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Clinica Chimica Acta 372 (2006) 188–194
www.elsevier.com/locate/clinchim

Prediction of serum total antioxidant activity from the concentration of individual serum antioxidants

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Received 3 March 2006; received in revised form 13 April 2006; accepted 13 April 2006
Available online 6 June 2006

Abstract

Background: Redox mechanisms are implicated in the pathogenesis of many diseases and several assays of total antioxidant capacity (TAOC) have been reported. Large epidemiological databases contain information on individual serum antioxidants as well as disease-specific phenotypic data. However, antioxidants work co-operatively in biological systems and it is important to be able to translate individual antioxidant measures into those of global antioxidant defence. Models therefore need developing to quantify contributions made by individual species to global antioxidant defence.
Objective: To develop a predictive model that translates individual antioxidant concentrations into an index of TAOC, enabling interrogation of epidemiological databases that contain information about individual antioxidants, but not about TAOC.
Methods: Sera from 256 volunteers were simultaneously assayed for key antioxidants and TAOC by enhanced chemiluminescence ($TAOC_{ECL}$). A predictive model was developed for serum TAOC using multiple linear regression analysis.
Results: The model explained 86% of $TAOC_{ECL}$ variability in serum. The strongest predictor of $TAOC_{ECL}$ was uric acid (1 SD increase associated with $TAOC_{ECL}$ increase of 103 μmol/l Teq–95% CI: 96.8–109), followed by vitamins A, C, E.
Conclusions: The reported model represents a powerful tool for interrogating databases where individual serum antioxidant concentrations are known, and TAOC measures are required.

Keywords: Total antioxidant activity; Uric acid; Vitamin A; Vitamin C; Vitamin E; Multiple linear regression analysis

1. Introduction

There is a considerable interest in the contribution of free radical mediated tissue damage to the pathogenesis of inflammatory diseases and malignancy and the antioxidant defence systems that protect against excessive radical activity to maintain tissue homeostasis [1,2]. Antioxidant mechanisms are important in protecting molecules, cells and tissues against the threat of oxidative damage in an aerobic environment [3]. The scavenging or "chain breaking" antioxidants are believed to confer substantial protection on vital cell structures, due to their cellular and extracellular ubiquity and rapid rates of sacrificial oxidation [3]. Such antioxidants work in concert through redox-cycling reactions, regenerating each other from their respective radical species [2,4]. For example, reduced glutathione (GSH) will regenerate α-tocopherol and vitamin C from their radicals, preventing further lipid peroxidation and cellular damage [5]. In recent years the impact of dietary antioxidants upon cellular and molecular processes has received substantial press [6].

The task of quantifying antioxidant activity can be approached in two different ways. Firstly, the concentrations of all of the individual molecules that are currently recognized as antioxidants can be measured. This approach presents several important problems. First, it is time consuming, expensive in terms of other resources and technically demanding. Second, it fails to yield information about their combined effectiveness i.e. the "total" antioxidant activity may be greater than the sum of the individual antioxidants because of the previously described co-operative interactions. Third, it does not account for the influence of

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0009-8981/$ - see front matter © 2006 Published by Elsevier B.V.
doi:10.1016/j.cca.2006.04.015

antioxidant substances that are as yet undiscovered or technically difficult to assay. Therefore, assays of "total" antioxidant activity have been developed to provide an overview of the biological interactions between individual antioxidant species and how efficiently these translate into host cell protection during periods of oxidative stress [7–17]. Such assays provide a measure of the capacity of biological systems to withstand oxidative stress. Predictably, there are some limitations to assays of total antioxidant activity (TAOC), principally that they provide limited information on specific mechanisms of radical removal and hence the contribution of individual antioxidant species to the pathobiology of specific diseases. Furthermore, the different systems used to measure TAOC appear sensitive to different antioxidants and the index of oxidative damage used to define the course of radical-induced oxidation also differs, hence "between assay" data are not necessarily comparable [18]. Nevertheless, such assays do permit reliable measurement of the ability to withstand specific oxidative stresses in vivo [19] and can be modified for use with several biological media [20].

Large epidemiological datasets now exist describing a variety of biological and clinical parameters in distinct populations, such as the National Health And Nutrition Examination Survey (NHANES) III database in the US [21]. These are powerful resources with the potential to further our understanding of the pathogenic processes underlying important diseases. However, none have provided measurements of TAOC, but have recorded the different serum antioxidants independently. The capacity to translate data on individual antioxidants to a measure of TAOC would provide a valuable investigative tool, with which to gain insight into the role and importance of global antioxidant defence systems in various disease processes, where oxidative stress is implicated in disease pathogenesis.

We have developed and reported an enhanced chemiluminescent (ECL) assay for measuring total antioxidant activity of biological tissues and fluids [9,20]. The aims of this study were (i) to describe mathematically the relationship between a variety of individual antioxidants and the total antioxidant activity measurement of serum samples, (ii) to determine the main contributors to variations in the $TAOC_{ECL}$ measurement, and (iii) to derive an equation to allow us to retrospectively derive total antioxidant activity values in samples for which levels of individual antioxidants are known. Such a predictive model will facilitate the retrospective interrogation of large epidemiological databases that already contain individual antioxidant concentrations to provide further information about the potential contribution of total antioxidant activity to disease susceptibility.

2. Materials and methods

2.1. Participants and blood samples

Blood samples ($n=256$) were obtained from a mixture of healthy subjects recruited from laboratory staff and the local community and from hospital outpatients attending a hypertension and diabetic clinic. Following an overnight fast, blood was taken from a forearm vein after 5 min rest in the sitting position. The blood was allowed to clot at 4 °C and immediately centrifuged to separate serum, which was rapidly cooled and frozen at −70 °C prior to analysis. For vitamin C assays, equal volumes of serum and 10% w/v metaphosphoric acid were vortex mixed to precipitate serum proteins before the deproteinized serum was frozen at −70 °C until analysis (within one month). No study subject was taking antioxidant vitamins at the time of blood sampling and visibly haemolysed samples were discarded and not assayed. All subjects gave their informed consent to participate in the study, which was approved by the South Birmingham Local Research Ethics Committee.

2.2. Experimental methods

Enhanced chemiluminescence (ECL) reagents were obtained from Amersham International (Amersham, Bucks, UK). These consisted of 'Amerlite' signal reagent buffer (pH 8.5, 30 ml), tablets A and B (containing luminol, *para*-iodophenol and sodium perborate) and horseradish peroxidase conjugate (anti-mouse immunoglobulin HRP-linked whole antibody from sheep). The water-soluble tocopherol analogue trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) was obtained from the Aldrich Chemical Company (Poole, Dorset, UK) and was used to make up standard antioxidant solutions for assay calibration. All other chemicals were obtained from the Sigma Chemical Co. (St. Louis, US) unless otherwise stated. HPLC grade methanol and water were obtained from BDH Chemicals (Poole, Dorset, UK) and GOLD label spectrophotometric grade petroleum ether was obtained from Aldrich Chemical Co. (Milwaukee, US). All other materials for HPLC were analytical grade also from BDH.

The total antioxidant activity (TAOC) of serum samples was determined by enhanced chemiluminescence (ECL) according to the method of Whitehead et al [9]. Briefly, the assay depends on the fact that a stable 'glowing' light emission is produced when the chemiluminescent substrate luminol is oxidized by hydrogen peroxide in the presence of both the catalyst horseradish peroxidase (HRP) and an enhancer phenol such as *para*-iodophenol [22]. The generation of light emission by ECL depends on the continuous production of free radical intermediates derived from both *para*-iodophenol and luminol and is abolished by radical-scavenging (chain-breaking) antioxidants (Fig. 1). Light emission is restored when all of the added antioxidants have been consumed in the reaction (Fig. 2). Light emission was measured using a conventional luminometer based on a side-window photomultiplier tube (EMI/Type 9781A, 94 μA lumen/L). The antioxidant activity of test serum solutions was determined by comparison of the period of light suppression it produced (t-serum) with that produced by a standard trolox solution (t-trolox) and was expressed as *micromoles of trolox equivalents/litre* (μmol/l Teq). The within-batch and between-batch coefficient of variation (CV) for serum samples was 1.7% and 5.0% respectively.

The urate concentration of serum and other samples was measured using an assay based on the enzymatic oxidation of urate in the presence of the enzyme uricase as previously described [23]. Serum vitamin C (ascorbate) concentration was determined by a modification of the isocratic reversed-phase HPLC method of Nagy and Degrell [24]. The within batch and between batch CVs were 2.8% and 5.3% respectively. Following sample deproteinization