Glycation and Skin Aging

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M.A. Farage et al. (eds.), *Textbook of Aging Skin*,
DOI 10.1007/978-3-662-47398-6_128

Abstract

Our skin, just like our whole body is submitted to aging. Important changes occur: skin gets dryer, thinner, age spots appear. It becomes less elastic and more rigid, fine lines and wrinkles appear, and complexion changes. Skin aging is characterized by all these visible signs, which depend on many factors. One of them has been studied for many years and is known to be one of the mechanisms involved in body aging: the glycation reaction.

One of the causes of skin aging is the appearance of AGEs (advanced glycosylation end roducts). AGEs cause biomecanics properties alterations and biological changes involving activation of synthesis of molecules (macromolecules of the extracellular matrix, cytokines) and the activation of the matrix metalloproteinases or MMPs (matrix-degrading enzymes). The effect of UV on some AGEs (e.g., pentosidine) generates reactive oxygen species (ROS) in the matrix with induced additional deleterious effects. AGEs can be formed intracellularly also and consequently change the biological homeostasis of the cell. Taken together, these modifications induced by AGEs stress the importance of glycation in skin aging.

Introduction

Our skin, just like our whole body, is submitted to aging. Important changes occur: skin gets dryer, thinner, age spots appear. It becomes less elastic and more rigid, fine lines and wrinkles appear, and complexion changes. Skin aging is characterized by all these visible signs, which depend on many factors. One of them has been studied for many years and is known to be one of the mechanisms involved in body aging: the glycation reaction. Indeed, the glycation reaction leads to products called AGEs (advanced glycosylation end products) known to form crosslinks and to accumulate in tissues. The nonenzymatic glycation of proteins is a common factor in the pathophysiology related to aging disorders and diseases such as diabetes mellitus (DM). In elderly subjects, the nonenzymatic glycation is high, not only because of possible hyperglycemia but also due to long-term exposure to normoglycemic conditions. The glycation of proteins has been described at a cutaneous level [1] and in organs such as the kidney, blood vessels, and lens [2].

The Glycation Reaction

This reaction is also known as the Maillard reaction described in the early 1900s by Louis Camille Maillard. Maillard discovered that amino acids heated in the presence of reducing sugars developed a yellow-brown coloration [3].

The reaction of glycation is a nonenzymatic reaction between sugar and free amine function of amino acids (lysine, arginine) in proteins. This reaction occurs not only in the skin. Indeed, AGEs (advanced glycation end products) are also found in the kidney, lens, vessels, etc. This reaction takes place in proteins with long half-life and/or low renewal.

In 1981, Monnier and Cerami connect the browning reaction in nonenzymatic glycosylation of proteins (for the reaction occurring between glucose and the amino groups of proteins without intervention of enzyme) with glycation in aging of the lens, collagen, and more generally of the extracellular matrix [4].

There are several factors that can modulate the accumulation of AGEs: renewal of proteins, concentration and type of molecule, availability and reactivity of amino acids on protein to initiate the reaction, degradation of AGEs, and their removal by the body [5].

Mechanism of the Glycation Reaction

The aldehydic group of the reducing sugar such as glucose reacts with a free amino group of amino acid (lysine, arginine) proteins. This reaction leads to unstable Schiff base which turns into Amadori product (which will undergo rearrangements and fragmentation) to eventually

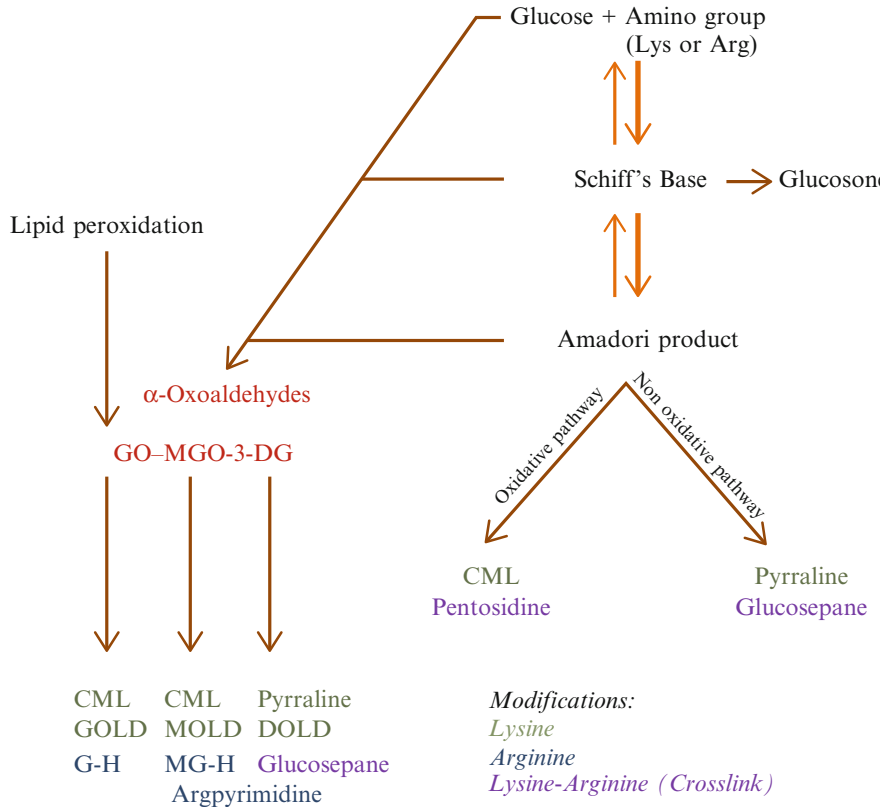

Fig. 1 **Schematic representation of glycation reaction**. *CML* carboxymethyl-lysine, *CEL* carboxyethyl-lysine, *GOLD* glyoxal-lysine dimer, *MOLD* methylglyoxal- lysine dimer, *DOLD* 3-deoxyglucosone-lysine dimer, *G-H* G-hyydroimidazolone, *MG-H* MG-hydroimidazolone, *GO* glyoxal, *MGO* methylglyoxal, *3-DG* 3-deoxyglucosone (Adapted from Singh [28, 29])

produce advanced glycosylation end products or AGEs.

There are different metabolic pathways that can lead to the appearance of AGEs. Briefly, there are three steps in the glycation reaction: an early stage which consists in the formation of a Schiff base, an intermediate step in which are found intermediate molecules known as "propagators," and at the end the formation of AGEs (Fig. 1).

Many AGEs have been identified to date. Structures are varied, forming linear chains or cyclic structures on the surface of proteins or even crosslinks between two protein chains (Fig. 2). The same AGE and intermediate product can be produced from various metabolites. The intermediate products such as glyoxal, methylglyoxal, and 3-deoxyglucosone (3 DG) are known as the dicarbonyl compounds or oxoaldehydes [6–8]. Methylglyoxal (MGO) can be generated by glycolysis, oxidation of threonine, ketone groups [9] or ascorbic acid [10]. 3-DG is formed by a nonoxidative rearrangement and hydrolysis of the product of Amadori [8] and by the fructose-3-phosphate, which is a metabolite of the polyols pathway [11]. CML appears from the oxidation of the Amadori product (fructose-lysine) catalyzed by transition metals or peroxynitrite, ascorbic acid, lipid peroxidation, and different pathways involving the formation of glyoxal and glycoaldehyde [12]. The pentosidine is formed with pentose, ascorbic acid, or by oxidation and fragmentation of the fructose-lysine [13, 14]. The nature of the

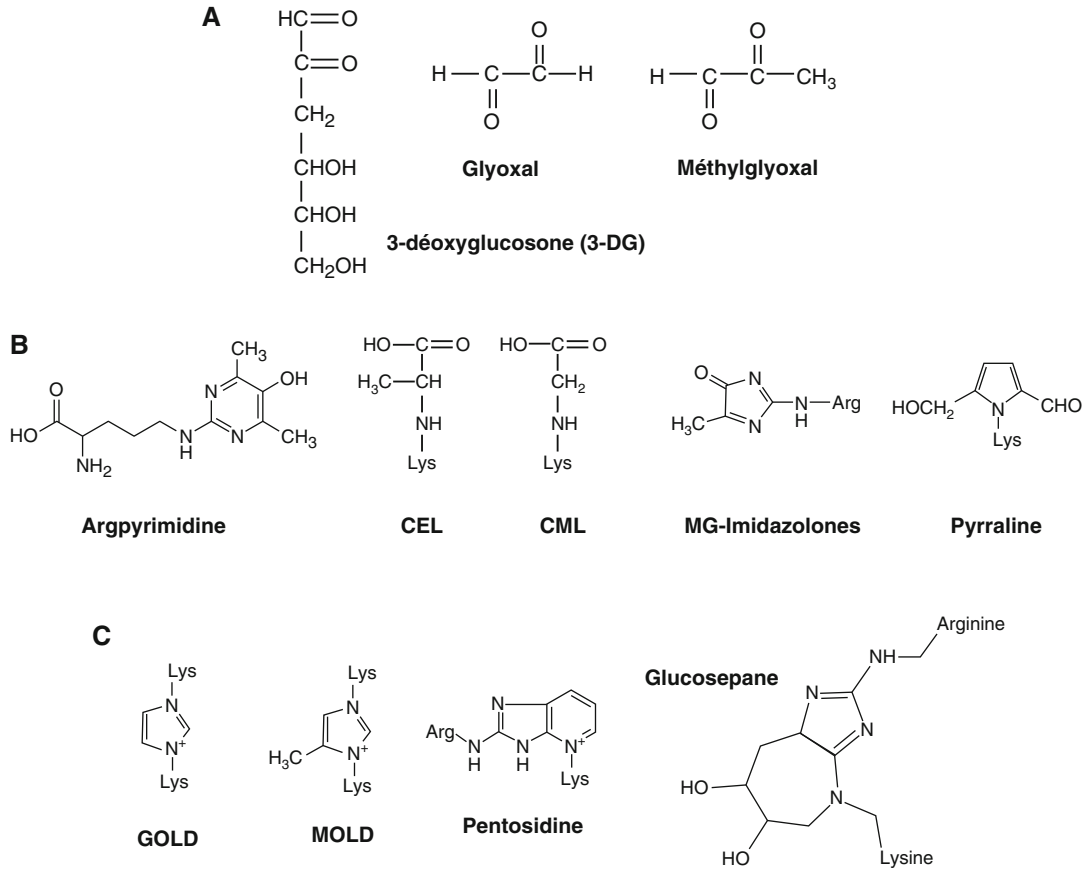

Fig. 2 **Chemical structure of α-oxoaldehydes and AGEs identified in skin**. (**a**) Carbonyls group or α-oxoaldehydes which lead to AGEs. (**b**) AGEs without crosslinks. (**c**) AGEs with crosslinks

metabolite and the oxidative environment is an important parameter to be considered in the development of the glycation end products. The formation of AGEs can be catalyzed by metals of transitions [15].

In addition, it should be noted that compounds such as methylglyoxal or deoxyglucosone derivatives in interaction with lysine can produce the allysine which in the presence of hydrogen peroxide (H_2O_2) leads to 2-aminoadipic acid stable by oxidation [16] and the decarbamylation of arginine product form ornithine which accumulates with age [17]. Ornithine is the deguanidinylation product of arginine resulting from the reaction with oxoaldehydes including methylglyoxal, glyoxal, and also the glucosepane. Ornithine can be produced from different AGEs involving arginine [18].

Glycation Modulation by Oxidative Stress

The majority of the steps leading to glycation end products is accompanied by an oxidative stress (except apparently the pathway leading to glucosepane [19]) and often referred to as glycoxidation [20]. The glycation reaction generates oxygen radicals in the initial, intermediate, and advanced steps. The Amadori product and glycated proteins can react with oxygen to form superoxide ion [21] or hydrogen peroxide [22]. Namiki described unstable imine formed in the initial steps of the reaction likely to be oxidized and to lead to the appearance of oxoaldehydes [23] such as glyoxal, methylglyoxal, and 3-deoxyglucosone. Oxidation of glucose (catalyzed by transition

metals) can generate hydrogen peroxide and cetoaldehydes [24].

Glycation pathways lead to AGE compounds some of which are common to those derived from lipid peroxidation and especially polyunsaturated fatty acids (named ALEs for advanced lipoxydation products) [25, 26]. CML may serve as a biomarker of general oxidative stress resulting from both carbohydrate and lipid oxidation reactions [27].

The RAGE Receptor (Advanced Glycation End-Products Receptor)

AGEs have the ability to bind to specific membrane proteins [28]. There are several types of receptors for AGEs: AGE receptor 1 or AGE-R1 (protein OST-48, complex oligosaccharyl transferase). AGE-R1 is able to bind AGEs, remove oxidative stress [30] and the induced inflammatory response [31]. Copurified with AGE-R1, AGE-R2 (protein 80 K - H, membrane substrate of protein kinase) has been described as a protein involved in intracellular signaling of multiple receptors [32]. AGE-R2 was found associated with another protein that can bind AGEs: AGE-R3 (Galectin 3). AGE-R3 performs different functions including the internalization and degradation of AGEs [33]. Recently, an inverse correlation between Galectin 3 and AGEs localization in human skin was described, suggesting a protection against accumulation of AGEs in wound healing [34]. AGE-R3 is colocalized with AGE-R1 and AGE-R2 and are overexpressed in contact with AGEs [35]. Macrophage scavenger receptor (MSR) enables macrophagic cells to internalize and degrade the AGEs. The MSR are of two types: MSR-AII (macrophage scavenger receptor class A type II) [36] and MSR-BI (class B scavenger receptor type I), or CD36 [37].

However, the well-known receptor and probably the most studied is RAGE, the receptor for AGEs (Fig. 3). RAGE is a member of the immunoglobulin superfamily. The extracellular domain of RAGE is composed by one variable part (V) and two constant parts (C). The RAGE is a multiligand receptor: CML, AGE peptides, AOPPs (advanced oxidation protein products), HMGB1, S100A12/B/A6, amyloid β products [38]. The cytoplasmic domain of RAGE is linked to the extracellular domain by a simple membrane domain. The intracellular domain is short (< amino acids 50) and highly charged. This cytoplasmic domain binds to diaphanous-1 (mDia-1 or mammalian diaphanous-1), a binding which is required for cell activation after binding AGEs-RAGE [38] (Fig. 3a). The extracellular domain of the RAGE can be cleaved via the action of ADAM10 (A Disintegrine And Metallopeptidase 10) and releases the soluble receptor (sRAGE). A second soluble receptor, esRAGE (endogenous secretory RAGE), can be released resulting from alternative splicing of mRNA coding for the RAGE (Fig. 3b). sRAGE and esRAGE are supposed to act as a decoy receptor for AGEs (competitive binding of AGEs) and could facilitate their elimination. sRAGE would decrease the binding between AGEs and the cell surface, therefore preventing the activation of the cell. The binding AGEs-RAGE leads to a loop of activation, in which inflammatory stimuli activate NFκB, which induces the expression of RAGE, followed again by NFκB activation. NFκB stimulates multiple cell signaling pathways that lead to increased production of many growth factors and cytokines, influence cell growing, gene expression, inflammation, and extracellular matrix synthesis [38–40] (Fig. 3c). RAGE activation induces also oxidative stress by activating NADPH-oxidase, decreasing SOD, catalase activity, and also GSH (intracellular antioxidative systems) which by consequence reduces Glo1 activity [41–42]. The stimulation of esRAGE, the increasing of sRAGE, and blocking of mDia-1 link could be opportunities to inhibit the response of the cell to AGEs. Recently it has been shown that high levels of sRAGE were correlated with longevity of humans [43].

Glycation in Skin

One of the causes of skin aging is the appearance of AGEs (advanced glycosylation end products). AGEs cause biological changes involving activation of synthesis of molecules (macromolecules of

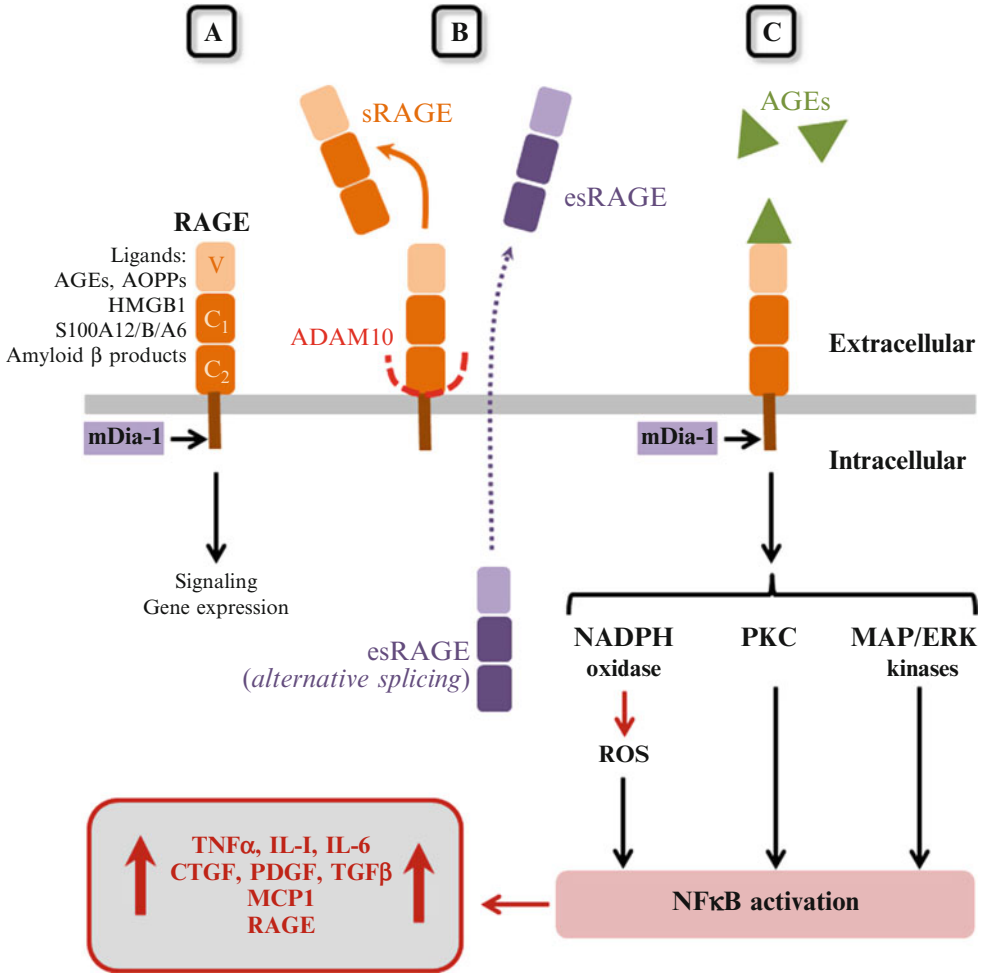

Fig. 3 Schematic representation of Advanced Glycation End Products Receptor: RAGE. Modulation, signaling pathways, and consequences. (**a**) RAGE structure. (**b**) Soluble RAGE. (**c**)Signaling pathways and consequences (Adapted from Yan [38] and Barlovic [40])

the extracellular matrix, cytokines) and the activation of the matrix metalloproteinases or MMPs (matrix-degrading enzymes). The effect of UV on some AGEs (e.g., pentosidine) generates reactive oxygen species (ROS) in the matrix with induced additional deleterious effects. AGEs can be formed intracellularly also and consequently change the biological homeostasis of the cell.

Accumulation of AGEs in Skin

AGEs are known to accumulate in human skin during chronological aging [44]. This accumulation of AGEs in tissues may also be dependent of the protein turnover. Thus in human skin, the fact that collagen has a half-life of 15 years makes it a potential target for the reaction of glycation and accordingly for the accumulation of AGEs [45]. Verzijl has shown using HPLC analysis that the appearance of AGEs in skin seems to be linear during chronological aging. This observation has been confirmed using AGEs immunostaining [46] and by autofluorescence measurement of skin using AGE-Reader which is correlated with the amount of pentosidine [47].

Previously, Sell showed that the accumulation of AGEs (pentosidine) in the skin was inversely proportional to longevity of the species, suggesting that the alteration of the processes controlling the speed of the collagen glycoxidation may be under genetic control, and within the same species according to the considered tissue (and renewal) the rate of accumulation of pentosidine is different [48]. In the skin, the quantities of CML, CEL, or pentosidine are increased by a factor of 3–4 between 20 and 80 years [45]. Glycation collagen accumulates at the rate of 3.7 % annually [49].

However, if the accumulation is linear, structures and levels may be different. All the structures identified as AGEs do not coexist in human skin. For example, the identification of the AGEs in human skin of subject aged 80 years allowed to highlight various structures (linear or crosslinking forming bonds between protein) [50]. The most important AGEs in concentration in skin (from the most highly concentrated to the least concentrated) are: glucosepane, fructosyl-lysine, CML, pentosidine, and CEL. If some AGEs are in significant quantities like glucosepane (on average 1000 pmol/mg protein), others on the contrary are in low concentrations, less than 10 pmol/mg protein (e.g., GOLD = glyoxal-lysine dimer).

If AGEs have been strongly evidenced in the dermis, they were recently observed in epidermis of human skin [51]. In addition to the chronobiological accumulation of AGEs, solar irradiation accelerates their formation [46, 52] (see section "Modulation of glycation by UV light").

AGES Alter Physical Parameters of Skin

Glycation is known to change the organization of collagen fibers and to induce expansion of the molecular packing of collagen [53]. The presence of AGEs in skin changes the mechanical properties thereof in part through the formation of crosslinks. It has been shown that mechanical parameters, using the multiaxial test mode, were altered in elderly diabetic (noninsulin-dependent diabetes mellitus) subjects compared to nondiabetic subjects of the same age (>74-year-old age group) [54]. This modification of the mechanical parameters is equivalent to that obtained in vitro by incubation for 4 weeks of normal human skin with 0.5 M glucose-6-phosphate [54]. The intermolecular crosslinks of adjacent collagen fibers changes its biomechanical properties [55]. More recently, Wilson et al. described that age-related intermolecular and intramolecular collagen crosslinks interfere with fibrillogenesis, change collagen monomer structure and macroscopic properties. Indeed, these modifications influence the ability of the cells to contract and remodel the collagen constructs [56]. This result was obtained with collagen from rat tail tendon; however, the process with human skin collagen should be similar. In 2008, Corstjens has suggested that the accumulation of AGEs in human skin of elderly subjects and/or overweight could contribute to loss of elasticity [49]. In dermal equivalents containing collagen modified by glycation and fibroblasts used in reconstructed skin models, the properties of contraction are also altered. Dermal equivalents containing collagen modified by glycation show a reduction of contraction as compared to control without glycation [57, 58]. In addition, intracellular glycation has also been shown to reduce collagen gel contraction [59]. In diabetic subjects of type 2, with an increase in the concentrations of fructose-lysine and pentosidine, the plantar skin shows an increase of its thickness and its elastic property is reduced compared to nondiabetic control group [60].

The formations of AGEs on chains of collagen change the global charge. In consequence, the contact with cells and proteins is altered and affect the structure reactivity [61].

Yoshinaga et al. show by optical microscopy that aggregates of CML-modified α-elastin are larger than the unmodified α-elastin. Comparison of the elastic modulus and rupture elongation between unmodified and CML-modified elastic fiber sheets reveals decreased elastic modulus and rupture elongation of the glycated sheets [62].

AGEs could also increase the yellowish change in the skin. In acellular dermis model only a slight yellowish change was produced by the treatment with 200 mM ribose or 10 mM glyoxal. [63].

If changes in the mechanical properties are essentially observed for the dermis or its equivalent, there are also changes in the epidermis. Indeed, the presence of AGEs has been reported in epidermis. Pentosidine identified in the stratum corneum modifies the viscoelasticity properties and could be implicated in the ulceration pathology [60]. Glycated stratum corneum and epidermis-dermis differentially regulate the permeability of hydrophilic molecules [64].

The mechanical changes of skin induced by AGEs thus participate in alterations in elastic properties of skin observed during aging.

Effects of AGEs on Skin Cell Viability

The CML-collagen injection into mouse skin (scalp site) triggers a process of apoptosis of fibroblasts at the site of injection [65]. The same authors also obtained this result with human dermal fibroblasts cultured in the presence of CML-collagen. A time course experiment determined that CML-induced apoptosis was not detected before 6 hours and increased after this time. In addition CML-collagen induced a dose dependent increase in fibroblast apoptosis mediated by RAGE. The CML-collagen-induced apoptosis is highly dependent on the presence of caspases 3, 8, and 9. In addition, after extraction of the fibroblasts cultured in the presence of CML-collagen, the level of mRNA coding for genes involved in apoptosis was altered (P53 gene was upregulated six-fold while Bcl-2 gene was downregulated by two-fold). The proapoptotic FOXO1 transcription factor induced by CML-collagen stimulated the fibroblast apoptosis and reduced by 75 % if FOXO1 is silenced [66]. The use of inhibitors helped to highlight that the CML-collagen-induced apoptosis was dependent on reactive oxygen species (ROS), of nitric oxide (NO), ceramide, p38, and JNK MAP kinase activation, inducing FOXO1 and caspase 3. Similar results involving the effect of the ROS inducing changes of proliferation and cell death have been reported [67]. In the culture medium of cultured fibroblasts in the presence of glyoxal and methylglyoxal, the concentration of hydrogen peroxide increases by a factor of 2 causing a growth arrest without apoptotic process [68]. The involvement of receptor RAGE and growth factor receptors (EGFR, FGFR-1, and FGFR-2) were likely to be involved in apoptosis and also in maintaining the effect after exposure to AGEs [67]. This is correlated with the results of Ravelojaona and colleagues that demonstrate a cytotoxic effect when fibroblasts are cultured in the presence of AGEs. This effect persisted when fibroblasts were transferred into a new medium devoid of glycation end products. The authors suggest that the persistence of the toxicity is maintained by RAGE [69]. However, recent results show that apoptosis is not necessarily due to the presence of RAGE. Indeed, 3-deoxyglucosone (3DG), a highly reactive precursor α-dicarbonyl of AGEs, induces oxidative stress and activation of caspase 3 without the intervention of the RAGE. Apoptosis induced by the 3DG would be via integrin α1β1 [41].

AGEs could provoke cellular senescence. Indeed, as a function of passages, fibroblasts accumulate pentosidine and the number of cells decreases. These results suggest an alteration of antiglycoxidation defense systems of the cell when the passages increase allowing accumulation of pentosidine and altering the properties of fibroblasts [70]. When fibroblasts are submitted to AGEs and perform successive passages, β-galactosidase positive cells increase as compared to fibroblasts which were not in contact with the AGEs. The increase in the number of fibroblasts with a senescent phenotype is a function of the AGE contact time [71]. We showed in our laboratory that the type of AGEs can be important also in the cellular senescence process. Indeed, the exposure of fibroblasts to CML or MG-H1 during one week caused an increase in β-galactosidase positive cells after successive passages. The number of senescent cells increase according to the time spent in culture, as expected, and this increase is higher when the cells are preexposed to AGE products. It seems that the senescent potential effect was higher with MG-H1 as compared with CML (Fig. 4). Incubation of fibroblasts with glyoxal or methylglyoxal also causes this increase in senescence. The

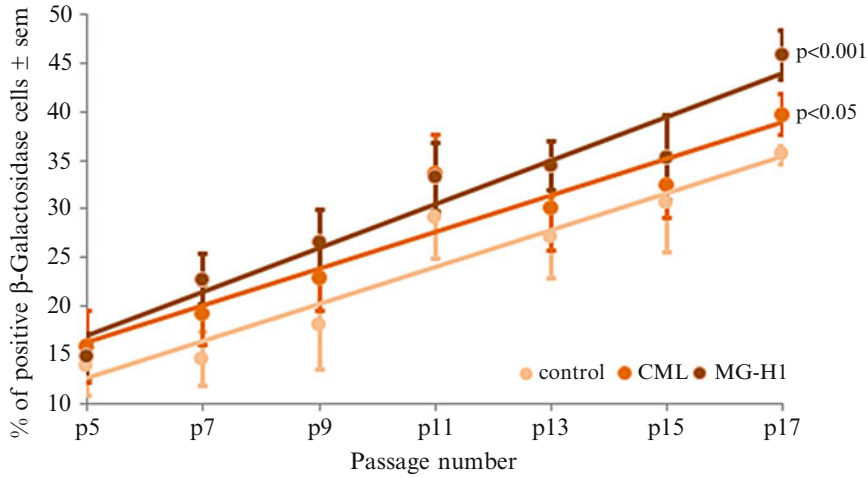

Fig. 4 **Percentage of senescent cells (positive β-galactosidase cells) in function of passage number**. Each point represents the mean of human skin fibroblasts cultures from four donors treated or not with CML (600 μM) or MG-H1 (600 μM) during 1 week before the successive passages

reversion of the phenomenon does not appear before 72 h after replacing fibroblasts in a new culture medium without AGEs [68]. Like fibroblasts, normal human keratinocytes show a decrease of viability in presence of glucose or glyoxal. The proportion of β-galactosidase-positive cells increased significantly in number by 52 % in 100 mM glucose and by 44 % in 100 μM glyoxal-treated keratinocytes and in the same time glycoxidation level of total proteins was 58 and 68 % higher, respectively [72]. The AGEs effect on the keratinocyte viability has also been reported and associated to loss of their migratory and proliferation abilities [73].

Solar irradiation can affect the viability also. The viability of dermal fibroblasts cultured in presence of AGEs and exposed to UVA is reduced [74].

This effect of AGEs on cell viability (senescence and apoptosis) is particularly important because it could contribute to cell loss observed during aging of the skin.

Effect of AGEs on the Synthesis of the Dermal Matrix and Epidermal Cells

The bibliography described essentially the glycation modifications on the collagens and elastin; however, any protein is susceptible to be modified and in consequence to participate to dermal dysfunction [46, 55, 62, 75]. Glycation end products affect the physiology of fibroblasts in terms of mRNA and protein expression. Indeed when fibroblasts are cultured in the presence of AGEs, the synthesis of extracellular matrix proteins is altered. Thus, the synthesis of collagen type I is increased by 28 % and synthesis of hyaluronic acid is reduced by 40–50 %. Modulation of this synthesis is based on the concentration of AGEs in the culture medium [76]. Unlike Okano et al. it has been observed a decrease in the synthesis of type I procollagen. The same authors also observed alteration of type I procollagen mRNA expression in the presence of β2 microglobulin or bovine serum albumin (BSA) as amended by glycation [77]. More recently, other authors have described other changes on the extracellular matrix mRNA expression using microarrays: downregulation of fibronectin, chain α2 of type I collagen, chain α1 of type III collagen, and decorin [78]. If Molinari described a reduction of these mRNAs, however, other authors observed overexpression of mRNA for the chain α2 of type I procollagen and chain α1 of type III procollagen [79]. Using a reconstructed skin system modified by glycation, an increase of

type I procollagen [80], type III procollagen, and type VII collagen [81, 82] synthesis was also observed.

In addition, specific AGEs influence soluble factor releasing like growth factor or proinflammatory molecules [80]. VEGF (vascular endothelial growth factor) was reduced which could lead to the increasing scarcity of vessels reported in skin aging [83] or enhanced MCP1 (monocytes chemoattractant protein type I) known to be involved in matrix protein synthesis [84] or in inflammatory response [85, 86].

Indirectly, glycation in the dermis could modify the biology of epidermis. Indeed, a persistence of β1 integrin subunit was observed in the suprabasal layer of epidermis and an increase of α6 expression in the basal layer mediated by soluble factor synthesis from fibroblasts [58, 81]. These integrin subunits have been reported to be associated with epidermal stem cells [87], or a dedifferentiation process [88] or a hyperproliferative process [89].

Recently CML was detected in human epidermis associated with keratin 10 [51] and probably with other members of the keratin family [90]. Other authors identified previously the presence of glycated proteins in the stratum corneum of diabetic subject [91] and more specifically pentosidine in the stratum corneum of plantar epidermis [60]. As a consequence, AGEs could modify the epidermal physiology like keratinocyte migration [73]: by increasing MMP9 expression [92, 93], by induction of terminal differentiation markers [92], or by reducing the synthesis of antimicrobial peptides like defensin β2 and β3 [94, 95]. These modifications could be involved in wound-healing defect or infection in diabetic subjects.

It has been recently reported that changes in the dermal matrix caused by collagen I glycation also affects the epidermal compartment. Indeed, glycation of collagen induces the synthesis of carboxymethyllysine in both dermal and epidermal compartments. The aging phenotype consisting of poor stratification of epidermal layers and vacuolization of keratinocyte cytoplasm, increasing expression of cell–cell adhesion markers, such as desmoglein and E-cadherin or upregulation of keratin 10 and 14 were observed in glycated skins [96]. Recently, a system of reconstructed skin treated by glyoxal to induce CML showed an alteration of capillary and nerve networks associated with a lack of both loricirin and filaggrin in epidermis reflected an epidermal terminal differentiation defect [97].

If all experiments show that the AGEs alter the expression and synthesis of extracellular matrix molecules, the results are not necessarily the same (either increased or decreased). This can be explained by the different AGEs structures generated in protein solutions modified by glycation. All these extracellular matrix molecules are essential actors in the stability of the dermal matrix and their alteration can change the balance and have a role in aging of the skin.

Effect of AGEs on the Degradation of the Dermal Matrix

AGEs can also modify the expression and synthesis of enzymes which are responsible for ECM degradation. AGEs has been showed to alter the elastase-type matrix metalloproteinase (ET-MMT) activity in human fibroblasts: ET-MMT activity was reduced in a dose-dependent manner (by −27 % and −41 % for 1.25 and 10 mg of AGEs per ml) while no effect was detected on the secretion of MMP1 in the culture medium. Dysfunctions of dermal fibroblasts are induced by AGEs [76]. The modulation of MMPs (matrix metalloproteinases) by AGEs in fibroblasts cultures was observed in another study. Indeed, Molinari et al. have observed an upregulation of mRNA coding for MMP8 and 9 (202 % and 160 %, respectively, as compared to the control) [78]. A decrease of mRNA MMP3 expression in fibroblasts has been observed after contact with CML [98] and also the MMP3 expression in in vitro skin 3D model containing CML in the dermis [80]. Using a 3D system, containing collagen modified by glycation and fibroblasts without keratinocytes, MMP1 synthetized by fibroblasts was decreased but no modification of pro-MMP2. However, MMP2 activation (observed by zymography method)

was strongly inhibited by AGEs without modification of tissue inhibitors of metalloproteinase (TIMP-1 and 2) production [57]. In a full thickness reconstructed skin system, AGEs induced overexpression of MMPs synthesis and activity which could be correlated with a decrease of the thickness dermis probably degraded by these MMPs [81].

Concept of AGE's Biological Specificity in Skin

The AGEs family is characterized by different chemical structure like linear chain or cyclic structure or crosslinks between proteins. AGEs bound to lysine or arginine residues could have opposite effects concerning the expression of biological markers (blocking of Lys or Arg and/or leading to modifications of charge). Indeed, it seems that AGEs-crosslinks (like pentosidine) induce a downregulation of mRNA coding for matrix molecules [80] which could explain in part the volume reduction of dermal molecules which is observed during aging skin like collagen [99], proteoglycans, and glycosaminoglycans [100]. This concept of specific reactivity has been previously notified without structure identification. Indeed, Ohashi et al. described with monocytes a response depending on the BSA-AGE preparation. BSA-AGEs obtained after incubation with D-glyceraldehyde or D-glycoladehyde stimulate the RAGE expression and increase cytokine production while with BSA-AGEs obtained by methylglyoxal or glyoxal no effect are detected [101]. In the same way, fibroblasts cultivated in presence of methylglyoxal induce an upregulation of mRNA Col1A1, Col3A1, TGFβ1, and β1 integrin as opposed to incubation with 3-deoxyglucosone which provoke a downregulation of these mRNA [102]. In addition, Abe et al. demonstrated a different invasive potential with tumoral melanocytes in function of the AGEs preparation type [103]. In addition, our results seem to show a different effect concerning the senescence intensity when human dermal fibroblasts were cultivated in presence of CML or MG-H1 (Fig. 4).

Glycation and the Monocyte Lineage in Skin

The effect of AGEs on monocytes and macrophages has been studied; the most important effects are proliferation, apoptosis, and differentiation. Hou et al. reported that AGEs delayed apoptosis of monocytes and induced monocytic differentiation into macrophage morphology [104]. Also, dendritic cell maturation of monocyte-derived cells by AGEs was reported [105]. AGES could affect the number of monocyte-derived cells ($CD45^+$, CD14+) in the dermis and lead to dendritic cells/macrophages differentiation [106]. Interestingly, Gunin et al. observed a monocyte cell increase in the dermis with aging [107]. AGEs exert a chemotactic effect toward the monocytes [108] and endothelial cells in contact with AGEs released the chemokines MCP-1 – monocyte chemoattractant protein type 1 [109–111]. AGEs stimulate the synthesis of factors or proinflammatory cytokines by monocytes and macrophages [104, 111–114] or increase the extracellular matrix degradation induced by metalloproteinase, e.g., MMP-9 [115, 116]. Both receptors, SRA and RAGE were expressed by $CD14^+$ cells [106]. RAGE was reported to induce the secretion of MMPs [116, 117] and inflammatory factors by monocytes or macrophages [113, 114]. Overexpression of SRA suppressed RAGE-induced MAPK signaling, whereas RAGE activation in macrophages favors a proinflammatory phenotype in absence of SRA [118]. As a consequence, the accumulation of these cells in skin could favor an inflammation process, a loss of dermal matrix balance, and skin homeostasis.

Interaction of AGEs with Cell Membranes

AGEs seem to have an important interaction with cell membranes. After incubation of fibroblasts in the presence of AGEs, a level of AGEs in the cellular lysate associated to liposomes and an increase in membrane fluidity was observed [76]. In addition, the lactate dehydrogenase

(LDH) release from fibroblasts measured in the culture media in the presence of AGEs was found to be increased in a dose-dependent manner without affecting cell viability corresponding to a loss of membrane permeability.

AGEs and Intracellular Activity

If AGEs alter extracellular matrix in skin, also intracellular proteins are modified by AGEs products. Kueper et al. reported that vimentin (intermediate filament) was the major target for CML in human skin fibroblasts. Crosslinked by AGEs, vimentin was redistributed into a perinuclear aggregate. This rearrangement of CML-vimentin was identified as an "aggresome". The consequence was a reduction of contraction properties on collagen gel by fibroblasts. A treatment of fibroblasts by glyoxal exhibited CML modification in vimentin. Like this, the contractile capacity of three-dimensional collagen gel as compared to untreated fibroblasts was decreased [59]. In another study, the same author demonstrated that methylglyoxal induced also the aggregation of vimentin. Vimentin could be modified, not only by CML and CEL but also by pentosidine and pyrraline [119]. The accumulation of modified vimentin is observed in fibroblasts from human facial skin biopsies of aged donors [59]. The skin of the face being exposed to UV, we can hypothesize that these "aggresome" formations could be directly related to the oxidative stress induced by them via the generation of α-dicarbonyl compounds such as glyoxal. Interestingly, Shin et al. reported that expression of CML-vimentin increased in HDMEC (human dermal microvascular endothelial cells) during culture and passage, an effect which was reversed by intense pulsed light treatment [120].

AGEs can also modify other constituents of the cell. Indeed, the enzymatic activity of proteasome (intracellular proteolytic system involved in the removal of altered proteins) can be reduced by glycation after glyoxal treatment on dermal fibroblasts [121]. Also, the proteinase activities of the proteasome decline during aging, probably due to posttranslational modifications of the subunits forming the proteasome complex. An age-related increase in glycated α7 subunit of the proteasome was observed after serial passing of human skin fibroblasts [122]. Glycation of the proteasome has also been reported for keratinocytes. After glucose treatment, proteasome glycation increased by +61 % with a synchronous decrease of its activity (−44 %) [72]. AGE-modified proteins, with a decrease in proteasome activity and content, were found in keratinocytes from old donors [123].

Also alterations of antioxidant (SOD and Catalase) enzyme activities were observed [68, 124] with increased oxidative reactions in the cells.

In addition, HScP 70 (heat shock cognate protein 70) is a target for AGE modification in senescent human dermal fibroblasts [125].

DNA of the cells is also sensitive to glycation. To mimic the cellular carbonyl stress, keratinocytes and fibroblasts from human skin were cultivated in presence of glyoxal or methylglyoxal. Both dicarbonyl compounds caused growth inhibition of cells (concentration dependent) and in addition this treatment provoked CML accumulation in histones (<0.10 mmol CML/mol lysine from untreated and 1.3 mmol CML/mol lysine from glyoxal treated keratinocytes) and DNA strand cleavage. Interestingly, at the molecular level the effects of α-dicarbonyl compounds were different. Indeed, glyoxal caused DNA strand breaks, while methylglyoxal produced extensive DNA-protein crosslinking [126].

Modulation of Glycation by UV Light

It is now well known that ultraviolet radiations and especially UVA have a deleterious effect on dermis and fibroblasts [127]. The dermal extracellular matrix is sensitive to UVA. UVA induces an oxidant stress in the dermis environment which could be related to existing crosslinking on collagen [128]. In vitro, the viability of dermal fibroblasts cultured in the presence of AGEs and exposed to UVA decreases and the rate of lipid

peroxides in fibroblasts and liposomes increases [74]. This loss of viability can be explained by the production of radical oxygen species like superoxide anion radicals ($\cdot O^-_2$) and hydroxyl radicals (·OH) after AGEs irradiation. The hydroxyl radical is derived from the production of hydrogen peroxide after irradiation of AGEs via the Fenton reaction. Hydrogen peroxide (H_2O_2) increases in an AGEs concentration-dependent and UVA dose-dependent manner. Pentosidine-rich compounds exposed to UVA release H_2O_2 [129] and provoke cellular deleterious effects like cell damage leading to LDH accumulation outside the cell. In addition, the enzymatic system able to eliminate H_2O_2 declines with age. The activity of catalase in stratum corneum declines in an age-dependent manner on sun-exposed sites and the creatine kinase activity decreases after in vitro glycation by methylglyoxal [130], and in addition the inactivations of catalase and superoxide dismutase by sugars of different glycating abilities have been described [124].

Pentosidine is established as photosensitizer-AGEs because associated to UVA it leads to the formation of $_1O^2$. Consequently, AGE sensitization can be implicated in photodamage of glycated lens proteins and chronologically aged human skin. Photosensitization of skin cell as photooxidative stress by UVA-irradiation of AGE modified proteins has been demonstrated in cultured human skin fibroblasts and keratinocytes [131]. Due to accumulation of skin AGEs during aging, involvement of AGE photosensitization in skin photooxidative stress may contribute to UVA-induced photoaging and carcinogenesis. Accumulation of AGEs was enhanced with UV preirradiated DED and incubated with sugar [46]. In vivo, CML and pentosidine accumulation in sun-exposed skin especially in the aged group has been described. A vicious circle is envisioned in which the presence of AGEs in a tissue accelerates the formation of additional glycoxidation products following UV exposure [52]. Another study shows that AGE staining was increased in UV-exposed dermis as compared to UV-protected skin [132]. In the dermis of sun-exposed skin, the number and the intensity of CML positive cells in both fibroblasts and endothelial cells was higher compared to sun-protected site and significantly enhanced in older subjects [2]. Interestingly, low dose of UVA associated with the presence of AGEs in skin in vitro could provoke inflammation and matrix degradation by synthesis of IL1α and upregulation of mRNA MMPs [133].

In solar elastosis, a colocalization of elastin and CML has been observed [75]. The oxidation induced via ultraviolet could promote the emergence of CML (a glycoxidation product) at this particular zone. This accumulation of AGEs which correlates with the presence of elastin was also observed by Jeanmaire et al. [46]. The CML-modified elastin is more resistant to degradation by elastase [62]. After irradiation of in vitro skin containing AGEs, upregulations of mRNA coding for tropoelastin, elastase, and MMP12 were observed emphasizing the possible direct implication in the elastosis process [133]. In addition, in monolayer culture of fibroblasts we showed an increase of tropoelastin synthesis after stimulation by MG-H1. No effect was observed after CML stimulation (Fig. 5).

Also CML was detected in human epidermis associated with different members of the keratin family after UVB exposition [90]. AGEs were enhanced in the stratum corneum and in the nuclear of epidermal cells of UV-exposed as compared to UV-protected skin [132]. An age-dependent adaption and protective mechanisms of the epidermis has been suggested against sunlight-associated oxidative stress like CML formation [134].

RAGE in Skin Aging

The distribution pattern of AGEs receptor (RAGE) is modified in epidermis and dermis in function of chronological aging and photo-aging (sun-protected or sun-exposed site). In young skin from breast (sun-protected), RAGE was more expressed in the upper part of the epidermis and dermis as opposed to old donor where RAGE was preferentially expressed in lower parts. In the sun-exposed face (old donor) the distribution of

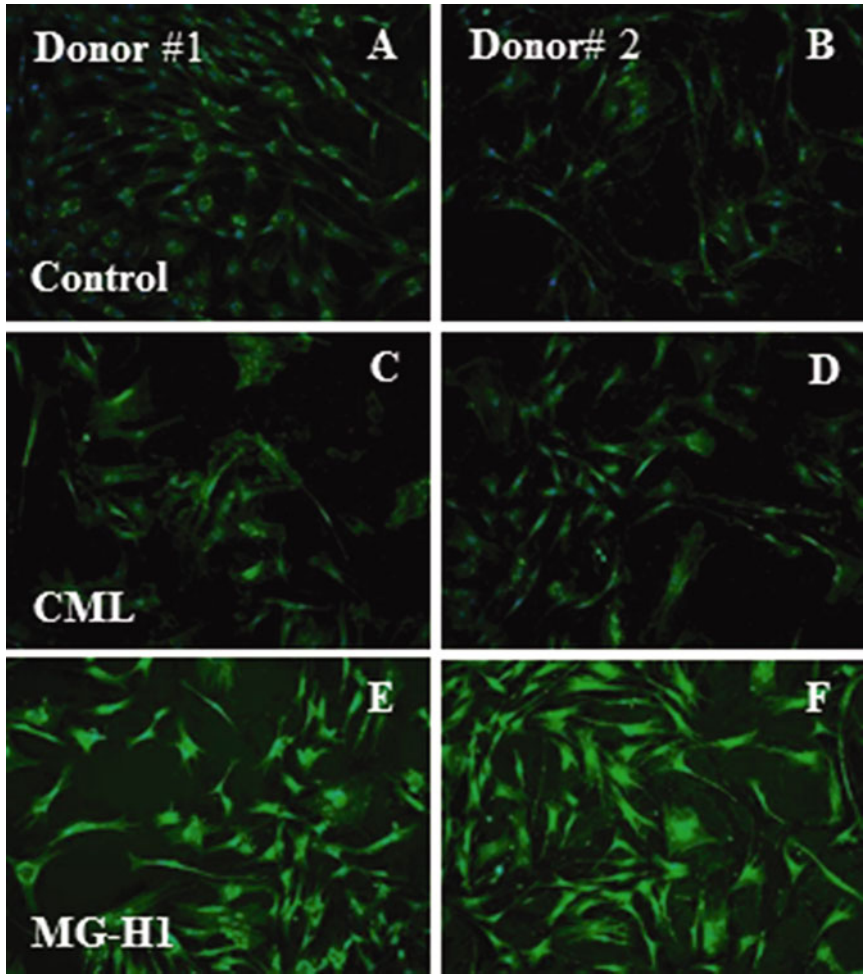

Fig. 5 Stimulation of the tropoelastin synthesis by AGEs. Immunostaining of tropoelastin (**a**–**f**) on human dermal fibroblasts from two different donors – donor#1 (**a**, **c**, **e**) and donor#2 (**b**, **d**, **f**) treated with CML (**c**, **d**) or MG-H1 (**e**, **f**) or without treatment (**a**, **b**). 100× magnification

RAGE was almost similar to old skin from breast except for the upper dermis where RAGE was more expressed which can be attributed to photo-aging. RAGE is highly expressed in skin and upregulated in sun-exposed sites [98]. Interestingly, human foreskin fibroblasts stimulated by CML and tumor necrosis factor-alpha (TNFα) resulted in upregulation of RAGE expression and CML induced profibrogenic markers like connective tissue growth factor (CTGF), transforming growth factor-beta 1 (TGFβ1), and chain α1 of type I procollagen. CML could not be the only AGEs structure responsible for the induction of RAGE. Indeed, Buetler et al. demonstrated that CML do not form the necessary structure to interact with RAGE. This could be explained by the method of CML preparation which would generate other structures which can react with RAGE [135]. Such observation was previously reported by Twigg et al. since CTGF was induced by AGE-BSA stimulated human dermal fibroblasts but not by the RAGE-specific ligand CML-BSA [136]. However, it was interesting to note that a weak expression of RAGE in fibroblasts [137] and absence of induction in the expression of mRNA RAGE in keratinocytes have been reported [92].

It was highlighted that the presence of AGEs could induce the differentiation of normal human

keratinocytes (increase of keratin 10 and involucrin) and increases the expression of MMP9 via CD36 receptor expression (but not other types of receptors). This interaction could contribute to explain the mechanism involved in some pathologies related to diabetes such as perforating dermatosis (biopsies of these patients express strongly the CD36 and MMP9) [92]. A more recent study has also shown the involvement of AGEs in wound-healing defects related to diabetes via an increase in the rate of MMP9 (protein and mRNA) as shown on cultures of keratinocytes [73] mediated by RAGE, ERK1/2, p38MAPK pathways, and also activation of NFκB. In the mouse model, RAGE expression in keratinocytes is involved in acute inflammation and supports the role of RAGE in paracrine communication between keratinocytes and stromal immune cells like monocytes and macrophages [138]. Also RAGE was described to be involved in skin pathologies. Indeed, e.g., RAGE is involved in tumoral pathology like melanoma [103] or promoted the development of immune mediated disorders, like psoriasis, through the regulation of many proinflammatory genes [139]. Recently, the implication of RAGE in squamous cell carcinoma (SCC) has been described. The proliferation and migratory activity of normal keratinocytes and SCC was induced by S100A8/A9 and abolished by blocking RAGE [140].

Particular Case of Melanocyte/Melanoma

AGEs could also influence melanocyte physiology. Indeed, an indirect effect mediated by MCP1 released by fibroblasts in contact with AGEs has been reported. Melanocyte MCP1 receptor could induce tyrosinase expression and increase activity [141]. In addition, upregulation of MCP1 mRNA [110] and MCP1 protein [142] is induced by endothelial cells after contact with AGEs. Previously, it has been described that in parallel with AGEs inhibition, a reduction of tyrosinase activity was observed, suggesting a possible relationship between them [143]. However, in the Japanese population, the AGEs index does not seem to indicate an alteration in the melanin amount [144]. More recently, Leblanc-Noblesse et al. described the correlation between AGEs and solar lentigo. In this study, CML was enhanced in the dermis of solar lentigo as compared to the adjacent photoexposed zone. Autofluorescence measurement (AGE Reader) of the skin was linked to depigmentation [145].

In pathologies such as melanoma (described in the mouse model), overexpression of RAGE might be responsible for the development of tumors and the metastatic ability of cells; the use of anti-RAGE antibodies reduces this effect [103]. This observation has been reported more recently in humans in the case of melanoma where the expression of the RAGE and S100 ligand are strongly increased [146]. Also, binding extracellular S100P to RAGE or coupling the intracellular S100P with ezrin (a cytoskeletal protein) was involved in tumor growth, invasion, and metastasis. The coordinate upregulation of S100P, RAGE, and ezrin may provoke the malignant transformation of melanoma [147].

How to Fight Against AGEs?

Since AGEs are known to have an impact on aging and certain diseases (including diabetes-related), work has been undertaken in order to find ways to reduce the reaction of glycation, accumulation of AGEs, and its effects on tissues. Many dedicated publications [148–150] have detailed various molecules and strategies to protect from glycation. The main possibilities to protect from glycation with some examples are described below:

Prevention

Different types of strategies [149] or molecules [148] already exist to prevent glycation: (i) competition with protein amino groups (for example, aspirin can react with the amino group by acetylation, anti-inflammatory molecules like ibuprofen and diclofenac have a protective effect against glycation by protecting the enzymes from

inactivation via the glycation as catalase for example); (ii) binding to the protein to reduce the accessibility of the amino group, the elimination of the open form of the sugar in the reaction of glycation (amino acids, polyamines, peptides as carnosine which can also react with the protein carbonylated and prevent the formation of crosslinks) and (iii) binding to a reactive intermediate to prevent the appearance of the terminal product (aminoguanidine can react with the product of Amadori thus blocking the following reactions. However, the aminoguanidine can also react directly with the sugar, to eliminate methylglyoxal and other dicarbonyls and to act as a chelator of metals. Another example is the metformin which could act by removing the intermediate reagents).

The use of plant extracts is also a source of glycation inhibitors, most often associated with antioxidant activities of the molecule families contained in extracts like blueberry. Using blueberry extract in reconstructed skin as glycation inhibitor, a return to a normal pattern concerning the biological markers previously modified by the presence of AGEs has been observed [58]. Flavonoids (antioxidants present in vegetal foods) at micromolar concentrations are very potent inhibitors of pentosidine formation in collagens [151]. Other vegetable substances containing the puerarine and chlorogenic acid have also inhibitory activity [152]. Consumable plants are also sources of AGEs inhibition (in vitro) like ginger, cumin, black pepper, green tea [153]. Tea polyphenols like epicatechin and theaflavin can also trap the methylglyoxal and reduce the accumulation of AGEs [154].

Crosslinks Breakers

Thiazolium salts have been studied and the first results suggested they were able to break crosslinks (especially of the di-ketone crosslinks) [155, 156]. If the exact mechanism of action remains disputed [157], experimental results showed a restoration of the flexibility of arteries after administration of thiazolium salts to animals characterized by experimentally induced diabetes [158].

Prevention of the Consequences of Glycation

Modulation of RAGE expression could be a means to reduce the incidence of AGEs [159]. For instance, nifedipine can inhibit overexpression of RAGE by removing the appearance of reactive oxygen species [160].

Intracellular Defense Systems

Several systems of defense against glycation are described in the literature. This role is played by several enzymes: the fructosylamine oxidase (amadoriases), fructosamine 3 kinase (FN3K), and the glyoxalase system.

Fructosylamine Oxidase or Amadoriases: Horiuchi has isolated fructosylamine oxidase (FAO) from *Corynebacterium* sp. [161]. Subsequently various FAO have been isolated and cloned from different microorganisms. It may be noted that two enzymes have been isolated from *Aspergillus* sp. (amadoriase I and amadoriase II). FAO oxidizes the Amadori product and generates H_2O_2. The Amadori product obtained after oxidation breaks down spontaneously through hydrolysis. The result is a free amine, glucosone, and H_2O_2. In higher organisms the FAO has not been identified. FAO can remove AGEs only from products with low molecular weight but is not active on the BSA glycated proteins as example. Two hypotheses can explain this activity: (i) small PM products can be easily placed near the active site of the enzyme and (ii) the charges brought by the protein affect the protein enzyme interaction [162].

Fructosamine 3-Kinase (***FN3K***): Szwergold identified 3-phosphate fructose in the lens of diabetic rats [163]. Then the fructoamine 3-kinase was identified from a lysate of erythrocytes [164]. Fructosamine 3 kinase phosphorylates Amadori products and generates 3-phosphate fructose which breaks down into a residue lysine, a phosphate and 3-deoxyglucosone (3DG). The fructosamine 3 kinase (FN3K) gene is expressed in all tissues. The enzyme was intracellular and ATP dependent; the deglycation of the product of

Amadori in the extracellular matrix is, therefore, not possible. The existence of a fructosamine 3-kinase-related-protein (FN3KRP) has been described in addition to the FN3K with a similar mechanism [165]. It can be assumed that the use of assets protecting these enzymes or their disappearance during the aging process (if this is the case) would be beneficial for the cell.

Glyoxalase: The glyoxalase system present in the cytosol of cells catalyzes the conversion of methylglyoxal in D-lactate via an intermediary S-D-lactoylglutathione [42]. The system consists of two enzymes, glyoxalase I (Glo1) and glyoxalase II (Glo2) and the GSH (glutathione). The main substrate of Glo1 is the methylglyoxal, but glyoxal(the hydroxypyrivaldehyde) and 4, 5-doxovalerate are also potential substrates. Glo1 and the glyoxalase system prevent the α-oxoaldehydes formation inside the cells. Enzymatic defense (glyoxalase) decreases during aging (particularly Glo1). Oxidative stress is closely linked with glycation because GSH depletion in oxidative stress also decreases activity in situ of Glo1 and thus increases the concentrations of glyoxal and methylglyoxal and therefore accumulation of AGEs as well as possible increase of radical oxygen.

In the aging of *C. elegans*, an accumulation of MG-H1 (methylglyoxal hydroimidazolone) in the mitochondria was observed. If Glo1 is stimulated, MG-H1 appearance is prevented and the life of *C. elegans* increases. If Glo1 is silenced the lifespan of *C. elegans* decreases. The decline of Glo1 with aging has been also highlighted in rodents and humans. The Glo1 activity is directly proportional to the concentration of GSH. In lens, the concentration of GSH decreases with age while MG-H1 increases, thus the protective role of GSH in aging is not only related to its antioxidant function but also its role as cofactor in the glyoxalase system. Recent results suggest that hyperglycemia could decrease the expression of Glo1 by increased activity and activation of RAGE [166, 167]. It was reported that the genomic expression of Glo1 is variable; therefore, the significance of the expression of Glo1 could be an important factor to be considered in research on aging.

DJ-1/PARK7

Recently has been reported a new antiglycating enzyme activity. This enzyme is DJ-1/PARK7. The Parkinsonism-associated protein DJ-1/Park7 is described as a multifunctional oxidative stress response protein. DJ-1 is a protein deglycase that repairs methylglyoxal- and glyoxal-glycated amino acids and proteins by acting on early glycation intermediates and releases deglycated proteins and lactate or glycolate, respectively [168].

Diet

It is established that the origin of AGEs in the body is not only endogenous but also exogenous. Glycation reagents (named glycotoxines) are present in aqueous tobacco extract and smoke in a form that can quickly react with proteins to form AGEs and this reaction is inhibited by aminoguanidine [169]. It is also known that food and the way used for cooking can be a source of exogenous AGEs by ingestion. Recently, Uribarri has published a list of foods with AGEs, named dAGEs for dietary AGEs. Heat (cooking mode) increases the formation of new dAGEs by ten- to hundredfold as compared to the raw food. The cooking mode or the use of acidic ingredients (e.g., lemon juice) can reduce the formation of the dAGEs [170].

If the cooking mode affects the appearance of AGEs, caloric restriction can also regulate the concentration of AGEs [171]. Animal caloric restriction studies have shown an increase in life expectancy, a decline in the rate of insulin and glucose [172], or even a reduction of oxidative damage [173]. In addition, some caloric restriction studies have been conducted in humans in whom a decrease of atherosclerosis risk, diabetes, and a reduction of inflammatory processes were observed [174]. Most of these changes can be linked to the reduction of the rate of glycation.

Taken together, these findings suggest that healthy diet associated with selected cooking modes could limit the addition of an exogenous supply of AGEs.

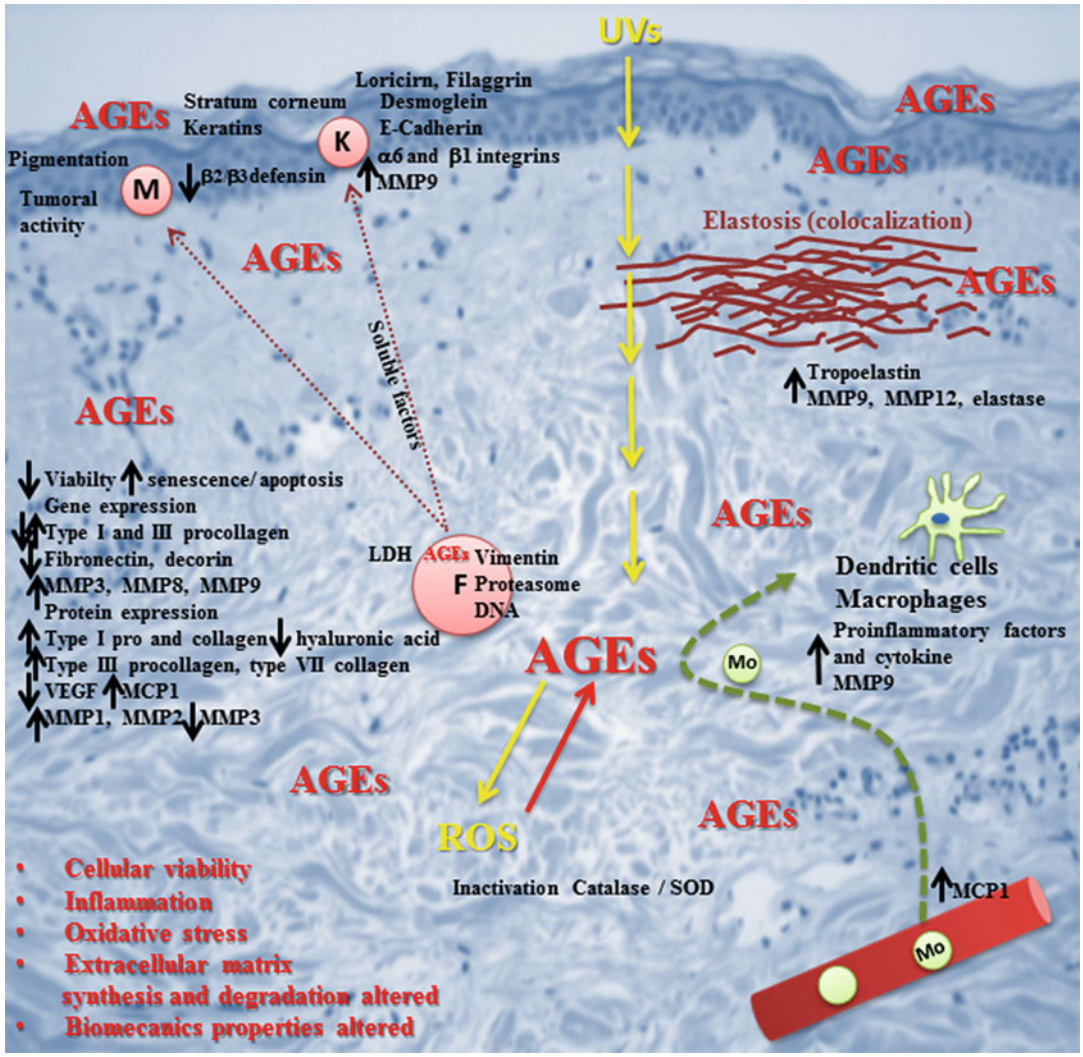

Fig. 6 **Schematic effects of AGEs in the skin**. *F* fibroblasts, *K* feratinocytes, *M* melanocyte, *Mo* monocytes, *LDH* lactate deshydrogenase, *MMP* matrix metalloproteinase, *VEGF* vascular endothelial growth factor, *MCP1* monocyte chemoattractan protein type 1, *ROS* reactive oxygen species

Conclusion

Taken together, these reports brought together in this chapter allow us to stress the importance of glycation in skin aging. Most skin alterations caused by AGEs (Fig. 6) are correlated with the major biochemical changes and signaling pathways involved in the generation of intrinsically and extrinsically aged skin [175]. Skin aging is characterized by the progressive degradation of skin components, the development of an inflammatory environment, and it's self-maintaining due to the progressive accumulation of AGE products.

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