Administration of N-acetylcysteine causes beneficial posttranslational modifications of transthyretin in hemodialysis patients

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Introduction and Aim

The thiol antioxidant N-acetylcysteine (NAC) may mediate interactions with protein-associated cysteine residues, however, information on protein level in vivo are missing. Therefore, in the present study we aimed to analyze N-acetylcysteine-induced modifications of the protein transthyretin (TTR) in plasma from hemodialysis patients in a randomized, placebo-controlled study in vivo and after administration to plasma in vitro. TTR was selected due to its low molecular weight and the free cysteine residue in the polypeptide chain, which is known to be extensively modified by formation of mixed disulfides.

Results

Clinical characterisitics of hemodialysis patients are shown in Table 1. The administration of NAC during a hemodialysis session resulted in a substantial increase of native TTR from median 15% (range 8.8-30%) to median 40% (37-50) and a reduction of S-cysteinylated TTR [51% (44-60) vs. 6.6% (2.4-10)]. Additionally the pronounced formation of a TTR-NAC adduct was detected. However, all these modifications seemed to be reversible (Figure 1). Additionally, in vitro incubation of plasma with NAC confirmed the in vivo results and indicated that changes in PTM pattern of TTR were a function of NAC concentration (Figure 2).

Table 1 Clinical characteristics hemodialysis patients

	NAC treatment			Placebo		
	predialysis	postdialysis	P	predialysis	postdialysis	P
N (m/ f)	6 (5/ 1)					
Age (years)	71 (46-75)					
Body weight (kg)	69.7 (50.0-93.6)	69.2 (49.0-91.4)	0.031	71.9 (50.0-92.6)	70.0 (49.5-91.5)	0.031
SBP (mm Hg)	127 (90-161)	137 (77-160)	0.750	140 (90-153)	131 (57-173)	0.438
DBP (mm Hg)	70 (40-75)	65 (49-71)	0.917	64 (46-80)	60 (33-85)	0.281
Creatinine (µmol/L)	588 (406-769)	282 (174-345)	0.031	773 (306-876)	289 (127-522)	0.031
BUN (µmol/L)	28.6 (17.6-36.7)	9.60 (5.90-13.7)	0.031	28.2 (15.3-49.8)	9.40 (5.40-33.0)	0.031
TTR (µmol/L)	5.78 (2.81-13.1)	5.58 (2.59-12.3)	0.844	8.29 (4.59-11.0)	6.93 (3.85-8.52)	0.063

Data are presented as median (range). Abbreviations used: BUN, blood urea nitrogen; DBP, diastolic blood pressure; NAC, N-acetylcysteine; SBP, systolic blood pressure; TTR, transthyretin.

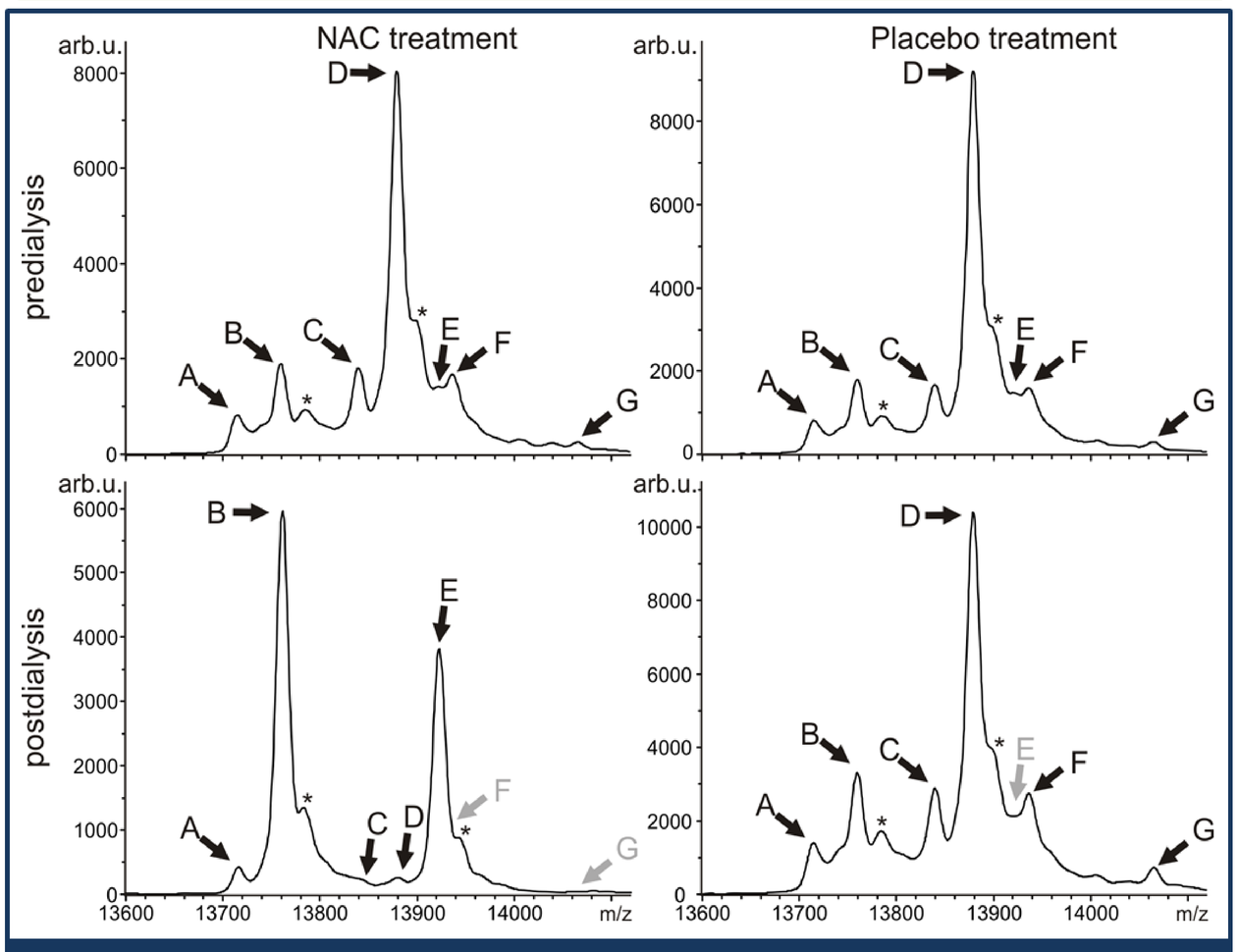

Figure 1 Changes in posttranslational modification pattern of TTR in plasma of hemodialysis patients during dialysis sessions with NAC and placebo, respectively

Letters indicate TTR variants with A TTR modified by cleavage of cysteine side chain to form glycine; B unmodified TTR; C sulfonated TTR; D cysteinylated TTR; E NAC-TTR adduct; F glutatthionylated TTR.

Methods

Plasma levels of TTR were determined by a non-commercial enzyme-linked immunosorbent assay (ELISA) using polyclonal rabbit anti-human TTR antibodies. Spectra of immunoprecipitated TTR were obtained using an AutoflexSpeed matrix assisted laser desorption ionization-time of flight (MALDI-TOF) mass spectrometer (Bruker-Daltonik, Bremen, Germany). The samples were analyzed in triplicates. For ionization, a Smartbeam-II laser was used and 1500 shots per spot were collected. For spectra calibration an external standard was used. Spectra were evaluated using the software flexAnalysis. TTR variants were expressed as relative amounts of the summed intensity of all observed TTR variants.

Conclusion

We conclude that the interaction of N-acetylcysteine with proteins may explain altered protein functions due to beneficial modification of cysteine residues in hemodialysis patients.

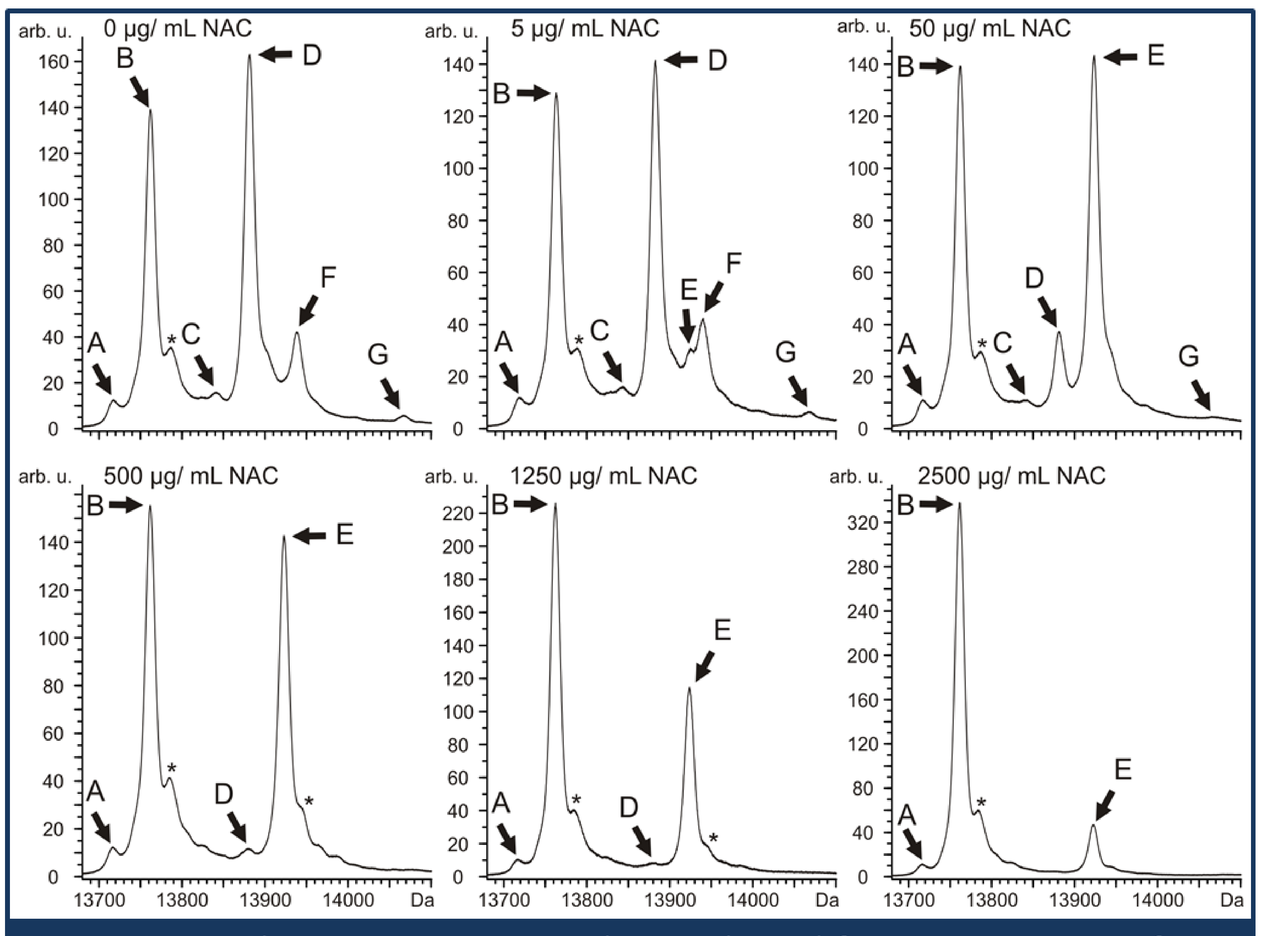

Figure 1 Changes in posttranslational modification pattern of TTR in human plasma after incubation with different concentrations of NAC.

Letters indicate TTR variants with A TTR modified by cleavage of cysteine side chain to form glycine; B unmodified TTR; C sulfonated TTR; D cysteinylated TTR; E NAC-TTR adduct; F glutatthionylated TTR.