Review

AGEs and their interaction with AGE-receptors in vascular disease and diabetes mellitus. I. The AGE concept

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Abstract

Objective: This is the first part of a bipartite review that summarizes the rising knowledge on the molecular mechanisms underlying the action of advanced glycation endproducts (AGEs) and their contribution to diabetic complications and vascular disease. While the first part presented here focusses on AGE formation, the second part will describe the AGE-protein/receptor interactions and their role in mediating AGE-dependent intracellular signalling.

Results: Nonenzymatic glycation, in which reducing sugars are covalently attached to free amino groups and ultimately form AGEs, has been found to occur during normal aging and at accelerated rate in diabetes mellitus. Oxidation, accompanying glycation in vivo, further supports chemical modifications. AGE formation and protein crosslinking are irreversible processes that alter the structural and functional properties of proteins, lipid components and nucleic acids. AGE modifications do not only change the physicochemical properties of the afflicted molecules, but also induce cellular signalling, activation of transcription factors and subsequent gene expression in vitro and in vivo.

Conclusions: AGEs elicit a wide range of cell-mediated responses that might contribute to the pathogenesis of diabetic complications, vascular and renal disease and Alzheimer’s disease. Substances that inhibit AGE formation, reduce oxidative stress or destroy already formed crosslinks may limit the progression of disease and may offer new tools for therapeutic interventions in the therapy of AGEs mediated disease.

Keywords: Advanced glycation endproducts; Diabetes mellitus; Vascular disease

1. Introduction

Advanced Glycation Endproducts (AGEs) [1,2] accumulate during aging [3] and at accelerated rate during the course of diabetes [1,2,4]. They are believed to induce changes in endothelial cell properties relevant in the pathogenesis of vascular disease. Thus, AGEs are not only markers, but also mediators of chronic vascular complications.

2. Chemistry of ‘advanced glycation endproducts’ formation

Reactive derivatives of nonenzymatic glucose–protein condensation reactions, as well as lipids and nucleic acids exposed to reducing sugars, form a heterogeneous group of irreversible adducts called ‘Advanced Glycation Endproducts’ (AGEs). AGEs were originally characterized by their yellow–brown fluorescent color and their ability to form crosslinks to and between amino groups [5]. The term ‘AGEs’ is now used for a broad range of advanced products of the Maillard reaction [6–8] including compounds as (3,4)Nε-(carboxymethyl)lysine (CML) and pyrraline, which do neither show color and fluorescence, nor occur as crosslinks in proteins [9]. The formation of AGEs in vitro and in vivo is dependent on the turnover rate of the chemically modified target, time and sugar concentration.

In the classical pathway of AGE formation (Fig. 1), a nonenzymatic reaction (also called ‘Maillard’ reaction [6,7]) between glucose or other reducing sugars and N-terminal amino acid residues and/or ε-amino groups of proteins forms initially a Schiff base adduct. Rearrange-
Fig. 1. (a) Scheme of advanced glycation endproduct (AGEs) formation in the ‘Maillard’ reaction: Reducing sugars condense with amino groups of macromolecules to form reversible Schiff base adducts. Intramolecular rearrangements lead to chemically stabilized Amadori products. Further rearrangements, dehydration and condensation reactions result in the irreversible formation of advanced glycation endproducts AGEs that are capable of physically crosslink amino groups. (b) Structure of advanced glycation endproducts (AGEs): The term ‘AGEs’ is used for a variety of structurally different compounds that result from advanced glycation. The different structures of yet identified AGEs as FFI, AFPG, pentosidine, pyrraline and CML are given.

ment of this aldmine leads to the formation of reversible Amadori adducts such as fructose–lysine. Dehydration, successive β-eliminations and condensation reactions finally result in the production of irreversible ‘crosslinks’ that persist for the lifetime of the modified substrate [10]. A similar reaction takes place when glycerolaldehyde interacts with proteins and thereby mediates latent cross-linking potential to α-hydroxyaldehydes [11]. From all natural occurring sugars, glucose has the slowest glycation rate, while intracellular sugars as fructose, threose, glucose-6-phosphate and glycerolaldehyde-3-phosphate form AGEs at much faster rate [12–14].

Oxidation, accompanying glycation in vivo, supports the formation of more permanent, irreversible chemical modifications, such as the glycoxidation products (3,4)N-carboxymethyllysine (CML) [15,16] and pentosidine [16,17]. CML forms from the Amadori product of glucose or threose through metal-catalyzed oxidative fragmentation [15,18] and through autoxidation of glucose [19,20] or ascorbate [21]. In the latter case, autoxidation converts ascorbate to dehydroascorbate, which is rapidly degraded to L-threose. Condensation reactions between two molecules of threose and the ε-aminogroup of lysine lead to the formation of formyl-threosyl-pyrrole (FTP) [21] (Fig. 2). Oxidative cleavage of the Amadori product threosyl-lysine generates CML. CML is also formed independent of the presence of fructose–lysine during metal-catalyzed oxidation of LDL [22] and peroxidation of polyunsaturated fatty acids [22]. In addition, CML can be formed from the precursors glyoxal and glycolaldehyde largely independent from glucose autoxidation by an intramolecular Cannizzaro reaction [19].

Intermediates contributing to chemical modifications during AGE formation are dicarbonyl intermediates as 1-, 3-, and 4-deoxyalduloses [23,24]. The potent cross-linking intermediate desoxyglucosone, which rapidly increases AGE-modifications in proteins [25], is formed most probably only under nonoxidative conditions [26]. In addition, the dicarbonyl intermediates arabinose and glyoxal are generated through metal-catalyzed autoxidation of glucose [26]. From those, glyoxal has been demonstrated to be most important in AGE formation, since it easily
Fig. 2. Proposed mechanism for the formation of ascorbate-specific advanced glycation end products. Autoxidation converts ascorbate to dehydroascorbate, which is rapidly degraded to threose. Condensation of two threose molecules with the ε-amino group of lysine residue results in the formation of formyl-threosyl-pyrrole (FTP) [21].

forms crosslinks with arginine and lysine (resulting in the formation of pentosidine and CML) [26]. Beside its formation from triosephosphates, the reactive α-oxoaldehyde methylglyoxal can also be formed enzymatically in the acetal/acetone metabolism by elimination of phosphate from dihydroxyacetone phosphate and subsequent oxidation [27]. Reduction of methylglyoxal by aldose reductase results in the formation of acetal (95%) and β-lactatadly-hyde (5%). Further reduction of acetal results in the generation of 1,2-propandiol, that has been demonstrated to accumulate in badly controlled diabetes mellitus. Since methylglyoxal and acetal are potent crosslinking agents, the aldose reductase/polyol pathway also promotes AGE formation [28,29].

Metal-catalyzed autoxidation of glucose in the presence or absence of protein is paralleled by the generation of reactive oxygen species (ROS) as superoxide radicals [30] that can undergo dismutation to hydrogen peroxides [31]. Incubation of proteins, low density lipoproteins (LDL) or phosphatidylcholine liposomes with glucose under oxidizing conditions in the presence of transition metals (e.g. copper) also results in increased protein fragmentation, carbohydrate incorporation and generation of thiobarbituric acid reactive species and hydrogen peroxides [32,33]. In the transition metal mediated ‘Fenton reaction’ [34] hydrogen peroxides can produce highly reactive hydroxyl radicals (Fig. 3) [31,32]. Thus, not only glycation but also oxidative damage of macromolecules as peroxidation of LDL [32] accounts for the alterations observed during ’AGE’ing [16,35]. A recent report therefore supposes that formation of glycoxidation products in vivo does not only depend on the relative glucose concentrations, but also on the local oxidative environment [33].

Oxidative modifications and chemical modification resulting from advanced glycation have been demonstrated to affect the clearance of LDL [36]. Increased levels of AGEs on LDL from diabetic individuals [36] indicate that LDL-AGE formation accompanies LDL oxidation and therefore might be a primary mechanism for the pathogenic modification of LDL [37]. This view is emphasized by the observation that lysosomal degradation of AGE-LDLs in macrophages is much slower than degradation of acetylated or oxidized LDL [38,39]. Recently it has been suggested that glucose directly acts with phosphatidylethanolamine and phosphatidylserine to form AGEs on phospholipids (Fig. 4) [37]. Intramolecular oxidation-reduction reactions might then oxidize fatty acid residues independently of the presence of transition metals or free-radical generating systems.

Fig. 3. Metal catalyzed autoxidation of glucose to protein-reactive dicarbonyls is paralleled by the formation of superoxide (O₂⁻). Superoxide free radicals can dismutate to hydrogen peroxides, that are converted to reactive hydroxyl radicals in the presence of transition metals [31,32].
AGES formed on matrix components of the vessel wall or the kidney can further cross-link and trap plasma proteins, lipoproteins and immunoglobulins [2,37,40]. Since small soluble AGE peptides, released into the circulation after macrophage dependent degradation of AGE proteins, readily react and covalently bind to proteins as collagen and plasma LDL in vitro [41], it is supposed that reactive intermediates released from naturally degraded tissue AGEs can bind again and thereby form ‘second’ generation AGEs [37]. If the clearance of circulating low-molecular weight AGE-modified substances is delayed, the degraded AGE macromolecules therefore include reactive substances, which further permit the formation of new crosslinks [37] (Fig. 5).

3. The cellular actions of AGEs

AGE formation was originally thought to specifically tag senescent proteins, thereby providing a specific signal for recognition, degradation and removal of senescent macromolecules [2,42]. Low molecular weight AGE-rich peptides (LMW-AGE) were identified as degradation products and are presumably released into the circulation to be cleared by the kidneys [43,44]. Recent studies, however, demonstrate that interactions of AGE modified proteins with different AGE-receptor complexes not only serve to degrade AGE-proteins, but also activate signal transduction pathways (Table 1), that induce the synthesis and release of cytokines and growth factors that might initiate tissue repair and protein turnover [45], but also contribute to the development of vascular disease and diabetic complications [1,2]. A large number of studies recently confirmed the close correlation between AGE formation and physiologic changes observed in vascular disease, diabetes, atherosclerosis and aging.

Since intracellular sugars are much more reactive than glucose [46], intracellular AGE formation occurs extremely fast. In vitro experiments demonstrated that 1 week incubation of endothelial cells in the presence of high glucose results in an 13.8-fold increase in the intracellular AGE-content [47]. In parallel, the mitogenic activity of high glucose cultivated endothelial cells markedly decreased. The observed loss in mitogenic activity was due to post-translational modifications of basic fibroblast growth factor (bFGF) by AGEs representing the major AGE-modified protein in endothelial cells [47].
Fig. 5. Small, soluble AGE-peptides, released into the circulation after endogenous degradation of AGEs by macrophages, can covalently bind to target proteins as collagen or plasma LDL and thereby form ‘second-generation’ AGEs.

AGE formation on the extracellular matrix results in decreased elasticity, increased thickness, rigidity, breaking time and narrowing the vessel lumen. AGEs formed on vascular matrix proteins mediate defects in the vasodilatory response by inactivating nitric oxide (NO) [67]. In parallel, AGEs induce the expression of the potent vasoconstrictor endothelin-1 and change endothelial function towards vasoconstriction [68].

Binding of AGEs to their cellular binding sites [74] results in depletion of cellular antioxidant defense mechanisms (e.g. glutathione, vitamin C) [70] and the generation of oxygen free radicals. As consequence, increased cellular oxidative stress lead to the activation of the free radical sensitive transcription factor NF-κB in vitro and in vivo (Fig. 6) and thus promotes the expression of NF-κB regulated genes such as the procoagulant Tissue Factor [55,61] or the adhesion molecule VCAM-1 [63,64], that has been associated with early stages of atherosclerosis. Induction of Tissue Factor expression and the parallel AGE-dependent reduction in thrombomodulin activity [55] changes the dynamic endothelial balance from an anti-coagulant to a procoagulant state resulting in focal thrombosis and vasoconstriction. AGE-dependent induction of VCAM-1 can prime the vasculature for enhanced interaction with circulating monocytes [63]. Thus, excessive deposition of AGEs might attract monocytes to bind to the vessel surface, to transmigrate the vessel wall and to release mediators that potentially contribute to the development of vascular lesions (Table 2).

4. Effects of ‘advanced glycation endproducts’ in vivo

AGEs formation proceeds slowly under normal ambient sugar concentrations, but is enhanced in the presence of hyperglycemia and/or under conditions, where the protein and lipid turnover is prolonged. First evidence for post-translational glucose modifications of proteins came from structural studies on human hemoglobin (Hb), in which the variant HbA1c was found to carry an glucose-Amadori product at its N-terminal valine [75]. Thus, determination of HbA1c became a measure for the amount of Amadori products present on red blood cells. An increased HbA1c level indicates sustained hyperglycemia [76] and indirectly advanced glyation [77].

The structure of the different crosslinked AGEs, that are generated in vivo, has not yet been completely determined. Because of their heterogeneity and the complexity of the chemical reactions involved, only some AGE structures have been structurally characterized in vivo. CML and pentosidine have been found to accumulate in tissue collagen of the human skin with age and at accelerated rate in diabetes mellitus [78,79]. CML has also been described in human lens [80] and urine [81]. In addition, pyrraline-specific antibodies detected AGEs in sclerosed glomeruli of kidneys from old nondiabetic animals as well as in diabetic kidneys [82]. Increased pyrraline contents of plasma proteins have also been described in diabetes mellitus [83]. However, there are still doubts whether pyrraline forms under physiological conditions [27,82–84]. Dicarbonyl intermediates have been found to be elevated in plasma and urine of patients with diabetes mellitus [85,86] and a 3- to 6-fold increase in methylglyoxal has been reported in serum of patients with NIDDM and IDDM, respectively [87].

Beside these structurally characterized AGEs, a large number of studies describes the detection of uncharacterized AGEs in serum and tissue proteins, identified by ELISA and RRA techniques [88,89] or immunohistochemistry using antibodies developed to proteins browned by glucose [90–92]. The AGEs recognized by these antibodies were defined by (i) yellow–brown color, (ii) fluorescence, (iii) crosslinking and/or (iv) their interaction with AGE-specific receptors, but were not structurally defined. Although CML might represent the majority of the structures recognized by these antibodies [9], it cannot be excluded that other brown-fluorescent and crosslinking AGE structures are also detected [9]. Using these antibodies, increased serum levels of AGEs in very young prepubertal
and pubertal IDDM diabetic patients were detected shortly after manifestation of diabetes [93]. This indicates that the pathological process leading to diabetic late complications starts at very early timepoints. AGES were further detected in the retinal vasculature of AGE-injured [45,100]. Consistently, serum AGE levels correlate with the progressive loss of kidney function and are up to 8-fold elevated in individuals with end-stage renal disease requiring dialysis [44]. Diabetic patients with uremia have also an increased risk for cardiovascular complications [2]. Inefficient clearance of degraded low molecular weight AGE-rich peptides (LMW-AGES) and recirculation of these ‘toxic’ middle molecules might therefore not only account for uremia [94–96,101], but also perpetuate extrarenal vascular damage in these patients. AGES were also detected in diabetic red blood cells [77] and in liver histones of diabetic rats [102]. The latter one might account for increased theratogenity associated with diabetes [102]. The recently identified guanine advanced glycation endproduct N^\gamma-(1-carboxyethyl)guanine is also supposed to contribute to mutations and DNA transpositions [103]. Further confirmatory evidence for AGES being central mediators of late diabetic complications was provided by animal models, in which an 8 weeks lasting administration of AGES to euglycemic rats resulted in glomerular and arteriolar basement thickening, mesangial expansion and glomerulosclerosis with proteinuria and albuminuria and vascular dysfunction [51,58,104]. Significant AGE deposits were also detected in the retinal vasculature of AGE-infused rats as well as in rats with long-term diabetes [105]. Short-term administration of in vitro prepared AGE-albumin produced vascular defects as vascular permeability and leakage, unresponsiveness to vasodilatory agents, subendothelial mononuclear recruitment [55,89,93], activation of the transcription factor NF-κB [69] and subsequent VCAM-1 gene expression [63,64,106] and thus underlined the concept of AGES as modulators of the vascular tone in diabetes and vascular disease.

Besides diabetes mellitus, increased AGE levels were described in diseases associated with amyloidosis as haemodialysis-associated β2-microglobulin containing amyloidosis [107] and Alzheimer’s disease [108–114]. Furthermore, AGES were detected in healthy persons with a long history of smoking [115], in patients with vascular disorders in the absence of diabetes [115] and in aortic atherosclerotic lesions in patients lacking a history of diabetes [92]. In the latter, extracellular deposition and intracellular accumulation of AGES in the intimal lesions
correlated with age and progression of atherogenesis [92] and strongly implicates that the presence of redox active AGEs might accelerate oxidation and lipid peroxidation and thereby increase the process of atherogenesis [116].

Consistently, long term infusion of physiological amounts of AGE-modified serum albumin into nondiabetic rabbits resulted in AGE accumulation in aortic tissues associated with intimal changes and focal expression of the adhesion molecules VCAM-1 and ICAM-1 [64]. Thus, it is hypothesized that continuous AGE deposition might potentially promote the development of atherosclerotic lesions [37]. This view is emphasized by the finding that AGES were detected in atherosclerotic lesions of euglycemic LDL-receptor deficient rabbits in areas rich in lipids and lipoproteins [117] and in lung collagen of old euglycemic rats [128].

5. Exogenous ‘advanced glycation endproducts’

Recent studies demonstrate that AGES are found in cooked food and remain active in the circulation after oral uptake. The renal clearance of food AGES is markedly impaired in individuals with diabetes compared to non-diabetics and therefore might represent an additional risk for AGE toxicity [129][130] (Fig. 7). Another source of orally administered AGES seems to be smoking, since significantly increased serum AGE levels have been observed in diabetic smokers compared to diabetic non-smokers [131]. Therefore it seems reasonable that ‘breaking the curse of the AGES’ [132] is not exclusively restricted to therapeutic interventions with old and new drugs, but can at least in part be supported by an antioxidant diet and a healthy lifestyle.

6. Pharmacological inhibition of ‘advanced glycation endproducts’ and possible therapeutic interventions

6.1. Aminoguanidine-HCl and other inhibitors of advanced glycation

Aminoguanidine is a small nucleophilic hydrazine compound. Its terminal amino group reacts specifically with non-protein-bound glucose-derived intermediates of early glycation products such as 3-deoxyglucosone. This results in the formation of 3-amino-5- and 3-amino-6-substituted triazines [133,134] (Fig. 8), which prevent the further rearrangement of intermediates to protein-protein and protein-lipid crosslinks. Since the first description of aminoguanidine action [135], a large number of studies have confirmed that aminoguanidine prevents AGE formation and AGE-related complications in vivo [2,37,45,136] (Table 3).

Animal models demonstrated an aminoguanidine-mediated decrease of AGE accumulation in the large arteries [139], the glomerular basement membrane [141,142] and in the retina of diabetic animals [124] and improved abnormalities of the diabetic peripheral nerve [147]. Furthermore, a recent study demonstrated that continuous application of aminoguanidine to non-diabetic rats reduced the age-associated increase in serum and tissue AGES and protected from the aging-dependent progressive decline in cardiovascular and renal function [149]. Although aminoguanidine is regarded as a virtually nontoxic compound [LD₅₀ = 1800 mg/kg in rodents] [141], recent studies demonstrated that high doses of aminoguanidine generate hydrogen peroxides and inhibit catalase, whereas inhibition of catalase is dependent upon the endogenous H₂O₂ production [150]. Chronic administration of aminoguanidine therefore might promote side effects relating to inhibition of catalase as acatalasaemia or suppressed iodine uptake by the thyroid [150].
Table 2
Consequences of AGE formation and deposition

1. AGEs in atherosclerosis
Accumulation in the vascular matrix → narrowing and occlusion [96,117]
Vascular endothelial dysfunction → procoagulant state, vasoconstriction, hypertension [55,67,104]
Glyoxidation of LDL → slow degradation of LDL, lipid peroxidation, oxidative stress [36]
Monocyte activation → vascular cell proliferation, cytokine release, oxidative stress [36,56–58]
Trapping of plasma proteins → initiation of complement activation, oxidation [2,118]

2. AGEs in renal disease
Matrix expansion, vascular leakage and basement membrane thickening → glomerular hypertrophy and glomerular sclerosis [51,58,104]
Glomerular sclerosis → albuminuria [51]
Delayed clearance of AGE-peptides → uremic complications [101,119,120]

3. AGEs in diabetic neuropathy
Accumulation in vasa nervorum → wall thickening and occlusion, ischemia [121]
Vascular endothelial dysfunction → occlusion and ischemia [104]
Glycation of myelin → myelin damage [5,122]
Glycation of growth factors (NGF, FGF) → loss of function [47,123]

4. AGEs in diabetic retinopathy
Increased endothelial cell permeability → vascular leakage and retinal damage [2,105,124–126]
Vessel wall thickening → occlusion, ischemia [2,105,124–127]
Coagulation → occlusion, ischemia [2,105,124–127]
Induction of autocrine VEGF synthesis → angiogenesis, neovascularisation [49]

First clinical studies evaluating its efficacy in diabetic patients have been started in the United States and in Canada. A small study, in which 30 diabetic patients were randomized and treated with aminoguanidine or placebo for 28 days, showed that aminoguanidine treatment significantly decreased hemoglobin-AGE, while HbA1c-values were not affected [77]. Another significant effect of aminoguanidine has been the lowering of triglycerides, LDL-cholesterol and VLDL-cholesterol [41]. The latter observation is consistent with the hypothesis that AGE modifications significantly delay LDL-clearance by macrophages [36]. However, at present it remains unclear, whether the therapeutic effects of aminoguanidine are only due to inhibition of AGE formation.

Recently, the hypoglycemic drug OPB-9195 has been described to act as potent inhibitor of advanced glycation [151]. The thiazolidine derivate reduced AGE formation and AGE-derived crosslinks in vitro and in vivo at significantly lower doses than those of aminoguanidine [151]. OPB-9195 prevented the progression of diabetic glomerular sclerosis in a diabetic rat model even under conditions of persistent hyperglycemia [151]. Up to date, however, clinical studies are missing to evaluate the therapeutic benefits of OPB-9195 in ameliorating diabetic nephropa-

Fig. 7. Potential effects of orally absorbed exogenous AGEs: The renal clearance of food AGEs is impaired in diabetics with renal insufficiency. Therefore, the oral intake of AGEs might represent an additional risk for AGE toxicity [129].
Fig. 8. Mechanism of aminoguanidine-mediated inhibition of cross-linking: The hydrazine compound aminoguanidine can bind to non-protein-bound reactive intermediates of early glycation products and thereby prevents protein–protein or protein–lipid crosslinking by forming triazine compounds.

6.2. AGE-crosslink ‘breakers’

While aminoguanidine prevents ongoing AGE formation, it will probably be not effective in patients with a long history of disease that already resulted in extensive tissue-AGE accumulations. The need to remove irreversibly bound AGEs from connective tissues and matrix compounds led recently to the design of AGE-cleaving agents as the prototypic AGE crosslink ‘breaker’ 1\(\text{N}\)-phenacylthiazoliumbromide (PTB) [156] (Fig. 9). Based on the hypothesis that the majority of AGE crosslinks formed from glucose involve an \(\alpha\)-diketone moiety, PTB specifically attacks the carbon–carbon bond of \(\alpha\)-diketones and thereby breaks the carbon–carbon bond between two carbonyls of an AGE crosslink [156] (Fig. 9). First experiments demonstrated the ability of PTB to reduce AGE crosslinks in vitro and in vivo [156]. Although further studies are needed to proof directly the evidence for the formation of \(\alpha\)-diketones in vivo, this new class of ‘crosslink breakers’ provides a rational basis for a broad clinical application [156].

6.3. Antioxidants

Under physiological conditions, free radicals are rapidly eliminated by antioxidative defense mechanisms such as the glutathione redox system, the vitamin C/vitamin E

Table 3
Effects of aminoguanidine

1. Effects on the development of atherosclerotic lesions
   Prevention of collagen-to-collagen cross-linking [135,137]
   Decreased collagen stability [138]
   Increased elasticity and decreased fluid filtration in large arteries [139]
   Decreased trapping of lipoproteins [2,37]
   Inhibition of glycation and oxidation of LDL [36,37,140]
   Decrease in LDL-cholesterol, VLDL-cholesterol, total cholesterol and triglycerides [37,41]

2. Effects in kidney disease
   Reduction of AGE accumulation in the renal glomerulus [141–143]
   Prevention of diabetes-induced thickening of basement membranes [142]
   Prevention of diabetic nephropathy in animal models [144]
   Reduction of albuminuria in diabetic and hypertensive animals [141–143]

3. Effects on the diabetic peripheral nerve
   Preservation of nerve conduction velocity [145,146]
   Normalisation of nerve action potential amplitude and peripheral nerve blood flow [147]

4. Effects on diabetic retinopathy
   Prevention of lens protein cross-linking [148]
   Prevention of AGE-formation and formation of acellular capillaries in the retina [124]
   Reduction of microaneurysms [124]
   Reduction of pericyte drop-out [124]
   Inhibition of hypertension dependent accelerated diabetic retinopathy [125]
Fig. 9. Proposed mechanism for the N-phenacylthiazolium bromide (PTB) mediated ‘crosslink break’: PTB can selectively cleave diketone bridges of two adjacent carbonyl groups, that might form intermolecular crosslinks with amino acid side chains and thus appears to reverse crosslinking in vitro and in vivo [156].

cycle and the α-lipoic acid/dihydrolipoic acid redox pair [157]. In diabetic patients antioxidant capacity is decreased, resulting in an increased susceptibility to oxidative stress [158–162]. Reactive oxygen radicals play a central role in the generation of intracellular AGEs by sugar adduct autoxidation [32,78,163,164]. Antioxidants as α-tocopherol, α-lipoic acid, deferioxamine or dimethylsulfoxide have been demonstrated to inhibit hyperglycemia-induced reactive oxygen species and AGE formation in vitro. Aspirin administration seems to inhibit protein glucose modifications in diabetic animals and to demonstrate protective effects in age-related cataractogenesis in humans [165].

In addition, AGEs bound to their endothelial surface receptor RAGE have been demonstrated to induce the oxidative-stress sensitive transcription factor NF-κB [69]. Reactive oxygen species dependent NF-κB activation can be inhibited in the presence of antioxidants (α-lipoic acid, vitamin E, N-acetylcysteine (Nac) or pyrrolinedithiocarabamate (pDTC) in vitro and in vivo [157]. Thus, antioxidants do not only reduce the formation of AGEs [166], but also suppress AGE-mediated intracellular effects [70].

The dual concept of antioxidant protection was underlined by experiments in which the peroxidation-suppressing protooncogene bcl-2 was overexpressed [167]. Overexpression of bcl-2 protected cells from lipid peroxidation and AGE formation [167]. This effect might be due to a yet unknown peroxidase or reductase activity of bcl-2. However, it has been shown that the bcl-2 related protein bcl-XL did not only protect cells from oxidative stress, but also from NF-κB activity by binding to its carboxy-terminal end. Thereby bcl-XL modifies NF-κB transactivating capacity even after translocation of NF-κB into the nucleus. The dual effects of antioxidants and the oxidation-suppressing protooncogene bcl-2 on AGE mediated cellular activation might therefore offer additional strategies for the potential prevention of AGE mediated disease.

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