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Review article

Immunogenicity of long-lasting recombinant factor VIII products

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ABSTRACT

Replacement therapy for patients with hemophilia A using plasma-derived or recombinant factor VIII (FVIII) is complicated by the short half-life of the FVIII products and by the occurrence of neutralizing antibodies in a substantial number of patients. In the recent years, enormous efforts have been invested to develop new generations of coagulation factors with extended half-lives. Presumably, the use of long-lasting FVIII products should reduce the frequency of administration to the patients and drastically improve their quality of life. The question of their immunogenicity remains however unanswered as yet. The present review proposes a summary of the different strategies developed to enhance the half-life of FVIII, including fusion of FVIII to the Fc fragment of the human IgG1 or to human serum albumin, or attachment of polyethylene glycol. Based on the available literature, we hypothesize on the potential benefits or risks associated with each of the latter strategies in terms of immunogenicity of the newly derived hemostatic drugs.

1. Introduction

The past decades have witnessed extraordinary improvement in the treatment of bleeding disorders. Progressing from the Stone Age, with the use of whole blood and cryoprecipitate infusion, we have evolved to a modern time where recombinant coagulation factors are administered. Congenital disorders such as hemophilia A, that display life-threatening bleeding manifestations, have benefitted utmost from the development of new generation therapeutic factor VIII (FVIII), owing to the implication and commitment of the pharmaceutical field. Simultaneously, clinical studies as well as the implication of clinicians and basic researchers have promoted new treatment regimens (prophylaxis rather than "on-demand" treatment) that reduce the risks for hemarthrosis and arthropathy episodes and have improved the quality of life of hemophilic patients.

Despite the quality of FVIII in terms of efficacy and viral safety, the short half-life of FVIII imposes frequent administrations to provide an optimal protection of the patients from bleeding episodes. This is understandably associated with a limited adherence of the patients to prophylaxis regimen, thereby resulting in a lack of complete protection and higher treatment costs. Accordingly, the most recent developments for therapeutic coagulation factors have focused on extending their half-life in the blood: recombinant factors with longer residual time in circulation would cumulate the benefice of reducing the frequency of administration, thus improving the compliance of patients, and increasing the bleed-free timespan of the patients, thus reducing the risks for minor arthropathy-prone joint bleeds. In this context, several strategies are being exploited to optimize the pharmacokinetics of therapeutic FVIII, that include coupling of the effector protein to dimeric Fc

Abbreviations: APC, antigen-presenting cells; BcR, B cell receptor; EMA, European Medicines Agency; Fc, crystallizable fragment; FcγR, receptor for IgG Fc portion; FcRn, neonatal Fc receptor; FVIIa, activated factor VII; FVIII, factor VIII; GLP-1, glucagon-like peptide 1; HA1, hemagglutinin; HSA, human serum albumin; IgG, immunoglobulin G; IgM, immunoglobulin M; LRP-1, lipoprotein receptor protein 1; MHC, major histocompatibility complex; PEG, polyethylene glycol; PTPs, previously treated patients; PUPs, previously untreated patients; Tregs, regulatory T cells; VWF, von Willebrand factor.

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fragments of human immunoglobulin G, to polyethylene glycol (PEG) or to human serum albumin (HSA).

The treatment of patients with hemophilia A using exogenous FVIII is complicated by the immunogenicity of the infused FVIII. Indeed, up to 30% of patients with severe hemophilia A, and up to 5% of patients with mild/moderate forms of the disease, develop anti-FVIII IgG antibodies following replacement therapy. The induced anti-FVIII IgG presumably affect the pharmacokinetics of the exogenously administered coagulation factors but, more critically, inhibit their pro-coagulant activity. The development of inhibitory anti-FVIII IgG, or 'FVIII inhibitors', represents a major clinical burden as well as a major societal concern owing to the additional costs that are associated with inhibitor management. To our knowledge, there is no available study that clearly establishes a correlation between the residual time of a molecule and its immunogenicity in humans. It is thus hazardous to predict the immunogenicity of long-acting FVIII products. Besides, FVIII exhibits a degree of immunogenicity that is unexpected given the fact that FVIII has no known pro-inflammatory role. In this review, we summarize the rationale for the different strategies developed to enhance the half-life of FVIII. Based on the available evidence, we further anticipate the consequences and limitations of coupling FVIII to 'half-life enhancers' for its immunogenicity in hemophilia A patients.

2. Coupling therapeutic FVIII to human Fc fragments

The interaction of the Fc domain of immunoglobulins with the neonatal Fc receptor (FcRn) has been known for years as a physiological mechanism that protects IgGs from catabolism and confers them a long half-life in the blood [1]. Therefore, IgGs of the IgG1, IgG2 and IgG4 sub-classes are known to circulate in the body with a half-life of 3 weeks. The FcRn is expressed at many sites and by different cell types in mammals, where it mediates IgG transcytosis and IgG recycling in a pH-dependent manner [1]. In particular, the FcRn expressed in the vascular endothelium participates in protection of IgG from degradation. Endothelial cells widely express FcRn and are able to internalize blood-borne molecules by pinocytosis. Under acidic pH in the early endosomes, the engulfed IgG binds the FcRn through their C_H2 domain. Upon further acidification of the endosomes, IgG–FcRn complexes are redirected from the lysosomal degradation compartment to the cell surface. At neutral pH, the bound IgG dissociates from FcRn and is released in the circulation. This property has been exploited to increase the residence time of coagulation factors *in vivo*. Besides, owing to the conformation of the Fc domain, Fc-fused proteins are generally endowed with improved biophysical characteristics in terms of stability and solubility.

In particular, a new generation of recombinant FVIII, referred to as Eloctate™ in North America, has been developed by fusing the dimeric C_H2–C_H3 domains of the human IgG1 Fc to the recombinant B domain-deleted FVIII. The post-translational modifications on the FVIII moiety are similar to that found on the commercially available B domain-deleted FVIII product, and the dimeric Fc carries two additional asparagine-glycosylated moieties. The addition of the Fc domain substantially increases the plasma half-life of FVIII and thus reduces the dosing frequency necessary to achieve prophylaxis in the patients. Thus, studies in pre-clinical models of hemophilia A have shown that fusion of the Fc to B domain-deleted FVIII confers a 2-fold increased half-life over recombinant FVIII, while preserving the specific activity of FVIII [2]. Indeed, the functionality of the Fc-fused FVIII in correcting whole blood clotting time has been demonstrated in FVIII-deficient mice, a model of severe hemophilia A, as well as in hemophilic dogs. The Fc-fused FVIII demonstrates a 1.5-fold extended half-life in human clinical trials as well as reduced annualized bleeding rates as compared to its non-fused counterpart [3–5].

From an immunogenicity standpoint, available data from clinical trials suggest that FVIII-Fc is a safe molecule since no inhibitor development has been reported under prophylaxis regimen [5,6]. It is important to note however that clinical trials have been performed only in previously treated patients (PTPs) with severe hemophilia A, who are at a lower risk of developing an alloimmune response to exogenous FVIII as compared to previously untreated patients (PUPs) [7]. Ongoing clinical trials performed in PUPs in Europe, which are required by the European Medicines Agency (EMA) prior to release of the drug on the European market, shall bring additional evidence in the coming years.

Presentation of Fc-fused FVIII as a magic drug devoid of immunogenicity needs to be confronted to the facts that therapeutic human monoclonal antibodies do induce neutralizing antibodies in a substantial number of patients [8] and the fused Fc domain has been used for enhancing the adjuvant properties of vaccine antigens [9,10]. Because the Fc portion of FVIII not only interacts with FcRn, but also with a number of receptors for the Fc portion of IgG (FcγR), as well as receptors for Fc-linked glycans [11], there are little chances that the FVIII-Fc fusion molecule will be immunologically inert. Yet, recent work by Krishnamoorthy et al. shows that the immune response to FVIII-Fc in FVIII-deficient mice, is less frequent, or less intense, than in the case of FVIII lacking the Fc fragment, when FVIII is injected at 50 IU/kg [12]. However, at higher concentrations, all FVIII products show the same degree of immunogenicity. The rest of this chapter is an attempt to anticipate the interactions of FVIII-Fc with cells of the innate and adaptive immune system, and potential immunological consequences of infusing Fc-fused FVIII in patients.

2.1. Fc-mediated immune interactions

Navarrete et al. have previously observed that the marginal zone in the spleen is the primary site of FVIII accumulation in hemophilic mice [13]. Recently published data comparing the half-life and biodistribution of recombinant FVIII and FVIII-Fc intravenously administered to FVIII-deficient mice suggest that the accumulation of both molecules in the spleen does not show drastic differences [14]. The data also show that, as is the case for exogenous recombinant FVIII, the half-life and biodistribution of FVIII-Fc is largely dependent upon its interaction with endogenous von Willebrand factor (VWF). Interestingly, in view of the plethora of Fc receptors and Fc-binding cellular proteins expressed on immune cells, one may anticipate that FVIII-Fc is more prone to interact with immune cells than its native FVIII counterpart. In particular, one might foresee a synergistic effect of Fc-dependent and FVIII-dependent interactions with different types of endocytic receptors expressed by the same cells, although any repercussion in terms of immune activation or immune tolerance induction remains speculative.

Dendritic cells, monocytes, macrophages and neutrophils are the primary immune cells participating in the initiation of immune responses. Provided the constitutive expression of various Fcγ receptors on these immune cells [15,16], it is anticipated that FVIII-Fc will interact with the latter cell types via its Fc domain, in addition to accumulating at the site of vascular injury. In this context, we may expect a differential assessment of FVIII and FVIII-Fc by the immune system, although predicting whether this will lead to increased or reduced immunogenicity is impossible. Dendritic cells are known to simultaneously express both the activating and inhibitory FcγR. Presumably, FVIII-Fc-mediated signaling through the inhibitory FcγRIIB on dendritic cells should favor peripheral tolerance (Fig. 1B), either by inhibiting dendritic cell activation or by limiting the processing of the exogenously

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