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Ascorbate and dehydroascorbic acid as biomarkers of oxidative stress: validity of clinical data depends on vacutainer system used

Jens Lykkesfeldt*

Section of Biomedicine, Department of Veterinary Disease Biology, Faculty of Life Sciences, University of Copenhagen, DK-1870 Frederiksberg C, Denmark

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Abstract

Ascorbate and dehydroascorbic acid are frequently used as biomarkers of oxidative stress, but their lack of stability ex vivo and rapid postsampling interconversion continue to result in erroneous reference values. One problem is the large variety of vacutainer devices used for blood sampling purposes and the basic question of plasma vs serum as matrix. This study acquired blood samples by using 9 different and commonly used vacutainer systems followed by acidic stabilization and analysis by a well-validated method with the purpose of identifying acceptable means of collecting samples for proper ascorbate/dehydroascorbic acid analysis. In comparison, K_3-EDTA vacutainers were superior in maintaining low ex vivo oxidation of vitamin C.

Keywords: Ascorbate; Dehydroascorbic acid; Human plasma; Stability; Vacutainer systems

Abbreviations: ASC, ascorbate; DHA, dehydroascorbic acid.

1. Introduction

The universal antioxidant ascorbate (ASC) and its oxidized form dehydroascorbic acid (DHA) remain subjects of much interest both as biomarkers of oxidative stress and because further insight into vitamin C's role as cofactor in biological reactions continues to emerge. Optimized methodology ensuring precise and accurate quantification of ASC and DHA is a necessary prerequisite for continued progress in this field. However, proper analysis of ASC and, in particular, DHA in biological samples poses a significant challenge in clinical biochemistry. Particularly important issues include the lability of the compounds and rapid nonenzymatic interconvertion ex vivo. The stabilization of ASC and DHA in plasma and serum samples has been studied previously [1-7]. However, the techniques used for blood sampling may also have a substantial influence of the data obtained.

Vacutainer devices are commonly used for quick, safe, and reproducible blood sampling. In clinical studies, blood samples are commonly collected in, for example, heparin standard tubes, unless a specific protocol outlines differently. However, although numerous anticoagulant combinations for plasma and clotting systems for serum exist, no systematic test of vacutainers has been published with the purpose of limiting the loss of ASC and DHA and ex vivo formation of DHA from ASC.

In the present study, 8 commonly used vacutainer formulations were tested for their ability to maintain the in vivo equilibrium of ASC to DHA, with reference to a standard heparin sampling.

2. Methods and materials

2.1. Subjects

Blood samples were obtained from 7 apparently healthy individuals after informed consent but without collection of any data on the individual. The use of human blood for

* Tel.: +45 35 33 31 63; fax: +45 35 35 35 14.
E-mail address: jopl@life.ku.dk.

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quality control purposes is not subject to ethical approval in Denmark.

Blood samples for plasma were immediately centrifuged and stabilized with 10% (wt/vol) *meta*-phosphoric acid (Sigma, Brøndby, Denmark) containing 2 mmol/L Na_2-EDTA as reported previously and stored at −80°C until analysis [4].

2.2. Analytical methods

The following vacutainer types were used: Li-heparin (reference; plasma; BD 367869), K_3-EDTA (plasma; BD 368270), K_2-EDTA (plasma; BD 368861), citrate (plasma; BD 367704), fluoride + heparin (plasma; BD 367764), fluoride + Na-EDTA (plasma; BD 368521), fluoride + oxalate (plasma; BD 368921), GEL (serum; BD 367955), and Empty (serum; BD 367614) (all from BD, Brøndby, Denmark). The stability of ASC and DHA in *meta*-phosphoric acid–stabilized plasma has been studied previously and found adequate in preserving the in vivo equilibrium, and thus, provides a valid analytical platform to test the vacutainer systems [4]. Blood samples for serum were kept on ice in the dark for precisely 4 hours to allow clotting, after which the serum was separated by centrifugation and worked up as described previously. Ascorbate and total vitamin C (ASC + DHA) after reduction were quantified by high-performance liquid chromatography with coulometric determination as described elsewhere, and DHA was assessed by subtraction of ASC from total vitamin C using uric acid as an endogenous internal standard [4]. The within- and between-day coefficients of variation for the complete assay were less than 1.5% and 3.5%, respectively [8]. When the vacutainers contained liquid, the concentrations were corrected for the dilution factor. There were no significant differences in Δurate between sample pairs, demonstrating that the urate levels between tubes did not itself influence the results (data not shown).

2.3. Statistical analyses

Differences between vacutainer systems were analyzed by paired *t* test using heparin as a reference tube. For DHA, comparisons were also made to the K_3-EDTA vacutainer system that showed the comparably best ability to prevent postsampling oxidation judged by DHA levels. Paired statistical analyses in Fig. 1 were based on values normalized to the mean of the corresponding heparin tube concentrations. A P value less than .05 was considered statistically significant.

3. Results and discussion

Table 1 presents the concentrations of ASC and DHA in the tested vacutainer systems, whereas Fig. 1 shows the normalized data relative to those of heparin. Among the plasma samples, no significant differences were found in ASC/total vitamin C levels between heparin and the 2 types of EDTA tubes, showing that these vacutainers are optimal for obtaining ASC data. Previous reports have suggested either heparin or EDTA as superior in preserving ASC [1,2,7]. However, in our experience and as shown in Table 1, EDTA and heparin vacutainer tubes are equally suited for this purpose.

Plain tubes for serum also produced valid ASC data after 4 hours on ice. Previous experiments have shown substantial postsampling oxidation in plasma and whole blood stored on ice up to 2 hours [3]. In contrast to these results, the plasma from tubes containing fluoride as anticoagulant as well as the serum obtained from the GEL tube showed a significantly decreased ASC and total vitamin C level (Table 1). Citrate tubes also gave lower total Vitamin C levels compared with heparin. The decrease in total vitamin C levels after reduction of the DHA present suggests that degradation of DHA is increased when using these tubes. A significantly altered recovery of urate was also observed (Fig. 1).

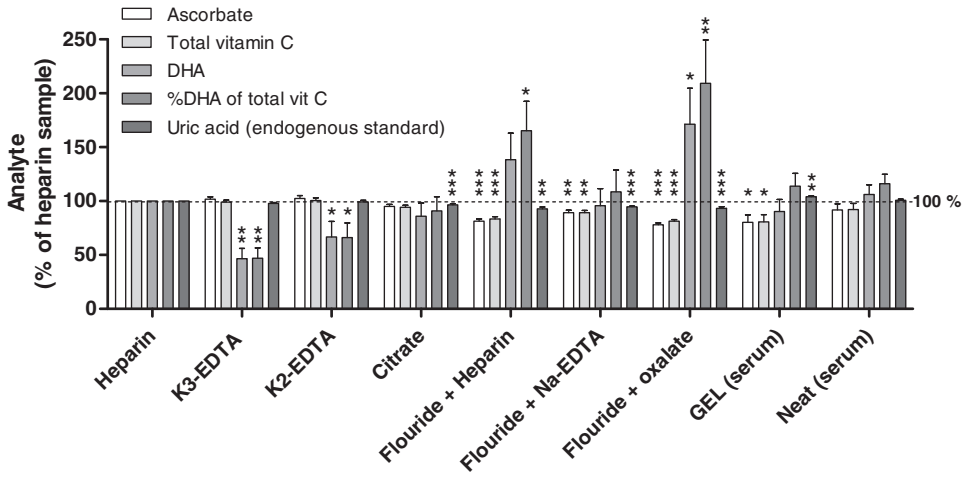

Fig. 1. Relative concentrations of ASC, total vitamin C, DHA, and uric acid in various vacutainers normalized to that of blood sampled in a vacutainer containing Li-heparin. *$P < .05$, **$P < .01$, and ***$P < .001$ by paired *t* test based on values normalized to the mean of the corresponding heparin tube concentrations.