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Effect of diflunisal on clusterin levels in ATTRwt amyloidosis

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Background

Wild-type transthyretin (ATTRwt) amyloidosis is a disease that features wild-type transthyretin tetramer dissociation and formation of amyloid fibrils that frequently infiltrate cardiac tissue causing heart dysfunction in older adults. While amino acid replacements cause disruption of the native state of transthyretin (TTR) in hereditary TTR amyloidosis, the factor(s) responsible for TTR destabilization and accumulation of amyloid fibrils in ATTRwt amyloidosis remains undefined. One such factor may be clusterin (CLU), a plasma circulating glycoprotein known to exhibit chaperone-like activity. Here, we report 1) baseline serum CLU concentrations in a large cohort of patients with an unequivocal diagnosis of ATTRwt amyloidosis, 2) CLU levels at 1-year follow-up in untreated patients and those receiving the TTR tetramer stabilizing agent, diflunisal, and 3) associations between CLU levels and clinical measures.

Material and Methods

Serum samples from patients with biopsy-proven ATTRwt amyloidosis featuring cardiac involvement were obtained from the Boston University Amyloidosis Center IRB-approved repository; healthy control sera were purchased from Bioreclamation (Westbury, NY). All sera (patients and controls) were collected from Caucasian males ≥ 60 years of age. ATTRwt patients were assessed at baseline and 1-year follow-up evaluations. In the study group receiving drug, treatment with diflunisal was initiated post-baseline visit; untreated patients did not receive diflunisal. Serum clusterin concentrations were measured by sandwich ELISA on a 96-well plate format using a commercially available human clusterin ELISA kit (R&D Systems, MN). GraphPad Prism software was used for data calculations and generation of plots. CLU levels are presented as mean $\pm$ SD. Deviations from normal distribution were assessed using the Shapiro-Wilk test. *P*-values comparing groups were determined by unpaired, two-sided t-test. Spearman's or Person's correlation coefficients were used to assess associations between CLU levels and baseline values for ~ 30 routinely measured laboratory and echocardiographic parameters. *P*-values comparing linear associations were obtained by linear regression analysis.

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Disclosure statement

The authors report no conflict of interest

Results

The study group at baseline consisted of 122 patients, none of whom had been treated with diflunisal prior to initial evaluation. Serum CLU concentration in this group was 165.1 ± 25.7 ug/mL; this value was significantly lower when compared to a race-, gender- and age-matched healthy control group ($n=25$; 189.2 ± 33.1 ug/mL, $p=0.002$, Fig. 1A). In a subset ($n=31$) of this cohort, CLU was measured at 1-year follow-up in a diflunisal-treated group ($n=13$) and patients not receiving drug ($n=18$). In the patients receiving diflunisal, serum CLU at 1-year was higher (163.1 ± 15.7 ug/mL) than at baseline (157.1 ± 25.5 ug/mL); conversely, a lower level of CLU (152.4 ± 22.6 ug/mL) was measured in the untreated group at follow-up. While significant differences from baseline or between the treated and untreated groups at 1-year were not reached, opposing trends in serum CLU concentrations of the groups at annual follow-up were clearly noted (Fig. 1B). CLU was found to strongly correlate with TTR ($r=0.519$, $p<0.001$). Significant inverse correlations of CLU with B-type natriuretic peptide (BNP, $r=-0.188$, $p=0.04$), left ventricular end-systolic diameter (LVESD, $r=-0.223$, $p=0.01$) and left ventricular mass index (LVMI, $r=-0.290$, $p=0.001$), as well as positive correlations of CLU with modified body mass index ($r=0.221$, $p=0.01$), triglycerides ($r=0.352$, $p<0.001$) and albumin ($r=0.251$, $p=0.005$) were also demonstrated.

Discussion and conclusions

We have previously reported that CLU levels were significantly lower in a small group of clinically-diagnosed ATTRwt patients ($n=36$) compared to age-, gender- and race-matched controls [1]. The present study confirms this result in a much larger population ($n=122$) of ATTRwt patients with biopsy-proven disease. Moreover, we report a temporal increase in serum CLU in patients treated with diflunisal at 1-year follow-up compared to baseline; in contrast, patients who were not taking diflunisal demonstrated decreased CLU levels at annual evaluation. Validation of these findings on larger groups at follow-up will increase the statistical power of our analyses and potentially shed light on the role of CLU in the pathobiology of ATTRwt amyloidosis. Interestingly, the strong positive correlation between CLU and TTR levels found at baseline may support a pathological mechanism in which soluble tetrameric TTR decreases as more of the native protein dissociates and forms aggregated non-native species, overwhelming the protein folding capacity of CLU and causing a reduction in circulating levels of the chaperone. Our data may indicate that diflunisal contributes to a partial recovery of soluble CLU levels. These results may be in accordance with the reported benefits for patients with hereditary ATTR amyloidosis treated with the TTR tetramer stabilizer [2]. Finally, inverse associations of CLU with echocardiographic measures (BNP, LVESD and LVMI) indicate that decreasing serum levels of the protein are concomitant with declining cardiac function, while direct correlations with BMI, triglycerides and albumin are consistent with previous reports [3] and suggest the presence of comorbidities (low-grade inflammation, metabolic syndrome).

References

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Figure 1.

A. Baseline serum CLU concentrations. CLU levels were significantly lower in patients compared to the levels found for healthy controls (165.1 vs 189.2 ug/mL). **B. Serum CLU levels at 1-year follow-up.** Serum CLU was 152.4 ± 22.6 ug/mL in patients not being treated with diflunisal, while a higher concentration of 163.1 ± 15.7 ug/mL was measured in patients on the drug. Baseline CLU (overall) for the entire group was 157.1 ± 25.5 ug/mL.