

No Benefit of Hemodiafiltration over Hemodialysis in Lowering Elevated Levels of Asymmetric Dimethylarginine in ESRD Patients

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Key Words

Asymmetric dimethylarginine · Hemodiafiltration · Hemodialysis · β_2 -Microglobulin

Abstract

Background: It has been suggested that hemodiafiltration (HDF) is more efficient than hemodialysis (HD) in lowering plasma levels of the endogenous nitric oxide synthase inhibitor asymmetric dimethylarginine (ADMA), which is a strong and independent predictor of overall mortality in ESRD patients. **Methods/Patients:** Twenty ESRD patients (11 women) were studied during both a single online HDF session and a single HD session. In each patient, ADMA, L-arginine, SDMA, β_2 -microglobulin and urea were measured at several time points. **Results:** Although HDF was clearly superior to HD in decreasing plasma β_2 -microglobulin, there was no difference in the elimination characteristics of ADMA. However, HDF but not HD eliminated the nitric oxide synthase substrate L-arginine, making HD superior in increasing the L-arginine/ADMA ratio. **Conclusion:** Neither HD nor HDF

sufficiently removes the putative uremic toxin ADMA. The clinical significance of HD better improving the L-arginine/ADMA ratio (parameter of NO production) as compared to HDF needs to be determined. Copyright © 2006 S. Karger AG, Basel

Introduction

Convective dialysis therapies (i.e. hemofiltration and hemodiafiltration, HDF) are receiving renewed interest due to the availability of feasible and affordable technology. Since the introduction of these techniques in the 1970s, it has been claimed that superior clearance of 'middle' molecules will result in improved hemodynamics and better survival. However, a systematic review of the analyses of four trials consisting of 336 patients found that all-cause mortality was significantly higher with HDF than with hemodialysis (HD) [1]. This is in contrast to observational studies showing a survival benefit for patients treated with a high volume of convection, and caution must be exercised due to the paucity of data. In the context of mortality of dialysis patients, asymmetric dimethylarginine (ADMA) has recently sparked interest.

M.K. and J.T.K. contributed equally to the study.

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ADMA is the predominant endogenous inhibitor of nitric oxide synthase (NOS). Elevated concentrations of ADMA correlate with traditional and nontraditional risk factors, as recently reviewed [2]. Furthermore, cohort studies in both the general population [3–5] and in patients on HD [6] demonstrated a strong and independent link between ADMA, all-cause mortality and cardiovascular events. Lowering ADMA is therefore thought to be beneficial. Since ADMA has a low molecular weight (about 202 Da), comparable with that of urea (60 Da), renal replacement therapy seems to be the option of choice for removing ADMA in ESRD patients. However, dialysance and thus removal of ADMA during regular HD is lower than would be expected with regard to its molecular weight, in part owing to the significant protein-binding of ADMA [7]. Therefore, it has been suggested that convective means of renal replacement therapy such as HDF would be more efficient than conventional HD with regard to ADMA elimination [7] and that the former method would therefore be successful in reducing elevated plasma ADMA levels [8]. The aim of the study was to investigate the different effects of HDF and HD on plasma ADMA and L-arginine levels and to additionally assess differences in the L-arginine/ADMA ratios.

Subjects and Methods

Patients

The studied group consisted of 20 chronic HD patients (9 men and 11 women), mean age 54 ± 15 years. Patients were dialyzed three times a week for 4 h and their dialysis treatment lasted for 3 years (range 1–17 years). In the most recent 5 months (2–19 months), half of these patients were treated with HDF. All patients fulfilled the criteria of adequate dialysis ($Kt/V > 1.2$ according to Daugirdas II formula). The etiology of renal failure was as follows: glomerulonephritis in 5 cases, interstitial nephritis in 9 cases, polycystic kidney disease in 4 cases and diabetic nephropathy and hypertensive nephropathy in 1 case each. Their residual diuresis was 500 ml (0–600 ml). Their albumin was 41 (39–43) g/l, hemoglobin 112 (106–119) g/l and hematocrit 33.4 (31.7–35.7)% (measured before dialysis session). Patients received an average weekly erythropoietin dose of 60 IU/kg body weight. The majority of patients had hypertension and were treated with moderate doses of antihypertensive drugs. Two of the patients were type 2 diabetics on oral antidiabetics or diet, 5 patients had ischemic heart disease and 3 patients were being treated for dyslipidemia. All patients were in stable clinical status, had no acute cardiac problems and there were no signs of acute infection. All patients were studied after the long dialysis interval. Current drug regimes were not altered throughout the study.

Each patient was tested during one 4-hour HD with low-flux polysulfon membranes (F6 1.3 m² or F7 1.5 m², Fresenius Medical Care, Germany) and during one 4-hour postdilution on-line HDF

with high-flux polysulfon membranes (HF80, 1.8 m² Fresenius Medical Care, Germany) and a substitution volume of 78.1 ± 11.2 ml/min. Blood flow rate and dialysate flow rate was maintained at 300 and 500 ml/min, respectively. Dialysis was performed with bicarbonate solution. Patients received heparin during dialysis at a dose of $6,100 \pm 1,500$ IU and mean ultrafiltration rate per session was 1.9 l. Endotoxin in dialysate was below detection limit (limulin amebocyte lysate test, performed once in 3 months).

The study was approved by local Institutional Ethical Committee (approval No. 26/03) and all patients provided informed consent prior to entering the study. The study is registered as a clinical trial in The Cochrane Renal Group Registry (<http://www.cochrane-renal.org/dbsearch.php>) and its ID number is CRG110500021.

Samples

Blood samples from the arteriovenous fistula (arterial line) were drawn before dialysis (time 0) and at 15, 120 and 240 min (end) of the session. Blood was centrifuged for 10 min at 1,450 g and plasma was frozen at -80°C . Analysis of samples was performed within 6 months.

Laboratory Parameters

Determination of L-Arginine, ADMA and Symmetric Dimethylarginine in Plasma

The determination of L-arginine, ADMA and symmetric dimethylarginine (SDMA) in plasma samples was carried out by high performance liquid chromatography-tandem mass spectrometry, according to the procedure described previously [9]. The limits of detection were 0.4 $\mu\text{mol/l}$ for L-arginine, 0.02 $\mu\text{mol/l}$ for ADMA and 0.01 $\mu\text{mol/l}$ for SDMA. The relative standard deviations of the quantification results were 4.5% for L-arginine, 5.5% for ADMA and 3.9% for SDMA.

Other Parameters

Routine biochemical parameters were determined with automated analysers using standard clinical-chemistry methods recommended by the International Federation of Clinical Chemistry. β_2 -Microglobulin was assessed with Microparticle Enzyme Immunoassay with AxSYM analyzer (Abbott, USA).

Statistical Analysis

Results are expressed as means \pm standard deviation. Analysis of variance, Mann-Whitney test and t test were used for statistical evaluation. Results were considered as statistically significant at $p < 0.05$.

Results

HDF significantly lowered β_2 -microglobulin plasma levels (from 30.3 ± 12.5 to 9.5 ± 4.5 mg/l; $p < 0.001$), while HD had no effect on β_2 -microglobulin (31.8 ± 12.1 to 36.6 ± 13.5 ; n.s.; fig. 1). ADMA was lowered by both HD (from 0.81 ± 0.25 to 0.52 ± 0.11 $\mu\text{mol/l}$, $p < 0.001$) and HDF (from 0.79 ± 0.21 to 0.48 ± 0.09 $\mu\text{mol/l}$; $p < 0.001$; fig. 2). Since HDF tended to eliminate the NOS

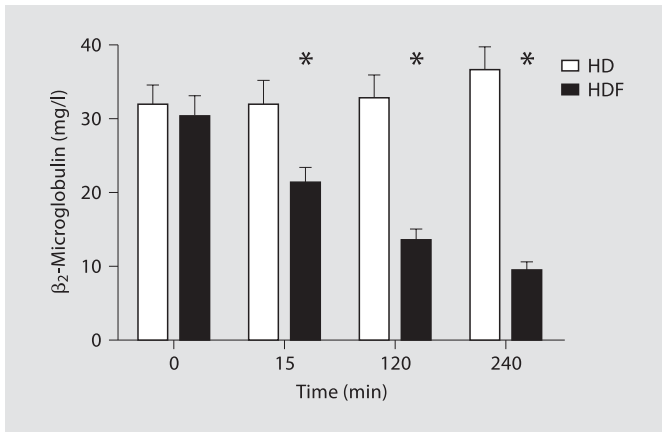


Fig. 1. Influence of HD and HDF on β_2 -microglobulin levels. * $p < 0.001$, comparison between HD and HDF.

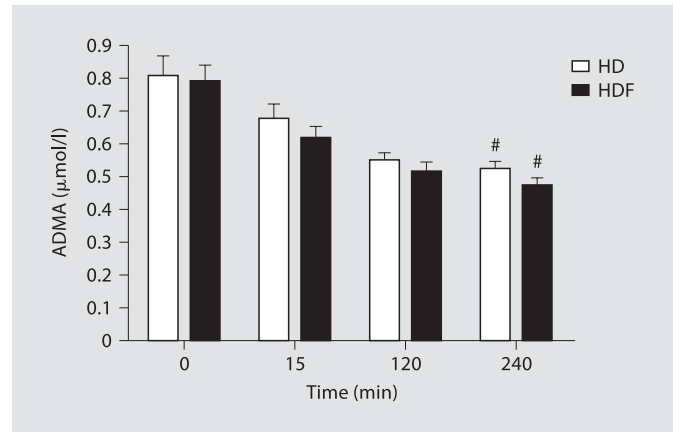


Fig. 2. Influence of HD and HDF on plasma ADMA. # $p < 0.001$, comparison between 0 and 240 min.

substrate L-arginine (from 85.6 ± 35.3 to $63.2 \pm 29.1 \mu\text{mol/l}$; $p < 0.05$) more than HD (from 75.7 ± 36.6 to $71.8 \pm 40.3 \mu\text{mol/l}$; n.s.; fig. 3a), the crucial L-arginine/ADMA ratio (parameter of NO production) showed more significant improvement during HD (increase by $46 \pm 11\%$) than during HDF (increase by $25 \pm 11\%$; fig. 3b). While ADMA decreased modestly (by 35% in HD and by 40% in HDF), SDMA – despite being of similar molecular weight – was much more easily removed by both HD (by 44%) and HDF (by 48%) (fig. 4). Urea decrease was much more pronounced during both HD (by 70%) and HDF (by 73%) as compared to the two methylarginines (fig. 5).

Discussion

Our data show for the first time that single HD and HDF sessions have the same effect on plasma ADMA levels. Furthermore, due to the enhanced removal of L-arginine during HDF in contrast to HD, the latter one has a more favorable effect on the crucial L-arginine/ADMA ratio (parameter of NO production). In contrast, HDF significantly lowered β_2 -microglobulin while HD had no effect on plasma β_2 -microglobulin.

The finding that HDF was very effective in reducing elevated levels of β_2 -microglobulin by almost 69%, is in line with recent data on postdilution HDF [10]. Although HDF was strikingly superior in eliminating β_2 -microglobulin, we did not see a significant difference in the reduction of plasma ADMA levels by HD (35%) or HDF

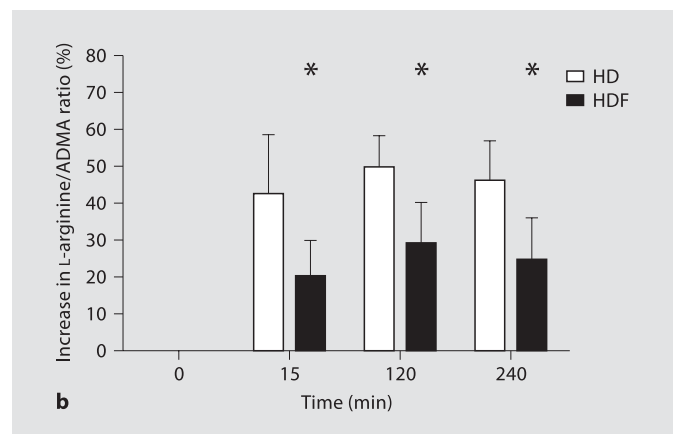
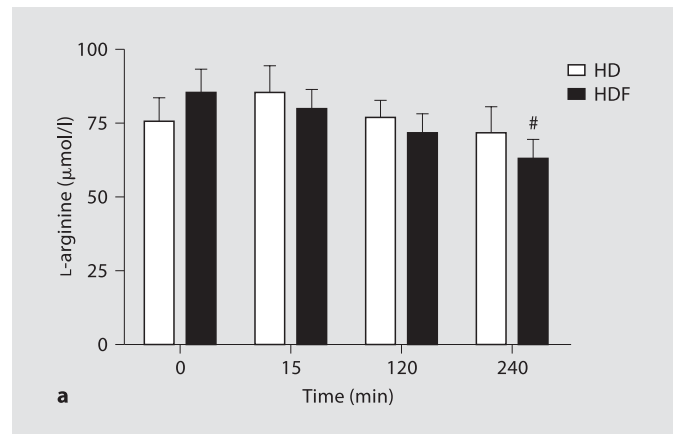


Fig. 3. a Influence of HD and HDF on plasma L-arginine. # $p < 0.05$, comparison between HDF 0 min and HDF 240 min. **b** Influence of HD and HDF on the plasma L-arginine/ADMA ratio. * $p < 0.05$, comparison between HD and HDF.

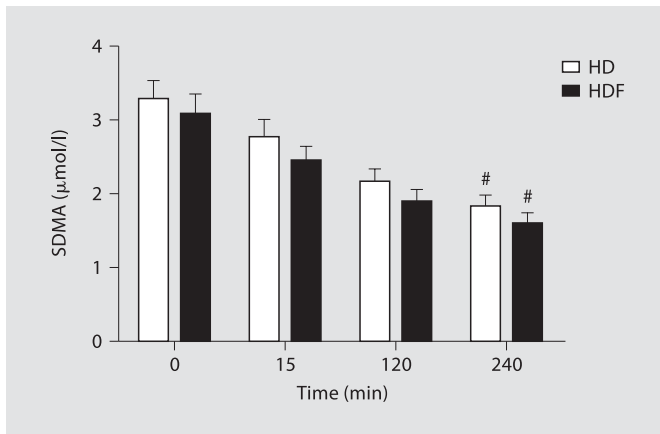


Fig. 4. Influence of HD and HDF on plasma SDMA. # $p < 0.001$, comparison between 0 and 240 min.

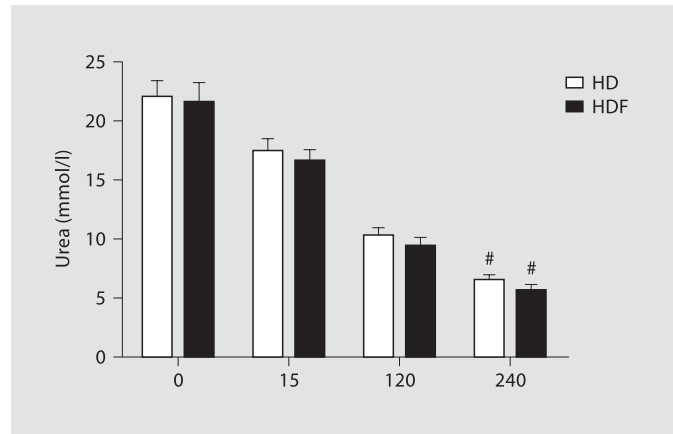


Fig. 5. Influence of HD and HDF on plasma urea. # $p < 0.001$, comparison between 0 and 240 min.

(40%). For both HD and HDF, the degree of ADMA reduction is in the range previously reported, i.e. between 23 [11] and 65% [12]. However, several studies did not see a significant decrease in ADMA at all when comparing pre- and postdialysis ADMA levels [7, 13, 14]. Such decrease was particularly absent in patients prone to hypotension [15]. The discrepancy between studies might be due to different blood-draw timing as well as different analytical methods used.

This study confirms recent data which suggest that the dialysance and thus removal of ADMA is lower than would be expected with regard to its molecular weight, in part due to significant protein binding of ADMA [7]. In an earlier study, we found about 37 μmol , i.e. 12% of the ADMA produced per day in the collected dialysate after a regular HD [7]. This is in line with a study by Achan et al. [16] who found that in healthy men only 50 μmol per day are excreted via urine. It is therefore reasonable to assume that dialysis of any kind will not be more effective with respect to elimination of ADMA than the normal kidney. However, besides plain removal, dialysis could alter activity of dimethylarginine dimethylaminohydrolase (DDAH), the enzyme hydrolyzing about 80% of ADMA produced daily. Investigating this rather complex scenario of oxidative stress, inflammatory cytokines and pH changes is however beyond the scope of this study.

HDF is considered to be beneficial for the dialysis patients by decreasing procedure-related problems like hypotension. Removal of ADMA and the consequent excess production of NO has been suggested to lower blood

pressure in hypertensive patients [8] and play a crucial role in dialysis-related hypotension [15]. Our data suggest that the beneficial effect of only HDF on cardiovascular stability during dialysis is not due to different effects of these treatment methods on plasma ADMA levels. Furthermore, exogenous ADMA has long-lasting biological effects, a half-life of more than 20 min and only minor effects on blood pressure [17]. This makes ADMA removal less likely to have an immediate effect on blood pressure. Indeed, recent experimental and clinical data suggest that the thermal effects of HDF appear to be the main blood pressure-stabilizing factor [18, 19]. The design of our study did not allow evaluating long-term effects of both treatment methods. However, Beerenhout et al. [18] did not see any difference in plasma ADMA levels 1 year after patients had been randomly assigned to either predilution on-line HDF or low-flux HD. Taken together, the available data cast doubt on the theory that the beneficial effect of HDF, that still has to be proven [1], might be attributable to enhanced elimination of ADMA as it has been suggested [8]. Although ADMA is a strong independent predictor of cardiovascular and all cause mortality in ESRD patients [6], the beneficial effect of lowering ADMA on morbidity and mortality, if any, still needs to be determined [2]. Interestingly, a recent study by Chan et al. [20] showed that even a significant increase in dialysis dose, a significant reduction in blood pressure and an improvement of endothelial function after switch from regular to nocturnal HD were not associated with a change in plasma ADMA levels. The results of the Dutch Study CONTRAST which will randomly assign 800 pa-

tients to either low-flux HD or HDF will help answer the question whether convective means of renal replacement therapy do improve the dismal cardiovascular morbidity and mortality of our patients [21].

A second finding of our study was the fact that both HD and HDF improved the L-arginine/ADMA ratio. This is an important ratio since it is considered a parameter of NO production. Interestingly HD improved the L-arginine/ADMA ratio significantly better than HDF. Although single trials increasing the L-arginine/ADMA ratio by supplementing L-arginine have been promising [22], the clinical significance of our finding needs to be determined since there are conflicting data on whether or not this will translate into clinical benefit for the patients [23].

Finally we could show that SDMA although of similar molecular weight, was much more easily removed by both HD (by 44%) and HDF (by 48%) than ADMA. This is in line with previous studies that addressed that point [11, 14, 15]. It also points to the pivotal role of DDAH in the elimination of ADMA.

In summary neither HD nor HDF sufficiently lowered the plasma level of the putative uremic toxin ADMA. ADMA behaves differently to solutes with comparable molecular weight such as urea and it is worse eliminated than its structural isomer SDMA. Thus neither diffusive nor convective means of renal replacement therapy provide good strategies to eliminate ADMA. In contrast, HDF but not HD reduces the plasma level of L-arginine, the substrate for NOS. Future therapeutic strategies should be aimed at increasing the activity of DDAH, the enzyme that degrades about 80% of the ADMA produced in man. This might be possible by using modified, e.g. vitamin-coated, dialysis membranes [24].

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