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Erythrocyte glycohydrolases in subjects with trisomy 21: Could Down's syndrome be a model of accelerated ageing?

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Abstract

We studied some erythrocyte glycohydrolases, erythrocyte membrane fluidity, plasma hydroperoxides and total antioxidant defences in 23 Down syndrome (DS) individuals in comparison with healthy age-matched and elderly controls.

With regard to erythrocyte plasma membrane fluidity, plasma hydroperoxides and total plasma oxidative defences, DS subjects resembled the age-matched controls more than the elderly ones. Membrane glycohydrolases in DS, however, presented a pattern partly similar to age-matched controls and partly to elderly controls. Concerning cytosol glycohydrolases, DS subjects had lower levels of hexosaminidase and *N*-acetyl-β-D-glucosaminidase, the latter specific for the hydrolysis of GlcNAc residues O-linked to proteins. In general, erythrocyte membrane and cytosol glycohydrolases decreased during erythrocyte ageing in DS subjects and in all controls. The increased levels of the same enzymes in DS plasma might be attributed to an alteration of their release-uptake mechanisms between the two different compartments, on account of the higher plasma hydroperoxide levels.

These findings indicate that erythrocyte ageing in DS differs partially from that of age-matched and elderly controls. In any case, the accelerated ageing seen in DS is no fully comparable to physiological ageing.

Keywords: Erythrocyte; Ageing; Down syndrome; Glycohydrolases; Oxidative stress

1. Introduction

Down's syndrome (DS), the most frequent chromosomal alteration in man, involves an extra copy of chromosome 21 or part of it (Sever et al., 1970). DS children suffer from many diseases, such as cardiovascular diseases, increased susceptibility to infections, leukaemia, endocrine alterations, immune defects, nutritional disturbances and mental retardation (Baird and Sadovnick, 1988). The reason for mental retardation is not known. Alterations of chromosome 21 are also seen in Alzheimer's disease (AD) (Wisniewski et al., 1985; Mann and Esiri, 1989), together with several of the neuropathological features also present in DS subjects in the fourth decade of life (Wisniewski et al., 1985; Mann and Esiri, 1989; Licastro et al., 2005); these include reduction of nerve cells, changes in the phospholipid composition of membranes, neuritic plaques, neurofibrillary tangles, degeneration of the basal forebrain cholinergic neurons and dementia.

DS and AD share some of the physiopathological characteristics of ageing and DS is considered a precocious and/or accelerated model of senescence (Walford et al., 1981). In the development of AD (Jeandel et al., 1989), like in other age-related chronic degenerative diseases, such as diabetes and atherosclerosis (Baldini et al., 1989; Stringer et al., 1989; Rabibi et al., 1993; Ames et al., 1993), oxidative stress is crucial. The theory that oxidative stress is also the reason for the accelerated senescence of DS subjects is widely supported (Busciglio and Yankner, 1995; De Haan et al., 1997). In DS there is a rise of reactive oxygen species (ROS) and a drop in total plasma antioxidant defences (Carratelli et al., 2001).

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In addition, in DS and AD the erythrocytes may show alterations (Bosman et al., 1993; Kantar et al., 1992; Jayakumar et al., 2003). Some erythrocyte membrane glycohydrolases are involved in specific chronic-degenerative pathologies (Goi et al., 1999; Venerando et al., 2002). Studies on erythrocytes of different ages showed that these enzymes can serve as a useful early tool for evaluating alterations associated with ageing in healthy subjects (Goi et al., 2005).

On the basis of these considerations, we studied some erythrocyte characteristics of DS subjects in comparison with age-matched and elderly individuals, with a view to clarifying how peroxidative processes are involved in ''early ageing''. We therefore analyzed: (a) glycohydrolase activity in erythrocyte plasma membrane and cytosol; (b) plasma membrane erythrocyte fluidity; (c) plasma hydroperoxides; (d) total plasma antioxidant defences.

2. Materials and methods

2.1. Materials

Commercial chemicals were of the highest grade available. The water routinely used was freshly redistilled in a glass apparatus. 1,6-Diphenyl-1,3,5-hexatriene (DPH) and its cationic derivative 1-[4-(trimethyl-amino)-phenyl]-6-phenyl-1,3,5-hexatriene (TMA-DPH) were purchased from Molecular Probes Inc. (Eugene, OR, U.S.A.); 4-methylumbelliferone (MU), 4-methylumbelliferyl-α- and -β-glycosides, *N*-acetyl-D-neuraminic acid (NeuAc), crystalline bovine serum albumin, *N*-2-hydroxyethylpiperazine-*N*-2-ethane sulphonic acid (HEPES) and bovine serum albumin were from Sigma Chemical Co. (St. Louis, MO, U.S.A.). Dowex 2 × 8 resin (200–400 mesh) was obtained from Bio-Rad Laboratories (Richmond, VA, U.S.A.).

2.2. Subjects

Twenty-three DS individuals, aged 44.1 ± 12.5 [18–58] years, 9 over 50 years (7 men and 2 women), 14 under 50 years (11 men and 3 women), were assessed by clinical examination and karyotype analysis. They had a mild but variable degree of mental retardation; they were free of other pathological conditions at the moment of the study and were in good health status. All the DS subjects were infection-free and none had taken any drugs in the 2 weeks before blood sampling, they were without dyslipidemia, anaemia, diabetes, thyroid pathologies, hypertension or any family history of coronary heart disease, no smokers or drinkers, with a body mass index (BMI) < 25. None of them were following a special diet or taking vitamin and trace element supplements. This study was conducted in accordance with the Declaration of Helsinki, 1975, amended in 1983. All relatives were aware of the aims of this investigation and gave their signed informed consent.

Controls, females and males, were divided into two groups: group A comprised 80 people age-matched with DS subjects, 39.6 ± 10.6 [21–60] years, adult volunteer blood donors at the National Tumour Institute, Milan, Italy; group B were 55 healthy elderly people, 66.8 ± 13.4 [61–93] years, attending the Department of Experimental Pathology, Immunology Unit, University of Bologna, Italy, for routine blood and biochemical tests.

The following exclusion criteria were applied to controls: hypertension, diabetes mellitus, cardio- or cerebrovascular events, respiratory and hepatic diseases, malignant neoplasm, major organ failure, disability, insufficient cognition (MMSE score < 20) and alcoholism. None of the controls was following a special diet or taking vitamin and trace element supplements. The data are mean ± S.D. with ranges in square brackets.

2.3. Blood samples

Heparinized venous blood was taken in the morning between 7 and 10 a.m. after fasting overnight and used for haematological procedures and to obtain erythrocytes and plasma. Plasma aliquots were immediately frozen in liquid nitrogen and stored at −80 °C until further assays. Erythrocytes were separated from leukocytes and platelets by filtration through a column of α-cellulose and microcrystalline cellulose (2:1, w/w), according to the method of Beutler et al. (1977). The haematocrit of filtered blood was adjusted to approximately 30% and the suspension was used immediately. Unsealed ghost membranes were prepared at 4 °C according to Steck and Kant (1974), employing hypotonic treatment (from 5.0 to 1.25 mmol/L PBS, pH 7.2); cytosol was obtained by pooling the three supernatants.

2.4. Fractionation of erythrocytes according to age: young, middle-aged and senescent

Human erythrocytes were divided into young, middle-aged and senescent erythrocytes by the Percoll discontinuous density gradient/centrifugation method described by Salvo et al. (1982), and modified by Mosca et al. (1991). Each fraction was characterized for cell age according to Fehr and Knob (1979), by measuring MCV with a cell counter (Model Z_{BI}, Coulter Electronics, Hialeah, FL, U.S.A.). The erythrocytes were stored at 4 °C (no longer than 1 h), and homogeneously suspended in HEPES buffered isotonic saline solution immediately before use.

2.5. Enzyme assays

The activities of resealed membrane sialidases (E.C. 3.2.1.18) were routinely determined by fluorimetric methods (Venerando et al., 1997). Glycohydrolases, hexosaminidase (E.C. 3.2.1.52), β-D-glucuronidase (E.C. 3.2.1.31), β-D-glucosidase (E.C. 3.2.1.21), β-D-galactosidase (E.C. 3.2.1.23), α-D-glucosidase (E.C. 3.2.1.20), α-D-galactosidase (E.C. 3.2.1.22), α-D-mannosidase (E.C. 3.2.1.24) and α-L-fucosidase (E.C. 3.2.1.51), were assayed fluorimetrically in plasma membrane by the method of Goi et al. (2000), in cytosol by the method of Venerando et al. (2003) and in plasma by the method of Lombardo et al. (1980). To determine the activity of *N*-acetyl-β-D-glucosaminidase (O-GlcNAcase) (E.C. 3.2.1.52), which is active only toward GlcNAc derivatives, the assay employed 4-methylumbelliferyl-*N*-acetyl-β-D-glucosaminide (4MU-GlcNAc) as substrate, with GalNAc (50 mmol/L) as competitive inhibitor (Dong and Hart, 1994). Enzyme activities in membrane were expressed as μUnits/mg protein, and in plasma and cytosol as mU/L and μU/mL, respectively.

2.6. Ghost fluorescence anisotropy

We assessed the membrane fluidity of the hydrocarbon core and the region of phospholipid head groups by measuring, respectively, the steady-state anisotropy of DPH, as previously described (Cazzola et al., 2003), and TMA-DPH. The DPH and TMA-DPH probes were excited at a wavelength of 340 nm, and the emission wavelength was set at 420 nm. Samples were then excited with vertically polarized light and we measured the intensity of emitted light, vertically (Iv) and horizontally (Ih) polarized with respect to the exciting light. Anisotropy was calculated using the equation rs = (Iv − Ih)/(Iv + 2Ih), where rs is inversely related to membrane fluidity.

2.7. Plasma peroxidation

Plasma lipid hydroperoxide levels were determined colorimetrically (Yalcin et al., 1993) and expressed as H_2O_2 equivalents. The kinetics of plasma oxidation were determined at 37 °C by monitoring the development of fluorescence at 430 nm, setting the excitation at 360 nm as described by Cervato et al. (1999). The fluorimetric kinetic profile of each plasma sample, divided into latency, propagation and steady-state phases, gives an indication of the lag-time, expressed in minutes and calculated as the intercept of the linear regression of the propagation phase and the latency phase.

2.8. Other methods

Protein content was determined by the method of Lowry et al. (1951), using crystalline bovine serum albumin as standard; when HEPES buffer was present, we used the Coomassie Brilliant Blue Bradford method (1976). Total sialic acid