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Research paper

The impact of eculizumab on routine complement assays

Maria A.V. Willrich[a,*], Bruna D. Andreguetto[b], Meera Sridharan[c], Fernando C. Fervenza[d], Linda J. Tostrud[a], Paula M. Ladwig[a], Ann M. Rivard[a], MeLea D. Hetrick[a], Ryan N. Olson[a], Sandra C. Bryant[e], Melissa R. Snyder[a], David L. Murray[a,*]

[a] Department of Laboratory Medicine and Pathology, Mayo Clinic, Rochester, MN, USA
[b] Department of Clinical Pathology, University of Campinas, Campinas, Sao Paulo, Brazil
[c] Division of Hematology, Mayo Clinic, Rochester, MN, USA
[d] Division of Nephrology, Mayo Clinic, Rochester, MN, USA
[e] Department of Health Sciences Research, Mayo Clinic, Rochester, MN, USA

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ABSTRACT

Background: Eculizumab (ECU) blocks complement C5 cleavage, preventing the formation of C5a and the cytolytic effects of the membrane attack complex. The presence of ECU in blood impacts routine complement tests used to monitor treatment.

Methods: Residual serum samples with normal total complement (CH50) and residual citrate plasma with normal PT/APTT were spiked with ECU at varied concentrations ranging from 25 to 600 μg/mL. In addition, seventy-one samples from patients on ECU were obtained. Artificial and patient samples were analyzed for CH50 and C5 function (Wako Diagnostics), C5 concentration (Quidel), AH50 (Wieslab ELISA) and sMAC (Quidel). ECU concentration was measured by mass spectrometry for all patients.

Results: Complement blockage by ECU was evident in spiked artificial samples. At 25 μg/mL ECU, partial complement blockage was observed in CH50, AH50 and C5 function in serum. Complete blockage defined by undetectable AH50 (< 10%) occurred at 100 μg/mL ECU. C5 concentrations remained the same regardless of ECU. sMAC results stayed around 81% of baseline in serum and 47% in citrate plasma with 50μg/mL ECU. Patient samples had ECU ranging from < 5 to 1220 μg/mL. In all patients with ECU > 100 μg/mL, C5 function was < 29 U/mL.

Conclusions: The spiked sera and patient samples showed complement blockage with CH50, AH50 and C5 function assays when ECU > 100 μg/mL. CH50, AH50 or C5 function assays can serve as indicators for the pharmacodynamic effects of eculizumab. Allied to ECU concentration, laboratory studies may be helpful to tailor therapy.

1. Background

Eculizumab (ECU, Soliris; Alexion Pharmaceuticals Inc., Cheshire, CT, USA) is a humanized hybrid IgG2/IgG4 kappa monoclonal antibody (mab) against the terminal pathway complement component C5 (Rother et al., 2007). ECU binds to C5, inhibiting its cleavage into C5a and C5b, thereby preventing the release of the inflammatory mediator C5a and the formation of the terminal complement cytolytic- pore C5b–C9 (soluble membrane attack complex, sMAC). It has been approved by the FDA to treat rare diseases such as paroxysmal nocturnal hemoglobinuria (PNH), atypical hemolytic uremic syndrome (aHUS) and refractory myasthenia gravis. In addition, though not FDA approved, ECU has also been used to treat cases of C3 glomerulopathies refractory to conventional treatment (Bomback et al., 2012; Zuber et al., 2012).

ECU therapy is initiated with an induction protocol of weekly intravenous infusions for 4 weeks (600 mg for PNH and 900 mg for aHUS or myasthenia gravis) followed by a maintenance biweekly schedule of infusions thereafter (900 mg for PNH and 1200 mg for aHUS or myasthenia gravis). Therapeutic monitoring has not been widely available, and efficacy assessment is mostly based on improvement of clinical symptoms. In 2014, however, reports of pharmacodynamic monitoring of ECU to help guide treatment began to emerge (Noris et al., 2014; Peffault De Latour et al., 2015). *In vivo* studies have demonstrated complete blockade of the terminal pathway of complement with ECU serum concentrations above 35 μg/mL for PNH (Rother et al., 2007;

* Corresponding authors at: Department of Laboratory Medicine and Pathology, Mayo Clinic, 200 First Street SW, Rochester, MN 55905, USA.
E-mail addresses: willrich.mariaalice@mayo.edu (M.A.V. Willrich), murray.david@mayo.edu (D.L. Murray).

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Wong et al., 2013; Wong and Kavanagh, 2015) and 50 μg/mL for aHUS (Legendre et al., 2013a). At these concentrations, in theory the bivalent mab can neutralize approximately 70–100 μg/mL of C5 (Legendre et al., 2013b; Szarvas et al., 2014). Currently the dosing regimen and intervals are designed to be life-long, and to completely block the complement system, without any type of personalized approach. As one of the more expensive drugs in use (yearly cost over $500,000), the cost of a life-long treatment alone may limit its use in clinical practice worldwide. Assessing complement blockage using routine complement assays may be one way to monitor and optimize treatment efficacy in patients undergoing ECU therapy.

The aims of this study are to evaluate impact of ECU on traditional complement assays thereby demonstrating which assays are optimal for monitoring therapy.

2. Materials and methods

Twenty-two residual serum samples from deidentified subjects with physician-ordered complement assays were obtained from the immunology clinical laboratory. All samples had normal total complement function as defined by total complement activity (CH50) results. Samples were handled after two freeze-thaw cycles and kept on ice (4° C) during all analyses. In addition, 6 residual waste citrate plasma samples were obtained. All plasma samples selected had normal prothrombin time (PT, 9.4–12.5 s) and activated partial thromboplastin time (APTT, 25–37 s) test results. Plasma samples were kept at room temperature for < 2 h after draw, and then frozen until testing. Immediately prior to testing, plasma samples were thawed at (4° C) and kept on ice during analysis. ECU vials were obtained from the institution's pharmacy and reconstituted as directed in the package insert. Samples were split into aliquots and spiked with ECU at 25, 50, 100, 150, 200, 400 or 600 μg/mL and allowed to incubate for 30 min. The sMAC assay (enzyme linked immunosorbent assay, Quidel) was performed on both the plasma and the serum samples. In addition, the following clinically available and validated routine complement assays were performed on all the serum specimens: total complement activity (CH50, liposome immunoassay, Wako Diagnostics), alternative pathway activity (AH50, enzyme linked immunosorbent assay, Wieslab Immunediagnostik), C5 function (C5Fx, liposome immunoassay using C5 depleted human serum), and C5 complement component quantitation (C5Ag, nephelometry, Quidel) (Frazer-Abel et al., 2016). This study was approved by the Mayo Clinic Institutional Review Board (IRB 15-000488/Bio 00012866).

In order to evaluate the impact of ECU on the complement assays, results obtained in the baseline (neat) specimens were compared to results obtained in serum spiked with the different concentrations of ECU. All data was summarized and presented as mean ± standard deviation (SD), median and ranges or percentages, as appropriate. For comparisons, generalized estimating equations were used to adjust for the repeated measures design of this part of the study using a normal distribution with the identity link and exchangeable correlation structure for the correlation within each sample at various doses of ECU. Dependent variables were CH50, AH50, C5Fx and C5Ag with independent variable of ECU concentration. In addition, for the sMAC dependent variable, the natural log of ECU concentration was used as the independent variable to meet the normal distributional assumptions.

Thirteen paired serum and citrate plasma samples from patients undergoing therapy with ECU who had a physician-ordered complement alternative pathway serological assessment were tested for CH50, AH50, C5Fx and C5Ag (IRB 16–005201). Another 58 samples from 28 unique patients undergoing ECU therapy had physician-ordered testing for C5Ag and C5Fx as part of their routine management (IRB 15–000488). The serum samples were pulled from frozen storage and ECU concentrations measured. ECU was measured by mass spectrometry using a laboratory developed method referred to as miRAMM (monoclonal immunoglobulin Rapid Accurate Mass Measurement), which quantifies intact kappa light chains from the therapeutic mab in human serum (Ladwig et al., 2017). Briefly, samples are treated with a camelid anti-IgG4 bead system (Capture Select, Thermo Fisher) which will bind to IgG4 molecules in the patient serum. This step significantly reduces background and removes non-IgG4 immunoglobulins as well as other proteins from the sample. After incubation, the mixture undergoes a reduction step with dithiothreitol (DTT) to release the light chains from the heavy chains by breaking the disulfide bonds that keep them together. The mixture is then diluted and while full scan data is collected, the intact light chain mass of a particular mab is deconvoluted by software monitoring of one or more ionized charge states. This is used for quantitation against a standard curve and the method measures total ECU (free and bound to C5). Samples are subjected to reverse phase chromatography on a Cohesive TLX4 Transcend multiplex HPLC system (Thermo Fisher) using a Poroshell 300SB C3 column, 2.1 × 75 μm (Agilent Biosystems). A QExactive plus Orbitrap (Thermo Fisher) high-resolution mass spectrometer is used to acquire the data, which is then analyzed with Tracefinder software version 4.0. To assess the association of C5Fx and C5Ag with ECU concentration, generalized estimating equations were used with independent variable natural log ECU concentration, C5Fx and C5Ag as the dependent variables, assuming a normal distribution with identity link and an exchangeable correlation structure. For C5Fx, the modeling was done only for those patients with ECU < 390 μg/mL, since the previous titration experiments showed that C5Fx would be less than the limit of quantitation (LOQ) of the assay at ECU concentrations approaching 400 μg/mL. Patients with higher values were assigned a predicted value at the LOQ. For all analyses, significance was defined as p-value < .05. SAS (version 9.4, SAS Institute, Cary, NC) was used for all data analysis.

3. Results

3.1. The impact of eculizumab in complement function assays

There was a significant decrease in CH50, AH50 and C5Fx measurements with ECU in the spiked normal samples (all $p < .0001$, Fig. 1). ECU is partially blocking the complement cascade at 25 μg/mL and causes complete blockade of C5 function at 100 μg/mL.

Before spiking ECU, CH50 mean ± SD results were 54 ± 5 U/mL (reference interval, RI: 30–75 U/mL, and limit of quantitation, LOQ, 3 U/mL). At spiked concentrations of 25 μg/mL of ECU, the average activity dropped to 72% ± 16% of the original result, with 3 individual results below the lower RI. At 50 μg/mL of ECU, results were 32% ± 18% of the original CH50. At 100 μg/mL of ECU, 17% ± 8% of the original measurement was detected, and all individual CH50 results were below the lower RI. CH50 activity continued to decrease with 150 μg/mL of ECU and at 600 μg/mL, 9 out of the 11 samples had undetectable CH50 (< 3 U/mL) with the other 2 showing only 2% ± 2% of the original CH50 result (Fig. 1A, Table 1).

As expected, alternative pathway activity measured by the AH50 assay significantly decreased with ECU ($p < .0001$). Before spiking with ECU, the AH50 values were 97 ± 30% (RI: ≥46%, LOQ 10%). The trend observed for CH50 was followed closely in the AH50 Wieslab ELISA assay. At 25 μg/mL of ECU the activity decreased to 60% ± 24% of the original AH50 result, with 5 individual results below the lower RI. At 50 μg/mL, the residual activity was 11% ± 24% compared to the neat results and all but one sample had results below the RI. At 100 μg/mL, all samples had results below the LOQ of < 10%, which was replicated at ECU concentrations of 150 μg/mL or greater (Fig. 1B).

C5 function measured with C5Fx prior to ECU spike was 48 ± 6 U/mL (RI: 29–53 U/mL, LOQ 5 U/mL) and dropped to 70% ± 14% with 25 μg/mL of ECU. At 50 μg/mL of ECU, all but one sample had results below the RI. At 100 μg/mL 18% ± 8% of the original C5Fx was measured, with all results below the lower RI. Finally, at 200 μg/mL 8 out of 11 samples had undetectable C5Fx < LOQ of the assay, and at