Oxidative stress in Portuguese children with Down syndrome

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Abstract -
Background - Individuals with Down syndrome have an accelerated process of ageing which is thought to be associated with oxidative stress.
Aim - Since Zn/Cu superoxide dismutase is increased by about 50% in children with Down syndrome, glutathione and other less known antioxidant mechanisms were studied to determine whether there were changes in reactive oxygen species.
Methods - Plasma reduced and oxidised glutathione and red blood cells enzymes including acid phosphatase, methemoglobin reductase and transmembrane reductase were evaluated in Portuguese children with Down syndrome and their siblings, who were used as a control group.
Results - No significant differences were found between the study and control groups. A negative correlation was noted between total glutathione and acid phosphatase in the siblings without Down syndrome, but not in the children with Down syndrome.
Conclusion - Although it is claimed that the production of hydrogen peroxide is enhanced in children with Down syndrome, their antioxidant mechanisms do not seem to be significantly different compared with their siblings. This may result in an excess of reactive oxygen species that could help to explain accelerated ageing in children with Down syndrome. Further studies will be needed to shed light on these mechanisms.

Keywords: Down syndrome, ageing, oxidative stress, glutathione

Introduction

Individuals with Down syndrome seem to have an accelerated process of ageing (Carmeliet, David & Cassiman, 1991; Prasher, 1993; Pueschel, 1990), evidenced by early onset of cataracts and the risk of developing Alzheimer's disease. Ageing is believed to be a condition associated with free radical production (Busciglio & Yanker, 1995; Crastes de Paulet, 1990; Rubin, Gatchalian, Rimon & Brooks, 1994).

During oxidative stress, harmful reactive oxygen species are generated (Crastes de Paulet, 1990). Superoxide radicals are converted to hydrogen peroxide by Zn/Cu superoxide dismutase (SOD) (De-Haan, Cristiano, Innello & Kola, 1995; Gerli *et al.*, 1990). Thereafter, several enzyme systems, including glutathione peroxidase (GPX) and catalase (CAT), independently convert it to water (De-Haan *et al.*, 1995; Gerli *et al.*, 1990), see Figure 1.

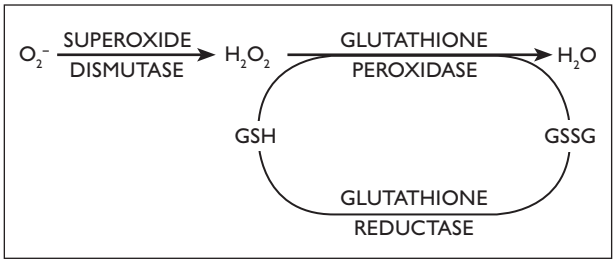

Figure 1. Glutathione peroxidase (GPX) role in oxidative stress and its interaction with superoxide dismutase (SOD).

The SOD gene locus resides on chromosome 21 and as a consequence of gene dosage excess, SOD activity has been shown to be increased by about 50% in all tissues of patients with Down syndrome (Brooksbank & Balzas, 1983; Ceballos-Picot, 1993; De La Torre *et al.*, 1996). An imbalance between SOD activity and GPX was proposed as an important determinant of molecular ageing, since the

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resultant hydroxyl radicals which are highly reactive could cause damage to macromolecules such as DNA, protein and lipids (Bar-Peled, Korkotian, Segal & Groner, 1996; De-Haan, *et al.*, 1996). These free radicals are normally neutralised by free radical scavengers and other antioxidant enzyme systems (Gerli *et al.*, 1990).

Glutathione (GSH) represents a well-known major physiological mechanism of response to oxidative stress, interacting with SOD, as shown in Figure 1 (De-Haan *et al.*, 1996; Ishikawa & Sies, 1989). Acid phosphatase (ACP1) is a tyrosine phosphatase protein, and is affected by reactive oxygen species. It has a role as flavin mononucleotide (FMN) phosphatase and subsequently interferes with glutathione reductase activity (Chiarugi, *et al.*, 1996; Fuchs, Shekels & Bernlohr, 1992; Gerli, *et al.*, 1990; Magenis, *et al.*, 1975; Mohreweiser & Novotny, 1982).

Methemoglobin reductase (MHR) is an erythrocyte cytosolic system responsible for reactivating haemoglobin's ability to transport oxygen after it has been converted into methemoglobin by reactive oxygen species (Board & Pidcock, 1981; Hatherill, Till & Ward, 1991). Transmembrane reductase of ferricyanide (TMR) expresses the existence of an erythrocyte transmembrane redox system responsible for, among others, recycling antioxidants (Hatherill *et al.*, 1991; May, Qu & Morrow, 1996; Orringer & Roer, 1979).

Our purpose was to study some of these systems including plasma GSH and red cell ACP1, MHR and TMR activities in a sample of Portuguese children with Down syndrome. Considering the possibility of intervening factors from the environment, we decided to study the siblings of children with Down syndrome as a control group. We believe that by doing this the two groups would have similar environmental exposure, nutrition and social, cultural and genetic background.

Population and methods

During a visit to the Child Development Centre of the Paediatric Department of the Santa Maria University Hospital of Lisbon, the aims of the study were explained to the parents of children with Down syndrome. They were then asked to give consent for their children to be enrolled in our study and to bring their other children on the next visit.

All children attending the clinic during the time period of the study were invited to take part. All but three families with non-Down syndrome siblings agreed to participate. The three families not participating were from a distant part of the country. Two groups were defined: 60 children with Down syndrome and 29 siblings without Down syndrome. The mean age of the children with Down syndrome was 3.6 years (SD 3.33; range 0.5 to 12 years), and in the population of siblings without Down syndrome the mean age was 7.3 years (SD 4.48; range 1 to 17 years). There was a significant age difference between the two groups, t(82) = -4.153, p = .0001. The sex distribution in the population with Down syndrome was 43% females and 57% males; in the sibling group the distribution was 51% and 48%, respectively.

Blood samples of children with Down syndrome and their siblings were collected by venipuncture and were analysed blind by the Genetic Laboratory of the Faculty of Medicine of Lisbon, between the 1st of April of 1995 and the 1st of April of 1996. Each of the tests was performed by an experienced technician, replicated three times and the mean was used as the test result.

Plasma GSH/GSSG was measured by an adapted fluorimetric assay of Hissin and Hilf (1976) and expressed as µg/g protein. Red blood cell TMR was performed using a method modified by Orringer and Roer (1979), expressed as mmol/l cell/h. MHR in the same cells was evaluated by a spectrophotometric assay described by Board and Pidcock (1981), expressed as µmol/g Hb/min, and ACP1 activity was measured by the method of Magenis, *et al.* (1975), expressed as µmol/g Hb/min.

Data are presented as mean (standard error of the mean) and analysed with the paired t Student test and significance was accepted for p value < 0.05. The statistical software "Primer of Biostatistics, version 3.02" was used.

Results

Plasma glutathione levels (reduced and oxidised forms) are presented in Table 1. The siblings without Down syndrome had higher concentrations of GSH (reduced form), GSSG (oxidised form) and total GSH, but the difference was not statistically significant.

Red blood cell activity of ACP1, TMR and MHR is presented in Table 2. All enzymatic systems show more enhanced activity in the population without Down syndrome than in the children with Down syndrome, however, there was no statistically significant difference between the two groups.

	Children with Down syndrome (n=60) (µg/g protein) mean ± sem	**Siblings without Down syndrome (n=29) (µg/g protein) mean ± sem**	**t**	**p value**
GSH	34.02 ± 2.03	37.71 ± 3.40	-0.98	> .05
GSSG	2.32 ± 0.17	2.74 ± 0.30	-1.28	> .05
Total	36.34 ± 2.03	40.45 ± 3.47	0.67	> .05
GSH/Total	0.93 ± 0.05	0.92 ± 0.05	-9.01	> .05

Table 1. Glutathione concentrations (reduced and oxidised) in children with Down syndrome and their siblings.

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Correlation analysis between ACP1 and TMR and MHR activities was performed and Pearson coefficients are presented in Table 3. In our samples the r-values showed weak, non-significant, correlations. Correlation analysis between ACP1 and GSH concentrations was performed and the Pearson coefficients are presented in Table 4. The r-value showed a negative and significant correlation, only in the children without Down syndrome, for ACP1 and GSH/GSH+GSSG. None of the other results were significant.

	Children with Down syndrome		Siblings without Down syndrome		t	p value
	n	mean ± sem	n	mean ± sem		
ACP1 (μmol/g Hb/min)	30	294.87 ± 31.95	16	332.93 ± 43.91	-.70	> .05
MHR(μmol/g Hb/min)	42	32.90 ± 2.62	16	34.48 ± 2.95	-.34	> .05
TMR(mmol/l cell/h)	60	4.88 ± 0.40	27	4.98 ± 0.61	-.23	> .05

Table 2. Red cells acid phosphatase (ACP1), methemoglobin reductase (MHR) and transmembrane reductase (TMR) activities in children with Down syndrome and their siblings.

	Children with Down syndrome	Siblings without Down syndrome
	r	r
ACP1 vs THR	-.16	-.31
ACP1 vs MHR	-.12	.11
MHR vs TMR	.24	.14

All correlation coefficients non significant at the 5% level.

Table 3. Correlation coefficients (Pearson's r) between red cells acid phosphatase (ACP1), transmembrane reductase (TMR) and methemoglobin reductase (MHR) in children with Down syndrome compared with their siblings.

	Children with Down syndrome	Siblings without Down syndrome
	r	r
ACP1 vs GSH	.10	-.40
ACP1 vs GSH/Total	-.14	-.76*

* $p < .05$, strong negative significant correlation

Table 4. Correlation coefficients (Pearson's r) between red cells acid phosphatase (ACP1) and absolute (GSH) and relative (GSH/total) reduced glutathione concentrations in children with Down syndrome compared with their siblings.

Discussion

There was a high level of agreement to take part in the study by the families and the high rate of involvement of siblings demonstrated willingness to contribute to greater knowledge in this area.

TMR, MHR and ACP1 are, as stated above, important during oxidative stress, preventing the formation or converting reactive oxygen species, in order to reduce tissue damage. In the current samples, no difference was found in the two groups between these systems. Thus, in children with Down syndrome no special changes in the anti-oxidant system seem to have been produced to compensate for the higher levels of reactive oxygen species. However, we must consider that this study has some limitations since SOD should have also been measured.

Another limitation is that using siblings as a comparison group, the age difference between the two groups can introduce some bias, and this must be taken into account in interpreting the results.

In children without Down syndrome there is a negative correlation between ACP1 and relative GSH concentrations, since ACP1 modulates glutathione reductase activity. In the group of children with Down syndrome this correlation was not found. This might be associated with a metabolic imbalance, resulting in the accumulation of reactive species, which may be responsible for the premature ageing and tissue damage found in Down syndrome. Further studies will be needed to help clarify these mechanisms.

Abbreviations

ACP1 – acid phosphatase

CAT – catalase

FMN – flavin mononucleotide

GPX – glutathione peroxidase

GSH – plasma reduced glutathione

GSSG – oxidised glutathione

MHR – methemoglobin reductase

SOD – Zn/Cu superoxide dismutase

TMR – transmembrane reductase of ferricyanide

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