Free Radical Research, Volume 38 Number 10 (October 2004), pp. 1093–1100

Taylor & Francis healthsciences

Free Radic Res Downloaded from informahealthcare.com by Library of Health Sci-Univ of Il on 11/28/11
For personal use only.

Effects of Hemodialysis, Dialyser Type and Iron Infusion on Oxidative Stress in Uremic Patients

CHRISTOPH MÜLLER[a], GERHARD EISENBRAND[a], MARTINA GRADINGER[b], THOMAS RATH[b], FRANZ WERNER ALBERT[b], JÖRG VIENKEN[c], RAJINDER SINGH[d], PETER B. FARMER[d], JEAN-PIERRE STOCKIS[e] and CHRISTINE JANZOWSKI[a,*]

[a]*Department of Chemistry, Division of Food Chemistry and Environmental Toxicology, Technical University of Kaiserslautern, Kaiserslautern, Germany;* [b]*Westpfalz-Klinikum Kaiserslautern, Kaiserslautern, Germany;* [c]*BioScience Department, Fresenius Medical Care, Bad Homburg, Germany;* [d]*Cancer Biomarkers and Prevention Group, Biocentre, University of Leicester, Leicester, UK;* [e]*Department of Mathematics, Technical University of Kaiserslautern, Kaiserslautern, Germany*

Accepted by Professor F.J. Kelly

(Received 12 February 2004; In revised form 17 August 2004)

Uremic patients undergoing hemodialysis (HD) are considered to face an elevated risk for atherosclerosis and cancer. This has been attributed in part to an increased oxidative stress. In this pilot study, oxidative cell damage in blood of HD-patients was compared to those of controls: total DNA damage (basic and specific oxidative DNA damage), modulation of glutathione levels (total and oxidized glutathione) and of lipid peroxidation were monitored via the Comet assay (with and without FPG), a kinetic photometric assay and HPLC quantification of plasma malondialdehyde (MDA), respectively. In some samples, leukocytes were analysed for malondialdehyde–deoxyguanosine-adducts (M_1dG) with an immunoslot blot technique.

HD-patients ($n = 21$) showed a significant increase of total DNA damage ($p < 10^{-12}$), compared to controls ($n = 12$). In a subset of patients and controls, GSSG levels and M_1dG, however, only increased slightly, while tGSH and MDA levels did not differ. The influence of different low flux HD-membranes was tested in a pilot study with nine patients consecutively dialysed on three membrane types for four weeks each. In addition to the individual disposition of the patient, the dialyser membrane had a significant impact on oxidative stress. Total DNA damage was found to be almost identical for polysulfone and vitamin E coated cellulosic membranes, whereas a slight, but significant increase was observed with cellulose-diacetate ($p < 0.001$). In patients receiving iron infusion during HD, MDA-formation ($n = 11$) and total DNA damage ($n = 10$) were additionally increased ($p < 0.005$).

Our results show an increased oxidative damage in HD-patients, compared to healthy volunteers. Significant influences were found for the dialyser membrane type and iron infusion.

Keywords: Oxidative stress; Hemodialysis; Dialyser membrane; Iron infusion; Comet assay

Abbreviations: AGE, advanced glycation end products; ANOVA, analysis of variance; BHT, butylated hydroxytoluene; DTNB 5, 5′-dithiobis(2-nitrobenzoic acid); ESRD, end-stage renal disease; FPG, formamidopyrimidine-DNA *N*-glycosylase; GSH, reduced glutathione; GSSG, oxidised glutathione; HD, hemodialysis; M_1dG, malondialdehyde–deoxyguanosine-adduct; MDA, malondialdehyde; ROS, reactive oxygen species; SSA, 5-sulfosalicylic acid; tGSH, total glutathione; TI%, tail intensity in percent; TNB, 5-thio-2-nitrobenzoate

INTRODUCTION

Chronically increased formation of reactive oxygen species (ROS) is associated with severe adverse health effects, such as degenerative diseases including atherosclerosis, Alzheimer's disease, Parkinson's disease, cancer, diabetes and end-stage renal disease (ESRD).[1,2]

Chronically increased oxidative stress seems to play a key role in hemodialysis (HD) patients concerning their increased risk for cardiovascular diseases and accelerated atherosclerosis; the latter occurs 10–20 years earlier, compared to other population groups.[3,4] It is the decisive factor leading to death in 60% of cases. Moreover, the risk for infections and cancer, especially of kidney,

*Corresponding author. Address: Christine Janzowski, Erwin Schrödinger-Str. 52/316, 67663 Kaiserslautern, Germany. Tel.: +49-631-205-2532. Fax: +49-631-205-3085. E-mail: janzo@rhrk.uni-kl.de

ISSN 1071-5762 print/ISSN 1029-2470 online © 2004 Taylor & Francis Ltd
DOI: 10.1080/10715760400011452

RIGHTSLINK

urinary bladder, thyroid and other endocrine organs, is distinctly increased in ESRD patients.[5,6]

It is well known that the balance between pro- and anti-oxidative processes is impaired by HD treatment. Pro-oxidative events in these patients may be further associated with their uremic status. In addition, the administration of iron/erythropoietin to treat anaemia may additionally contribute to oxidative stress, in parallel with both, an enhanced elimination of antioxidants during HD and malnutrition because of dietary restrictions.[3,7] Correspondingly, it was found that the antioxidative capacity of blood decreases in HD-patients during the time course of therapy. This is reflected by reduced activities of antioxidative enzymes.[8] Plasma levels of oxidized glutathione (GSSG) are significantly increased and as a result the ratio of reduced glutathione (GSH) to total glutathione (tGSH) is decreased. This has been observed for chronic ambulatory peritoneal dialysis patients.[9] Furthermore, antioxidants such as vitamin E in blood cells, plasma levels of uric acid and other antioxidants have been found to be decreased.[10,11]

Different markers of oxidative stress, such as products of lipid peroxidation like isoprostane 8-epi-$PGF_{2\alpha}$,[12] advanced glycation end products (AGEs)[13,14] and oxidative protein modifications[15,16] are increased in HD-patients. Increased induction of micronuclei has a marker for oxidative stress has been described in lymphocytes of HD-patients.[17]

In HD-patients, DNA has been hardly used as a target to quantify oxidative cell damage, although oxidative DNA damage is known as a sensitive marker in other human studies.[18,19] Only the determination of 8-hydroxy-deoxyguanosine by HPLC/ECD has been described in leukocytes of HD-patients.[20] The efficacy of oxidative DNA damage as a marker was also confirmed using the Comet assay with formamidopyrimidine-DNA *N*-glycosylase (FPG) for HD-patients.[21]

In the present study, oxidative DNA damage in leukocytes was compared for HD-patients and volunteers. The relevance of HD related factors was investigated by testing different dialysers and after the iron infusion. In addition to oxidative DNA damage (Comet assay with FPG), malondialdehyde (MDA in plasma), the MDA–deoxyguanosine-adduct (M_1dG in leukocytes), and the antioxidants uric acid (in plasma) and glutathione (total glutathione: tGSH and oxidized glutathione: GSSG, in whole blood) were included to correlate the extent of oxidative stress in different blood compartments.

MATERIALS AND METHODS

This study was approved by the local ethic committee (Rheinland-Pfalz 837.188.00 (2554)). All investigated subjects gave their informed consent.

Subjects

Twelve volunteers (healthy non-smokers aged 48–62 years), recruited from our university and the Westpfalz-Klinikum Kaiserslautern, and 21 clinically stable HD-patients (aged 41–87 years, of which 20 were non-smokers) of the Westpfalz-Klinikum without kidney function, which were dialysed three times a week for four to five hours, were studied. Types of kidney disease were glomerulonephritis (6 subjects), polycystic kidney disease (1 subject), haemolytic uremic syndrome (1 subject), amyloidosis (1 subject), interstitial nephritis (1 subject), diabetic nephropathy (3 subjects), shrunken kidney (1 subject), nephrectomy (1 subject) and unknown genesis (6 subjects). Patients were treated with established therapeutic regimens, including antihypertensive, anticoagulative, antiarrythmic and cholesterol-lowering drugs. Furthermore, patients received anti-anaemia treatment encompassing erythropoietin and iron preparations. In addition, some individuals also received vitamin D/calcium and other vitamin preparations.

Blood Sampling, Processing, Storage

Venous blood samples (2.7 ml) were collected in EDTA-tubes and stored at room temperature during further sampling (comparison of dialysers, effect of iron infusion) prior to being utilised in the Comet assay. Glutathione was determined in whole blood. Immediately after collection, blood (0.3 ml) was mixed with 5-sulfosalicylic acid (SSA 10% w/v, 1.2 ml) for protein precipitation, followed by shock-freezing and storage in liquid nitrogen until analysis. For MDA and uric acid determination, blood was centrifuged (5 min, 300*g*). Butylated hydroxytoluene (BHT, 0.05% w/v) was immediately added to the plasma (0.2 ml) and the mixture was stored at −70°C until analysis. All determinations of biomarkers were performed blinded.

Comparison of HD-patients and Volunteers

Venous blood drawn from 21 HD-patients (immediately before the start of the HD session, with different dialysers) and from 12 volunteers was analysed for basic and total DNA damage. In sub-studies, plasma MDA (13 patients, 12 volunteers), tGSH and GSSG (10 patients, 6 volunteers) and M_1dG (6 patients, 4 volunteers) were investigated.

Comparison of Dialysers

In a sub-study, 9 patients aged 54–87 years, selected from the above described group, were consecutively

Free Radic Res Downloaded from informahealthcare.com by Library of Health Sci-Univ of Il on 11/28/11
For personal use only.

RIGHTSLINK

dialysed with three low flux HD membranes: polysulfone (PSu-F8, 1.8 m^2 surface area; Fresenius Medical Care, Bad Homburg, Germany; cellulose-diacetate (DICEA 170, 1.7 m^2; Baxter Healthcare, Mc Gaw Park, Illinois, USA); vitamin E-coated cellulose, E15, 1.5 m^2, Terumo Ltd, Tokyo, Japan) in four week periods each. After a two week adaptation period blood samplings were performed in two HD sessions immediately before the start (pre-HD), during (15′, 30′, 60′, 120′), at the end, 10′ after HD session (only MDA, post 10′) and 20′ after HD session (post 20′). The change of membrane types was conducted randomly.

Effect of Iron Infusion

A subset of 11 patients (MDA-formation), aged 55–87 years with renal anaemia, was comparatively investigated in two subsequent HD sessions, with and without i.v. iron infusion (5 ml sodium ferric gluconate complex in sucrose containing 62.5 mg Fe, Ferrlecit®, Aventis Pharma). Ten of these patients were additionally investigated for basic and total DNA damage by Comet assay. The iron was infused 5 min before the end of HD within 1 min. Blood was collected immediately prior to iron infusion, and 5 and 25 min after iron infusion. For control, HD's were performed under identical conditions but without iron infusion.

Comet Assay

Alkaline single cell gel electrophoresis was performed according to Singh *et al.*[22] and Collins *et al.*[23] with slight modifications.[24] Aliquots of blood (6 μl) were mixed with low melting agarose (LMA, 65 μl), distributed onto a frosted glass microscope slide, precoated with a layer of normal melting agarose (2 gels per slide), coverslipped and kept at 4°C for solidification. After removing the cover glass, slides were immersed in a lysis solution for 1 h at 4°C. After cell-lysis, slides were washed three times in enzyme buffer, drained and covered with 50 μl of either enzyme buffer (1 slide) or FPG enzyme (2 slides), sealed with a cover glass and incubated for 30 min at 37°C. The FPG protein recognises oxidised purine bases, cuts them out and nicks the DNA at the respective sites, resulting in additional DNA damage.[23] Consequently, the DNA damage observed after FPG treatment reveals total DNA damage, consisting of basic strand breaks and oxidised bases.

DNA was allowed to unwind (pH 13.5, 20 min, 4°C) and horizontal gel electrophoresis (Bio-Rad Sub Cell GT) was conducted at 4°C for 20 min (25 V, 300 mA, 0.89 V/cm) using a Bio-Rad 300 power supply. Then slides were washed three times with TRIS-buffer (0.4 M, pH 7.5), stained with ethidium bromide (40 μl, 10 μg/ml) and viewed microscopically with a Zeiss Axioskop 20, equipped with a filter set 15 (excitation: BP 546/12; emission: LP 590). Slides were analysed by computerised image analysis (Perceptive Instruments, Suffolk, Great Britain), scoring 2 × 50 images per slide (2 gels per slide). DNA migration is directly expressed as mean tail intensity (TI%) from 1 or 2 slides. Data are presented as basic DNA damage (without FPG treatment), total DNA damage (with FPG treatment) and specific oxidative DNA damage (subtraction of basic from total DNA damage). The coefficient of variation of the method (processing with FPG) was 7.3%.

Determination of MDA

Using the reagents of a MDA detection kit (Sobioda, France), sample or standard (10 μl each) were mixed with thiobarbituric acid/perchloric acid (80 μl, 2:1, v/v) and heated for 60 min at 95°C. After cooling and centrifugation, butanol (200 μl) was added and mixed (1 min). After further centrifugation (5 min, 2600*g*), The butanol phase (150 μl) was used to measure the thiobarbituric acid derivative by HPLC/fluorescence (Jasco HPLC system: PU 1580, DG 1580-53, LG 1580-02, FP 1520, AS 1550; excitation: 532 nm, emission: 553 nm, Merck LichroSpher 100; 5 μm, RP-18 250 × 4 mm, isocratic flow of 12.5 mM Na_2HPO_4, pH 7.4/methanol (57:43, v/v), 1.2 ml/min, retention time of MDA: 3.2 min). The coefficient of variation of the method was 3.4%.

Determination of Uric Acid

Uric acid was measured photometrically in plasma after derivatisation to a quinone imine dye, following the manufacturer's instructions [Sigma Diagnostics]. The coefficient of variation of the method was 7.8%.

Determination of Total and Oxidized Glutathione

Total glutathione (tGSH: GSH + GSSG) and GSSG were measured in blood by photometric determination of 5-thio-2-nitrobenzoate (TNB), in a kinetic assay, according to Gallagher *et al.*[25] with slight modifications. Samples were thawed and centrifuged (12000*g*), supernatants (final dilution: 1:50 in 5% w/v SSA, 20 μl) or glutathione standards (range 1–32 μM) were reacted with NADPH/DTNB solution (700 μl phosphate buffer, 100 μl 6 mM 5,5′-*dit*hiobis(2-*n*itro*b*enzoic acid), 20 μl 20 mM NADPH and 150 μl distilled water) and glutathione reductase (10 μl, 0.5 U) in the cuvette. TNB formation rate (ΔE/10 min) was monitored at 412 nm and compared with standards. For measuring GSSG, supernatants (final dilution: 1:25 in 5% SSA, 250 μl) or standards (range 1–32 μM) were reacted with triethanolamine

Free Radic Res Downloaded from informahealthcare.com by Library of Health Sci-Univ of Il on 11/28/11
For personal use only.

RIGHTSLINK

(60 μl) and vinylpyridine (10 μl) for 1 h in a thermomixer at 26°C. Aliquots of 100 μl were assayed according to standardised tGSH-determination. The coefficient of variations of *t*-GSH determination and GSSG determination were 2.5 and 2.4%, respectively.

Determination of Malondialdehyde–DNA-adduct M_1dG

The malondialdehyde–DNA-adduct M_1dG was quantified according to Leuratti *et al.*[26] and Singh *et al.*[27] Briefly, after isolation from leukocytes DNA was blotted onto a nitrocellulose membrane and fixed in a drying oven. Then, unspecific binding sites of the membrane were blocked with milk powder. After incubation with primary antibody (kindly provided from L. Marnett, Vanderbilt University, Nashville, Tennessee, USA), M_1dG was determined by chemiluminescence reaction of the secondary antibody labelled with horse-radish peroxidase. The amount of DNA, quantified after staining with propidium iodide, was used to calculate the level of $M_1dG/\mu g$ DNA. The coefficient of variation of the method was 14.8%.

Statistics

Natural logarithms of data were taken and used for analyses, yielding median and mean values in close proximity, indicating that the transformed data had a near normal distribution. Differences between volunteers and pre-HD-patients were analysed with the Welch two-sample *t*-test. Statistical differences of markers within a group of patients or controls were determined with the paired *t*-test. For analysis of independent predictors (dialyser membrane, dialysis time point, individual patient and the interaction between these variables) for total DNA damage and lipid peroxidation, an analysis of variance (ANOVA) was performed. The effects of different dialyser membrane materials and of iron infusion on total DNA damage and on lipid peroxidation (MDA-formation) were investigated using *F*-test, paired *t*-test and Welch two-sample *t*-test. The influence of membrane material was analysed including all blood sampling time points.

RESULTS

Comparison of HD-patients and Volunteers

Total DNA damage in leukocytes in terms of tail intensity (TI%) of 12 volunteers and 21 pre-HD patients is shown in Fig. 1A. Basic DNA damage in volunteers (without FPG treatment) ranged between 0.2 to 0.5TI% (mean ± SD: 0.3 ± 0.1TI%). In the presence of FPG, mean TI% was increased about two-fold, reflecting the background oxidative DNA damage, equalling 0.5TI% (SD: 0.3TI%).

In pre-HD patients, basic DNA damage (mean ± SD: 0.6 ± 0.3TI%) only marginally differed from those of controls. Total DNA damage of patients (mean ± SD: 5.4 ± 2.6TI%), however, was drastically increased compared to the respective basic damage and to the total DNA damage of controls (with FPG) ($p < 10^{-12}$ for each comparison). This was clearly attributable to significantly increased specific oxidative DNA damage. Age of patients was unrelated to both, basic DNA damage (correlation coefficient $R = -0.04$) and total DNA damage ($R = -0.03$). This applied as well to controls (correlation of age to basic DNA damage, $R = -0.09$ and of age to total DNA damage, $R = -0.22$).

In contrast to specific oxidative DNA damage, plasma MDA-formation in patients and controls (Fig. 1B) did not significantly differ under these conditions. First results on M_1dG levels indicated higher levels in patients ($n = 6$) compared to the controls ($n = 4$, Fig. 1C).

Mean plasma concentration of uric acid in pre-HD-patients (mean ± SD: 310 ± 96 μM, 10 patients) was slightly increased compared to controls (mean ± SD: 241 ± 83 μM, 6 controls). In the course of HD, uric acid concentrations continuously diminished down to approximately 30% of the starting concentration (data not shown).

The results of glutathione determination showed similar levels of tGSH in blood of pre-HD-patients (mean ± SD: 1038 ± 234 μM, $n = 10$) and of controls (mean ± SD: 1098 ± 213 μM, $n = 6$); GSSG levels, however, were slightly higher ($p < 0.05$) in pre-HD-patients (mean ± SD: 53 ± 14 μM, $n = 10$) vs. controls (mean ± SD: 34 ± 12 μM, $n = 6$). During HD, tGSH levels significantly decreased within the first 60 min down to 907 μM (SD: 208 μM, $n = 10$), whereas in the following time period a slight increase back to 1052 μM (SD: 170 μM, $n = 10$, 10 min after end of HD) was observed (significance: pre-HD vs. 60 min HD and 60 min HD vs. 10 min post HD: $p < 0.02$ each). GSSG concentrations decreased down to 42 μM (SD: 15 μM, $n = 6$) between the time points "pre-HD" and "60 min HD".

Comparison of Dialyser Membranes/Crossover Study

Basic DNA damage in leukocytes was found unchanged during the time course of HD (data not shown). Time dependent formation of total DNA damage (with FPG) resulting from HD with the low flux membranes is shown in Fig. 2A. During HD, distinct changes in total DNA damage were not observed. Almost identical TI% were obtained for polysulfone membrane PSu-F8 and for vitamin-E

Free Radic Res Downloaded from informahealthcare.com by Library of Health Sci-Univ of Il on 11/28/11
For personal use only.

RIGHTSLINK

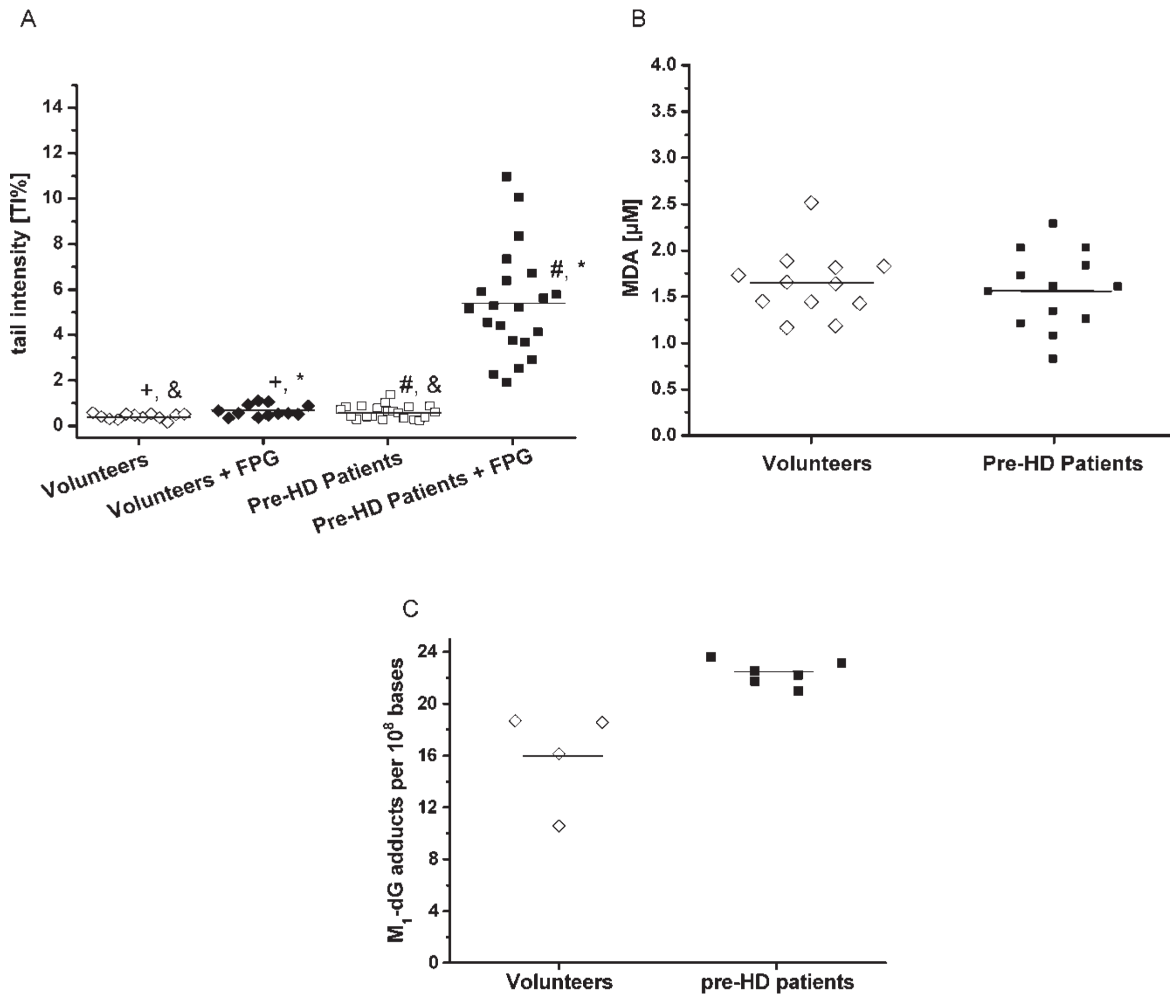

FIGURE 1 (A) Basic and total DNA damage in leukocytes (12 volunteers, 21 patients pre-HD data); (B) Plasma MDA-formation (12 volunteers, 13 patients pre-HD data); (C) Leukocyte M_1dG levels (4 volunteers, 6 patients pre-HD data). The lines represent means. (A): $+p < 0.005$; #: $p < 10^{-12}$ (paired t-test); *: $p < 10^{-12}$; &: $p < 0.05$ (Welch two-sample t-test).

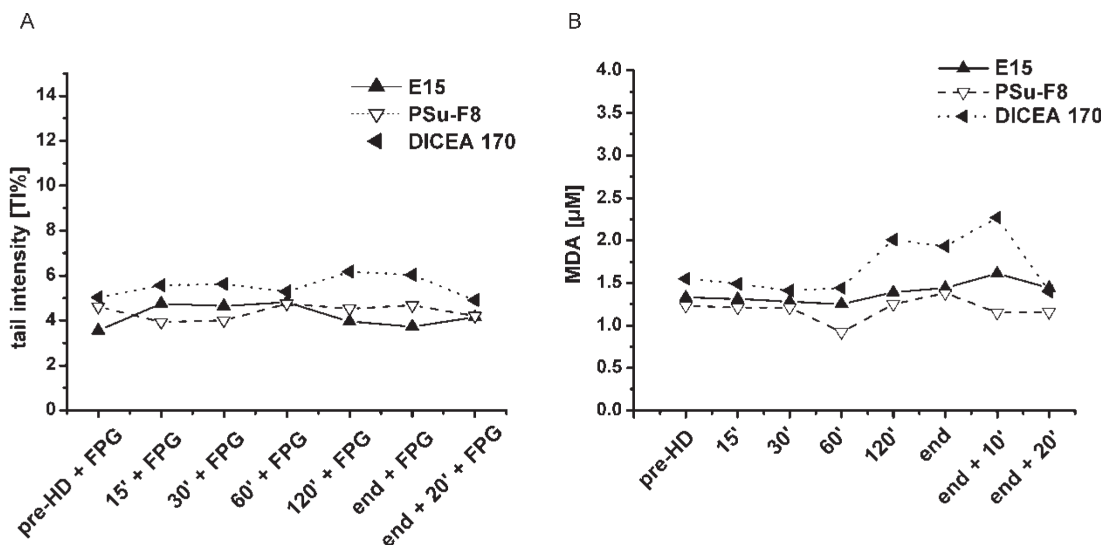

FIGURE 2 Comparison of dialysers/crossover study: (A) Total DNA damage in leukocytes during HD with different membrane types. Significance (Welch two-sample t-test) of total DNA damage: PSu-F8/E15: not significant ($p = 0.21$), DICEA 170/PSu-F8: $p < 0.001$, DICEA 170/E15: $p < 0.001$. (B) Plasma MDA-formation during HD with different membrane types.

Free Radic Res Downloaded from informahealthcare.com by Library of Health Sci-Univ of Il on 11/28/11
For personal use only.

TABLE I Independent predictors in the comparison of the dialyser membranes/crossover study (3 dialyser membranes, 9 patients, 7 time points, ANOVA)

Variable	*p*-value
(a) Total DNA damage	
Dialyser membrane	<0.001
Patient	<0.001
Dialysis time point	0.97
Interaction: dialyser membrane–patient	<0.001
Interaction: dialyser membrane–dialysis time point	0.21
Interaction: patient–dialysis time point	0.16
(b) MDA-formation	
Dialyser membrane	<0.001
Patient	<0.001
Dialysis time point	<0.001
Interaction: dialyser membrane–patient	<0.001
Interaction: dialyser membrane–dialysis time point	<0.01
Interaction: patient–dialysis time point	<0.001

bonded membrane E15 ($p = 0.21$). With the diacetylated cellulose membrane DICEA 170 a low, but significant increase of TI% occurred, compared to PSu-F8 ($p < 0.001$) and E15 ($p < 0.001$) (means ± SD of all time points and all patients DICEA170: 5.6 ± 0.4TI%; PSu-F8: 4.5 ± 0.3TI%, E15: 4.5 ± 0.4TI% each). MDA-formation was slightly higher with E15 compared to PSu-F8 ($p < 0.001$).

ANOVA for the influence of dialyser membrane, individual patient and dialysis time point on total DNA damage is shown in Table I. The dialyser membrane type and the individual patient significantly affected total DNA damage. The time point of dialysis, however, did not seem to play a role. The interaction between the variables dialyser membrane type and individual patient were considered to have a distinct impact on the marker total DNA damage. For MDA levels, statistical analysis of predictors showed that all variables tested significantly affected the extent of lipid peroxidation (Table I).

Effect of Iron Infusion

Basic DNA damage was almost identical with and without iron infusion (Fig. 3A, columns 1, 3, 5). Total DNA damage of both blood samplings did not differ at the time point "prior to iron infusion" (columns 2). For iron infusion treatment a tendency for increased total DNA damage (columns 4) was apparent already after 5 min becoming significant at 25 min after application, as compared to the respective HD without iron (columns 6, $p < 0.005$). MDA-formation was similar in both HD conditions at the time point "prior to iron infusion" (Fig. 3B). Iron infusion, however, resulted in a significant increase of MDA-formation ($p < 0.005$), which became observable already at earlier time points, compared to total DNA damage.

DISCUSSION

HD-patients, compared to volunteers, showed a strongly increased oxidative DNA damage. Moreover, as specific oxidative DNA damage largely exceeded basic DNA damage in the blood samples of the patients in all sub-studies, it had the predominant impact on the extent of total DNA damage.

Increased oxidative DNA damage was observed before the start of individual HD sessions. Similar findings have been described by Tarng *et al.*[20] who used 8-hydroxy-2′-deoxyguanosine as a marker for oxidative DNA damage. Oxidative stress in leukocytes can result from the underlying disease as well as from repeated exposure to HD membranes. As a result of the bioincompatibility of the membrane, complement activation is known to occur and white blood cell-activation is observed. This in turn leads to degranulation and activation of adhesion molecules. During these processes, ROS are formed, including

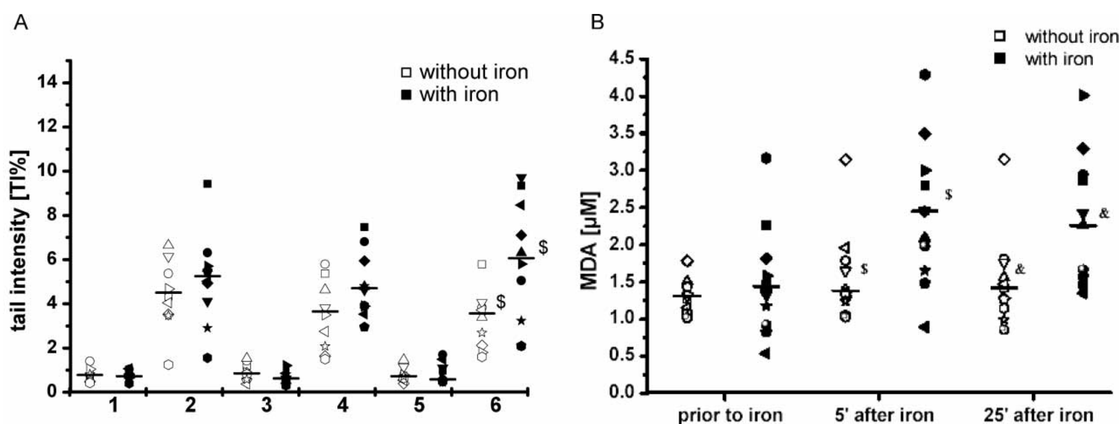

FIGURE 3 Effect of iron infusion: (A) Basic and total DNA damage in leukocytes during HD without and with iron infusion. (1) prior to iron infusion; (2) prior to iron infusion, +FPG; (3) 5′ after iron infusion; (4) 5′ after iron infusion, +FPG; (5) 25′ after iron infusion; (6) 25′ after iron infusion, +FPG. Significance: $: $p < 0.005$; the lines represent medians. (B) Plasma MDA-formation during HD prior to iron infusion and after iron infusion. Significance: $: $p < 0.05$; &: $p < 0.005$; The lines represent medians.

Free Radic Res Downloaded from informahealthcare.com by Library of Health Sci-Univ of Il on 11/28/11
For personal use only.

RIGHTSLINK

Free Radic Res Downloaded from informahealthcare.com by Library of Health Sci-Univ of Il on 11/28/11
For personal use only.

hydroxyl radical, hydrogen peroxide, hypochlorous acid and nitrogen oxides, followed by an enhancement of inflammatory/immune reactions.[15,28] An increase in basic DNA damage in HD-patients was also observed by Stopper *et al.*[17,29] who reported an elevated micronuclei rate under similar conditions. The DNA-adduct M_1dG represents a long-term accumulating endpoint of oxidative cell damage (resulting from MDA-formation by ROS attack on lipids or on the sugar/phosphate backbone of DNA). This may explain the observed, diverging findings concerning the DNA-adduct M_1dG and the short-lived MDA in plasma.

In the course of individual HD's, a distinct increase in basic and total DNA damage has not been found, even though the antioxidant uric acid was diminished in plasma by about 70%. A similar HD related excretion of uric acid has been described by Jackson *et al.*[10] Thus, the extracellular uric acid seems to play only a minor role in intracellular ROS detoxification. The observed depletion of tGSH and GSSG during HD may be explained at least in part by reactions of GSH with cellular electrophiles, preferentially generated from lipid peroxidation. Elimination of tGSH via dialysis as a consequence of cellular membrane leakage seems not to be relevant because tGSH and GSSG diminished to a different extent.

In the pilot crossover study with nine patients and three different dialysers, significant differences in oxidative DNA damage have been observed in the time course of HD. The synthetic polysulfone and the vitamin E-coated cellulose membrane induce less oxidative stress, compared to the semi-synthetic diacetylated cellulose membrane. Apparently, the remaining two hydroxyl-groups in the cellobiose unit of cellulose-di-acetate still suffice to induce complement activation and may thus stimulate oxidative stress effects. MDA-formation was lower with the polysulfone than with the vitamin E-coated cellulosic membrane, even though the polysulfone membrane had a greater surface ($1.8\,m^2$), compared to the vitamin E coated cellulosic membrane ($1.5\,m^2$). With respect to induction of oxidative DNA breakage and MDA-formation, the membranes maximally differed by about 50 and 70%, respectively. This strongly suggests a higher biocompatibility of synthetic lipophilic polysulfone membranes. Tarng *et al.*[30] using 8-hydroxy-deoxyguanosine as a marker for oxidative DNA damage, described similar minimal pro-oxidative effects for polysulfone and vitamin E coated dialyser membranes whereas an unmodified cellulose membrane was found to be less biocompatible. The ANOVA showed that the dialyser membrane, the individual disposition of the patient and the interaction between these factors had a significant impact on oxidative DNA damage. For MDA-formation all variables investigated were considered to play a role, indicating a high variability of this marker.

The results of the iron infusion study show no differences in DNA damage (basic and total) and in MDA-formation prior to iron infusion in individual patients at different HD sessions (Fig. 3A, columns $1 + 2$, "prior to iron infusion"; Fig. 3B, column 1). This reveals a rather constant low oxidative status, indicating good clinical stability of the patients. In the case of iron application basic DNA breakage was not increased. However, total DNA damage and MDA-formation in plasma showed strong and time dependent increase (maximal difference of means: approximately 50 and 70%) following iron infusion. The results on MDA-formation are in line with data published by Roob *et al.*[31] who observed a rapid increase with a maximum at 30 min after start of iron application and a rather low linear elimination from the plasma pool. Apparently, plasmatic iron binding proteins (transferrin) are not capable to scavenge free iron ions fast enough to suppress Fenton type reactions.[32] The comparison of oxidative DNA breakage and formation rates of MDA shows that lipid peroxidation is induced faster than oxidative DNA modifications. This may simply reflect the fact that preferentially plasmatic or cell membrane lipoproteins are targeted by iron mediated oxidative reactions resulting in MDA formations, whereas DNA is somewhat more protected in the nucleus by the nuclear membrane and tight packaging into chromatin.[33]

In conclusion, HD-patients are permanently exposed to an elevated oxidative stress, when compared to controls. In the time course of HD sessions, some influence of the membrane polymer type on oxidative DNA damage has been observed. Iron infusion caused increased oxidative cell damage of similar extent than observed for the diacetylated cellulose membrane. Since the increased ROS formation resulting from bioincompatibility of dialyser membrane types and from iron infusion represents a long-term adverse factor, these results may be considered to be relevant for accelerated atherosclerosis and the higher risk of cancer in HD-patients. In addition to the development and use of membranes with better biocompatibility, other preventive factors such as the intake of dietary antioxidants may therefore offer some promise for prevention.

Acknowledgements

We are indebted to Kirsten Würth, and Maria Mork for their excellent assistance in the determination of glutathione and uric acid.

We thank the nursing staff of the Westpfalz-Klinikum Kaiserslautern and Dr M. Riedel for assistance in blood sampling and organisation.

RIGHTSLINK

The gift of M_1dG and of the respective antibody for immunoslot blot by Lawrence J. Marnett, Vanderbilt University, Nashville, Tennessee, USA is gratefully acknowledged.

We thank A.R. Collins, University of Oslo, Norway for providing the FPG protein.

References

[1] Halliwell, B. and Gutteridge, J.M.C. (1999) *Free Radicals in Biology and Medicine*, 3rd Ed. (Oxford University Press).

[2] Loft, S. and Poulsen, H.E. (1996) "Cancer risk and oxidative DNA damage in man", *J. Mol. Med.* **7**, 297–312.

[3] Canaud, B., Cristol, J.-P., Morena, M., Leray-Moragues, L., Bosc, J.-Y. and Vaussenat, F. (1999) "Imbalance of oxidants and antioxidants in haemodialysis patients", *Blood Purif.* **17**, 99–106.

[4] Locatelli, F., Pozzoni, P., Tentori, F. and Del Vecchio, L. (2003) "Epidemiology of cardiovascular risk in patients with chronic kidney disease", *Nephrol. Dial. Transplant.* **18**, 2vii–9vii.

[5] Maisonneuve, P., Agodoa, L., Gellert, R., Stewart, J.H., Buccianti, G., Lowenfels, A.B., Wolfe, R.A., Jones, E., Disney, A.P.S., Briggs, D., McCredie, M. and Boyle, P. (1999) "Cancer in patients on dialysis for end-stage renal disease: an international collaborative study", *Lancet* **354**, 93–99.

[6] Vamvakas, S., Bahner, U. and Heidland, A. (1997) "Erhöhte Krebsinzidenz bei terminaler Niereninsuffizienz: potentielle pathogenetische Mechanismen", *Schweiz. Med. Wochenschr.* **127**, 597–604.

[7] Himmelfarb, J., Stenvinkel, P., Ikizler, T.A. and Hakim, R.M. (2002) "The elephant in uremia: oxidant stress as a unifying concept of cardiovascular disease in uremia", *Kidney Int.* **62**, 1524–1538.

[8] Ceballos-Picot, I., Witko-Sarsat, V., Merad-Boudia, M., Nguyen, A.T., Thévenin, M., Jaudon, M.C., Zingraff, J., Verger, C., Jungers, P. and Descamps-Latscha, B. (1996) "Glutathione antioxidant system as a marker of oxidative stress in chronic renal failure", *Free Radic. Biol. Med.* **21**, 845–853.

[9] Canestrari, F., Buoncristiani, U., Galli, F., Giorgini, A., Albertini, M.C., Carobi, C., Pascucci, M. and Bossu, M. (1995) "Redox state, antioxidative activity and lipid peroxidation in erythrocytes and plasma of chronic ambulatory peritoneal dialysis patients", *Clin. Chim. Acta* **234**, 127–136.

[10] Jackson, P., Loughrey, C.M., Lightbody, J.H., McNamee, P.T. and Young, I.S. (1995) "Effect of hemodialysis on total capacity and serum antioxidants in patients with chronic renal failure", *Clin. Chem.* **41**, 1135–1138.

[11] Pastor, M.C., Sierra, C., Bonal, J. and Teixid, J. (1993) "Serum and erythrocyte tocopherol in uremic patients. Effect of hemodialysis versus peritoneal dialysis", *Am. J. Nephrol.* **13**, 238–243.

[12] Walter, M.F., Blumberg, J.B., Dolnikowsi, G. and Handelman, G.J. (2000) "Streamlined method for plasma esterified isoprostanes, using HPLC purification and measurement by GC/MS", *Anal. Biochem.* **280**, 73–79.

[13] Nawroth, P.P., Bierhaus, A., Vogel, G., Hofmann, M.A., Zumbach, M., Wahl, P. and Ziegler, R. (1999) "Nichtenzymatische Glykolierung und oxidativer Stress bei chronischen Erkrankungen und Diabetes mellitus", *Med. Klin.* **94**, 29–38.

[14] Weiss, M.F. (1999) "Pathogenic role of advanced glycation end-products (AGEs): an overview", *Perit. Dial. Int.* **19**(Suppl. 2), 47–52.

[15] Handelman, G.J. (2000) "Evaluation of oxidant stress in dialysis patients", *Blood Purif.* **18**, 343–349.

[16] Ziouzenkova, O., Asatryan, L., Akmal, M., Tetta, C., Wratten, M.L., Loseto-Wich, G., Juergens, G., Heinecke, J. and Sevanian, A. (1999) "Oxidative cross-linking of ApoB100 and hemoglobin results in low density lipoprotein modification in blood. Relevance to atherogenesis caused by hemodialysis", *J. Biol. Chem.* **274**, 18916–18924.

[17] Stopper, H., Meysen, T., Böckenförde, A., Bahner, U., Heidland, A. and Vamvakas, S. (1999) "Increased genomic damage in lymphocytes of patients before and after long-term maintenance hemodialysis therapy", *Am. J. Kidney Dis.* **34**, 433–437.

[18] Collins, A.R. (1999) "Oxidative DNA damage, antioxidants and cancer", *Bioassays* **21**, 238–246.

[19] ESCODD (European Standards Committee on oxidative DNA damage) (2002) "Comparative analysis of baseline 8-oxo-7,8-dihydroguanine in mammalian cell DNA, by different methods in different laboratories: an approach to consensus", *Carcinogenesis* **23**, 2129–2133.

[20] Tarng, D.-C., Huang, T.-P., Wei, Y.-H., Liu, T.-Y., Chen, H.-W., Chen, T.W. and Yang, W.-C. (2000) "8-hydroxy-2′-deoxyguanosine of leukocyte DNA as a marker of oxidative stress in chronic hemodialysis patients", *Am. J. Kidney Dis.* **36**, 934–944.

[21] Janzowski, C., Mueller, C., Riedel, M., Morgenstern, T., Albert, F.W., Vienken, J. and Eisenbrand, G. (2001) "Oxidative DNA damage of peripheral leukocytes in hemodialysis patients", *Proc. Am. Assoc. Cancer Res.* **42**, 468.

[22] Singh, N.P., McCoy, M.T., Tice, R.R. and Schneider, E.L. (1988) "A simple technique for quantitation of low levels of DNA damage in individual cells", *Exp. Cell Res.* **175**, 184–191.

[23] Collins, A.R., Dušinská, M., Gedik, C.M. and Štětina, R. (1996) "Oxidative damage to DNA: Do we have a reliable biomarker?", *Environ. Health Perspect.* **104**, 465–469.

[24] Glaab, V., Eisenbrand, G., Collins, A.R. and Janzowski, J. (2001) "DNA damaging potential, mutagenicity and glutathione depletion of 2-cyclohexene-1-one in mammalian cells, compared to food relevant 2-alkenals", *Mutat. Res.* **497**, 185–197.

[25] Gallagher, E.P., Kavanagh, T.J. and Eaton, D.L. (1994) "Glutathione, oxidized glutathione, and mixed disulfides in biological samples", *Methods Toxicol. 1B*, 349–366.

[26] Leuratti, C., Singh, R., Lagneau, C., Farmer, P.B., Plastaras, J.P., Marnett, L.J. and Shuker, D.E.G. (1998) "Determination of malondialdehyde-induced DNA damage in human tissues using an immunoslot blot assay", *Carcinogenesis* **19**, 1919–1924.

[27] Singh, R., Leuratti, C., Josyula, S., Sipowicz, M.A., Diwan, B.A., Kasprzak, K.S., Schut, H.A.J., Marnett, L.J., Anderson, L.M. and Shuker, D.E.G. (2001) "Lobe-specific increases in malondialdehyde DNA adduct formation in the livers of mice following infection with *Helicobacter hepaticus*", *Carcinogenesis* **22**, 1281–1287.

[28] Himmelfarb, J., Lazarus, M. and Hakim, R. (1991) "Reactive oxygen species production by monocytes and polymorphonuclear leukocytes during dialysis", *Am. J. Kidney Dis.* **17**, 271–276.

[29] Stopper, H., Boullay, F., Heidland, A., Vienken, J. and Bahner, U. (2001) "Comet-assay analysis identifies genomic damage in lymphocytes of uremic patients", *Am. J. Kidney Dis.* **38**, 296–301.

[30] Tarng, D.-C., Huang, T.-P., Wei, Y.-H., Liu, T.-Y., Chen, H.-W., Sung, Y.-J. and Wei, Y.-H. (2000) "Effect of vitamin E-bonded membrane on the 8-hydroxy-2′-deoxyguanosine level in leukocyte DNA of hemodialysis patients", *Kidney Int.* **58**, 790–799.

[31] Roob, J.M., Khoschsorbur, G., Tiran, A., Horina, J.H., Holzer, H. and Winklhofer-Roob, B.M. (2000) "Vitamin E attenuates oxidative stress induced by intravenous iron in patients on hemodialysis", *J. Am. Soc. Nephrol.* **11**, 539–549.

[32] Zanen, A.L., Adriaansen, H.J., van Bommel, E.F., Posthuma, R. and de Jong, G.H. (1996) "The oversaturation of transferrin after intravenous ferric gluconate (Ferrlecit®) in haemodialyis patients", *Nephrol. Dial. Transplant.* **11**, 820–824.

[33] Ziouzenkova, O., Asatryan, L., Tetta, C., Wratten, M.L., Hwang, J. and Sevanian, A. (2002) "Oxidative stress during *ex vivo* hemodialysis of blood is decreased by a novel hemolipodialysis procedure utilizing antioxidants", *Free Radic. Biol. Med.* **33**, 248–258.

Free Radic Res Downloaded from informahealthcare.com by Library of Health Sci-Univ of Il on 11/28/11
For personal use only.

RIGHTSLINK