The Role of Hyperglycemia in Skin Wrinkle Formation: Mediation of Advanced Glycation End-Products

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Abstract: Aging is a process of changes that occur within a system over a period of time. It is due to the variation in the continuous activities that maintain life. Changes that occur in aging can be seen in various parts of the body system but most especially in the skin. This study reviews the role and mechanism of Advanced Glycation End products (AGES) in skin wrinkle formation. Literature searches were conducted. Literatures concerning hyperglycemia and skin wrinkles were used focusing on the mechanisms of AGES. This study reveals that AGEs increases the stiffness and reduces the elasticity of the skin increases oxidative stress with resultant destruction of skin fibroblasts by increasing vascular permeability, inhibiting vascular dilatation and enhancing oxidative stress.

Key words: Hyperglycemia, skin wrinkles, aging, oxidative stress, advanced glycation end products, Nigeria

INTRODUCTION

Researches have revealed that free radicals are the main culprits of skin aging. These radicals are component of oxygen and nitrogen from ultraviolent radiation exposure, environmental pollution, smoke, nutrition, oxidation, glycosylation and chronic inflammations (Fu *et al.*, 2004). Aging in skin is characterized by thinning of skin, loss of elasticity and decrease metabolic activity (Forbers *et al.*, 2005) which is observed as winkles, cramping, loss of moisture and support, droopy and other imperfections.

Skin and skin wrinkles: The skin is a connective tissue composed of various components including protein fibers, collagen and elastin and matrix inclusions. It has two layers, the epidermis and dermis. The dermis contains the fibers as its main component which forms specific scaffolding to support other skin structures.

Collagen, a protein molecule that support other skin structures has a triple-helical structure that arises from an unusual abundance of three amino acids, glycine, proline and hydroxyproline (NCBI). Collagen and keratin provide the strength of the skin.

Researches show three primary biochemical reactions that take place in the skin to contribute to the structural changes associated with aging and skin changes. These include the loss of the ability of the skin antioxidant defense system to fight oxidative stress and associated oxygen species (ROS) otherwise known as free radicals,

increase in the destructive Matrix Metalloproteinase (MMP) and associated decrease in collagen synthesis and the formation of Advanced Glycation End-products (AGES) which result in prematurely aged skin (Pinnell, 2003). On the other hand, the normal skin structure is maintained by the physiological metabolic processes which maintain the chemical constituents of the skin cells. As people age, collagen degradation occurs leading to structural changes in the skin usually seen as wrinkles.

A wrinkle is a ridge or crease of a surface. It usually refers to folds on the skin of an organism and these folds are generally random with no repeating pattern. Precedent upon the biochemical reactions that take place in the skin, most especially on the collagen fiber structure, winkles or folding of the skin may be observed. Although, wrinkles, a potent symptom of skin aging could be found all over the body but it was discovered that much more are found in areas where there has been sun exposure. This effect is known as photo-aging. Overtime, wrinkles have been associated majorly with photo-aging processes, oxidation, inflammation and glycosylation within the body cells (Watson *et al.*, 2001). Although, many products and procedures promise to reduce wrinkles, some do little or nothing while others achieve a fair amount of success.

Glycation/glycosylation: Forbers *et al.* (2005) defined glycation as the irreversible attachment of reducing sugars to the free amino groups of proteins. Glycation, also known as glycosylation usually refers to a non-enzymatic glycosylation. Its physiologic roles are thought to include the identification of senescent proteins (AGES)

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(Forbers *et al.*, 2005). Various people define it under divers viewpoints. Ezine defined it as large aggregate of damage proteins that accumulate over time and proceed to damage adjacent proteins in a domino like fashion.

It was also defined as a non-enzymatic medicated reaction that takes place between the amino group in proteins and a sugar such as glucose (the same glucose that provide energy for the cells). This was confirmed by the non-enzymatic Maillard or browning biochemical reaction between reducing sugars and amine residues on proteins (Hodge, 1953; Maillard, 1912).

In addition to this non-enzymatic reaction, glycation may also be caused by other toxic by-products such as Malondialdehyde (MDA) and Methylglyoxal (M), these are involved in lipid peroxidation, the destruction of healthy lipids by free radicals and also glycosylated materials interfere with mitochondrial function which in turn stimulates production of inflammatory chemicals such as Tumor Necrotic Factors (TNF) and the Nuclear Factor kappa-b (NFkb). These inflammatory agents attack other skin cells materials resulting in chronic skin micro-inflammatory which do not only make the skin red, rough and itchy but contribute to wrinkles and skin blemishes (Giacomoni *et al.*, 2000).

Although, it has been known that photo-aging leads to cross-linking of the collagen and elastin in the skin, it has only been in recent years that scientist began to understand more about the process that expresses the highly susceptibility of collagen and elastic proteins to an internal chemical reactions within the body called Glycation. Consequently, muscle weakness, heart disease and brain disease are also associated with glycation.

ADVANCED GLYCATION END-PRODUCTS (AGES)

AGEs are the result of a chain of chemical reactions for an initial glycation. The intermediate products are known as amadori products schiff base and maillard reaction products named after the researchers who first discovered them. Thus glycation products accumulates during aging of many slowly renewing tissues including skin (Hartog *et al.*, 2005).

Types of AGEs: Various AGEs have been discovered to be formed from the reactive intermediates like 3-deoxyglucosone, methylglyoxal and glyoxal under anaerobic glycolytic conditions. Methlyglyoxal (MGO) produces the following AGEs: N-(carboxyethyl) lysine, a homologue of N-carboxyllysine (CML), redox active di-imine cross-links between lysine residues, arginine imidazole aducts, Methylglyoxal Lysine Dimmer (MOLD) (Fu *et al.*, 1994). In addition to MGO-induced AGES,

oxidative reactions also produce the AGEs mention above. Other types of AGEs are pentosidine, Glyoxal Lysine Dimmer (GOLD), N-Carboxymethylysine (CML) (Thornalley *et al.*, 1995, 1999). Research findings stated that during the pathogenesis of diabetes-induced AGEs formation, hyperglycemia results in higher cellular glucose levels in those cells unable to reduce glucose intake (e.g., endothelial cells) (Dominicza, 2003; Brownlee, 2001, 2005). This in turn results in increased levels of reduced Nicotinamide Adenine Dinucleotide (NADH) and reduced Flavin Adenine Dinucleotide (FADH) increasing the proton gradient beyond a particular threshold at which complex III prevents further increase by stopping the electron transport chain (Topol and Califf, 2006).

Finally this results in mitochondrial production of ROS (Reactive Oxygen Species), activating PARP1 and Damaging Deoxyribonucleic Acid (DNA). PARP1 in turn, activates Adenosine Diphosphate (ADP) which ribosylates Glyceraldehyde-3-phosphate Dehydrogenase (GAPDH), a protein involved in glucose metabolism leading to the accumulation of metabolite earlier in the metabolism pathway. These metabolites activate multiple pathogenic mechanisms, one of which includes increased production of AGEs (Topol and Califf, 2006). It appears that the determination of the circulating levels of AGEs *in vivo* may provide a powerful tool to predict progression to complication of diseases in the future (Nathan *et al.*, 2003; Hartog *et al.*, 2005).

Sources of AGEs: Intracellular formation of AGEs from reactive carbonyl intermediates (such as glycolytic intermediate. 3-deoxyglycosone and the sugar phosphates methglyoxal and glyoxal occur at a much faster rate than glucose-derived AGE formation. This appears to be triggered by increased oxidative stress induced in response to intracellular hyperglycemia although glucose appears to be fundamental for the generation of those reactive intermediates. Thus, AGEs are produced in the body as a result of imbalanced metabolic processes.

Glucose-derived AGE formation or exogenous AGE: These are AGEs derived from food components with high sugar content or diet lacking retinoids or antioxidants. According to research, AGEs molecules are found in many regularly consumed foods especially high glycemic foods and are also created in carbohydrate-rich foods when they are cooked (Bai *et al.*, 1992).

It was noted that the consumption of sugar, white bread, bakery products and other AGE rich foods adds to the total level of endogenous AGEs already produced in the body and that modern food processing also results in foods with increased AGE levels. This is also evident in

infant formulas which is high levels of AGEs indicating that people are exposed to AGEs from very early 6 years (Bai *et al.*, 1992). In addition, sugar intake in humans causes extensive cross-linking evidenced by enhanced glycation and AGEs (Kim *et al.*, 2002; Topol and Califf, 2006).

Hodge (1953) reported that diets that lack retinoids are said to enhance the activities of AGEs since its presence leads to the production of new collagen. In addition to diets, curing of tobacco also produces AGEs (Koslchinsky *et al.*, 1997). Therefore, it is concluded that the destructive compounds result in the reaction of carbohydrates with the free amino group of proteins.

Hence, it is now thought that exogenous AGEs acquired from diets and cigarette smoking contributes to the overall AGEs burden particularly in diabetes. Indeed, long-term storage or prolonged treating of food stuffs in the presence of sugars generates a number of biologically reactive AGEs capable of interacting with AGE receptors involved in the inflammatory responses and fibrogenesis (Koslchinsky *et al.*, 1997).

Formation of AGEs: The formation of AGEs is shown in (Fig. 1). It was discovered that when glucose or aldehyde molecules interact with skin protein they may form glycosylated protein which are generally more susceptible to damage caused by free radicals. A glycosylated protein may interact with another glycosylated unit causing cross-linking which is an irreversible union between two molecules. In this way, neither of the molecules is free to perform its ordinary functions and this contributes to the overall damage. An additional problem is that the cross-linking unit may then interact with other oxidized molecules resulting in large and harmful molecules called AGEs (Forbers *et al.*, 2005).

It was described that when sugars combine with proteins during maillard reaction, it produces a schiff base which is then converted into another harmful substance called an amadori product. However, the final products of this reaction are rearranged and transformed to AGEs. These Schiff bases, Amadori product and Maillard reaction products are earlier discovered as intermediate in the glycation pathway.

These AGEs accumulate in many tissues with aging and this is especially true in individual whose limbic-hypothalamic-pituitary axis is down regulated. This is because the body becomes inefficient at regulating a normal blood glucose concentrations and maintaining proper insulin levels by blocking intracellular uptake of both insulin and glucose, paring the way for increased glycation and production of AGE. In addition to the production of AGEs, reactive oxygen species have been reported to be formed during the formation of AGEs thereby causing a self-perpetuating cycle of ROS/AGE formation in disease such as diabetes. The proposed sources of ROS in the maillard reaction are many fold including the auto-oxidation of glucose (Wolff pathway); Schiff basics (Namiki pathway) and Amadori adducts (Hodge pathway) as well as AGEs protein themselves (Maillard, 1912).

Effects of AGEs and glycation: The most visible effects of AGEs and glycation is skin damage. It was noted that the accumulation of AGEs in collagen of the skin and the resulting structural alterations result in impaired tissues properties (increased stiffness and reduced elasticity). Thus, AGEs perpetuate the damage to the skin collagen, elastin and intracellular matrix and increase the death rate of the fibroblasts through apoptosis (Forbers *et al.*, 2005).

Dietary AGEs have been shown to initiate a number of destructive effects. They increase free radical production and deplete levels of glutathione, a critical antioxidant (Bai *et al.*, 1992). Increased consumption of dietary AGEs can alter low density lipoprotein, LDL in a way that increases its negative effects (Yan *et al.*, 2007). Studies showed that subjects consuming high AGEs diet has significantly increased vascular cell adhension molecule-1, a molecule that encourage blood cells to stick to arterial walls and block the arteries. This provides evidence to support the role of AGEs in diabetic-induced heart disease. Studies revealed that exposure to dietary AGEs increases LDL-induced vascularized toxicity and this can be prevented by dietary AGE restriction (Martin *et al.*, 2003). The importance of exogenous AGEs is especially apparent in the presence of impaired clearance of AGEs seen in patients with renal disease. There is now evidence to suggest that exposure to high levels of exogenous AGEs may directly contribute to the development of albuminuria and atherosclerosis in other normal animals (Valssara, 2001). Therefore, the total state
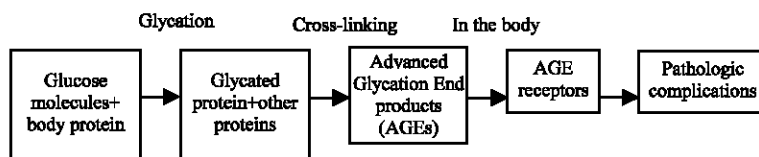

Fig. 1: Formation of AGEs from glycation

of oxidation and peroxidative stress on the healthy body and the accumulation of AGE-related damage is proportional to dietary intake of exogeneous (preformed) AGEs, the consumption of sugars with a propensity towards glycation such as fructose and galactose. (Koslchinsky *et al.*, 1997; Yan *et al.*, 2007).

This could be a premise upon which it is concluded that AGEs formed after the consumption and impaired metabolism of glucose and fructose are known to have a destructive effect on skin fibroblasts. In addition to these, AGEs also encourages the development of insulin resistance and elevated blood glucose transforming LDL-cholesterol into harmful LDL type B subparticle which are destructive to the cardiovascular system. It also appears that AGEs are capable of individually contributing to diabetic complications thus it is evident that advance glycation is clearly one pathway by which injury may be included in diabetes (Koslchinsky *et al.*, 1997), thus confirming that AGEs play a significant role in a majority of the chronic, degenerative diseases associated with aging.

Mechanism of action of AGEs: AGEs affect nearly all type of cells and molecules in the body and are thought to be major factors in aging and age-related chronic diseases. They also have a range of pathological effects including increasing vascular permeability, inhibition of vascular dilatation by interfering with nitric oxide, oxidizing LDL (Brenner *et al.*, 2001), binding cells including macrophages, endothelial and mesangial cells to induce the secretions of variety of cytokines and enhancing oxidative stress (Mogensen *et al.*, 2000; Brenner *et al.*, 2001).

It was discovered that when AGEs form in the skin they activate a receptor site and form a complex known as Receptor AGE (RAGE) which signals cellular process that are related to inflammation and subsequent disease.

AGE-RECEPTOR IN INTERACTIONS AS MEDIATORS OF DAMAGE

Researches have revealed that the effects of AGEs appear in part to be mediated via interactions with specific receptors and binding proteins. These receptors are present on most renal cell types including endothelial cells, proximal tubular cells, mesangial cells and podocytes (Bucciarelli *et al.*, 2002; Soulis *et al.*, 1997). The AGE Receptors for AGEs (RAGE): AGE-RI (055-480, P60), AGE-R2 (80K-H, protein kinase C substrate) AGE-R3 (gelatin-3), lysozyme as well as the Macrophage Scavenger Receptors (MSR); SCR-II and CD-361 and the recently identified members of the ezrin-radixin-meosin family (Szweda *et al.*, 2002; Valssara, 2001). Other

mutiligand receptors such as megalin may be have the ability to bind AGEs in the proximal tubule (Soulis *et al.*, 1997). Expression of these receptors appears to be tightly regulated under physiological conditions.

However, there is marked up-regulation in response to metabolic states such as diabetes, dyslipidemia and uremia, possibly due to high levels of AGEs (Soulis *et al.*, 1997). Recently, the study of RAGE has been further complicated by the recognition of three functionally distinct splice variants. These are soluble RAGE and full-length RAGE. It is better divided into 3 variants viz:

- The full-length RANGE receptors
- The N-terminal variant that does not contain the AGE-binding domain but soluble in nature
- The C-terminal splice variant, soluble RANGE which does not contain the trans-membrane and effectors domains (Bucciarelli *et al.*, 2002)

Hence, the balance between synthesis of soluble RAGE and full-length RAGE may be an impotant determinant of AGE-induce dysfuntion. Therefore, many of the AGE receptors are multi-specific and thus able to bind and be activated by a range of molecules including non-AGE moieties. AGE-receptors have the ability to bind a plethora of structurally district AGE. A common characteristic of most known RAGE ligands is a net negative charge at physiological pH. Polyanionic molecules such as heparin, fucoidan and dextran sulfate competes out the interaction between AGEs and RAGE.

NON-RECEPTOR MEDIATED ACTIONS OF AGE ACCUMULATION

AGE modification of proteins has important structural and functional consequences with proteins of the extracellular matrix such as elastin and collagen which are more pore to AGE accumulation due to their slow turnover. This is because protein within collagen are slowly metabolized and this makes them more susceptible to changes that occur when they are exposed to AGEs. Therefore, AGE mediated intramolecular and intermolecular cross-linking of collagen alters its surface change and packing density and thus leads to a decrease in enzymatic proteolysis and degradation rate, ultimately favouring accumulation of extracellular matrix.

Futhermore, AGE modification disturbs normal protein function including a reduction in self assembly as well as altered interactions with other matrix proteins which leads to changes in cellular adhesion and cell growth. In addition to these, AGEs are shown to accumulate on beta-amyloid plagues which are linked to the development of Alzheimers diseases where AGEs trigger chronic oxidative stress.

CONCLUSION

In conclusion, AGEs causes impaired structural alterations of the skin, increases peroxidative stress with resultant destruction of skin fibroblasts by increasing vascular permeability, inhibiting vascular dilatation and enhancing oxidative stress.

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