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Characterization of Anti-Emicizumab Antibodies Using Repository Samples Obtained in Clinical Studies of Emicizumab Conducted in Japan

Naoki Matsumoto[1] Hiroto Abe[1] Ryohei Kawasaki[1] Yoshihito Tashiro[1] Mariko Noguchi-Sasaki[1] Suguru Harada[1] Koichiro Yoneyama[2] Tomomi Niino[2] Tetsuhiro Soeda[1] Yasushi Yoshimura[1]

[1] Chugai Pharmaceutical Co., Ltd., Yokohama, Kanagawa, Japan
[2] Chugai Pharmaceutical Co., Ltd., Chuo-ku, Tokyo, Japan

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Address for correspondence Tetsuhiro Soeda, PhD, Medical Affairs Division, Product Research Department, Chugai Pharmaceutical Co., Ltd., 216 Totsuka, Yokohama, Kanagawa 244-8602, Japan (e-mail: soedatth@chugai-pharm.co.jp).

Hemophilia A (HA) is a rare bleeding disorder caused by a lack of functional coagulation factor VIII (FVIII). Treatments for patients with HA (PwHA) can be achieved by administration of the missing functional FVIII. Other coagulation factors called bypassing agents are also used for PwHA with inhibitors. Emicizumab is a humanized bispecific monoclonal antibody which mimics the cofactor function of FVIIIa by binding to activated factor IX (FIXa) and factor X (FX), and it is used for routine prophylaxis in PwHA with or without inhibitors to prevent bleeding.[1,2] Multiple clinical studies have confirmed the efficacy of emicizumab,[3–7] although treatment with monoclonal antibodies may result in the development of antidrug antibodies (ADAs).[8] A recent report revealed that in seven phase 3 trials of emicizumab, 34 of 668 PwHA developed ADAs against emicizumab, and 4 of them developed neutralizing ADAs with decreased emicizumab exposure.[9] Decreased emicizumab efficacy due to ADAs has also been reported.[10–14] In these cases, decreased efficacy was caused by neutralizing and/or clearing abilities of the ADAs. However, the characteristics of ADAs have not been well understood. In this research, we aimed to evaluate the characteristics of ADAs by analyzing neutralizing activity and epitopes using repository samples from healthy volunteers (HVs) and PwHA who tested positive for ADAs in clinical studies of emicizumab conducted in Japan.

To assess the neutralizing activity of ADAs in repository samples, we established a neutralizing activity assay for ADAs against emicizumab. The neutralizing activity of ADAs was quantified as the inhibited fraction of spiked emicizumab concentration in a modified one-stage clotting assay in vitro. Animal-derived ADAs neutralized the spiked emicizumab in a concentration-dependent manner, and differences in sensitivity were observed in 50, 5, and 0.5 µg/mL emicizumab-spiked conditions (►**Fig. 1A**). These results thus indicated that this neutralization assay could detect a broad range of neutralizing activity by ADAs using a certain amount of spiked emicizumab.

We then analyzed the ADA-positive repository samples collected from 6 HVs (HVs A–F) and 4 PwHA (PwHA G–J) from 3 clinical studies: 2 HVs from a phase 1 study,[15] 4 HVs from a bioavailability study,[16] and 4 PwHA from a phase 1/2 study[17] (►**Table 1**). Informed consent about storing and using the repository samples had been obtained in the clinical studies, and this research was approved by an internal ethics committee. In the clinical studies, four HVs showed shorter elimination half-lives of emicizumab (C: 10.1 days, D: 14.1 days, E: 6.93 days, F: 9.27 days) than those for the other two HVs (A: 31.1 days, B: 30.0 days), indicating that ADAs of the former four HVs affected emicizumab pharmacokinetics (PK) (►**Table 1**). No PwHA showed decreased emicizumab exposure, indicating that their ADAs did not affect PK (►**Table 1**).

To measure the neutralizing activity of ADAs, FVIII activity in plasmas from HVs was neutralized by adding two anti-FVIII monoclonal antibodies to mimic the FVIII-deficient condition in vitro.[18] In ►**Fig. 1B**, we show the results of neutralizing activity in the most sensitive 0.5 µg/mL emicizumab-spiked condition. Neutralizing activity was detected in HVs D, E, and F and PwHA J; among them, PK was affected in three cases (HVs D, E, and F) but not in PwHA J. On the other hand, neutralizing activity was not detectable in HVs A, B, and C and PwHA G, H, and I; among them, PK was affected in HV C, but not in the others (HVs A and B and PwHA G, H, and I). The lack of detectable neutralizing activity in these six cases might be accounted for by low titers of ADAs, the interference of the

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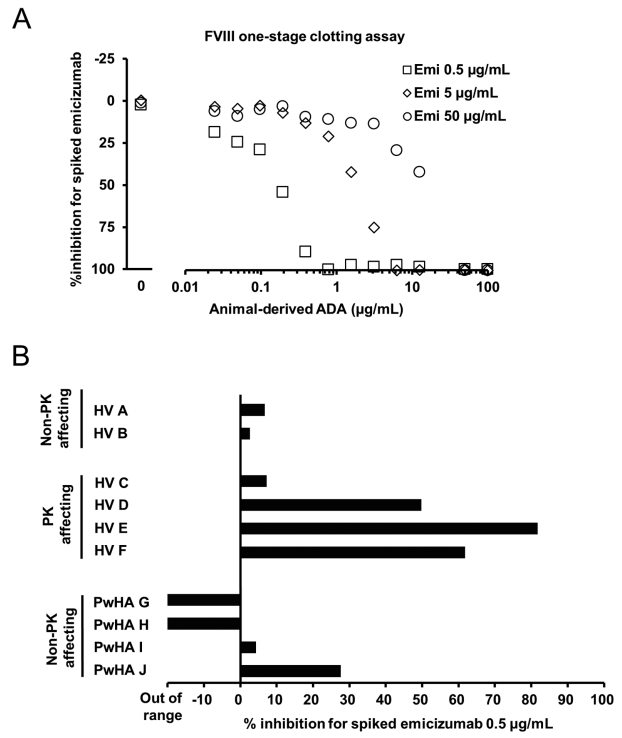

Fig. 1 Measurement of neutralizing activity of animal-derived ADAs against emicizumab and ADAs from clinical repository samples. (**A**) The neutralizing activity of the animal-derived monoclonal ADAs against emicizumab was measured using FVIII-deficient control plasma spiked with 50, 5, and 0.5 μg/mL emicizumab in one-stage clotting assay. (**B**) The neutralizing activity of ADAs in plasmas from 6 HVs or 4 PwHA was measured using FVIII-deficient control plasma spiked with 0.5 μg/mL emicizumab in a one-stage clotting assay. The neutralizing activity of the representative sample (shown as the sampling day below) from HVs or PwHA is shown; HV A (day 1), HV B (day 113), HV C (day 112), HV D (day 113), HV E (day 110), HV F (day 113), PwHA G (day 589), PwHA H (day 505), PwHA I (day 197), PwHA J (day 1). Among the samples in which plasma emicizumab concentration is below the limit of quantification, the sample collected at the latest day from the beginning of emicizumab treatment is shown as the representative. Emicizumab concentration of HV A, B, C, D, E, F, and PwHA J is below the limit of quantification, and plasma emicizumab concentration of PwHA G, H, and I is 14.0, 16.9, 0.0853 μg/mL, respectively.

emicizumab remaining in the samples, and the possibility that their ADAs were really not neutralizing.

We further characterized the ADAs by performing epitope analysis with a previously reported electrochemiluminescence (ECL) immunoassay.[12] Predominant epitopes of each ADA on the emicizumab molecule were identified when the ECL signal reduction ratio was above the confirmatory assay cut point. For HV A and PwHA I, epitope analysis was not performed since their ADA titers were too low. In the other eight cases, various epitopes recognized by ADAs were detected, but none were in the fragment crystallizable (Fc) region (►**Table 1**). Epitopes of ADAs in HVs D, E, and F, whose ADAs were PK-affecting and neutralizing, were commonly complement-determining region (CDR) 1 and 3 of the common light chain (cLC). Epitopes of ADAs in PwHA H were also CDR1 and three of cLC, although ADAs of this case were not PK-affecting nor neutralizing. ADAs of HV B and PwHA G were also not PK-affecting nor

Table 1 Summary of ADA characteristics in ADA-positive subjects from three clinical studies

Subjects	Clinical study	ADA response	Detectable neutralizing activity	$t_{1/2}$ (d)	PK affecting	Epitope recognition (predominant)		
						FIXa arm	FX arm	Fc
HV A	Bioavailability	Treatment-unaffected	No	31.1	No	Not analyzed		
HV B	Phase 1	Treatment-unaffected	No	30.0	No	Fab region	–	–
HV C	Bioavailability	Treatment-induced	No	10.1	Yes	Fab region	Fab region	–
HV D	Bioavailability	Treatment-induced	Yes	14.1	Yes	CDR1 and 3 of light chain	CDR1 and 3 of light chain	–
HV E	Bioavailability	Treatment-induced	Yes	6.93	Yes	CDR1 and 3 of light chain	CDR1 and 3 of light chain	–
HV F	Phase 1	Treatment-induced	Yes	9.27	Yes	CDR1 and 3 of light chain	CDR1 and 3 of light chain	–
PwHA G	Phase 1/2	Treatment-induced	No	N/A	No	Fab region	Fab region	–
PwHA H	Phase 1/2	Treatment-induced	No	N/A	No	CDR1 and 3 of light chain	CDR1 and 3 of light chain	–
PwHA I	Phase 1/2	Treatment-induced	No	N/A	No	Not analyzed		
PwHA J	Phase 1/2	Treatment-boosted	Yes	N/A	No	Fab region	Fab region	–

Abbreviations: ADAs, antidrug antibodies; CDR, complement-determining region; Fab, fragment antigen-binding; Fc, fragment crystallizable; FIXa, activated factor IX; FX, factor X; HV, healthy volunteer; PK, pharmacokinetics; PwHA, patients with hemophilia A; $t_{1/2}$, elimination half-life; N/A, not available.
Note: ADA response was determined based on the harmonized common terminology for immunogenicity.[8]

neutralizing, yet they had different epitopes; fragment antigen-binding (Fab) region of the FIXa arm only in HV B and Fab regions of the FIXa and FX arms in PwHA G. In HV C, ADA epitopes were Fab regions of the FIXa and FX arms, although the ADAs of this case were PK-affecting but not neutralizing. On the other hand, ADAs in PwHA J also recognized the Fab regions of the FIXa and FX arms, yet the ADAs were not PK-affecting but neutralizing. Overall, neutralizing activity of ADAs was detected in HVs D, E, and F and PwHA J. ADAs of HVs D, E, and F were PK-affecting and their epitopes were commonly cLC.

So far, several reports about emicizumab ADAs have been published. Neutralizing ADAs that affect PK have been reported in several clinical cases.[10,12,19] There have been reports of cases of ADAs with undetectable neutralizing activity that do not affect PK,[9] cases of neutralizing ADAs that do not decrease emicizumab exposure,[9] and one case of ADAs that affect PK but lack detectable neutralizing activity.[13] Although our results were derived from a limited number of subjects, we confirmed all these reported patterns of characteristics in ADAs against emicizumab.

In conclusion, we characterized ADAs against emicizumab and elucidated various patterns of ADAs using repository samples obtained from six HVs and four PwHA. We hope that our results will promote the clearer understanding of ADAs and their characteristics.

Author Contribution

N.M. and H.A. analyzed the data and wrote the manuscript; N.M., H.A., R.K., M.N.-S., S.H., K.Y., T.N., T.S., and Y.Y. contributed to/designed the research; N.M., H.A., and Y.T. performed the research; K.Y., T.N., and T.S. participated in writing the manuscript. All authors reviewed and approved the final version submitted.

Conflict of Interest

All authors are current employees at Chugai Pharmaceutical Co., Ltd. N.M., H.A., R.K., Y.T., S.H., and Y.Y. hold stocks of Chugai Pharmaceutical Co., Ltd. K.Y. and T.S. are inventors of patents related to Chugai Pharmaceutical Co., Ltd.

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