Genomic Damage in End-Stage Renal Failure: Potential Involvement of Advanced Glycation End Products and Carbonyl Stress

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In patients with chronic renal failure, genomic damage has been shown by numerous biomarkers, such as micronuclei frequency and comet assay (single-cell gel electrophoresis) in peripheral lymphocytes, 8-hydroxy 2'-deoxyguanosine (8-OH-dG) content in leukocytes, mitochondrial DNA deletions in skeletal muscle tissue and hair follicles, as well as in DNA repair mechanisms in freshly isolated lymphocytes after ultraviolet light exposure. In the pathogenesis of DNA damage—besides genetic influences, enhanced reactive oxygen species (ROS), and lipid peroxidation—the genotoxic potential of advanced glycation end products (AGEs) and reactive carbonyl compounds deserve special attention. In fact, reactions of glucose with DNA can lead to mutagenic DNA AGEs. In vitro, incubation of tubulus cells with various AGEs and methylglyoxal induces DNA damage, which is suppressed by antioxidants. This underlines the role played by oxidative stress in DNA damage.

Semin Nephrol 24:474-478 © 2004 Elsevier Inc. All rights reserved.

KEYWORDS genomic damage, micronuclei, comet assay, AGEs, reactive carbonyl compounds

Reactive oxygen species (ROS) are generated by cellular respiration, intracellular signal transduction, phagocytosis, and metabolism of xenobiotics. Enhanced levels of ROS lead to an increased reactivity with biomolecules (eg, DNA, proteins, and lipids). DNA damage is of central interest because of its genetic consequences. The spectrum of DNA lesions is far-reaching and includes oxidative DNA base modifications, abasic sites, deoxyribose damage, single- and double-strand breaks, genomic instability, and inhibition of repair systems. DNA lesions may induce mutations and represent one of the mechanisms of carcinogenesis. They also have been linked to aging, neurodegenerative diseases, diabetes, and atherosclerosis. Patients with end-stage renal failure are exposed to an increased oxidative stress, as evidenced by decreased levels of the antioxidant system, as well as an increased ROS formation. Consequently, an enhanced incidence of genomic damage with potential cancer development has to be expected. In fact, in numerous studies a high incidence of cancer has been shown in end-stage renal failure patients. This article summarizes the current data on DNA damage in patients with chronic renal failure. Moreover, our recent studies on the potential genotoxicity of advanced glycation end products (AGEs) and reactive carbonyl compounds are presented.

Micronuclei Frequency

Micronuclei (MN) are sensitive indicators of exogenously or endogenously caused genetic damage and have become an important end point in genotoxicity testing. They are chromatin-containing structures in the cell cytoplasm, surrounded by a membrane without any detectable link to the cell nucleus. They are formed by exclusion of whole chromosomes or chromatin fragments during mitosis. We found a significant increase of MN by using the method of Fenech in peripheral lymphocytes of patients with severe renal fail-
ure before maintenance hemodialysis (MHD) as compared with age-matched healthy control subjects (28.2 ± 9.4 versus 15.3 ± 4.7 MN/1,000 binucleate). A further increase of MN frequency (44.3 ± 13.7 MN/1,000 binucleate) was observed in patients on MHD (>100 mo) (Fig 1).

**Figure 1** MN in end-stage renal disease. MN frequencies in binucleate (BN) lymphocytes. Co, healthy individuals (n = 23); Pre, predialysis (n = 19); HD, hemodialysis (n = 16). (Reprinted from Stopper et al\textsuperscript{11} with permission.)

**Single-Cell Gel Electrophoresis (Comet Assay)**

Another useful method for quantifying DNA damage is the alkaline comet assay, which has gained fast acceptance because of its simplicity and sensitivity.\textsuperscript{12} In this test, cells are embedded in agar and exposed to an electrical field. From cells with damaged DNA, more of the genetic material can migrate in the electrical field than from cells with intact nuclear DNA. The presence of single- or double-strand breaks, alkali labile sites, and relaxed chromatin cause the resulting DNA fragments or loops to move ahead of the intact nuclear DNA. A comet-like structure is formed because smaller fragments and relaxed loops move faster than larger fragments and intact DNA. Comets are quantified microscopically after appropriate DNA staining. We applied this assay in peripheral lymphocytes according to the method described by Singh et al,\textsuperscript{13} with slight modifications in patients with advanced renal failure.\textsuperscript{14} Although in the age-matched control group of healthy subjects the DNA damage averaged 10.5% ± 0.8%, a marked increase was observed in patients with chronic renal insufficiency in relation to the decrease of glomerular filtration rate. In patients with a creatinine level greater than 6 mg/dL, the mean DNA damage increased to 17.7% ± 3.0%. During MHD therapy, the DNA damage averaged 16.7% ± 4.3%. A continuous increase occurred in the long-term treatment, with the highest values reached after more than 10 years. Mean values are shown in Figure 2.

By using the comet assay, DNA damage also has been shown in lymphocytes of rats with chronic renal failure induced by subtotal nephrectomy. Interestingly, the severe

**Figure 2** DNA damage (%) in patients with chronic kidney disease. Comet assay analysis of DNA damage in peripheral lymphocytes. Co, controls (n = 21); Pre, predialysis (n = 23); Pre Crea > 6, subgroup with serum creatinine levels greater than 6 mg/dL (n = 12); HD, hemodialysis (n = 26); HD > 10a, subgroup with treatment duration greater than 10 years (n = 8); HDF, hemodiafiltration (n = 15). (Reprinted from Stopper et al\textsuperscript{14} with permission.)
damage could be ameliorated by long-term therapy with the angiotensin II type I receptor antagonist, losartan.\textsuperscript{15}

8-Hydroxy 2'-Deoxyguanosine

8-hydroxy 2'-deoxyguanosine (8-OH-dG) is an important parameter for the evaluation of oxidative DNA damage and is measured by high-performance liquid chromatography (HPLC) electrochemical detection or gas chromatography/mass spectrometry. It is an established mutagenic\textsuperscript{16} and induces G\textsuperscript{3}T transversions, commonly observed in mutated oncogenes and tumor suppressor genes.\textsuperscript{2} In peripheral leukocytes of patients with chronic renal failure, a gradual increase of 8-OH-dG was observed with the progression of the disease. Markedly increased levels, as compared with healthy control subjects, have been found in patients on MHD as well as on peritoneal dialysis therapy.\textsuperscript{17,18} As determinants of leukocyte 8-OH-dG content, a relationship to intracellular ROS production as well as to serum iron concentration could be documented. The 8-OH-dG content in leukocytes is increased further by the kind of hemodialysis treatment. The use of biocompatible complement- and leukocyte-activating membranes was followed by higher levels of 8-OH-dG as compared with synthetic and vitamin A–bonded membranes, as a consequence of the enhanced ROS generation.\textsuperscript{17}

Mitochondrial DNA Deletions

Mitochondria are a major intracellular source of ROS and free radicals. Because of a lack of protective histones and a low efficiency of DNA repair, mitochondrial DNA seems to be particularly sensitive to ROS.\textsuperscript{19} In patients with end-stage renal failure, the frequency of 4,977-bp deletions in the skeletal muscle tissue was enhanced significantly, as compared with normal aged controls (77\% versus 22\%).\textsuperscript{20} Similar data were obtained when mitochondrial DNA deletions were analyzed by the polymerase chain reaction technique in hair follicles of patients with end-stage renal failure.\textsuperscript{21}

DNA Repair Mechanisms

A decrease in ultraviolet light–induced DNA repair has been shown in freshly isolated lymphocytes from patients with pre–end-stage renal failure not enrolled in the MHD program.\textsuperscript{22} After initiation of the replacement therapy, a tendency to an increased DNA repair has been described, followed by a suppression after long-term MHD therapy.\textsuperscript{23} Interestingly, in patients who had developed cancer during the treatment, a reduced repair was observed even in the early stages of MHD.

According to these data in end-stage renal failure (both in the predialysis and dialysis phases), noticeable genomic damage occurs, which could be of importance in carcinogenesis and, possibly, in the accelerated atherosclerosis observed in these patients. However, this concept has not as yet been confirmed in long-term prospective investigations.

Advanced Glycation End Products and Reactive Carbonyl Compounds as Mediators of DNA Damage

In the pathogenesis of DNA damage, a plethora of factors could be involved. Besides genetic influences, enhanced ROS formation, lipid peroxidation products, hypomethylation,
and iron overload could be contributory factors. In particular, the role played by AGEs and reactive carbonyl compounds has to be taken into account. Both are markedly enhanced in end-stage renal failure. In the past few years it has been shown that AGEs and reactive carbonyl compounds not only cause progressive and irreversible modifications of proteins but also possess genotoxic potential. In fact, glucose reacts with DNA in a similar way to proteins, resulting in the formation of DNA-bonded AGEs, thereby various mutagenic effects of DNA AGEs, such as deletions, insertions, and transposon activation, were shown in bacterial model systems. Glucose reacts with the 2-NH₃ group of guanosines in particular, yielding carboxymethylguanosine as a major product. We recently studied the in vitro genotoxicity of various AGEs in cultured LLC-PK₁ cells, a porcine cell line with properties of proximal tubular cells. Besides AGE bovine serum albumin (BSA) and carboxymethyllysine BSA, the AGE precursor, methylglyoxal-BSA—an α-dicarbonyl compound—was assessed. The comet assay was applied as an indicator of DNA damage and ethylmethanesulfonate was used as a positive control. Incubation of the LLC-PK₁ cells with the various AGEs led to a dose-dependent formation of comets, with a saturation response at higher doses. When the cells were pretreated with the proteases trypsin or bromelain, the AGE-induced damage was abolished, suggesting that the observed genotoxicity of AGEs might be receptor-mediated. Proteases most likely cleave the external domain of the cellular receptor of AGEs, resulting in its inactivation (Fig 3).

Binding of AGEs to receptors enhances the formation of ROS. Also, methylglyoxal has been shown to produce marked oxidative stress, which may be mediated in part by glutathione depletion of the cell. Therefore, the effect of various antioxidants prevented the AGE-induced DNA damage.

In line with these data in kidney cells, DNA damage recently has been shown also in human skin cells after exposure to the α-dicarbonyl compounds glyoxal and methylglyoxal. Treatment of the human keratinocytes with glyoxal resulted in extensive DNA strand breaks, whereas methylglyoxal induced cross-linked DNA and led to oxygen-dependent cleavage of plasmid DNA. Moreover, the investigators gave evidence for glycation of nuclear protein by reactive carbonyl stress, as indicated by an accumulation of carboxymethyllysine in the histones.

**Conclusion**

The data discussed earlier show that in end-stage renal failure, a high incidence of DNA damage is documented. Its pathogenesis may include the markedly increased AGE levels as well as carbonyl compounds. The genotoxic effects were prevented in vitro studies by antioxidants. Whether this effect can be reproduced in end-stage renal failure patients remains to be confirmed.

**References**