A Comparative Study of Advanced Glycation End Products for Correlation between their Structures and Inflammatory Cellular Responses

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（異なる構造を持つ終末糖化産物による刺激がヒト培養細胞の炎症応答に及ぼす影響の解析）

Advanced Glycation End Products (AGEs) are produced from the non-enzymatic reaction of reducing sugars with amino groups in a protein. They are found in an endogenous condition as well as heat-processed food, and approximately fifty glycation adducts have been identified so far (Arena et al., 2014). A binding to a receptor for AGEs (RAGE) of endogenous AGEs triggers superoxide anion production and NFκB activation, which leads to expressions of several inflammatory genes related to diabetic complications and metabolic syndrome. Although AGEs from dietary sources possess the same chemical structures as endogenous AGEs, an effect of dietary AGEs on health upon ingestion has been still controversial. It is known that dietary AGEs are easily excreted from our body (Bergmann et al., 2001) and that carboxymethyl lysine, one of the known glycation adducts, does not affect an endothelial function in healthy individuals (Semba et al., 2014). In contrast, dietary AGEs is also known to promote insulin resistance and diabetes (Cai et al., 2012). A possible reason why observations in terms of health effects of dietary AGEs fluctuated was because a single glycation adduct or a single sugar-derived AGEs would be mostly monitored regardless of its RAGE-binding ability. The other factor to be considered would be a difference in the existing form of endogenous and dietary AGEs in vivo. Namely, endogenous and dietary AGEs exist on a protein and a peptide, respectively. Therefore, to clarify the impact of the dietary AGEs on health, I posed two fundamental questions to be answered as follows: Do all glycation adducts similarly bind to RAGE? Does a backbone structure, to which a glycation adduct is connected, affect RAGE-binding and subsequent

cellular response? In this thesis, the possibility of the health effect of dietary AGEs was examined through answering these two questions.

In Chapter 2, for answering the first question above, it was examined if glycation adducts bound to RAGE. I took methylglyoxal (MGO) derived AGEs formed on Bovine Serum Albumin (BSA) as an example. At the same time, lysine-methylated BSA was also used for the preparation of MGO-derived arginine specific glycation adducts for comparison. These AGEs samples were coated on the wells in the microplate, and successively, purified RAGE (Kumano-Kuramochi et al., 2017) and antibodies recognizing specific glycation adducts were incubated in the wells for screening of RAGE-binding glycation adducts. Anti-carboxyethyl lysine (CEL) and anti-pentosidine (PEN) antibodies suppressed RAGE binding to AGEs prepared on BSA, suggesting that CEL and PEN would bind to RAGE. However, anti-MG-H1 antibody did not significantly suppress the RAGE binding to AGEs on BSA, whereas any RAGE binding to AGEs on methylated BSA was not observed. Therefore, these results indicate that individual glycation adducts would have a varied role in RAGE binding, and furthermore, that this developed in vitro method could be used for estimation of a cellular response triggered by RAGE binding of AGEs.

In Chapter 3, for answering the second question posed in Chapter 1, it was examined if a backbone structure of AGEs modulated RAGE binding and subsequent cellular response. Protein-linked AGEs were prepared through incubation of BSA with dietary sugars, and the resultant Protein-linked AGEs were used as a model of endogenous AGEs. Then, peptide-linked AGEs was prepared by enzymatic hydrolysis of protein-linked AGEs and was used as a model of dietary AGEs. When these samples were applied to the plate assay as developed in chapter 2, peptide-linked AGEs generally exhibited lower binding to RAGE. In fact, superoxide production in human umbilical vein endothelial cells (HUVEC) was also evaluated after addition of protein-

and peptide-linked AGEs to the culture medium, resulting in lower induction of superoxide anions was observed in peptide-linked AGEs than protein-linked AGEs. Some peptide-linked AGEs prepared with specific sugars still kept an ability to induce superoxide anion. Production of superoxide anion was suppressed by addition of an anti-RAGE antibody to the medium containing peptide-linked AGEs as observed in protein-linked AGEs. These results indicate that some dietary AGEs could affect a cellular function via RAGE if those AGEs were absorbed after digestion. In addition, these results also suggest that glycation adducts could be classified into three classes based on RAGE binding, including glycation adducts which never bound to RAGE, glycation adducts which bound to RAGE with the assistance of peptide backbone, and glycation adducts which bound to RAGE even without the assistance of peptide backbone.

Chapter 4 includes the summary and future perspectives regarding dietary AGEs. In conclusion, glycation adducts would exhibit RAGE binding property different from one another and could be divided into three classes based on RAGE binding and superoxide anion production in HUVEC. For consideration of a possible health effect of dietary AGEs in vivo, glycation adducts, which bound to RAGE without the assistance of peptide backbone, would be of great significance. Although further study on dietary AGEs using animal model will be necessary, these results suggest that some dietary AGEs could affect a cellular function. Scientific evidence obtained would contribute to the improvement of food processing in the future.

References

Arena, S., Salzano, A. M., Renzone, G., D'Ambrosio, C., & Scaloni, A. (2014). Non-enzymatic glycation and glycoxidation protein products in foods and diseases: An interconnected, complex scenario fully open to innovative proteomic studies. *Mass Spectrometry Reviews*, *33*(1), 49–77. https://doi.org/10.1002/mas.21378

Bergmann, R., Helling, R., Heichert, C., Scheunemann, M., Mäding, P., Wittrisch, H., Henle, T. (2001). Radio fluorination and positron emission tomography (PET) as a new approach to study the in vivo distribution and elimination of the advanced glycation endproducts Nε-carboxymethyllysine (CML) and Nε-carboxyethyllysine (CEL). *Nahrung/Food*, *45*(3), 182–188. https://doi.org/10.1002/1521-3803(20010601)45:3<182::AID-FOOD182>3.0.CO;2-Q

Cai, W., Ramdas, M., Zhu, L., Chen, X., Striker, G. E., & Vlassara, H. (2012). Oral advanced glycation endproducts (AGEs) promote insulin resistance and diabetes by depleting the antioxidant defenses AGE receptor-1 and sirtuin 1. *Proceedings of the National Academy of Sciences*. https://doi.org/10.1073/pnas.1205847109

Kumano-Kuramochi, M., Tatematsu, K., Ohnishi-Kameyama, M., Maeda-Yamamoto, M., Kobori, T., Sezutsu, H., & Machida, S. (2017). Co-expression of BirA with biotin bait achieves in vivo biotinylation of overexpressed stable N-glycosylated sRAGE in transgenic silkworms. *Scientific Reports*, *7*(1), 356. https://doi.org/10.1038/s41598-017-00420-4

Semba, R. D., Gebauer, S. K., Baer, D. J., Sun, K., Turner, R., Silber, H. A., Novotny, J. A. (2014). Dietary Intake of Advanced Glycation End Products Did Not Affect Endothelial Function and Inflammation in Healthy Adults in a Randomized Controlled Trial. *The Journal of Nutrition*. https://doi.org/10.3945/jn.113.189480