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


Affiliations below.

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Abstract:
Emicizumab, a factor (F) VIII function-mimetic bispecific antibody, is used for the treatment with patients with hemophilia A (PwHA). Although the immunogenicity of emicizumab is low, potential of immunogenicity is still remained. Despite some cases of anti-drug antibodies (ADAs) reported, the characteristics of ADAs have not been fully elucidated. In this research, we evaluated the characteristics of ADAs by using repository samples collected in phase 1, phase 1/2 and bioavailability studies conducted in Japan. Ten plasma/serum samples from 6 healthy volunteers (HVs) and 4 PwHA who tested positive for ADAs in the clinical studies were used for the assessment of neutralizing activity, epitope analysis and pharmacokinetics (PK). Neutralizing activity of ADAs was observed in 3 HVs and 1 PwHA. Among these, 3 HVs developed ADAs which bound to the complement-determining region (CDR)1, 3 of the common light chain (cLC) of emicizumab and associated with shorter half-life. Epitopes of ADAs in 1 PwHA were on the Fab-regions of emicizumab, and the ADAs were not associated with decreased exposure in this PwHA. Neutralizing activity was undetectable in 3 HVs and 3 PwHA. Among these, ADAs in 2 HVs and 2 PwHA recognized the Fab-regions or the CDR 1, 3 of the cLC, and 1 of these 2 HVs showed shorter half-life of emicizumab. In conclusion, our analysis of ADAs demonstrated the various characteristics of ADAs, such as ADAs with either neutralizing activity or affected pharmacokinetics, or both properties.

Corresponding Author:
Tetsuhiro Soeda, Chugai Pharmaceutical Co., Ltd. Chugai Life Science Park Yokohama, Product Research Department, Yokohama, Japan, soedatth@chugai-pharm.co.jp

Affiliations:
Naoki Matumoto, Chugai Pharmaceutical Co., Ltd. Chugai Life Science Park Yokohama, Yokohama, Japan
Hiroto Abe, Chugai Pharmaceutical Co., Ltd. Chugai Life Science Park Yokohama, Yokohama, Japan
Ryohei Kawasaki, Chugai Pharmaceutical Co., Ltd. Chugai Life Science Park Yokohama, Yokohama, Japan
[Yasushi Yoshimura, Chugai Pharmaceutical Co., Ltd. Chugai Life Science Park Yokohama, Yokohama, Japan [..]]
Characterization of Anti-Emicizumab Antibodies Using Repository Samples Obtained in Clinical Studies of Emicizumab Conducted in Japan

Naoki Matsumotoi, Hiroto Ahe, Ryohi Kawasakii, Yoshimoto Tashiro, Mariko Noguchi-Sasaki, Suguru Harada, Koichiro Yoneyama, Tomomi Niino, Tetsuhiro Soeda, Yasushi Yoshimura

1Chugai Pharmaceutical Co., Ltd., 216 Totsuka, Yokohama, Kanagawa 244-8602, Japan

2Chugai Pharmaceutical Co., Ltd., 2-1-1 Nihonbashi-Muromachi, Chuo-ku, Tokyo 103-8324, Japan

Corresponding Author: Tetsuhiro Soeda, Product Research Department, Medical Affairs Division, Chugai Pharmaceutical Co., Ltd., Yokohama, Kanagawa 244-8602, Japan (e-mail: soeda.t@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 human 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. Clinical studies have confirmed the efficacy of emicizumab, although treatment with monoclonal antibodies may result in the development of anti-drug antibodies (ADAs). A recent report revealed that, in 7 phase 3 trials of emicizumab, 34 of 668 PwHA developed ADAs against emicizumab, and 4 of them developed neutralizing ADAs with decreased emicizumab exposure. Decreased emicizumab efficacy due to ADAs has also been reported. 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 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 (Figure 1A). These results thus indicated that this neutralization assay could detect a broad range of neutralizing activity by ADAs.
certain amount of spiked emicizumab.

We then analyzed the ADA-positive repository samples collected from 6 HVs (HV A-F) and 4 PwHA (PwHA G-J) from 3 clinical studies. HVs from a bioavailability study, 4 HVs from a phase 1 study, 154 HVs from a phase 1 study (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 ethics committee. In the clinical studies, 4 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 2 HVs (A: 31.1 days, B: 30.0 days), indicating that ADAs of the former 4 HVs affected emicizumab pharmacokinetic (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 2 anti-FVIII monoclonal antibodies to mimic the FVIII-deficient condition in vitro. In Figure 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, F, and PwHA J; among them, PK was affected in 3 cases (HV 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 (HV A and B and PwHA G, H, and I). The lack of detectable neutralizing activity in these 6 cases
might be accounted for by low titers of ADAs, the interference of the 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) assay. 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 8 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 3 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 neutralizing, yet they had different 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.\textsuperscript{12, 19} There have been reports of cases of ADAs with undetectable neutralizing activity, cases of neutralizing ADAs that do not decrease emicizumab exposure, one case of ADAs that affect PK but lack detectable neutralizing activity.\textsuperscript{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 6 HVs and 4 PwHA. We hope that our results will promote the clearer understanding of ADAs and their characteristics.

**Conflict of Interest**

All authors are current employees at Chugai Pharmaceutical Co., Ltd. NM, HA, RK, YT, SH, and YY hold stocks of Chugai Pharmaceutical Co., Ltd. KY and TS are inventors of patents related to Chugai Pharmaceutical Co., Ltd.

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Figure 1 Measurement of neutralizing activity of animal-derived emicizumab and ADAs from clinical repository samples.

(A) The neutralizing activity of the animal-derived emicizumab was measured using FVIII-deficient control plasma spiked with 50, 5, and 0.5 μg/mL emicizumab in one-stage clotting assay. (B) 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 (day1), 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...
below the limit of quantification, and plasma emicizumab concentration of PwHA G, H, I is 14.0 µg/mL, 16.9 µg/mL, 0.0853 µg/mL, respectively.

Table 1 Summary of ADA characteristics in ADA-positive subjects from 3 clinical studies

<table>
<thead>
<tr>
<th>Subjects</th>
<th>Clinical study</th>
<th>ADA response</th>
<th>Detectable neutralizing activity</th>
<th>t1/2 (day)</th>
<th>PK affecting</th>
<th>Epitope recognition (predominant)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>FIXa arm</td>
</tr>
<tr>
<td>HV A</td>
<td>Bioavailability</td>
<td>Treatment-unaffected</td>
<td>No</td>
<td>31.1</td>
<td>No</td>
<td>Not analyzed</td>
</tr>
<tr>
<td>HV B</td>
<td>Phase 1</td>
<td>Treatment-unaffected</td>
<td>No</td>
<td>30.0</td>
<td>No</td>
<td>Fab region</td>
</tr>
<tr>
<td>HV C</td>
<td>Bioavailability</td>
<td>Treatment-induced</td>
<td>No</td>
<td>10.1</td>
<td>Yes</td>
<td>Fab region</td>
</tr>
<tr>
<td>HV D</td>
<td>Bioavailability</td>
<td>Treatment-induced</td>
<td>Yes</td>
<td>14.1</td>
<td>Yes</td>
<td>CDR1 and 3 of light chain</td>
</tr>
<tr>
<td>HV E</td>
<td>Bioavailability</td>
<td>Treatment</td>
<td>Yes</td>
<td>6.93</td>
<td>Yes</td>
<td>CDR1 and 3</td>
</tr>
<tr>
<td>Abbreviations: ADAs; anti-drug 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</td>
<td>ADA response was determined based on the harmonized immunogenicity.</td>
<td></td>
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</tr>
</tbody>
</table>
Figure 1

A

FVIII one-stage clotting assay

% inhibition for spiked emicizumab

Animal-derived ADA (µg/mL)

B

Non-PK affecting

HV A
HV B

PK affecting

HV C
HV D
HV E
HV F

Non-PK affecting

PwHA G
PwHA H
PwHA I
PwHA J

% inhibition for spiked emicizumab 0.5 µg/mL