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Protective effects of D-glucaro 1,4-lactone against oxidative/nitrative modifications of plasma proteins

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Abstract

Objective: The protective effects of D-glucaro 1,4-lactone (1,4-GL) against oxidative/nitrative protein damage (determined by parameters such as levels of protein carbonyl groups and nitrotyrosine residues) to human plasma treated with peroxynitrite ($ONOO^-$) or hydroperoxide (H_2O_2) were studied in vitro. We also investigated the effects of 1,4-GL on the level of total free thiol groups and low-molecular-weight thiols (glutathione and homocysteine) in plasma treated with $ONOO^-$ (0.1 mM).

Methods: Levels of carbonyl groups and nitrotyrosine residues in human plasma proteins were measured by ELISA and a competition ELISA, respectively. High-performance liquid chromatography (HPLC) was used to analyze free thiols from plasma.

Results: Exposure of plasma to $ONOO^-$ (0.1 mM) resulted in an increase of the level of carbonyl groups and nitrotyrosine residues in plasma proteins and in a distinct decrease in total thiols and low-molecular-weight thiols (glutathione and homocysteine) measured by high-performance liquid chromatography. In the presence of 1,4-GL (0.4–6.4 mM), a distinct decrease in carbonyl group formation and tyrosine nitration in plasma proteins and changes in plasma thiols caused by 0.1 mM of peroxynitrite were observed. Moreover, 1,4-GL inhibited plasma protein oxidation induced by H_2O_2 (2 mM).

Conclusion: The obtained results indicate that in vitro 1,4-GL has inhibitory effects on $ONOO^-$- or hydroperoxide-mediated oxidative stress in human plasma and changes plasma redox thiol status. The mechanism of the antioxidative action of 1,4-GL present in plasma is not known yet.

Keywords: Plasma; Peroxynitrite; Hydroperoxide; Nitrotyrosine; Carbonyl groups; D-glucaro 1,4-lactone; Thiols

Introduction

D-glucaro 1,4-lactone (1,4-GL) is formed in the gastrointestinal tract from D-glucaric acid or its salts and is transported to blood where it can have effects on blood components [1]. D-glucaric acid is a natural, non-toxic compound found in large amounts in many different fruits and vegetables, with the highest concentrations in oranges, apples, grapefruits, and cruciferous vegetables; it also may be produced in small amounts by mammals (including humans) [2]. 1,4-GL possesses detoxifying and anticarcinogenic properties, attributed to an ability to increase glucuronidation and excretion of potentially toxic compounds [3]. 1,4-GL with some biological properties is the most pharmacologically active metabolite of D-glucaric acid. It is a β-glucuronidase inhibitor [4]. The aim of the present study was to estimate the direct effects of 1,4-GL on changes induced by strong biological oxidants, i.e., peroxynitrite ($ONOO^-$) and hydroperoxide (H_2O_2), in plasma proteins and on the level of total free thiol groups and low-molecular-weight thiols such as glutathione and homo-

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cysteine (HCSH) in plasma. The defense mechanisms against oxidative stress ($ONOO^-$ and H_2O_2 action) are very important for biological activities of human plasma components. Moreover, the role of exogenous antioxidants (present in the human diet) in the defense against oxidative stress in human plasma is still unknown. Therefore, the protective effects of 1,4-GL against the oxidative/nitrative damage of human plasma proteins and low-molecular-weight thiols (glutathione and HCSH as important components of plasma redox thiol status) induced by $ONOO^-$ (0.1 mM) and H_2O_2 (2 mM) were studied. The concentration of $ONOO^-$ used in our experiments was relatively high. The lifetime of $ONOO^-$ at physiologic pH is very short, with its half-time being of the order of 1 s. Exposure to a bolus of 250 μM of $ONOO^-$ is equivalent to 7 min of exposure to a steady-state $ONOO^-$ concentration of 1 μM. This concentration could be readily formed at sites of inflammation, where production rates of nitric oxide radicals ($NO^\bullet$) and superoxide radicals considerably increase [5].

Material and methods

Materials

Peroxynitrite was synthesized according to the method of Pryor and Squadrito [6]. Freeze fractionation (−70°C) of the peroxynitrite solution formed a yellow top layer, which was retained for further studies. The top layer typically contained 80–100 mM of peroxynitrite as determined spectrophotometrically at 302 nm in 0.1 M of NaOH (ϵ_{302nm} = 1679 M/cm). Some experiments were also performed with decomposed $ONOO^-$, which was prepared by allowing the $ONOO^-$ to decompose at neutral pH (7.4) in 100 mM of potassium phosphate buffer (15 min at room temperature). This allowed us to compare the effects of $ONOO^-$ with those of compounds derived from its decomposition.

1,4-GL, hydroperoxide, rabbit anti-dinitrophenylhydrazine antibodies, anti-rabbit antibodies, and anti-goat/sheep antibodies coupled with peroxidase were purchased from Sigma (St. Louis, MO, USA). Sheep anti-nitrotyrosine polyclonal antibodies were obtained from Oxis (Portland, OR, USA). Biotinylated anti-goat/mouse/rabbit antibodies and streptavidin-biotinylated horseradish peroxidase were from DAKO (Glostrup, Denmark). All other reagents were of analytical grade and were provided by commercial suppliers.

Incubation of plasma with 1,4-GL and oxidants

Human blood from healthy volunteers was collected into sodium citrate (at a final concentration of 5 mmol/L) and immediately centrifuged (3000 *g*, 15 min) to obtain plasma. Peroxynitrite was added to plasma as a bolus to the final concentration of 0.1 mM and the samples were immediately vigorously mixed. Some samples of human plasma were preincubated (2 min at room temperature) with 1,4-GL at final concentrations of 0.4–6.4 mM and then treated with only one concentration of $ONOO^-$ (at a final concentration of 0.1 mM) or hydroperoxide (at a final concentration of 2 mM).

Determination of nitrotyrosine in the proteins of human plasma by a competition enzyme-linked immunosorbent assay

Detection of nitrotyrosine-containing proteins by a competition enzyme-linked immunosorbent assay (C-ELISA) method in plasma (control, 1,4-GL- or/and $ONOO^-$-treated plasma) was performed according to the procedure of Khan et al. [7] as described previously [8]. The nitro-fibrinogen (at concentrations of 0.5 μg/mL and 3–6 mol nitrotyrosine/mol protein) was prepared for use in the standard curve. The linearity of the C-ELISA method was confirmed by the construction of a standard curve ranging from 10 to 500 nM of a nitrotyrosine-fibrinogen equivalent. The concentrations of nitrated proteins that inhibit anti-nitrotyrosine antibody binding were estimated from the standard curve and are expressed as nitro-fibrinogen equivalents. The amount of nitrotyrosine present in fibrinogen after treatment with peroxynitrite (at a final concentration of 1 mM) was determined spectrophotometrically (at pH 11.5, ϵ_{430nm} = 4400 M/cm) [7].

Detection of carbonyl groups in human plasma proteins by ELISA

Detection of carbonyl groups by ELISA (using anti-dinitrophenylhydrazine antibodies) in plasma (control, 1,4-GL and $ONOO^-$- or H_2O_2-treated plasma) was carried out according to the procedure of Buss et al. [9] as described previously [8]. Human plasma proteins reacted with dinitrophenylhydrazine and then proteins were non-specifically adsorbed to an ELISA plate. The peroxynitrite-treated fibrinogen (10 nmol of carbonyl groups/mg fibrinogen) was prepared for use in the standard curve. The linearity of the ELISA method was confirmed by the construction of a standard curve ranging from 0.1 to 10 nmol of carbonyl group per milligram of fibrinogen. The amount of carbonyl groups present in fibrinogen after treatment with peroxynitrite (at a final concentration of 1 mM) was determined spectrophotometrically as described by Levine et al. [10].

Determination of thiols

The classic technique high-performance liquid chromatography (HPLC) was used to analyze thiols (glutathione and homocysteine) from human plasma treated