Effect of Different Hemodialysis Regimens on Genomic Damage in End-Stage Renal Failure

Nicole Schupp,* Helga Stopper,* Przemyslaw Rutkowski,†
Kristin Kobras,* Michael Nebel,‡ Udo Bahner,§ Jörg Vienken,¶ and August Heidland‖

Patients with end-stage renal disease display enhanced genomic damage that may have pathophysiologic relevance for cancer development and cardiovascular complications. We investigated to what extent the genomic damage in peripheral blood lymphocytes can be modulated #1: by initiation of standard hemodialysis (SHD) in formerly conservatively treated end-stage renal disease patients, #2: by a switch from SHD to hemodiafiltration, and #3: daily dialysis (DHD). Genomic damage was evaluated by the micronuclei (MN) frequency test and the comet assay (CA). In a prospective study we found that initiation of SHD did not induce significant changes of genomic damage in peripheral blood lymphocytes whereas the change to hemodiafiltration improved the percentage of DNA in the tail as measured by CA without modulating the MN frequency. In a cross-sectional investigation the degree of genomic damage as evaluated by MN frequency was significantly lower in a patient group treated by DHD as compared with a group treated by SHD. In the DHD patients there also was a significant decrease of the plasma concentrations of urea and the advanced glycation end products imidazolone A, carboxymethyllysine, and of advanced glycation end product–associated fluorescence.
Semin Nephrol 26:28-32 © 2006 Elsevier Inc. All rights reserved.

KEYWORDS hemodiafiltration, daily hemodialysis, genomic damage, comet assay, micronuclei, AGEs

An enhancement of genomic damage in end-stage renal disease (ESRD) has been shown by the micronuclei (MN) frequency test[1] and the formation of so-called tails in the comet assay (CA) (single-cell gel electrophoresis).[2] In addition, the determination of the 8-hydroxy 2′-deoxyguanosine (8-OH-dG) content in leukocyte DNA,[3] the analysis of sister chromatid exchange and chromosome aberrations,[4] as well as the demonstration of mitochondrial DNA deletions in skeletal muscle[5] and hair follicles[6] support the observation of increased DNA damage in ESRD. Tarng et al[7] also described that a polymorphism of the human OGG1 gene (1245C→G, genotype 1245GG) is a determinant of genomic damage in leukocytes.

The consequences of DNA damage are far reaching. If these alterations are left unrepaired or are repaired with errors, mutations of critical genes may occur. In various experimental and human malignant diseases, higher DNA modifications were found in the cancerous tissue as compared with the surrounding histologically normal tissue.[8] In renal cell tumors of ESRD patients, mutations of mitochondrial DNA have been observed, which differ from those known for renal cell tumors of the general population.[9] DNA damage also may be involved in the initiation and progression of cardiovascular diseases. In the aging human heart muscle, oxidative DNA modifications with deletions in mitochondrial DNA have been observed, associated with muscle weakness.[10] Enhanced oxidative DNA damage also has been described in human atherosclerosis. In particular, in atherosclerotic plaques of the

*Institute of Pharmacology and Toxicology, University of Würzburg, Würzburg, Germany.
‖Department of Internal Medicine, University of Würzburg, Würzburg, Germany.
†Department of Nephrology, Transplantology and Internal Medicine, Medical University, University Gdansk, Poland.
‡Kuratorium for Dialysis and Kidney Transplantation, Cologne, Germany.
§Kuratorium for Dialysis and Kidney Transplantation, Würzburg, Germany.
¶Fresenius Medical Care, Bad Homburg, Germany.
Address reprint requests to Professor August Heidland, Department of Internal Medicine, University of Würzburg, Josef Schneider-Strasse 2, 97080 Würzburg, Germany. E-mail: August.Heidland@t-online.de

Table 1 Genomic Damage in Peripheral Lymphocytes and Blood Chemical Parameters of Healthy Controls and of Patients Switched From Predialysis to SHD (SHD1) and of Patients Switched From SHD (SHD2) to HDF

	Predialysis (n = 5)	SHD1 (n = 5)	SHD2 (n = 7)	HDF (n = 7)	Control
MN/1,000 BN	34.3 ± 9.3	30.3 ± 7.3	31.0 ± 11.5	30.7 ± 12.0	16.5 ± 4.5*
DNA in tail (%)	14.5 ± 0.5	13.9 ± 1.0	13.7 ± 0.5	12.4 ± 0.9†	9.7 ± 0.2‡
Creatinine, mg/dL	6.6 ± 1.7	7.8 ± 1.7	8.2 ± 2.0	8.6 ± 1.9	
Urea, mg/dL	129 ± 66.1	106 ± 62.4	93 ± 51.5	119 ± 30.0	

NOTE. Mean ± SD shown.
*$P \leq .05$ control level versus predialysis, SHD1/2, and HDF.
†$P \leq .05$ HDF compared with SHD1/2 and predialysis levels.
‡$P < .01$ control level versus predialysis, SHD1/2, and HDF.

carotid artery, a strong immunoreactivity against the oxidative DNA damage marker 8-OH-dG and increased DNA strand breaks in the CA have been found in all cell types of the plaque.[11]

With regard to the high incidence of cancer[12,13] and cardiovascular morbidity/mortality in ESRD patients[14,15] and the involvement of genomic damage in the development of these disturbances, DNA damage should be a target of therapeutic interventions.

Until now there has been little information regarding whether the degree of genomic damage can be modulated in ESRD. When studying the effect of different dialysis membranes, 8-OH-dG levels in leukocyte DNA have been shown to be highest in patients when using cellulose dialyzers as compared with vitamin E–bounded cellulose dialysers and with the synthetic polymethyl methacrylate and polysulphone membranes.[16]

In this report we speculate whether DNA damage in peripheral blood lymphocytes (PBLs) of ESRD patients can be influenced 1: by initiation of hemodialysis (SHD) in hitherto conservatively treated patients, 2: by a switch from standard hemodialysis (SDH) to hemodiafiltration (HDF) therapy or 3: by short daily dialysis (DHD) compared with SHD. The first 2 studies were performed longitudinally, the third study was performed in a cross-sectional manner because only a limited number of patients are treated this way.

Biomonitoring of genomic damage was done in PBLs by measurement of the MN frequency and the CA. The MN frequency test is a very sensitive and widely accepted marker for in vitro and in vivo genotoxicity investigations.[17-19] MN are DNA-containing particles that arise during mitosis and result from unrepaired DNA double-strand breaks, leading to chromatin fragments or whole chromosomes incorrectly distributed during mitosis. The CA is a direct method used to detect the presence of DNA single- or double-strand breaks and alkali-labile damage in individual cells.[20] It has proven to be very sensitive as well.

In our studies we also included measurements of various circulating advanced glycation end products (AGEs) that are involved in many uremic complications and exert genotoxic effects in vitro.[21] We analyzed the plasma levels of imidazolone A, carboxymethyllysine (CML; only in DHD/SHD patients), and the AGE-associated fluorescence before and after

changing the treatment modalities. Imidazolone A was determined by enzyme-linked immunosorbent assay by using a noncommercial, monoclonal antibody that is specific for imidazolone A.[22] CML was measured by the same method using the CML-specific monoclonal antibody 4G9 (Roche Diagnostic GmbH, Penzberg, Germany). AGE-associated fluorescence was analyzed by fluorescence spectroscopy according to Münch et al.[23]

Genomic Damage in PBLs of Conservatively Treated ESRD Patients Switching to SHD

In earlier investigations decreased levels of DNA repair mechanisms were found in conservatively treated predialysis patients, whereas the initiation of SHD was associated with a normal or even slightly enhanced DNA repair.[24,25] With regard to these findings we hypothesized that the initiation of SHD should decrease the degree of DNA damage as compared with the predialysis period.

Therefore, a prospective study of genomic damage was performed in a small group of 5 stable ESRD patients (unpublished data). In all 5 patients, MN frequency and the percentage of DNA in the tail were analyzed at monthly intervals during the predialysis phase ($\approx$6 mo) and after the initiation of SHD ($\approx$7 mo). In line with our earlier cross-sectional study,[1,2] in the predialysis patients the number of MN and the percentage of DNA in the tail was significantly enhanced as compared with healthy controls (Table 1). However, the initiation of SHD did not induce significant changes, although a tendency to less genomic damage was found. Thus, the formerly observed normal or slightly enhanced DNA repair after initiation of SHD[25] evidently was insufficient for correcting DNA damage in our patients. The missing significance of the treatment change could be caused, at least in part, by the fact that the degree of uremic toxicity after initiation of SHD therapy was not better than during the predialysis period because of a progressive deterioration of residual renal function. In fact, there was no significant difference in plasma creatinine and urea concentrations, or in the plasma levels of circulating AGEs (Tables 1 and 2).

The persistent high degree of genomic damage in the

Table 2 Levels of AGEs in the Plasma of Predialysis, SHD, and HDF Patients and of Healthy Controls

	Predialysis	SHD	HDF	Control
Fluorescence AGEs, AU/mg protein	14.5 ± 5.4	14.1 ± 6.4	13.5 ± 2.6	5.5 ± 0.9*
Imidazolone A, mU/mg protein	66.1 ± 3.0	72.4 ± 1.0	74.4 ± 2.0	43.2 ± 0.9*

NOTE. Mean ± SD shown.
*P ≤ .05 control versus predialysis, SHD, and HDF.

SHD patients may be a risk factor for the excess incidence of cancer and cardiovascular morbidity/mortality.

Genomic Damage of SHD Patients Switched to HDF

The effect of HDF was analyzed because this kind of treatment combines the diffusion mechanism of SHD with the convective component of hemofiltration. Therefore, it offers an improved solute clearance for both low and high molecular weight uremic toxins by enhancing convective clearance through highly permeable membranes.[26] In a group of 7 stable ESRD patients who were treated initially with SHD, genomic damage was evaluated monthly for 3.5 months and then after the switch to HDF for approximately 7 months (unpublished data). The results showed that the change of therapy provided heterogeneous effects: although there was a significant decrease in the genomic damage analyzed by the CA, no alterations in the MN frequency were observed (Table 1). One possible explanation for these inconsistent DNA data is the different sensitivity of the CA and the MN frequency test to DNA repair mechanisms. Genomic damage analyzed with the CA can be repaired by the cells whereas the formation of MN cannot be reversed. Therefore, the MN frequency provides a record of accumulated genomic damage over the lifespan of the lymphocyte, which is approximately 3 to 4 years. Subsequently, it is conceivable that the observation period after the switch to HDF in this group may not have been long enough to observe significant changes in the MN frequency test. This assumption is supported by a recent cross-sectional, pair-wise investigation in patients on long-term SHD and on long-time HDF therapy, in whom a significantly lower level of DNA damage was observed both in the CA and in the MN frequency test in the latter group (unpublished data).

With regard to the uremic retention products, plasma creatinine, blood urea concentration, and circulating AGE levels remained unchanged. The data from the AGE measurements are in line with earlier studies that showed that their removal by SHD or HDF is poor. Our findings, however, do not exclude decreasing toxic plasma peptide levels such as β_2-microglobulin after the initiation of HDF, which were not analyzed in the current study.

The fact that HDF only slightly improved the degree of genomic damage underlines the need for further improvement of this kind of renal replacement therapy.

Effect of DHD Therapy on Genomic Damage

In predialysis patients, a direct relationship between the severity of renal impairment and the degree of genomic damage has been shown.[2] We hypothesized that an improved uremic state of ESRD induced by DHD should be associated with fewer DNA lesions. In fact, in these patients, the plasma levels of various uremic toxins including circulating AGEs are lower owing to shorter interdialytic intervals and an increased removal of small solutes.[27-29] In a cross-sectional study, the genomic damage of 2 patient groups with ESRD, treated with either SHD or DHD, was analyzed.[30] The SHD group consisted of 12 patients and the DHD group, 13 patients. Dialysis was performed with synthetic noncomple-

Table 3 Plasma Parameters of Inflammation and Oxidative Stress and Levels of AGEs in the Plasma of SHD Patients, DHD Patients, and Healthy Controls

	DHD	SHD	Control
Interleukin-6, pg/mL	10.63 ± 7.73	7.90 ± 3.61	1.41 ± 0.38*
Neopterin, ng/mL	66.69 ± 18.71†	83.25 ± 10.78	1.38 ± 0.47*
Homocysteine, μmol/L	26.27 ± 4.81	32.58 ± 20.63‡	13.9 ± 9.1*
Imidazolone A, mU/mg protein	46.38 ± 11.96§	82.99 ± 42.31	24.54 ± 7.62‖
CML, ng/mg protein	2.01 ± 1.05†	3.15 ± 1.26	0.014 ± 0.0004
Fluorescent AGEs, AU/mg	14.26 ± 3.74†	19.09 ± 7.83	4.00 ± 1.22*
Urea, mg/dL	115 ± 53.5†	159 ± 48.7	
Creatinine, mg/dL	8.25 ± 2.29	9.30 ± 3.67	

NOTE. Mean ± SD shown.
*P < .01 (control versus SHD and DHD).
†P ≤ .05 (DHD versus SHD).
‡P ≤ .05 (SHD versus control).
§P < .01 (DHD versus SHD).
‖P < .01 (control versus SHD).

ment-activating polyethersulphone membranes. Twelve healthy patients served as controls. In each patient the number of MN/1,000 binucleated cells (BN) cells was determined 3 to 4 times in 1- to 2-month intervals. The CA could not be performed in this study because of technical reasons. As expected, the results in the SHD group showed markedly increased MN levels compared with healthy controls. On the contrary, in the DHD group (Table 3) the levels were significantly lower and were comparable with healthy controls. This amelioration was associated with significantly lower plasma concentrations of urea and of the AGEs CML, imidazolone A, and of AGE-associated fluorescence, which is in line with the former prospective study by Floridi et al[29] in DHD patients. The improved uremic state therefore could be the determinant of the decreased genomic damage.

Among the factors involved in the amelioration, circulating AGE levels have to be mentioned because their genotoxic effect was shown in in vitro studies.[21] AGEs also can react with DNA in a similar way as with proteins, resulting in the formation of DNA-bonded AGEs.[31] Moreover, the genotoxicity of accumulated carcinogenic heterocyclic amines, such as the dietary pyrolysis products of tryptophane and glutamic acid and imidazoquinoline compounds, has to be discussed in uremia. In DHD patients, lower plasma levels are expected because of the higher rate of small-solute clearance that could contribute, at least in part, to improve the genomic damage.

Another important factor for genomic damage is the occurrence of hypomethylation. In ESRD, an intracellular accumulation of the natural methyltransferase inhibitor S-adenosylhomocysteine, which impairs methylation-dependent repair processes, has been shown.[32] Adenosylhomocysteine levels were related directly to the blood concentration of homocysteine in ESRD. In our study homocysteine levels were markedly increased in both groups compared to controls. In DHD patients they were slightly (19%) lower than in SHD patients and therefore could play only a minor role in explaining the decreased DNA damage.

The question of whether the amelioration of genomic damage in PBLs by DHD is associated with a corresponding improvement of high cancer incidence and cardiovascular complications needs to be analyzed in long-term epidemiologic studies.

References

1. Stopper H, Meysen T, Böckenförde A, et al: Increased genomic damage in lymphocytes of patients before and after long-term maintenance hemodialysis therapy. Am J Kidney Dis 34:433-437, 1999
2. Stopper H, Boullay F, Heidland A, et al: Comet-assay analysis identifies genomic damage in lymphocytes of uremic patients. Am J Kidney Dis 38:296-301, 2001
3. Tarng DC, Huang TP, Wei YH, et al: 8-hydroxy-2′-deoxyguanosine of leukocyte DNA as a marker of oxidative stress in chronic hemodialysis patients. Am J Kidney Dis 36:934-944, 2000
4. Cengiz K, Block AM, Hossfeld DK, et al: Sister chromatid exchange and chromosome abnormalities in uremic patients. Cancer Genet Cytogenet 36:55-67, 1988
5. Lim PS, Cheng YM, Wei YH: Large-scale mitochondrial DNA deletions in skeletal muscle of patients with end-stage renal disease. Free Radic Biol Med 29:454-463, 2000
6. Liu CS, Ko LY, Lim PS, et al: Biomarkers of DNA damage in patients with end-stage renal disease: Mitochondrial DNA mutation in hair follicles. Nephrol Dial Transplant 16:561-565, 2001
7. Tarng DC, Tsai TJ, Chen WT, et al: Effect of human ogg1 1245c->g gene polymorphism on 8-hydroxy-2′-deoxyguanosine levels of leukocyte DNA among patients undergoing chronic hemodialysis. J Am Soc Nephrol 12:2338-2347, 2001
8. Loft S, Poulsen H: Cancer risk and oxidative DNA damage in man. J Mol Med 74:297-312, 1996
9. Nagy A, Wilhelm M, Kovacs G: Mutations of mtDNA in renal cell tumours arising in end-stage renal disease. J Pathol 199:237-242, 2003
10. Hayakawa M, Hattori K, Sugiyama S, et al: Age-associated oxygen damage and mutations in mitochondrial DNA in human hearts. Biochem Biophys Res Commun 198:979-985, 1992
11. Martinet W, Knaapen M, De Meyer G, et al: Elevated levels of oxidative DNA damage and DNA repair enzymes in human atherosclerotic plaques. Circulation 106:927-932, 2002
12. Maisonneuve P, Agodoa L, Gellert R, et al: Cancer in patients on dialysis for end-stage renal disease: An international collaborative study. Lancet 354:93-99, 1999
13. Teschner M, Garte C, Ruckle-Lanz H, et al: Incidence and spectrum of malignant disease among dialysis patients in north bavaria. Dtsch Med Wochenschr 127:2497-2502, 2002
14. Herzog C, Ma J, Collins A: Poor long-term survival after acute myocardial infarction among patients on long-term dialysis. N Engl J Med 339:799-805, 1998
15. Beddhu S, Allen-Brady K, Cheung AK, et al: Impact of renal failure on the risk of myocardial infarction and death. Kidney Int 62:1776-1783, 2002
16. Tarng DC, Huang TP, Liu TY, et al: Effect of vitamin E-bonded membrane on the 8-hydroxy 2′-deoxyguanosine level in leukocyte DNA of hemodialysis patients. Kidney Int 58:790-799, 2000
17. Fenech M: The cytokinesis-block micronucleus technique: A detailed description of the method and its application to genotoxicity studies in human populations. Mutat Res 285:35-44, 1993
18. Miller B, Potter-Locher F, Seelbach A, et al: Evaluation of the in vitro micronucleus test as an alternative to the in vitro chromosomal aberration assay: Position of the gum working group on the in vitro micronucleus test. Gesellschaft fuer umwelt-mutations-forschung. Mutat Res 410:81-116, 1998
19. Stopper H, Eckert I, Wagener P, et al: Formation of micronuclei and inhibition of topoisomerase II in the comet assay in mammalian cells with altered DNA methylation. Recent Results Cancer Res 143:183-193, 1997
20. Kassie F, Parzefall W, Knasmuller S: Single cell gel electrophoresis assay: A new technique for human biomonitoring studies. Mutat Res 463:13-31, 2000
21. Stopper H, Schinzel R, Sebekova K, et al: Genotoxicity of advanced glycation end products in mammalian cells. Cancer Lett 190:151-156, 2003
22. Niwa T, Katsuzaki T, Miyazak S, et al: Immunohistochemical detection of imidazolone, a novel advanced glycation end product, in kidneys and aortas of diabetic patients. J Clin Invest 99:1272-1280, 1997
23. Münch G, Keis R, Wessels A, et al: Determination of advanced glycation end products in serum by fluorescence spectroscopy and competitive ELISA. Eur J Clin Chem Clin Biochem 35:669-677, 1997
24. Malachi T, Zevin D, Gafter U, et al: DNA repair and recovery of RNA synthesis in uremic patients. Kidney Int 44:385-389, 1993
25. Vamvakas S, Bahner U, Becker P, et al: Impairment of DNA repair in the course of long-term hemodialysis and under cyclosporine immunosuppression after renal transplantation. Transplant Proc 28:3468-3473, 1996
26. Canaud B, Bosc JY, Leray H, et al: On-line haemodiafiltration: State of the art. Nephrol Dial Transplant 13:3-11, 1998 (suppl 5)
27. Ting GO, Kjellstrand C, Freitas T, et al: Long-term study of high-comorbidity ESRD patients converted from conventional to short daily hemodialysis. Am J Kidney Dis 42:1020-1035, 2003
28. Williams AW, Chebrolu SB, Ing TS, et al: Early clinical, quality-of-life, and biochemical changes of "daily hemodialysis" (6 dialyses per week). Am J Kidney Dis 43:90-102, 2004

29. Floridi A, Antolini F, Galli F, et al: Daily haemodialysis improves indices of protein glycation. Nephrol Dial Transplant 17:871-878, 2002
30. Fragedaki E, Nebel M, Schupp N, et al: Genomic damage and circulating age levels in patients undergoing daily versus standard hemodialysis. Nephrol Dial Transplant (in press)
31. Pischetsrieder M, Seidel W, Münch G, et al: N2(1-carboxylethyl)deoxy-guanosine, a nonenzymatic glycation adduct of DNA, induces single-strand breaks and increases mutation frequencies. Biochem Biophys Res Commun 264:544-549, 1999
32. Perna AF, Ingrosso D, De Santo NG, et al: Mechanism of erythrocyte accumulation of methylation inhibitor S-adenosylhomocysteine in uremia. Kidney Int 47:247-253, 1995