Lipid peroxidation in Down syndrome caused by regular trisomy 21, trisomy 21 by Robertsonian translocation and mosaic trisomy 21

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Abstract

Background: It has been suggested that an increase in oxidative stress in individuals with Down syndrome (DS) may cause adverse effects in the cell membranes through the oxidation of polyunsaturated fatty acids.

Methods: We examined erythrocyte malondialdehyde (MDA) levels in 100 individuals of both sexes (34 males and 66 females) with DS, aged from newborn to 29 years. The cytogenetic analysis revealed 90 individuals with regular trisomy 21, four individuals with trisomy 21 by Robertsonian (Rb) translocation, and six individuals with mosaic trisomy 21. DS individuals were divided into six age groups. The control group consisted of 100 healthy individuals of both sexes (40 males and 60 females) who were age-matched with DS subjects.

Results: No significant differences were found in erythrocyte MDA levels between the sexes in any of the age groups for the DS group and the control group. We confirmed significantly higher erythrocyte levels of MDA in individuals with DS compared to the control group. A significant difference was observed in erythrocyte MDA levels between DS individuals with trisomy and controls for all age groups, and in individuals with DS due to Rb translocation trisomy. However, in DS individuals with mosaicism, MDA levels depended on the percentage of diploid and trisomy cells.

Conclusions: Our results confirm an increase in lipid peroxidation in patients with DS.

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Keywords: Down syndrome; free radicals; lipid peroxidation; malondialdehyde; oxidative stress.

Introduction

Down syndrome (DS), a genetic abnormality associated with the presence of three copies of chromo-

some 21, reported by Lejeune et al. (1), is one of the most important human congenital diseases, occurring in 1 out of 700–1000 live births (2). The disease is associated with immune system disorders, autoimmune processes, increased incidence of heart defects, and gastrointestinal anomalies. Higher incidence of leukaemia and other haematological disorders is common in affected individuals. However, the most important features of DS are mental retardation and accelerated ageing. Numerous studies link both of these abnormalities to disturbances in oxygen metabolism. Reactive oxygen species (ROS) are substances that are released during oxidative metabolism. ROS include the superoxide anion ($O_2^{\cdot-}$), hydrogen peroxide (H_2O_2), and the hydroxyl radical ($OH^{\cdot}$) (3). The reaction of ROS with macromolecules can lead to DNA mutations, changes in the structure and function of proteins, and peroxidative cell-membrane lipids (4). Oxidative stress may result from an excess of Cu/Zn superoxide dismutase (SOD-1) activity in DS individuals due to an extra copy of the SOD-1 gene located on chromosome 21.

Lipid peroxidation is an autocatalytic process that is a common consequence of cell death, and is an important biological consequence of oxidative cellular damage. ROS have been suggested to exert their cytotoxic effect by peroxidation of polyunsaturated fatty acids of the membrane, changing the permeability of cellular membranes, increasing their fluidity, their rigidity and in some cases making them lose their integrity and increasing the risk of membrane rupture. Malondialdehyde (MDA) is the most abundant individual aldehyde resulting from lipid peroxidation; thus, MDA can be considered a marker of the lipid peroxidation (5). In this way, higher MDA levels could contribute to oxidative stress (6). In addition, MDA itself, because of its high chemical reactivity, can generate other cellular alterations, both structural and functional. MDA is generally measured by the 2-thiobarbituric acid (TBA) method, although this is not specific for free MDA, since many other substances that can occur in biological material react positively with TBA. In this study MDA was determined by high-performance liquid chromatography (HPLC). This method is highly specific and accurate, and distinguishes between true MDA and other aldehydes that may react with TBA (7).

In this work, we investigated MDA levels, an end-product of lipid peroxidation, in a sample of DS individuals and a group of healthy subjects age-matched to the DS individuals to establish any change in levels due to oxidative stress, and matching oxidative stress

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to different types of DS: regular trisomy 21, trisomy 21 caused by translocation and mosaic trisomy 21.

Materials and methods

Sample of DS individuals

We examined 100 individuals (34 males and 66 females) with DS, aged from newborn to 29 years. These individuals had no associated anomalies. In all individuals, DS diagnosis was confirmed by cytogenetic analysis, which identified 90 subjects with regular trisomy 21, four subjects with Robertsonian (Rb) translocation trisomy t(q21q21) and six subjects with mosaic. They were registered as outpatients in the Social Paediatric Unit at Niño Jesús Hospital and the Paediatric Service at San Rafael Hospital, both in Madrid (Spain). The study was approved by the Ethics Committee of the Superior Council of Scientific Investigation in Spain. Each participant or a legal guardian signed an informed consent form detailing the analysis and handing of the data.

Control group

The control group comprised 100 subjects with a normal diploid complement of chromosomes and clinically healthy individuals of both sexes (40 males and 60 females), who were age-matched with DS individuals. The subjects of the control group were recruited from kindergarten, primary and secondary schools in Madrid (Spain). Biological samples were taken after prior written consent was given by parents.

The individuals with DS and control subjects were divided into six age groups: group 1, newborn–1 year ($n=20$); group 2, 2–4 years ($n=20$); group 3, 5–9 years ($n=18$); group 4, 10–14 years ($n=15$); group 5, 15–19 years ($n=15$); and group 6, 20–29 years ($n=12$).

Blood sampling

Blood samples were obtained by venipuncture. Fresh blood (5 mL) was collected in tubes containing lithium heparin and stored at 0–4°C. All assays were carried out within 24 h of sampling.

Blood samples were taken according to the principles of the Helsinki declaration.

Chromosome analysis

Chromosome analysis was performed on cultured peripheral blood lymphocytes. Three different handling techniques were used to examine the chromosomes in detail: GTG, CBG and RHG bands.

MDA determination

MDA levels were measured in erythrocytes according to the method of Bull and Marnett (8). Blood was centrifuged at $2500\times g$ for 10 min and the plasma was then removed as completely as possible. Erythrocytes were washed three times with 0.9% NaCl solution and subsequently haemolysed by addition of distilled water. The supernatant solution was diluted by addition of acetonitrile (v/v), followed by vigorous mixing. The mixture was centrifuged at $3000\times g$ for 5 min and the resulting supernatant solution was filtered (0.2- μ m pore membrane).

HPLC was performed on an LKB Bromma 2151 system (Pharmacia LKB Bromma, Bromma, Sweden) equipped with an LKB Bromma 2151 model diode-array UV detector and ChromJet integration Spectra-Physics data processor (PEMED) Production Engineering-Medical Equipment Division (Denver, Colorado, USA), which allowed analysis of the peak purity. An ODS Hypersil column (25 cm $\times$ 4.6 mm, 5 μ m) (Shandon Scientific Ltd., Runcorn, Cheshire, UK) was used as the stationary phase. The chromatographic conditions employed were: mobile phase, PO₄HNa₂ and myristyl trimethylammonium bromide buffer (pH 7.4)/acetonitrile; flow rate, 0.4 mL/min; UV detection, 268 nm; chart speed, 0.5 cm/min; attenuation, 6; temperature, ambient; and injection volume, 10 μ L.

The column was equilibrated at the beginning of each daily series of measurements with at least 50 mL of the eluant. The MDA peak in the chromatogram was identified by comparison with a reference chromatogram of freshly prepared free MDA. The concentration of MDA was calculated from the area, based on a calibration chromatogram performed with a standard solution of MDA prepared by acid hydrolysis as described by Esterbauer et al. (9).

Statistical analysis

Data were processed using standard statistical software such as SPSS 10.0 (SPSS Inc., Chicago, IL, USA). Results are expressed as mean $\pm$ SD. The distribution of the groups was analysed using the Kolmogorov-Smirnov test. As both groups showed a normal distribution, parametric statistical methods were used. For comparison of the groups, Student's t-test and analysis of variance (ANOVA) were performed. Differences were considered significant for $p<0.05$.

Results

No statistically significant differences were found in erythrocyte MDA levels between the sexes in any age group for the DS group and the control group. As shown in Table 1, erythrocyte MDA levels increased with age in both individuals with trisomy 21 and control subjects. Our results show that MDA levels were

Table 1 Erythrocyte MDA levels in individuals with DS (regular trisomy 21) and in the control group according to age.

Age, years	DS individuals		Control group		p
	n	MDA, nm/mg Hb	n	MDA, nm/mg Hb	
Newborn–1	16	112.37 $\pm$ 5.14	20	86.92 $\pm$ 5.14	<0.05*
2–4	17	136.25 $\pm$ 5.06	20	114.36 $\pm$ 5.32	<0.05*
5–9	18	161.39 $\pm$ 5.34	18	132.68 $\pm$ 3.09	<0.05*
10–14	14	184.75 $\pm$ 4.16	15	156.11 $\pm$ 5.02	<0.05*
15–19	14	228.19 $\pm$ 5.28	15	189.36 $\pm$ 4.71	<0.05*
20–29	11	263.36 $\pm$ 6.15	12	217.22 $\pm$ 5.30	<0.05*

*Statistically significant differences.

Table 2 Erythrocyte MDA levels in individuals with DS due to mosaic.

Karyotype	Age	Normal cellular line, %	Trisomic cellular line, %	MDA, nm/mg Hb	MDA, nm/mg Hb ^a
47,XX+21	15 months	91	9	107.38	86.92±5.14
47,XX+21	2 years	65	35	121.05	114.36±5.32
47,XX+21	3 years	19	81	129.31	114.36±5.32
47,XY+21	19 months	20	80	113.41	86.92±5.14
47,XX+21	11 years	95.70	4.30	169.05	156.11±5.02
47,XX+21	22 years	98	2	219.14	217.22±5.31

^aMean±SD MDA level in age-matched control group.

significantly higher ($p<0.05$) in DS individuals with regular trisomy compared to controls of all ages.

Table 2 shows erythrocyte MDA levels in six individuals diagnosed with DS caused by mosaic and in age-matched controls. The results show that MDA levels depend on the percentage distribution between diploid and trisomic cellular lines. Compared to individuals belonging to the control group of similar age, we observed that, in general, the higher the percentage of diploid cells, the lower are erythrocyte MDA levels. Lower erythrocyte MDA levels could contribute to lower oxidative stress.

Table 3 shows erythrocyte MDA levels in four individuals with DS due to Rb translocations of chromosome 21 compared to age-matched individuals with regular trisomy 21. The erythrocyte MDA levels obtained in these cases are similar to those observed in individuals with DS due to regular trisomy 21.

Discussion

No statistically significant differences were found in erythrocyte MDA levels between the sexes in any age group within the DS group and the control group. Our findings are in agreement with those reported by Gil et al. (10), who assayed biological variations in erythrocyte MDA levels in healthy subjects from newborns to old people aged 80 years and older. However, Inal et al. (11) found higher MDA levels in women than in men aged 40–69 years, perhaps due to menopause (12).

As shown in Table 1, erythrocyte MDA levels seem to increase with age in individuals with DS caused by regular trisomy 21 and in control subjects. Our results show that peroxidative damage increases with the ageing process, with a positive correlation between MDA levels and age (DS individuals: $\text{MDA level} = 30.20 \times \text{age} + 75.84$; $r^2 = 0.9885$; $p < 0.05$; controls: $\text{MDA level} = 25.69 \times \text{age} + 59.72$; $r^2 = 0.9935$; $p < 0.05$). Similar findings have been reported by Inal et al. (11). Rodriguez et al. (13) indicated that plasma MDA levels

increase with age in healthy subjects, while Nohl (14) reported accumulation of lipid peroxidation products during ageing.

Interestingly, our results show that MDA levels were significantly higher ($p < 0.05$) in DS individuals with regular trisomy compared to control groups of all ages. Our findings are in agreement with urine MDA levels in DS patients reported by Jovanovic et al. (15) and serum MDA in DS patients reported by Muchová et al. (16). However, our data show some differences when compared with erythrocyte MDA levels calculated by Muchová et al. (16) for 37 DS patients using the TBA reaction. These discrepancies may be due to the different methods used to determine MDA or differences in the study populations.

Zitnanova et al. (17) detected no change in 4-hydroxy-2-nonenal (4-HNE) levels in plasma of patients with DS compared to healthy controls. 4-HNE, another end product of lipid peroxidation, is not a stable product of oxidative damage to lipids. It may be continuously removed or detoxified by various pathways (18).

Table 2 shows erythrocyte MDA levels in the six individuals analysed with DS caused by mosaic and in age-matched controls. The results indicate that MDA levels depend on the percentage distribution between diploid and trisomic cell lines. Compared to control subjects of similar ages, we observed that, in general, the higher the percentage of diploid cells, the lower are erythrocyte MDA levels; these lower levels could contribute to lower oxidative stress.

Table 3 shows erythrocyte MDA levels in four individuals with DS due to Robertsonians translocations of chromosome 21. The erythrocyte MDA levels obtained in these cases are similar to those observed in individuals with DS due to regular trisomy 21. These results are in accordance with those reported by Garber et al. (19) and Jeziorowska et al. (20), who reported elevated SOD-1 activity in red blood cells in cases of translocation trisomy 21 t(q21q21).

Higher mean levels of biomarkers of oxidative stress indicate an increased rate of oxidative damage

Table 3 Erythrocyte MDA levels in individuals with DS due to translocations.

Karyotype	Age	Translocation	MDA, nm/mg Hb	MDA, nm/mg Hb ^a
46,XY,der (14;21) (q10;q10)	3 months	Robertsonians 14: 21	109.07	112.37±7.11
46,XY,der (14;21) (q10;q10)	17 years	Robertsonians 14: 21	229.01	128.19±5.28
46,XY,der (14;21) (q10;q10)+21	1 year	Robertsonians 21: 21	111.39	112.37±7.11
47,XY,t (10;21) (q22;q22)+21	17 years	Reciprocal 10: 21	228.04	228.19±5.28

^aMean±SD MDA level in age-matched individuals with regular trisomy 21.

in individuals with DS characterised by excess SOD-1 activity in all cells (21). It has been argued (22, 23) that excess SOD-1 can catalyse surrogate reactions, a peroxidase function that generates hydroxyl radicals from hydrogen peroxide. The hydroxyl radical is highly damaging. It reacts with DNA and RNA to produce altered base products, cross-links and strand breaks, with proteins to inactivate enzymes, and with lipids to initiate lipid peroxidation by oxidation of polyunsaturated fatty acids (24). The involvement of hydroxyl radicals in DS could be caused by the increase in erythrocyte MDA levels, an end product of lipid peroxidation.

In summary, our data are consistent with the hypothesis that an imbalance in antioxidant enzymes may have adverse effects on cell membranes through the indiscriminate oxidation of susceptible molecules such as polyunsaturated fatty acids. Cells need to maintain a balance between the first- and second-step antioxidant enzymes to prevent the excessive generation of hydroxyl radicals. We conclude that erythrocyte MDA levels in DS with regular trisomy 21 and in DS with Rb translocation trisomy 21 (which are similar to regular trisomy 21 because the Rb translocation includes gene for SOD-1) are age-dependent and depend on the oxidative imbalance of the antioxidant enzymes in these individuals. Further studies involving individuals with mosaic DS will help to elucidate the relationship between erythrocyte MDA levels and the consequences of oxidative stress.

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