Study on glyceraldehyde content and its novel reactants on collagen in the body

（生体内のグリセルアルデヒド含量とコラーゲンとの新規反応物に関する研究）

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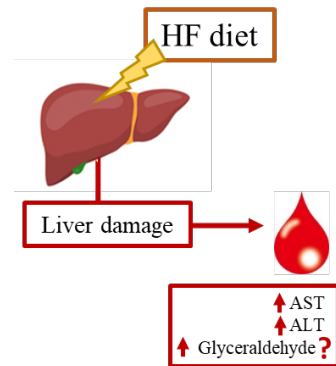Martin　　Morales　Agustin

Introduction

Glyceraldehyde is an intermediary metabolite in carbohydrate metabolism. Glyceraldehyde has been proposed as one of the primary sources of toxic advanced glycation end products (AGEs). AGEs can modify intracellular signalling and gene expression associated with reactive oxygen species and also release pro-inflammatory compounds, contributing to the pathology of diseases such as diabetes or aging. However very little is known of physiological or pathological levels of glyceraldehyde in body, and its effect in the formation and toxicity of these AGEs. Existing quantification methods of glyceraldehyde are not reliable or sensitive enough. The objectives of the present study were to develop reliable method for glyceraldehyde quantification and examine effects of diets on liver and blood glyceraldehyde contents.

A method using 1-phenyl-3-methyl-5-pyrazolone was optimized in order to achieve high and reproducible recovery of derivative for LC-MS/MS quantification. Briefly, strong basic and acid conditions were substituted by 7 % ammonia treatment and removal of ammonia by evaporation. To supress undesirable absorption of PMP-derivatives to column, 20 mM sodium acetate was used for liquid partition process. These changes extensively increased glyceraldehyde stability during derivatization and improved PMP-glyceraldehyde signal in sample injected in LC-MS/MS system. This method was tested in plasma and liver samples,

good linearity was observed and accuracy above 98 %.

Glyceraldehyde was measured in human plasma after the ingestion of 100 g of rice following overnight fasting. Glyceraldehyde remained consistent before and after food consumption. However, the plasma glyceraldehyde level in patients with type 2 diabetes was positively correlated with plasma glucose level ($p < 0.0001$) and glycated haemoglobin ($p < 0.01$) and inversely correlated with HDL levels. These results indicate that glyceraldehyde content in plasma can be affected by diseases such as diabetes and therefore have an effect in protein glycation in the vascular system or other tissues.

Same as in human plasma, the glyceraldehyde levels stayed unchanged between fasting and non-fasting groups in both mice and rats. On the other hand, the liver glyceraldehyde levels significantly increased after food consumption ($p < 0.05$). Next, mice were fed 60% high fat diet for 10 weeks. Surprisingly, high fat diet (low carbohydrate diet) feeding significantly increased plasma glyceraldehyde level in mice ($p < 0.005$). A concomitant increase in liver damage markers ALT and AST was also found in mice fed with high fat diet. Glyceraldehyde leaking for damaged hepatocytes could be a possible explanation for the elevation in plasma in mice fed with high fat diet.

Mice were also administered a single dose of fructose or glucose and glyceraldehyde was measured in plasma and liver after 30, 60 and 180 min of ingestion. In liver glyceraldehyde was quickly converted into methylglyoxal after the ingestion of fructose but not glucose. Same results were replicated in rats fed with high fructose drink for 10 weeks. Fructose consumption did not significantly change glyceraldehyde levels in plasma or liver.

Collagens are well-recognized targets for glycation because they have long half-lives in bone, skin and cartilage. In order to investigate the possible glyceraldehyde-derived AGEs formed in collagen, glyceraldehyde was incubated with collagen at 37°C for 48 h. A novel compound was found in the proteolytic digest of collagen. Incubation of lysine with glyceraldehyde elucidated the origin of this compound as reactants of lysine residues in collagen. Derivatization with AccQ and PMP revealed that this compound has an amino and an aldehyde group respectively. Finally, nuclear magnetic resonance analysis revealed the structure: this compound was composed of the lysine chain and two glyceraldehyde molecules forming a pyrrole ring with an aldehyde group and a hydroxymethyl group. This novel compound was also found in kidney of animals injected with 2 mg/g body weight of glyceraldehyde.
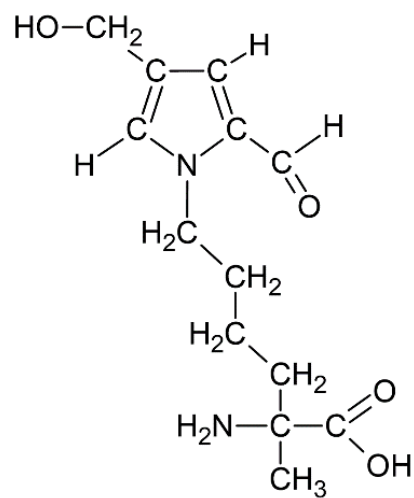
Conclusion

This study optimized a method for the measurement of glyceraldehyde using LC-MS/MS and first provides quantitative data of glyceraldehyde in body. The present study indicates that glyceraldehyde levels in body can be affected by some diseases such as diabetes and special diets like high fat diet. Interestingly liver damage seems to be involved in the increase of glyceraldehyde in plasma in the context of a high fat diet. These results must be taken into consideration when assessing the toxic effects of glyceraldehyde as an AGE promoter. Finally, a new glyceraldehyde-derived AGE with aldehyde group was identified in collagen, which suggests that glyceraldehyde might crosslink collagen and affect its turnover.

学術論文発表リスト

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