Blood Cells, Molecules and Diseases 75 (2019) 11–12

Contents lists available at ScienceDirect

Blood Cells, Molecules and Diseases

journal homepage: www.elsevier.com/locate/bcmd

Letter to the Editor

Anti-factor VIII inhibitors against A2 and C2 domains in hemophilia A patients from India

ARTICLE INFO

Editor: Mohandas Narla

To the Editor,

Development of inhibitors is perhaps the most serious adverse effect of factor replacement therapy in hemophilia A patients. These inhibitors are observed in 5–40% of hemophilia patients, though the incidence in India has been observed to be low i.e. 8.2%; presumably due to the reduced frequency of transfusion [1]. There are several mechanisms by which the inhibitors inactivate factor VIII (FVIII) which include interference with the activation of FVIII or its binding with von Willebrand factor (VWF), phospholipids or assembly of the intrinsic FXase complex. Some reports also suggest that FVIII inhibitors act like enzymes having catalytic activity [2]. Inhibitors are mainly directed to A2 and C2 domains of FVIII, but they have also been reported against other domains and often to multiple domains in a single patient (Fig. 1). Considering the heterogeneity of these inhibitors, there is a need to understand the pathophysiology of the inhibitory mechanisms for more targeted therapeutic interventions.

In the present report, we analysed 70 plasma samples from 57 FVIII inhibitor positive patients (55 congenital hemophilia A and 2 acquired hemophilia A) referred to the Comprehensive Hemophilia Care Centre in Mumbai. FVIII epitope specificity of the all samples were analysed by ELISA using overlapping synthetic peptides spanning the A2 and C2 domains of FVIII as described by Gharagozlou et al. [3].

Before screening for epitope specificities, inhibitor status was confirmed both by Nijmegen modified Bethesda assay and ELISA. The inhibitor levels in these 70 plasma samples from 57 patients ranged from 2 to 256 BU/mL. All the plasma samples were found to be positive for IgG antibodies by ELISA.

For epitope specific peptide ELISA, 20 amino acid long peptides (with the exception of the carboxyl terminal peptide, which included only 13 residues) overlapped by 10 residues were used as previously described [3]. The Nunc™ CovaLink™ (Denmark) plates were coated with 50 µL of a solution of individual overlapping synthetic peptides (10 µg/mL in Phosphate Buffered Saline). Plates were blocked using 3% gelatine. Diluted plasma samples were then added, followed by HRP-goat anti-human IgG conjugate. The substrate Tetramethylbenzidine (TMB) was subsequently added and the absorbance was measured at 450 nm.

Seven out of 57 patients (12.2%) were found to be positive for antibodies against A2 domain, 10 (17.54%) against C2 domain and 18 (31.57%) against both A2 and C2 domains and 27 out of 57 patients (47.36%) were negative for both the domains. In three patients there was a change in domain specificity with time.

The FVIII molecule has 6 domains (A1-A2-B-A3-C1-C2) with a heavy chain comprising of A1 and A2 domains along with a B domain, which is insignificant for the procoagulant function of FVIII and a light chain consisting of A3, C1, and C2 domains. The A2 domain and a2 acidic region cover residues 373–710 and 711–740 respectively. These regions i.e. A2, a2 along with A1, a1 comprise the heavy chain. The C2 domain

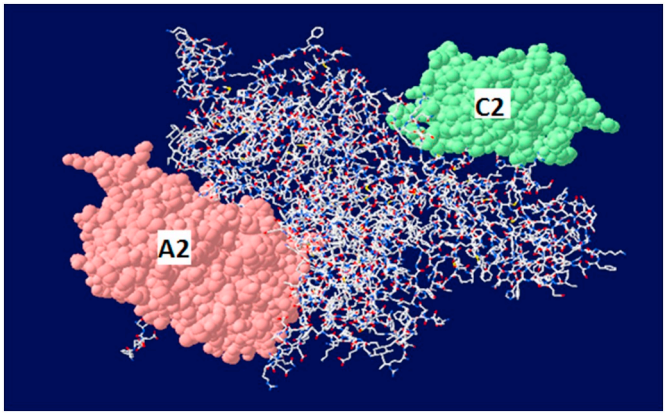

Fig. 1. Crystal structure of B domain-deleted factor VIII (PDB 2R7E) highlighting the A2 and C2 domains using Swiss-Pdb viewer 4.1.0.

https://doi.org/10.1016/j.bcmd.2018.11.004
Received 11 October 2018; Received in revised form 13 November 2018; Accepted 13 November 2018
Available online 16 November 2018

covers residues 2173–2332 and comprises the light chain [4]. Inhibitors can arise against any of these domains and affect specific functional attributes associated with the domain. Three patients showed variation in domain specificities when tested at different times. These findings were similar to the study conducted by Fulcher et al. [5], wherein few samples showed epitope spreading during treatment. Whether switching of domains is related to the type of treatment or specific immunological context is not clear, though similar switchover phenomenon has been observed in patients with different subclasses of IgG antibodies [6].The unique observation in the current study is that unlike other studies, inhibitors are found to react with the entire domain rather than specific peptides. Though in other autoimmune disorders, there is a difference in clinical manifestations of those cases with and without epitope spreading [7], no such differences in the clinical severity was observed in these cases. Most of our patients are treated with multiple products ranging from whole blood, fresh frozen plasma (FFP), cryoprecipitate, plasma derived or recombinant factor products. Whether the heterogeneity in the treatment products leads to such broad range specificity is not clear, though wide differences are observed in inhibitor titres [8] with different treatment products.

Thus in conclusion, majority of our patients showed specificities against A2 and/or C2 domains and all of them were specific to a functional domain rather than a specific epitope. The ELISA assay using overlapping peptides is a reliable technique for epitope mapping of FVIII inhibitors.

Acknowledgements

This work was supported by the Indian Council of Medical Research (ICMR) [Grant number 56/6/2013 HAE-BMS], Delhi, India.

Conflict of interest

None declared.

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