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Advanced Glycation Endproducts, (AGEs): Formation, complication and pharmacological evaluation to its inhibition.

Abstract:-

9 Glycation sometimes called non enzymatic glycosylation is result of sugar molecules bonding to a protein or
10 lipid molecule without controlling action of an enzyme. During the process of glycation, early glycation
11 products are formed first, which subsequently rearrange into final AGE structures through a series of very
12 complex chemical reactions and formed (MOLD), (GOLD) and (DOLD). AGEs are implicated in many age related
13 diseases such as type-II (diabetic mellitus), cardiovascular disease (the endothelial cell, collagen, fibrinogen are
14 damaged), Alzheimer diseases (amyloid protein are side product of the reaction progressing to AGEs), Cancer
15 (acryl-amide and other side product are related), peripheral neuropathy (the myelin is attached), and other
16 sensory losses such as deafness (due to demyelination),and blindness (mostly due to micro-vascular damage
17 in the retina),this range of diseases is the result of very basic level at which glycation interfere with
18 molecular and cellular functioning throughout the body. Pharmacologically influence the process of non-
19 enzymatic glycation and AGE product formation Inhibit the formation of AGEs are purported to have
20 therapeutic potentials in patients with diabetes and age-related diseases. The oxidation process is believed to
21 play an important role in AGEs formation The best cross-link inhibitors currently available are carnosine,
22 aminoguanidine, metformin and acarbose, whereas others are now becoming available. No cross-link breakers
23 are commercially obtainable as yet, but these will be marketed within 2-3 years. Soon after, combinations of
24 inhibitors and breakers are due to follow.

25 **Key words:** - AGEs, MOLD, GOLD, Amadori reaction, NEG, CML (carboxyl methyl lysine), β-
26 amyloid.

INTRODUCTION:-

28 The aldehyde or ketone groups of reducing sugars react non-enzymatically with the free
29 amino groups of proteins, lipids and nucleic acids leading to the formation of advanced
30 glycation end products (AGEs)¹. In this reaction, sugars react reversibly with the free amino
31 group of proteins to form unstable Schiff bases, which then undergo an intra molecular
32 rearrangement to form a stable Amadori product. These Amadori products are believed to
33 undergo a series of reactions to form heterogeneous complex fluorophores and chromophores
34 collectively referred to as advanced Maillard products or advanced glycation end products
35 (AGEs)². The production of these AGEs are implicated in many age related diseases such as
36 type-II (diabetic mellitus), cardiovascular disease (the endothelial cell, collagen, fibrinogen
37 are damaged), Alzheimer diseases (amyloid protein are side product of the reaction

38 progressing to AGEs), Cancer (acryl-amide and other side product are related), peripheral
39 neuropathy (the myelin is attached), and other sensory losses such as deafness (due to
40 demyelination),and blindness (mostly due to micro-vascular damage in the retina),this
41 range of diseases is the result of very basic level at which glycation interfere with
42 molecular and cellular functioning throughout the body³ . An important part of tissue
43 damage and of cell death associated with chronic hyperglycemia, and diabetes is mediated by
44 free radicals. E.C.M. (Extra cellular matrix), proteins such as collagen, elastin, actin, and
45 myosin are the backbone for architectural and functional stability of tissues cell and organs.
46 When AGEs accumulations particularly high in E.C.M., proteins are result in intra and inter
47 molecular cross-linking and later has been hypotized to stiffening of these proteins and
48 believed to play an important role in etiology of various AGEs related diseases⁴The present
49 review will focus on AGEs, related complications and on their inhibition by various
50 therapeutic compounds.

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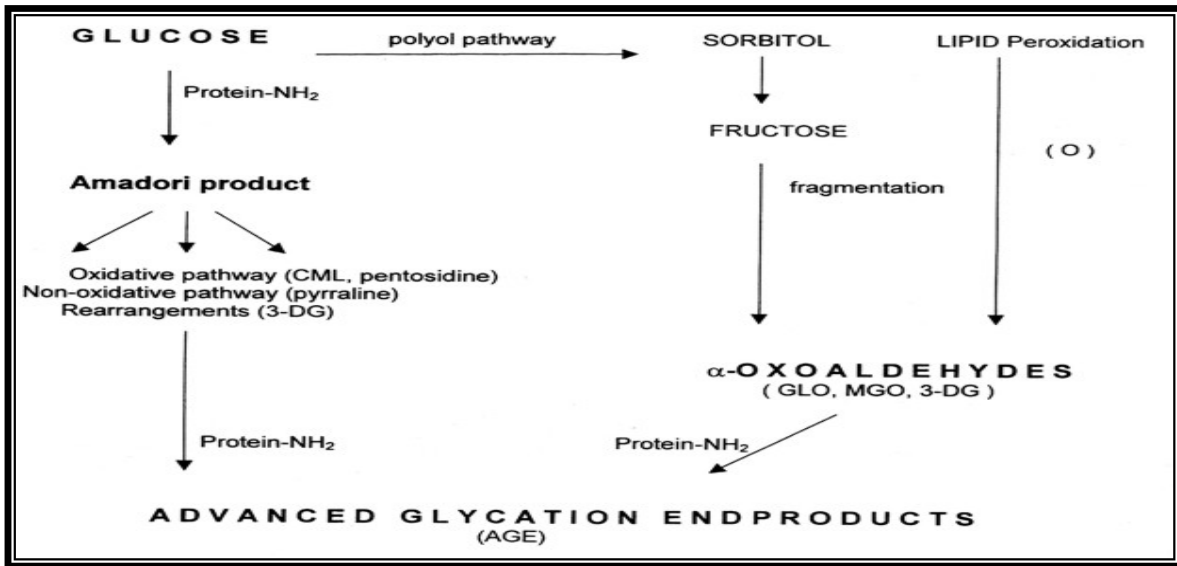
52 **Biochemistry of Non-enzymatic glycation**

53 Non-enzymatic glycation is a process by which glucose is chemically bound to amino groups
54 of proteins but without the help of enzymes. It is a classical covalent reaction in which, by
55 means of N-glycoside bonding, the sugar-protein complex is formed through a series of
56 chemical reactions described by a chemist Maillard. Maillard reactions are complex and
57 multi-layer, and can be analyzed in three steps. The sugar-protein complex is formed first
58 (Amadori rearrangement). It is an early product of non-enzymatic glycation, an intermediary
59 which is a precursor of all later compounds. The second step includes the formation of
60 numerous intermediary products, some of which are very reactive and continue with
61 glycation reaction. The third, final phase consists of polymerization reaction of the complex
62 products formed in the second step, whereby heterogeneous structures named advanced
63 glycation end products (AGE) are formed⁵. It was believed that the primary role in Maillard
64 reactions was exclusively played by high glucose concentration. However, recent data show
65 that, in spite of the fact that sugars are the main precursors of AGE compounds, numerous
66 intermediary metabolites⁶, i.e. alpha-oxo-aldehydes, also creatively participate in
67 nonenzymatic glycation reactions. Such intermediary products are generated during
68 glycolysis (methylglyoxal) or along the polyolic pathway, and can also be formed by auto-
69 oxidation of carbohydrates (glyoxal). Alpha-oxo-aldehydes modify AGEs surprisingly fast, in
70 contrast to classical Maillard reactions, which are very slow (Fig. 1).

71 Figure 1:- Schematic presentation of potential pathway leading to AGE formation

- 72 a. AGE arise from decomposition of Amadori products
- 73 b. fragmentation products of polyol pathway
- 74 c. as glycol-oxidative products,
- 75 d. which all react with amino groups of protein
- 76 e. which all react with amino groups of protein

77 Fig no -1 Glycation reaction



78 **GLO=glyoxal; MGO=methylglyoxal; 3-DG=3-deoxyglucosone; CML=carboxymethyl-lysine**

79

80 In physiological conditions, glycation can be detected in the process of aging, and the
81 reactions are significantly faster and more intensive, with frequently increased glucose
82 concentrations. In diabetology, the importance of these processes manifests in two essential
83 issues: 1) effect of protein glycation on the change of their structure and function, and 2) use
84 of glycated protein level as a parameter of integrated glycemia⁷. A classical example of non-
85 enzymatic glycation is the formation of glycated hemoglobin, or more precisely, HbA1c. As
86 the degree of non-enzymatic glycation is directly associated with the level of blood glucose,
87 the percentage of HbA1c in diabetes can also be greatly increased. HbA1c was the first
88 glycated protein studied, however, soon it was discovered that other various structural and
89 regulatory proteins also are subject to non-enzymatic glycation to form glycation end
90 products⁸.

91

92

93 **Types of Advanced glycation end products (AGEs)**

94 During the process of glycation, early glycation products are formed first, which
95 subsequently rearrange into final AGE structures through a series of very complex chemical
96 reactions. Protein modification with AGE is irreversible, as there are no enzymes in the body
97 that would be able to hydrolyze AGE compounds⁹. These structures then accumulate during
98 the lifespan of the protein on which they have been formed. In some cases oxidation is also
99 involved, so that it is possible to distinguish between compounds formed by glycation by
100 others formed by glycoxydation. From glucose the non oxidative pathway could give rise to
101 pyrroline; in the oxidative pathway to pentosidine and N6-carboxymethyllysine (CML)¹⁰.
102 Glyceraldehyde can also be involved. It is formed from glyceraldehyde-3-phosphate, an
103 intermediate of glycolysis, through the polyol pathway, or from fructose, during its
104 transformation by fructokinase. A glyceraldehyde derived AGE is the so called
105 glyceraldehyde-derived pyridinium compound (GLAP), a compound that has been seen to
106 induce oxidative cellular dysfunction. Glyceraldehyde derived AGEs have been shown
107 initially in AD brain and in the cytosol of neurons¹¹. Later, GLAP has been detected in the
108 plasma protein and in collagen obtained from streptozotocin-induced diabetic rats¹². When
109 glycol-oxidation occurs, new compounds are formed, such as MG and glyoxal. These in turn
110 can also react with proteins. In this case MG reacts mainly with Arg, less so with Lys and
111 Cys (contrary to what occurs in the glycation with glucose). One compound obtained is CML,
112 formed from fructoselysine, one of the Amadori products, in the presence of metal ions.
113 However, now CML is suggested to be a marker of oxidation rather than of glycation, as it
114 can also be formed during lipid per-oxidation besides malondialdehyde and hydroxynonenal
115 adducts to lysine. Moreover, the methylglyoxal-lysine dimer (MOLD), the glyoxallysine
116 dimer (GOLD) and the deoxyglucosone-lysine dimer (DOLD), argpyrimidine and its
117 tetrahydroderivative) are also formed (fig-2). Other compounds formed are pentosidine and
118 vesperlysines (A, B, C). Pentosidine derives from lysine and arginine. It has been found in
119 several tissues, such as plasma and erythrocytes. The pentose which is mainly used appears to
120 be ribose. Vesperlysines A has been shown in the lens of diabetic subjects¹³. It derives from
121 ascorbate, ribose and threose. Pyrroline is also formed from 3-deoxyglucosone and lysine.

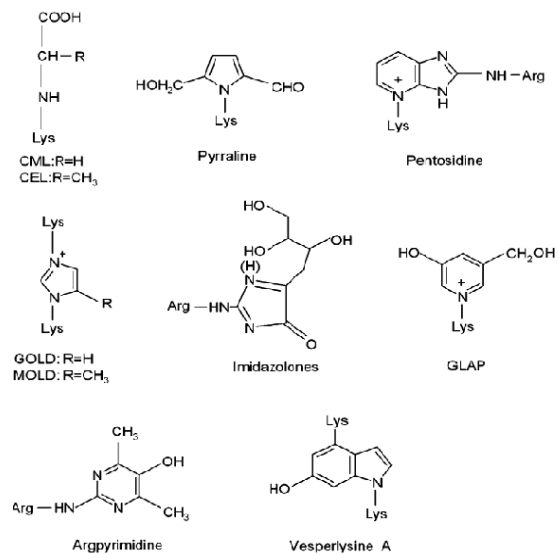


Fig. 2. Chemical structure of various AGEs: CML (N-carboxymethyllysine); CEL (N-carboxylethyllysine); GOLD (glyoxal lysine dimer); MOLD (methylglyoxal lysine dimer); GLAP (glyceralkdehyde-derived pyridinium compound); vesperlysine A

122

123 AGEs, and oxidation

124

125 An important part of tissue damage and of cell death associated with chronic hyperglycemia,
 126 and diabetes is mediated by free radicals. In hyperglycemic diabetic patients, exaggerated
 127 oxidative stress is due both to an excess in free oxygen species production, secondary to
 128 increased oxidation of substrates (sugars, non-saturated fats, and glycated proteins), to
 129 increased glucose auto-oxidation, and to a decrease in antioxidants. In animal models of
 130 diabetes, hyper-production of free radicals is responsible for endothelial dysfunction, *via* a
 131 decrease in NO (nitric oxide) production, thus decreasing vasorelaxation of smooth muscle
 132 cells¹⁴. The links between oxidative stress and AGEs may explain in part the relation
 133 between hyperglycemia and both endothelial dysfunction and tissue damage. Oxidized LDL
 134 is responsible for decreased NO production, by a reduction in NO synthase¹⁵. AGEs quench
 135 the NO, and thus contribute to defective vasodilatation observed in animal models. AGEs
 136 induce apoptosis in cultured human umbilical vein endothelial cells¹⁶. Experimentally, we
 137 have shown that the interaction between AGEs and RAGE induces an activation of oxidative
 138 stress, and stimulates the production and release of cytokines, which amplifying thus tissue
 139 damage¹⁷.

140

141 **AGE receptors**

142 The level of AGE proteins reflects kinetic balance of two opposite processes: the rate of AGE
143 compound formation, and the rate of their degradation by means of receptors. AGE receptors
144 participate in the elimination and change of aged, reticular and denatured molecules of
145 extracellular matrix as well as of other AGE molecules. However, in diabetes mellitus AGE
146 protein accumulation may exceed the ability of their elimination due to chronic
147 hyperglycemia and excessive glycation process¹⁸.

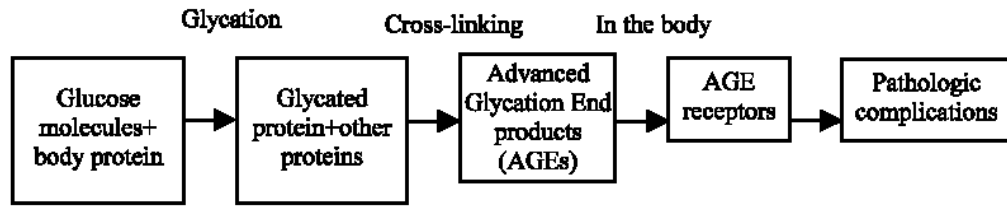
148 The first structures were identified as possible AGE receptors using radiolabelled AGE
149 proteins. Human and murine monocytes, lymphocytes bind specifically AGEs with a
150 dissociation coefficient between 50 and 200 nmol/L. Receptor proteins which bind AGEs,
151 have been isolated from cell membrane and have been purified. They have different apparent
152 molecular weights according to the cell type: 40 KD for kidney, 36-83 KD for macrophage
153 cell line, 60-90 KD for liver cells. AGEs binding protein have been purified from endothelial
154 cells and characterized. Two polypeptides were obtained from pulmonary endothelial cells,
155 one was described as the receptor for AGEs (RAGE) and the second has a very high
156 homology to lactoferrin (LF1)¹⁹. RAGE in a truncated form has a molecular weight of 35 Kd
157 and belongs to the immunoglobulin super-family. RAGE gene is located on chromosome six
158 in the MHC region (6p 21-3). Human, rat and bovine RAGE have a high degree of homology,
159 but slight differences in glycosylation sites and susceptibility to proteases may explain their
160 different pharmacological parameters²⁰. RAGE has also some homology with molecules of
161 the immunoglobulin super-family (MUC, CD20). RAGE is expressed by different cell types:
162 monocyte/ macrophage, T-lymphocytes, endothelial cells, smooth muscle cells, mesangial
163 cells, neuronal cells. RAGE expression is potentiated by hyperglycemia or TNF- α treatment.
164 RAGE binds different ligands such as amphoterin, β -amyloid substances or calgranulin
165 polypeptides²¹. Carboxymethyl lysine (CML) is the AGE which after binding to RAGE, is a
166 stronger inducer of vascular cell adhesion molecule (VCAM-1)²².

167 **Consequences of engagement of the receptor RAGE**

168

169 The finding that enhanced expression of tissue factor in AGEs-stimulated macrophages
170 retrieved from (gp91phox) null mice was suppressed compared to wild-type macrophages,
171 strongly suggests important roles for NADPH oxidase in AGEs-mediated processes²³.
172 Importantly, recent studies indicating that endothelial cells express a gp91phox-containing
173 NADPH oxidase support our hypothesis that activation of this enzyme provides source of
174 ROIs upon AGEs engagement of RAGE in endothelial cells. In those studies by Gorkach *et*

175 *al.*, it was shown that NADPH oxidase was a major source in the arterial wall, as its
176 activation was associated with impaired bioavailability of endothelium-derived NO²⁴. RAGE
177 is a multi-ligand receptor of the immunoglobulin super-family. In addition to AGEs, RAGE
178 serves as a cell surface receptor for amyloid β - peptide (A β), a cleavage product of the β -
179 amyloid precursor protein which accumulates in Alzheimer's disease and β sheet fibrils^{25, 26}.
180 *In vivo*, blockade of RAGE in a murine model of systemic amyloidosis suppressed amyloid
181 induced nuclear translocation of NF- κ B and cellular activation. RAGE is also a signal
182 transduction receptor for EN-RAGES, and related members of the S100/cal granulin family
183 of pro-inflammatory cytokines. The S100/cal granulin family is comprised of closely-related
184 polypeptides released from activated inflammatory cells, including polymorphonuclear
185 leukocytes, peripheral blood-derived mononuclear phagocytes and lymphocytes. Their
186 hallmark is accumulation at sites of chronic inflammation, such as psoriatic skin disease,
187 cystic fibrosis, inflammatory bowel disease, and rheumatoid arthritis. Ligation of RAGE by
188 ENRAGES mediated activation of endothelial cells, macrophages and lymphocytes. In
189 parallel with suppression of the inflammatory phenotype, inhibition of RAGE-S100/cal
190 granulin interaction decreased NF- κ B activation and expression of pro-inflammatory
191 cytokines in tissues, suggesting that receptor blockade changed the course of the
192 inflammatory response. Previous studies further indicated that RAGE was likely a receptor
193 for amphoterin, a molecule linked to neurite outgrowth in developing neurons of the central
194 and peripheral nervous system²⁷. These studies suggested that amphoterin-RAGE was linked
195 to cellular migration and invasiveness. Consistent with this concept, the expression of
196 amphoterin and RAGE is increased in murine and human tumors. Blockade of RAGE *in vivo*
197 suppressed local growth and distant spread of implanted tumors, as well as the growth of
198 tumors forming endogenously in susceptible mice. Consistent with an important role for
199 RAGE-mediated signal transduction in these processes, blockade of RAGE/RAGE signaling
200 on amphoterin coated matrices suppressed activation of p44/42, p38 and SAPK/JNK kinases
201²⁸. In settings characterized by increased accumulation and expression of RAGE and its
202 ligands, such as diabetic atherosclerotic lesions and periodontium, chronic disorders such as
203 rheumatoid arthritis and inflammatory bowel disease, and Alzheimer disease, enhanced
204 inflammatory responses have been linked to ongoing cellular perturbation. One consequence
205 of ligand-RAGE-mediated activation of MAP kinases and NF- κ B is increased transcription
206 and translation of vascular cell adhesion molecule (VCAM-1). At the cell surface,
207 endothelium stimulated by a range of mediators, such as endotoxin, tumor necrosis factor α
208 (TNF α), AGEs display increased adhesion of pro-inflammatory mononuclear cells, at least in
209 part, *via* VCAM-1. Recent studies have suggested that the pro-inflammatory effects of
210 VCAM-1 are not limited to cellular adhesion events, as binding of ligand to VCAM-1 in
211 endothelial cell lines and primary cultures induced activation of endothelial NADPH oxidase,
212 a process shown to be essential for lymphocyte migration through the stimulated cells. These
213 findings suggest that activation of RAGE at the cell surface may initiate a cascade of events
214 including activation of NADPH oxidase and a range of pro-inflammatory mediators such as
215 VCAM-1. In diabetes, although oxidant stress responses are essential to eliminate pathogenic
216 periodontal pathogens, ongoing AGE/EN-RAGE-mediated cellular activation in infected
217 periodontium has been linked to increased generation of pro-inflammatory cytokines and
218 tissue-destructive matrix metallo-proteinases, processes leading to destruction of alveolar
219 bone²⁹. The various role of AGEs receptors in the pathogenesis of later diabetic
220 complications summarized in table-1.
221



222

223 **Fig-3:-Formation of AGEs from glycation**

224 **Table -1:- Role of AGEs and AGE receptors in the pathogenesis of diabetic complications**
 225

Serial No.	Different diabetic condition	Role of AGEs and AGE receptors and it's mechanism
1.	Diabetic atherosclerosis	<ul style="list-style-type: none"> • Vascular tissue AGE accumulation → protein crosslinking → oxidative damage • Increased vascular matrix → thickening and narrowing of lumen • Increased endothelial cell permeability and procoagulant activity → thrombosis • Mononuclear cell chemotaxis/activation → cytokine and growth factor release • Increased macrophage uptake of AGE-LDL → atheroma
2.	Diabetic kidney disease	<ul style="list-style-type: none"> • Increased cell permeability → vascular leakage and retinal damage • Increased vessel wall thickening → occlusion → retinal ischemia → neovascularization • Increased intravascular coagulation → occlusion → retinal ischemia → neovascularization
3.	Diabetic retinopathy	<ul style="list-style-type: none"> • Increased cell permeability → vascular leakage and retinal damage • Increased vessel wall thickening → occlusion → retinal ischemia → neovascularization • Increased intravascular coagulation → occlusion → retinal ischemia → neovascularization
4.	Diabetic neuropathy	<ul style="list-style-type: none"> • Increased AGEs in vasa nervorum → wall thickening and occlusion • Increased vascular permeability and thrombosis → occlusion → neuronal ischemia • Increased AGE myelin accumulation → myelin damage

		<ul style="list-style-type: none">• Increased macrophage activity → myelin and vascular degeneration
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226

227 **AGEs in diabetic vasculopathy and atherosclerosis**

228 Atherosclerotic cardiovascular disease is the major cause of morbidity and mortality in
229 diabetes. The mechanisms by which diabetes so dramatically increases atherosclerosis are yet
230 poorly understood. AGEs also play a significant role in atherosclerosis. For instance,
231 reticulated and irreversible LDL from the circulation binds to AGE-modified collagen of the
232 blood vessel walls. In the majority of blood vessels, such reticular binding delays normal
233 outflow of LDL particles that have penetrated the vessel wall, thus enhancing cholesterol
234 deposition in the intima. Such AGE reticulation increases lipoprotein deposition regardless of
235 the plasma LDL level. This is followed by an accelerated development of atherosclerosis.³⁰ It
236 has been well documented that lipids and lipoproteins are deeply involved in the atherogenic
237 process. Diabetes can lead to several lipoprotein modifications that can affect their interaction
238 with arterial wall cells, thereby contributing to the increased risk of atherosclerosis. The
239 modifications of lipoproteins include oxidation and glycation. Approximately 2% to 5% of
240 apo B in the plasma of diabetic persons are glycated, compared with about 1% in the plasma
241 from non diabetic control subjects. AGEs have recently been reported to be associated with
242 LDL, and an elevated level of AGE-LDL was found in patients with diabetes and renal
243 insufficiency as compared with the LDL obtained from normal controls. This observation
244 suggests that the formation of AGE might occur more rapidly than previously believed, or
245 that AGE-LDL may enter plasma from extravascular tissues such as arterial wall. The
246 presence of AGEs on apo B stimulated investigation of the consequences of this modification
247 on LDL metabolism. Glycated LDL interacts poorly with LDL receptor, thereby increasing
248 its residence time in plasma and presumably in the extracellular space of the arterial wall.
249 Furthermore, there is a significant relationship between the extent of apo B-AGE and
250 impairment in the plasma LDL clearance³¹. AGE lipoproteins, like other advanced glycation
251 modified proteins, bind to specific receptors on macrophages and other cell types, and can
252 stimulate the release of cytokines and growth factors which may play a role in atherogenesis.
253 Thus, a reduction in the level of glycation of lipoproteins as well as of the arterial wall
254 extracellular matrix might alter the interaction of lipoproteins with the matrix and reduce
255 their retention in the arterial wall where they are able to exert their atherogenic damage³².

256 **AGEs and renal failure**

257 Persistent hyperglycemia has a central role in the development of diabetic nephropathy that is
258 clinically manifested by proteinuria progressing to renal insufficiency, and
259 histopathologically by mesangial expansion and glomerular basement membrane
260 thickening³⁰. A possible link between elevated glucose level and diabetic nephropathy
261 resides in the glycation process producing AGEs. This modification may impair the original
262 function of either protein and may affect normal processes of turnover and clearance. AGEs
263 can induce an excess crosslinking of collagen molecules in the glomerular plasma membrane
264 affecting the assembly and architecture of the glomerular basement membrane and mesangial
265 matrix, and can potentially act on mesangial cells *via* growth factors, causing cells to
266 synthesize more extracellular matrix. All these processes may lead to enhanced deposition of
267 extracellular matrix proteins in the mesangial, interfere with the mesangial clearance of
268 macromolecules, and alter macrophage function, thus contributing to mesangial expansion
269 and glomerular occlusion³³.

270 Circulating serum AGE level is markedly increased in patients with diabetes and renal
271 insufficiency. Serum AGEs include both serum proteins that have been modified by advanced
272 glycation and low molecular weight AGE peptides. Using specific immunoassay, serum AGE
273 peptide levels have been found to correlate with renal function. In fact, close correlation has
274 been demonstrated between serum AGE levels and creatinine clearance. In normal controls,
275 AGE peptide clearance has been estimated to 0.72 ml/min. Diabetic persons with normal
276 glomerular filtration rate can clear AGE peptides at the same rate. However, progressive loss
277 of renal function is associated with increasing circulating AGE peptide levels. Current renal
278 replacement therapies, hemo-dialysis or peritoneal dialysis, are relatively inefficient in
279 removing AGEs from the serum of diabetic patients. In these patients, AGE peptides persist
280 at up to 8-fold normal level. In contrast, serum AGE peptide levels rapidly decrease and
281 remain within the normal range in patients undergoing kidney transplantation³⁴.

282 Skin AGEs levels detected by immunochemistry correlate with severity of nephropathy and increase
283 in early stages of renal involvement³⁵. A longitudinal study in type 1 diabetic patients followed during
284 2.5 years has indicated the predictive value of AGE serum levels for the development of the
285 morphological changes in the kidney³⁶. AGEs infusion in normal rats during 5 months results in
286 increased AGEs renal tissue content and in alterations similar to diabetic nephropathy: increase in
287 glomerular volume, in basement membrane thickness and in mesangial extracellular matrix³⁷. An

288 effect of AGEs on renal gene expression has been evidenced³⁸. Administration of AGE-modified
289 albumin during 4 weeks to normal mice induces glomerular hypertrophy as well as an increase in
290 glomerular extracellular matrix, α 1 (IV) collagen, laminin B1 and transforming growth factor β 1
291 (TGF β 1) mRNA levels. This response seems to be specific to AGEs because all these changes can be
292 prevented by aminoguanidine co-administration. The role of AGEs in diabetic nephropathy
293 development has been investigated in streptozotocin-induced diabetic rats compared to non diabetic
294 control rats, and diabetic rats co-treated with aminoguanidine³⁹. After thirty two weeks, diabetic rats
295 exhibit increased fluorescence in glomeruli and renal tubes, which was prevented by
296 aminoguanidine⁴⁰. Diabetic rats develop albuminuria over the 32-week period⁴¹. This increase was
297 attenuated by aminoguanidine, but not by antioxidant and by aldose reductase inhibitor⁴². Other
298 inhibitors of renal AGEs accumulation, as ALT-946, are also effective in preventing and retarding
299 diabetic nephropathy in animal models⁴³. However, studies with aminoguanidine (pimagedine) are no
300 more in progress in human diabetics at the present time. Treatment with ALT-711 and
301 aminoguanidine, which both attenuate renal AGE accumulation, abrogated these increases in
302 PKC expression. However, translocation of phosphorylated PKC-alpha from the cytoplasm to
303 the membrane was reduced only by ALT-711. ALT-711 treatment attenuated expression of
304 vascular endothelial growth factor and the extracellular matrix proteins, fibronectin and
305 laminin, in association with reduced albuminuria. Aminoguanidine had no effect on VEGF
306 expression, although some reduction of fibronectin and laminin was observed. These findings
307 implicate AGEs as important stimuli for the activation of PKC, particularly PKC-alpha, in the
308 diabetic kidney, which can be directly inhibited by ALT-711.

309 **AGEs and diabetic retina**

310 Diabetic retinal complications result from retinal capillaries functional and morphological
311 alterations: increased permeability to albumin and macromolecules, vascular dysfunction,
312 loss of pericytes, and basement membrane thickening. The arguments in favor of a central
313 role for AGEs in these alterations have been discussed above. These alterations lead to
314 macular edema secondary to the leakage of macromolecules, and progressive capillary
315 closures related to microthrombosis. Capillary closures are responsible for non-perfused areas
316 (ischemic retinopathy), which induce the secretion of Vascular Endothelial Growth Factor
317 (VEGF) and the development of neo-vessels (proliferative retinopathy). In diabetic patients,
318 pentosidine skin concentrations have been shown to be associated with the development of
319 proliferative retinopathy⁴⁴. The oxidatively formed CML is increased in diabetic rats both in
320 neuroglial and vascular retinal components, while imidazole-type AGEs are restricted to
321 microvessels, co-localizing with the expression of RAGE⁴⁵. In rats with streptozotocin-

322 induced diabetes, treatment with aminoguanidine prevents diabetic retinopathy, resulting in
323 an 80% reduction in pericytes loss, in an absence of micro-aneurysms development, and of
324 endothelial cell proliferation. The accumulation of AGEs in pre-capillary arterioles is
325 inhibited by treatment with aminoguanidine⁴⁶. Aminoguanidine prevents the development of
326 retinopathy in the diabetic spontaneous hypertensive rat (SHR), and completely suppresses
327 the deposit of PAS positive material in arterioles, and microthrombosis formation⁴⁷. Evidence
328 of this role relies on the results of studies indicating that the deleterious effects of AGEs on
329 retinal capillary pericytes and endothelial cells are inhibited by RAGE-antibodies⁴⁸. The role
330 of AGEs mediated by VEGF in vascular dysfunction related to pseudo-hypoxemic changes
331 has been suggested by recent experiments⁴⁹. These effects are prevented by neutralizing
332 VEGF antibodies and markedly reduced by aminoguanidine. Moreover, an association
333 between accumulation of CML in human diabetic retina, proliferative and non-proliferative
334 retinopathy, and expression of VEGF has been reported⁵⁰.

335 **AGEs in diabetic neuropathy**

336 The major causative link between clinical diabetic neuropathy and peripheral nerve changes
337 is hyperglycemia. One of the important biochemical pathways involved, with a potential role
338 in diabetic neuropathy, is glycation leading to AGE modification of nerve proteins⁶⁴. AGEs
339 have been stained in the endoneurial, particularly on the axons, endoneurial capillaries, and
340 perineurium of diabetic patients with neuropathy. Axonal cytoskeleton proteins have essential
341 roles in axonal structure and function⁵¹. Nonenzymatic glycation of axonal proteins causes
342 alteration in structure and transport, leading to axonal atrophy and degeneration.
343 Additionally, studies have shown that glycation of myelin occurs in both peripheral nerve and
344 brain. The AGEs are accumulated in the perineurium, endothelial cells and pericytes of
345 endoneurial microvessels, as well as myelinated and unmyelinated fibers. At the sub microscopic level, the
346 AGEs deposit appear focally as irregular aggregates in the cytoplasm of endothelial cells,
347 pericytes, axoplasm and Schwann cells of both myelinated and unmyelinated fibers⁶⁸.
348 Diabetic polyneuropathy is a complication that affects most patients with long standing
349 hyperglycemia, deteriorating their quality of life. In the last few years, new therapeutic
350 approaches have been developed that can improve symptoms and neutralize function and
351 which may prevent and in some cases stop nerve damage and even promote nerve fiber
352 regeneration⁵².

353 **Non-receptors AGEs complication**

354 **AGEs, extracellular matrix, and vessel wall components**

355

356 Capillary basement membrane thickening and hypertrophy of extra vascular matrix are
357 common features of diabetic microvascular complications. The link between high plasma
358 glucose levels and tissue damage is due, at least in part, to the formation and accumulation of
359 AGEs in tissues⁵³. AGEs accumulate in extracellular matrix proteins as a physiological
360 process during aging . However, this accumulation happens earlier, and with an accelerated
361 rate in diabetes mellitus than in non-diabetic individuals⁵⁴. Increased serum and tissue levels
362 of AGEs, due to a reduced removal by kidney, have been evidenced in end-stage renal failure
363 and are more important in diabetic than in non-diabetic patients . A highly significant
364 correlation has been shown between the importance of the AGEs deposits and the severity of
365 diabetic complications . *In vitro* and *in vivo* studies have indicated that AGEs induce
366 irreversible cross-links in long-living matrix structural proteins, such as type IV collagen,
367 laminin, and fibronectin . AGEs are implicated in the basement membrane thickening through
368 these alterations, *via* a reduction in susceptibility of matrix proteins to proteolytic
369 degradation. These architectural changes alter also the functional properties of the basement
370 membrane, including permeability. Advanced glycation of proteoglycans induces a decrease
371 in electronegative charges and therefore modifies selective filtration properties of the
372 basement membrane⁵⁵. Mesangial expansion is an important part of diabetic nephropathy.
373 The role of AGEs in the over expression of TGF- β 1, which has been implicated in the
374 pathogenesis of diabetic vasculopathy and of vascular remodeling, has been studied in a
375 model of mesenteric vessels of streptozotocin-induced diabetic rat . Vascular hypertrophy
376 was observed, together with an increase in TGF- β 1 and in α 1 (IV) collagen gene expression.
377 AGEs and extracellular matrix were present in abundance in diabetic, but not in control rats.
378 Treatment of diabetic rats with the AGEs formation inhibitor aminoguanidine results in a
379 significant reduction in pathological changes and in over expression of TGF β 1 and α 1
380 collagen genes.⁵⁶

381 **Pharmacologic inhibition of AGE**

382

383 Attempts have been made, with greater or lesser efficacy, to pharmacologically influence the
384 process of non-enzymatic glycation and AGE product formation⁵⁷. Inhibit the formation of
385 AGEs are purported to have therapeutic potentials in patients with diabetes and age-related

386 diseases. The oxidation process is believed to play an important role in AGEs formation.
387 Further oxidation of Amadori product leads to the formation of intermediate carbonyl
388 compounds that can react with the nearby lysine or arginine residues to form protein crosslink
389 and AGEs. The reactive carbonyl compounds may also be generated from the metal ion-
390 catalyzed auto-oxidation of glucose⁵⁸(Rahbar and Figarola, 2003; Voziyan *et al.*, 2003)
391 Therefore, agents with antioxidative or metal-chelating property may retard the process of
392 AGEs formation by preventing further oxidation of Amadori product and metal-catalyzed
393 glucose oxidation. In addition, they block soluble receptors (sRAGEs) or specific receptors
394 (RAGEs) which recognize AGEs. Some soluble receptors circulate freely, whereas specific
395 ones can be found on macrophages, fibroblasts and endothelial cells. When an AGE molecule
396 interacts with a RAGE it forms an adduct which is then prone to create more damage through
397 oxidation and increased metal toxicity. In this regard, several natural and synthetic
398 compounds known to possess antioxidative property which, have been shown to prevent
399 AGEs formation *in vitro* and *in vivo*⁵⁹

400 **Medicinal plants based AGEs inhibitors**

401 several phytochemicals known to possess anti-oxidative property, such as, curcumin, rutin,
402 garcinol and flavonoid-rich extracts, have been shown to prevent AGEs formation *in vitro* and
403 *in vivo*⁶⁰. Arbutin (hydroquinone- β -D-glucopyranoside) is a naturally occurring compound
404 found in various plant species of diverse family such as Ericaceae (*Arctostaphylos* spp.)⁶¹,
405 Betulaceae(*Betula alba*) and Rosaceae (*Pyrus communis* L.) (Petkou *et al.*, 2002)⁶⁹ in right
406 referrence]. Arbutin possessed an *in vitro* antiglycation activity⁶².(Aroma J., 2005).⁷⁰
407 Babu *et al.* (1994)⁶³, Sheikh *et al.* (2004)⁶⁴, and Choi *et al.* (2006)⁶⁵ were under taken studies in
408 Glycation inhibitory reaction particularly in medicinal plants like *W. Somnifera*⁶³, *Allium sativum*⁶⁴,
409 and *Plantago asiatica*⁶⁵. Puerariafuran⁶⁶, a New Inhibitor of advanced glycation end products (AGEs)
410 Isolated from the roots of *Pueraria lobata* was reported by JANG *et al.* (2006)⁶⁶. Chaiyasut *et al.*
411 (2007) was observed that *P. emblica* extract showed higher inhibitory effect on AGEs formation than
412 *K. parviflora* and *G. wintii* extracts⁶⁷. Rebecca *et al.* (2008) were tested whether poly-phenolic
413 substances in extracts of commercial culinary herbs and spices would inhibit fructose-mediated
414 protein glycation. Extracts of 24 herbs and spices were tested for the ability to inhibit glycation of
415 albumin. The most potent inhibitors included extracts of cloves, ground Jamaican allspice, and
416 cinnamon. Potent herbs tested included sage, marjoram, tarragon, and rosemary. The
417 concentration of phenolics that inhibited glycation by 50% was typically 4–12 μ g/ml. Relative to total

418 phenolic concentration extracts of powdered ginger and bay leaves were less effective than
419 expected, and black pepper was more effective⁶⁸.

420 **Commercial AGEs inhibitors**

421 There are several commercially available inhibitors of cross-linking. Examples of these
422 include carnosine, aminoguanidine, metformin, acarbose, and pyridoxamine. Some of these
423 (like acarbose and metformin) are already in use as anti-diabetic drugs but new research
424 coming to light is now emphasizing their additional anti-cross-linking effects. Other not yet
425 widely available inhibitors are Tenilsetam, OPB9195, phenazinediamine (2,3-
426 diaminophenazone), and several hundred others still in development⁶⁹. The Alteon
427 Corporation alone has identified over 850 separate cross-link inhibitors.

428 **Carnosine**

429 The dipeptide carnosine (beta alanyl- L-histidine) is a naturally-occurring agent found in
430 muscle and nervous tissue. Carnosine has been hailed as one of the most promising cross-link
431 inhibitors. It has multiple actions and as such it has been called a pluripotent agent. One way
432 carnosine works is by scavenging for free carbonyl groups. Carnosine is one of the few cross-
433 link inhibitors that is not only active against protein-to-protein cross-linking but also against
434 protein-to-DNA cross-linking⁷⁰. Another important carnosine activity is 'carnosinylation',
435 which is a process whereby carnosine attaches to the protein bearing a carbonyl group, thus
436 blocking the carbonyl from attaching to another protein. It is just like placing a piece of paper
437 (carnosine) between two proteins bearing glue (carbonyls). In other words, carnosine reacts
438 with carbonylated proteins to form carnosine-carbonyl-protein adducts. These adducts are
439 then removed by proteolysis and degradation. Conveniently, carnosine also stimulates and
440 enhances the process of proteolysis. Carnosine has a direct antioxidant action, and it also has
441 a sparing effect on other antioxidants such as glutathione. It is a strong chelator of copper
442 thereby reducing the copper-mediated damage during AGE activity. Finally, it has a possible,
443 yet unconfirmed, bond-breaking capability by dissolving certain bonds (S-S bonds) on cross-
444 linked proteins⁷¹. At the clinical level, carnosine reduced urinary products of free radical and
445 glycosylation metabolism in humans. One of the most important developments regarding
446 carnosine is its ability to prevent and cure age-related cataract, and possibly glaucoma and
447 other age-related eye conditions. People taking 50 mg-100 mg of carnosine a day have not

448 reported any side effects whereas those taking higher doses (1000 mg to 1500 mg a day) have
449 reported occasional histamine-related allergic reactions⁷².

450 **Metformin**

451 Metformin (brand names Glucophage ®, Metforal ®) is a standard anti-diabetic drug
452 (dimethyl-biguanide) used worldwide both against insulin-dependent and against non-insulin-
453 dependent diabetes. Metformin lowers cholesterol, reduces body fat, stimulates antioxidant
454 defenses⁷³ and it is also an effective inhibitor of glycation. It reduces the formation of AGEs,
455 particularly those affecting collagen. In that respect, it prevents diastolic stiffness in the
456 myocardium of diabetic dogs. It has direct anti-glycation effects and improves cross-linking
457 induced damage to nerves in diabetic rats. Its main mechanism of action is its carbonyl
458 trapping ability, as will be explained below. In a clinical trial examining 57 people with type
459 2 diabetes, treatment with metformin was shown to reduce the concentration of
460 methylglyoxal in a dose dependent manner⁷⁴. Methylglyoxal, and the related compound,
461 glyoxal, are both reactive carbonyl agents (alpha-dicarbonyls) which are blocked by the
462 guanidine molecule, (remember that metformin is a guanidine-containing drug). Specifically,
463 the guanidine moiety of metformin combines with methylglyoxal dicarbonyls to form
464 guanidine-dicarbonyl adducts which are then eliminated from the tissues⁷⁵. With reduced
465 amounts of carbonyl groups in the tissues, the likelihood of cross-linking is reduced. This
466 mechanism of action is similar to that of aminoguanidine (below), which, as the name
467 suggests, it is also a guanidine-containing molecule. More recent experiments show
468 metformin to have widespread activities as a cross-link inhibitor. It reduces cross-linking of
469 fibrin proteins which take part in the clotting of blood. Metformin reduces fibrin cross-linking
470 and therefore, ultimately, reduces the risk of thrombosis⁷⁶. In summary, with regards to
471 glycation, metformin has a dual effect. It lowers blood glucose, (a well-known and
472 established activity) plus, as new research is revealing, it is an effective inhibitor of cross-
473 linking through carbonyl trapping.

474 **Aminoguanidine**

475 As with the case of metformin, aminoguanidine is also a guanidine-containing agent, and it
476 therefore acts as a carbonyl trapping agent⁷⁷. Aminoguanidine too works by forming
477 guanidine-dicarbonyl adducts, thereby reducing the numbers of free carbonyl groups. In
478 particular, it is active against certain aldehydes which contribute to cross-linking, (e.g. alpha-

479 oxoaldehyde, and malondialdehyde). Aminoguanidine is active mainly during the early stages
480 of glycosylation. It is an effective inhibitor of cross-linking initiated by glucose molecules, but
481 not as effective in situations involving ribose-related cross-linking. In any case, it prevents
482 collagen cross-linking in tendons and skin ⁷⁸ which shows its potential for prevention of
483 muscle and joint age-related stiffness, and skin ageing (wrinkles). It limits the development
484 of diabetic complications in animals and it has shown promising actions in improving
485 diabetic nephropathy in double blind human trials . In addition, it is a weak copper chelator.
486 Copper chelation is important in AGE induced damage, as high amounts of free copper are
487 more likely to increase AGE-induced injury. Aminoguanidine prevents cardiac enlargement in
488 animal studies by reducing the risk of glycation-induced damage to cardiac collagen. Also, it
489 prevents cross-linking between lipoproteins, (proteins carrying fat molecules) and therefore
490 reduces the risk of blockage of the arteries, particularly the small arteries that feed the nerves
491 ⁷⁹. It is such a strong carbonyl scavenger that it can sometimes result in excessive removal of
492 carbonyl groups (which, in small quantities, are necessary for the normal functioning of the
493 metabolism). Side effects are rare and mild and include nausea or headache. There are two
494 main varieties of aminoguanidine, the hydrochloride and the bicarbonate variety. Although
495 the bicarbonate variety is more commonly available, the hydrochloride version is believed to
496 be the most active (bioavailable) as it is more soluble. Aminoguanidine may be used together
497 with carnosine which is active both in early and late stages of glycosylation, or together with
498 metformin, particularly in diabetics.

499 **Acarbose**

500 Alpha-glicosidases are enzymes which facilitate the breakdown of complex carbohydrates,
501 (such as starch) into smaller sugar molecules which are then absorbed through the intestinal
502 wall. Acarbose blocks this, therefore inhibiting the absorption of certain sugar molecules such
503 as maltose and sucrose, while allowing the absorption of glucose and lactose, which are
504 needed for energy. In this way the overall absorption of carbohydrates is reduced and this
505 lessens the risk of glycation-induced damage and AGE formation. Acarbose's main activities
506 include a reduction of blood lipids (reduced uptake of triglycerides), an aid to weight loss, as
507 well as being an important anti-glycation activity ⁸⁰. Several studies have shown that
508 Acarbose reduces the formation of glycated proteins (including the glycated haemoglobin
509 A1c which is a marker for diabetes). Animal models show an ability of acarbose to slow down
510 the rate of protein glycation and delay renal, brain and eye complications of diabetes ⁸¹. Other

511 studies confirm its effectiveness in protecting against nephropathy, neuropathy and
512 retinopathy in diabetes, by its ability to lower AGE formation⁸². With regard to the kidney-
513 protecting effects of acarbose, it was shown that one possible mechanism could be its ability
514 to protect the glomerular membranes, (where filtering of urine takes place in the kidney)
515 against the effects of cross-linking⁸³. Acarbose is safe but it may have side effects such as
516 abdominal pain and cramps, bloatedness and diarrhea. These are due to excessive amounts of
517 unabsorbed carbohydrates in the bowel. The usual dose is 50 mg to 100 mg daily but the
518 maximum should be kept to 300 mg a day to prevent these side effects. For greater benefits, it
519 may be worth using acarbose together with other cross-link inhibitors such as carnosine. (Ed.-
520 Acarbose is best taken by chewing the tablets, usually just before or during meals).

521 **Tenilsetam** (3-2-thienyl-2-piperazinone)

522 This is manufactured by Cassella, a subsidiary of Aventis, and has traditionally been used as
523 a brain stimulant (nootropic). New research has examined its anti-AGE actions and its
524 significant glycosylation-inhibiting benefits. It works like most cross-link blockers, namely
525 by carbonyl trapping. In addition, Tenilsetam has antioxidant activities and copper chelating
526 properties⁸⁴. A double blind, placebo-controlled trial performed over a decade ago showed
527 that Tenilsetam increases brain performance, (increased rate of information processing,
528 improved cognition and memory)⁸⁵. Re-evaluation of these results shows that the
529 effectiveness of Tenilsetam may be due to a reduction of AGEs in the brain. Particularly, it
530 blocks the reactive sites on glycated proteins and does not allow these to be cross-linked.
531 With a low rate of AGE formation in the brain, the damage caused by inflammation is
532 reduced and brain activities improve⁸⁶. More recent experiments show that Tenilsetam
533 reduces AGEs in diabetic rats, reduces amyloid aggregates (amyloid is the result of brain
534 protein cross-linking), prevents oxidation injury to the brain and has an overall anti-dementia
535 effect⁸⁷. Due to its brain protective effects it may be used by diabetics who are concerned
536 about age-related dementia or those who want to improve brain function plus cover them
537 against cross-linking.

538

539 **Pyridoxamine**

540 All of these are naturally occurring. Pyridoxamine (brand name Pyridorin(, made by
541 BioStratum) is found in animal sources, whereas pyridoxine is also found in plant sources.
542 All three variants have a certain degree of anti-cross-linking actions, but pyridoxamine is the
543 strongest and most significant. Trials are in progress to evaluate the product's safety and
544 efficacy in preventing diabetic complications. Pyridoxamine prevents the formation of AGEs
545 by 25-50% and ameliorates diabetes-related kidney dysfunction, (it improves albuminuria,
546 plasma creatinine and hyperlipidemia). It works by trapping reactive carbonyl groups⁸⁸ and
547 exhibits free radical scavenging properties ⁸⁹. It is most effective in the later stages of
548 glycosylation and therefore, for full protection, it may be used together with aminoguanidine
549 which is active in the early stages of glycosylation. In fact, comparison studies with
550 aminoguanidine suggest that, although both are effective against AGEs, pyridoxamine may
551 be a more versatile agent to use against glycosylation, in order to avoid the low risk of
552 potential toxicity problems with aminoguanidine mentioned above⁹⁰.Pyridoxamine does not
553 affect the levels of blood glucose. It inhibits both methylglyoxal and glycoaldehydes which
554 are most active following lipid peroxidation. It forms methylglyoxal-pyridoxamine dimers
555 which are inactive and eliminated easily ⁹¹.There have been reports of neurotoxicity from
556 using very high doses of pyridoxine, but the use of pyridoxamine is thought to be free from
557 these side effects. The reason is that pyridoxamine needs to be phosphorylated (i.e. it needs
558 the addition of phosphate on the main molecule) before it can become active.

559 **OPB-9195**(2-isopropylidenehydrazono-4-oxo-thiazolidin-5-ylacetanilide)

560 A relatively new compound, first described in 1997, this carbonyl-trapping agent is a
561 synthetic thiazolium derivative which inhibits cross-linking and improves kidney function. It
562 is made by a Japanese company, Otsuka Pharmaceuticals Ltd. It works by blocking carbonyl
563 groups, reducing the overall rate of AGE formation and, in addition, it reduces lipoxidation
564 end-products such as malondialdehyde (MDA) ⁹².It was studied in relation to diabetic
565 complications in rats. It reduced AGEs, restored nerve conduction velocity, limited free
566 radical formation and reduced the rate of DNA damage ⁹³. OPB-9195 modulates the
567 production of toxic cytokines (TNF alpha and interleukin 6), and increases the rate of
568 elimination of abnormal proteins ⁹⁴. OPB-9195 protects against vascular tissue damage and
569 prevents intimal (internal arterial) thickening ⁹⁵.Other experiments showed it to be active in
570 protecting against diabetic nephropathy in rats, through an AGE inhibiting action. It does not

571 reduce blood glucose levels, and therefore it may need to be taken with metformin or
572 acarbose when it becomes available.

573 Other potential cross-link inhibitors are:

- 574 • Pentoxifylline (brand name Trendal^(®)) which is normally used to improve circulation
575 to the extremities .
- 576 • Pioglitazone , This is used in diabetes, to sensitise the cells to the actions of insulin,
577 and it is best used together with Metformin. It has weak activity during early
578 glycation but it becomes more active in the end stages⁹⁶.
- 579 • Kinetin (furfuriladenine) brand name Kinerase(. In a study, kinetin inhibited carbonyl
580 activity and reduced AGEs by up to 68% ⁹⁷.

581 **Cross-link Breakers**

582 The most important cross-link breaker is the drug ALT-711, an orally active compound. This
583 is a thiazolium product (dimethyl-3-phenacyl-thiazolium chloride) manufactured by the
584 Alteon Corporation in the US. A related compound is PTB (dimethyl-Phenacyl-Thiazolium
585 Bromide), which has actions similar to the chloride variety. ALT-711 is not an enzyme as
586 such, but it has enzymatic properties. It has been shown to actually break the covalent bonds
587 between cross-linked proteins and free the proteins which are then able to function again
588 normally. Particularly, ALT-711 breaks the bonds between $-O=C - C=O-$, (the first $-O=C$
589 group belonging to one protein and the second $C=O-$ belonging to another). When the bond
590 between C-C is broken, the first protein has a $-COOH$ group and the second protein has a
591 CHO group. Although, in theory, the bonds may then re-form, (because the carbonyl group is
592 still active on the freed protein), ALT-711 has benefits which persist after the drug is stopped
593 (Alteon Corporation, personal communication). In other words, if the proteins are cross-
594 linked again, ALT-711 will divide them once more, and if they are then rebound, it will keep
595 on separating them. For this reason, it may be necessary to use a combination of the cross-
596 link inhibitor carnosine together with ALT-711 for full protection against cross-linking. In
597 that situation, when the C-C bond is broken, carnosine will immediately bind to the carbonyl
598 group (i.e. it will 'carnosinate' the protein) and therefore cross-linking of that particular
599 protein will not take place for the second time. The ALT-711 molecule will then be free to
600 seek out other cross-linked proteins to work on. ALT-711 can reverse aortic stiffening in
601 rodents, canines and primates. A 40% reduction on age-related left ventricular stiffness (in

602 dogs) was reported after just one month of treatment ⁹⁸. Other experiments support its
603 effectiveness against hypertension, cardiovascular stiffness and heart failure ⁹⁹.It has also
604 been studied in a number of human clinical trials. It was found to be effective in reversing
605 some of the complications of diabetes, improving myocardial and arterial stiffness, heart
606 failure, and reducing blood pressure. In July 2001 Alteon has started the placebo-controlled
607 SAPPHIRE (Systolic And Pulse Pressure Haemodynamic Improvement Restoring Elasticity)
608 phase Iib clinical trial for systolic hypertension. It includes 450 patients aged over 50 years,
609 and it involves 40 centres throughout the United States. The results are expected during 2003.
610 A second, phase Iib SILVER (Systolic hypertension Interaction with Left Ventricular
611 Remodelling) trial is a companion to the first and has enrolled 180 patients with left
612 ventricular hypertrophy¹⁰⁰.

613 Preliminary reports are optimistic, showing that ALT711 is effective at reducing clinical
614 symptoms, (statistically significant reduction of blood pressure and an increase in large artery
615 compliance, achieved after an eight week treatment period). The drug was well tolerated and
616 few side effects were reported. Other trials are in progress aiming to study ALT711 in
617 relation to diabetes and skin ageing. Far from being unique, ALT711 is in a group of 375
618 other cross-link breakers developed by Alteon in near future.

619 **Conclusion**

620 Increased non enzymatic protein glycation, formation of AGEs and their accumulation in
621 tissue and serum have an important role in the pathogenesis diabetic complication. Long
622 lived extra cellular matrix (E.C.M.) proteins have highlighted importance of intra cellular
623 glycation. The diabetic complication can be reduced by reducing glycation synthesis,
624 crosslink formation and tissue accumulation of AGEs or by blocking AGEs receptors
625 blocker.The best cross-link inhibitors currently available are carnosine, aminoguanidine,
626 metformin and acarbose, whereas others are now becoming available. No cross-link breakers
627 are commercially obtainable as yet, but these will be marketed within 2-3 years. Soon after,
628 combinations of inhibitors and breakers are due to follow.

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