30

Aging: Drugs to Eliminate Methylglyoxal, a Reactive Glucose Metabolite, and Advanced Glycation Endproducts

Indu Dhar and Kaushik Desai
Department of Pharmacology, College of Medicine, University of Saskatchewan, Saskatoon, SK, Canada

1. Introduction

The aging process not only affects the whole body, but also affects individual cells. While the age-related changes in the body are popularly recognized as wrinkling of the skin, indicating alterations in basement membrane proteins, the processes of cellular aging are less well defined. The underlying common theme of cellular aging and whole body aging seems to be an increase in oxidative stress. Advanced glycation endproducts (AGEs), which are widely accepted to alter basement membrane proteins, also increase oxidative stress. Reactive dicarbonyls, such as methylglyoxal (MG), formed during glycolysis and other metabolic processes are precursors of AGEs formation and triggers of oxidative stress. MG, AGEs and oxidative stress are very likely to induce DNA damage and be at the root of cellular aging. Thus, a strategy to prevent an elevation of MG, formation of AGEs and the associated oxidative stress has great therapeutic potential to slow the aging process at the cellular and the whole body level.

2. The ageing process

The process of aging is accepted as an inevitable normal part of the life cycle of each and every living organism. Aging can be grossly defined as an overall decline in biological functions. Thus, aging involves gradual changes in the body such as reduced immunity, loss of muscle strength, stiffening of the arterial wall, loss of elasticity and wrinkling of the skin, and decline in memory, all of which result in increasing weakness, risk of developing diseases, and ultimately death. These changes take place at the cellular, organ and the whole organism level. The whole process of aging unfolds very clearly in species with a long life span such as human beings. Cellular aging ultimately translates into whole body aging.

Hayflick et al. [1] first described cellular senescence in the sixties when they showed that normal cells had a limited ability to proliferate in culture. Cellular senescence is believed to be initiated by increased cellular stress [2, 3]. Factors contributing to cellular stress and aging include dysfunctional telomeres (telomere length) [4, 5], DNA damage [6] and mitogenic or oncogenic stimuli and signals [2, 4, 5]. The factors such as age and oxidative

stress affect telomere length and telomerase activity which in turn affects cellular senescence [4]. Oxidative stress has been shown to damage DNA and affect life span [7-10]. A controversial view of cellular senescence is that it is an important protective mechanism against transformation of the cell into a malignant phenotype, in which case it would affect only mitotically active cells [2, 3]. The molecular mechanisms involved in cellular senescence are still being unraveled and will not be considered further in this review. The focus of this review will be on MG, a reactive dicarbonyl metabolic intermediate produced in the body, AGEs, and oxidative stress, all of which are interrelated and affect cellular as well as whole body aging. We will discuss some compounds that can scavenge MG, prevent the formation of AGEs (inhibitors) or break the existing AGEs (AGE breakers).

3. Theories of aging

Aging has been attributed to a number of different causes which have been presented in the form of different theories. These theories are based broadly on two different ideas, one of which is programmed life processes (program theories, e.g. Biological Clock theory, Limited Number of Proliferation theory), and the other one is of errors, mainly at the DNA and gene level, in life processes (error theories, e.g. Disease theory, Cross-linking theory, Rate of living theory, Free radical theory). A number of theories of aging are based on the combination of these two ideas, i.e. program theories and error theories [11-14].

Changes at the cellular level ultimately affect the whole body. The cell is a dynamic centre of ongoing metabolic activity driven by almost constant use of oxygen. Reasonably, the metabolic activity may affect survival or the death of the cell. The 'Rate of living theory' implicates the role of metabolism in aging, which is based on the observation that animals with higher metabolic rates often have shorter life spans. Since the metabolic processes and oxygen consumption can also generate oxidative stress, an excess of which is deleterious for the cell, the 'Free radical theory' of aging has become one of the more popular theories. The free radical theory proposes a connection between the metabolic rate and aging through an increased oxidative stress generation.

4. Free radical theory of aging

Max Rubner proposed the 'rate of living theory' early in the 20th century [15]. He observed that larger animals, which generally have slower metabolic rates, live longer than smaller animals with faster metabolic rates [15]. Even though it is now common knowledge that metabolism is associated with the generation of free radicals, it was Commoner *et al*. [16] who discovered the formation of free radicals *in vivo*. Commoner et al. [16] found that an increase in an organism's metabolic activity can increase the concentration of endogenous free radicals. Free radicals are atoms or molecules with an unpaired electron in an orbit, making them highly reactive. The high reactivity of free radicals makes them deleterious for cells because they react with proteins, lipids, DNA and other biomolecules, and disrupt their structure and function. Free radicals can be derived from oxygen mainly in the form of superoxide anions ($O_2^{\bullet -}$) and hydroxyl radicals ($^{\bullet}OH$), which are known as reactive oxygen species (ROS). Free radicals can also be in the form of highly reactive non-radicals which do not have an unpaired electron in their orbit, such as hydrogen peroxide (H_2O_2). Normally, the cells and the body have adequate antioxidant defenses which can neutralize free radicals

and prevent the generation of oxidative stress resulting from an excess of free radicals [17-22]. The formation of free radicals and the function of antioxidants have been nicely explained in reviews by Haliwell [20, 21].

The free radical theory of aging was proposed by Denham Harman in 1956 [23]. The free radical theory of aging attributes the aging process to cumulative cellular damage inflicted by the reaction of free radicals with key functional cellular and tissue constituents resulting in impaired function, disease and death [23]. The discovery of an antioxidant enzyme, superoxide dismutase (SOD) [24], which plays a key role to eliminate superoxide anion levels, provided some validity to the free radical theory, which was not initially accepted by many.

The mitochondrial respiratory chain is a major source of free radicals, mainly in the form of superoxide anions, which cause damage to the mitochondria and reduce life span [25, 26]. The damage inflicted by ROS, especially to DNA [7], rather than the metabolic rate, showed a greater correlation with life span [8]. Damage to DNA was formulated into the somatic mutation theory, which states that genetic mutations caused by an excess of free radicals could lead to accelerated aging [7, 9, 10].

The fact that increased production of ROS in the mitochondria can reduce life span was supported by several studies. Thus, Ku *et al*., [27] showed that the rates of mitochondrial superoxide anion and hydrogen peroxide generation were inversely correlated to maximum life span potential when they compared seven different mammalian species with different life spans ranging from 3.5 to 30 years. Similarly, ROS production was higher in heart mitochondria of the rat, which has a life span of about 4 yrs, than in the long-lived pigeon, which has a longer life span of 35 yrs [28]. Theoretically, therefore, if the free radical production is diminished, the life span should increase. This has been demonstrated in several species. Thus, over expression of SOD and catalase in the worm *Caenorhabditis elegans* (*C. Elegans*) through *age-1* alleles, increases their oxidative defenses and life span by 65% longer on average [29, 30]. Increased activity of SOD and reduced oxidative stress in the transgenic *Drosophila* (*Drosophila melanogaster*) flies also slows the aging process and results in a longer life [31, 32]. Also, over expression of catalase in the peroxisome, the mitochondria or the nucleus in transgenic mice, reduced oxidative damage, hydrogen peroxide production, and delayed the development of cardiac pathology and cataract formation along with an average increase of 5.5 months in the life span [33]. The observation that long-lived animals have lower levels of antioxidant enzymes was explained as being due to a lower rate of production of oxygen radicals [34].

Interestingly, some of the studies in rodents did not produce the expected results. For example, the administration of antioxidants [35], or over expression of CuZn SOD and catalase in mice [36], or SOD in rats [37], did not increase their life spans. In rodents, one reason for the lack of additional protective effects, which are normally associated with an increase in antioxidants, could be their ability to synthesize vitamin C [38], which might already be providing the required protection. This was verified by knocking out the vitamin C synthesizing enzyme, L-gluconolactone oxidase (GLO) in mice, which then have to depend on dietary vitamin C [39]. GLO knockout mice had damaged aortic walls when they were fed a diet low in vitamin C, which underlined the importance of the constitutive antioxidant function of vitamin C in rodents [39]. Thus, studies in rodents do not provide unequivocal support for the free radical theory of aging [40].

Another way of increasing free radical production and oxidative stress is by increasing total caloric intake, which can be easily done be feeding an excess of glucose. A correlation between life span and dietary caloric intake was reported in rats and mice by McCay *et al*. [41]. One quantitative estimate was provided in the study by Weindruch and Walford [42] who showed that a 40% reduction in dietary caloric intake extended maximum life span by one third. A high dietary caloric intake causes an increased rate of DNA damage [43], due to a high metabolic rate which in turn results in higher amounts of superoxide anion, hydrogen peroxide and hydroxyl radical formation [44].

5. Methylglyoxal

Chemically, MG, or pyruvaldehyde, is a highly reactive electrophilic α,β-dicarbonyl compound [45, 46] (Fig. 1). MG has been proposed to be formed mainly during glycolysis, through spontaneous nonenzymatic transformation of triose phosphates [45, 47-49] (Fig. 2). MG synthase has been proposed to convert the triose phosphate intermediate, dihydroxyacetone phosphate (DHAP), into MG, especially when inadequate inorganic phosphate is available [50, 51]. Other sources of MG, which are believed to produce lower amounts of MG, include intermediates of protein and fatty acid metabolism, such as aminoacetone produced from L-threonine and glycine [52, 53], and acetone [54, 55], respectively (Fig. 2). Semicarbazide-sensitive amine oxidase (SSAO) catalyzes the breakdown of aminoacetone [52, 55, 56], while acetone and acetol mono-oxygenase (AMO) converts acetone to acetol and acetol to MG, respectively [54] (Fig. 2). SSAO is found in substantial amounts in the vascular smooth muscle cells and the plasma [55].

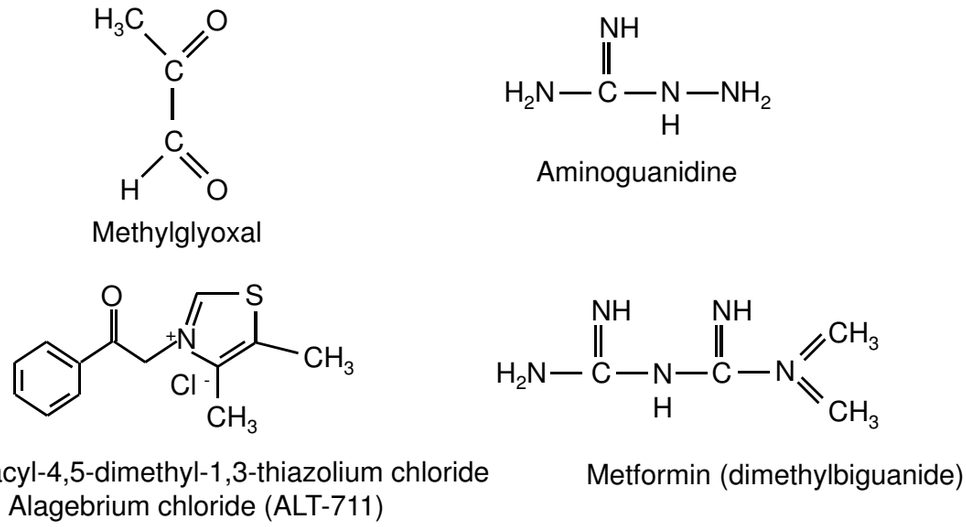


Fig. 1. Structure of methylglyoxal (MG) and three compounds with an ability to bind MG or inhibit the formation of advanced glycation endproducts (AGEs) or break formed AGEs. These compounds are discussed in this review.

After MG is formed, it is rapidly degraded to D-lactic acid by the highly efficient and ubiquitous glyoxalase system, which consists of two key enzymes, glyoxalase I

(lactoylglutathione lyase) and glyoxalase II (hydroxyacylglutathione hydrolase) [57-59] (Fig. 2). Reduced glutathione (GSH) plays a key role by binding MG and presenting it to glyoxalase I. Thus, adequate availability of GSH is important in keeping MG levels low in the body. For this reason enzymes involved in the synthesis and recycling of GSH, such as glutathione peroxidase and glutathione reductase are also important in the metabolism of MG [60-62].

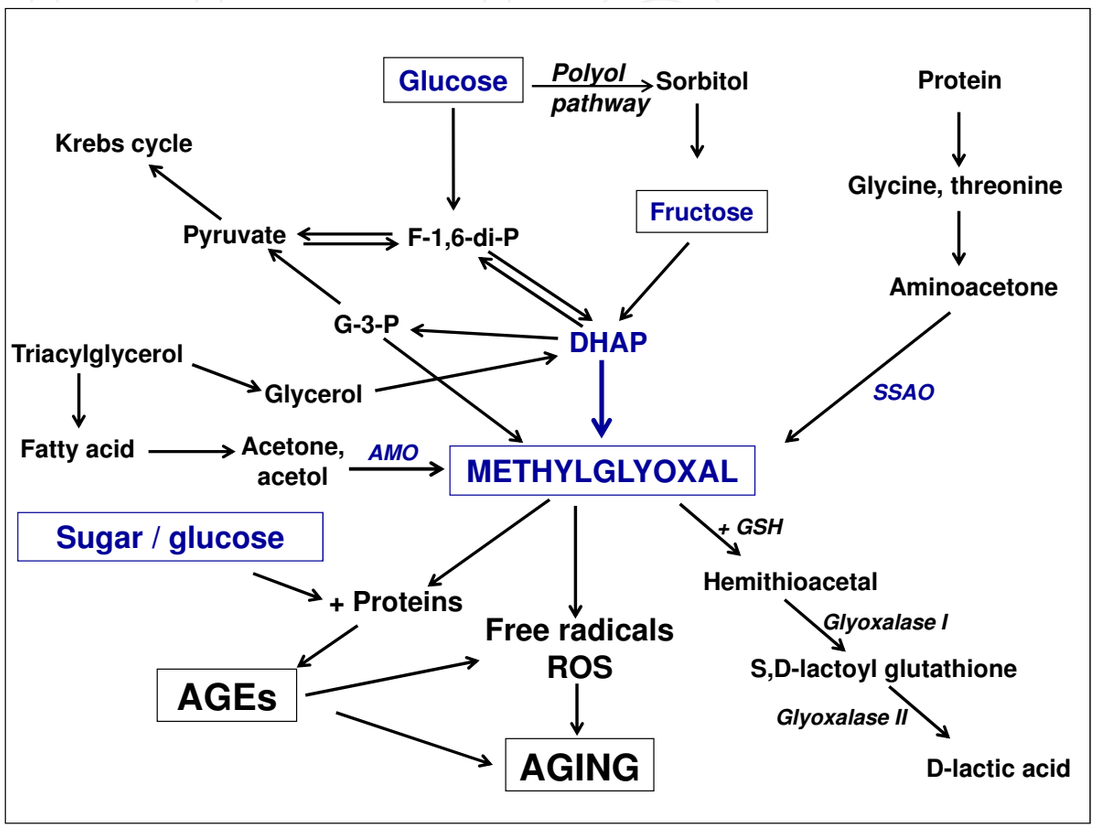


Fig. 2. A schematic of key sources and steps of methylglyoxal (MG) formation from intermediates of glucose, protein and fat metabolism, and its degradation by the glyoxalase enzymes. Abbreviations: AGEs – advanced glycation endproducts; AMO – amine oxidase; DHAP – dihydroxacetone phosphate; FA – fatty acid; F-1-P – fructose-1-phosphate; F-1,6-di-P – fructose-1,6-diphosphate; F-6-P – fructose-6-phosphate; G-3-P – glyceraldehyde-3-phosphate; G-6-P – glucose-6-phosphate; ROS – reactive oxygen species; SSAO – semicarbazide-sensitive acetone/acetol mono-oxygenase; GSH, reduced glutathione.

Despite the efficient glyoxalase system, MG levels can increase significantly in the plasma and different organs such as the aorta and the kidneys [61, 63-66]. We have shown that MG levels are elevated in the plasma, aorta and kidney of fructose-fed Sprague-Dawley rats and spontaneously hypertensive rats (SHR) [61, 63-65]. Patients with type 1 and type 2 diabetes have 2-6 fold higher plasma levels of MG compared to healthy people [67, 68]. MG possibly plays a role in the pathogenesis of insulin resistance and type 2 diabetes as shown by several *in vitro* [69-71], and by our recent *in vivo* study in acute [66] and chronic MG-treated

Sprague-Dawley rats [72]. Elevated MG levels are linked to the development of micro-vascular complications of diabetes such as retinopathy and nephropathy, and other conditions such as atherosclerosis and neurodegenerative diseases [73-77]. MG levels are high in the cerebrospinal fluid of patients with Alzheimer's disease [76].

6. Advanced glycation endproducts

Unwanted chemical modification of physiologic constituent molecules of the body, which leads to the formation of harmful chemical entities, seems to be an unavoidable part of metabolic processes of the body. One type of modification, known as glycation, a nonenzymatic reaction, is a serious hazard of excess glucose availability in the body. The chemical interaction leading to the formation of AGEs starts when a reducing sugar condenses with the amino groups of proteins at their N terminus or on lysyl side chains (ε-amino groups) [78]. This nonenzymatic glycation involves a series of post-translational modifications. Glycation begins with the aldehyde or the ketone carbonyl group of the sugar combining with the protein to form an unstable aldimine intermediate or a Schiff base. Later on the Schiff base undergoes an Amadori rearrangement to form a stable Amadori product, a l-amino-l-deoxyfructose derivative with a stable ketoamine linkage, which can get cyclized to form a ring structure [78-80]. The Amadori product can undergo oxidation, degradation or rearrangement and form AGEs, a heterogenous group of products. Auto oxidation of glucose (Wolff pathway) [81] or of the Schiff bases (Namiki pathway) [82] can lead to formation of reactive diacrbonyls, but these pathways which are readily observed at high glucose concentrations *in vitro*, are not predominant *in vivo* [83]. The Maillard reaction, also known as the "browning reaction", involves oxidation of the glycated product which forms a brown coloured product. Glucose, fructose and glucose-6-phosphate are all involved in glycation, albeit at different rates of reaction with glucose, the most important contributor, reacting at a comparatively slower rate than the other two [6]. Increased glucose levels, as seen in diabetic patients, causes more AGEs formation than in healthy people. These AGEs affect the normal function of several proteins and enzymes, and are responsible for aging [74, 80] and the complications of diabetes such as nephropathy and retinopathy [79]. Another way by which the glycation reaction causes damage is through the formation of reactive α-dicarbonyl compounds, such as MG, glyoxal and 3-deoxyglucosone (3-DG), when the sugar molecule undergoes fragmentation [78].

MG can also cause AGEs formation [78, 79]. In fact, MG and two other dicarbonyl metabolic intermediates, 3-DG and glyoxal, are believed to be major sources of intracellular and plasma AGEs formation [79, 84, 85], which are commonly implicated in the aging process. Any MG which is not degraded by the glyoxalase system or aldose reductase, reacts non-enzymatically with arginine or lysine residues of proteins [45] to form irreversible AGEs. This glycation is not random, but it depends on the structural configuration and (or) physical locations of the target proteins [86, 87]. The AGEs produced by the reaction between MG and arginine are hydroimidazolone Nε-(5-hydro-5-methyl-4-imidazolon-2-yl)-ornithine and argpyrimidine [88], whereas the AGE, Nε-carboxyethyllysine (CEL) [89, 90] is formed when MG reacts with lysine. Further crosslinking of these AGEs produces fluorescent products such as pentosidine and cross-line, and non-fluorescent ones such as argpyrimidine, methylglyoxal-lysine dimer (MOLD), glyoxal-lysine dimer (GOLD) and

imidazolones [91, 92]. The presence of these AGEs can be detected immunohistochemically in tissues [93].

7. Methylglyoxal, AGEs, oxidative stress and aging

The damage inflicted by oxidative stress and the formation of intracellular AGEs likely contribute to cellular aging. From this point of view, both increased MG and AGEs would cause accelerated cellular aging. MG would be a double-edged sword because it is a potent inducer of oxidative stress [17, 62, 94, 95], as discussed below, and it is a major precursor of AGEs formation. AGEs also induce oxidative stress.

8. Methylglyoxal and oxidative stress

The role of MG in inducing oxidative stress is well established [17]. Several studies have helped to develop an integrated view of the multiple pathways activated by MG to increase oxidative stress (Fig. 3). The reader is referred to our earlier review on MG and oxidative stress [17]. MG increases the formation of superoxide [94, 96-99], hydrogen peroxide and peroxynitrite [94, 95, 98, 100], proinflammatory cytokines, such as interleukin 1β (IL-1β) [101], interleukin-6 (IL-6), interleukin-8 (IL-8) and tumor necrosis factor-α (TNF-α) [67, 101], in different cell types such as VSMCs [62, 94, 95], endothelial cells [102], rat kidney mesangial cells [97], rat hepatocytes [100], neutrophils [67, 98], platelets [99], cultured neural cells from rat hippocampus [101], cultured cortical neurons [103], and SH-SY5Y neuroblastoma cells [104].

MG has been shown to increase the activity of several prooxidant enzymes such as NADPH oxidase [94, 97] (Fig. 3), p38 MAPK [98, 102], and increase the of expression of JNK and PPAR-α [104].

Excess superoxide can react with nitric oxide (NO) to form peroxynitrite ($ONOO^-$) [105] (Fig. 3). Peroxynitrite is a strong oxidant and nitrating agent. Because of its oxidizing properties, peroxynitrite can damage a wide range of molecules including DNA and proteins in cells [105].

Besides directly increasing free radical production, MG can increase oxidative stress by reducing antioxidants (Fig. 3) such as GSH [104, 106, 107], glutathione peroxidase [108], glutathione reductase [60, 62, 108, 109], and manganese superoxide dismutase (MnSOD) [96], in different cells such as erythrocytes [106, 107], VSMCs [62, 96], and endothelial cells [109]. Reduced antioxidants in turn impair the detoxification of MG, increase its half-life and set up a vicious cycle to cause further oxidant damage. Glutathione peroxidase removes hydrogen peroxide with the help of GSH which in turn is converted to oxidized glutathione (GSSG). Glutathione reductase recycles GSSG to GSH [62, 110] (Fig. 3).

An increased production of ROS was also observed in monocytes treated with MG-modified albumin [111]. Thus, MG induced thrombosis and inflammation by activating monocytes, induced apoptosis of neutrophils, and caused platelet-neutrophil aggregates [112].

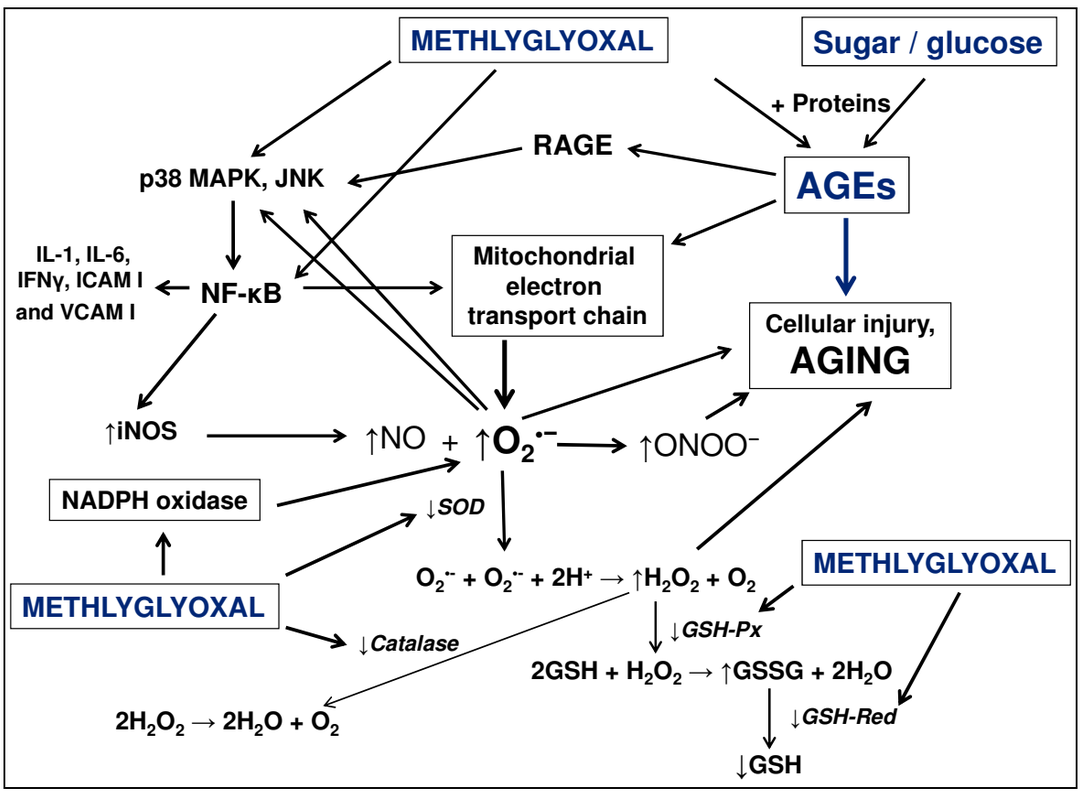


Fig. 3. A schematic of oxidative stress pathways activated by methylglyoxal and advanced glycation endproducts and their implication in aging. Abbreviations: AGEs, advanced glycation end products; GSH-Px, glutathione peroxidase; GSH-Red, glutathione reductase; GSH, reduced glutathione; GSSG, oxidized glutathione; H_2O_2, hydrogen peroxide; ICAM 1, intercellular adhesion molecule 1; IFNγ, interferon γ; IL1, interleukin 1; JNK, JUN N-terminal kinase; MG, methylglyoxal; NF-κB, nuclear factor-kappaB; NO, nitric oxide; $O_2^{\bullet-}$, superoxide anion; $ONOO^-$, peroxynitrite; p38 MAPK, p38 mitogen activated protein kinase; RAGE, receptor for advanced glycation endproduct; SOD, superoxide dismutase; VCAM 1, vascular cell adhesion molecule 1.

Metabolic activity in the mitochondria is at the centre of the free radical theory of aging. Mitochondria, which are the major sites of ATP and energy production in the cell, also generate about 85% of total intracellular superoxide when electrons escape, mainly from complex I and complex III, and react with oxygen [23, 113-116].

MG increases mitochondrial superoxide production [116, 117]. Treatment of rat aortic VSMCs (A-10 cells) with MG (30 μM) significantly increased mitochondrial superoxide production by 69.9% compared with untreated cells. The AGEs cross-link breaker, alagebrium (50 μM), and SOD mimetic 4-hydroxy-tempo (Tempol, 500 μM) significantly decreased MG-induced mitochondrial superoxide production by 57% and 85.8%, respectively. Mitochondrial nitrotyrosine formation was also increased by MG [96].

In *in vivo* studies elevated MG levels are associated with increased oxidative stress. For example, we have shown that in 13 wk old SHR with elevated blood pressure, significantly elevated plasma and aortic MG levels are associated with increased levels of superoxide,

and significantly reduced GSH levels, glutathione peroxidase, and glutathione reductase activities, compared with age-matched Wistar Kyoto (WKY) rats [61]. Similarly, in diabetes mellitus and hypertension, increased MG levels are associated with increased oxidative stress [61, 65, 67, 68, 118].

An excess of MG, CEL and CML indicate carbonyl overload and are associated with oxidative stress [73, 79, 119-123].

Glycated proteins and AGEs also induce oxidative stress (Fig. 3) through several mechanisms. AGEs induce production of cytokines and growth factors [124-130]. AGEs bind to the receptor for AGEs (RAGE) and scavenger receptors to induce oxidative stress in various cells including VSMCs, endothelial cells, and mononuclear phagocytes [128, 131]. In endothelial cells AGEs increase expression of vascular cell adhesion molecule-1 (VCAM-1), intercellular adhesion molecule-1 (ICAM-1), and increase activity of NF-κB to increase oxidative stress [126, 132].

9. Methylglyoxal and aging

The accumulation of AGEs in extracellular tissue proteins, such as the basement membrane and matrix proteins of blood vessels and skin, is a well known phenomenon characteristic of aging and age-related diseases. Several studies demonstrate aging associated increase in AGEs. Thus, accumulation of AGEs in the vessel walls results in a gradual loss of elasticity, which makes older subjects more susceptible to cardiovascular diseases [133, 134]. MG-induced AGEs, such as CEL and CML, increased with age in human lens and cause cataract formation [90]. In a study on 172 subjects serum levels of CML, 8-isoprostanes and C-reactive protein, which are markers of oxidative stress, were higher in elderly people (>60 years old) compared with younger people (<45 years old) [135]. One reason why AGEs accumulate during the aging process could be due to an age-related decrease in antioxidant enzymes. Thus, Mailankot et al. [136] reported that the activity and expression of glyoxalase I protein, which is involved in MG degradation, decreased with age in the anterior epithelial cells of human lens, which causes an accumulation of MG. Similarly, an age-dependent decrease in catalase activity in the skin may be responsible for elevated MG and peroxynitrite production [137].

However, it is doubtful whether extracellular AGEs accumulation plays a causative role in aging. On the other hand AGEs formation inside the cell, such as AGE-nucleotides in DNA, may contribute to cellular senescence [6, 60, 74, 80]. DNA integrity is an important determinant of lifespan and errors in DNA repair would lead to substitutions, deletions, insertions, and transpositions of nucleotides, with increased risk of carcinogenesis and reduced life span. Animals with a longer lifespan and more efficient DNA repair have delayed carcinogenesis [80]. In this regard MG-induced DNA damage can have a more direct effect on aging.

Studies directly implicating MG in the aging process are very few and this is one area where there is a knowledge gap. The study by Morcos et al in the worm *C. elegans* highlights the role of MG, glyoxalase I and MG-induced ROS formation in aging and life span [138]. They showed that the activity of glyoxalase I was markedly reduced with age resulting in accumulation of MG-derived adducts and oxidative stress markers, which further inhibited

the expression and the activity of glyoxalase I. Over expression of glyoxalase I decreased MG-induced modification of mitochondrial proteins and ROS production, and prolonged the lifespan of *C. elegans*; whereas CeGly knock-out produced the opposite effect [138].

Scheckhuber et al. studied the degradation of MG by the glyoxalase system enzymes and its effect on growth and lifespan in filamentous ascomycete and a model of aging, Podospora anserine (*P. anserina*) [139]. Using genetic manipulation of the two enzymes of the glyoxalase system, they found that up-regulation of both components of the glyoxalase system was effective in increasing lifespan in P. anserina.

Oxidative stress-induced cellular senescence was demonstrated in the study by Sejeresen and Rattan [140]. They treated human skin fibroblasts with MG (400 μM), or glyoxal (1.0 mM), and found the appearance of various senescent phenotypes within three days. These phenotypes showed growth arrest, had increased hydrogen peroxide and the glyoxal-induced AGE, Nε-carboxymethyl lysine (CML) protein levels, and altered SOD and catalase antioxidant enzyme activities [140]. Sejeresen and Rattan proposed this model to study cellular senescence *in vitro* [140].

In this review we have highlighted some important facts that oxidative stress is a major factor in the cellular and whole body aging processes, AGEs are strongly associated with aging and MG is a major precursor of AGEs formation, and both MG and AGEs are potent inducers of oxidative stress. Based on these facts, it is highly likely that MG may have a major role in the aging process through induction of oxidative stress as depicted in the scheme in Fig. 3. In fact, elevated MG levels may be responsible for causing accelerated aging in many tissues and organs of the cardiovascular system, nervous system, and other systems of the human body. A strategy to prevent aging should include targeting MG by reducing excessive formation, inhibit AGEs formation, and remove excessively formed MG and AGEs from the body.

10. Anti-MG and anti-AGEs compounds

The deleterious effects of MG and AGEs can be prevented by compounds that can do one or all of the following: (i) bind and neutralize reactive aldehydes, especially MG, (ii) prevent the formation of AGEs, (iii) neutralize formed AGEs, (iv) break down formed AGEs.

Considering these multiple ways of preventing the effects of MG or AGEs these compounds will be termed as "anti-MG" or "anti-AGE". Unfortunately, specific anti-MG or anti-AGE compounds are not yet available. The ones available are non-specific and have one or more other effects, which limits their usefulness. A number of the available compounds happen to have anti-MG as well as anti-AGE effects.

The possible sites at which anti-AGEs and anti-MG drugs can work are shown in Fig. 4, which outlines the various stages of AGEs formation.

Site 1. The first step in glycation is the binding of a sugar to the free amino groups of a protein. Drugs that bind to the amino group of proteins, such as aspirin, can prevent the binding of the sugar to the amino group. This group of compounds is likely to produce non-specific effects.

Site 2. Compounds can be used to bind aldose and ketose sugars to neutralize them and prevent them from reacting with proteins. E.g. Aminoguanidine reacts with the carbonyl group of glucose and prevents AGEs formation.

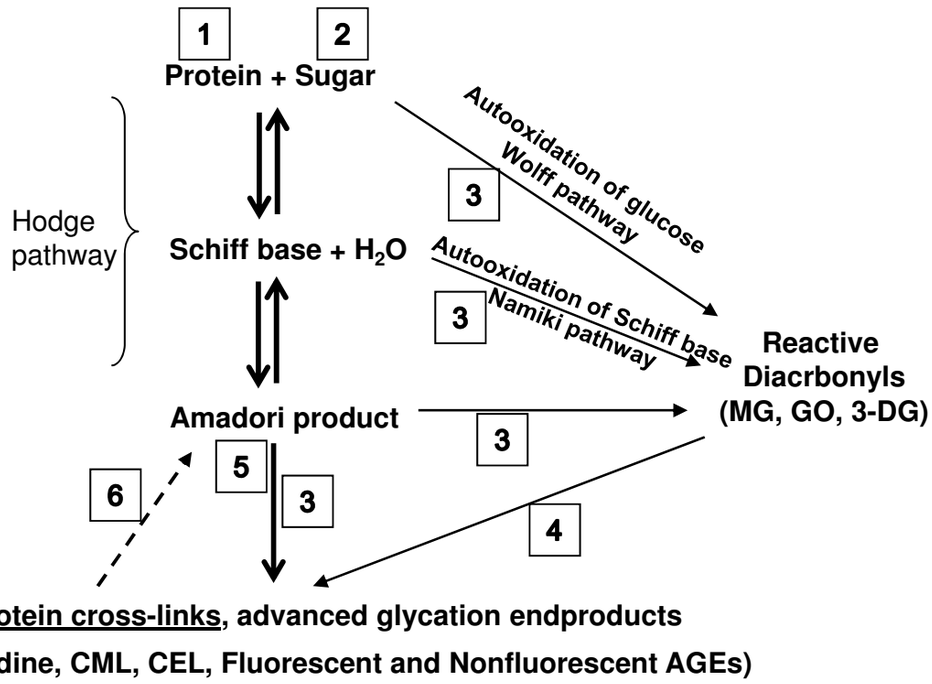


Fig. 4. Stages of formation of advanced glycation endproducts (AGEs) from glycation of proteins. Nonenzymatic glycation of protein leads to reversible formation of Schiff bases, which lead to further reversible formation of Amadori adducts and ultimate formation of stable irreversible AGEs. Solid vertical arrows show these steps which form the classical Hodge pathway of AGEs formation. Auto oxidation of glucose (Wolff pathway) or of the Schiff base (Namiki pathway) forms reactive dicarbonyls such as methylglyoxal (MG), glyoxal (GO) or 3-deoxyglucosone (3-DG) which is mostly seen *in vitro*, rather than *in vivo*. The dicarbonyls, which are also formed from other metabolic pathways, also contribute significantly to AGEs formation, as explained in the text. The various sites at which anti-AGEs and anti-MG compounds can act are indicated by numbers and discussed in the text. Based on the scheme proposed by Khalifah et al. [83]

Site 3. AGEs and reactive aldehydes also generate reactive oxygen species which adds to their damaging effects [17, 141, 142]. Antioxidants such as vitamin C or E [143] and metal chelators such as penicillamine [144] can be used to quench ROS and metal ions.

Site 4. Reactive aldehydes such as MG, glyoxal, glycoaldehyde and glucosones, which are formed during nutrient metabolism and AGEs formation, are a major source of AGEs formation. Reactive aldehydes can be neutralized by compounds such as aminoguanidine [145, 146] and metformin [147, 148].

Site 5. Amadori adducts, formed in the intermediate stages of AGEs formation can either be quenched by compounds such as aminoguanidine, or degraded enzymatically by enzymes such as amadoriase and human fructosamine-3-kinase, which belong to this group [149, 150]. Amadoriases have not been detected in higher organisms [151, 152].

Site 6. The final group of compounds acts on formed AGEs and are therefore, known as AGE breakers or cross-link breakers. E.g. phenacylthiazolium bromide (PTB) [153] and

alagebrium (previously known as ALT-711), which are thiazolium compounds. AGEs which do not have cross-links such as pentosidine [154], GOLD and MOLD [155] will not be affected by these drugs.

Some of the more common compounds with anti-MG or anti-AGEs effects are described below.

Aminoguanidine is one of the popular and widely used AGEs inhibitor [145] and MG scavenger. Despite being a guanidine derivative it shares many common properties with hydrazine and is classified as a hydrazine [156]. As described above, aminoguanidine acts at site 2 as well as site 4 (Fig. 4) meaning that it prevents AGEs formation by combining with the carbonyl group of glucose as well as by scavenging reactive dicarbonyls formed during various metabolic processes [146]. The inhibitory effect of aminoguanidine is mainly at the Amadori stage [83]. Aminoguanidine is not a specific AGEs inhibitor or MG scavenger and it has other actions. Aminoguanidine potently inhibits histaminases [157, 158] and prevents deamination of histamine and putrescine. Aminoguanidine also inhibits nitric oxide synthase (NOS) [159, 160] and prevents the formation of nitric oxide (NO), a dynamic signaling molecule in the body [161], from L-arginine. Aminoguanidine also binds to the enzyme S-adenosylmethionine decarboxylase and increases synthesis of polyamines such as spermidine and spermine from ornithine [162]. Aminoguanidine can also bind pyridoxal and cause vitamin B6 deficiency, which in turn can result in adverse reactions to aminoguanidine [163]. A number of *in vitro* and *in vivo* studies have described the inhibitory effects of aminoguanidine on AGEs formation [145, 146, 164-167]. The doses used *in vivo* range from 25 mg/kg/day [145, 166] to 50 mg/kg/day [165] and up to 100 mg/kg/day [167]. Thus, aminoguanidine is far from an ideal MG scavenger and AGEs inhibitor. In clinical trials aminoguanidine was found to be too toxic for use in patients. Two double-masked, multiple-dose, placebo-controlled, randomized clinical trials, ACTION I and ACTION II [168, 169] investigated the therapeutic potential of aminoguanidine in preventing the progression of renal damage in patients with diabetic nephropathy. The ACTION I trial involving 690 participants did not show a statistically significant difference between the placebo group and the combined aminoguanidine dose groups, even though patients treated with aminoguanidine showed a tendency of having a lower risk of doubling of serum creatinine [168]. Due to safety concerns and an apparent lack of efficacy, the External Safety Monitoring Committee for the ACTION II trial involving 599 participants recommended early termination [169]. Patients with diabetes may have impaired red blood cell-deformability, which could cause microvascular and kidney damage. A one year trial with aminoguanidine and erythropoietin on 12 patients on dialysis restored red blood cell-deformability to near-normal levels, an effect attributed to inhibition of AGEs formation by aminoguanidine [170]. As mentioned earlier the toxic effects of aminoguanidine have limited its therapeutic potential. For example, like hydrazine, aminoguanidine may be associated with drug-induced systemic lupus erythematosus and abnormal liver function tests, and it can cause flu-like syndromes and vasculitis [169]. Aminoguanidine can also cause damage to DNA through hydroxyl- and hydrogen peroxide-formation in the presence of Fe^{+3} [171].

Metformin is an oral dimethylbiguanide antihyperglycemic agent, which can also inhibit AGEs formation [172], through its action in the post-Amadori stages [83, 173]. Metformin has also been proposed to have a MG scavenging effect attributed to its guanidino group,

which binds with MG to form an inactive product, triazepinone [147, 148, 174, 175]. The MG-scavenging effect of metformin resulted in significantly reduced elevated MG levels in type 2 diabetes patients treated with high doses of metformin, between 1,500 and 2,500 mg/day [172]. We have shown that fructose-fed Sprague-Dawley rats had significantly elevated serum MG and blood pressure, and increased levels of MG, hydrogen peroxide and the MG-derived AGE, CEL, in the aorta, all of which were attenuated by metformin [65]. Metformin has been proposed to protect against MG-induced increased atherogenicity of low density lipoprotein (LDL) [176]. The use of metformin as a MG scavenger and AGEs inhibitor is limited, and hopefully more studies showing its anti-MG, anti-AGEs effectiveness will promote its use for this purpose. Metformin can be considered to have a good therapeutic potential in this regard since it is already in clinical use for type 2 diabetes as an insulin sensitizing agent.

Pioglitazone, a thiazolidinedione, is a peroxisome proliferator-activated receptor gamma (PPARγ) agonist which acts as an insulin sensitizer and is used in type 2 diabetes. Pioglitazone has been proposed to have anti-AGEs effect by inhibiting glycation, AGEs formation and protein cross-linking [177, 178]. Studies employing pioglitazone as an anti-MG or anti-AGEs compound are not forthcoming and more evidence is needed to make definitive statements about the therapeutic potential of pioglitazone in this regard.

N-acetylcysteine (NAC) is a MG scavenging and antioxidant compound [179, 180]. There are good reasons for using NAC as an anti-MG compound: NAC can increase GSH levels [181], which is an efficient MG scavenger and antioxidant [70, 179, 180], NAC is a cysteine containing thiol compound and MG binds with high affinity to cysteine [180, 181], and NAC is already used clinically for other conditions such as acetaminophen overdose [179, 181]. More studies employing NAC as an anti-MG drug should provide interesting results and may help to establish the potential of NAC in this regard.

A widely used class of antihypertensive drugs has been proposed to have anti-AGE effects. Angiotensin receptor blockers (ARBs) and angiotensin converting enzyme inhibitors (ACEIs) have been shown to protect against kidney damage, an effect claimed to be independent of their blood pressure lowering action [182-185], but proposed to be due to an anti-AGE effect. Clinical trials evaluating AGEs lowering effects of ARBs and ACEIs at blood pressure lowering doses can add to the therapeutic utility of these drugs.

A number of compounds have been investigated for anti-AGE effects in limited studies, but have not been widely used as such in experimental studies in animals or humans. These compounds include the cycloxygenase inhibitors, aspirin [186-188], ibuprofen [189], diclofenac [190], xanthine derivative, pentoxifylline [191, 192], which is used for claudication in peripheral vascular disease, metal chelators, D-penicillamine [144] and desferoxamine [83, 144], thiamine pyrophosphate and pyridoxamine [193-195]. Many more studies are necessary for these compounds in order to draw definitive conclusions about their anti-MG or anti-AGE effects.

A number of deglycating enzymes have been discovered, especially in microorganisms, which remove the sugar bound to the protein molecule, and possibly provide these bacteria with energy substrates derived from glycated products [196]. These enzymes, known as amadoriases, include fructosylamine oxidases [197, 198], fructosamine-3-kinase [149, 150], fructoselysine-6-kinase [199], fructoselysine-3-epimerase (FrlC) [200], and glucoselysine-6-

phosphate deglycase [201]. The use of these enzymes or development of stable analogues for deglycation therapy remains speculative .

A number of AGE inhibitors were synthesized and screened for their AGEs inhibitory effects by Rahbar et al. [173, 202, 203]. These are derivatives of aryl ureido and aryl carboxaminido phenoxy isobutyric acids and were derived from some known AGEs inhibitors. These compounds act at multiple stages of the AGEs formation process. Some of these compounds have AGEs breaking properties. More studies are needed for these compounds to establish their specificity and safety for therapeutic use.

11. AGEs breakers

The AGEs which have undergone cross-linking are very stable products and their concentration, especially in long-lived matrix proteins, increases with age. AGEs inhibitors are ineffective against formed AGEs and compounds which can break the cross-links are required. The AGEs breaker compounds can prove invaluable to slow down the aging process, and in the treatment of established stages of diseases such as diabetes, Alzheimer's, atherosclerosis and rheumatoid arthritis.

The first AGEs breaking compound reported was phenacylthiazolium bromide (PTB) in 1996. PTB breaks the covalent cross-links of AGEs [204]. Administration of PTB (10 mg/kg/day, intraperitoneal for 4 wks) reduced the amount of IgG bound to the surface of red blood cells in diabetic rats [153]. However, PTB is not stable.

The search for a stable derivative of PTB resulted in the synthesis of ALT 711 (4,5-dimethylthiazolium) [205]. ALT 711 (now known as alagebrium) reduced arterial stiffness in streptozotocin-induced diabetic rats [205]. In aging rats, ALA (10 mg/kg for 16 weeks) also increased glutathione peroxidase and superoxide dismutase activities and reduced oxidative stress [206]. Alagebrium improved impaired cardiovascular function in older rhesus monkeys [207]. Alagebrium demonstrated promising results and a good safety profile in phase 2 clinical trials. In a clinical study involving 93 subjects, 50 yrs and older, with evidence of vessel stiffness (pulse pressure ≥60 mm Hg, systolic blood pressure ≥140 mm Hg, and large artery compliance ≤1.25 mL/mm Hg), alagebrium (210 mg, once per day for 56 days) improved arterial compliance [208]. In another group of 13 patients aged 65±2 yrs with systolic hypertension (systolic blood pressure > 140 mmHg, diastolic blood pressure <90 mm Hg), alagebrium (210 mg/kg twice a day for 8 weeks) reduced vascular fibrosis and markers of inflammation [209]. Alagebrium (administered for 16 weeks) decreased left ventricular mass and improved left ventricular diastolic filling in another trial in 23 patients with diastolic heart failure [210, 211].

However, in a recent study [212] involving 102 patients (aged 62 ± 11 years) with heart failure (left ventricular ejection fraction (LVEF ≤0.45), alagebrium (400 mg/day/36 wks) did not improve exercise tolerance and systolic dysfunction, and no changes were observed in a number of secondary endpoints. Thus, the authors could not verify the claims that alagebrium has beneficial effects in systolic heart failure [212].

Alagebrium has been reported to be a weak inhibitor of thiamine diphosphokinase and is unlikely to interfere with thiamine metabolism at therapeutic concentrations [213]. However, the authors urge caution when new AGE-crosslink breakers based on thiamine are designed, to make sure they are not potent inhibitors of thiamine diphosphokinase.

In the studies described above alagebrium has been studied mainly for its chronic effects on AGEs as an AGEs breaker. We investigated whether alagebrium also has acute preventive effects against the reactive dicarbonyl, MG, in 12 wk old male Sprague-Dawley rats [66]. Our results showed that alagebrium also has acute (< 6 h) MG scavenging ability [66]. AGEs are formed slowly over a time ranging from 24 h to up to 7 days and more. Therefore, the attenuation of MG-induced acute effects (seen within 6 h of MG administration) is most likely due to scavenging of MG by alagebrium. Thus, alagebrium significantly attenuated the significant increases in MG levels in the plasma, and different organs (measured 2 h after administration), and also attenuated MG-induced impaired glucose tolerance and the reduced insulin-stimulated glucose uptake by adipose tissue. In an *in vitro* assay in which MG (10 μM) was incubated with or without alagebrium (100 μM) for different times at 37° C, alagebrium significantly reduced the amount of detectable MG [66]. Our results strongly indicate an acute MG scavenging effect of alagebrium which can add to its AGEs breaking ability. More direct evidence of interaction of alagebrium and MG using mass spectrometry would be very useful. We have also recently shown that alagebrium significantly attenuated the deleterious effects of chronic MG administration for 4 wks on glucose tolerance and pancreatic islet β-cell function in male Sprague-Dawley rats [72].

In conclusion, MG and AGEs are very likely to be involved in the initiation and or progression of the aging process. Commonly available pharmacological compounds to investigate these roles of MG and AGEs, such as aminoguanidine, are non-specific, whereas some of the newer compounds appear promising in inhibiting AGEs formation at multiple steps in the pathway in *in vitro* studies. However, more *in vivo* studies are required before their therapeutic potential can be established. A more dedicated effort is necessary to identify newer anti-MG and anti-AGEs compounds which are more specific and safer before more can be done about their therapeutic potential.

12. References

[1] Hayflick, L. The limited in vitro lifetime of human diploid cell strains. *Exp. Cell Res.* 37: 614-636; 1965.

[2] Kovacic, J. C.; Moreno, P.; Hachinski, V.; Nabel, E. G.; Fuster, V. Cellular senescence, vascular disease, and aging: Part 1 of a 2-part review. *Circulation* 123:1650-1660; 2011.

[3] Kovacic, J. C.; Moreno, P.; Nabel, E. G.; Hachinski, V.; Fuster, V. Cellular senescence, vascular disease, and aging: Part 2 of a 2-part review: Clinical vascular disease in the elderly. *Circulation* 123:1900-1910; 2011.

[4] Matthews, C.; Gorenne, I.; Scott, S.; Figg, N.; Kirkpatrick, P.; Ritchie, A.; Goddard, M.; Bennett, M. Vascular smooth muscle cells undergo telomere-based senescence in human atherosclerosis: Effects of telomerase and oxidative stress. *Circ. Res.* 99:156-164; 2006.

[5] Campisi, J.; d'Adda di Fagagna, F. Cellular senescence: When bad things happen to good cells. *Nat. Rev. Mol. Cell Biol.* 8:729-740; 2007.

[6] Bucala, R.; Model, P.; Cerami, A. Modification of DNA by reducing sugars: A possible mechanism for nucleic acid aging and age-related dysfunction in gene expression. *Proc. Natl. Acad. Sci. U. S. A.* 81:105-109; 1984.

[7] Beckman, K. B.; Ames, B. N. The free radical theory of aging matures. *Physiol. Rev.*78:547-581; 1998.
[8] Finkel, T.; Holbrook, N. J. Oxidants, oxidative stress and the biology of ageing. *Nature* 408:239-247; 2000.
[9] Barja, G.; Herrero, A. Oxidative damage to mitochondrial DNA is inversely related to maximum life span in the heart and brain of mammals. *FASEB J.* 14:312-318; 2000.
[10] Mandavilli, B. S.; Santos, J. H.; Van Houten, B. Mitochondrial DNA repair and aging. *Mutat. Res.* 509:127-151; 2002.
[11] Vina, J.; Borras, C.; Miquel, J. Theories of ageing. *IUBMB Life*59:249-254; 2007.
[12] Weinert, B. T.; Timiras, P. S. Invited review: Theories of aging. *J. Appl. Physiol.* 95:1706-1716; 2003.
[13] Semsei, I. On the nature of aging. *Mech. Ageing Dev.* 117:93-108; 2000.
[14] Medvedev, Z. A. An attempt at a rational classification of theories of ageing. *Biol. Rev. Camb. Philos. Soc.* 65:375-398; 1990.
[15] [Anonymous]. Max rubner--1854-1932. energy physiologist. *JAMA* 194:86-87; 1965.
[16] Commoner, B.; Townsend, J.; Pake, G. E. Free radicals in biological materials. *Nature* 174:689-691; 1954.
[17] Desai, K. M.; Wu, L. Free radical generation by methylglyoxal in tissues. *Drug Metabol. Drug Interact.* 23:151-173; 2008.
[18] McCord, J. M. The evolution of free radicals and oxidative stress. *Am. J. Med.* 108:652-659; 2000.
[19] Cadenas, E. Biochemistry of oxygen toxicity. *Annu. Rev. Biochem.* 58:79-110; 1989.
[20] Halliwell, B. Antioxidant defence mechanisms: From the beginning to the end (of the beginning). *Free Radic. Res.* 31:261-272; 1999.
[21] Halliwell, B. Reactive oxygen species in living systems: Source, biochemistry, and role in human disease. *Am. J. Med.* 91:14S-22S; 1991.
[22] Gilca, M.; Stoian, I.; Atanasiu, V.; Virgolici, B. The oxidative hypothesis of senescence. *J. Postgrad. Med.* 53:207-213; 2007.
[23] Harman, D. Aging: A theory based on free radical and radiation chemistry. *J. Gerontol.* 11:298-300; 1956.
[24] McCord, J. M.; Fridovich, I. Superoxide dismutase. an enzymic function for erythrocuprein (hemocuprein). *J. Biol. Chem.* 244:6049-6055; 1969.
[25] Harman, D. Aging: Overview. *Ann. N. Y. Acad. Sci.* 928:1-21; 2001.
[26] Gruber, J.; Schaffer, S.; Halliwell, B. The mitochondrial free radical theory of ageing--where do we stand? *Front. Biosci.* 13:6554-6579; 2008.
[27] Ku, H. H.; Brunk, U. T.; Sohal, R. S. Relationship between mitochondrial superoxide and hydrogen peroxide production and longevity of mammalian species. *Free Radic. Biol. Med.* 15:621-627; 1993.
[28] Herrero, A.; Barja, G. Sites and mechanisms responsible for the low rate of free radical production of heart mitochondria in the long-lived pigeon. *Mech. Ageing Dev.* 98:95-111; 1997.
[29] Johnson, T. E.; Tedesco, P. M.; Lithgow, G. J. Comparing mutants, selective breeding, and transgenics in the dissection of aging processes of caenorhabditis elegans. *Genetica* 91:65-77; 1993.
[30] Larsen, P. L. Aging and resistance to oxidative damage in caenorhabditis elegans. *Proc. Natl. Acad. Sci. U. S. A.* 90:8905-8909; 1993.

[31] Tower, J. Transgenic methods for increasing drosophila life span. *Mech. Ageing Dev.* 118:1-14; 2000.

[32] Arking, R.; Burde, V.; Graves, K.; Hari, R.; Feldman, E.; Zeevi, A.; Soliman, S.; Saraiya, A.; Buck, S.; Vettraino, J.; Sathrasala, K.; Wehr, N.; Levine, R. L. Forward and reverse selection for longevity in drosophila is characterized by alteration of antioxidant gene expression and oxidative damage patterns. *Exp. Gerontol.* 35:167-185; 2000.

[33] Schriner, S. E.; Linford, N. J.; Martin, G. M.; Treuting, P.; Ogburn, C. E.; Emond, M.; Coskun, P. E.; Ladiges, W.; Wolf, N.; Van Remmen, H.; Wallace, D. C.; Rabinovitch, P. S. Extension of murine life span by overexpression of catalase targeted to mitochondria. *Science* 308:1909-1911; 2005.

[34] Perez-Campo, R.; Lopez-Torres, M.; Cadenas, S.; Rojas, C.; Barja, G. The rate of free radical production as a determinant of the rate of aging: Evidence from the comparative approach. *J. Comp. Physiol. [B].*168:149-158; 1998.

[35] Huang, T. T.; Carlson, E. J.; Gillespie, A. M.; Shi, Y.; Epstein, C. J. Ubiquitous overexpression of CuZn superoxide dismutase does not extend life span in mice. *J. Gerontol. A Biol. Sci. Med. Sci.* 55:B5-9; 2000.

[36] Perez, V. I.; Van Remmen, H.; Bokov, A.; Epstein, C. J.; Vijg, J.; Richardson, A. The overexpression of major antioxidant enzymes does not extend the lifespan of mice. *Aging Cell.* 8:73-75; 2009.

[37] Mehlhorn, R. J. *Oxidants and antioxidanrs in aging. in: Physiological basis of aging and geriatrics.* Boca Raton, FL:CRC; 2003.

[38] Linster, C. L.; Van Schaftingen, E. Vitamin C. biosynthesis, recycling and degradation in mammals. *FEBS J.* 274:1-22; 2007.

[39] Maeda, N.; Hagihara, H.; Nakata, Y.; Hiller, S.; Wilder, J.; Reddick, R. Aortic wall damage in mice unable to synthesize ascorbic acid. *Proc. Natl. Acad. Sci. U. S. A.* 97:841-846; 2000.

[40] Muller, F. L.; Lustgarten, M. S.; Jang, Y.; Richardson, A.; Van Remmen, H. Trends in oxidative aging theories. *Free Radic. Biol. Med.* 43:477-503; 2007.

[41] McCay, C. M.; Crowell, M. F.; Maynard, L. A. The effect of retarded growth upon the length of life span and upon the ultimate body size. *J. Nutr.* 10:63-79; 1935.

[42] Weindruch, R.; Walford, R. L. *The retardation of aging and disease by dietary restriction.* Springfield IL: Charles C. Thomas.; 1988.

[43] Simic, M. G.; Bertgold, D. S. *Urinary biomarkers of oxidative DNA base damage and human caloric intake. in: Biological effects of dietary restriction.* New York: Springer-Verlag.; 1991.

[44] Szatrowski, T. P.; Nathan, C. F. Production of large amounts of hydrogen peroxide by human tumor cells. *Cancer Res.*51:794-798; 1991.

[45] Thornalley, P. J. Pharmacology of methylglyoxal: Formation, modification of proteins and nucleic acids, and enzymatic detoxification--a role in pathogenesis and antiproliferative chemotherapy. *Gen. Pharmacol.* 27:565-573; 1996.

[46] Kalapos, M. P. Methylglyoxal in living organisms: Chemistry, biochemistry, toxicology and biological implications. *Toxicol. Lett.* 110:145-175; 1999.

[47] Phillips, S. A.; Thornalley, P. J. The formation of methylglyoxal from triose phosphates. investigation using a specific assay for methylglyoxal. *Eur. J. Biochem.* 212:101-105; 1993.

[48] Sato, J.; Wang, Y. M.; van Eys, J. Methylglyoxal formation in rat liver cells. *J. Biol. Chem.* 255:2046-2050; 1980.

[49] Kalapos, M. P. The tandem of free radicals and methylglyoxal. *Chem. Biol. Interact.* 171:251-271; 2008.

[50] Hopper, D. J.; Cooper, R. A. The regulation of escherichia coli methylglyoxal synthase; a new control site in glycolysis? *FEBS Lett.* 13:213-216; 1971.

[51] Ray, S.; Ray, M. Isolation of methylglyoxal synthase from goat liver. *J. Biol. Chem.* 256:6230-6233; 1981.

[52] Lyles, G. A.; Chalmers, J. The metabolism of aminoacetone to methylglyoxal by semicarbazide-sensitive amine oxidase in human umbilical artery. *Biochem. Pharmacol.* 43:1409-1414; 1992.

[53] Deng, Y.; Yu, P. H. Simultaneous determination of formaldehyde and methylglyoxal in urine: Involvement of semicarbazide-sensitive amine oxidase-mediated deamination in diabetic complications. *J. Chromatogr. Sci.* 37:317-322; 1999.

[54] Casazza, J. P.; Felver, M. E.; Veech, R. L. The metabolism of acetone in rat. *J. Biol. Chem.* 259:231-236; 1984.

[55] Yu, P. H.; Wright, S.; Fan, E. H.; Lun, Z. R.; Gubisne-Harberle, D. Physiological and pathological implications of semicarbazide-sensitive amine oxidase. *Biochim. Biophys. Acta* 1647:193-199; 2003.

[56] Lyles, G. A.; Chalmers, J. Aminoacetone metabolism by semicarbazide-sensitive amine oxidase in rat aorta. *Biochem. Pharmacol.* 49:416-419; 1995.

[57] Vander Jagt D.L. *The glyoxalase system. in: Glutathione: Chemical, biochemical and medical aspects - part A*. John Wiley & Sons Inc.; 1989.

[58] Thornalley, P. J. Glyoxalase I--structure, function and a critical role in the enzymatic defence against glycation. *Biochem. Soc. Trans.* 31:1343-1348; 2003.

[59] Thornalley, P. J. The glyoxalase system in health and disease. *Mol. Aspects Med.* 14:287-371; 1993.

[60] Blakytny, R.; Harding, J. J. Glycation (non-enzymic glycosylation) inactivates glutathione reductase. *Biochem. J.* 288 (Pt 1):303-307; 1992.

[61] Wang, X.; Desai, K.; Chang, T.; Wu, L. Vascular methylglyoxal metabolism and the development of hypertension. *J. Hypertens.* 23:1565-1573; 2005.

[62] Wu, L.; Juurlink, B. H. Increased methylglyoxal and oxidative stress in hypertensive rat vascular smooth muscle cells. *Hypertension* 39:809-814; 2002.

[63] Wang, X.; Chang, T.; Jiang, B.; Desai, K.; Wu, L. Attenuation of hypertension development by aminoguanidine in spontaneously hypertensive rats: Role of methylglyoxal. *Am. J. Hypertens.* 20:629-636; 2007.

[64] Wang, X.; Desai, K.; Clausen, J. T.; Wu, L. Increased methylglyoxal and advanced glycation end products in kidney from spontaneously hypertensive rats. *Kidney Int.* 66:2315-2321; 2004.

[65] Wang, X.; Jia, X.; Chang, T.; Desai, K.; Wu, L. Attenuation of hypertension development by scavenging methylglyoxal in fructose-treated rats. *J. Hypertens.* 26:765-772; 2008.

[66] Dhar, A.; Desai, K. M.; Wu, L. Alagebrium attenuates acute methylglyoxal-induced glucose intolerance in sprague-dawley rats. *Br. J. Pharmacol.* 159:166-175; 2010.

[67] Wang, H.; Meng, Q. H.; Gordon, J. R.; Khandwala, H.; Wu, L. Proinflammatory and proapoptotic effects of methylglyoxal on neutrophils from patients with type 2 diabetes mellitus. *Clin. Biochem.* 40:1232-1239; 2007.

[68] McLellan, A. C.; Thornalley, P. J.; Benn, J.; Sonksen, P. H. Glyoxalase system in clinical diabetes mellitus and correlation with diabetic complications. *Clin. Sci. (Lond)* 87:21-29; 1994.

[69] Jia, X.; Olson, D. J.; Ross, A. R.; Wu, L. Structural and functional changes in human insulin induced by methylglyoxal. *FASEB J.* 20:1555-1557; 2006.

[70] Jia, X.; Wu, L. Accumulation of endogenous methylglyoxal impaired insulin signaling in adipose tissue of fructose-fed rats. *Mol. Cell. Biochem.*306:133-139; 2007.

[71] Riboulet-Chavey, A.; Pierron, A.; Durand, I.; Murdaca, J.; Giudicelli, J.; Van Obberghen, E. Methylglyoxal impairs the insulin signaling pathways independently of the formation of intracellular reactive oxygen species. *Diabetes* 55:1289-1299; 2006.

[72] Dhar, A.; Dhar, I.; Jiang, B.; Desai, K. M.; Wu, L. Chronic methylglyoxal infusion by minipump causes pancreatic beta-cell dysfunction and induces type 2 diabetes in sprague-dawley rats. *Diabetes* 60:899-908; 2011.

[73] Baynes JW Thorpe SR. Role of oxidative stress in diabetic complications: A new perspective on an old paradigm. *Diabetes* 48:1-9; 1999.

[74] Baynes, J. W. From life to death--the struggle between chemistry and biology during aging: The maillard reaction as an amplifier of genomic damage. *Biogerontology* 1:235-246; 2000.

[75] Baynes, J. W.; Thorpe, S. R. Glycoxidation and lipoxidation in atherogenesis. *Free Radic. Biol. Med.* 28:1708-1716; 2000.

[76] Kuhla, B.; Luth, H. J.; Haferburg, D.; Boeck, K.; Arendt, T.; Munch, G. Methylglyoxal, glyoxal, and their detoxification in alzheimer's disease. *Ann. N. Y. Acad. Sci.* 1043:211-216; 2005.

[77] Yu, P. H. Involvement of cerebrovascular semicarbazide-sensitive amine oxidase in the pathogenesis of alzheimer's disease and vascular dementia. *Med. Hypotheses* 57:175-179; 2001.

[78] Singh, R.; Barden, A.; Mori, T.; Beilin, L. Advanced glycation end-products: A review. *Diabetologia*44:129-146; 2001.

[79] Vlassara, H.; Bucala, R.; Striker, L. Pathogenic effects of advanced glycosylation: Biochemical, biologic, and clinical implications for diabetes and aging. *Lab. Invest.* 70:138-151; 1994.

[80] Baynes, J. W. The role of AGEs in aging: Causation or correlation. *Exp. Gerontol.* 36:1527-1537; 2001.

[81] Wolff, S. P.; Bascal, Z. A.; Hunt, J. V. "Autoxidative glycosylation": Free radicals and glycation theory. *Prog. Clin. Biol. Res.* 304:259-275; 1989.

[82] Glomb, M. A.; Monnier, V. M. Mechanism of protein modification by glyoxal and glycolaldehyde, reactive intermediates of the maillard reaction. *J. Biol. Chem.* 270:10017-10026; 1995.

[83] Khalifah, R. G.; Baynes, J. W.; Hudson, B. G. Amadorins: Novel post-amadori inhibitors of advanced glycation reactions. *Biochem. Biophys. Res. Commun.* 257:251-258; 1999.

[84] Kilhovd, B. K.; Giardino, I.; Torjesen, P. A.; Birkeland, K. I.; Berg, T. J.; Thornalley, P. J.; Brownlee, M.; Hanssen, K. F. Increased serum levels of the specific AGE-compound methylglyoxal-derived hydroimidazolone in patients with type 2 diabetes. *Metabolism* 52:163-167; 2003.

[85] Mostafa, A. A.; Randell, E. W.; Vasdev, S. C.; Gill, V. D.; Han, Y.; Gadag, V.; Raouf, A. A.; El Said, H. Plasma protein advanced glycation end products, carboxymethyl

cysteine, and carboxyethyl cysteine, are elevated and related to nephropathy in patients with diabetes. *Mol. Cell. Biochem.* 302:35-42; 2007.

[86] Jana, C. K.; Das, N.; Sohal, R. S. Specificity of age-related carbonylation of plasma proteins in the mouse and rat. *Arch. Biochem. Biophys.* 397:433-439; 2002.

[87] Poggioli, S.; Bakala, H.; Friguet, B. Age-related increase of protein glycation in peripheral blood lymphocytes is restricted to preferential target proteins. *Exp. Gerontol.* 37:1207-1215; 2002.

[88] Shipanova, I. N.; Glomb, M. A.; Nagaraj, R. H. Protein modification by methylglyoxal: Chemical nature and synthetic mechanism of a major fluorescent adduct. *Arch. Biochem. Biophys.* 344:29-36; 1997.

[89] Glomb, M. A.; Rosch, D.; Nagaraj, R. H. N(delta)-(5-hydroxy-4,6-dimethylpyrimidine-2-yl)-l-ornithine, a novel methylglyoxal-arginine modification in beer. *J. Agric. Food Chem.* 49:366-372; 2001.

[90] Ahmed, M. U.; Brinkmann Frye, E.; Degenhardt, T. P.; Thorpe, S. R.; Baynes, J. W. N-epsilon-(carboxyethyl)lysine, a product of the chemical modification of proteins by methylglyoxal, increases with age in human lens proteins. *Biochem. J.* 324 (Pt 2):565-570; 1997.

[91] Chellan, P.; Nagaraj, R. H. Protein crosslinking by the maillard reaction: Dicarbonyl-derived imidazolium crosslinks in aging and diabetes. *Arch. Biochem. Biophys.* 368:98-104; 1999.

[92] Konishi, Y.; Hayase, F.; Kato, K. Novel imidazolones compound formed by the advanced maillard reaction of 3-deoxyglucosone and arginine residues in proteins. *Biosci Biotechnol Biochem* 58:1953-1955; 1994.

[93] Uchida, K.; Khor, O. T.; Oya, T.; Osawa, T.; Yasuda, Y.; Miyata, T. Protein modification by a maillard reaction intermediate methylglyoxal. immunochemical detection of fluorescent 5-methylimidazolone derivatives in vivo. *FEBS Lett.* 410:313-318; 1997.

[94] Chang, T.; Wang, R.; Wu, L. Methylglyoxal-induced nitric oxide and peroxynitrite production in vascular smooth muscle cells. *Free Radic. Biol. Med.* 38:286-293; 2005.

[95] Dhar, A.; Desai, K.; Kazachmov, M.; Yu, P.; Wu, L. Methylglyoxal production in vascular smooth muscle cells from different metabolic precursors. *Metabolism* 57:1211-1220; 2008.

[96] Wang, H.; Liu, J.; Wu, L. Methylglyoxal-induced mitochondrial dysfunction in vascular smooth muscle cells. *Biochem. Pharmacol.* In press; 2009.

[97] Ho, C.; Lee, P. H.; Huang, W. J.; Hsu, Y. C.; Lin, C. L.; Wang, J. Y. Methylglyoxal-induced fibronectin gene expression through ras-mediated NADPH oxidase activation in renal mesangial cells. *Nephrology (Carlton)* 12:348-356; 2007.

[98] Ward, R. A.; McLeish, K. R. Methylglyoxal: A stimulus to neutrophil oxygen radical production in chronic renal failure? *Nephrol. Dial. Transplant.* 19:1702-1707; 2004.

[99] Leoncini, G.; Poggi, M. Effects of methylglyoxal on platelet hydrogen peroxide accumulation, aggregation and release reaction. *Cell Biochem. Funct.* 14:89-95; 1996.

[100] Kalapos, M. P.; Littauer, A.; de Groot, H. Has reactive oxygen a role in methylglyoxal toxicity? A study on cultured rat hepatocytes. *Arch. Toxicol.* 67:369-372; 1993.

[101] Di Loreto, S.; Caracciolo, V.; Colafarina, S.; Sebastiani, P.; Gasbarri, A.; Amicarelli, F. Methylglyoxal induces oxidative stress-dependent cell injury and up-regulation of interleukin-1beta and nerve growth factor in cultured hippocampal neuronal cells. *Brain Res.* 1006:157-167; 2004.

[102] Akhand, A. A.; Hossain, K.; Mitsui, H.; Kato, M.; Miyata, T.; Inagi, R.; Du, J.; Takeda, K.; Kawamoto, Y.; Suzuki, H.; Kurokawa, K.; Nakashima, I. Glyoxal and methylglyoxal trigger distinct signals for map family kinases and caspase activation in human endothelial cells. *Free Radic. Biol. Med.* 31:20-30; 2001.

[103] Kikuchi, S.; Shinpo, K.; Moriwaka, F.; Makita, Z.; Miyata, T.; Tashiro, K. Neurotoxicity of methylglyoxal and 3-deoxyglucosone on cultured cortical neurons: Synergism between glycation and oxidative stress, possibly involved in neurodegenerative diseases. *J. Neurosci. Res*.57:280-289; 1999.

[104] Amicarelli, F.; Colafarina, S.; Cattani, F.; Cimini, A.; Di Ilio, C.; Ceru, M. P.; Miranda, M. Scavenging system efficiency is crucial for cell resistance to ROS-mediated methylglyoxal injury. *Free Radic. Biol. Med.* 35:856-871; 2003.

[105] Pacher, P.; Beckman, J. S.; Liaudet, L. Nitric oxide and peroxynitrite in health and disease. *Physiol. Rev.* 87:315-424; 2007.

[106] Nicolay, J. P.; Schneider, J.; Niemoeller, O. M.; Artunc, F.; Portero-Otin, M.; Haik, G.,Jr; Thornalley, P. J.; Schleicher, E.; Wieder, T.; Lang, F. Stimulation of suicidal erythrocyte death by methylglyoxal. *Cell. Physiol. Biochem.* 18:223-232; 2006.

[107] Beard, K. M.; Shangari, N.; Wu, B.; O'Brien, P. J. Metabolism, not autoxidation, plays a role in alpha-oxoaldehyde- and reducing sugar-induced erythrocyte GSH depletion: Relevance for diabetes mellitus. *Mol. Cell. Biochem.* 252:331-338; 2003.

[108] Park, Y. S.; Koh, Y. H.; Takahashi, M.; Miyamoto, Y.; Suzuki, K.; Dohmae, N.; Takio, K.; Honke, K.; Taniguchi, N. Identification of the binding site of methylglyoxal on glutathione peroxidase: Methylglyoxal inhibits glutathione peroxidase activity via binding to glutathione binding sites arg 184 and 185. *Free Radic. Res.* 37:205-211; 2003.

[109] Paget, C.; Lecomte, M.; Ruggiero, D.; Wiernsperger, N.; Lagarde, M. Modification of enzymatic antioxidants in retinal microvascular cells by glucose or advanced glycation end products. *Free Radic. Biol. Med.* 25:121-129; 1998.

[110] Juurlink, B. H. Management of oxidative stress in the CNS: The many roles of glutathione. *Neurotox Res.* 1:119-140; 1999.

[111] Rondeau, P.; Singh, N. R.; Caillens, H.; Tallet, F.; Bourdon, E. Oxidative stresses induced by glycoxidized human or bovine serum albumin on human monocytes. *Free Radic. Biol. Med.* 45:799-812; 2008.

[112] Gawlowski, T.; Stratmann, B.; Stirban, A. O.; Negrean, M.; Tschoepe, D. AGEs and methylglyoxal induce apoptosis and expression of mac-1 on neutrophils resulting in platelet-neutrophil aggregation. *Thromb. Res.* 121:117-126; 2007.

[113] Chance, B.; Oshino, N.; Sugano, T.; Mayevsky, A. Basic principles of tissue oxygen determination from mitochondrial signals. *Adv. Exp. Med. Biol.* 37A:277-292; 1973.

[114] Turrens, J. F.; Boveris, A. Generation of superoxide anion by the NADH dehydrogenase of bovine heart mitochondria. *Biochem. J.*191:421-427; 1980.

[115] Turrens, J. F. Superoxide production by the mitochondrial respiratory chain. *Biosci. Rep.* 17:3-8; 1997.

[116] Rosca, M. G.; Mustata, T. G.; Kinter, M. T.; Ozdemir, A. M.; Kern, T. S.; Szweda, L. I.; Brownlee, M.; Monnier, V. M.; Weiss, M. F. Glycation of mitochondrial proteins from diabetic rat kidney is associated with excess superoxide formation. *Am. J. Physiol. Renal Physiol.* 289:F420-30; 2005.

[117] Rabbani, N.; Thornalley, P. J. Dicarbonyls linked to damage in the powerhouse: Glycation of mitochondrial proteins and oxidative stress. *Biochem. Soc. Trans.* 36:1045-1050; 2008.

[118] Vander Jagt, D. L. Methylglyoxal, diabetes mellitus and diabetic complications. *Drug Metabol. Drug Interact.* 23:93-124; 2008.

[119] Ando, K.; Beppu, M.; Kikugawa, K.; Nagai, R.; Horiuchi, S. Membrane proteins of human erythrocytes are modified by advanced glycation end products during aging in the circulation. *Biochem. Biophys. Res. Commun.* 258:123-127; 1999.

[120] Li, Y. M.; Steffes, M.; Donnelly, T.; Liu, C.; Fuh, H.; Basgen, J.; Bucala, R.; Vlassara, H. Prevention of cardiovascular and renal pathology of aging by the advanced glycation inhibitor aminoguanidine. *Proc. Natl. Acad. Sci. U. S. A.*93:3902-3907; 1996.

[121] Kilhovd, B. K.; Giardino, I.; Torjesen, P. A.; Birkeland, K. I.; Berg, T. J.; Thornalley, P. J.; Brownlee, M.; Hanssen, K. F. Increased serum levels of the specific AGE-compound methylglyoxal-derived hydroimidazolone in patients with type 2 diabetes. *Metabolism* 52:163-167; 2003.

[122] Degenhardt, T. P.; Thorpe, S. R.; Baynes, J. W. Chemical modification of proteins by methylglyoxal. *Cell. Mol. Biol. (Noisy-Le-Grand)* 44:1139-1145; 1998.

[123] Sugimoto, K.; Nishizawa, Y.; Horiuchi, S.; Yagihashi, S. Localization in human diabetic peripheral nerve of N(epsilon)-carboxymethyllysine-protein adducts, an advanced glycation endproduct. *Diabetologia* 40:1380-1387; 1997.

[124] Lo, T. W.; Westwood, M. E.; McLellan, A. C.; Selwood, T.; Thornalley, P. J. Binding and modification of proteins by methylglyoxal under physiological conditions. A kinetic and mechanistic study with N alpha-acetylarginine, N alpha-acetylcysteine, and N alpha-acetyllysine, and bovine serum albumin. *J. Biol. Chem.* 269:32299-32305; 1994.

[125] Oya, T.; Hattori, N.; Mizuno, Y.; Miyata, S.; Maeda, S.; Osawa, T.; Uchida, K. Methylglyoxal modification of protein. chemical and immunochemical characterization of methylglyoxal-arginine adducts. *J. Biol. Chem.* 274:18492-18502; 1999.

[126] Kikuchi, S.; Shinpo, K.; Takeuchi, M.; Yamagishi, S.; Makita, Z.; Sasaki, N.; Tashiro, K. Glycation--a sweet tempter for neuronal death. *Brain Res. Brain Res. Rev.* 41:306-323; 2003.

[127] Westwood, M. E.; Thornalley, P. J. Induction of synthesis and secretion of interleukin 1 beta in the human monocytic THP-1 cells by human serum albumins modified with methylglyoxal and advanced glycation endproducts. *Immunol. Lett.* 50:17-21; 1996.

[128] Wautier, M. P.; Chappey, O.; Corda, S.; Stern, D. M.; Schmidt, A. M.; Wautier, J. L. Activation of NADPH oxidase by AGE links oxidant stress to altered gene expression via RAGE. *Am. J. Physiol. Endocrinol. Metab.* 280:E685-94; 2001.

[129] Basta, G.; Lazzerini, G.; Massaro, M.; Simoncini, T.; Tanganelli, P.; Fu, C.; Kislinger, T.; Stern, D. M.; Schmidt, A. M.; De Caterina, R. Advanced glycation end products activate endothelium through signal-transduction receptor RAGE: A mechanism for amplification of inflammatory responses. *Circulation* 105:816-822; 2002.

[130] Chen, J.; Brodsky, S. V.; Goligorsky, D. M.; Hampel, D. J.; Li, H.; Gross, S. S.; Goligorsky, M. S. Glycated collagen I induces premature senescence-like phenotypic changes in endothelial cells. *Circ. Res.* 90:1290-1298; 2002.

[131] Thornalley, P. J. Cell activation by glycated proteins. AGE receptors, receptor recognition factors and functional classification of AGEs. *Cell. Mol. Biol. (Noisy-Le-Grand)* 44:1013-1023; 1998.

[132] Bierhaus, A.; Chevion, S.; Chevion, M.; Hofmann, M.; Quehenberger, P.; Illmer, T.; Luther, T.; Berentshtein, E.; Tritschler, H.; Muller, M.; Wahl, P.; Ziegler, R.; Nawroth, P. P. Advanced glycation end product-induced activation of NF-kappaB is suppressed by alpha-lipoic acid in cultured endothelial cells. *Diabetes* 46:1481-1490; 1997.

[133] Asif, M.; Egan, J.; Vasan, S.; Jyothirmayi, G. N.; Masurekar, M. R.; Lopez, S.; Williams, C.; Torres, R. L.; Wagle, D.; Ulrich, P.; Cerami, A.; Brines, M.; Regan, T. J. An advanced glycation endproduct cross-link breaker can reverse age-related increases in myocardial stiffness. *Proc. Natl. Acad. Sci. U. S. A.* 97:2809-2813; 2000.

[134] Scuteri, A. Slowing arterial aging: How far have we progressed? *J. Hypertens.* 25:509-510; 2007.

[135] Uribarri, J.; Cai, W.; Peppa, M.; Goodman, S.; Ferrucci, L.; Striker, G.; Vlassara, H. Circulating glycotoxins and dietary advanced glycation endproducts: Two links to inflammatory response, oxidative stress, and aging. *J. Gerontol. A Biol. Sci. Med. Sci.* 62:427-433; 2007.

[136] Mailankot, M.; Padmanabha, S.; Pasupuleti, N.; Major, D.; Howell, S.; Nagaraj, R. H. Glyoxalase I activity and immunoreactivity in the aging human lens. *Biogerontology* 2009.

[137] Corstjens, H.; Declercq, L.; Hellemans, L.; Sente, I.; Maes, D. Prevention of oxidative damage that contributes to the loss of bioenergetic capacity in ageing skin. *Exp. Gerontol.* 42:924-929; 2007.

[138] Morcos, M.; Du, X.; Pfisterer, F.; Hutter, H.; Sayed, A. A.; Thornalley, P.; Ahmed, N.; Baynes, J.; Thorpe, S.; Kukudov, G.; Schlotterer, A.; Bozorgmehr, F.; El Baki, R. A.; Stern, D.; Moehrlen, F.; Ibrahim, Y.; Oikonomou, D.; Hamann, A.; Becker, C.; Zeier, M.; Schwenger, V.; Miftari, N.; Humpert, P.; Hammes, H. P.; Buechler, M.; Bierhaus, A.; Brownlee, M.; Nawroth, P. P. Glyoxalase-1 prevents mitochondrial protein modification and enhances lifespan in caenorhabditis elegans. *Aging Cell.* 7:260-269; 2008.

[139] Scheckhuber, C. Q.; Mack, S. J.; Strobel, I.; Ricciardi, F.; Gispert, S.; Osiewacz, H. D. Modulation of the glyoxalase system in the aging model podospora anserina: Effects on growth and lifespan. *Aging (Albany NY)* 2:969-980; 2010.

[140] Sejersen, H.; Rattan, S. I. Dicarbonyl-induced accelerated aging in vitro in human skin fibroblasts. *Biogerontology* 10:203-211; 2009.

[141] Chappey, O.; Dosquet, C.; Wautier, M. P.; Wautier, J. L. Advanced glycation end products, oxidant stress and vascular lesions. *Eur. J. Clin. Invest.*27:97-108; 1997.

[142] Gillery, P. Advanced glycation end products (AGEs), free radicals and diabetes. *J. Soc. Biol.* 195:387-390; 2001.

[143] Kutlu, M.; Naziroglu, M.; Simsek, H.; Yilmaz, T.; Sahap Kukner, A. Moderate exercise combined with dietary vitamins C and E counteracts oxidative stress in the kidney and lens of streptozotocin-induced diabetic-rat. *Int. J. Vitam. Nutr. Res.* 75:71-80; 2005.

[144] Wondrak, G. T.; Cervantes-Laurean, D.; Roberts, M. J.; Qasem, J. G.; Kim, M.; Jacobson, E. L.; Jacobson, M. K. Identification of alpha-dicarbonyl scavengers for cellular protection against carbonyl stress. *Biochem. Pharmacol.* 63:361-373; 2002.

[145] Brownlee, M.; Vlassara, H.; Kooney, A.; Ulrich, P.; Cerami, A. Aminoguanidine prevents diabetes-induced arterial wall protein cross-linking. *Science* 232:1629-1632; 1986.

[146] Edelstein, D.; Brownlee, M. Mechanistic studies of advanced glycosylation end product inhibition by aminoguanidine. *Diabetes* 41:26-29; 1992.

[147] Beisswenger, P.; Ruggiero-Lopez, D. Metformin inhibition of glycation processes. *Diabetes Metab.* 29:6S95-103; 2003.

[148] Ruggiero-Lopez, D.; Lecomte, M.; Moinet, G.; Patereau, G.; Lagarde, M.; Wiernsperger, N. Reaction of metformin with dicarbonyl compounds. possible implication in the inhibition of advanced glycation end product formation. *Biochem. Pharmacol.* 58:1765-1773; 1999.

[149] Szwergold, B. S.; Kappler, F.; Brown, T. R. Identification of fructose 3-phosphate in the lens of diabetic rats. *Science* 247:451-454; 1990.

[150] Delpierre, G.; Rider, M. H.; Collard, F.; Stroobant, V.; Vanstapel, F.; Santos, H.; Van Schaftingen, E. Identification, cloning, and heterologous expression of a mammalian fructosamine-3-kinase. *Diabetes* 49:1627-1634; 2000.

[151] Saxena, A. K.; Saxena, P.; Monnier, V. M. Purification and characterization of a membrane-bound deglycating enzyme (1-deoxyfructosyl alkyl amino acid oxidase, EC 1.5.3) from a pseudomonas sp. soil strain. *J. Biol. Chem.* 271:32803-32809; 1996.

[152] Takahashi, M.; Pischetsrieder, M.; Monnier, V. M. Isolation, purification, and characterization of amadoriase isoenzymes (fructosyl amine-oxygen oxidoreductase EC 1.5.3) from aspergillus sp. *J. Biol. Chem.* 272:3437-3443; 1997.

[153] Vasan, S.; Zhang, X.; Zhang, X.; Kapurniotu, A.; Bernhagen, J.; Teichberg, S.; Basgen, J.; Wagle, D.; Shih, D.; Terlecky, I.; Bucala, R.; Cerami, A.; Egan, J.; Ulrich, P. An agent cleaving glucose-derived protein crosslinks in vitro and in vivo. *Nature* 382:275-278; 1996.

[154] Grandhee, S. K.; Monnier, V. M. Mechanism of formation of the maillard protein cross-link pentosidine. glucose, fructose, and ascorbate as pentosidine precursors. *J. Biol. Chem.* 266:11649-11653; 1991.

[155] Frye, E. B.; Degenhardt, T. P.; Thorpe, S. R.; Baynes, J. W. Role of the maillard reaction in aging of tissue proteins. advanced glycation end product-dependent increase in imidazolium cross-links in human lens proteins. *J. Biol. Chem.* 273:18714-18719; 1998.

[156] Nilsson, B. O. Biological effects of aminoguanidine: An update. *Inflamm. Res.* 48:509-515; 1999.

[157] SCHULER, W. The inhibition of histaminase. *Experientia* 8:230-232; 1952.

[158] Ivy, A. C.; Ivy, E. K.; Karvinen, E.; Lin, T. M. Effect of an histaminase inhibitor (aminoguanidine) on the gastric secretory response to exogenous histamine. *Am. J. Physiol.* 186:231-238; 1956.

[159] Corbett, J. A.; Tilton, R. G.; Chang, K.; Hasan, K. S.; Ido, Y.; Wang, J. L.; Sweetland, M. A.; Lancaster, J. R.,Jr; Williamson, J. R.; McDaniel, M. L. Aminoguanidine, a novel inhibitor of nitric oxide formation, prevents diabetic vascular dysfunction. *Diabetes* 41:552-556; 1992.

[160] Laszlo, F.; Evans, S. M.; Whittle, B. J. Aminoguanidine inhibits both constitutive and inducible nitric oxide synthase isoforms in rat intestinal microvasculature in vivo. *Eur. J. Pharmacol.* 272:169-175; 1995.

[161] Moncada, S.; Palmer, R. M.; Higgs, E. A. Nitric oxide: Physiology, pathophysiology, and pharmacology. *Pharmacol. Rev.* 43:109-142; 1991.

[162] Stjernborg, L.; Persson, L. Stabilization of S-adenosylmethionine decarboxylase by aminoguanidine. *Biochem. Pharmacol.* 45:1174-1176; 1993.

[163] Miyata, T.; van Ypersele de Strihou, C. Angiotensin II receptor blockers and angiotensin converting enzyme inhibitors: Implication of radical scavenging and transition metal chelation in inhibition of advanced glycation end product formation. *Arch. Biochem. Biophys.* 419:50-54; 2003.

[164] Sajithlal, G. B.; Chithra, P.; Chandrakasan, G. Advanced glycation end products induce crosslinking of collagen in vitro. *Biochim. Biophys. Acta* 1407:215-224; 1998.

[165] Corman, B.; Duriez, M.; Poitevin, P.; Heudes, D.; Bruneval, P.; Tedgui, A.; Levy, B. I. Aminoguanidine prevents age-related arterial stiffening and cardiac hypertrophy. *Proc. Natl. Acad. Sci. U. S. A.* 95:1301-1306; 1998.

[166] Seyer-Hansen, M.; Andreassen, T. T.; Oxlund, H.; Jorgensen, P. H. The influence of aminoguanidine on borohydride reducible collagen cross-links and wound strength. *Connect. Tissue Res.* 26:181-186; 1991.

[167] Ino-ue, M.; Ohgiya, N.; Yamamoto, M. Effect of aminoguanidine on optic nerve involvement in experimental diabetic rats. *Brain Res.* 800:319-322; 1998.

[168] Bolton, W. K.; Cattran, D. C.; Williams, M. E.; Adler, S. G.; Appel, G. B.; Cartwright, K.; Foiles, P. G.; Freedman, B. I.; Raskin, P.; Ratner, R. E.; Spinowitz, B. S.; Whittier, F. C.; Wuerth, J. P.; ACTION I Investigator Group. Randomized trial of an inhibitor of formation of advanced glycation end products in diabetic nephropathy. *Am. J. Nephrol.* 24:32-40; 2004.

[169] Freedman, B. I.; Wuerth, J. P.; Cartwright, K.; Bain, R. P.; Dippe, S.; Hershon, K.; Mooradian, A. D.; Spinowitz, B. S. Design and baseline characteristics for the aminoguanidine clinical trial in overt type 2 diabetic nephropathy (ACTION II). *Control. Clin. Trials* 20:493-510; 1999.

[170] Brown, C. D.; Zhao, Z. H.; Thomas, L. L.; deGroof, R.; Friedman, E. A. Effects of erythropoietin and aminoguanidine on red blood cell deformability in diabetic azotemic and uremic patients. *Am. J. Kidney Dis.* 38:1414-1420; 2001.

[171] Suji, G.; Sivakami, S. DNA damage by free radical production by aminoguanidine. *Ann. N. Y. Acad. Sci.* 1067:191-199; 2006.

[172] Beisswenger, P. J.; Howell, S. K.; Touchette, A. D.; Lal, S.; Szwergold, B. S. Metformin reduces systemic methylglyoxal levels in type 2 diabetes. *Diabetes*48:198-202; 1999.

[173] Rahbar, S.; Figarola, J. L. Novel inhibitors of advanced glycation endproducts. *Arch. Biochem. Biophys.* 419:63-79; 2003.

[174] Baynes, J. W. Role of oxidative stress in development of complications in diabetes. *Diabetes* 40:405-412; 1991.

[175] Ota, K.; Nakamura, J.; Li, W.; Kozakae, M.; Watarai, A.; Nakamura, N.; Yasuda, Y.; Nakashima, E.; Naruse, K.; Watabe, K.; Kato, K.; Oiso, Y.; Hamada, Y. Metformin prevents methylglyoxal-induced apoptosis of mouse schwann cells. *Biochem. Biophys. Res. Commun.* 357:270-275; 2007.

[176] Rabbani, N.; Godfrey, L.; Xue, M.; Shaheen, F.; Geoffrion, M.; Milne, R.; Thornalley, P. J. Glycation of LDL by methylglyoxal increases arterial atherogenicity: A possible contributor to increased risk of cardiovascular disease in diabetes. *Diabetes* 60:1973-1980; 2011.

[177] Rahbar, S.; Natarajan, R.; Yerneni, K.; Scott, S.; Gonzales, N.; Nadler, J. L. Evidence that pioglitazone, metformin and pentoxifylline are inhibitors of glycation. *Clin. Chim. Acta* 301:65-77; 2000.

[178] Hirasawa, Y.; Matsui, Y.; Ohtsu, S.; Yamane, K.; Toyoshi, T.; Kyuki, K.; Sakai, T.; Feng, Y.; Nagamatsu, T. Involvement of hyperglycemia in deposition of aggregated protein in glomeruli of diabetic mice. *Eur. J. Pharmacol.* 601:129-135; 2008.

[179] Millea, P. J. N-acetylcysteine: Multiple clinical applications. *Am. Fam. Physician* 80:265-269; 2009.

[180] Vasdev, S.; Ford, C. A.; Longerich, L.; Parai, S.; Gadag, V.; Wadhawan, S. Aldehyde induced hypertension in rats: Prevention by N-acetyl cysteine. *Artery* 23:10-36; 1998.

[181] Lauterburg, B. H.; Corcoran, G. B.; Mitchell, J. R. Mechanism of action of N-acetylcysteine in the protection against the hepatotoxicity of acetaminophen in rats in vivo. *J. Clin. Invest.* 71:980-991; 1983.

[182] Forbes, J. M.; Cooper, M. E.; Thallas, V.; Burns, W. C.; Thomas, M. C.; Brammar, G. C.; Lee, F.; Grant, S. L.; Burrell, L. M.; Jerums, G.; Osicka, T. M. Reduction of the accumulation of advanced glycation end products by ACE inhibition in experimental diabetic nephropathy. *Diabetes* 51:3274-3282; 2002.

[183] Izuhara, Y.; Nangaku, M.; Inagi, R.; Tominaga, N.; Aizawa, T.; Kurokawa, K.; van Ypersele de Strihou, C.; Miyata, T. Renoprotective properties of angiotensin receptor blockers beyond blood pressure lowering. *J. Am. Soc. Nephrol.* 16:3631-3641; 2005.

[184] Miyata, T.; van Ypersele de Strihou, C.; Ueda, Y.; Ichimori, K.; Inagi, R.; Onogi, H.; Ishikawa, N.; Nangaku, M.; Kurokawa, K. Angiotensin II receptor antagonists and angiotensin-converting enzyme inhibitors lower in vitro the formation of advanced glycation end products: Biochemical mechanisms. *J. Am. Soc. Nephrol.* 13:2478-2487; 2002.

[185] Nangaku, M.; Miyata, T.; Sada, T.; Mizuno, M.; Inagi, R.; Ueda, Y.; Ishikawa, N.; Yuzawa, H.; Koike, H.; van Ypersele de Strihou, C.; Kurokawa, K. Anti-hypertensive agents inhibit in vivo the formation of advanced glycation end products and improve renal damage in a type 2 diabetic nephropathy rat model. *J. Am. Soc. Nephrol.* 14:1212-1222; 2003.

[186] Rao, G. N.; Lardis, M. P.; Cotlier, E. Acetylation of lens crystallins: A possible mechanism by which aspirin could prevent cataract formation. *Biochem. Biophys. Res. Commun.* 128:1125-1132; 1985.

[187] Lin, P. P.; Barry, R. C.; Smith, D. L.; Smith, J. B. In vivo acetylation identified at lysine 70 of human lens alphaA-crystallin. *Protein Sci.*7:1451-1457; 1998.

[188] Ajiboye, R.; Harding, J. J. The non-enzymic glycosylation of bovine lens proteins by glucosamine and its inhibition by aspirin, ibuprofen and glutathione. *Exp. Eye Res.* 49:31-41; 1989.

[189] Raza, K.; Harding, J. J. Non-enzymic modification of lens proteins by glucose and fructose: Effects of ibuprofen. *Exp. Eye Res.* 52:205-212; 1991.

[190] van Boekel, M. A.; van den Bergh, P. J.; Hoenders, H. J. Glycation of human serum albumin: Inhibition by diclofenac. *Biochim. Biophys. Acta* 1120:201-204; 1992.

[191] Regensteiner, J. G.; Stewart, K. J. Established and evolving medical therapies for claudication in patients with peripheral arterial disease. *Nat. Clin. Pract. Cardiovasc. Med.* 3:604-610; 2006.

[192] Rahbar, S.; Natarajan, R.; Yerneni, K.; Scott, S.; Gonzales, N.; Nadler, J. L. Evidence that pioglitazone, metformin and pentoxifylline are inhibitors of glycation. *Clin. Chim. Acta* 301:65-77; 2000.

[193] Booth, A. A.; Khalifah, R. G.; Hudson, B. G. Thiamine pyrophosphate and pyridoxamine inhibit the formation of antigenic advanced glycation end-products: Comparison with aminoguanidine. *Biochem. Biophys. Res. Commun.* 220:113-119; 1996.

[194] Nagaraj, R. H.; Sarkar, P.; Mally, A.; Biemel, K. M.; Lederer, M. O.; Padayatti, P. S. Effect of pyridoxamine on chemical modification of proteins by carbonyls in diabetic rats: Characterization of a major product from the reaction of pyridoxamine and methylglyoxal. *Arch. Biochem. Biophys.* 402:110-119; 2002.

[195] Stitt, A.; Gardiner, T. A.; Alderson, N. L.; Canning, P.; Frizzell, N.; Duffy, N.; Boyle, C.; Januszewski, A. S.; Chachich, M.; Baynes, J. W.; Thorpe, S. R. The AGE inhibitor pyridoxamine inhibits development of retinopathy in experimental diabetes. *Diabetes* 51:2826-2832; 2002.

[196] Monnier, V. M. Bacterial enzymes that can deglycate glucose- and fructose-modified lysine. *Biochem. J.*392:e1-3; 2005.

[197] Horiuchi, T.; Kurokawa, T.; Saito, N. Purification and properties of fructosylamino acid oxidase from corynebacterium sp. 2-4-1. *Agri Biol Chem* 53:103-110; 1989.

[198] Horiuchi, T.; Kurokawa, T. Purification and properties of fructosylamine oxidase from aspergillus sp. 1005. *Agri Biol Chem* 55:333-338; 1991.

[199] Wiame, E.; Delpierre, G.; Collard, F.; Van Schaftingen, E. Identification of a pathway for the utilization of the amadori product fructoselysine in escherichia coli. *J. Biol. Chem.* 277:42523-42529; 2002.

[200] Wiame, E.; Van Schaftingen, E. Fructoselysine 3-epimerase, an enzyme involved in the metabolism of the unusual amadori compound psicoselysine in escherichia coli. *Biochem. J.*378:1047-1052; 2004.

[201] Wiame, E.; Lamosa, P.; Santos, H.; Van Schaftingen, E. Identification of glucoselysine-6-phosphate deglycase, an enzyme involved in the metabolism of the fructation product glucoselysine. *Biochem. J.* 392:263-269; 2005.

[202] Rahbar, S.; Kumar Yernini, K.; Scott, S.; Gonzales, N.; Lalezari, I. Novel inhibitors of advanced glycation endproducts. *Biochem. Biophys. Res. Commun.* 262:651-656; 1999.

[203] Rahbar, S.; Yerneni, K. K.; Scott, S.; Gonzales, N.; Lalezari, I. Novel inhibitors of advanced glycation endproducts (part II). *Mol. Cell Biol. Res. Commun.* 3:360-366; 2000.

[204] Ulrich, P.; Zhang, X. Pharmacological reversal of advanced glycation end-product-mediated protein crosslinking. *Diabetologia* 40 Suppl 2:S157-9; 1997.

[205] Wolffenbuttel, B. H.; Boulanger, C. M.; Crijns, F. R.; Huijberts, M. S.; Poitevin, P.; Swennen, G. N.; Vasan, S.; Egan, J. J.; Ulrich, P.; Cerami, A.; Levy, B. I. Breakers of advanced glycation end products restore large artery properties in experimental diabetes. *Proc. Natl. Acad. Sci. U. S. A.* 95:4630-4634; 1998.

[206] Guo, Y.; Lu, M.; Qian, J.; Cheng, Y. L. Alagebrium chloride protects the heart against oxidative stress in aging rats. *J. Gerontol. A Biol. Sci. Med. Sci.* 2009.

[207] Vaitkevicius, P. V.; Lane, M.; Spurgeon, H.; Ingram, D. K.; Roth, G. S.; Egan, J. J.; Vasan, S.; Wagle, D. R.; Ulrich, P.; Brines, M.; Wuerth, J. P.; Cerami, A.; Lakatta, E. G. A cross-link breaker has sustained effects on arterial and ventricular properties in older rhesus monkeys. *Proc. Natl. Acad. Sci. U. S. A.* 98:1171-1175; 2001.

[208] Kass, D. A.; Shapiro, E. P.; Kawaguchi, M.; Capriotti, A. R.; Scuteri, A.; deGroof, R. C.; Lakatta, E. G. Improved arterial compliance by a novel advanced glycation end-product crosslink breaker. *Circulation* 104:1464-1470; 2001.

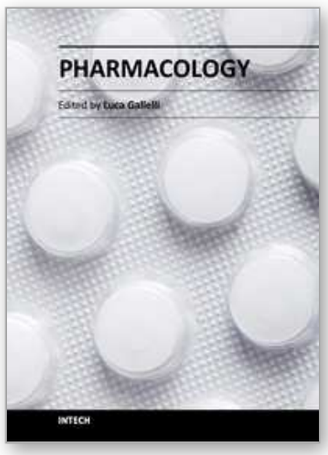
[209] Zieman, S. J.; Melenovsky, V.; Clattenburg, L.; Corretti, M. C.; Capriotti, A.; Gerstenblith, G.; Kass, D. A. Advanced glycation endproduct crosslink breaker (alagebrium) improves endothelial function in patients with isolated systolic hypertension. *J. Hypertens.* 25:577-583; 2007.

[210] Little, W. C.; Zile, M. R.; Kitzman, D. W.; Hundley, W. G.; O'Brien, T. X.; Degroof, R. C. The effect of alagebrium chloride (ALT-711), a novel glucose cross-link breaker, in the treatment of elderly patients with diastolic heart failure. *J. Card. Fail.* 11:191-195; 2005.

[211] Bakris, G. L.; Bank, A. J.; Kass, D. A.; Neutel, J. M.; Preston, R. A.; Oparil, S. Advanced glycation end-product cross-link breakers. A novel approach to cardiovascular pathologies related to the aging process. *Am. J. Hypertens.* 17:23S-30S; 2004.

[212] Hartog, J. W.; Willemsen, S.; van Veldhuisen, D. J.; Posma, J. L.; van Wijk, L. M.; Hummel, Y. M.; Hillege, H. L.; Voors, A. A.; for the BENEFICIAL investigators. Effects of alagebrium, an advanced glycation endproduct breaker, on exercise tolerance and cardiac function in patients with chronic heart failure. *Eur. J. Heart Fail.* 13:899-908; 2011.

[213] Krautwald, M.; Leech, D.; Horne, S.; Steele, M. L.; Forbes, J.; Rahmadi, A.; Griffith, R.; Munch, G. The advanced glycation end product-lowering agent ALT-711 is a low-affinity inhibitor of thiamine diphosphokinase. *Rejuvenation Res.* 14:383-391; 2011.



Pharmacology
Edited by Dr. Luca Gallelli

ISBN 978-953-51-0222-9
Hard cover, 720 pages
Publisher InTech
Published online 14, March, 2012
Published in print edition March, 2012

The history of pharmacology travels together to history of scientific method and the latest frontiers of pharmacology open a new world in the search of drugs. New technologies and continuing progress in the field of pharmacology has also changed radically the way of designing a new drug. In fact, modern drug discovery is based on deep knowledge of the disease and of both cellular and molecular mechanisms involved in its development. The purpose of this book was to give a new idea from the beginning of the pharmacology, starting from pharmacodynamic and reaching the new field of pharmacogenetic and ethnopharmacology.

How to reference
In order to correctly reference this scholarly work, feel free to copy and paste the following:

Indu Dhar and Kaushik Desai (2012). Aging: Drugs to Eliminate Methylglyoxal, a Reactive Glucose Metabolite, and Advanced Glycation Endproducts, Pharmacology, Dr. Luca Gallelli (Ed.), ISBN: 978-953-51-0222-9, InTech, Available from: http://www.intechopen.com/books/pharmacology/aging-drugs-to-eliminate-methylglyoxal-a-reactive-glucose-metabolite-and-advanced-glycation-endprodu

INTECH
open science | open minds

InTech Europe
University Campus STeP Ri
Slavka Krautzeka 83/A
51000 Rijeka, Croatia
Phone: +385 (51) 770 447
Fax: +385 (51) 686 166
www.intechopen.com

InTech China
Unit 405, Office Block, Hotel Equatorial Shanghai
No.65, Yan An Road (West), Shanghai, 200040, China
中国上海市延安西路65号上海国际贵都大饭店办公楼405单元
Phone: +86-21-62489820
Fax: +86-21-62489821

IntechOpen