Original Article

Methylglyoxal: its presence and potential scavengers

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Hyperglycemia is the most important factor for the onset and progress of diabetic complications. A growing body of evidence indicates that the increase in reactive carbonyl intermediates such as methylglyoxal (MG) is a consequence of hyperglycemia in diabetes. Several studies have shown that higher levels of MG are present in diabetic patients' plasma compared to non-diabetics. Glyoxal (GO) and MG, the two major α-dicarbonyl compounds found in humans, are very reactive and lead to nonenzymatic glycation *in vivo*. Glycation is a complex series of reactions between reducing sugars and amino compounds, and it will lead to the formation of advanced glycation end products (AGEs). AGEs and dicarbonyl species are both linked to possible clinical significance in chronic and age-related diseases. It is well-known that tea is rich in polyphenolic compounds and that it has potential health benefits, including the prevention of diabetes. We have shown in a previous study that all tea polyphenols have very good MG trapping abilities. In this study, using time course, we have further indicated that one molecule form black tea, theaflavins-3,3′-digallate, can trap two molecules of MG under simulated physiological conditions. In addition, we have discovered that commercial carbonated beverages contain extremely high levels of MG. The potential hazardous effects of dietary MG on humans remain to be explored.

Key Words: reactive carbonyl species, diabetes, tea polyphenols, theaflavins, carbonated beverages

INTRODUCTION

Diabetes is a heterogeneous disorder that is generally accompanied by multiple complications. It involves the of glucose and lipid metabolism in peripheral tissues to the biological activities of insulin and inadequate insulin secretion by pancreatic β cells. Epidemiological and large prospective clinical studies have confirmed that hyperglycemia is the most important factor for the onset and progress of diabetic complications, both in type 1 (insulin-dependent) and type 2 diabetes mellitus.[1] Increasing evidence identifies the formation of advanced glycation end products (AGEs) as a major pathogenic link between hyperglycemia and diabetes related complications.[2]

Nonenzymatic glycation is a complex series of reactions between reducing sugars and amino compounds. As the first step of AGEs formation, the free amino groups of proteins in the tissues react with a carbonyl group of reducing sugars, such as glucose, to form fructosamines via a Schiff base by Amadori rearrangement. Both Schiff base and Amadori product further undergo a series of reactions through dicarbonyl intermediates [e.g., glyoxal (GO), methylglyoxal (MG) and 3-deoxyglucosone], to form AGEs.[2] GO and MG, the two major α-dicarbonyl compounds found in the human body, are extremely reactive and readily modify lysine, arginine, and cysteine residues of proteins.[3] Reactive carbonyl compounds such as GO and MG have recently attracted much attention because of their possible clinical significance in chronic and age-related diseases.

A growing body of evidence indicate that the increase in reactive carbonyl intermediates is a consequence of hyperglycemia in diabetes. Carbonyl stress leads to increased modification of proteins, followed by oxidant stress and tissue damage.[4] Several studies have shown that diabetic patients have higher levels of GO and MG in their plasma compared to non-diabetics.[5-7] In a most recent report,[7] the amount of MG from diabetic patients was found to be 16-27 μg/dL, while the amount found in non-diabetics was 3.0-7.0 μg/dL. Thus, decreasing the levels of GO and MG may provide a useful approach for preventing the formation of AGEs.

The ability to prevent diabetic-related complications by using tea and its polyphenols has been tested in several studies. For instance, oral administration of tea catechins retarded the progression of functional and morphological changes in the kidney of STZ-induced diabetic rats.[8] The detailed mechanisms for the prevention of diabetic complications by tea and its polyphenols require further studies. Many studies have shown that those effects could partially be due to the inhibition of AGEs formation. Rutter *et al*.[9] reported that green tea extract was able to delay collagen aging in C57Bl/6 mice by blocking AGEs formation and collagen cross-linking.

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Meanwhile, tea polyphenols may inhibit the formation of AGEs by trapping reactive dicarbonyl compounds. A recent study explored the inhibitory effect of tea polyphenols, such as catechins, epicatechin (EC), epicatechin 3-gallate (ECG), epigallocatechin (EGC), and epigallocatechin 3-gallate (EGCG), on different stages of protein glycation, including MG-mediated protein glycation.[10] EGCG exhibited a significant inhibitory effect of 69.1% on MG-mediated protein glycation.

We have previously demonstrated that MG can be effectively trapped by green tea catechins and black tea theaflavins, with theaflavins being the most effective.[11] In the present study, the time course of MG trapping by theaflavins-3,3′-digallate (TF-3) was investigated. In addition, we have identified commercial beverages as a major source of dietary MG.

MATERIALS AND METHODS

The time course of TF3 and MG was monitored by High-performance liquid chromatography (HPLC). The reactions were started by setting the vials with equal amounts of TF3 and MG (1.15 mM, 2 mL of each) in 37 ^{o}C water bath. After the reaction was stopped by settling the reaction vials in the ice bath, the sample was divided into two. HCl was added to one of the samples to stop further MG trapping by TF3 before the measurement by HPLC. The MG concentration of the other sample was quantified via HPLC, equipped with UV detector at 313 nm, after derivatization with 1,2-diaminobenzene with the available standard, 2-methylquinoxaline.

HPLC analysis of derivatized MG was performed on a Dionex UHiMate 3000 with a UV detector with a C18 column of 150 ×4.60 mm i.d. (Phenomenex luna 3u C18 100A). The column elution started at a constant flow rate of 0.8 mL/min with 8.0% of B and 92% of A (water with 0.2% acetic acid), followed by a progressive, linear increases in B (100% acetonitrile) to 40% at 10 min, 48% at 12 min, 60% at 13 min, 28% at 70 min. The mobile phase was then re-equilibrated to 8% of B for 5 minutes. The injection amount was 15 μL. The wavelength used for MG detection was 313 nm. The compounds were detected at peak of 11 min. For HPLC analysis of TF3, the same HPLC system and column as described for MG analysis were use. The column elution started at a constant flow rate of 0.8 mL/min with 8.0% of B and 92% of A (water with 0.2% acetic acid), followed by progressive, linear increases in B (100% acetonitrile) to 12% at 10 min, 18% at 40 min, 21% at 41 min, 28% at 70 min. The mobile phase was then re-equilibrated to 8% of B at 71 min for 5 minutes. The injection amount was 15 μL. The wavelength used for TF3 detection was 280 nm. The compounds were detected at peak of 33 min.

All regular and diet carbonated beverages (15) and regular and diet tea drinks (16) were obtained from a local supermarket in New Jersey. MG, 40 wt% in water, 1,2-diaminobenzene, 2-methylquinoxaline (2-MQ; 97%) and 2,3-hexanedione were purchased from Sigma-Aldrich (St. Louis, MO, USA). Methylene chloride and acetaldehyde were purchased from Fisher Scientific (Fairlawn, NJ, USA).

Preparation of samples: 1 mL of 0.02 mmole/mL 1,2-diaminobenzene was added to 4 mL of beverage solution, then mixed with 0.5 mL of 2,3-hexanedione (internal standard) at 5 μm/mL. The reaction mixture was kept for 15min at 60 ^{o}C, and after cooling in the ice bath, 1 mL 0.4 mmole/mL acetaldehyde was added and incubated in a 60 °C water bath for 15 min, to react with the rest of the derivatization agent. The mixture was cooled by ice bath and extracted three times with 4 mL methylene chloride. The organic phase was concentrated by nitrogen gas to 0.5 mL and 1 μL of it containing quinoxaline was directly injected into the GC.

GC Analysis: The analyses of derivative volatiles were performed with an Agilent Gas Chromatograph (6850 Series, Agilent Technologies, Palo Alto, CA, USA) equipped with an Agilent autosampler (7683 Series Injector) and a flame ionization detector (FID). The column was HP-1 MS dimethylpolysiloxane silica capillary (30 m ×0.25 mm id, film thickness 1.00 μm, Agilent, Wilmington, DE, USA). The injector temperature was 250°C and detector temperature was 280°C with hydrogen, air, and makeup gas (helium) flow rates at 30.0, 300, and 5.0 mL/min, respectively. The injector was in 1:1 split mode. The 1.0 mL/min constant carrier gas (helium) flow rate was set. The GC oven temperature was programmed as follows: the initial oven temperature 40 °C was held for 1 min and increased to 250 °C at a rate of 4 °C/min and held for 5 min. Total run time was 60 min.

RESULTS

In a previous study we have shown that among all tea polyphenols, black tea theaflavins would trap the MG most efficiently.[17] To better understand the reaction trend of TF3 and MG, a time course study was carried out with the same molar amount of TF3 and MG in an hour and showed that TF3 started the MG scavenging reaction rapidly. At the first measurement, at three minutes, more than one third of the MG had been trapped. The reaction of MG with TF3 was almost completed during the time allocated for our experiment. In addition, TF3 decreased simultaneously as MG decreased (Fig. 1).

Because of their high sugar contents, soft drinks are potential sources of MG. The levels of MG in different commercial regular and diet carbonated beverages as well as regular and diet tea drinks were measured. Table 1 shows the level of MG in 10 regular carbonated beverages and 11 regular tea drinks that all contained high fructose

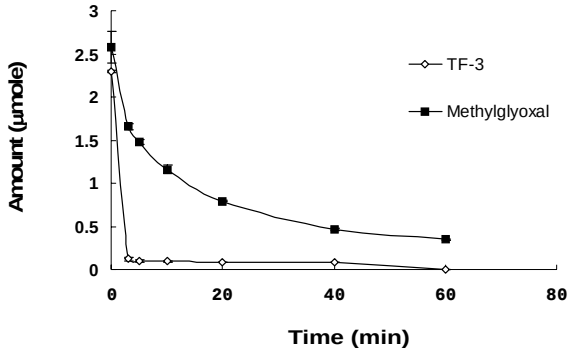

Figure 1. The time course study of TF3 and MG in 1:1 molar ratio in 60 minutes. Decreases in MG as compared to the initial point were presented, and the values were expressed as mean ± STDEV (n=3).

Table 1. MG levels in different regular and diet carbonated beverages and tea drinks

Sample	MG level μg/100 mL beverage	HFCS
Carbonated Beverages		
RC1	267±5.6	+
RC2	79.8±2.4	+
RC3	80.9±3.7	+
RC4	79.4±1.1	+
RC5	80.3±2.2	+
RC6	245±4.3	+
RC7	71.2±0.5	+
RC8	93.5±3.9	+
RC9	93.1±0.7	+
RC10	96.4±0.6	+
DC1	43.1±0.3	-
DC2	20.9±0.2	-
DC3	60.2±0.4	-
DC4	40.8±1.1	-
DC5	43.2±0.9	-
Tea Beverages		
RT1	55.4±0.7	+
RT2	48.1±1.1	+
RT3	53.7±1.4	+
RT4	58.8±2.3	+
RT5	50.1±0.6	+
RT6	85.4±2.7	+
RT7	98.1±3.2	+
RT8	81.1±0.9	+
RT9	59.7±2.8	+
RT10	64.9±3.5	+
RT11	32.1±0.5	+
DT1	26.2±0.4	-
DT2	27.0±0.6	-
DT3	27.0±0.7	-
DT4	42.0±0.9	-
DT5	42.5±1.0	-

RC: regular carbonated beverage; DC: diet carbonated beverage; RT: regular tea drink; DT: diet tea drink

corn syrup, as well as 5 diet carbonated beverages and tea drinks.

In regular carbonated beverages, the MG levels ranged between 71.2-267.2 μg/100 mL, which was 3-4 folds higher than that of diet carbonated beverages. The major difference between regular and diet beverages are the usage of high fructose corn syrup (HFCS) as a sweetener in regular carbonated beverages. HFCS could be the major resource of MG in beverages, which is under study in our lab. It is also interesting to observe that regular tea drinks contain much less MG as compared to regular carbonated beverages. It was suggested that tea polyphenols may function as MG scavengers in beverages. Discussion

MG can be generated both *in vitro* and *in vivo*. The rate of MG formation is approximately 120 μM/day, which is about 0.1% of the flux of glucose under normal conditions measured in *in vitro* red blood cells.[11,12] Even with such a small fraction, MG is of importance and of threat because of its high reactivity. MG presence in most food and beverages may come from sugars, the intermediates of Maillard reaction and lipids. Intake of MG has been shown to induce hypertension in animal studies.[13-16] Our study on the measurement of MG levels in commercial beverages shows that regular carbonated beverages containing HFCS have astonishingly high levels of MG. It can range from 4 to 25 folds higher than the blood MG levels in diabetic patients. The health concerns about MG presence are thus questioned here.

In our previous study, we have shown that black tea theaflavins have stronger MG trapping efficiency than the major green tea catechin, EGCG (Fig. 2).

In addition, the major adduct between EGCG and MG has been identified (as shown in Figure 3).[17] The 1:1 adduct formation between EGCG and MG dominantly occurs at the C8-position in the A ring of EGCG. TF3 is a condensation product of EGCG and ECG, and it contains two A rings of catechin moiety. If the reaction of TF3 with MG follows a similar mechanism to that of EGCG,

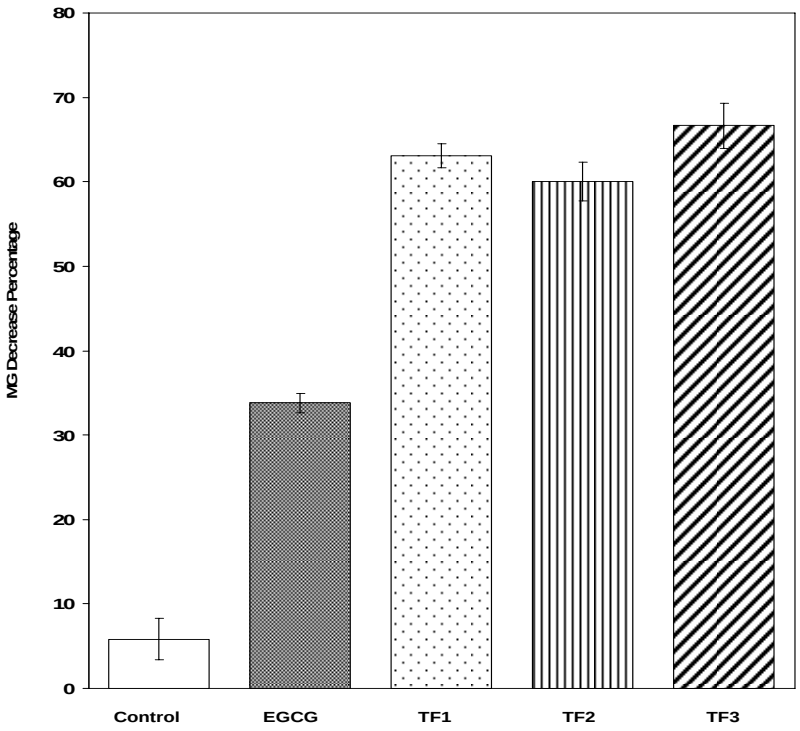

Figure 2. Decreases in the percentage of MG after 1 hour at 37 °C water bath incubation. INC: methylglyoxal only incubation sample; EGCG: epigallocatechin-3 gallate; TF1: theaflavins; TF2: theaflavin 3-gallate and theaflavins-3′-gallate; TF3: theaflavins-3,3′-digallate

two molecules of MG can be trapped by TF3. From the time course of the reaction of TF3 and MG, the decline of TF3 was even sharper than MG, which not only suggests that one theaflavin molecule is capable of trapping two MG molecules, but also that the first trapping step is faster than the second one.

Our study indicates that tea catechins including black tea TF3 are able to effectively trap MG, the most important reactive carbonyl species, *in vitro*. Whether tea catechins can trap reactive carbonyl species *in vivo* and thus reduce the formation of AGEs and prevent the development of diabetic complications needs to be further studied. It is also interesting to note that although regular tea drinks contain about the same amount of high fructose corn syrup as the regular carbonated beverages, they have much low levels of MG. It is possible the tea polyphenols such as catechins or theaflavins can either prevent the formation of MG or trap MG during the manufacturing and storage of tea drinks.

AUTHOR DISCLOSURES

Di Tan, Yu Wang, Chih-Yu Lo and Chi-Tang Ho, no conflicts of interest.

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