J Am Soc Nephrol 11: 1744–1752, 2000

Advanced Glycation and Lipoxidation End Products: Role of Reactive Carbonyl Compounds Generated during Carbohydrate and Lipid Metabolism

TOSHIO MIYATA,* KIYOSHI KUROKAWA,* and
CHARLES VAN YPERSELE DE STRIHOU†

**Molecular and Cellular Nephrology, Institute of Medical Sciences and Department of Internal Medicine, Tokai University School of Medicine, Isehara, Japan; and †Service de Nephrologie, Universite Catholique de Louvain, Brussels, Belgium.*

The uremic syndrome is usually attributed to the retention of a variety of compounds as a result of a deficient renal clearance. These retention solutes are taken to induce biochemical disorders characteristic of uremic complications. However, as recently noted by Vanholder and De Smet (1), only a few of them have an established role as uremic toxins.

Most studies on uremic toxins have focused on disorders of enzymatic biochemistry and/or on toxins that are the result of enzymatic biochemistry. In this review, we concentrate on another aspect of uremic toxicity, related to nonenzymatic biochemistry of proteins. We investigate the causal role of various reactive carbonyl compounds (RCO) accumulating in the serum; postulate the existence of "carbonyl stress" in uremia and analyze its clinical consequences; and, finally, discuss therapeutic perspectives.

Accumulation of Advanced Glycation End Products/Advanced Lipoxidation End Products in Uremia

The advanced glycation of proteins has been initially investigated by food and nutrition biochemists (2). The Maillard reaction, a nonenzymatic process, is initiated when proteins are exposed to glucose or other carbohydrates. It generates first reversible Schiff base adducts and subsequently more stable Amadori rearrangement products. Through a series of oxidative and nonoxidative reactions, it eventually yields the irreversible advanced glycation end products (AGE) linked with amino groups, *e.g.*, lysine residues, of several proteins.

In human pathology, irreversible advanced glycation of proteins is a part of the ageing process. It is markedly amplified in diabetes, as a consequence of hyperglycemia: AGE levels are indeed correlated with those of fructoselysine, taken as a surrogate marker of prevailing plasma glucose concentration (3–5). Of interest, they are also correlated with the severity of diabetic complications, a finding supporting their clinical relevance (6–8).

The subsequent discovery that AGE also accumulate in uremic patients was surprising because most uremic individuals have normal blood glucose levels. Sensitive and specific chemical methods including HPLC (9) and gas chromatography/mass spectrometry (GC/MS) (10) were developed to quantify AGE, such as pentosidine (11) and carboxymethyllysine (CML) (12). The levels of these two AGE adducts in plasma proteins of hemodialysis patients proved markedly higher than in control or diabetic subjects (13,14). Other AGE adducts also accumulate in uremia, such as glyoxal-lysine dimer, methylglyoxal-lysine dimer, and imidazolone (15).

Among dialysis patients, those with diabetes and those without had similar plasma pentosidine and CML levels (13,14). In contrast to nonuremic diabetic patients, neither pentosidine nor CML correlated with fructoselysine levels in uremic subjects. Thus, it became clear that factor(s) other than hyperglycemia are critical for AGE formation in uremia. The fact that more than 90% of plasma pentosidine and CML are albumin adducts (13,14) suggests that its accumulation does not result from a decreased renal clearance of AGE-modified proteins.

The second approach to irreversible protein modification in uremia derives from studies of lipid metabolism, especially lipid peroxidation. Proteins are modified not only by carbohydrates but also by lipids (16). For instance, proteins modified by malondialdehyde, which is derived from the oxidation of polyunsaturated fatty acids such as arachidonate, accumulate in hemodialysis patients (14). Malondialdehyde as well as other lipid peroxidation product modified proteins are called advanced lipoxidation end products (ALE) (17).

Uremia is thus characterized by irreversible nonenzymatic protein modifications by carbohydrates or lipids, *i.e.*, AGE/ALE (Table 1). Of note, the levels of AGE and ALE rise concomitantly in uremic serum: Plasma CML, an AGE species, is highly correlated with plasma malonyldialdehyde-lysine, an ALE species, in patients on chronic hemodialysis (14). This observation points to a common cause in the genesis of AGE/

Received October 29, 1999. Accepted December 29, 1999.

Correspondence to Dr. Toshio Miyata, Molecular and Cellular Nephrology, Institute of Medical Sciences and Department of Internal Medicine, Tokai University School of Medicine, Bohseidai, Isehara, Kanagawa 259-1143, Japan. Phone: +81 463 93 1936; Fax: +81 463 93 1938; E-mail: t-miyata@is.icc.u-tokai.ac.jp

1046-6673/1109-1744
Journal of the American Society of Nephrology

Copyright © 2000 by the American Society of Nephrology

Table 1. Accumulation of AGE/ALE in hemodialysis patients[a]

AGE/ALE	Precursor(s)	Reference
Pentosidine	Carbohydrate	13, 18
Carboxymethyllysine	Carbohydrate, lipid, amino acid	14
Malondialdehydelysine	Lipid	14
Imidazolone	Carbohydrate	19
Glyoxal-lysine dimer	Carbohydrate, lipid, amino acid	15
Methylglyoxal-lysine dimer	Carbohydrate, lipid, amino acid	15

[a] AGE, advanced glycation end product; ALE, advanced lipoxidation end product.

ALE. This conclusion is further supported by a recent report that, in the skin of renal failure patients, lipid peroxidation and advanced glycation of matrix collagen increase in close relation to each other (20).

Accumulation of Reactive Carbonyl Compounds in Uremia: Carbonyl Stress

Both AGE and ALE are formed by carbonyl amine chemistry between protein residues and reactive carbonyl compounds (RCO) (17). These RCO are constantly produced by the metabolism of carbohydrates, lipids, and amino acids, all of which are abundantly present throughout the body. RCO, *e.g.*, glyoxal, methylglyoxal, arabinose, glycoaldehyde, 3-deoxyglucosone, and dehydroascorbate, are formed from carbohydrates and ascorbate (21–25). They react nonenzymatically with protein amino groups and eventually yield AGE, *e.g.*, CML, pentosidine, pyrraline, imidazolone, glyoxal-lysine dimer, and methylglyoxal-lysine dimer. Similarly, RCO, *e.g.*, glyoxal, malondialdehyde, hydroxynonenal, and acrolein, are generated by lipid peroxidation of polyunsaturated fatty acids (16,26). In addition, RCO, such as glyoxal, methylglyoxal, acrolein, and glycoaldehyde, are produced during the myeloperoxidase catalyzed metabolism of amino acids (27). These RCO react with proteins and form ALE as well as AGE.

Could the raised levels of AGE and ALE in uremia accrue from an accumulation of carbohydrate- and lipid-derived RCO? Total RCO have been measured in uremic plasma with the 2,4-dinitrophenylhydrazine (DNPH) method. DNPH is known to combine with RCO and to yield hydrazones. The yield of hydrazone is indeed several times higher in uremic than in normal plasma (28). The accumulation of total as well as of individual RCO has also been documented by several groups (17,28–31). Table 2 summarizes the various RCO thus far found to be raised in uremic plasma.

The production of low molecular weight AGE precursors in uremic plasma has also been demonstrated by *in vitro* incubation experiments (28). Plasma samples were incubated under air for several weeks while the generation of pentosidine in the medium was monitored. Protein-linked pentosidine levels rise much more in uremic than in control plasma. Most precursors of this newly formed pentosidine have a molecular weight below 5000 Da. Indeed, when plasma is ultrafiltrated through a filter with a 5000 Da cutoff, the difference in pentosidine generation between uremic and control plasma ultrafiltrate is sustained. This conclusion is further supported by the observation that the pentosidine yield is higher in pre- than in postdialysis plasma samples. Finally, addition of inhibitors of the carbonyl amine reaction, such as aminoguanidine or 2-isopropylidenehydrazono-4-oxo-thiazolidin-5-ylacetanilide (OPB-9195), represses the production of pentosidine, pointing to the RCO nature of the pentosidine precursor(s). Taken together, these results confirm the accumulation of RCO in uremic plasma and suggest that they are indeed precursors of AGE.

In vivo pentosidine levels in plasma are correlated with the level of *in vitro*-generated pentosidine after incubation of the same plasma samples (28). This correlation suggests that the prevailing plasma pentosidine level mirrors the level of its RCO precursor(s) and might be used as a marker of their accumulation.

The accumulation in uremic plasma of various RCO derived from either carbohydrates or lipids and the subsequent carbonyl modification of proteins suggest that chronic uremia may be characterized as a state of carbonyl stress (17). Under

Table 2. Accumulation of RCO in hemodialysis patients[a]

RCO	Precursor(s)	Reference
3-Deoxyglucosone	Carbohydrate	29
Dehydroascorbate	Ascorbic acid	31
Glyoxal	Carbohydrate, lipid, amino acid	30
Methylglyoxal	Carbohydrate, lipid, amino acid	30
Malondialdehyde	Lipid	14
Arabinose	Carbohydrate	Miyata *et al.*, unpublished observation

[a] RCO, reactive carbonyl compound.

carbonyl stress, not only AGE derived from carbohydrates, but also ALE derived from lipids accumulate in parallel in plasma as well as in tissue proteins.

Causes of Uremic Carbonyl Stress

Two competing but not mutually exclusive hypotheses should be considered to account for the uremic carbonyl stress: an increased generation or a decreased removal (detoxification or clearance) of RCO.

Oxidative Stress

Production of RCO is known to be increased by oxidative stress. Several reports point to an increased oxidative stress in uremia, characterized by an augmented production of oxidants and a decreased level of antioxidants. The evidence includes increased serum ratios of oxidized to reduced ascorbate (31), glutathione (32), and albumin (33); increased serum levels of advanced oxidation protein products (34) and of protein carbonyls (35); decreased serum activity of glutathione-dependent enzymes (36,37); increased lipid peroxidation products (14); and accumulation of dialyzable oxidants (38). The uremic oxidative stress might be further worsened by some modalities of renal replacement therapy: The hemodialysis treatment activates complement and leukocytes, which release reactive oxygen species (39,40).

The oxidative stress modifies proteins either directly through the oxidation of amino acids by reactive oxygen species (41) or indirectly by an increased generation of RCO (17). Carbohydrates and lipids targeted by reactive oxygen species yield, as mentioned earlier, increased amounts of RCO involved eventually in the formation of AGE and ALE ($k_1 + k_3$ in Figure 1). A causal role of the oxidative stress in AGE and ALE formation is supported by the correlation existing in uremic serum between pentosidine and oxidative markers such as dehydroascorbate (31) and advanced oxidation protein products (34).

The relevance of these abnormalities to intracellular events remains to be thoroughly documented. The detection of AGE and ALE by immunohistochemistry in vascular and renal tissues suggests that a local oxidative stress may contribute to pathologic lesions, such as fatty streak and thickened intima of arterial walls in atherosclerosis (42,43) and expanded mesangial area and nodular lesions in diabetic nephropathy (44,45).

The oxidative stress hypothesis, however, is not wholly satisfactory. Indeed, RCO such as 3-deoxyglucosone and methylglyoxal are derived from nonoxidative chemistry. 3-Deoxyglucosone is formed nonoxidatively by rearrangement and decomposition of Amadori compounds or by anaerobic metabolic reactions leading to formation of fructose-3-phosphate, which decomposes spontaneously to 3-deoxyglucosone (24). The more reactive RCO, methylglyoxal, is also formed during anaerobic metabolism of acetone and amino acids (22). Both RCO react with proteins and form AGE. Raised levels of 3-deoxyglucosone and methylglyoxal and of their protein adducts in uremia (15,29) demonstrate that nonoxidative chemistry is also involved in the generation of the carbonyl stress ($k_4 + k_5$).

Impaired RCO Detoxification

The rise in RCO in uremia might also be accounted for by a decreased removal. RCO are detoxified by several enzymatic pathways, such as aldose reductase, aldehyde dehydrogenases, and the glyoxalase pathway (22). Redox coenzymes, reduced glutathione (GSH) and nicotinamide adenine denucleotide (phosphate) (NAD(P)H), contribute to their activity. RCO such as methylglyoxal and glyoxal react reversibly with the thiol

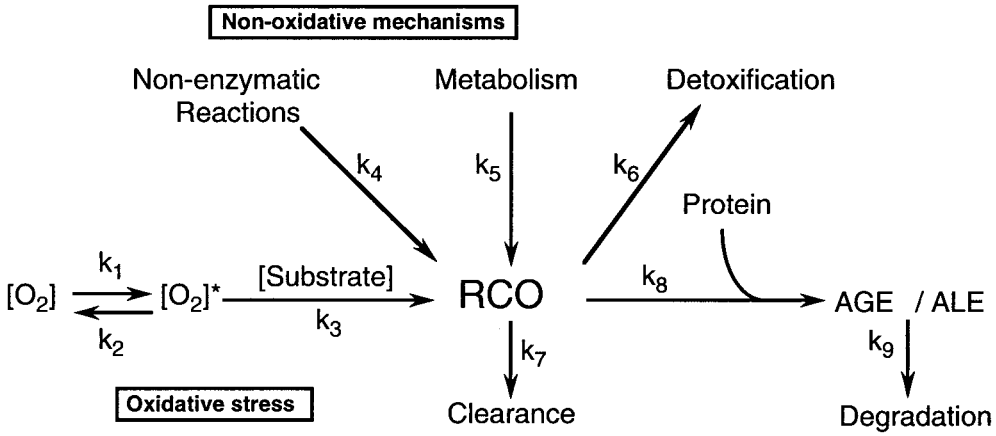

Figure 1. Routes to generation and detoxification of reactive carbonyl compounds (RCO). The level of oxidative stress is depicted as the balance between the overall rate of formation of reactive oxygen species (ROS) ($k_1 \times [O_2]$) and the rate of their inactivation by antioxidant defenses ($k_2 \times [O_2]^*$). ROS react with carbohydrates, polyunsaturated fatty acids, or amino acids ($k_3 \times [O_2]^* \times$ [Substrate]) to yield RCO. RCO are also formed by nonenzymatic reactions (k_4: *e.g.*, 3-deoxyglucosone) and by anaerobic metabolism (k_5: *e.g.*, methylglyoxal). RCO are detoxified ($k_6 \times$ [RCO]) by a variety of metabolic pathways (*e.g.*, the glyoxalase pathway) or probably cleared from the body (k_7). The RCO subsequently react with protein ($k_8 \times$ [RCO] $\times$ [Proteins]) to form advanced glycation end products (AGE)/advanced lipoxidation end products (ALE). The increase in RCO in uremia results from an increase in their production ($k_3 + k_4 + k_5$) and/or a decrease in their removal ($k_6 + k_7$). The AGE/ALE formation depends on the balance between their rates of formation (k_8) and degradation (k_9).

group of glutathione and are subsequently detoxified by glyoxalases I and II into lactate and glutathione. NAD(P)H replenishes glutathione by increasing the activity of glutathione reductase. Decreased levels of glutathione and NAD(P)H can therefore result in augmented levels of a wide range of RCO (k_6).

It is of interest to know in this context that the glutathione concentration in red blood cells and the serum activity of glutathione-dependent enzymes are significantly reduced in uremic patients (32,36,37). The hypothesis that a decreased thiol storage capacity contributes to the accumulation of RCO in uremia is supported by recent evidence. Glutathione peroxidase activity is indeed inversely correlated with the pentosidine levels in the plasma of hemodialysis patients (37). Of course, other as yet unexplored enzymatic mechanisms might contribute to a decreased removal of RCO and thus to the uremic carbonyl stress.

It should be pointed out that the decreased thiol concentration in uremia might reflect its consumption during detoxification of reactive oxygen species generated under uremia-associated oxidative stress. Still, as yet unproven in uremia, a nonoxidative pathway to decrease thiol concentration has been recognized in diabetes. The polyol pathway is activated by hyperglycemia and consumes NAD(P)H for the reduction of glucose to sorbitol, a process catalyzed by aldose reductase (46). The decrease of NAD(P)H availability for GSH reductase lowers GSH levels. This process depends on glucose concentration but is independent of oxidative stress. It remains to be seen whether the decrease of thiol concentration in uremia derives only from oxidative stress or from other nonoxidative pathways.

Decreased Glomerular Filtration of RCO

RCO derived from both oxidative and nonoxidative chemistry of both carbohydrates and lipids have a rather low molecular weight. A decreased renal clearance may contribute to the uremic carbonyl stress (k_7). In fact, the pentosidine level in both uremic and diabetic patients is strongly influenced by residual renal function (8,13,47). If pentosidine levels are taken as markers of RCO precursors (28), these data point to a significant role of the failing kidney in raising plasma RCO levels.

Implications of Carbonyl Stress

The consequences of the uremic carbonyl stress and its attendant AGE and ALE modification of proteins are discussed below.

Cellular Effects of RCO

Several lines of evidence suggest that RCO interfere with various cellular functions independently of their effect on AGE and ALE modification of proteins. RCO are biologically active, initiate a variety of cellular responses, and induce structural and functional alterations of proteins (17,48).

For example, renal failure is associated with resistance to the action of calcitriol (1,25-dihydroxyvitamin D) (49), which is partly attributed to the inhibition by unknown uremic toxins of the interaction between the vitamin D receptor and vitamin D response elements (50). Subsequently, Patel and coworkers (51) demonstrated that RCO capable of Schiff base formation with lysine residues of the vitamin D receptor inhibit its interaction with the vitamin D response element.

In another model, exposure *in vitro* of cultured mesothelial and endothelial cells to methylglyoxal increases mRNA and protein synthesis of vascular endothelial growth factor (VEGF) (52). VEGF also increases *in vivo* in the peritoneal tissue of rats given repeated intraperitoneal loads of methylglyoxal (52).

Carbonyl stress also influences the intracellular signaling by multiple pathways. First, AGE, upon interaction with the receptor for AGE, trigger the signaling involving ras pathway (53). Second, exposure of fibroblasts to glyoxal activates protein kinases such as c-Src and increases intracellular tyrosine phosphorylation of several cellular proteins (54). This effect is mediated by the formation of Schiff base on cell surface protein since it is prevented by an inhibitor of the carbonyl amine reaction, OPB-9195 (54). Third, hydroxynonenal causes a capping of epidermal growth factor (EGF) receptor on the cell surface, mimics the effect of EGF on the downstream signaling pathways that involve mitogen-activated protein kinases, and contributes to oxidative stress-induced apoptotic cell death (55). Fourth, hydroxynonenal also triggers oxidative stress-induced apoptotic cell death by activating caspase-3 through a Fas-independent but GSH-dependent redox pathway (56). Finally, methylglyoxal induces apoptosis in Jurkat leukemia T cells by activating c-Jun N-terminal kinase (57).

AGE and ALE Biologic Effects

Carbonyl stress is also implicated in the development of several uremic complications. Dialysis-related amyloidosis is a serious bone and joint destruction associated with chronic renal failure (58). The cause of β_2-microglobulin amyloid fibril deposition is not fully elucidated. The role of elevated plasma β_2-microglobulin levels is disputed because no difference has been found between dialysis patients with and those without clinical evidence of complications (59). Efforts have therefore been directed toward identification of chemical modifications of β_2-microglobulin. Although these studies are incomplete, immunohistochemical and chemical analyses indicate that β_2-microglobulin amyloid deposits are modified by carbonyl stress. Long-lived β_2-microglobulin amyloid plaques react with anti-AGE (CML, pentosidine, and imidazolone) and anti-ALE (malondialdehyde-lysine and hydroxynonenal-protein adduct), as well as anti-receptor for AGE antibodies (60–65). β_2-Microglobulin amyloid fibrils isolated from plasma and urine of uremic patients also reacts with these antibodies (9,60).

Atherosclerosis is a major complication of chronic renal failure. The levels of AGE in randomly collected arterial tissues are higher in dialysis patients than in healthy subjects (66). AGE and ALE are detectable by immunohistochemistry in the fatty streak and in the thickened neointima (43). The staining patterns of AGE and ALE correspond with that for protein carbonyls, a biomarker of oxidative protein damage (42). The formation of AGE/ALE in vascular lesions is not

specific to uremia, but might be a phenomenon common to most, if not all, types of vascular damage, regardless of whether the vascular injury is caused by metabolic or mechanical factors: AGE/ALE are identified in vascular lesions not only in uremic patients but also in aged and diabetic subjects (42), and in nonuremic rats whose carotid artery has been injured by a balloon (43). Colocalization of AGE/ALE and protein carbonyls in the vascular tissue indicates a wide range of chemical modifications in vascular matrix proteins.

The quality of the peritoneal membrane deteriorates progressively with peritoneal dialysis (PD) duration (67). Membrane alterations, characterized by interstitial fibrosis, disappearance of mesothelial cells, vascular wall thickening, vasodilation, and increased angiogenesis develop progressively, together with changes in permeability characteristics. The glucose content of PD fluid has been incriminated. Glucose is degraded during heat sterilization into a variety of RCO, such as methylglyoxal, glyoxal, and 3-deoxyglucosone (68–70). These RCO might play a role in the development of AGE and ALE accumulating in the mesothelial layers and in the vascular walls of the peritoneum (70,71). Interestingly, AGE such as CML and pentosidine colocalize in the peritoneum with VEGF (52,72). These findings support the implication of peritoneal modification by RCO in the liberation of VEGF, a potent factor enhancing vasodilation, vascular permeability, angiogenesis, and nitric oxide synthase production (73), all of which may contribute to peritoneal membrane deterioration.

In these three examples, it remains to be seen whether the presence of AGE and ALE merely result from the long-term accumulation of protein modifications and is therefore an inert surrogate marker for carbonyl stress, or, alternatively, whether it plays an active role in the pathogenesis of these complications. Recent studies support the latter hypothesis. AGE- and ALE-modified proteins prepared *in vitro* initiate a range of inflammatory responses, including stimulation of monocyte chemotaxis (74,75), secretion of inflammatory cytokines from macrophages (74–76), stimulation of collagenase secretion from synovial cells (74), stimulation of osteoclast-induced bone resorption (77), proliferation of vascular smooth muscle cells (78), stimulation of aggregation of platelets (79), stimulation of VEGF (80) and platelet-derived growth factor (81) production from endothelial cells, induction of insulin-like growth factor I from monocytes (82), inhibition of antibacterial activity of lysozyme and lactoferrin (83), and quenching nitric oxide activity (84).

Therapeutic Approaches

Carbonyl stress is clearly implicated in the development of several uremic complications. Its manipulation should provide new therapeutic insights. Among them are redox modulation, the use of inhibitors of carbonyl amine chemistry, and the improvement of dialyzer membrane biocompatibility.

Redox Therapy

The decrease in thiol concentration impairs the detoxification of RCO and potentiates the formation of AGE and ALE. Repletion of thiol might prove possible and useful by supplementation of glutathione, *N*-acetylcysteine, or cysteine. Addition of these thiol compounds in both uremic and normal plasma as well as in glucose-based PD fluid lowers the generation of AGE after incubation *in vitro* (our unpublished observation). Several other compounds may also prove helpful. Vitamin E and ubiquinol relieve the demands on the activity of glutathione. Lipoic acid is reduced by mitochondrial dehydrogenases to dihydrolipoate, which subsequently reacts with reactive oxygen species and replenishes glutathione (85). Inhibitors of aldose reductase are other candidates to replenish glutathione, as they prevent the polyol pathway activation and replenish NAD(P)H and glutathione available for both aldose reductase and glutathione reductase (86). Administration of lipoic acid (87) or of the aldose reductase inhibitor Statil (88) to rats with streptozotocin-induced diabetes increases thiol concentration and/or decreases methylglyoxal in tissues and blood.

Carbonyl Stress Inhibitor

AGE/ALE are formed by carbonyl amine chemistry between RCO and proteins. Trapping of RCO with substances such as aminoguanidine (89) and OPB-9195 (90) should inhibit the formation of AGE and ALE. Indeed, we have demonstrated that both compounds inhibit the *in vitro* formation of AGE from a variety of individual precursors such as ribose, glucose, and ascorbate, as well as that of ALE, malondialdehyde-lysine, and hydroxynonenal-protein adduct from arachidonate (91). Pentosidine generation in uremic plasma (28) and in glucose-rich PD fluid (91) incubated for 4 wk is also inhibited by aminoguanidine and OPB-9195.

On a molar basis, OPB-9195 is more effective than aminoguanidine because the latter's hydrazine nitrogen atom has a decreased nucleophilicity due to the proximity of the guanidinium cation. OPB-9195 as well as aminoguanidine might act by an antioxidative mechanism inhibiting the production of RCO from the various substrates. Alternatively, it may trap the available RCO and thus prevent AGE formation. The observation that OPB-9195 markedly decreases *in vitro* the level of RCO present in glucose-based PD fluid (91) strongly supports the latter hypothesis. Trapping may result from the reaction of the hydrazine nitrogen atom of aminoguanidine and OPB-9195 with carbonyl groups, leading to the eventual formation of hydrazones.

Interestingly, OPB-9195 corrects several biologic effects of RCO. In murine thymocytes and fibroblasts, it inhibits the phosphorylation of tyrosine residues of a number of intracellular proteins induced by glyoxal (54). Given to Otsuka-Long-Evans-Tokushima-Fatty (OLETF) rats, a model of non-insulin-dependent diabetes mellitus, OPB-9195 reduces urinary albumin excretion and improves the morphology of glomeruli (90). Furthermore, oral administration of OPB-9195 to rats, after balloon injury of their carotid arteries, effectively reduces neointima proliferation in arterial walls, an early and major step in the development of atherosclerotic lesions (43).

The development of less toxic and more specific carbonyl stress inhibitors should prove an important new therapeutic

avenue. Such compounds immobilized in cartridges might enhance extraction of RCO from blood during dialysis therapy.

Membrane Biocompatibility

An unexpected observation has yielded an additional therapeutic insight. A cross-sectional and longitudinal study of dialyzed patients has shown that both protein-linked and free pentosidine levels are lower in the plasma of patients given polysulfone dialysis than in those treated with several other membranes (47,92), including the equally biocompatible toward the complement and leukocyte system high-flux AN69 membrane and the less biocompatible low-flux cuprophane membrane. Pentosidine levels are lower in Belgian, Japanese, and German patients given dialysis with polysulfone membranes produced by two different companies.

There is at present no obvious explanation for these results. The effect is unrelated to the porosity or to the clearing ability of the membrane for pentosidine: Hemodialysis itself does not modify protein-linked pentosidine levels (more than 90% of pentosidine is linked to nondiffusible albumin), and the clearance of free pentosidine (379 Da) during a single dialysis session is similar for all membranes (18). The lower level of pentosidine probably reflects a lower generation of RCO, precursors of pentosidine through an as yet undefined mechanism. Much research remains to be done to demonstrate the clinical relevance of these observations.

Until recently, membrane biocompatibility has been discussed in relation to acute, enzymatic biochemistry, such as leukocyte and complement activation, and production of cytokines. However, long-term, nonenzymatic biochemistry may also be equally important in terms of membrane biocompatibility.

Conclusion

Research on AGE and ALE has led to new insights in nonenzymatic biochemistry in renal failure. It has revealed the accumulation of RCO derived from carbohydrates and lipids, the so-called carbonyl stress. Carbonyl stress alters the structure and function of cellular and matrix proteins and might underlie the development of long-term complications. It may prove amenable to therapeutic interventions. Carbonyl stress should therefore be considered a major contributor to uremic toxicity.

Acknowledgments

This study was supported by grants from Research for the Future Program of the Japan Society for the Promotion of Science (96L00303) and from the Japanese Ministry of Health and Welfare for Research on Health Services (H10–079).

References

1. Vanholder R, De Smet R: Pathophysiologic effects of uremic retention solutes [Review]. *J Am Soc Nephrol* 10: 1815–1823, 1999
2. Baynes JW, Monnier VM: The Maillard reaction in aging diabetes and nutrition. *Prog Clin Biol Res* 304: 1–410, 1989
3. Brownlee M, Cerami A, Vlassara H: Advanced glycosylation end products in tissue and the biochemical basis of diabetic complications. *N Engl J Med* 318: 1315–1321,1988
4. Dyer DG, Dunn JA, Thorpe SR, Bailie TK, Lyons TJ, McCance DR, Baynes JW: Accumulation of Maillard reaction products in skin collagen in diabetes and aging. *J Clin Invest* 91: 2463–2469, 1993
5. Makita Z, Vlassara H, Rayfield E, Cartwright K, Friedman E, Rodby R, Cerami A, Bucala R: Hemoglobin-AGE: A circulating marker of advanced glycosylation. *Science* 258: 651–653, 1992
6. Sell DR, Lapolla A, Odetti P, Forgarty J, Monnier VM: Pentosidine formation in skin correlates with severity of complication in individuals with long standing IDDM. *Diabetes* 41: 1286–1292, 1992
7. McCance DR, Dyer DG, Dunn JA, Dunn JA, Bailie KE, Thorpe SR, Baynes JW, Lyons TJ: Maillard reaction products and their relation to complications in insulin-dependent diabetes mellitus. *J Clin Invest* 91: 2470–2478, 1993
8. Sugiyama S, Miyata T, Ueda Y, Tanaka H, Maeda K, Kawashima S, van Ypersele de Strihou C, Kurokawa K: Plasma level of pentosidine, an advanced glycation end product, in diabetic patients. *J Am Soc Nephrol* 9: 1681–1688, 1998
9. Miyata T, Taneda S, Kawai R, Ueda Y, Horiuchi S, Hara M, Maeda K, Monnier VM: Identification of pentosidine as a native structure for advanced glycation end products in β_2-microglobulin forming amyloid fibrils in patients with dialysis-related amyloidosis. *Proc Natl Acad Sci USA* 93: 2353–2358, 1996
10. Fu MX, Requena JR, Jenkins AJ, Lyons TJ, Baynes JW, Thorpe SR: The advanced glycation end product, N^{ϵ}-(carboxymethyl) lysine, is a product of lipid peroxidation and glycoxidation reactions. *J Biol Chem* 271: 9982–9986, 1996
11. Sell DR, Monnier VM: Structure elucidation of a senescence cross-link from human extracellular matrix. *J Biol Chem* 264: 21597–21602, 1989
12. Ahmed MU, Thorpe SR, Baynes JW: Identification of N^{ϵ}-carboxymethyllysine as a degradation product of fructoselysine in glycated protein. *J Biol Chem* 261: 4889–4894, 1986
13. Miyata T, Ueda Y, Shinzato T, Iida Y, Tanaka S, Kurokawa K, van Ypersele de Strihou C, Maeda K: Accumulation of albumin-linked and free-form pentosidine in the circulation of uremic patients with end-stage renal failure: Renal implications in the pathophysiology of pentosidine. *J Am Soc Nephrol* 7: 1198–1206, 1996
14. Miyata T, Fu MX, Kurokawa K, van Ypersele de Strihou C, Thorpe SR, Baynes JW: Autoxidation products of both carbohydrates and lipids are increased in uremic plasma: Is there oxidative stress in uremia? *Kidney Int* 54: 1290–1295, 1998
15. Odani H, Shinzato T, Usami J, Matsumoto Y, Brinkmann Frye E, Baynes JW, Maeda K: Imidazolium cross-links derived from reaction of lysine with glyoxal and methylglyoxal are increased in serum proteins of uremic patients: Evidence for increased oxidative stress in uremia. *FEBS Lett* 427: 381–385, 1998
16. Esterbauer H, Schuer RJ, Zollner H: Chemistry and biochemistry of 4-hydroxynonenal, malondialdehyde and related aldehyde. *Free Radical Biol Med* 11: 81–128, 1991
17. Miyata T, van Ypersele de Strihou C, Kurokawa K, Baynes JW: Alterations in non-enzymatic biochemistry in uremia: Origin and significance of "carbonyl stress" in long-term uremic complications. *Kidney Int* 55: 389–399, 1999
18. Miyata T, Ueda Y, Yoshida A, Sugiyama S, Iida Y, Jadoul M, Maeda K, Kurokawa K, van Ypersele de Strihou C: Clearance of pentosidine, an advanced glycation end product, by different

modalities of renal replacement therapy. *Kidney Int* 51: 880–887, 1997
19. Takayama F, Aoyama I, Tsukushi S, Miyazaki T, Miyazaki S, Morita T, Hirasawa Y, Shimokata K, Niwa T: Immunohistochemical detection of imidazolone and N^{ϵ}-(carboxymethyl) lysine in aortas of hemodialysis patients. *Cell Mol Biol* 44: 1101–1109, 1998
20. Meng J, Sakata N, Imanaga Y, Tachikawa Y, Chihara J, Takebayashi S: Evidence for a link between glycoxidation and lipoperoxidation in patients with chronic renal failure. *Clin Nephrol* 51: 280–289, 1999
21. Glomb MA, Monnier VM: Mechanism of protein modification by glyoxal and glycolaldehyde, reactive intermediates of the Maillard reaction. *J Biol Chem* 276: 10017–10025, 1995
22. Thornalley PJ: Advanced glycation and development of diabetic complications: Unifying the involvement of glucose, methylglyoxal and oxidative stress. *Endocrinol Metab* 3: 149–166, 1996
23. Wells-Knecht KJ, Zyzak DV, Litchfield JE, Thorpe SR, Baynes JW: Mechanism of autoxidative glycosylation: Identification of glyoxal and arabinose as intermediates in the autoxidative modification of protein by glucose. *Biochemistry* 34: 3702–3709, 1995
24. Hayashi T, Namiki M: Role of sugar fragmentation in the Maillard reaction. In: *Amino-Carbonyl Reaction in Food and Biological Systems*, edited by Fujimaki M, Namiki M, Kato H, Amsterdam, The Netherlands, Elsevier, 1986, pp 29–38
25. Dunn JA, Ahmed MU, Murtiashaw MH, Richardson JM, Walla MD, Baynes JW: Reaction of ascorbate with lysine and protein under autoxidizing conditions: Formation of N^{ϵ}-(carboxymethyl) lysine by reaction between lysine and products of autoxidation of ascorbate. *Biochemistry* 29: 10964–10970, 1990
26. Uchida K, Kanematsu M, Sakai K, Matsuda T, Hattori N, Suzuki D, Miyata T, Noguchi N, Niki E, Osawa T: Protein-bound acrolein: Potential markers for oxidative stress. *Proc Natl Acad Sci USA* 95: 4882–4887, 1998
27. Anderson MM, Hazen SL, Hsu FF, Heinecke JW: Human neutrophils employ the myeloperoxidase-hydrogen peroxide-chloride system to convert hydroxy-amino acids into glycolaldehyde, 2-hydroxypropanol, and acrolein: A mechanism for the generation of highly reactive α-hydroxy and α, β-unsaturated aldehydes by phagocytes at sites of inflammation. *J Clin Invest* 99: 424–432, 1997
28. Miyata T, Ueda Y, Yamada Y, Saito A, Jadoul M, van Ypersele de Strihou C, Kurokawa K: Carbonyl stress in uremia: Accumulation of carbonyls accelerate the formation of pentosidine, an advanced glycation end product. *J Am Soc Nephrol* 9: 2349–2356, 1998
29. Niwa T, Takeda N, Miyazaki T, Yoshizumi H, Tatematsu A, Maeda K, Ohara M, Tomiyama S, Niimura K: Elevated serum levels of 3 deoxyglucosone, a potent protein-cross-linking intermediate of the Maillard reaction, in uremic patients. *Nephron* 69: 438–443, 1995
30. Odani H, Shinzato H, Matsumoto Y, Usami J, Maeda K: Increase in three α, β-dicarbonyl compound levels in human uremic plasma: Specific in vivo determination of intermediates in advanced Maillard reaction. *Biochem Biophys Res Commun* 256: 89–93, 1999
31. Miyata T, Wada Y, Cai Z, Iida Y, Horie K, Yasuda Y, Maeda K, Kurokawa K, van Ypersele de Strihou C: Implication of an increased oxidative stress in the formation of advanced glycation end products in patients with end-stage renal failure. *Kidney Int* 51: 1170–1181, 1997
32. Canestrari F, Galli F, Giorgini A, Albertini MC, Galiotta P, Pascucci M, Bossu M: Erythrocyte redox state in uremic anemia: Effects of hemodialysis and relevance of glutathione metabolism. *Acta Haematol* 91: 187–193, 1994
33. Kumano K, Yokota S, Go M, Suyama K, Sakai T, Era S, Sogami M: Quantitative and qualitative changes of serum albumin in CAPD patients. *Adv Perit Dial* 8: 127–130, 1992
34. Witko-Sarsat V, Friedlander M, Capeillere-Blandin C, Capeillere-Blandin C, Nguyen-Khoa T, Nguyen AN, Zingraff J, Jungers P, Descamps-Latscha B: Advanced oxidation protein products as a novel marker of oxidative stress in uremia. *Kidney Int* 49: 1304–1313, 1996
35. Odetti P, Garibaldi S, Gurreri G, Aragno I, Dapino D, Pronzato MA: Protein oxidation in hemodialysis and kidney transplantation. *Metabolism* 45: 1319–1322, 1996
36. Yeung JH: Effects of glycerol-induced acute renal failure on tissue glutathione and glutathione-dependent enzymes in the rat. *Methods Find Exp Clin Pharmacol* 13: 23–28, 1991
37. Ueda Y, Miyata T, Hashimoto T, Yamada H, Izuhara Y, Sakai H, Kurokawa K: Implication of altered redox regulation by antioxidant enzymes in the increased plasma pentosidine, an advanced glycation end product, in uremia. *Biochem Biophys Res Commun* 245: 785–790, 1998
38. Roselaar SE, Nazhat NB, Winyard PG, Jones P, Cunningham J, Blake DR: Detection of oxidant in uremic plasma by electron spin resonance spectroscopy. *Kidney Int* 48: 199–206, 1995
39. Nguyen AT, Lethias C, Zingraff J, Herbelin A, Naret C, Descamps-Latscha B: Hemodialysis membrane-induced activation of phagocyte oxidative metabolism detected in vivo and in vitro within microamounts of whole blood. *Kidney Int* 28: 158–167, 1985
40. Cheung AK: Biocompatibility of hemodialysis membranes. *J Am Soc Nephrol* 1: 150–161, 1990
41. Stadtman ER, Oliver CN: Metal catalyzed oxidation of proteins: Physiological consequences. *J Biol Chem* 266: 2005–2008, 1991
42. Miyata T, Inagi R, Asahi K, Yamada Y, Horie K, Sakai H, Uchida K, Kurokawa K: Generation of protein carbonyls by glycoxidation and lipoxidation reactions with autoxidation products of ascorbic acid and polyunsaturated fatty acid. *FEBS Lett* 437: 24–28, 1998
43. Miyata T, Ishikawa S, Asahi K, Inagi R, Suzuki D, Horie K, Tatsumi K, Kurokawa K: 2-Isopropylidenehydrazono-4-oxo-thiazolidin-5-ylacetanilide (OPB-9195) inhibits the neointima proliferation of rat carotid artery following balloon injury: Role of glycoxidation and lipoxidation reactions in vascular tissue damage. *FEBS lett* 445: 202–206, 1999
44. Horie K, Miyata T, Maeda K, Miyata S, Sugiyama S, Sakai H, van Ypersele de Strihou C, Monnier VM, Witztum JL, Kurokawa K: Immunohistochemical colocalization of glycoxidation products and lipid peroxidation products in diabetic renal glomerular lesions. *J Clin Invest* 100: 2995–3004, 1997
45. Suzuki D, Miyata T, Saotome N, Horie K, Inagi R, Yasuda Y, Uchida K, Izuhara Y, Yagame M, Sakai H, Kurokawa K: Immunohistochemical evidence for an increased oxidative stress and carbonyl modification of proteins in diabetic glomerular lesions. *J Am Soc Nephrol* 10: 822–832, 1999
46. Travis SF, Morrison AD, Clements RSJ, Winegrad AI, Osaki FA: Metabolism of glucose in human erythrocytes by the polyol pathway. *J Clin Invest* 50: 2104–2112, 1972
47. Jadoul M, Ueda Y, Yasuda Y, Saito A, Robert A, Ishida N, Kurokawa K, van Ypersele de Strihou C, Miyata T: Influence of

hemodialysis membrane type on pentosidine plasma level, a marker of "carbonyl stress." *Kidney Int* 55: 2487–2492, 1999
48. Rhodes J: Covalent chemical events in immune induction: Fundamental and therapeutic aspects. *Immunol Today* 9: 436–441, 1996
49. Fukagawa M, Kaname S, Igarashi T, Ogata E: Regulation of parathyroid hormone synthesis in chronic renal failure in rats. *Kidney Int* 39: 874–881, 1991
50. Patel SR, Ke HQ, Vanholder R, Koenig RJ, Hsu CH: Inhibition of calcitriol receptor binding to vitamin D response elements by uremic toxins. *J Clin Invest* 96: 50–59, 1995
51. Patel SR, Koenig YRJ, Hsu CH: Effect of glyoxylate on the function of the calcitriol receptor and vitamin D metabolism. *Kidney Int* 52: 39–44, 1997
52. Inagi R, Miyata T, Yamamoto T, Suzuki D, Urakami K, Ishibashi Y, van Ypersele de Strihou C, Kurokawa K: Glucose degradation product methylglyoxal enhances the production of vascular endothelial growth factor in peritoneal cells: Role in the pathogenesis of peritoneal membrane dysfunction in peritoneal dialysis. *FEBS Lett* 17: 260–264, 2000
53. Yan SD, Schmidt AM, Anderson GM, Zhang J, Brett J, Zou YS, Pinsky D, Stern D: Enhanced cellular oxidant stress by the interaction of advanced glycation end products with their receptors/binding proteins. *J Biol Chem* 269: 9889–9897, 1994
54. Ahkand AA, Kato M, Suzuki H, Hamaguchi M, Miyata T, Kurokawa K, Nakashima I: Carbonyl compounds cross-link cellular proteins and activate protein-tyrosine kinase $p60^{c\text{-}src}$. *J Cell Biochem* 72: 1–7, 1999
55. Liu W, Ahkand AA, Kato M, Yokoyama I, Miyata T, Nakashima I: 4-Hydroxynonenal triggers an epidermal growth factor receptor-linked signal pathway for growth inhibition. *J Cell Sci* 112: 2409–2417, 1999
56. Liu W, Kato M, Ahkand AA, Hayakawa A, Suzuki H, Miyata T, Kurokawa K, Hotta Y, Ishikawa N, Nakashima I: 4-Hydroxynonenal induces a cellular redox status-related activation of the caspase cascade for apoptotic cell death. *J Cell Sci* 113: 635–641, 2000
57. Du J, Suzuki H, Nagase F, Akhand AA, Yokoyama T, Miyata T, Kurokawa K, Nakashima I: Methylglyoxal induces apoptosis in Jurkat leukemia T cells by activating c-Jun N-terminal kinase. *J Cell Biochem* 77: 333–344, 2000
58. Miyata T, Jadoul M, Kurokawa K, van Ypersele de Strihou C: β_2-Microglobulin in renal disease. *J Am Soc Nephrol* 9: 1723–1735, 1998
59. Gejyo F, Homma N, Suzuki Y, Arakawa M: Serum levels of β_2-microglobulin as a new form of amyloid protein in patients undergoing long-term hemodialysis. *N Engl J Med* 314: 585–586, 1986
60. Miyata T, Oda O, Inagi R, Iida Y, Araki N, Yamada N, Horiuchi S, Taniguchi N, Maeda K, Kinoshita T: β_2-Microglobulin modified with advanced glycation end products is a major component of hemodialysis-associated amyloidosis. *J Clin Invest* 92: 1243–1252, 1993
61. Miyata T, Inagi R, Wada Y, Ueda Y, Iida Y, Takahashi M, Taniguchi N, Maeda K: Glycation of human β_2-microglobulin in patients with hemodialysis-associated amyloidosis: Identification of the glycated sites. *Biochemistry* 33: 12215–12221, 1994
62. Niwa T, Miyazaki S, Katsuzaki T, Takamichi N, Takei Y, Miyazaki T, Morita T, Hirakawa Y: Immunohistochemical detection of advanced glycation end products in dialysis-related amyloidosis. *Kidney Int* 48: 771–778, 1995
63. Miyata T, Hori O, Zhang JH, Yan SD, Ferran L, Iida Y, Schmidt AM: The receptor for advanced glycation end products mediates the interaction of AGE–β_2-microglobulin with human mononuclear phagocytes via an oxidant-sensitive pathway: Implication for the pathogenesis of dialysis-related amyloidosis. *J Clin Invest* 98: 1088–1094, 1997
64. Niwa T, Sato M, Katsuzaki T, Tomoo T, Miyazaki T, Tatemichi N, Takei Y, Kondo T: Amyloid β_2-microglobulin is modified with N^{ϵ}-(carboxymethyl) lysine in dialysis-related amyloidosis. *Kidney Int* 50: 1303–1309, 1996
65. Niwa T, Katsuzaki T, Miyazaki S, Momoi T, Akiba T, Miyazaki T, Nokura K, Hayase F, Tatemichi N, Takei Y: Amyloid β_2-microglobulin is modified with imidazolone, a novel advanced glycation end product, in dialysis-related amyloidosis. *Kidney Int* 51: 187–194, 1997
66. Makita Z, Radoff S, Rayfield EJ, Yang Z, Skolnik E, Delaney V, Friedman EA: Advanced glycosylation end products in patients with diabetic nephropathy. *N Engl J Med* 325: 836–842, 1991
67. Krediet RT: The peritoneal membrane in chronic peritoneal dialysis. *Kidney Int* 55: 341–356, 1999
68. Nilsson-Thorell CB, Muscalu N, Andren AH, Kjellstrand PT, Wieslander AP: Heat sterilization of fluids for peritoneal dialysis gives rise to aldehydes. *Perit Dial Int* 13: 208–213, 1993
69. Linden T, Forsback G, Deppisch R, Henle T, Wieslander A: 3-Deoxyglucosone, a promoter of advanced glycation end products in fluids for peritoneal dialysis. *Perit Dial Int* 18: 290–293, 1998
70. Miyata T, Horie K, Ueda Y, Fujita Y, Izuhara Y, Hirano H, Saito A, van Ypersele de Strihou C, Kurokawa K: Advanced glycation and lipoxidation of the peritoneal membrane in peritoneal dialysis: Respective contribution of serum and peritoneal dialysis fluid reactive carbonyl compounds. *Kidney Int* 2000, in press
71. Nakayama M, Kawaguchi Y, Yamada K, Hasegawa T, Takazoe K, Katoh N, Yamamoto H, Ogawa A, Kubo H, Shigematsu T, Sakai O, Horiuchi S: Immunohistochemical detection of advanced glycation end-products in the peritoneum and its possible pathophysiological role in CAPD. *Kidney Int* 51: 182–186, 1997
72. Combet S, Miyata T, Moulin P, Pouthier D, Goffin E, Devuyst O: Vascular proliferation and enhanced expression of endothelial nitric oxide synthase in human peritoneum exposed to long-term peritoneal dialysis. *J Am Soc Nephrol* 11: 717–728, 2000
73. Ferrara N, Davis-Smyth T: The biology of vascular endothelial growth factor. *Endocr Rev* 18: 4–25, 1997
74. Miyata T, Inagi R, Iida Y, Sato M, Yamada N, Oda O, Maeda K, Seo H: Involvement of β_2-microglobulin modified with advanced glycation end products in the pathogenesis of hemodialysis-associated amyloidosis: Induction of human monocyte chemotaxis and macrophage secretion of tumor necrosis factor-α and interleukin 1. *J Clin Invest* 93: 521–528, 1994
75. Miyata T, Iida Y, Wada Y, Ueda Y, Shinzato T, Monnier VM, Maeda K: Monocyte/macrophage response to β_2-microglobulin modified with advanced glycation end products. *Kidney Int* 49: 538–550, 1996
76. Iida Y, Miyata T, Inagi R, Sugiyama S, Maeda K: β_2-Microglobulin modified with advanced glycation end products induces interleukin-6 from human macrophages: Role in the pathogenesis of hemodialysis-associated amyloidosis. *Biochem Biophys Res Commun* 201: 1235–1241, 1994
77. Miyata T, Notoya K, Yoshida K, Maeda K, Taketomi S: Advanced glycation end products enhance osteoclast-induced bone resorption in cultured mouse unfractionated bone cells and in rats

implanted with devitalized bone particles. *J Am Soc Nephrol* 8: 260–270, 1997
78. Satoh H, Togo M, Hara M, Miyata T, Han K, Maekawa H, Ohno M, Hashimoto Y, Kurokawa K, Watanabe T: Advanced glycation end products stimulate mitogen-activated protein kinase and proliferation in rabbit vascular smooth muscle cells. *Biochem Biophys Res Commun* 239: 111–115, 1997
79. Hangaishi M, Taguchi J, Miyata T, Ikari Y, Togo M, Hashimoto Y, Watanabe T, Kimura S: Increased aggregation of human platelets produced by advanced glycation end products *in vitro*. *Biochem Biophys Res Commun* 248: 285–292, 1998
80. Kirstein M, Brett J, Radoff S, Ogawa S, Stern D, Vlassara H: Advanced protein glycosylation induces transendothelial human monocyte chemotaxis and secretion of platelet-derived growth factor: Role in vascular disease of diabetes and aging. *Proc Natl Acad Sci USA* 87: 9010–9014, 1990
81. Kirstein M, Aston C, Hintz R, Vlassara H: Receptor-specific induction of insulin-like growth factor I in human monocytes by advanced glycosylation end product-modified proteins. *J Clin Invest* 90: 439–446, 1992
82. Li YM, Tan AX, Vlassara H: Antibacterial activity of lysozyme and lactoferrin is inhibited by binding of advanced glycation-modified proteins to a conserved motif. *Nat Med* 1: 1057–1061, 1995
83. Bucala R, Tracey KJ, Cerami A: Advanced glycosylation products quench nitric oxide and mediate defective endothelium-dependent vasodilatation in experimental diabetes. *J Clin Invest* 87: 432–438, 1991
84. Lu M, Kuroki M, Amano S, Tolentino M, Keough K, Kim I, Bucala R, Adamis AP: Advanced glycation end products increase retinal vascular endothelial growth factor expression. *J Clin Invest* 101: 1219–1224, 1998
85. Packer L, Witt EH, Trischler HJ: Alpha-lipoic acid as a biological antioxidant. *Free Radical Biol Med* 19: 227–250, 1995
86. Gabbay KH: Hyperglycemia, polyol metabolism, and complications of diabetes mellitus. *Ann Rev Med* 26: 521–536, 1975
87. Nagamatsu M, Nickander KK, Schmelzer JD, Raya A, Wittrock DA, Tritschler H, Low PA: Lipoic acid improves nerve blood flow, reduces oxidative stress, and improves distal nerve conduction in experimental diabetic neuropathy. *Diabetes Care* 18: 1160–1167, 1995
88. Phillips SA, Mirrlees D, Thornalley PJ: Modification of the glyoxalase system in streptozotocin-induced diabetic rats. *Biochem Pharm* 46: 805–811, 1993
89. Brownlee M, Vlassara H, Kooney, Ulrich P, Cerami A: Aminoguanidine prevents diabetes induced arterial wall protein cross-linking. *Science* 232: 1629–1632, 1986
90. Nakamura S, Makita Z, Ishikawa S, Yasumura K, Fujii W, Yanagisawa K, Kawata T, Koike T: Progression of nephropathy in spontaneous diabetic rats is prevented by OPB-9195, a novel inhibitor of advanced glycation. *Diabetes* 46: 895–899, 1997
91. Miyata T, Ueda Y, Asahi K, Izuhara Y, Inagi R, Saito A, van Ypersele de Strihou C, Kurokawa K: Mechanism of the inhibitory effect of 2-isopropylidenehydrazono-4-oxo-thiazolidin-5-ylacetanilide (OPB-9195) on AGE and ALE formation. *J Am Soc Nephrol* 2000, in press
92. Stein G, Franke S, Sperschneider H: Decrease in serum pentosidine levels of ESRD patients during polysulfone haemodialysis. *Nephrol Dial Transplant* 14: 1606–1608, 1999