Clin Chem Lab Med 2007;45(1):59–62 © 2007 by Walter de Gruyter • Berlin • New York. DOI 10.1515/CCLM.2007.011

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

Ángela Casado*, Mª Encarnación López-Fernández and Rocío Ruíz

Departamento de Fisiopatología y Genética Molecular Humana, Centro de Investigaciones Biológicas (CSIC), Madrid, Spain

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 polyunsatured 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.
Clin Chem Lab Med 2007;45:59–62.

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 chromosome 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 ($O_2^{\bullet -}$), hydrogen peroxide (H_2O_2), and the hydroxyl radical ($OH^{\bullet}$) (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

*Corresponding author: Dra. Ángela Casado, Departamento de Fisiopatología y Genética Molecular Humana, Centro de Investigaciones Biológicas (CSIC), c/ Ramiro de Maeztu, 9, 28040 Madrid, Spain
Phone: +34-91-8373112, Fax: +34-91-5360432,
E-mail: acasado@cib.csic.es

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 handing 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-μm 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 × 4.6 mm, 5 μm) (Shandon Scientific Ltd., Runcorn, Cheshire, UK) was used as the stationary phase. The chromatographic conditions employed were: mobile phase, PO_4HNa_2 and myristyl trimethylammonium bromide buffer (pH 7.4)/acetonitrile; flow rate, 0.4 m/min; UV detection, 268 nm; chart speed, 0.5 cm/min; attenuation, 6; temperature, ambient; and injection volume, 10 μl.

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 ± 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 ± 5.14	20	86.92 ± 5.14	< 0.05*
2–4	17	136.25 ± 5.06	20	114.36 ± 5.32	< 0.05*
5–9	18	161.39 ± 5.34	18	132.68 ± 3.09	< 0.05*
10–14	14	184.75 ± 4.16	15	156.11 ± 5.02	< 0.05*
15–19	14	228.19 ± 5.28	15	189.36 ± 4.71	< 0.05*
20–29	11	263.36 ± 6.15	12	217.22 ± 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

[a] Mean±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: MDA level $=30.20\times$age$+75.84$; $r^2=0.9885$; $p<0.05$; controls: MDA level $=25.69\times$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;10)	3 months	Robertsonians 14: 21	109.07	112.37±7.11
46,XY,der (14;21) (q10;10)	17 years	Robertsonians 14: 21	229.01	128.19±5.28
46,XY,der (14;21) (q10;10)+21	1 year	Robertsonians 21: 21	111.39	112.37±7.11
47,XY,t (10;21) (q22;22)+21	17 years	Reciprocal 10: 21	228.04	228.19±5.28

[a] Mean±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.

Acknowledgements

This research project was supported by Foundation Inocente, Inocente, and was rewarded with the Ramón Trias Fargas award for research on Down syndrome. The authors wish to thank Dr. José Antonio Abrisqueta, Dr. Vitalino Aller and Dr. Juan Menaya [Human Genetics Unit, Centre for Biological Investigations (CSIC), Madrid] for their cytogenetic examinations. The authors express their gratitude to Dr. Jesús García and Dr. Julián Lirio (Social Paediatrics Unit, Niño Jesus Hospital, Madrid), and Dr. Alfonso Cañete and Dr. Romero (Paediatric Service, San Rafael Hospital, Madrid) for their valuable contributions to this work. They also thank Miss María Burgos for her excellent review of the English. Thanks are due to the Fundación Inocente, Inocente, which made it possible to carry out this work.

References

1. Lejeune J, Gautier M, Turpin R. Etude des chromosomes somatiques de 9 enfants mongoliens. CR Acad Sci Paris 1959;248:1721–8.
2. Hook EB. Down's syndrome-frequency in human populations and factors pertinent to variation in rates. In: de la Cruz FF, Gerald PS, editors. Trisomy 21 (Down syndrome): research perspectives. Baltimore: University Park Press, 1981:3–68.
3. Halliwell B, Gutteridge JM. Oxygen toxicity, oxygen radicals, transition metals and disease. Biochem J 1984; 219:1–14.
4. Halliwell B, Gutteridge JM. Free radicals in biology and medicine. Oxford: Clarendon Press, 1985.
5. Draper HH, Dhanakoti SN, Hadley M, Piche LA. Malondialdehyde in biological systems. In: Chow CK, editor. Cellular antioxidant defense mechanism. Boca Raton, FL: CRC Press, 1988:97–100.
6. Ambrosio G, Flaherty JJ. Oxygen radicals generated at reflow induce peroxidation of membrane lipids in reperfused heart. J Clin Invest 1991;87:2056–66.
7. Wong SH, Knight JA, Hopfer SM, Zaharia O, Leach CN, Sunderman FW. Lipoperoxides in plasma as measured by liquid-chromatographic separation of malondialdehyde-thiobarbituric acid adduct. Clin Chem 1987;33: 214–20.
8. Bull AW, Marnett LJ. Determination of malondialdehyde by ion-pairing high-performance liquid chromatography. Anal Biochem 1985;149:284–90.
9. Esterbauer H, Lang J, Zadravec S, Slater F. Detection of malondialdehyde by high-performance liquid chromatography. In: Packer L, editor. Oxygen radical in biological systems. Methods in enzymology, vol 105. London: Academic Press, 1984:319–27.
10. Gil P, Fariñas F, Casado A, López-Fernández ME. Malondialdehyde: a possible marker of ageing. Gerontology 2002;48:209–14.
11. Inal ME, Kanbak G, Sunal E. Antioxidant enzyme activities and malondialdehyde levels related to aging. Clin Chim Acta 2001;305:75–80.
12. Inal ME, Sunal E, Kanbak G, Zeytinoglu S. Effects of postmenopausal hormone replacement and α-tocopherol on the lipid profiles and antioxidant status. Clin Chim Acta 1997;268:21–9.
13. Rodriguez MM, Ruiz TA. Homeostasis between lipid peroxidation and antioxidant enzyme activities in healthy human aging. Mech Ageing Dev 1992;66:213–22.
14. Nohl H. Involvement of free radicals in ageing: a consequence or cause senescence. Br Med Bull 1991;49: 653–67.
15. Jovanovic SV, Clements D, MacLeod K. Biomarkers of oxidative stress are significantly elevated in Down syndrome. Free Radic Biol Med 1998;25:1044–8.
16. Muchová J, Sustrová M, Garaiová I, Liptákova A, Blazicek P, Kvasnicka P, et al. Influence of age on activities of antioxidant enzymes and lipid peroxidation products in erythrocytes and neutrophils of Down syndrome. Free Radic Biol Med 2001;31:499–508.
17. Zitnanova I, Korytar P, Sobotová H, Horáková L, Sustrová M, Pueschel S, et al. Markers of oxidative stress in children with Down syndrome. Clin Chem Lab Med 2006; 44:306–10.
18. Srivastava S, Chandra A, Wang LF, Seifert WE, Dague BB, Ansari NH, et al. Metabolism of the lipid peroxidation product 4-hydroxy-trans-nonenal in isolated perfused rat heart. J Biol Chem 1998;273:10893–900.
19. Garber P, Sinet P, Lejeune J. Copper zinc SOD activity in trisomy 21 by translocation. Lancet 1979;ii:914–8.
20. Jeziorowska A, Jakubowski L, Armatys A, Kaluzewski B. Copper/zinc superoxide dismutase (SOD-1) activity in regular trisomy 21, trisomy 21 by translocation and mosaic trisomy 21. Clin Genet 1982;22:160–4.
21. De la Torre R, Casado A, López-Fernández ME, Carrascosa D, Ramirez V, Sáez J. Overexpression of copper-zinc superoxide dismutase in trisomy 21. Experientia 1996;52:871–3.
22. Yim MB, Chock PB, Stadman ER. Enzyme function of copper, zinc superoxide dismutase as a free radical generator. J Biol Chem 1993;268:4099–105.
23. Yim MB, Chock PB, Stadman ER. Copper, zinc superoxide dismutase catalyzes hydroxyl radical production from hydrogen peroxide. Proc Natl Acad Sci USA 1990; 87:5006–10.
24. Pastor MC, Sierra C, Dolade M, Navarro E, Brandi N, Cabre E, et al. Antioxidant enzymes and fatty acid status in erythrocytes of Down syndrome patients. Clin Chem 1998;44:924–9.

Received August 2, 2006, accepted October 10, 2006