Protein oxidation parameters in type 2 diabetic patients with good and poor glycaemic control

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SUMMARY

Aim: In order to examine the influence of oxidative stress on protein oxidation, type 2 diabetic patients without clinical evidence of complications, either in good or poor glycaemic control, were studied.

Methods: Plasma protein carbonyl (PCO), total thiol (T-SH), and advanced oxidation protein products (AOPP) levels as markers of protein oxidation, and lipid hydroperoxide (LHP) levels as markers of lipid peroxidation were determined. Glycated haemoglobin (HbA_{1c}) levels were used as an index of glycaemic control. The subjects were divided into two groups according to their HbA_{1c} level at inclusion as follows: good HbA_{1c} Ω7%, and poor HbA_{1c} > 7%.

Results: Plasma PCO and AOPP levels of diabetic patients with poor glycaemic control were increased significantly compared with those of the diabetic patients with good glycaemic control. The decreased plasma T-SH level in the diabetic patients with poor glycaemic control was not statistically significant. On the other hand, plasma LHP levels were increased significantly in the diabetic patients with poor GC compared with those of the diabetic patients with good glycaemic control.

Conclusion: This study supports the hypothesis that poor glycaemic control is an important factor in generation of increased protein oxidation in type 2 diabetic patients clinically free of complications. Increase in plasma PCO, AOPP, and LHP levels in the diabetic patients with poor glycaemic control may contribute to the development of diabetic complications.

Key-words: Type 2 diabetes · Protein oxidation · Lipid peroxidation · Glycaemic control · Carbonyl groups.

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RÉSUMÉ

Paramètres d'oxydation des protéines chez des diabétiques de type 2 en équilibre glycémique correct et insuffisant

Objectif : Pour examiner l'influence du stress oxydatif sur l'oxydation des protéine, des diabétique de type 2 cliniquement indemnes de complications, en équilibre glycémique variable ont été étudiés.

Méthodes : Le dosage de marqueurs de l'oxydation des protéines (protéine carbonyle plasmatique [PCO], thiols totaux [T-SH] et produits de l'oxydation avancée des protéines [AOPP]) et de marqueurs de la peroxydation lipidique (hydroperoxydes lipidiques [LHP]) a été réalisé. L'équilibre glycémique a été évalué par le taux d'HbA_{1c}, en isolant 2 groupes de patients, équilibre glycémique correct (HbA_{1c} Ω7 %) et insuffisant (HbA_{1c} >7 %).

Résultats : Les concentrations de PCO, d'AOPP et de LHP, mais non celles des T-SH totaux plasmatiques des diabétiques de type 2 mal équilibrés étaient significativement plus basses que celles des diabétiques bien équilibrés.

Conclusion : Ces résultats sont en faveur de l'hypothèse que l'hyperglycémie chronique induit une augmentation de l'oxydation des protéines chez les diabétiques de type 2 indemnes de complications cliniques. Cette augmentation serait susceptible de contribuer au développement des complications vasculaires liées au diabète.

Mots-clés : Diabète de type 2 · Oxydation des protéines · Peroxydation lipidique · Contrôle glycémique · Groupes carbonyls.

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Introduction

Considerable evidence indicates that the maintenance of protein redox status is of fundamental importance for cell function, therefore structural changes in proteins are considered to be among the molecular mechanisms leading to diabetic complications [1]. Alterations in protein conformations can lead to increased aggregation, fragmentation, distortion of secondary and tertiary structure, susceptibility to proteolysis, and decrease of normal function [2,3].

Many different types of protein oxidative modification can be induced directly by reactive oxygen species (ROS) or indirectly by reactions of secondary by-products of oxidative stress. Protein modifications elicited by direct oxidative attack on Lys, Arg, Pro or Thr, by secondary reaction of Cys, His or Lys residues with reactive carbonyl compounds can lead to the formation of protein carbonyl (PCO) derivatives. PCO content is the most general and well-used biomarker of severe oxidative protein damage [4-6]. Several reports have been made on glycation-induced structural and functional modification of haemoglobin [7-9]. Compared to nonglycated haemoglobin, haemoglobin A_{1c} (HbA_{1c}) is more rapidly autooxidized [10]. In poorly controlled diabetes mellitus, glucose oxidation through the pentose phosphate pathway leads to the excessive formation of NADPH, which in turn can promote lipid peroxidation in the presence of the cytochrome P-450 system. Alternatively, inactivation or inhibition of antioxidant enzymes by glycosylation in poorly controlled diabetes mellitus may give rise to increased lipid peroxidation [11]. Lipid hydroperoxides (LHPs) are a large family of the first by-products of oxidized lipids, and their quantification could become a useful biomarker [12]. Dalle-Donne et al. [13] have reported that protein carbonyl PCO groups may be introduced proteins by secondary reaction of the nucleophilic side chains of cysteine, histidine, and lysine residues, and aldehydes produced during lipid peroxidation.

Albumin is the most abundant plasma protein and is a powerful extracellular antioxidant [14,15]. The measurement of plasma total thiol (T-SH) is a good reflection of excess free radical generation, since the conformation of albumin is altered, allowing thiol (-SH) groups to be oxidized [14–16]. Recently, a new marker of protein oxidation, advanced protein oxidation products (AOPP), has begun to attract the attention of various investigators [17-19]. AOPP are defined as dityrosine-containing cross-linked protein products and are considered as reliable markers to estimate the degree of protein oxidation [20]. Biochemical characterization of AOPP in plasma revealed that both the high- and low-molecular-weight AOPP peaks contain oxidized albumin in aggregate-forming or monomeric form [21].

The aim of this study was to reveal PCO, T-SH, and AOPP as markers of protein oxidation, as well as LHP levels as a marker of lipid peroxidation, and relation of HbA_{1c} levels with these markers in plasma of type 2 diabetic patients in good and poor glycaemic control.

Materials and methods

Subjects

Patients with type 2 diabetes according to the criteria of the Expert Committee were entered the study [22]. The study included 40 consecutive type 2 diabetic patients attending the İstanbul University, İstanbul Faculty of Medicine, Central Laboratory of Biochemistry over a one-year period. Type 2 diabetic patients were free from any clinical evidence of retinopathy, nephropathy, or neuropathy. Demographic (gender, age) and anthropometric data (weight, height), medical history (duration of disease), and type of treatment were recorded. Patients with coronary heart disease, or hypertension were excluded from the study. The sample groups consisted of 23 diabetic patients with good glycaemic control (GC) (HbA_{1c} Ω7%), and 17 diabetic patients with poor GC (HbA_{1c} > 7%). All subjects were non-smokers. None of the patients was known to suffer from any acute illness or chronic inflammatory condition at the time of study. Type 2 diabetic patients were not taking any medication that might have adverse effects on the tests performed, and they were advised for diabetes diet. A total of 7 subjects were treated with insulin therapy (after failure of oral hypoglycaemic agents), 22 subjects with oral hypoglycaemic agents (sulfonylureas or sulfonylureas plus biguanides), 5 with the combination of insulin and oral hypoglycaemic agents, and 6 with diet alone.

These patients continued their regular clinical and laboratory visits, including HbA_{1c} measurement, every 3 months. Blood samples were drawn during periodic routine control analyses. All samples were taken in the morning to avoid the confounding effect of diurnal variation of oxidative stress parameters as reported previously [23]. Venous blood samples were drawn in the fasting state and processed within 1 h of collection [24]. Blood samples were collected in tubes containing lithium heparin, EDTA or no additive depending on the analysis. For protein oxidation parameters, plasma samples containing lithium heparin were stored at – 70 ^{0}C until analysis, all other parameters were determined on the same day of collection. The ethical procedures were performed according to the World Medical Association Declaration of Helsinki [25].

Analytical methods

Apparatus. PCO, T-SH, AOPP, and LHP levels were measured by a spectrophotometer (Ultrospec 4050 LKB). Serum glucose, albumin, uric acid, total bilirubin, conjugated bilirubin, unconjugated bilirubin, unsaturated iron binding