ABSTRACT

Hyperglycemia has been implicated as a major risk factor for development of the complications of diabetes. Recent investigations have shown that advanced glycation end products (AGEs) are thought to play a crucial role in this process. Nε-(carboxymethyl)lysine (CML), the predominant antigenic advanced glycation end product in vivo, is known to be a product of both glycation and oxidative modification of glycated proteins. Glycation and oxidation of low density lipoproteins (LDL) are known to increase the atherogenic potential of LDL in type 2 diabetes mellitus (T2DM) by reducing its affinity for the LDL receptor, thus leading to reduced hepatic catabolism and increased accumulation of cholesteryl esters in macrophages and subsequently result in endothelial dysfunction.

The purpose of this study was to determine the levels of CML in patients with T2DM with and without coronary artery disease (CAD) and to find for a possible use of CML measurements as a prognostic biomarker of CAD in T2DM. This study aimed to clarify the role of Nε-(Carboxymethyl)lysine in the uptake of LDL by classic LDL receptors which can potentially give rise to increased risks of cardiovascular disease such as CAD in T2DM. Polyclonal anti-CML antibodies, developed in female New Zealand white rabbit, were used for measurement of serum CML. CML levels were measured by competitive ELISA in the serum of T2DM patients (with and without CAD), nondiabetic CAD patients, and age-and sex-matched healthy subjects. Serum levels of the lipid peroxidation product; malondialdehyde (MDA), lipid profile, and hemoglobin A1c
(HbA1c) tests were also assessed in the study groups. Correlations studies between CML levels and lipids, HbA1c, and lipid peroxidation were performed for the study populations. LDL fractions from the T2DM groups were isolated and labeled with the fluorescent 3,3'-dioctadecyloxa-carbocyanine perchlorate (DiI) dye. LDL particles from healthy subjects were subjected to oxidation, glycation, glycoxidation and carboxymethylation. Receptor-mediated uptake of labeled native-LDL and \textit{in vivo} and \textit{in vitro} modified-LDL was evaluated in human hepatoma (HepG2) cell line.

Serum levels of CML were significantly higher in T2DM with CAD patients than diabetic patients without CAD, nondiabetic CAD patients, and healthy control individuals. CML levels were highly correlated with MDA and strongly predicted CAD in patients with type 2 diabetes. In addition, LDL isolated from T2DM patients suffering from CAD complications revealed enhanced susceptibility to oxidation and had higher CML contents than T2DM subjects not having CAD. The cellular uptake of DiI-LDL from T2DM patients with CAD, glyoxidized-LDL and CML-LDL was significantly lower than that for LDL from diabetic patients without CAD, oxidized-, or glycated-LDL.

In conclusion, this study has demonstrated the effect of both diabetes and oxidative stress on the higher levels of circulating CML and showed that CML levels are associated with the development of CAD in patients with T2DM. It is also concluded that CML-LDL, rather than either AGE-LDL or oxidized-LDL, may be largely responsible for the defective uptake of LDL by the LDL receptor. Hence, CML may be used as an endogenous marker for early detection of CAD events in T2DM patients and may be considered as a new goal for the glycemic control in these patients.