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Plasmatic antioxidant capacity due to ascorbate using TEMPO scavenging and electron spin resonance

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Abstract

Background: Ascorbate is the most effective water-soluble antioxidant and its plasma concentration is usually measured by different methods including colorimetric assays, HPLC or capillary electrophoresis. Plasma antioxidant capacity is determined by indexes such as total reactive antioxidant potential, total antioxidant reactivity, oxygen radical absorbance capacity, etc. We developed an alternative method for the evaluation of the plasma antioxidant status due to ascorbate.
Methods: TEMPO kinetics scavenging analyzed by ESR spectroscopy was performed on plasma samples in different antioxidant situations. Plasma ascorbate concentrations were determined by capillary electrophoresis. Ascorbyl radical levels were measured by ESR.
Results: Plasma reactivity with TEMPO (PR-T) reflected plasma ascorbate levels. Average PR-T for normal plasmas resulted 85 ± 27 μmol/l ($n = 43$). PR-T during ascorbic acid intake (1 g/day) increased to an average value of 130 ± 20 μmol/l ($p < 0.001$, $n = 20$). PR-T correlated with the plasmatic ascorbate levels determined by capillary electrophoresis ($r = 0.92$), presenting as an advantage the avoiding of the deproteination step. Plasma ascorbyl radical levels increase from 16 ± 2 to 24 ± 3 nmol/l ($p < 0.005$, $n = 14$) after ascorbate intake.
Conclusions: PR-T could be considered as a measure of the plasmatic antioxidant capacity due to the plasma ascorbate levels and could be useful to investigate different antioxidant situations.
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Keywords: Plasma antioxidant capacity; TEMPO; Electron spin resonance; Ascorbate; Ascorbyl radical

1. Introduction

In healthy subjects, oxidative damage is prevented by several defense mechanisms, which include interrelated antioxidant compounds (ascorbic acid, reduced

Abbreviations: ESR, electron spin resonance; TEMPO, 2,2,5,5-tetramethyl-4-piperidin-1-oxyl; PR-T, plasma reactivity with TEMPO; CE, capillary electrophoresis.

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glutathione, α-tocopherol, uric acid, bilirubin, albumin, etc.) and enzymes (catalase, superoxide dismutase, gluthatione peroxidase). Reduced levels of antioxidants can be the cause or the consequence of oxidative stress [1,2].

In biological samples, the antioxidant capacity is frequently evaluated using different methodologies [3,4]. Most of these procedures require a constant source of radicals and a sensitive method to detect the increase of the steady-state reactive radical concentration when the antioxidants are consumed. These methods provide different indexes such as TRAP [5] (total reactive antioxidant potential), TAR (total antioxidant reactivity), TEAC [6] (Trolox equivalent antioxidant capacity), ORAC [7] (oxygen radical absorbance capacity), FRAP [8] (ferric reducing/antioxidant power), etc. These indexes have been used in the study of water-soluble and/or lipid-soluble antioxidants, in different biological samples and conditions giving information about the quantity and/or the quality of the sample's antioxidants [9,10]. In blood plasma, these indexes are conditioned by the uric acid level because it accounts for more than 40% of the measured antioxidant activity [3,11].

The partial consumption of a stable free radical, like DPPH [12] (1,1-diphenyl-2-picryl-hydrazyl) and nitroxides [13–15], is another procedure that provides information about the antioxidant status of a sample. Furthermore, these radicals have the advantage that they can be directly detected by electron spin resonance (ESR) spectroscopy due to their paramagnetic properties, being the ESR signal intensity proportional to the nitroxide concentration [16,17].

Nitroxides were used as models of persistent free radicals to study the antioxidant function of ascorbic acid in human erythrocytes and may be useful as indicators of redox metabolism [18]. Ascorbate is the most effective water-soluble antioxidant in human blood plasma and reacts with most of the oxidizing radicals that could arise in biological systems, protecting biological compounds from oxidative damage [19–21]. It is known that the reaction between the ascorbate anion and a nitroxide radical produces the respective hydroxylamine and ascorbyl radical [22,23].

The aim of this work is the development of an alternative method for the evaluation of the plasma antioxidant status due to ascorbic acid using the scavenging of the nitroxide radical TEMPO (2,2,5,5-tetramethyl-4-piperidin-1-oxyl) and electron spin resonance spectroscopy (ESR). The method was applied to evaluate the antioxidant status before and after several days of ascorbic acid intake.

2. Materials and methods

2.1. Chemicals

TEMPO, albumin, glutathione and ascorbate oxidase were from Sigma Chem Co. Sodium ascorbate was from Backer and Trolox was from Aldrich. All other chemicals were of analytical grade. Ascorbate solutions were freshly prepared just prior to use.

2.2. Plasma samples

Studies were performed on 43 apparently healthy, consenting, non-smoking and non-alcohol drinking, 20 to 50 year old adults. Subjects were consuming a self-selected diet and following a normal physical activity. For ascorbate supplementation studies, 20 apparently healthy consenting adults received 1 g ascorbate/day during 8 days.

Heparinized blood samples were centrifuged at 2000 rpm for 10 min. Plasma aliquots were stored at −20 °C for TEMPO scavenging, which was carried out within the following 2 h. To achieve additional ascorbate concentrations of 50, 100 and 150 μmol/l, plasma aliquots were added with exogenous ascorbate immediately before performing the assays. Ascorbyl radical ESR spectrum was recorded immediately after plasma separation. Plasma ascorbate concentration was determined by capillary electrophoresis (CE). All samples were processed twice.

2.3. Ascorbate–TEMPO kinetics

(a) Ascorbate solutions between 10 and 100 μmol/l in 100 mmol/l buffer phosphate saline pH 7.6 (PBS) were used. TEMPO solution was added to give a final concentration in the reaction mixture of 256 μmol/l. Each sample was placed in the ESR cavity using an ESR quartz flat cell and then TEMPO ESR spectra were recorded at 20 °C at given acquisition times between 3 and 120 min. Measurements were obtained

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