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Effect of lipid peroxides and antioxidants on glycation of hemoglobin: An in vitro study on human erythrocytes

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Abstract

Background: Glycation and lipid peroxidation are two important processes known to play a key role in complications of many pathophysiological process. We sought to assess the possibility of an interaction between these processes in vitro and to examine the effect of lipoic acid and taurine on the glycation of hemoglobin and lipid peroxidation.
Methods: Human erythrocytes in phosphate buffered saline (pH 7.4) were incubated with 5 or 50 mmol/l glucose. To study the effect of antioxidants on glycation of hemoglobin, erythrocytes were incubated with either lipoic acid or taurine and then exposed to glucose concentration of either 5 or 50 mmol/l. To clarify if lipid peroxides per se enhances the glycated hemoglobin level, an in vitro study was performed by incubating erythrocyte suspension containing either 5 or 50 mmol/l glucose with or without MDA. Lipid peroxides and glycated hemoglobin levels were determined in the glucose treated cells.
Results: Glycated hemoglobin levels were higher in erythrocytes incubated with 50 mmol/l glucose concentrations than in erythrocytes incubated with 5 mmol/l glucose. The increase in glycated hemoglobin levels was blocked significantly when erythrocytes were pretreated with either lipoic acid or taurine. Both the antioxidants used in the present study markedly reduced the MDA levels. The level of glycated hemoglobin in erythrocyte incubated in the presence of MDA was increased significantly when compared to erythrocyte incubated with glucose alone.
Conclusions: Lipid peroxides per se may have a role to play in glycation of hemoglobin and antioxidants (lipoic acid and taurine) can partially inhibit the formation of glycated hemoglobin by lowering the levels of lipid peroxides.

Keywords: Glycation; Malondialdehyde; Lipoic acid; Taurine

1. Introduction

Spontaneous nonenzymatic modifications of protein are commonly reported in tissues with slow turnover and they are considered by several authors as possible common mechanism involved in the progression of many pathological conditions [1,2]. Among the nonenzymatic processes, oxidative stress and nonenzymatic glycation have aroused a particular interest in the recent past [3,4].

Glycation is the nonenzymatic reaction of glucose with susceptible amino groups in the side chains of amino acid residues (usually lysine) in proteins. Although the initial reaction, the formation of fructose–lysine, is reversible, further rearrangement of the protein side chain can lead to more stable products [5]. It has been proposed that the tissue accumulation of advanced glycated end products participate in the alterations of structure and function of long-lived proteins responsible for cellular or tissue damage [6]. Similarly, free radicals that induce lipid peroxidation are another promoter event thought to be important in the development of several pathological conditions [7].

A combination of oxidative stress and glycation underlies most cases of diabetes, chronic renal failure (CRF) and atherosclerosis [1–4]. While the interplay of these two impairments is believed to be important in the development and progression of these pathophysiological states, the mechanisms involved are unclear.

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We previously reported a significant association between malondialdehyde and fructosamine in non-diabetic nephrotic syndrome and chronic renal failure patients [8,9]. In addition, we have also recently observed a parallel increase of glycated hemoglobin and malondialdehyde in hyperthyroid and chronic renal failure patients, with significant correlation between their respective increments [10,11]. These evidences support the hypothesis that glycation and oxidation processes may be mutually dependent in vivo. Therefore, further in vitro exploration of the role of lipid peroxidation and antioxidants on glycation of hemoglobin was deemed pertinent.

2. Materials and methods

Blood was collected from normal healthy volunteers into tubes containing EDTA according to a protocol approved by the Institution Human Ethics Review Committee. The blood was centrifuged and the clear plasma and buffy coat were discarded. The cells were washed with cold physiological saline and the cells were suspended to 10% hematocrit in phosphate-buffered saline (PBS containing 0.016 mol/l Na_2HPO_4, 0.001 mol/l NaH_2PO_4 and 0.14 mol/l NaCl, pH 7.4). Penicillin G and streptomycin were added to the incubating erythrocyte–PBS suspension to vitiate any microbial growth. All analyses were done in triplicate.

2.1. In vitro incubation with glucose

The washed erythrocytes suspended to 10% hematocrit in PBS were treated with freshly prepared stock glucose solution. Glucose concentrations were expressed in terms of the total cell suspension. Erythrocyte treated with 5 mmol/l glucose was considered as controls. The contents were incubated in a shaking water bath at 37 °C for 24 h. The percentage of hemolysis was <2% in all incubations. Glucose-treated erythrocytes were washed with PBS (pH 7.4) before biochemical analyses.

2.2. In vitro treatment with lipoic acid

In order to examine the protective role of lipoic acid against glycation of hemoglobin, erythrocytes were incubated with lipoic acid of different concentrations (50, 100, 150, 200 μmol/l) for 1 h at 37 °C. Nonenzymatic glycation of hemoglobin was initiated by incubating the pretreated (with lipoic acid) erythrocyte with glucose of either 5 or 50 mmol/l. The contents were incubated in a shaking water bath at 37 °C for 24 h. The percentage of hemolysis was <2% in all incubations.

2.3. In vitro treatment with taurine

Before treatment with glucose (concentration of either 5 or 50 mmol/l), erythrocytes were pretreated with taurine of 50, 100, 150 and 200 μmol/l for 1 h at 37 °C. The in vitro glycation of hemoglobin was carried out for 24 h at 37 °C in a shaking water bath. The percentage of hemolysis was <2% in all incubations. Erythrocytes were washed with PBS (pH 7.4) before biochemical analyses.

2.4. In vitro treatment with MDA

To examine if MDA per se has any role in glycation of hemoglobin, erythrocytes were incubated with different concentrations of glucose (5 and 50 mmol/l), with or without MDA of 0.01, 0.1, 1 and 10 mmol/l concentration. The contents were incubated in a water bath at 37 °C for 24 h. At the end of the incubation the cells were washed with PBS before biochemical analyses. The percentage of hemolysis was <2% in all incubations.

To exclude the possible influence of anionic products of MDA and hemoglobin in the estimation of glycated hemoglobin by ion exchange chromatography, erythrocytes were incubated with glucose (5 and 50 mmol/l) and 1 mmol/l MDA. The incubation was carried out for a period of 24 h at 37 °C. At the end of the incubation the cells were washed with PBS and glycated hemoglobin was estimated by affinity chromatography method.

2.5. Malondialdehyde synthesis

Malondialdehyde was prepared by acid hydrolysis of malondialdehyde bis (dimethyl acetate) [12]. Malondialdehyde bis (dimethyl acetate) was dissolved in 200 ml of double distilled water at 10^{-2} mol/l and incubated with 1 ml 1 mol/l HCl at 50 °C for 2 h. The reaction was stopped by cooling the solution at 4 °C. A portion of the acidic solution was diluted with 0.1 mol/l Tris–HCl (pH 8.0) and adjusted to pH 7.4 with 5 mol/l NaOH to prepare neutral 400 mmol/l MDA solution for use.

2.6. Measurement of glycated hemoglobin (HbA_{1C})

Glycated hemoglobin was measured by ion exchange chromatography using hemoglobin A_{1C} microcolumn (Bio-Rad, Hercules, CA; Catalog number 192 80000/192 8091) and expressed as the percentage of total hemoglobin.

To rule out the possible influence of MDA–hemoglobin adducts in the estimation of glycated hemoglobin by ion exchange chromatography, glycated hemoglobin was also estimated by affinity chromatography using the aminophenylboronic acid gels purchased from Millipore [13]. One milliliter of gel was packed in a plastic column. The gel was pre-equilibrated with 5 ml of phosphate buffer (50 mmol/l, pH 9.2) and hemolysate in a volume of 200 μl was loaded onto the column and then washed with 10 ml of the same buffer. Non-glycated hemoglobin was eluted in this fraction (peak 1) and glycated hemoglobin that remained attached to the column was eluted with the same phosphate buffer containing 100 mmol/l sorbitol (peak 2). Hemoglobin