The analysis of advanced glycation endproducts

Valorization

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More than 2000 years ago, Hippocrates, a well-known Greek physician, was one of the founding fathers of the modern pathology. He developed new methods to examine the human body and was one of the first who used body fluids (blood, phlegm, yellow bile and black bile) in order to understand the etiology and pathogenesis of a number of diseases. Today, researchers are still using body fluids (such as blood, plasma, serum, feces, saliva and urine) to investigate and understand diseases. One way of doing these investigations, is using measureable indicators to examine the physiological state of an individual. These indicators, also known as (disease-related) biomarkers, are a valuable tool to understand the mechanisms, and even more important, treat and prevent many diseases. Sensitive, specific and reliable state-of-the-art techniques are necessary to determine these biomarkers in many different matrices. In the present thesis we validated and developed new analytical methods to quantify specific biomarkers in order to investigate diabetes and cardiovascular disease (CVD).

Relevance of this thesis

In 2012, an estimated 1.5 million deaths were directly caused by diabetes[1] and even 17.5 million people died from CVD[2]. Individuals with diabetes are at an increased risk of cardiovascular events[3,4] and its associated complications leads to a permanent and significant loss of quality of life. Therefore, prevention of diabetes and in case of diabetes, prevention of cardiovascular disease, is of highest importance and clearly represents a medical and economical need[5]. Future strategies should therefore focus primarily on the prevention of diabetes. Given the enormous impact of cardiovascular disease, it is of utmost importance to find biomarkers for the identification of patients with diabetes which are at high risk of developing vascular complications and morbidity; i.e. to improve risk prediction[6]. In this thesis we focused on the analysis of advanced glycation endproducts (AGEs) and related biomarkers in relation to diabetes and CVD.

Advanced glycation endproducts and disease

AGEs are formed by the reaction of proteins with reactive metabolic intermediates derived from glucose and from lipid oxidation with the involvement of oxidative stress[7-9]. Increased endogenous formation and accumulation of AGEs is common in patients with diabetes, and, because of impaired clearance, in those with chronic kidney disease (CKD)[10] or end stage renal disease (ESRD)[11]. AGEs are implicated to play a role in the development of vascular disease[12-17], age-related disease[13,18,19] and diabetes[20]. Most recent, it has become apparent that dietary AGEs represent a significant source of circulating and tissue AGEs[21-25].

However, many of these AGE-related researches were based on techniques, such as enzyme-linked immunosorbent assay (ELISA). However, new and improved analytical techniques are necessary in order to investigate the role of these AGEs in disease.

Applications of biomarker analysis

In this thesis we have used ultra-performance liquid chromatography tandem mass spectrometry (UPLC-MS/MS) to determine AGEs and other related biomarkers, such as methylglyoxal, in healthy individuals, patients with type 2 diabetes mellitus (T2DM), patients with impaired glucose metabolism (IGM), patients with inflammatory bowel disease (IBD) and patients with end stage renal disease (ESRD). Moreover, this technique was used to determine the AGE content in foods. AGEs have traditionally been detected by ELISA[26-32]. For several reasons the use of antisera for quantitative immunoassays of protein-bound AGEs is questionable. Reproducibility and sensitivity of such an assay are not optimal, because the specificity of the antibodies is often difficult to define and, because of steric constraints, not all AGE epitopes on the protein may be available for interaction with the antibody [28,30]. Thus, AGE measurements with immunoassays should be interpreted with care. A better approach for the quantitative determination of specific AGEs is the use of a specific analytical technique. For the determination of AGE levels in both tissue and blood samples, high performance liquid chromatography (HPLC) measurements[33-36] and several mass spectrometry methods have been developed including gas chromatography mass spectrometry (GC–MS)[37,38], and liquid chromatography tandem mass spectrometry (LC–MS/MS)[32,36,39,40]. However, the simultaneous quantification of different AGEs in plasma or tissue, using a highly sensitive, selective and rapid analytical method, has not been reported yet.

In chapter 2, 4, 6 and 8 state-of-the-art techniques were described to analyze pentosidine, N^{ε}-(carboxymethyl)lysine (CML), N^{ε}-(carboxyethyl)lysine (CEL), 5-hydro-5-methylimidazolone (MG-H1), L-lactate, D-lactate, methylglyoxal, glyoxal and 3-deoxyglucosone. These techniques were successfully applied to investigate disease mechanisms.

In chapter 5 and 7 we describe the potential role of the biomarkers D-lactate and methylglyoxal in insulin resistance and the increased risk of diabetic complications. We found increased plasma concentrations of α-dicarbonyls and D-lactate in patients with T2DM as compared to non-diabetic controls. Moreover, an oral glucose tolerance test (OGTT) was performed in individuals with normal glucose metabolism (NGM), IGM and T2DM. The area under the curve (AUC) for the OGTT levels of the α-dicarbonyls and glucose were higher in individuals with IGM and T2DM as compared with NGM.

These findings underline the potential importance of α-dicarbonyl stress as a candidate to explain the increased risk of diabetic complications in individuals with postprandial hyperglycemia.

In chapter 8 we validated a new method to determine dietary AGEs (CML, CEL and MG-H1) in 190 food products as consumed in a Western diet. A dietary AGE database was presented and applied to calculate the dietary AGE intake of a Dutch cohort. In chapter 9 we used these data and demonstrated that higher intake of dietary AGEs is associated with higher levels of plasma and urinary AGEs. These findings are an important step in understanding the metabolic transit of these dietary AGEs. Several studies indicate that dietary AGEs are associated with poor health[13-15,23,29,42]. Future prospective studies should address whether dietary AGEs are associated with adverse outcomes such as accelerated development of cardiovascular disease, diabetes and other age-related diseases.

Future perspective

In the following years the UPLC-MS/MS applications, described in this thesis, will be used in many different studies. They create opportunities to investigate not only the glycation pathway but also other disease mechanisms in more detail. However, as new research questions arise, analytical methods will need to evolve as well. In this thesis we have made the first step in investigating the complex biochemical pathway of glycation and related mechanisms. We have shown that glucose is the key molecule which is responsible for initiating glycation and formation of reactive α-dicarbonyls. We have also shown that dietary AGEs are influencing circulating AGEs. However, several questions need to be answered: 1. What is the effect of AGEs for human health? and 2. Do dietary AGEs play a significant role in developing diseases, such as diabetes and CVD? To answer these questions, the effect of AGE inhibitors and AGE-diet interventions need to be investigated. These studies are ongoing. Biomolecule analysis with state-of-the-art UPLC-MS/MS techniques, such as the one described int this thesis, will be a fundamental part in these studies.

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