Progress in Neuro-Psychopharmacology & Biological Psychiatry 33 (2009) 76–80

Contents lists available at ScienceDirect

Progress in Neuro-Psychopharmacology & Biological Psychiatry

journal homepage: www.elsevier.com/locate/pnpbp

Oxidative stress and cognitive ability in adults with Down syndrome

Andre Strydom [a,*], Mark J. Dickinson [b], Simadevi Shende [c], Domenico Pratico [d], Zuzana Walker [a,c]

[a] Department of Mental Health Sciences, RFUCMS, UCL, Hampstead Campus, Rowland Hill Street, London, NW3 2PF, UK
[b] Enfield Primary Care Trust (Learning Disability Service), Cumbria Villa, Chase Farm Hospital, 1 The Ridgeway, Enfield, Middlesex, EN2 8JL, UK
[c] North Essex Mental Health Partnership Trust, St Margaret's Hospital, The Plain, Epping, Essex, CM6 6TN, UK
[d] Department of Pharmacology, School of Medicine, Temple University, MRB room 706A, 3420 North Broad Street, Philadelphia, PA 19140, USA

ARTICLE INFO

Article history:
Received 30 May 2008
Received in revised form 12 September 2008
Accepted 11 October 2008
Available online 25 October 2008

Keywords:
Down syndrome IQ
Glutathione peroxidase
Lipid peroxidation
Oxidative stress
Superoxide dismutase

ABSTRACT

Aims: We aimed to study the hypothesis that high levels of superoxide dismutase (SOD1), previously reported in Down syndrome, would be associated with poorer ability on cognitive tests. Compensatory rises in the activity of glutathione peroxidase (GPx) was expected to be associated with better ability, so that a high ratio between SOD1 and GPx was hypothesised to be the best predictor of poorer cognitive performance.
Methods: 32 adults with Down syndrome between the ages of 18 and 45 years donated blood samples for SOD1 and GPx assays and urine for Isoprostane 8,12-*iso*-iPF$_{2\alpha}$-VI assay, a specific biomarker of lipid peroxidation in vivo. Informants rated functional ability and memory function for all participants, and those adults with DS that was able to, also completed psychometric assessments of language ability and memory.
Results: Neither SOD1 nor GPx were related to the elevated markers of lipid peroxidation previously described in living adults with DS, and our hypothesis that an increased SOD1/GPx ratio would be correlated with worse performance on cognitive or functional measures was not supported. Contrary to our hypothesis, we found that low SOD1/GPx ratios were associated with worse memory ability, which remained after controlling for confounders such as sex, age or nutritional supplements.
Conclusions: The anti-oxidant system in DS is implicated in the cognitive phenotype associated with the chromosomal disorder, but the variations in the phenotype could result from several possible gene or gene product interactions. Much further research is required before it will be possible to counteract the oxidative stress associated with DS.

1. Introduction

Down syndrome, caused by the partial or complete triplication of chromosome 21, is the most common genetic cause of intellectual disability (ID) (also known as mental retardation). It is characterised by premature aging and is associated with early development of dementia (Hill et al., 2003). As a result of improvements in health and social care, the average life expectancy has increased dramatically (Day et al., 2005). However, by the age of 40, virtually all patients with Down syndrome have neuropathological and neuro-imaging changes characteristic of Alzheimer's disease (Roizen and Patterson, 2003) (Teipel and Hampel, 2006). Post mortem and in vitro studies have shown that oxidative stress plays a role in the pathogenesis of many of the clinical features of Down syndrome, and elevated levels of Isoprostane 8,12-*iso*-iPF2α (iPF2α), a specific marker of lipid peroxidation, have been demonstrated in living adults with DS (Pratico et al., 2000).

Cell damage can be induced by reactive oxygen species (ROS) through alteration of macromolecules such as polyunsaturated fatty acids in lipids of membranes, essential proteins, and DNA (Mazza et al., 2007). Under normal conditions, ROS, produced in vivo mainly by aerobic respiration, are cleared from the cell by the action of the antioxidant enzymes, superoxide dismutase (SOD), catalase, or glutathione peroxidase (GPx) (Zana et al., 2007). Cu Zn superoxide dismutase (SOD1) converts the superoxide radical to cytotoxic hydrogen peroxide which is further detoxified by glutathione peroxidase or catalase. As a result of over expression of SOD1 encoded by genes on chromosome 21 in Down syndrome, there may be an imbalance of the ratio of anti-oxidant enzymes (SOD1, GPx and catalase) which could result in oxidative damage to various molecules. There is variability in anti-oxidant enzyme levels within the DS population, which may be related to the degree of intellectual disability, premature aging and dementia seen in DS (Dickinson and Singh, 1993; Jovanovic et al., 1998; de Haan et al., 2003).

Our hypothesis was that high levels of SOD1, previously reported in Down syndrome, would be associated with higher levels of oxidative stress (lipid peroxidation) and poorer function or ability on tests. Compensatory rises in the activity of GPx was expected to be

Abbreviations: ABAS, adaptive behaviour assessment scale; BPVS, British picture vocabulary scale; CBS, cystathionine β synthase; DMR, Dementia Questionnaire for Persons with Mental Retardation; DS, Down syndrome; GPx, glutathione peroxidase; ID, intellectual disability; iPF2α, Isoprostane 8,12-*iso*-iPF$_{2\alpha}$-VI; MOMT, modified object memory task; ROS, reactive oxygen species; SOD1, Cu/Zn superoxide dismutase.

* Corresponding author.
E-mail address: a.strydom@medsch.ucl.ac.uk (A. Strydom).

0278-5846/$ – see front matter.
doi:10.1016/j.pnpbp.2008.10.006

associated with better ability, so that a high ratio between SOD1 and GPx was hypothesised to be the best predictor of poorer cognitive performance. In addition, higher levels of lipoperoxidation in urine samples were also expected to be associated with poorer cognitive performance.

2. Methods

2.1. Patient population

The study was approved by research ethics and informed consent was obtained from the carer and the participant. If participants lacked capacity to consent, assent was sought from their carers. Our sample size was calculated to be sufficient to detect moderate correlations based on previous studies (Brugge et al., 1992; Sinet et al., 1979) with a power of 80% and significance level of 0.05. Adults with Down syndrome between the ages of 18–45 years from London were recruited through local ID teams, residential care homes and the UK Down Syndrome Association. Those diagnosed with dementia or mental illness were excluded from the study. Down syndrome status was identified on the basis of the phenotype and patient records of karyotyping or blood chromosomal analysis.

2.2. Enzyme assays and biomarker of lipid peroxidation

Blood samples for enzyme analysis (SOD1 and GPx) and urine samples for analysis of lipid peroxidation was obtained. Blood reached the laboratory within 24 h of collection. Detection of SOD1 was based on how effectively the maximal production of superoxide by toxic stimulation of neutrophils with diolein can be switched off by an extract of patient's red blood cells (which contain SOD) with a normal range of 41–47%. SOD1 level was also measured using standard spectrophotometric methods. GPx activity was determined using the method of Paglia and Valentine based on the NADPH coupled reaction (Paglia and Valentine, 1967). The reference interval for red cell GPx was 67–90 international units of activity per gram of haemoglobin, and 240 to 410 enzyme units for SOD1. Urine spot samples were collected on the same day as the blood samples, stored within 6 h of collection at −80 °C, and analysed within 12 months. F_2-isoprostanes are isomers of the prostaglandin $F_{2\alpha}$ produced by reactive oxygen species attack on polyunsaturated fatty acids. They are chemically stable, sensitive and specific biomarkers of lipid peroxidation in vivo. Urine samples were used to measure Isoprostane 8,12-*iso*-iPF$_{2\alpha}$-VI, assayed by gas chromatography and mass spectrometry (Pratico, 1999).

2.3. Psychometric assessments

Psychometric assessments were completed within 6 weeks of collection of the blood sample. Functional ability was determined with the Adaptive Behaviour Assessment Scale (ABAS) (Harrison and Oakland, 2000). The British Picture Vocabulary Scale II (BPVS II) (Dunn and Dunn, 1997) was used to provide a measure of the approximate developmental level, and to measure vocabulary acquisition, an important facet of intelligence (Glenn and Cunningham, 2005). The cognitive subscale of the Dementia Questionnaire for Persons with Mental Retardation (DMR) (Evenhuis, 1996) was scored by carers to provide a proxy measure of memory function in all participants, including those adults with DS who were not able to complete assessments. Although the tool was originally designed to screen for dementia rather than to rate cognitive function, it has been found to correlate well with direct measurements of memory (Nelson et al., 2005). A Modified Object Memory Task (MOMT) was used as a supplementary directly assessed measure of delayed short term memory (Burt and Aylward, 2000). It involved naming a total of 6 objects in 2 stages and testing immediate recall followed by delayed recall of all 6 items after 5 min.

2.4. Data analysis

Data was coded and analysed with Statistical Package for the Social Sciences (SPSS) version 11 (SPSS inc, 2001). The SOD1/GPx activity ratio was calculated from the logarithms of the activities because of the different orders of magnitude (Pastor et al., 1998). Logarithmic transformation was also used to achieve approximate normal distributions for DMR scores. Correlations between the psychometric and functional assessments scores (BPVS, DMR, ABAS) and differences between the subgroups were examined using Pearson's correlations, *t*-tests or non-parametric statistics. DMR scores was entered as the dependent variable in a linear regression analysis with SOD1/GPx ratio as the dependent variable to investigate the association between these two variables with adjustment for age, sex and exogenous anti-oxidant supplementation. The significance level was set at 0.01.

3. Results

3.1. Demographics

32 individuals with DS, ranging from 18 to 45 years of age participated in this study. Demographic details are given in Table 1. None of the participants smoked. Of the 16 subjects who had karyotyping done, 13 had trisomy 21, 2 had translocations and one a mosaic. Participants with thyroid dysfunction were all receiving treatment, and none had clinical features of thyroid disorder. Those with epilepsy were diagnosed during childhood, and were stable on treatment. None of the participants had any other major neurological disorders. Hearing and vision deficits were not severe and did not prevent participation in any of the psychometric tests.

3.2. Psychometric assessments

Three of the 32 participants were unable to complete the BPVS II because they had very limited or no speech. Out of a possible 168, the

Table 1
Participant demographics

N=32	Frequency	Percent (%)
Sex		
Male	18	56
Female	14	44
Mean age	32.59±6.78	
Ethnicity		
White	24	75
Asian	3	9
Black	5	16
Cardiovascular disorder		
No cardiac disorder	25	78
Cardiac disorder	7	22
Visual disorder		
No visual disorder	15	47
Visual disorder	17	53
Hearing disorders		
No hearing disorder	19	59
Hearing disorder	13	41
Thyroid disorder		
No thyroid disorder	25	78
Thyroid disorder	7	22
Epileptic disorder		
No epileptic disorder	30	94
Epileptic disorder	2	6
Vitamins and supplements		
No	20	64.5
Yes	11	35.5
Missing	1	
Intellectual disability level		
Mild	10	31
Moderate	18	56
Severe	4	13