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INVITED REVIEW

Inhibition of AGEs formation by natural products

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Abstract Since advanced glycation end-products (AGEs) inhibitors such as benfotiamine, pyridoxamine and aminoguanidine significantly inhibit the development of retinopathy and neuropathy in streptozotocin-induced diabetic rats, treatment with AGEs inhibitors is believed to be a potential strategy for preventing lifestyle-related diseases such as diabetic complications and atherosclerosis. Furthermore, preventive medicine is the most important approach to preventing lifestyle-related diseases, and improving daily nutritional intake is thought to prevent the pathogenesis of such diseases. Therefore, AGEs inhibitors that can be obtained from daily meals are preferred to prescribed drugs. In this article, we describe a strategy for developing new AGEs inhibitors from natural products.

Keywords Advanced glycation end-products (AGEs) · Glycation · N^{ε}-(carboxymethyl)lysine (CML) · Aging · Diabetic complications

Introduction

Approximately 100 years have passed since French researcher Louis Camille Maillard's discovery of the reaction associated with browning during cooking and storing foods (Maillard 1912). However, the history of research on advanced glycation end-products (AGEs) in the human body has dramatically progressed in only the last 20 years. The reaction of proteins with glucose leads to the formation of AGEs via formation of early products such as Schiff base and Amadori products (Fig. 1). The level of hemoglobin A1c (HbA1c) is measured clinically as an index of blood glucose control and is an Amadori rearrangement product formed from the N-terminal valine residue of a hemoglobin β chain reacted with glucose. These early products are converted into AGEs, compounds that are characterized by fluorescence, a brown color and intra- or inter-molecular crosslinking.

Among the reported AGEs structures, N^{ε}-(carboxymethyl)lysine (CML) is the most thoroughly studied, both chemically and biologically (Nagai et al. 2010b). The CML concentration, adjusted for age and duration of diabetes, is increased in patients with severe complications, including nephropathy (Makino et al. 1995; Suzuki et al. 1996), retinopathy (Murata et al. 1997) and atherosclerosis (Kume et al. 1995; Sakata et al. 1999). The AGEs inhibitors aminoguanidine and pyridoxamine block CML formation and retard the development of early renal disease in streptozotocin-induced diabetic rats (Hammes et al. 1991; Degenhardt et al. 2002). These studies strongly suggest that there is an association between CML accumulation and the development of diabetic complications. However, since there are no simple and reliable methods for the analysis of AGEs, the clinical applications of AGEs measurements are limited.

To date, a variety of AGEs structures have been characterized (Fig. 2), including pyrraline (Hayase et al. 1989), pentosidine (Sell and Monnier 1989), crosslines (Ienaga et al. 1995), N^{ε}-(carboxymethyl)lysine (CML) (Ahmed et al. 1986), N^{ε}-(carboxyethyl)lysine (CEL) (Ahmed et al. 1997), GA-pyridine (Nagai et al. 2002), OP-lysine (Argirov et al. 2004), glyoxal-lysine dimer (GOLD), methylglyoxal-lysine dimer (MOLD) (Frye et al. 1998), methylglyoxal-

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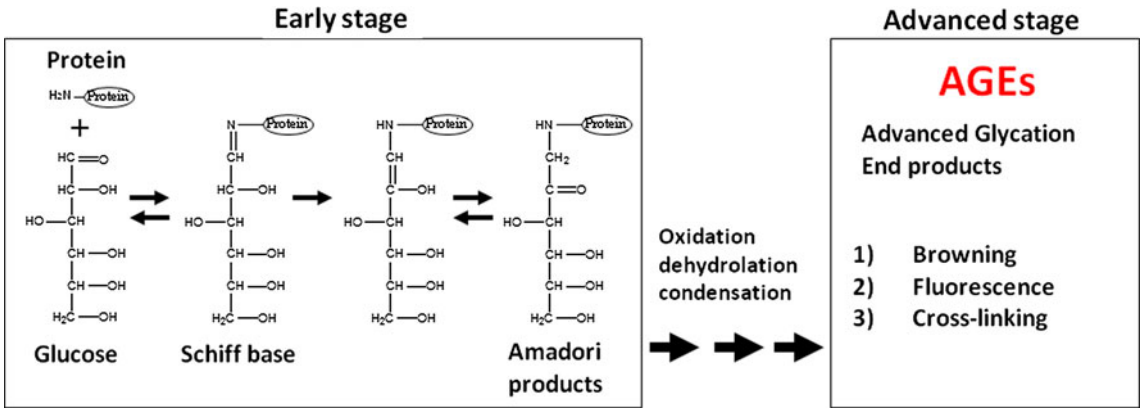

Fig. 1 Maillard reaction

derived hydroimidazolones (Thornalley et al. 2003) and Glucosepane (Lederer and Bühler 1999). Among these AGEs structures, determining the pentosidine content in tissues and biological fluids is widely employed to estimate the AGEs level in vivo due to pentosidine's acidic stability and characteristic autofluorescence. Furthermore, the pentosidine level measured in physiological samples is used as a marker for the early diagnosis of renal failure (Miyata et al. 1996). In the quantitative measurements of pentosidine reported to date, the rapid enzyme-linked immunosorbent assay (ELISA) has been widely used to estimate the plasma/serum pentosidine levels in a number of clinical samples because high performance liquid chromatography (HPLC) methods require multiple preparation steps before the analysis can be completed. However, the currently used clinical method for the analysis of the plasma/serum pentosidine levels using ELISA requires incubation of plasma/serum at 100 °C for 15 min in order to inactivate the protease (Sanaka et al. 2002). This step is required before the anti-pentosidine antibody can bind to the pentosidine. Pentosidine is generated artificially through the heating process (Nakano et al. 2011) as CML (Hayashi et al. 2002). Furthermore, autoantibodies against AGEs such as CML (Shibayama et al. 1999) and CEL (Mera et al. 2011) are detected, which may competitively interfere with ELISA when measuring the levels of AGEs in serum. Therefore, developing an easy-to-use and reliable detection system for the measurement of AGEs in physiological samples is necessary to clarify the physiological significance of glycation.

Development of new AGEs inhibitors from natural products

It is known that preventive medicine is the most important approach to preventing the development of lifestyle-related diseases such as atherosclerosis and diabetic complications, and improving daily nutritional intake is thought to prevent the pathogenesis of these diseases. We believe that the daily intake of AGEs inhibitors in natural products can thus play a beneficial role in preventing the pathogenesis of lifestyle-related diseases. Therefore, natural compounds were screened as potential inhibitors of AGEs formation. BSA was incubated with ribose in the presence or absence of natural compounds in sodium phosphate buffer, and the level of CML formation was measured. Consequently, several compounds including glycylrrhizin, glycylrrhetinic acid and quercetin pentaacetate, significantly inhibited CML formation, whereas other compounds, including epicatechin, acteoside and gallic acid, enhanced CML formation. Astragalosides, triterpenoid compounds isolated from Astragali Radix, also inhibit CML formation (Motomura et al. 2009). As shown in Fig. 3, the compounds that exhibit enhancing effects possess the same characteristic structure, namely a catechol group. On the other hand, natural compounds possessing a phenol group and an acetylated hydroxyl group on a catechol skeleton fail to enhance CML formation, indicating that a catechol group may be involved in enhancing the effects of CML formation. Although a high concentration (1 mM) of epicatechin, gallic acid and 4-MC exhibits enhancing effects, 0.01 mM of these compounds inhibits CML formation (Fujiwara et al. 2011), thus suggesting that the effects of catechol groups on CML formation are dependent on their concentration. Therefore, although low concentrations of catechol compounds inhibit CML formation due to their high antioxidative activity, high concentrations of catechol compounds enhance CML formation by producing hydrogen peroxide. It has been speculated that even high concentration of quercetin pentaacetate significantly inhibit CML formation, since the production of hydrogen peroxide is suppressed by the acetylation of the catechol group in this compound.

Akagawa et al. (2005) demonstrated that polyphenols such as catechins generate hydrogen peroxide during

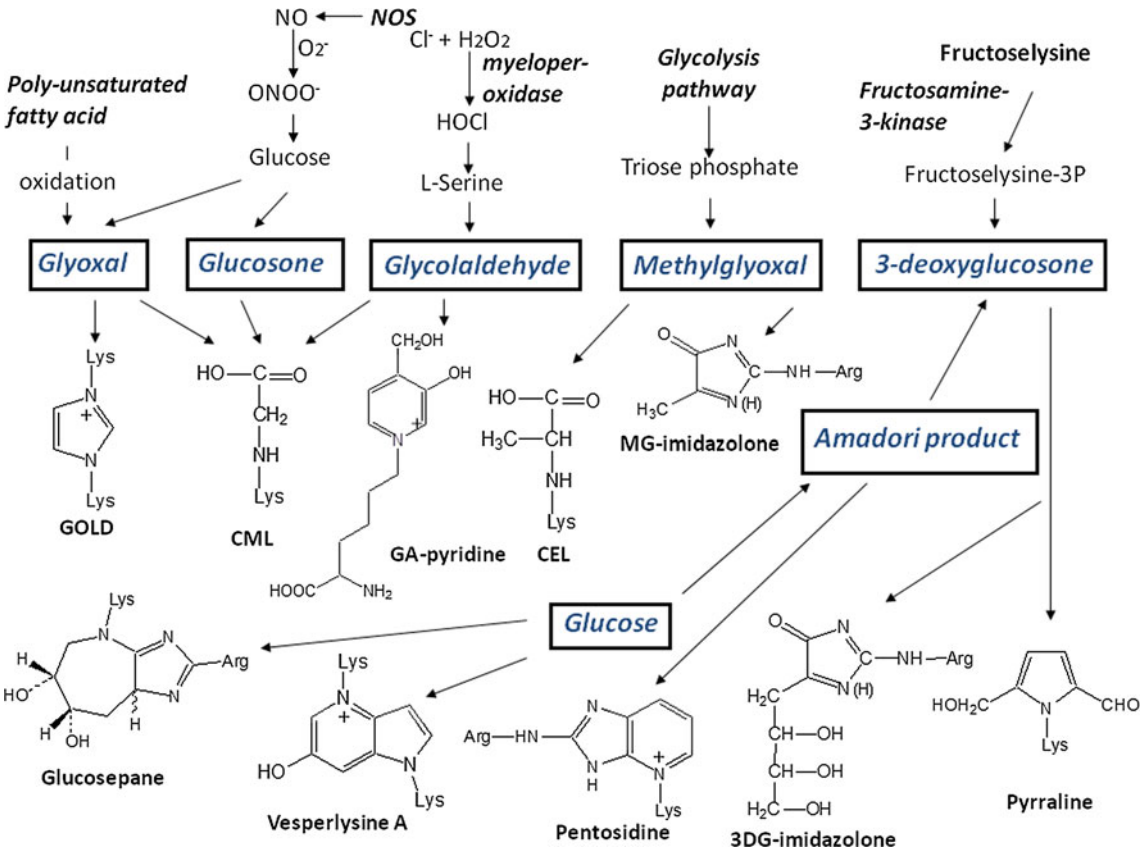

Fig. 2 Formation of AGEs structures

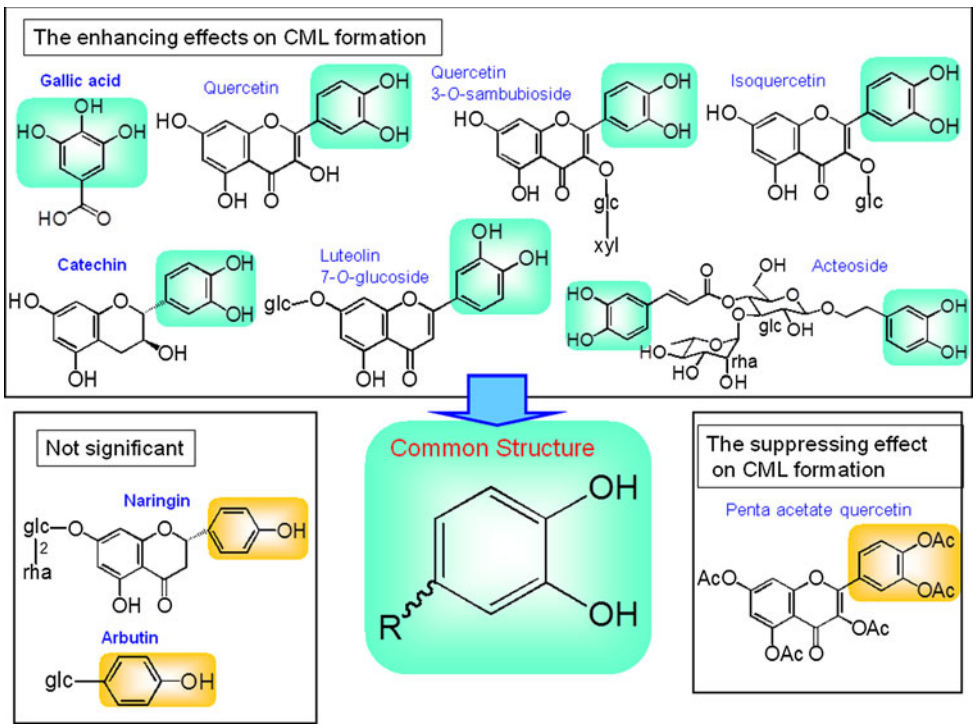

Fig. 3 Chemical structures of natural compounds that enhance or inhibit CML formation

autoxidation of catechins, thereby yielding quinones. Furthermore, the enhancing effects of catechins on CML formation are correlated with their yields of hydrogen peroxide. Furukawa et al. (Furukawa et al. 2003) reported that catechin causes oxidative DNA damage in the presence or absence of hydrogen peroxide. This suggests that oxidative DNA damage caused by catechin plays an important role in potential carcinogenicity. These reports and our study demonstrate that natural compounds containing catechol residues enhance CML formation and that flavonoid supplementation should be administered with care to prevent any unfavorable aspects of antioxidants. Furthermore, this phenomenon may explain the paradoxical effects that some flavonoids have on the redox status.

Inhibition of AGEs formation by improving carbohydrate and lipid metabolism

The blood glucose concentrations are elevated in both type 1 and type 2 diabetic patients, while ketone bodies, which are indicators of excessive fat metabolism, are generally elevated only in patients with poorly controlled type 1 diabetes. Under these circumstances, the level of total ketone bodies may exceed 10 mmol/l, while that in normal subjects is less than 0.5 mmol/l (Lebovitz et al. Lebovitz 1995). Ketoacidosis is a major complication of type 1 diabetes and also enhances endogenous AGEs formation by stimulating nitric oxide (NO) release from vascular endothelial cells (Avogaro et al. 1999) and promoting oxygen radical formation and lipid peroxidation (Jain and McVie 1999). Diabetic patients with frequent episodes of ketoacidosis suffer from increased incidences of vascular diseases, morbidity and mortality (Vignati 1985).

While investigating the pathway responsible for the formation of CEL, we found that incubating proteins with acetol, a metabolite formed from acetone by acetone monooxygenase and from methylglyoxal by aldehyde reductase, generates CEL. We then examined the inhibitory effects of citric acid on the formation of CEL in type 1 diabetic rats since citric acid is speculated to ameliorate the production of ketone bodies by improving the metabolism of carbohydrates. Citric acid was administered to type 1 diabetic rats at a dose (2 g/L in drinking water) commonly used for AGEs inhibitors such as aminoguanidine (AG) and pyridoxamine (PM) (Degenhardt et al. 2002). Consequently, the oral administration of citric acid in the diabetic rats did not affect the blood glucose concentrations or HbA1c levels (Fig. 4a), although it delayed the development of ketosis (Fig. 4b) and cataracts (Fig. 4c and Fig. 4d), inhibited the accumulation of CEL and CML in lens proteins and protected against albuminuria and ketosis (Nagai et al. 2010a). Although CML was not detectable, CEL was generated during the incubation of HSA with acetol in vitro. The inhibitory effect of the oral administration of citric acid on CML formation in lens proteins was weaker than that on CEL (Nagai et al. 2010a), suggesting that citric acid inhibited CML formation not by improving

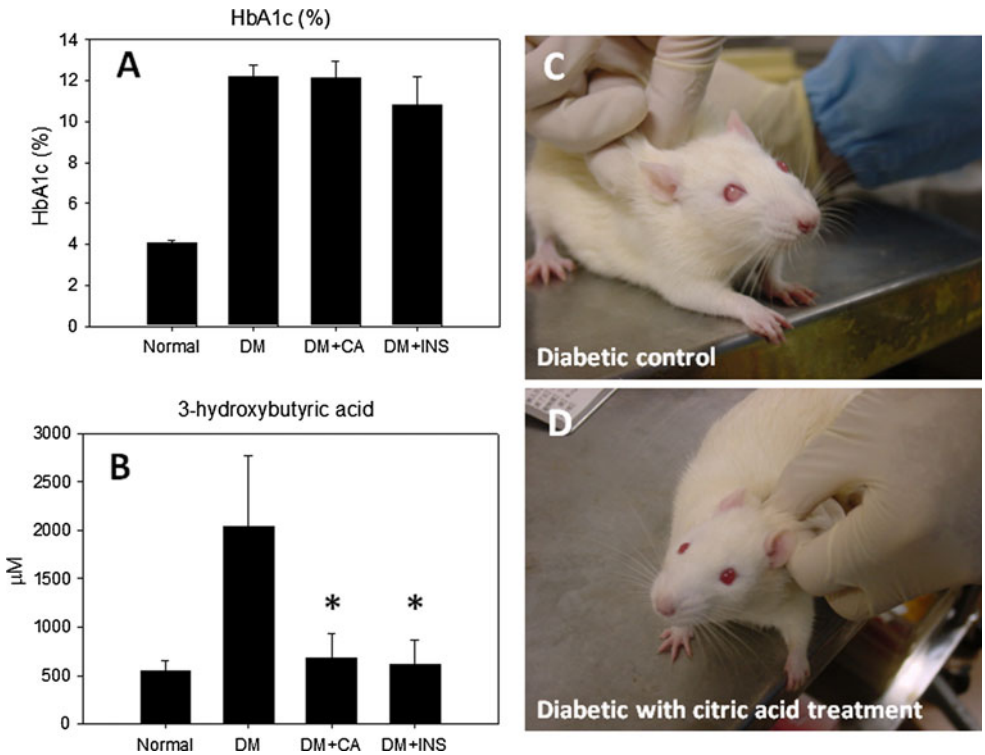

Fig. 4 Changes in biochemical parameters and cataractogenesis. The levels of HbA1c *A* and 3-hydroxybutyric acid *B* were measured. Diabetic with insulin therapy (DM + INS) and diabetic with citric acid (DM + CA). A comparison of cataractogenesis between diabetic controls *C* and diabetic rats treated with citric acid *D*. $*P < 0.05$ vs the diabetic control group

the metabolism of carbohydrates, but via its chelating activity (Nagai et al. 2012). Our results suggest that daily intake of citric acid from fruits such as lemons, limes and oranges and possibly through supplementation can play a beneficial role in limiting ketosis and the progression of diabetic complications. Other diseases complicated by ketosis, such as ketotic hypoglycemia of childhood, corticosteroid or growth hormone deficiency, intoxication with alcohol or salicylates and several inborn errors of metabolism (Mitchell GA et al. 1995), may also benefit from diets rich in citrus fruits or citrate supplementation. Taken together, we believe that the findings of the formation pathways of AGEs in vivo and treatment with AGEs inhibitors highlight a potential strategy for preventing many diseases.

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