Intradialytic Variations of Oxidative Stress Markers

Cristina Căpuşă[1], Gabriel Mircescu[1], Irina Stoian[2], Caterina Sântimbrean[1], Nicolae Ursea[1]
[1] "Dr Carol Davila" Hospital of Nephrology, Bucharest
[2] Biochemistry Dept. "Carol Davila" University of Medicine, Bucharest

Background

End-stage renal disease (ESRD) is known as a state of oxidants/antioxidants imbalance, but the mechanisms underlying oxidative stress are still matter of debate. Both uremic-related factors (retained uremic solutes) (1, 2) and dialytic procedure might be involved (3).

Hemodialysis (HD) therapy could influence the oxidative processes in regard of both reactive species generation (mainly as a result of membrane bioincompatibility) and antioxidant systems activity (4, 5). No undisputed opinion exists concerning the effects of HD on antioxidant defence. Hemodialysis-induced antioxidant substance losses contribute to the enhancement of oxidative stress (4, 5). On the other hand, the removal of some dialyzable oxidizing uremic toxins during HD might lead to the restoration of the antioxidant status (6).

The aim of the study was to investigate the acute effects of HD procedure on the oxidative stress associated with chronic renal failure (CRF) by assessing the variations of oxidative stress parameters during the HD session.

Materials and methods

Twelve ESRD patients (six men and six women; mean age: 49.5±14.7 years) on maintenance HD for at least 18 months (88.5±60 months) were included after informed consent was obtained. All patients were in a stable clinical condition and were dialyzed thrice weekly for 4.5 hours per session. Exclusion criteria were: active smokers, inflammatory and infectious conditions, malignancies, diabetes mellitus, active chronic liver diseases, and medications with antioxidative potentialities.

The HD session at which oxidative parameters were assessed was performed with single-used ethylenevinylalcohol (EVAL) membrane dialysers sterilized with gamma, bicarbonate-based dialysate and standard heparinization.

Blood samples were drawn after overnight fasting from the arteriovenous fistula just before (0min), at 40 minutes (40min) and at the end (270min) of the HD session, into standard vacuum tubes with heparin (for plasma and erythrocyte determinations) or without anticoagulant (for serum determinations). Samples were kept on ice, protected from light, and were processed within 30 minutes of sampling. Following centrifugation the plasma and serum aliquots were stored at -70°C until use. All oxidative stress assays were performed by spectrophotometry, according to previously described methods:

- plasma thiobarbituric acid reactive substances (TBARS) (7);
- plasma reactive dicarbonyl compounds (RDC) (8);
- total plasma free thiols (Pt-SH) (9);
- serum total antioxidant activity (TAA) (10);
- non-protein erythrocyte thiols (E-SH) (11);
- erythrocyte glutathione peroxidase activity (EGPx) (12);
- erythrocyte catalase activity (CAT) (13);
- erythrocyte superoxide dismutase activity (SOD) (14).

In order to correct for plasma volume changes that occur during HD as a result of ultrafiltration, ratios of oxidative stress parameters to hemoglobinemia were chosen for analysis.

Results were expressed as mean ± standard deviation and were compared using Student's *t* test and analysis of variance (ANOVA). *p* values less than 0.05 were considered significant.

Results

Intradialytic variations of the oxidative stress parameters to hemoglobinemia ratios are presented in Table I.

Since lipoperoxidation marker (plasma TBARS) increased significantly over the first 40 minutes (e.g. simultaneously with immune activation (15)), it is reasonably to assume the acute induction of oxidative stress during HD procedure as a result of blood to artificial materials interactions.

Plasma RDC concentrations decreased permanently during the HD session suggesting the dialytic removal of these low molecular weight intermediates which arise during irreversible non-enzymatic modifications of proteins from both carbohydrates and lipids by oxidative and non-oxidative chemistry.

Regarding extracellular antioxidant systems our study revealed a significant decrease in serum total antioxidant activity at the end of HD session and no intradialytic changes of plasma total free thiols levels.

Correspondence to: Cristina Căpuşă, Calea Grivitei no. 4, Bucharest 78104, Romania, tel: 40213129226
Fax: 40213129226, e-mail: cdavilla@rdsnet.ro

Table I. Intradialytic variations of the oxidative stress parameters (values expressed as ratio to hemoglobinemia)

Parameter	0min	40min	270min
TBARS/Hb	8.28±2.00	†12.46±3.00	†11.46±1.60
RDC/Hb	1.34±0.30	†1.08±0.14	†‡0.36±0.14
Pt-SH/Hb	1.20±0.23	1.22±0.18	1.18±0.16
TAA/Hb	0.13±0.02	0.12±0.02	†0.11±0.01
E-SH/Hb	0.86±0.27	0.82±0.20	†‡0.58±0.25
EGPx/Hb	0.38±0.05	0.41±0.05	0.37±0.09
CAT/Hb	22.81±5.60	15.67±13.0	17.27±4.90
SOD/Hb	37.00±5.60	33.58±6.50	33.16±10.3

$p<0.05$: † vs. 0min; ‡ vs. 40min.

These findings indicate a deficiency of the extracellular antioxidant defence during HD, probably accounted for intradialytic elimination of uric acid (the second most significant serum antioxidant after albumin).

Antioxidant erythrocyte enzyme activities did not shown notable intradialytic variations probably because they have a minor contribution to short-term antioxidant defence and the adaptive enzymatic induction in response to oxidative stress is a long-term process.

Non-protein erythrocyte thiols level, consisting mainly of reduced glutathione, was significantly lower at the end of HD session as compared with the other two moments investigated. This could reflect the enhanced consumption of intracellular glutathione due to augmented generation of reactive species during the HD session.

Conclusions

Our data point out:

1) the acute influence of hemodialysis procedure which consist in the enhancement of oxidative stress, as indirectly argued by increment in TBARS, reduction of TAA and E-SH;

2) the intradialytic removal of plasma reactive dicarbonyl compounds.

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