

Role of Oxidative Stress in Diabetic Complications

A New Perspective on an Old Paradigm

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Oxidative stress and oxidative damage to tissues are common end points of chronic diseases, such as atherosclerosis, diabetes, and rheumatoid arthritis. The question addressed in this review is whether increased oxidative stress has a primary role in the pathogenesis of diabetic complications or whether it is a secondary indicator of end-stage tissue damage in diabetes. The increase in glycoxidation and lipoxidation products in plasma and tissue proteins suggests that oxidative stress is increased in diabetes. However, some of these products, such as 3-deoxyglucosone adducts to lysine and arginine residues, are formed independent of oxidation chemistry. Elevated levels of oxidizable substrates may also explain the increase in glycoxidation and lipoxidation products in tissue proteins, without the necessity of invoking an increase in oxidative stress. Further, age-adjusted levels of oxidized amino acids, a more direct indicator of oxidative stress, are not increased in skin collagen in diabetes. We propose that the increased chemical modification of proteins by carbohydrates and lipids in diabetes is the result of overload on metabolic pathways involved in detoxification of reactive carbonyl species, leading to a general increase in steady-state levels of reactive carbonyl compounds formed by both oxidative and nonoxidative reactions. The increase in glycoxidation and lipoxidation of tissue proteins in diabetes may therefore be viewed as the result of increased carbonyl stress. The distinction between oxidative and carbonyl stress is discussed along with the therapeutic implications of this difference. *Diabetes* 48:1-9, 1999

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AGE, advanced glycation end product; CML, N^ε-(carboxymethyl)lysine; 3-DG, 3-deoxyglucosone; GSH, glutathione; HNE, 4-hydroxynonenal; MDA, malondialdehyde; MGO, methylglyoxal; RAGE, receptor for AGE.

Although the Diabetes Control and Complications Trial has identified hyperglycemia as a risk factor for development of diabetic complications (1), there is no consensus regarding the pathogenic link between hyperglycemia and complications. There are a number of equally tenable hypotheses on the origin of complications, including but not limited to, the Maillard, or advanced glycation end product (AGE) hypothesis (2,3), the aldose reductase hypothesis (4), oxidative stress (5-8), reductive stress (pseudohypoxia) (9,10), true hypoxia (11), carbonyl stress (12,13), altered lipoprotein metabolism (12,14,15), increased protein kinase C activity (16), and altered growth factor (17) or cytokine (18) activities. All of these hypotheses have strong proponents in academe, in medicine, and in the pharmaceutical industry. The list is long, perhaps because each hypothesis is a different reflection of an underlying common pathogenic mechanism, or perhaps because different tissues are sensitive to different mechanisms. The various hypotheses overlap and intersect with one another: AGE formation and altered polyol pathway activity may lead to oxidative stress, oxidative stress may accelerate AGE formation, reductive stress may lead to activation of protein kinase C, AGEs may induce oxidative stress and growth factor expression, and so on. The long list is a strong indication of the uncertainties in our understanding of the pathogenesis of diabetic complications.

In this article, we will present a current perspective on one of the above hypotheses, the oxidative stress hypothesis. Because oxidative stress and the AGE hypothesis are inextricably intertwined, we will also address the role of the AGE hypothesis in diabetic complications. The question is not so much whether oxidative stress is increased in diabetes, but whether oxidative stress has a primary role in the pathogenesis of diabetic complications. Oxidative stress and resultant tissue damage are hallmarks of chronic disease and cell death: diabetes is no exception. At issue is whether oxidative stress occurs at an early stage in diabetes, preceding the appearance of complications, or whether it is merely a common consequence of the tissue damage, reflecting the presence of complications. The question is important: should we focus on the application of antioxidant therapy for managing the progression of complications, or on the intermediate steps in the transition from chronically poor glycemic control to the development of complications? Obviously, we need to focus on both stages of the disease, but we will argue that treatment of diabetes with antioxidant therapy is like applying water to

a burning house, certainly helpful in limiting the conflagration, but also a little late in the process. The water is certainly helpful in a salvage effort to quench the fire and limit damage, but understanding the origin of the fire, e.g., electrical wiring, flammable chemicals, or arson, provides a target that might have been addressed to prevent the damage in the first place.

Hyperglycemia is clearly recognized as the primary culprit in the pathogenesis of diabetic complications (1), but while hyperglycemia and poor metabolic control must be addressed, they are likely, for motivational, educational, and economic reasons, to remain a chronic problem. Even intensive glycemic control is associated with progression of complications (1). Recognizing this, what are the critical intermediate steps that link hyperglycemia to tissue damage? If oxidative damage is a late event, how can the multistep progression from hyperglycemia to overt complications be retarded or prevented, even in patients with poor control? We will argue that one of the critical pathogenic consequences of hyperglycemia in diabetes is a deficit in detoxification of reactive carbonyl compounds. The increase in reactive carbonyls derived from both oxidative and nonoxidative reactions (defined as carbonyl stress) leads to increased chemical modification of proteins, and then, at a late stage, to oxidative stress and tissue damage. Intervention, we will argue, should begin at the level of carbonyl stress, long before the appearance of overt oxidative stress and damage. Antioxidant therapy may not only be too late, but it may also miss a large fraction of the target, nonoxidatively derived carbonyl compounds, which contribute to tissue damage. To provide a background for this discussion, we will begin with a brief overview.

HISTORICAL PERSPECTIVE

The AGE hypothesis proposes that chronic accelerated chemical modification of proteins by reducing sugars in diabetes

alters the structure and function of tissue proteins, contributing to pathophysiology and precipitating the development of diabetic complications (1,2). The original focus of the hypothesis was on the formation of brown and fluorescent cross-link structures, exemplified by pentosidine (Table 1). Over time, however, the hypothesis has evolved to recognize a wide variety of chemical structures formed during carbohydrate-protein interaction, including structures that are not brown or fluorescent, that are not cross-links, and that may increase, but do not necessarily accumulate, in tissue proteins in aging or diabetes (Table 1). There is also a consensus today that AGEs are not necessarily end products, but that they may be active intermediates in the cross-linking of proteins and formation of reactive oxygen species. Some of these compounds, such as pentosidine, N^ε-(carboxymethyl)lysine (CML), and pyrraline, have been detected and measured in tissue proteins by chemical and chromatographic methods, while others, such as the crossline cross-links and arginine imidazolone adducts (Table 1), have been detected chemically in proteins modified in vitro, but only by immunochemical methods in tissues.

Until 1987, the Amadori adduct was considered to be an essential intermediate in the formation of AGEs and a direct precursor of reactive dicarbonyl species such as 1- and 3-deoxyglucosone (3-DG). Wolff and colleagues (19,20) argued, however, that metal-catalyzed oxidation of glucose itself, described as autooxidative glycosylation, was the major route for formation of Maillard products in tissues, and also proposed that ascorbate and other carbohydrates, including fructose and metabolic intermediates, might be equally important sources of AGEs. Today, there is general agreement that there are multiple sources and mechanisms of formation of AGEs in vivo, involving oxidative and nonoxidative chemistry of reducing sugars, Schiff bases, Amadori adducts, ascorbic acid, and metabolic intermediates (13,21,22).

TABLE 1
Current list of AGEs identified in tissue proteins by chemical or immunological methods

Assay technique	HPLC and GC/MS	HPLC	HPLC	Immunochemical	HPLC and LC/MS	Immunochemical*
Fluorescent	-	+	-	+	-	+/-†
Cross-link	-	+	-	+	+	-
Accumulates with age	+	+	-	?	+	?
Increased in diabetes	+	+	+	+	?	+
Oxidation product	+/-‡	+	-	+	+/-‡	+/-§

*Antibodies do not distinguish between the imidazolone and dehydroimidazolone derivatives. †The dehydroimidazolone, but not the imidazolone, is fluorescent. ‡N^ε-(carboxymethyl)lysine (CML) and glyoxal-lysine dimer (GOLD) are considered oxidation products, while N^ε-(carboxyethyl)lysine (CEL) and methylglyoxal-lysine dimer (MOLD) may be formed from MGO, which is formed primarily from triose phosphates produced during anaerobic glycolysis. §Dehydroimidazolone is an oxidation product of the imidazolone. HPLC, high-performance liquid chromatography.

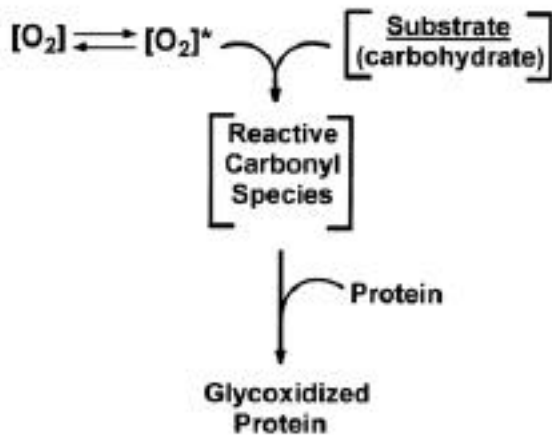


FIG. 1. Role of oxidative stress in formation of CML and pentosidine. The rate of formation of CML, pentosidine, and other glycooxidation products is considered to be a second-order process, dependent on the prevailing level of oxidative stress, which is represented by the steady-state level of reactive oxygen species ($[O_2]^*$), and the concentration of oxidizable substrate, e.g., glucose. Analysis of age-adjusted levels of CML and pentosidine in diabetic skin collagen suggests that the increase in these AGEs in diabetes can be explained by the increase in glycemia (substrate stress) in diabetes without invoking an increase in oxidative stress (25). The increase in CML and pentosidine in uremia is considered to be the result of an increase in oxidative stress (36).

The glycooxidation hypothesis, introduced in 1991, proposed a general role for oxidative stress in the formation of AGEs (5). Glycooxidation products, such as pentosidine and CML, were described as a subclass of AGEs formed by oxidative reactions. Because these were inert end products of the Maillard reaction and were the only AGEs known to accumulate with age in lens proteins and collagen, oxidation was described as a fixative of irreversible Maillard reaction damage to proteins. The glycooxidation hypothesis intertwined the glucose and free-radical theories of aging, proposing that glycooxidation was a source of permanent, cumulative, oxidative damage to long-lived proteins in aging and diabetes. In subsequent studies, increased age-adjusted levels of pentosidine and CML were detected in diabetic skin collagen and correlated with the severity of diabetic complications, including nephropathy, retinopathy, and vascular disease (23–26). The correlation between glycooxidative damage in skin and affected tissues was consistent with a systemic derangement in chemistry and metabolism in diabetes.

Dyer et al. (25) treated the rate of accumulation of glycooxidation products in skin collagen as the second-order product of the degree of hyperglycemia and the status of oxidative stress (Fig. 1). Based on this analysis, they concluded that the level of pentosidine and CML in collagen in diabetes could be explained by a patient's age, duration of diabetes, and long-term mean glycemic control without invoking an increase in oxidative stress. Wells-Knecht et al. (27) also concluded that oxidative stress was not increased in diabetes, based on their observation that, although levels of the oxidized amino acids methionine sulfoxide and α -tyrosine increased with age in human skin collagen, age-adjusted levels of these compounds were not increased in diabetes. Dyer et al. (25) suggested that individuals with higher set points for oxidative stress might still be at greater risk for development

of complications, in much the same way that diabetic patients with hypertension or hyperlipidemia might be at greater risk for renal or vascular disease. None of these studies exclude localized tissue-specific increases in oxidative stress in renal, retinal, or vascular tissue in which complications develop, nor do they establish a clear role for AGEs or glycooxidation in the pathogenesis of diabetic complications: the results are only correlative. However, levels of AGEs appear to increase in concert in kidney, vascular tissue, and skin of diabetic animals within only a few weeks after induction of diabetes in animal models (5), suggesting that AGEs are formed at an early stage in the disease process and that the increase in their levels is systemic.

AGE-proteins are chemically damaged proteins. Therefore, it seems likely that, as with other damaged molecules, such as oxidized DNA or lipoproteins, biological mechanisms would have evolved for their recognition and turnover. A number of cell-surface AGE receptors have now been identified and are proposed to have a role in the uptake and catabolism of AGE-proteins in plasma, erythrocyte membranes, and the extracellular matrix. The best characterized among these are RAGE (receptor for AGE) (28) and the macrophage scavenger receptor (29,30). RAGE is widely distributed among cell types, including endothelial and smooth muscle cells and macrophages. It also is implicated in the transmission of oxidative stress to receptor-bearing cells, since binding of AGE-proteins to RAGE on cell surfaces induces an intracellular oxidative stress response *in vitro*, characterized by increased NF- κ B, and tissue-factor expression (31,32). However, the role of AGEs on AGE-proteins as direct sources of oxidative stress has been questioned recently, based on the observation that AGE-proteins prepared *in vitro* are highly oxidized and contain amino acid hydroperoxides and the superoxide generator dihydroxyphenylalanine (33). Binding interactions between AGEs and their receptors and the subsequent induction of oxidative stress may therefore be mediated by different chemical species on the modified proteins prepared *in vitro*. Diabetic erythrocytes also induce oxidative stress to endothelial cells, and these effects are blocked by soluble RAGE (34). However, it is not clear that AGEs on erythrocytes are a significant contributor to endothelial or vascular dysfunction in diabetes, in comparison with other possible mechanisms, such as increased red cell lipid peroxidation, decreased red cell deformability, and platelet hypercoagulability. Further, although both AGE receptors and scavenger receptors bind highly modified AGE-proteins prepared *in vitro*, natural AGE-proteins, such as AGE-LDL, are not efficiently removed from circulation (35), nor is erythrocyte survival significantly decreased in diabetes. High levels of AGEs also accumulate on plasma proteins in uremia, independent of diabetes (36). Although renal transplantation leads to a decrease in plasma AGEs, the kinetics of the decrease are consistent with the normal kinetics of turnover of plasma proteins (37). Thus, at this point, the ligands recognized by RAGE and several other putative AGE receptors (2,38) are still unknown, the activity and function of AGE receptors *in vivo* remain unclear, and the pathological significance of AGE-induced oxidative stress in biological systems is uncertain. It is possible that, like the scavenger receptor that has a role in the recognition and turnover of apoptotic cells (39), AGE receptors may have specialized functions in tissues, which may not

be obvious from analysis of the kinetics of turnover of erythrocytes or AGE-proteins in blood. Experiments with knockout mice lacking AGE receptors or with diabetic animals overexpressing these receptors should yield insight into their possible physiological functions in the development or deterrence of diabetic complications.

RECENT DEVELOPMENTS

A number of recent observations have had a substantial impact on the interpretation of the AGE hypothesis. Reddy et al. (40) identified CML as the major AGE epitope recognized by antibodies prepared against AGE-proteins, and anti-CML antibodies are now increasingly used as a tool for detecting glycoxidative damage to tissue proteins. We have also shown that CML is formed from polyunsaturated fatty acids during lipid peroxidation reactions, i.e., CML is a product of both glycoxidation and lipoxidation (41). Thus, the CML detected in atherosclerotic plaque from normoglycemic individuals (42) might be derived from peroxidation of lipids in lipoproteins, rather than from glucose. Lipids may also be the source of CML in the vascular wall, even in diabetic vascular disease. Indeed, lipids are more readily oxidized than glucose, and dyslipidemia is a common feature of diabetes. Bucala et al. (43) demonstrated the presence of AGE lipids in diabetic plasma lipoproteins by enzyme-linked immunosorbent assay and showed that levels of AGE lipids correlated with levels of malondialdehyde (MDA) adducts on LDL. Requena et al. (44) later suggested that the AGE lipid was N-(carboxymethyl)-phosphatidylethanolamine, based on cross-reactivity of anti-CML protein antibody with N-(carboxymethyl)-ethanolamine. Thus, glycoxidation and lipoxidation products appear to be formed together on both proteins and lipids. Diabetes is not only a disease of altered metabolism, but also of altered chemistry, of both carbohydrates and lipids.

In addition to diabetes, there are several other conditions characterized by increases in AGEs in tissue proteins (45). Sell and Monnier (46) originally reported increased levels of pentosidine in collagen from uremic patients, even in the absence of diabetes. Increases in AGEs (CML and pentosidine), lipoxidation products (MDA and 4-hydroxynonenal [HNE] adducts) on proteins, and protein oxidation products (chloro- and nitro-tyrosine, and protein carbonyls) occur together in plaque deposits in atherosclerosis (42,47,48), Alzheimer's disease (49,50), and dialysis-related amyloidosis (51,52), as well as in diabetes (53,54). The simultaneous

increases in CML, pentosidine, and MDA adducts to plasma proteins in uremia (36) also indicate that glycoxidative and lipoxidative damage are increased in concert in this disease. In contrast to diabetes, where the increase in glycoxidation products was attributed to the increase in glycemia (25) (substrate stress) (Fig. 1), the increase in CML, pentosidine, and MDA lysine in uremia was attributed to an increase in oxidative stress (36), since glycemia and lipemia were not increased in the uremic patients. The deposition of these compounds in amyloid plaque in hemodialysis-associated amyloidosis (51,52) suggests that inflammatory processes may exacerbate oxidative stress and tissue damage in this and other chronic diseases. Levels of amino acid oxidation products, such as methionine sulfoxide and α -tyrosine, have not been measured in tissues in uremia.

OXIDATIVE STRESS OR CARBONYL STRESS?

Beside substrate and oxidative stress, there is an alternative explanation for the increase in chemical modification of proteins in diabetes, uremia, and other diseases, i.e., carbonyl stress (Fig. 2), which is caused by a generalized increase in the concentration of reactive carbonyl precursors of AGEs, glycoxidation and lipoxidation products. Carbonyl stress may result from an increase in substrate stress and/or a decrease in the efficiency of detoxification of carbonyl compounds. The distinction between oxidative stress and carbonyl stress lies in the nature of the carbonyl compound, i.e., if the carbonyls are derived exclusively from oxidative reactions, then the condition would be described as oxidative stress. However, if the carbonyls are derived, in full or in part, from nonoxidative processes, then the conditions would be more appropriately described as carbonyl stress. Compared with oxidative stress, carbonyl stress is a more comprehensive term, since it includes increases in carbonyls derived from both oxidative and nonoxidative reactions. In fact, in both diabetes and uremia, there is an increase not only in glycoxidation and lipoxidation products, but also in products of reaction of proteins with dicarbonyl compounds formed by nonoxidative mechanisms.

The bottom row of Table 1 emphasizes that not all AGEs require oxidative chemistry for their formation in vivo. The concentration of the AGE precursor 3-DG, for example, is increased in both diabetic and uremic plasma (55-58), and 3-DG-arginine (imidazolone) adducts are increased in blood and tissue proteins in diabetes, in association with nephropathy

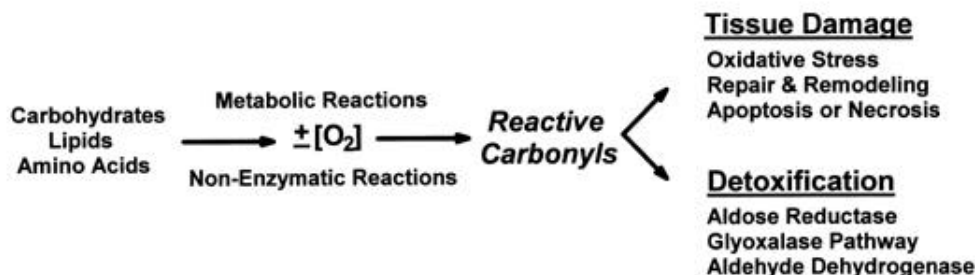


FIG. 2. Factors contributing to the development of carbonyl stress. Carbonyl stress is defined as the result of an increase in the steady-state concentration of reactive carbonyl species. These species are formed from small molecules by both enzymatic (metabolic) and nonenzymatic reactions and may or may not require oxygen for their formation. Their steady-state concentration is determined by the relative rates of their production and detoxification. An excess of reactive carbonyls leads to increased chemical modification of biomolecules, and thence to a series of biological responses, ranging from growth, repair, and remodeling to apoptosis and necrosis. Some of the events may be mediated by oxidative stress induced by the carbonyl compounds.

(59,60). However, 3-DG is formed by a nonoxidative rearrangement and hydrolysis, rather than oxidation, of Amadori adducts (61), or by nonoxidative β -elimination of phosphate from fructose-3-phosphate (62,63). Blood levels of methylglyoxal (MGO) are also increased in diabetes (64), along with increases in MGO-derived dilysine imidazolium cross-links (MOLD, Fig. 1) (65) and adducts to arginine residues (66) in plasma proteins. Levels of N^ε-(carboxyethyl)lysine, formed by reaction of MGO with lysine, are also increased in skin collagen in diabetes (J.W.B., unpublished observations). Like 3-DG, MGO is formed by nonoxidative mechanisms, primarily by β -elimination of phosphate from triose phosphate intermediates in anaerobic glycolysis (67). Thus, both oxidative AGEs (CML and pentosidine) and nonoxidative AGEs (3-DG and MGO derivatives) are increased in concert in proteins in diabetes and uremia. The simultaneous increase in tissue levels of both oxidative and nonoxidative AGEs indicates that diabetes and uremia are characterized by a general increase in carbonyl precursors (indicative of carbonyl stress), rather than a selective increase in only oxidatively derived carbonyls (indicative of oxidative stress). Even when steady-state levels of oxidation products, such as glyoxal or dehydroascorbate, are increased, this may result from deficiencies in detoxification or recycling systems, rather than from increased production as a result of oxidative stress.

Mechanistically, we propose that the increase in carbonyl stress in diabetes is caused by deficiencies in, or overload on, pathways for detoxification of dicarbonyl compounds: an imbalance between the rates of production and detoxification of reactive carbonyls. This would explain, for example, the increase in 3-DG and MGO in the absence of an increase in oxidative stress. Is substrate stress also increased? This seems likely during hyperglycemia in diabetes and during hyperlipidemia in atherosclerosis, but does not appear to be the case in uremia and amyloid diseases. Is oxidative stress also increased? Probably yes, but perhaps not until tissue damage is essentially irreversible. Even an increase in chemical modifications of protein by products of lipid peroxidation, such as MDA and HNE, may not be indicative of increased oxidative stress. The increase may result from an increase in substrate stress or a decrease in detoxification capacity, rather than an increase in oxidative stress. An increase in MDA and HNE adducts may be interpreted as evidence of increased oxidative damage, but does not necessarily imply an increase in oxidative stress. The distinction may not be purely academic. Consider that oxidative stress is widely invoked as a pathogenic mechanism for diabetes and atherosclerosis, yet there is limited evidence that antioxidant vitamin and drug supplements provide protection against the progression of these diseases, either in humans or in animal models. In contrast, intervention to decrease substrate concentrations (glucose in diabetes, cholesterol and triglycerides in atherosclerosis) has demonstrable effects on the risk for, and progression of, chronic disease. It is arguable that the set point for oxidative stress is unchanged in these diseases and that increased substrate stress and/or compromised detoxification systems set the stage for disease progression.

THE ORIGIN OF CARBONYL STRESS IN DIABETES

We propose that the generalized increase in reactive carbonyl compounds in diabetes results in part from substrate

stress, but also from a failure of or overload on detoxification pathways. Oxidative stress, we believe, is a secondary event in the pathogenic process. To clarify this argument, it is necessary to distinguish between antioxidant and detoxification activities. Consider, for example, the formation of MDA and HNE during lipid peroxidation reactions. Vitamin E, butylated hydroxytoluene, and probucol may act as antioxidants, inhibiting this reaction by trapping reactive oxygen intermediates (hydroxyl radical, superoxide, and organic radicals). In contrast, once MDA and HNE have been formed during lipid peroxidation reactions, the disposal of these reactive carbonyl intermediates is a detoxification function, perhaps conceptually a part of the antioxidant response, but occurring after the primary oxidative insult has occurred.

The relationship between antioxidant and detoxification functions can be illustrated by the dual function of glutathione (GSH) in the cell. GSH is commonly considered an antioxidant coenzyme, since it acts in this capacity when used by GSH peroxidase to reduce peroxides or superoxide, yielding oxidized glutathione (GSSG). However, GSH also has a discrete detoxification function in the glyoxalase pathway when it facilitates the rearrangement of dicarbonyls to hydroxyacids, e.g., MGO to D-lactate (67), or during NADPH-dependent reduction of HNE or MGO by aldose reductase (68,69). In both cases, GSH forms a hemithioacetal intermediate, acting as a carbonyl trap, not as an antioxidant; it is released from the conjugate as GSH, not GSSG. One might argue that both of these functions, the trapping of reactive oxygen and the detoxification of carbonyl products, are part of the antioxidant response. However, although MGO is increased in blood in diabetes, contributes to the increased formation of AGEs in diabetes, and is detoxified by the GSH-dependent glyoxalase system, MGO does not require oxidation for its formation from glycolytic intermediates. The disposal of MGO is, therefore, not an antioxidant function of GSH. Thus, the antioxidant and detoxification activities of GSH are discrete metabolic functions of the same coenzyme. Other antioxidant vitamin and coenzymes also have non-antioxidant functions: ubiquinol/ubiquinone in oxidative phosphorylation, carotene derivatives in vision, ascorbate as a coenzyme for proline hydroxylase, and perhaps vitamin E in its action on protein kinase C expression (16).

There are three general routes for detoxifying reactive carbonyls by converting them to less reactive metabolites. These include GSH-catalyzed rearrangement of dicarbonyls to hydroxyacids by the glyoxalase pathway, NADPH-dependent reduction to alcohols, and NAD⁺-dependent oxidation to carboxylic acids (Fig. 2). These three systems are partially redundant and compete with one another. For example, the fractional conversion of HNE to hydroxynonenol or hydroxynonenic acid, or of MGO to D-lactate or 1,2-propanediol, depends on the cell or organ system (67,70,71). 3-DG is apparently not a substrate for the glyoxalase system (72), and it appears to be converted primarily to 3-deoxyfructose (73) by aldehyde reductases (74). How does diabetes affect these detoxification pathways? Williamson (9) has proposed that metabolic imbalances in tissues, resulting from excess glucose metabolism, induce a state of pseudohypoxia or reductive stress, rather than oxidative stress, in tissues. Pseudohypoxia, like true hypoxia, is characterized by an increase in the cellular NADH/NAD⁺ ratio, however the redox shift in diabetes is attributed not to oxygen deprivation, but to excessive metabolism of glucose through

glycolysis and the polyol pathway or of lipids by β -oxidation. In some tissues, increased flux of glucose through the sorbitol pathway may also induce shifts in NADPH/NADP⁺ and GSH/GSSG concentrations, which may have a significant effect on detoxification pathways. These shifts, combined with increased levels of carbohydrate and lipid substrates, may lead to an increase in steady-state levels of reactive carbonyl compounds, characteristic of carbonyl stress.

The imbalances in amounts and ratios of reduced to oxidized forms of redox coenzymes are often difficult to interpret with respect to the status of oxidative stress. Hyperglycemia may lead to an increase in NADH by its effects on glycolysis, and at the same time to a decrease in NADPH and GSH through polyol pathway activity. However, the existence of reductive stress in diabetes is supported by actual measurements of redox cofactors in affected tissues and by demonstration of the beneficial effects of aldose reductase and sorbitol dehydrogenase inhibitors and of pyruvate administration on vascular function in diabetic animals (9,10,75): none of these treatments would be described as antioxidant therapy. The ratios of β -hydroxybutyrate to acetoacetate and of lactate to pyruvate are also increased in poorly controlled diabetes (J.W.B., unpublished observations), consistent with increases in the NADH/NAD⁺ ratio and development of reductive stress. Similarly, although treatment with GSH precursors or lipoic acid may retard the development of cataracts (in animal models) and neuropathy (in animal and human diabetes) (76), this may result from their effects on the detoxification, rather than the antioxidant function, of GSH. Overall, the increase in reactive carbonyl compounds derived from both lipids and carbohydrates by both oxidative and nonoxidative routes in diabetes is more consistent with increased production of carbonyls, coupled with limited or compromised detoxification capacity. In uremia, the increase in serum concentrations of carbonyls derived from both carbohydrates and lipids may result more directly from the failure of renal detoxification or excretion pathways. Interestingly, even though MGO and 3-DG are produced by nonoxidative pathways, both may induce oxidative stress and apoptosis in cells (77–79), illustrating a possible cause-effect relationship between carbonyl stress and oxidative stress.

IMPLICATIONS OF THE CARBONYL STRESS HYPOTHESIS

While the focus of this article is on a chemical hypothesis for diabetic complications, diabetes is, without question, primarily a metabolic disease, and its complications are sequelae of broad-based derangements in fuel metabolism. The carbonyl stress hypothesis is a mixture of metabolic and chemical hypotheses: altered metabolism and compromised detoxification lead to increased carbonyl formation, to increased chemical modification of proteins, and then to oxidative stress and tissue damage, which ultimately leads to the development of complications. The implication of the hypothesis is that the extent of chemical modification of proteins, e.g., by AGEs, glycooxidation and lipoxidation products, is sufficient to induce tissue dysfunction and pathology. Some of the arguments against this hypothesis are considered below within the framework of the AGE hypothesis, but apply to the broader range of chemical modifications produced by carbonyl compounds in general. These ideas, summarized here, are discussed in greater detail elsewhere (76,80).

First, AGEs (carbonyl products) are detectable at only trace concentrations in tissue proteins, even in severely compromised diabetic patients, weakening the argument that they have a quantitative role in the development of complications. Proponents of the AGE hypothesis argue, however, that the known AGEs are only the tip of the iceberg of carbohydrate-dependent chemical modification of proteins. The continuing detection and measurement of new AGEs (81) and lipid-derived modifications (82) of tissue proteins support this argument. Although the total concentration of known modifications of proteins still remains at the trace level, part of the challenge to identification of additional products is their heterogeneity and their instability to conditions required for their isolation and analysis.

Second, AGEs (carbonyl products) cannot be relevant to the pathogenesis of diabetic complications because older individuals have levels of AGEs in skin collagen similar to those in the collagen of younger diabetic patients with severe complications (23–26), yet older people do not necessarily have diabetes-like pathology. It is possible, however, that tissue damage early in life may have more severe consequences because of the greater potential for cell growth and division. In experiments in animal models, for example, young animals are significantly more sensitive to radiation-induced cataractogenesis (83,84). The increased sensitivity in young animals has been attributed not to a difference in damage sustained, which may actually be less in the young animals because of better defenses, but to the greater number of future lens epithelial cell divisions and potential for propagation of the genetic damage in the young animals. Thus, the timing of the damage may be important, and the residual cell division potential may be a critical consideration in the development of complications.

Third, AGEs (carbonyl products) cannot be responsible for diabetic complications because high levels of AGEs are present in both collagen and plasma proteins in uremia, yet uremic patients do not develop diabetic retinopathy (36). At the same time, uremic patients are at increased risk for cardiovascular disease, and it can be argued that in the absence of the diabetic milieu in uremia, the full impact of AGE-induced pathology is not manifest in the retina (or even in the vasculature). Hyperglycemia, hyperlipidemia, and their metabolic sequelae may be important for the full expression of AGE-induced pathology.

And finally, levels of AGEs (carbonyl products) are relatively low in tissues from diabetic animal models (85), compared with those in humans, arguing that AGEs cannot be a common cause of diabetic complications in animals and humans. However, differences in sensitivity may be explained by genetic or species-specific differences. It is possible that shorter-lived animals, with higher metabolic rates and lower DNA repair capacity, and possibly with less efficient detoxification potential, may be at greater risk compared with humans at the same steady level of reactive carbonyl compounds.

In defense of the carbonyl stress hypothesis, classes of compounds designed as AGE inhibitors, such as aminoguanidine, are acknowledged to have beneficial effects on the progression of a full range of diabetic complications in animal models (76,80). However, aminoguanidine acts as a general, rather than carbohydrate-specific, carbonyl scavenger, trapping both oxidative and nonoxidative AGE precursors, as well as intermediates, in lipid peroxidation reactions (86).

Potent analogs of aminoguanidine also inhibit cross-linking of collagen in diabetic rats (87), while the thiazolidine derivative OPB-9195 inhibits both AGE formation and nephropathy in diabetic rats (88). Booth and colleagues (89,90) have proposed that pyridoxamine, an inhibitor of AGE formation from Amadori adducts, may be useful in the treatment of diabetic complications. Indeed, we have observed recently that pyridoxamine is an effective inhibitor of albuminuria and end-stage renal disease in streptozotocin-diabetic rats (91). Like aminoguanidine, it is also a potent inhibitor of chemical modification of proteins during lipid peroxidation reactions (S.R.T., unpublished observations). The proposed mechanism of action of these AGE inhibitors remains to be confirmed; however, it appears that all of them are specific for both carbohydrate- and lipid-derived reactive carbonyl compounds. The elucidation of their mechanism of action should greatly improve our understanding of the role of carbonyl stress in diabetes.

FUTURE DIRECTIONS

The carbonyl stress hypothesis and the known AGEs, glycoxidation and lipoxidation products provide, at best, a limited perspective on the pathogenesis of diabetic complications. AGEs are, however, useful biomarkers of the extent of tissue damage, not only in diabetes but also in other chronic diseases (45). Once formed, both the carbonyl precursors and the end products themselves may exacerbate the pathogenic process. Despite the limitations of our knowledge, there is leading evidence that trapping of reactive carbonyl compounds—the chemical intermediaries between hyperglycemia/hyperlipidemia and complications—may be a valuable strategy for inhibiting or delaying diabetic complications. One of the major challenges in diabetes research is to define not only the cause-effect relationship between carbonyl stress and diabetic complications, but also to comprehend the effects of pharmaceutical agents that are beneficial in the management of diabetic complications but are not designed specifically to inhibit AGE formation. These include antihypertensive drugs, lipid-lowering agents, and ACE enzyme inhibitors for treatment of nephropathy and lipoic acid for treatment of neuropathy. The effects of these compounds on tissue levels of carbonyl compounds and the resultant chemical modifications of proteins should be explored. The intersection between the various hypotheses on the origin of diabetic complications may be reflected by the interplay between the effects of various drugs at the level of carbonyl stress and AGE formation.

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