Interplay of methylglyoxal and immune cells

Valorisation addendum

The burden of type 2 diabetes (T2D) is largely dependent on its cardiovascular complications. Current findings in this thesis implicate a role of methylglyoxal (MGO) and its interaction with immune cells in the development of cardiovascular disease. This chapter discusses the possibilities of MGO in immune cells as a potential risk marker and treatment target for T2D and cardiovascular disease, as well as potential treatment strategies.

Evaluation of methylglyoxal concentrations

The use of ultra-performance liquid chromatography tandem mass spectrometry (UPLC-MS/MS) allowed us to evaluate the precise concentrations of MGO in plasma, cells, and in different tissues. Universally labelled (D)+^{13}C glucose used in both human and mouse studies for the glucose tolerance test enabled us to evaluate the formation of MGO that was derived from exogenous glucose. We found that exogenous glucose during a glucose tolerance test directly contribute to postprandial MGO formation in blood and in tissues and that MGO formation is enhanced in obesity and T2D. These findings imply that postprandial MGO may be a potential marker for diabetes and its related diseases. Moreover, we showed for the first time that MGO is present in extremely high concentrations in circulating immune cells as compared to plasma. These data suggest an important role for intracellular formation of MGO rather than exogenously added MGO. Researchers should take this into account in future experimental design.

Overall, these findings provide important insights into the changes of postprandial MGO in the body in healthy and pathological conditions, and also imply that MGO in circulating immune cells deserve more attention in future research in the field of glycation in relation to T2D and its related complications.

Methylglyoxal as a treatment target

This thesis used a strong combination of animal studies, in vitro studies, and a large cohort study and identified MGO as a treatment target of cardiovascular disease, based on its effects on immune cells. We demonstrated in experimental studies that excess MGO may trigger trained immunity, i.e. a potential contributor to the high risk of cardiovascular disease in people with T2D. Under hyperglycaemic condition, trained immunity is induced in monocytes/ macrophages and promotes atherosclerosis[1]. Accumulation in immune cells of

MGO may lead to epigenetic changes and drive this hyperglycaemia-induced trained immunity. Although more measurements are needed regarding the epigenetic modifications, our findings suggested that MGO formation in innate immune cells is a potential target for the prevention of cardiovascular disease. Our investigation based on a population-based cohort (The Maastricht Study) also showed significant associations between fasting plasma MGO concentrations and higher numbers of circulating intermediate and non-classical monocytes, as well as intermediate monocyte exhaustion. Increased numbers of these cells are particularly relevant for the clinical outcomes of cardiovascular disease[2]. Exhausted monocytes that exposed to high levels of MGO may result in ineffective host defence, wound healing, and tissue repairment. These findings further support the need of targeting MGO in the treatment of diabetic vascular complications.

Potential future treatment strategies

There are several potential treatment strategies regarding MGO stress in T2D. The first is to achieve glycaemic control. Glucose-lowering drugs such as metformin, sulfonylurea, meglitinides, and thiazolidinediones are already used in the clinics[3]. Indeed, the use of metformin is associated with a reduction of MGO[4]. In addition, intensive lifestyle intervention such as a balanced diet and exercise, accompanied by clinically significant weight loss, also have been shown to improve glycaemic control and we previously demonstrated that weight loss is indeed associated with a reduction of MGO[5]. The second way to reduce MGO stress is a direct quenching of MGO. Pyridoxamine, a vitamin B6 analogue, is identified as an anti-glycating agent and has been shown to reduce MGO formation in high-fat diet-induced obese mice[6] and in a recently finished clinical trial in obese individuals[7]. In a clinical trial conducted at our department, we have showed that pyridoxamine has a favourable safety profile with no adverse effects[8]. In addition to pyridoxamine, we also demonstrated that quercetin is able to reduce the levels of MGO with 10%[9]. Third, enhancing GLO1 expression and activity to promote MGO metabolism can also help to reduce MGO stress. Cruciferous vegetables, which contain phenethyl isothiocyanate and sulforaphane, can be an option to stimulate GLO1[10] and to reduce the levels of MGO. In addition, *trans*-resveratrol and hesperetin coformulation, has recently been discovered as a GLO1 inducer, which significantly decreased plasma MGO levels in highly overweight subjects[11]. Future research may also consider to develop drugs that can be used after each meal to control postprandial endogenous production of MGO.

Methylglyoxal in immune cells as a potential risk marker

MGO in immune cells may serve as a potential risk marker for cardiovascular disease. Intracellular MGO levels in immune cells can be measured by 1) UPLC-MS/MS, 2) flow cytometry with a fluorescent MGO sensor probe, or 3) autofluorescence. Exact concentrations of MGO in total leukocytes can be directly analysed with UPLC-MS/MS and the combination with fluorescence activated cell sorting further enable the detection of MGO in specific immune cells. An alternative way to quantify MGO in circulating immune cells is with the use of the fluorescent sensor MBO (methyl diaminobenzene-BODIPY)[12], in combination with flow cytometry. Fluorescent intensities of the MBO probe reflect the relative levels of MGO in different immune cells. The strength of using UPLC-MS/MS is that the results are quantitative, and the detection of MGO can be achieved in both fresh and frozen samples. The use of flow cytometry requires fresh blood, is more efficient and faster, and is cheaper, but is semi-quantitative. In addition to the direct quantifications of MGO, we recently developed a novel way of measuring cellular autofluorescence with full spectrum cytometry. Autofluorescence is thought to reflect levels of MGO-derived advanced glycation endproducts, which may also be an option to indirectly detect MGO. Based on the availability of techniques in the laboratory to detect MGO, a choice can be made to study MGO in immune cells as a potential risk marker for cardiovascular disease.

References

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