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Urinary uric acid and antioxidant capacity in children and adults with Down syndrome

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Abstract

Objectives: To evaluate the urinary levels of uric acid (UA) and total antioxidant capacity (TAC) with and without UA relative contribution (TAC^{-UA}) in children and adults with Down syndrome (DS) and to prove the clinical use of TAC.

Design and methods: Urine samples were obtained from 32 individuals with DS and 29 controls. Two age groups were established (children and adults). Spectrophotometric methods were used for biochemical determinations.

Results: Children with DS had significantly higher UA/Cr and TAC/Cr levels than controls, whereas levels of TAC^{-UA}/Cr were lower in adults with DS than in controls ($P<0.05$ for all). In DS, levels of UA/Cr, TAC/Cr and TAC^{-UA}/Cr were higher in children than in adults ($P<0.05$ for all). Positive correlations between UA/Cr and TAC/Cr were found for all groups studied. Negative correlations with age were found for UA/Cr and TAC/Cr in children of both groups.

Conclusions: Our results proved that TAC is decreased in adults with DS. Besides, TAC^{-UA} seems to provide more reliable information about the antioxidant status, at least in DS.

Keywords: Down syndrome; Oxidative stress; Total antioxidant capacity; Uric acid; Urine; Aging

Introduction

Down syndrome (DS) or trisomy 21 is the most common chromosomal abnormality that comes to term in humans [1] occurring in about one in every 700-1000 live births [2]. It is associated with a wide variety of clinical features, including mental retardation, congenital heart disease, digestive problems, endocrine system deficits, cataracts, immune system disorders and increased risks of leukaemia and Alzheimer disease. Additionally, individuals with DS suffer from premature dementia and accelerated aging, and several studies have shown an increased oxidative stress in individuals with this pathology [3,4].

Antioxidant enzymatic alterations had been found in DS. The gene for Cu/Zn superoxide dismutase (SOD1) is coded on chromosome 21 and it is overexpressed (~50%) in DS [5] resulting in an increase of reactive oxygen species (ROS) due to an overproduction of hydrogen peroxide. ROS lead to oxidative damage of DNA, proteins and lipids. Moreover, the increase of enzymatic antioxidant defences such as SOD1 and catalase seems to be insufficient to prevent the exercise-induced oxidative damage in DS subjects which could be probably associated to a pro-oxidant status in this pathology [6]. Therefore, oxidative stress may play an important role in the pathogenesis of DS.

Oxidative damage can be monitored by the determination of different oxidative stress biomarkers. Some studies have shown higher levels of protein carbonyls, malondialdehyde, allantoin or 8-hydroxydeoxyguanosine in DS than in normal population [7–11]. Due to the ROS overproduction, a diminished

Abbreviations: ANOVA, analysis of variance; BCS, bathocuproinedisulfonic acid disodium salt; Cr, creatinine; Cu, copper; CUPRAC-BCS, copper(II) reduction assay with BCS as chelating agent; DS, Down syndrome; NS, not significant; ROS, reactive oxygen species; SD, standard deviation; SOD1, Cu/Zn superoxide dismutase; TAC, total antioxidant capacity; TAC^{-UA}, total antioxidant capacity without relative contribution of uric acid; TCA, trichloroacetic acid; UA, uric acid.

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antioxidants level may be found in DS because of their exhaustion. Separate measurement of different antioxidant molecules is not practical and their antioxidant effects are additive. Therefore, the total antioxidant capacity (TAC) is a useful measurement. Furthermore, the determination of any individual antioxidant could be less representative of the overall antioxidant capacity due to the possible *in vivo* interaction among different antioxidants. TAC is a measure of the amount (expressed in moles) of a given free radical scavenged by the non-enzymatic antioxidants which are present in a sample and in DS this parameter has not been studied in detail. Moreover, the studies are performed only with plasma or serum samples and in a reduced age range (usually only in children).

Uric acid (UA) is a powerful antioxidant and represents a high relative contribution of TAC in biological samples [12,13]. Several studies have reported elevated plasmatic levels of UA in DS, but urinary concentration of this antioxidant is not well known. UA has been associated to Alzheimer disease, cognitive decline, autism and sleep apnea [14–19], and these are pathologies related to DS [20–23]. Therefore, more studies are necessary on UA in DS to try to establish possible relations between this parameter and the pathogenesis of DS.

The present study was performed with urine samples due to the fact that they have several advantages over plasma, serum, saliva or cerebrospinal fluid spots for the determination of biochemical parameters. Urine collection is non-invasive, poses minimal infectious disease risk to participants and researchers and provides sufficient volume for multiple assays and future research. Furthermore, urine specimens are ideally suited for large studies because they can be collected and stored by participants, and compliance is high. Moreover, this sample is not usually used for the study of biochemical parameters in DS, being the first time that TAC had been evaluated in urine samples in this pathology. In addition, the study has been performed in children and also in adults. Since DS individuals suffer from accelerated aging, the age group of adults could be considered as a senescent group, as it was previously established by Bittles et al. [24].

The aim of this work was to compare the urinary levels of UA and antioxidant capacity, with and without UA contribution, in a sample of children and adults with DS with those of healthy age-matched controls in order to assess the role of oxidative stress in these subjects. Moreover, we evaluate the clinical use of urine TAC in a pathology with increased oxidative stress.

Materials and methods

Subjects

The study was performed in 32 individuals with Down syndrome and 29 healthy controls. Two age groups were established: group 1 (children group) consisted of 19 children with DS (13 male and 6 female, mean age = 7.6 ± 3.3 years ranging from 1 to 12) and 14 healthy age-matched controls (6 male and 8 female, mean age = 9.1 ± 3.0 years ranging from 5 to 13); group 2 (adult group) consisted of 13 adults with DS (7 male and 6 female, mean age = 48.8 ± 4.4 years ranging from 43 to 57) and 15 healthy age-matched controls (5 male and 10 female, mean age = 52.7 ± 5.3 years ranging from 43 to 61). All participants were non-smokers. Diagnosis of DS was confirmed by karyotyping. Informed consent was obtained from the participants or was given by parents. The study was approved by The Ethics Committee of the Spanish National Research Council.

Urine sample collection and preparation

First morning urine samples on an empty stomach were collected in a sterile flask without any preservative since, according to NCCLS approved guidelines on urinalysis and collection, transportation and preservation of urine specimens; chemical preservatives should be avoid for urinalysis [25] and in the same way Yilmaz et al. [26] showed that addition of preservatives (such as 6N HCl) is not necessary for measurement of uric acid in promptly assayed urine samples. The sterile flasks were covered with aluminium foil to keep out stray light.

All participants were appointed within the same timetable everyday and the group of samples collected each day was processed within 2 h of the collection. Samples were frozen at −20 °C until UA and TAC analysis. For creatinine (Cr) determination, samples were analyzed immediately, without previous freezing.

Reagents

Uric acid, creatinine, bathocuproinedisulfonic acid disodium salt (BCS) and copper(II) sulfate anhydrous were from Fluka (Buchs, Switzerland). *Candida* sp. uricase (EC 1.7.3.3) was from Sigma chemical Co. (St. Louis, MO) (ref. U0880). 2-Amino-2-(hydroxymethyl)-1,3-propanediol (Tris) was from Boehringer Mannheim GmbH (Mannheim, Germany). Trichloroacetic acid (TCA) was from Riedel-de Haën (Seelze, Germany). Other chemicals were from Panreac (Barcelona, Spain) and Probus (Barcelona, Spain). Deionized water was obtained from a Milli-RO water system (Millipore, Bedford, MA).

Biochemical determinations

UA was measured by the uricase spectrophotometric method of Duncan et al. [27] with modifications. The modifications consisted of the use of *Candida* sp. uricase, incubating the uricase solution mixture at 25 °C (maximum activity temperature of this enzyme) instead of 37 °C. Samples were preheated in a water bath at 60 °C during 5 min in order to dissolve the possible urates precipitates and were then diluted 1/10 with distilled water prior to analysis.

TAC was assayed by a quantitative colorimetric assay using the copper(II) reduction assay with BCS as chelating agent (CUPRAC-BCS) described by Campos et al. [28]. CUPRAC-BCS assay is based on the reduction of Cu(II) into Cu(I) by the action of the non-enzymatic antioxidants which are present in the sample. The chromogenic reagent BCS forms a stable complex with Cu(I) which has a maximum absorbance at