophilia occur. In such cases, assays of FVIII:C activity can show values below 1%, although sometimes, due to type II inhibitor kinetics, the values found are not lower than those historically recorded for the patient, but in-vivo recovery of activity is reduced and the half-life of the infused factor VIII is shortened. For this reason it is important to carry out a pharmacokinetic analysis at the start of replacement therapy and repeat it periodically in such patients in order to pick up possible changes in pharmacokinetic parameters. Although many of these patients develop the typical bleeding pattern of patients with severe classical hemophilia, about two-thirds of them develop a bleeding pattern similar to that observed in acquired hemophilia (8). These patients have a severe, life-threatening, bleeding tendency in which large ecchymoses, muscle bleeds, gastrointestinal and urogenital bleeding are commonly observed. Such bleeding resulted in the death of two of the 26 patients described by Hay et al. (8): one died from uncontrollable gastrointestinal hemorrhage and the other from retroperitoneal hemorrhage. The same study also provided important information regarding the natural history of the inhibitor: in fact, it disappeared in 15 of 26 cases spontaneously or following immune-tolerance induction and, in the remaining 11 cases, persisted after a median follow-up of 99 months. No links were found between the factor VIII genotype and the inhibitor titer or natural history.

Management of patients with mild/moderate hemophilia A and inhibitors

Bleeding episodes in patients with mild/moderate hemophilia and inhibitors are usually treated with activated coagulation factors, such as activated prothrombin complex concentrates (APCC) or recombinant factor VII activated (rFVIIa) (33, 43, 44), which have the advantage of avoiding anamnestic responses and moreover may permit the inhibitor to decline more rapidly than would be the case if human factor VIII were to be used (43). Some patients can be treated successfully with desmopressin, especially those whose basal factor VIII levels are not significantly lower than the historical levels or when the inhibitors are not directed to the patient's native factor VIII or once adequate circulating factor VIII levels have been restored (43). Moreover, desmopressin has been shown to minimize the risk of an anamnestic response as it releases endogenous factor VIII which is less immunogenic than exogenous factor VIII (11). In the series reported by Hay et al. (8), nine patients with adequate circulating factor VIII levels were successfully treated with desmopressin. Several other reports have described the successful use of desmopressin in the treatment of episodic minor bleeds and for minor surgical procedures in mild/moderate hemophiliacs with inhibitors (11, 19, 21, 45, 46). Robbins et al. (43) reported the effective long-term use of desmopressin prophylaxis in such patients in order to restore a mild bleeding phenotype and to avoid exposure to exogenous factor VIII.

As regards eradication of the inhibitor, published data on immune tolerance induction in patients with mild/moderate hemophilia and inhibitors are very scarce. In the series reported by Hay et al. (8), immune tolerance induction was attempted in eight patients using different regimens. The Malmö regimen (high-dose factor VIII combined with cyclophosphamide and intravenous immunoglobulin) was used successfully in two patients and with a partial response in a further two patients, the van Creveld regimen (low dose factor VIII every other day) was used unsuccessfully in one patient and with partial success in a further patient, and the Bonn regimen (high-dose factor VIII administration) was used unsuccessfully in one patient and with partial success in another patient. A possible explanation for the low success rate of immune tolerance induction in this subgroup of patients (only 25 percent, which is significantly lower than the success rate reported for severe hemophiliacs) is that the presence of endogenously synthesized factor VIII might prevent the development of tolerance.

Other reports have described the successful use of immunosuppressive therapy (47) and avoidance of re-exposure to factor VIII by using desmopressin and bypassing agents (43) to treat bleeding episodes. Finally, Wiestner et al. (48) reported a rapid response of factor VIII inhibitor to an immunosuppressive regimen including prednisone and rituximab, a monoclonal antibody against CD20-positive B cells, in a patient with mild hemophilia A.

However, the data currently available are insufficient to establish the optimal treatment of inhibitors in patients with mild/moderate hemophilia. Thus, in order to improve the knowledge not only about the eradication of inhibitors but also about the management of hemorrhagic episodes in mild/moderate hemophilia A inhibitor patients, a retrospective collection of data is currently being performed in France and Belgium by the Mild/moderate Hemophilia A Inhibitor (MHAI) Study Group (49).

Another open issue regards the prevention of inhibitor. In fact, some authors have suggested that all patients with mild hemophilia should be screened for their genetic mutation in order to identify those at particular high risk of developing inhibitors (40). Although DDAVP should be used whenever possible, if factor VIII concentrates must be used it is probably advisable that intensive courses of treatment and frequent changes of product type are avoided. Thus, in order to avoid factor exposure, DDAVP can be used in association with factor concentrates in particular situations, such as the postoperative period, when lower factor levels are required (50). Using bypassing agents rather than factor VIII concentrate might be another interesting option.

Conclusions

The development of inhibitors in patients with mild/moderate hemophilia A is a major complication because the immune response is frequently directed toward both exogenous normal factor VIII and to the patient's own mutated factor VIII, thus increasing the severity of the bleeding phenotype.

From an analysis of the literature data, it is clear that the incidence of inhibitor formation in patients carrying certain mutations, mostly located in the amino-terminal region of the A2 domain, the carboxy-terminal part of the C1 domain, and the amino-terminal part of the C2 domain, is comparable to that in patients with severe hemophilia. The mode and the intensity of factor VIII replacement also seem to play key roles in inhibitor development in such patients, although the paucity of the existing literature data prevents firm conclusions from being drawn on these associations.

Regarding the treatment, although bypassing agents (i.e. APCC and rFVIIa) have been demonstrated to be effective in the treatment of bleeding episodes, there are insufficient data to determine the optimal approach to immune tolerance induction in this group of patients, whose management remains controversial.

In conclusion, many aspects concerning inhibitors in patients with mild/moderated hemophilia remain to be clarified, including the pathogenesis and the optimal therapy. Only large, prospective studies will shed light on this far from rare complication.

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