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Rapid and sensitive determination of the intermediates of advanced glycation end products in the human nail by ultra-performance liquid chromatography with electrospray ionization time-of-flight mass spectrometry

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ABSTRACT

The resolution of the intermediate advanced glycation end products (AGEs) in the human nail was carried out by the combination of 4,5-dimethyl-1,2-phenylenediamine (DMPD) derivatives and ultra-performance liquid chromatography with electrospray ionization time-of-flight mass spectrometry (UPLC–ESI–TOF–MS). The reaction of the reagent with 3-deoxyglucosone (3-DG), methylglyoxal (MG), and glyoxal (GO) effectively proceeds at 60 °C for 2 h. The resulting derivatives were efficiently separated by a gradient program (a mixture of water and acetonitrile containing 0.1% formic acid) using a reversed-phase ACQUITY UPLC BEH C_{18} column (1.7 μm, 50 × 2.1 mm i.d.) and sensitively detected by TOF–MS. The detection limits (signal-to-noise ratio = 5) of the TOF–MS were 10 to 50 fmol. A good linearity was achieved from the calibration curve, which was obtained by plotting the peak area ratios of the analytes relative to the internal standard (IS) (i.e., 2,3-hexanedione) versus the injected amounts of 3-DG, MG, and GO ($r^2 > 0.999$), and the intra- and interday assay precisions were less than 6.89%. The derivatives of the compounds in the human nail were successfully identified by the proposed procedure. As we know, these three kinds of dicarbonyl intermediates in the formation of AGEs—3-DG, MG, and GO—were first found in human nail samples. Using these methods, the amounts of compound in the nails of healthy volunteers and diabetic patients were determined. When comparing the index from the diabetic patients with that from healthy volunteers, there is no significant difference in the content of the MG and GO in the nails. However, a statistically significant ($P < 0.001$) correlation was observed between the 3-DG concentrations. Because the proposed method provides a good mass accuracy and the trace detection of the dicarbonyl intermediates of AGEs in the human nail, this analytical technique could be a noninvasive technique to assist in the diagnosis and assessment of disease activity in diabetic patients. Here we present a novel, sensitive, and simple method for the simultaneous determination of dicarbonyl compounds in the human nail.

The 3-deoxyglucosone (3-DG),[1] methylglyoxal (MG), and glyoxal (GO) possessing a reactive dicarbonyl group is an important intermediate in the formation of advanced glycation end products (AGEs) (Fig. 1). The AGEs are particularly important in diabetes because they have been correlated with the development of diabetic complications. Patients with diabetes have a higher concentration of Amadori products because their formation is directly related to the concentration of glucose. In that plasma, 3-DG was significantly more increased in diabetic patients than in nondiabetic control subjects, and 3-DG levels were well correlated with plasma glucose and HbA1c levels in diabetic patients [1–4]. Glycated proteins can fragment into reactive species such as 3-DG, MG, and GO [5–9]. These dicarbonyl compounds are potent protein cross-linkers and precursors of AGEs. It is these ultimate AGEs that have been implicated in the development of complications of diabetes mellitus. Consequently, measurements of 3-DG, MG, and GO were likely to provide valuable insights into the role of this metabolite in the etiology of diabetic complications as well as the aging process [10–12].

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[1] *Abbreviations used:* 3-DG, 3-deoxyglucosone; MG, methylglyoxal; GO, glyoxal; AGE, advanced glycation end product; FL, fluorescence; UV, ultraviolet; GC, gas chromatography; MS, mass spectrometry; LC, liquid chromatography; OPD, *o*-phenylenediamine; DAN, 2,3-diaminonaphthalene; UPLC–ESI–TOF–MS, ultra-performance liquid chromatography with electrospray ionization time-of-flight mass spectrometry; IS, internal standard; DMPD, 4,5-dimethyl-1,2-phenylenediamine; HP, 2-hydrazinopyridine; HMP, 2-hydrazinol-methylpyridine; TFA, trifluoroacetic acid; SDS, sodium dodecyl sulfate; MeOH, methanol; ESI, electrospray ionization; CV, coefficient of variation; LOD, limit of detection; S/N, signal-to-noise ratio; HPLC, high-performance liquid chromatography; DAD, diode array detection.

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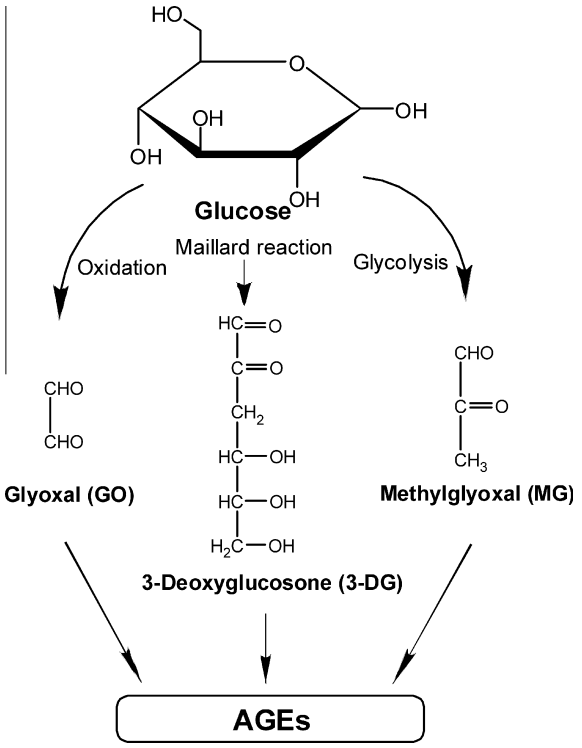

Fig.1. Pathway of AGE formation.

Tissue homogenates, peritoneal dialysis fluids, and plasma samples have been investigated extensively for the dicarbonyl intermediates of advanced glycation end product (AGE) assay in biological specimens [5,13–21]. The inherent problems of plasma and tissue homogenates, such as the fluctuation in composition during the day, should be considered. Hygienic practice during collection and handling is also another consideration. In contrast, the human nail is relatively clean and the samples can be quickly and noninvasively collected and easily stored. Analyzing the components of the human nail provides an important means for determining the individual past history of long-term chemical exposures because many substances have been detected in the nail [22–25]. Due to the stability of drugs in nails, many studies concerning nail analysis have dealt with drugs of abuse such as cocaine, itraconazole, and amphetamines [26,27]. During the past decade, interest in nail analysis has gradually shifted to other drug species such as doping agents and therapeutic drugs. According to recent reports, human nails may be used to obtain physiological information and may serve as a noninvasive biosample for the diagnosis of chronic disease. Certain kinds of endogenous biogenic amino acids have been detected in the human nail [23–25]. However, a method for determining the dicarbonyl intermediates of AGEs of the human nail has not been reported.

Various detection methods concerning the dicarbonyl intermediates of AGE analysis have been developed due to the importance of understanding diabetic complications in biological systems. However, the analysis of the dicarbonyl intermediates of AGEs in a real sample is very difficult due to no fluorescence (FL) and no effective absorption in the ultraviolet–visible (UV–vis) region. Therefore, derivatization using a suitable labeling reagent is a key step in the gas chromatography–mass spectrometry (GC–MS) [28–30] and liquid chromatography–mass spectrometry (LC–MS) [31–35] analyses of the dicarbonyl intermediates of AGEs. There are a significant number of precolumn labeling methods using fluorogenic and chromophoric reagents—such as *o*-phenylenediamine (OPD) [7,14,31,36], 2,3-diaminonaphthalene (DAN) [5,37], and 1,2-diamino-4,5-methylenedioxybenzene (DMB) [38]—for the analysis of the dicarbonyl intermediates of AGEs. The methods possessing excellent UV absorption or FL properties are certainly good, and subpicomole sensitivities can be achieved. However, the resolution of the dicarbonyl intermediates of AGEs in the human nail was very difficult even using the highly sensitive LC–FL and GC–MS. All of these reactions are usually the derivatization procedure and are time-consuming. The current study was undertaken to develop a reliable and sensitive method for the absolute quantitation of the dicarbonyl intermediates of AGEs in the human nail. Preliminary testing indicated that the detection of 3-DG, MG, and GO was difficult because the substance was unstable and existed in a very minute amount in the human nail. A higher sensitivity is essential in any assay to detect the dicarbonyl intermediates of AGEs. To achieve this goal, a new method was developed by substituting OPD with DAN [37,39].

According to this strategy, the aim of the current study was to inspect the usefulness of the human nail as a new noninvasive biological sample for the diagnosis of chronic disease and also to develop a reliable determination method to measure the free dicarbonyl intermediates of AGEs in the nail by ultra-performance liquid chromatography with electrospray ionization time-of-flight mass spectrometry (UPLC–ESI–TOF–MS). Therefore, this article describes the resolution of the dicarbonyl intermediates in the nail from diabetic patients.

Materials and methods

Materials and reagents

3-DG, MG, and GO were obtained from Dojindo (Kumamoto, Japan), Sigma–Aldrich (St. Louis, MO, USA), and Kanto Chemicals (Tokyo, Japan). 2,3-Hexanedione (Sigma–Aldrich) was used as the internal standard (IS). 4,5-Dimethyl-1,2-phenylenediamine (DMPD), 2-hydrazinopyridine (HP), 2-hydrazinol-methylpyridine (HMP), and *o*-phenylenediamine (OPD) were purchased from Tokyo Kasei (Tokyo, Japan). Trifluoroacetic acid (TFA), formic acid, hydrochloric acid, sodium dodecyl sulfate (SDS), methanol (MeOH), ethanol, and acetonitrile were of special reagent grade (Wako Pure Chemicals, Osaka, Japan). All other chemicals were of analytical reagent grade and were used without further purification. Deionized and distilled water was used throughout the study (Aquarius PWU-200 automatic water distillation apparatus, Advantec, Tokyo, Japan).

UPLC–ESI–TOF–MS

The UPLC–ESI–TOF–MS systems consisted of an ACQUITY Ultra-Performance Liquid Chromatography and Micromass LCT Premier XE Mass Spectrometer (high-sensitivity orthogonal TOF instrument, Waters, Milford, MA, USA). An ACQUITY UPLC BEH C_{18} column (1.7 μm, 50 × 2.1 mm i.d., Waters) was used as the analytical column. The column was maintained at 40 °C. The flow rate of the mobile phase was 0.6 ml/min. The TOF–MS was operated in the positive and negative ion modes using an electrospray ionization (ESI) source. The optimized conditions for the UPLC separation and TOF–MS detections are shown in Table 1.

Derivatizing dicarbonyl intermediates of AGEs with DMPD

3-DG, MG, GO, and 2,3-hexanedione (IS) were dissolved in water (2 μM each concentration). The solutions (100 μl each) and 100 μl of the DMPD (2 mM) in acetonitrile were mixed in 1.5-ml mini-vials. The vials were tightly capped and heated at 60 °C for 480 min using a dry heat block. The reaction mixture was