A Lesson Learned in Developing the Ligand Binding Assay for Quantification of Complement C3a

Mengmeng Zhai, Bing Yu, Hong Chen

Alliance Pharma, 17 Lee Boulevard, Malvern, Pennsylvania

Poster ID:
W1030-01-06

CONTACT INFORMATION: 610-296-3152 | INFO@ALLIANCEPHARMACO.COM

aaps National Biotechnology CONFERENCE

Advancing Biotechnology Innovation, Careers, and Community

PURPOSE

- All complement activation pathways lead to the activation of C3 and C3a release into the blood.
- C3a is an important biomarker of local inflammatory processes and a potential target for complement therapy.
- It is critical to establish a robust assay in house for quantification of human C3a.
- A wide range of C3a concentration was observed in human plasma, and yet their concentrations increased with time.
- A hypothesis was tested that C3a is continuously produced by testing the short term stability of C3a in human plasma

METHODS

The MSD Ligand Binding Assay:

- A biotinylated, neo-epitope specific anti-C3a desArg monoclonal antibody is coated onto streptavidin coated MSD plate.
- Human C3a, prepared by cleavage of C3 by a human C3 convertase, is used as reference material.
- C3a STD in Buffer, C3a QCs or C3a in human plasma samples is bound to the coated plate
- SulfoTag labeled mouse antibody specific to human C3/C3a is to detect the bound C3a or C3a desArg, a stable form of C3a.
- The ECL signal intensity is proportional to the amount of C3a, C3a desArg in the samples.

The Short Term Stability Test:

- Matrix quality controls (mQCs), prepared by pooling normal K2EDTA human plasma with pre-determined baseline C3a concentrations.
- C3a QCs in buffer were also prepared as controls.
- C3a matrix QCs, and the buffer QCs were stored at – 80°C freezer for at least 24 hours prior to the stability test (RT/4°C for up to 48 hours).
- All stability test samples were analyzed in human C3a MSD assay

RESULT(S)

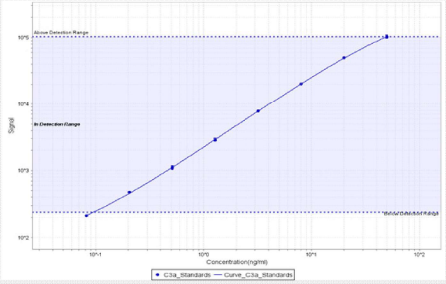

Fig. 1 Calibration curve for C3a quantification

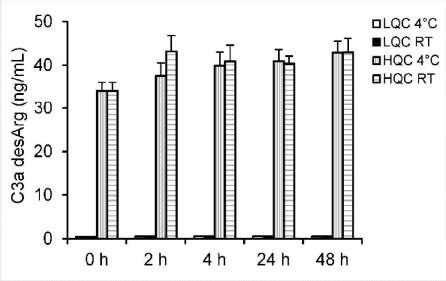

Fig. 2 Short-term stability of C3a QCs prepared in buffer at LQC and HQC levels. Data are presented as mean ± SD (n=3).

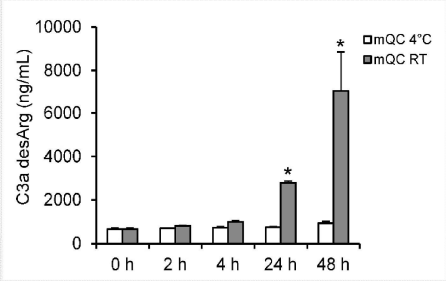

Fig. 3 Short-term stability of mQCs. Data are presented as mean ± SD (n=3). *p < 0.05 vs 0 h

Fig. 1 shows an 8-point calibration curve (50, 20, 8, 3.20, 1.28, 0.512, 0.205, and 0.082 ng/mL) with C3a concentration in X-axis and ECL signals in Y-axis

C3a in buffer were stable when stored at 4°C or RT over two days (Fig. 2).

C3a in human plasma (matrix QC) was relatively stable at 4°C within 24 hours but increased slightly at 48 hours (Fig. 3).

C3a in human plasma at RT were not stable, significantly increased by > 4 folds at 24 hours and by 10 folds at 48 hours (Fig. 3).

CONCLUSION(S)

- These short term stability tests demonstrated that C3 is continuously activated when human plasma samples are stored at RT.
- This test also implied that inappropriate handling of plasma samples may result in inaccurate measurement of C3a.
- Therefore, it is critically important to keep the thawed plasma samples cold before C3a assay and reduce the cumulative sample storage time at RT before measurement of the C3a.

Alliance PHARMA